Bedtools Documentation

Release 2.31.0

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Contents

Collectively, the **bedtools** utilities are a swiss-army knife of tools for a wide-range of genomics analysis tasks. The most widely-used tools enable *genome arithmetic*: that is, set theory on the genome. For example, **bedtools** allows one to *intersect*, *merge*, *count*, *complement*, and *shuffle* genomic intervals from multiple files in widely-used genomic file formats such as BAM, BED, GFF/GTF, VCF. While each individual tool is designed to do a relatively simple task (e.g., *intersect* two interval files), quite sophisticated analyses can be conducted by combining multiple bedtools operations on the UNIX command line.

bedtools is developed in the Quinlan laboratory at the University of Utah and benefits from fantastic contributions made by scientists worldwide.

CHAPTER 1

Tutorial

- We have developed a fairly comprehensive tutorial that demonstrates both the basics, as well as some more advanced examples of how bedtools can help you in your research. Please have a look.
- Robert Aboukhalil has developed sandbox.bio an excellent, web-based playground for the bedtools tutorial and other widely-used genomics tools.

CHAPTER 2

Important notes

- As of version 2.28.0, bedtools now supports the CRAM format via the use of htslib. Specify the reference genome associated with your CRAM file via the CRAM_REFERENCE environment variable. Bedtools will look for this environment variable when it needs to access sequence data from the CRAM file (e.g., *bamtofastq*).
- With the exception of BAM files, *bedtools* assumes all input files are TAB delimited.
- *bedtools* also assumes that all input files use UNIX line endings.
- Unless you use the *-sorted* option, *bedtools* currently does not support chromosomes larger than 512Mb
- When using the -sorted option with files whose chromosomes are not lexicographically sorted (e.g., sort -k1,1 -k2,2n for BED files), one must provide a genome file (*-g*) defining the expected chromosome order.
- *bedtools* requires that chromosome naming schemes are identical in files that you are comparing (e.g., 'chr1' in one file and '1' in another won't work).
- .fai files may be used as genome (*-g*) files.

CHAPTER 3

Interesting Usage Examples

In addition, here are a few examples of how bedtools has been used for genome research. If you have interesting examples, please send them our way and we will add them to the list.

- Coverage analysis for targeted DNA capture. Thanks to Stephen Turner.
- Measuring similarity of DNase hypersensitivity among many cell types
- Extracting promoter sequences from a genome
- Comparing intersections among many genome interval files
- RNA-seq coverage analysis. Thanks to Erik Minikel.
- Identifying targeted regions that lack coverage. Thanks to Brent Pedersen.
- Calculating GC content for CCDS exons.
- Making a master table of ChromHMM tracks for multiple cell types.

CHAPTER 4

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4.1 Overview

4.1.1 Background

The development of bedtools was motivated by a need for fast, flexible tools with which to compare large sets of genomic features. Answering fundamental research questions with existing tools was either too slow or required modifications to the way they reported or computed their results. We were aware of the utilities on the UCSC Genome Browser and Galaxy websites, as well as the elegant tools available as part of Jim Kent's monolithic suite of tools ("Kent source"). However, we found that the web-based tools were too cumbersome when working with large datasets generated by current sequencing technologies. Similarly, we found that the Kent source command line tools often required a local installation of the UCSC Genome Browser. These limitations, combined with the fact that we often wanted an extra option here or there that wasn't available with existing tools, led us to develop our own from scratch. The initial version of bedtools was publicly released in the spring of 2009. The current version has evolved from our research experiences and those of the scientists using the suite over the last year. The bedtools suite enables one to answer common questions of genomic data in a fast and reliable manner. The fact that almost all the utilities accept input from "stdin" allows one to "stream / pipe" several commands together to facilitate more complicated analyses. Also, the tools allow fine control over how output is reported. The initial version of bedtools supported solely 6-column BED files. *However, we have subsequently added support for sequence alignments in* BAM *format, as well as for features in* GFF , *"blocked" BED format, and* VCF *format*. The tools are quite fast and typically finish in a matter of a few seconds, even for large datasets. This manual seeks to describe the behavior and available functionality for each bedtool. Usage examples are scattered throughout the text, and formal examples are provided in the last two sections, we hope that this document will give you a sense of the flexibility of the toolkit and the types of analyses that are possible with bedtools. If you have further questions, please join the bedtools discussion group, visit the Usage Examples on the Google Code site (usage, advanced usage), or take a look at the nascent "Usage From the Wild" page.

4.1.2 Summary of available tools.

bedtools support a wide range of operations for interrogating and manipulating genomic features. The table below summarizes the tools available in the suite.

Utility	Description
annotate	Annotate coverage of features from multiple files.
bamtobed	Convert BAM alignments to BED (& other) formats.
bamtofastq	Convert BAM records to FASTQ records.
bed12tobed6	Breaks BED12 intervals into discrete BED6 intervals.
bedpetobam	Convert BEDPE intervals to BAM records.
bedtobam	Convert intervals to BAM records.
closest	Find the closest, potentially non-overlapping interval.
cluster	Cluster (but don't merge) overlapping/nearby intervals.
complement	Extract intervals _not_ represented by an interval file.
coverage	Compute the coverage over defined intervals.
expand	Replicate lines based on lists of values in columns.
fisher	Calculate Fisher statistic b/w two feature files.
flank	Create new intervals from the flanks of existing intervals.
genomecov	Compute the coverage over an entire genome.
getfasta	Use intervals to extract sequences from a FASTA file.
groupby	Group by common cols. & summarize oth. cols. (~ SQL "groupBy")
igv	Create an IGV snapshot batch script.
intersect	Find overlapping intervals in various ways.
jaccard	Calculate the Jaccard statistic b/w two sets of intervals.
links	Create a HTML page of links to UCSC locations.
makewindows	Make interval "windows" across a genome.
map	Apply a function to a column for each overlapping interval.
maskfasta	Use intervals to mask sequences from a FASTA file.
merge	Combine overlapping/nearby intervals into a single interval.
multicov	Counts coverage from multiple BAMs at specific intervals.
multiinter	Identifies common intervals among multiple interval files.
nuc	Profile the nucleotide content of intervals in a FASTA file.
overlap	Computes the amount of overlap from two intervals.
pairtobed	Find pairs that overlap intervals in various ways.
pairtopair	Find pairs that overlap other pairs in various ways.
random	Generate random intervals in a genome.
reldist	Calculate the distribution of relative distances b/w two files.
tools/sample	Sample random records from file using reservoir sampling.
shift	Adjust the position of intervals.
shuffle	Randomly redistribute intervals in a genome.
slop	Adjust the size of intervals.
sort	Order the intervals in a file.
tools/spacing	Sample random records from file using reservoir sampling.
tools/split	Split a file into multiple files with equal records or base pairs.
subtract	Remove intervals based on overlaps b/w two files.
tag	Tag BAM alignments based on overlaps with interval files.
unionbedg	Combines coverage intervals from multiple BEDGRAPH files.
window	Find overlapping intervals within a window around an interval.

4.1.3 Fundamental concepts.

What are genome features and how are they represented?

Throughout this manual, we will discuss how to use bedtools to manipulate, compare and ask questions of genome "features". Genome features can be functional elements (e.g., genes), genetic polymorphisms (e.g. SNPs, INDELs,

or structural variants), or other annotations that have been discovered or curated by genome sequencing groups or genome browser groups. In addition, genome features can be custom annotations that an individual lab or researcher defines (e.g., my novel gene or variant).

The basic characteristics of a genome feature are the chromosome or scaffold on which the feature "resides", the base pair on which the feature starts (i.e. the "start"), the base pair on which feature ends (i.e. the "end"), the strand on which the feature exists (i.e. "+" or "-"), and the name of the feature if one is applicable.

The two most widely used formats for representing genome features are the BED (Browser Extensible Data) and GFF (General Feature Format) formats. bedtools was originally written to work exclusively with genome features described using the BED format, but it has been recently extended to seamlessly work with BED, GFF and VCF files.

Existing annotations for the genomes of many species can be easily downloaded in BED and GFF format from the UCSC Genome Browser's "Table Browser" (http://genome.ucsc.edu/cgi-bin/hgTables?command=start) or from the "Bulk Downloads" page (http://hgdownload.cse.ucsc.edu/downloads.html). In addition, the Ensemble Genome Browser contains annotations in GFF/GTF format for many species (http://www.ensembl.org/info/data/ftp/index.html)

Overlapping / intersecting features.

Two genome features (henceforth referred to as "features") are said to overlap or intersect if they share at least one base in common. In the figure below, Feature A intersects/overlaps Feature B, but it does not intersect/overlap Feature C.

TODO: place figure here

Comparing features in file "A" and file "B".

The previous section briefly introduced a fundamental naming convention used in bedtools. Specifically, all bedtools that compare features contained in two distinct files refer to one file as feature set "A" and the other file as feature set "B". This is mainly in the interest of brevity, but it also has its roots in set theory. As an example, if one wanted to look for SNPs (file A) that overlap with exons (file B), one would use bedtools intersect in the following manner:

```
bedtools intersect -a snps.bed -b exons.bed
```

There are two exceptions to this rule: 1) When the "A" file is in BAM format, the "-abam" option must be used. For example:

```
bedtools intersect -abam alignedReads.bam -b exons.bed
```

And 2) For tools where only one input feature file is needed, the "-i" option is used. For example:

```
bedtools merge -i repeats.bed
```

BED starts are zero-based and BED ends are one-based.

bedtools users are sometimes confused by the way the start and end of BED features are represented. Specifically, bedtools uses the UCSC Genome Browser's internal database convention of making the start position 0-based and the end position 1-based: (http://genome.ucsc.edu/FAQ/FAQtracks#tracks1) In other words, bedtools interprets the "start" column as being 1 basepair higher than what is represented in the file. For example, the following BED feature represents a single base on chromosome 1; namely, the 1st base:

```
chr1    0       1    first_base
```

Why, you might ask? The advantage of storing features this way is that when computing the length of a feature, one must simply subtract the start from the end. Were the start position 1-based, the calculation would be (slightly) more complex (i.e. (end-start)+1). Thus, storing BED features this way reduces the computational burden.

GFF starts and ends are one-based.

In contrast, the GFF format uses 1-based coordinates for both the start and the end positions. bedtools is aware of this and adjusts the positions accordingly. In other words, you don't need to subtract 1 from the start positions of your GFF features for them to work correctly with bedtools.

VCF coordinates are one-based.

The VCF format uses 1-based coordinates. As in GFF, bedtools is aware of this and adjusts the positions accordingly. In other words, you don't need to subtract 1 from the start positions of your VCF features for them to work correctly with bedtools.

File B is loaded into memory (most of the time).

Whenever a bedtool compares two files of features, the "B" file is loaded into memory. By contrast, the "A" file is processed line by line and compared with the features from B. Therefore to minimize memory usage, one should set the smaller of the two files as the B file. One salient example is the comparison of aligned sequence reads from a current DNA sequencer to gene annotations. In this case, the aligned sequence file (in BED format) may have tens of millions of features (the sequence alignments), while the gene annotation file will have tens of thousands of features. In this case, it is wise to sets the reads as file A and the genes as file B.

Feature files *must* be tab-delimited.

This is rather self-explanatory. While it is possible to allow BED files to be space-delimited, we have decided to require tab delimiters for three reasons:

1. By requiring one delimiter type, the processing time is minimized.
2. Tab-delimited files are more amenable to other UNIX utilities.
3. GFF files can contain spaces within attribute columns. This complicates the use of space-delimited files as spaces must therefore be treated specially depending on the context.

All bedtools allow features to be "piped" via standard input.

In an effort to allow one to combine multiple bedtools and other UNIX utilities into more complicated "pipelines", all bedtools allow features to be passed to them via standard input. Only one feature file may be passed to a bedtool via standard input. The convention used by all bedtools is to set either file A or file B to "stdin" or "-". For example:

```
cat snps.bed | bedtools intersect -a stdin -b exons.bed
cat snps.bed | bedtools intersect -a - -b exons.bed
```

In addition, all bedtools that simply require one main input file (the -i file) will assume that input is coming from standard input if the -i parameter is ignored. For example, the following are equivalent:

```
cat snps.bed | bedtools sort -i stdin
cat snps.bed | bedtools sort
```

Most bedtools write their results to standard output.

To allow one to combine multiple bedtools and other UNIX utilities into more complicated "pipelines", most bedtools report their output to standard output, rather than to a named file. If one wants to write the output to a named file, one can use the UNIX "file redirection" symbol ">" to do so. Writing to standard output (the default):

```
bedtools intersect -a snps.bed -b exons.bed
chr1 100100 100101 rs233454
chr1 200100 200101 rs446788
chr1 300100 300101 rs645678
```

Writing to a file:

```
bedtools intersect -a snps.bed -b exons.bed > snps.in.exons.bed

cat snps.in.exons.bed
chr1 100100 100101 rs233454
chr1 200100 200101 rs446788
chr1 300100 300101 rs645678
```

What is a "genome" file?

Some of the bedtools (e.g., `genomecov`, `complement`, `slop`) need to know the size of the chromosomes for the organism for which your BED files are based. When using the UCSC Genome Browser, Ensemble, or Galaxy, you typically indicate which species / genome build you are working. The way you do this for bedtools is to create a "genome" file, which simply lists the names of the chromosomes (or scaffolds, etc.) and their size (in basepairs). Genome files must be tab-delimited and are structured as follows (this is an example for C. elegans):

```
chrI 15072421
chrII 15279323
...
chrX 17718854
chrM 13794
```

bedtools includes predefined genome files for human and mouse in the /genomes directory included in the bedtools distribution. Additionally, the "chromInfo" files/tables available from the UCSC Genome Browser website are acceptable. For example, one can download the hg19 chromInfo file here: http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/chromInfo.txt.gz

Paired-end BED files (BEDPE files).

We have defined a new file format (BEDPE) to concisely describe disjoint genome features, such as structural variations or paired-end sequence alignments. We chose to define a new format because the existing BED block format (i.e. BED12) does not allow inter-chromosomal feature definitions. Moreover, the BED12 format feels rather bloated when one want to describe events with only two blocks.

Use "-h" for help with any bedtool.

Rather straightforward. If you use the "-h" option with any bedtool, a full menu of example usage and available options (when applicable) will be reported.

BED features must not contain negative positions.

bedtools will typically reject BED features that contain negative positions. In special cases, however, BEDPE positions may be set to -1 to indicate that one or more ends of a BEDPE feature is unaligned.

The start position must be <= to the end position.

bedtools will reject BED features where the start position is greater than the end position.

Headers are allowed in GFF and BED files

bedtools will ignore headers at the beginning of BED and GFF files. Valid header lines begin with a "#" symbol, the work "track", or the word "browser". For example, the following examples are valid headers for BED or GFF files:

```
track name=aligned_read description="Illumina aligned reads"
chr5 100000 500000 read1 50 +
chr5 2380000 2386000 read2 60 -

#This is a fascinating dataset
chr5 100000 500000 read1 50 +
chr5 2380000 2386000 read2 60 -

browser position chr22:1-20000
chr5 100000 500000 read1 50 +
chr5 2380000 2386000 read2 60 -
```

GZIP support: BED, GFF, VCF, and BEDPE file can be "gzipped"

bedtools will process gzipped BED, GFF, VCF and BEDPE files in the same manner as uncompressed files. Gzipped files are auto-detected thanks to a helpful contribution from Gordon Assaf.

Support for "split" or "spliced" BAM alignments and "blocked" BED features

As of Version 2.8.0, five bedtools (`intersect`, `coverage`, `genomecov`, `bamToBed`, and `bed12ToBed6`) can properly handle "split"/"spliced" BAM alignments (i.e., having an "N" CIGAR operation) and/or "blocked" BED (aka BED12) features.

`intersect`, `coverage`, and `genomecov` will optionally handle "split" BAM and/or "blocked" BED by using the `-split` option. This will cause intersects or coverage to be computed only for the alignment or feature blocks. In contrast, without this option, the intersects/coverage would be computed for the entire "span" of the alignment or feature, regardless of the size of the gaps between each alignment or feature block. For example, imagine you have a RNA-seq read that originates from the junction of two exons that were spliced together in a mRNA. In the genome, these two exons happen to be 30Kb apart. Thus, when the read is aligned to the reference genome, one portion of the read will align to the first exon, while another portion of the read will align ca. 30Kb downstream to the other exon. The corresponding CIGAR string would be something like (assuming a 76bp read): 30M*3000N*46M. In the genome, this alignment "spans" 3076 bp, yet the nucleotides in the sequencing read only align "cover" 76bp. Without the `-split` option, coverage or overlaps would be reported for the entire 3076bp span of the alignment. However, with the `-split` option, coverage or overlaps will only be reported for the portions of the read that overlap the exons (i.e. 30bp on one exon, and 46bp on the other).

Using the -split option with bamToBed causes "spliced/split" alignments to be reported in BED12 format. Using the -split option with `bed12tobed6` causes "blocked" BED12 features to be reported in BED6 format.

Writing uncompressed BAM output.

When working with a large BAM file using a complex set of tools in a pipe/stream, it is advantageous to pass uncompressed BAM output to each downstream program. This minimizes the amount of time spent compressing and decompressing output from one program to the next. All bedtools that create BAM output (e.g. `intersect`, `window`) will now optionally create uncompressed BAM output using the `-ubam` option.

4.1.4 Implementation and algorithmic notes.

bedtools was implemented in C++ and makes extensive use of data structures and fundamental algorithms from the Standard Template Library (STL). Many of the core algorithms are based upon the genome binning algorithm described in the original UCSC Genome Browser paper (Kent et al, 2002). The tools have been designed to inherit core data structures from central source files, thus allowing rapid tool development and deployment of improvements and corrections. Support for BAM files is made possible through Derek Barnett's elegant C++ API called BamTools.

4.2 Installation

`bedtools` is intended to run in a "command line" environment on UNIX, LINUX and Apple OS X operating systems. Installing `bedtools` involves either downloading the source code and compiling it manually, or installing stable release from package managers such as homebrew (for OS X).

4.2.1 Installing stable releases

Downloading a pre-compiled binary

Note:

1. The following approach will only work for Linux (non-OSX) systems.

Starting with release 2.28.0, we provide statically-linked binaries thast should work right away on Linux systems. Go to the releases page and look for the static binary named *bedtools.static.binary*. Right click on it, get the URL, then download it with *wget* or *curl* and you should be good to go.

As an example. First, download *bedtools.static.binary* from the latest release here

```
mv bedtools.static.binary bedtools
chmod a+x bedtools
```

Compiling from source via Github

Stable, versioned releases of bedtools are made available on Github at the bedtools2 repository under the releases tab. The following commands will install `bedtools` in a local directory on an UNIX or OS X machine.

Note: 1. The bedtools Makefiles utilize the GCC compiler. One should edit the Makefiles accordingly if one wants to use a different compiler.

```
$ wget https://github.com/arq5x/bedtools2/releases/download/v2.29.1/bedtools-2.29.1.
↪tar.gz
$ tar -zxvf bedtools-2.29.1.tar.gz
$ cd bedtools2
$ make
```

At this point, one should copy the binaries in ./bin/ to either `usr/local/bin/` or some other repository for commonly used UNIX tools in your environment. You will typically require administrator (e.g. "root" or "sudo") privileges to copy to `usr/local/bin/`. If in doubt, contact you system administrator for help.

Installing with package managers

In addition, stable releases of `bedtools` are also available through package managers such as homebrew (for OS X), `apt-get` and `yum`.

Fedora/Centos. Adam Huffman has created a Red Hat package for bedtools so that one can easily install the latest release using "yum", the Fedora package manager. It should work with Fedora 13, 14 and EPEL5/6 (for Centos, Scientific Linux, etc.).

```
yum install BEDTools
```

Debian/Ubuntu. Charles Plessy also maintains a Debian package for bedtools that is likely to be found in its derivatives like Ubuntu. Many thanks to Charles for doing this.

```
apt-get install bedtools
```

Homebrew. Carlos Borroto has made BEDTools available on the bedtools package manager for OSX.

```
brew tap homebrew/science
brew install bedtools
```

MacPorts. Alternatively, the MacPorts ports system can be used to install BEDTools on OSX.

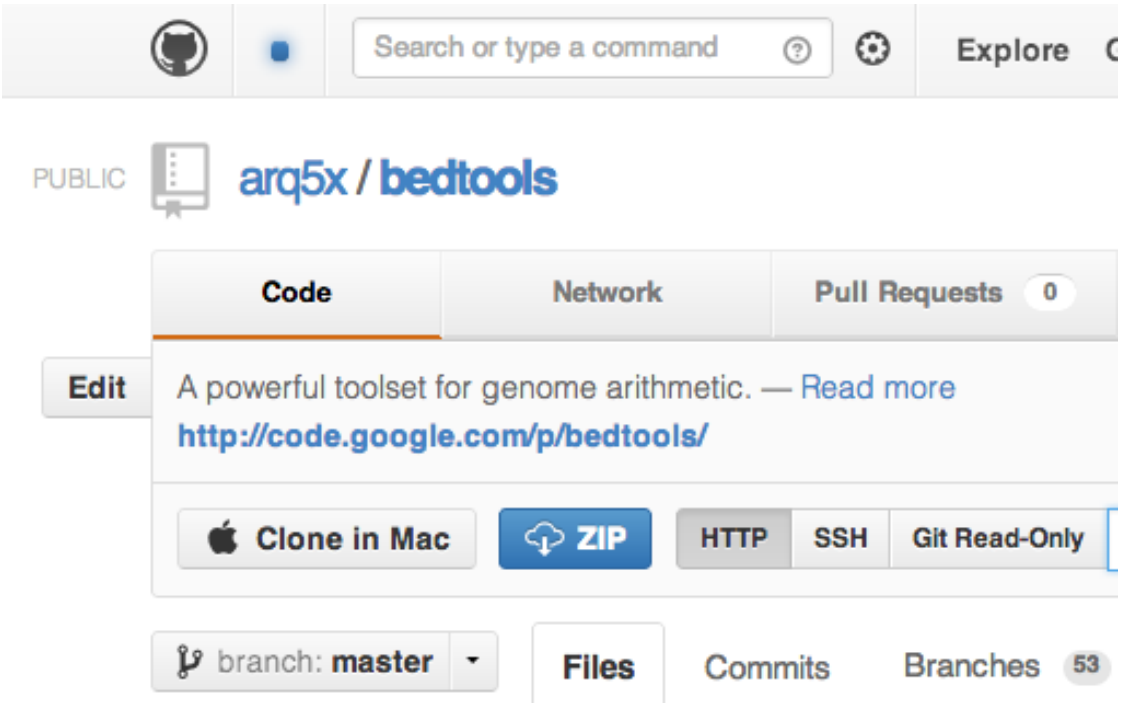
```
port install bedtools
```

4.2.2 Development versions

The development version of bedtools is maintained in a Github repository. Bug fixes are addressed in this repository prior to release, so there may be situations where you will want to use a development version of bedtools prior to its being promoted to a stable release. One would either clone the repository with `git`, as follows and then compile the source code as describe above:

```
git clone https://github.com/arq5x/bedtools2.git
```

or, one can download the source code as a `.zip` file using the Github website. Once the zip file is downloaded and uncompressed with the `unzip` command, one can compile and install using the instructions above.



4.3 Quick start

4.3.1 Install bedtools

```
curl http://bedtools.googlecode.com/files/BEDTools.<version>.tar.gz > BEDTools.tar.gz
tar -zxvf BEDTools.tar.gz
cd BEDTools
make
sudo cp bin/* /usr/local/bin/
```

4.3.2 Use bedtools

Below are examples of typical bedtools usage. Using the "-h" option with any bedtools will report a list of all command line options.

Report the base-pair overlap between the features in two BED files.

```
bedtools intersect -a reads.bed -b genes.bed
```

Report those entries in A that overlap NO entries in B. Like "grep -v"

```
bedtools intersect  -a reads.bed -b genes.bed -v
```

Read BED A from STDIN. Useful for stringing together commands. For example, find genes that overlap LINEs but not SINEs.

```
bedtools intersect -a genes.bed -b LINES.bed | \
  bedtools intersect -a stdin -b SINEs.bed -v
```

Find the closest ALU to each gene.

```
bedtools closest -a genes.bed -b ALUs.bed
```

Merge overlapping repetitive elements into a single entry, returning the number of entries merged.

```
bedtools merge -i repeatMasker.bed -n
```

Merge nearby repetitive elements into a single entry, so long as they are within 1000 bp of one another.

```
bedtools merge -i repeatMasker.bed -d 1000
```

4.4 General usage

4.4.1 Supported file formats

BED format

As described on the UCSC Genome Browser website (see link below), the browser extensible data (BED) format is a concise and flexible way to represent genomic features and annotations. The BED format description supports up to 12 columns, but only the first 3 are required for the UCSC browser, the Galaxy browser and for bedtools. bedtools allows one to use the "BED12" format (that is, all 12 fields listed below). However, only intersectBed, coverageBed, genomeCoverageBed, and bamToBed will obey the BED12 "blocks" when computing overlaps, etc., via the **"-split"** option. For all other tools, the last six columns are not used for any comparisons by the bedtools. Instead, they will use the entire span (start to end) of the BED12 entry to perform any relevant feature comparisons. The last six columns will be reported in the output of all comparisons.

The file description below is modified from: http://genome.ucsc.edu/FAQ/FAQformat#format1.

1. **chrom** - The name of the chromosome on which the genome feature exists.
   - *Any string can be used*. For example, "chr1", "III", "myChrom", "contig1112.23".
   - *This column is required*.
2. **start** - The zero-based starting position of the feature in the chromosome.
   - *The first base in a chromosome is numbered 0*.
   - *The start position in each BED feature is therefore interpreted to be 1 greater than the start position listed in the feature. For example, start=9, end=20 is interpreted to span bases 10 through 20,inclusive*.
   - *This column is required*.
3. **end** - The one-based ending position of the feature in the chromosome.
   - *The end position in each BED feature is one-based. See example above*.
   - *This column is required*.
4. **name** - Defines the name of the BED feature.
   - *Any string can be used*. For example, "LINE", "Exon3", "HWIEAS_0001:3:1:0:266#0/1", or "my_Feature".
   - *This column is optional*.
5. **score** - The UCSC definition requires that a BED score range from 0 to 1000, inclusive. However, bedtools allows any string to be stored in this field in order to allow greater flexibility in annotation features. For example, strings allow scientific notation for p-values, mean enrichment values, etc. It should be noted that this flexibility could prevent such annotations from being correctly displayed on the UCSC browser.

- *Any string can be used*. For example, 7.31E-05 (p-value), 0.33456 (mean enrichment value), "up", "down", etc.
- *This column is optional*.

6. **strand** - Defines the strand - either '+' or '-'.
   - *This column is optional*.
7. **thickStart** - The starting position at which the feature is drawn thickly.
   - *Allowed yet ignored by bedtools*.
8. **thickEnd** - The ending position at which the feature is drawn thickly.
   - *Allowed yet ignored by bedtools*.
9. **itemRgb** - An RGB value of the form R,G,B (e.g. 255,0,0).
   - *Allowed yet ignored by bedtools*.
10. **blockCount** - The number of blocks (exons) in the BED line.
    - *Allowed yet ignored by bedtools*.
11. **blockSizes** - A comma-separated list of the block sizes.
12. **blockStarts** - A comma-separated list of block starts.
    - *Allowed yet ignored by bedtools*.

bedtools requires that all BED input files (and input received from stdin) are **tab-delimited**. The following types of BED files are supported by bedtools:

1. **BED3**: A BED file where each feature is described by **chrom**, **start**, and **end**.

   For example: `chr1 11873 14409`
2. **BED4**: A BED file where each feature is described by **chrom**, **start**, **end**, and **name**.

   For example: `chr1 11873 14409 uc001aaa.3`
3. **BED5**: A BED file where each feature is described by **chrom**, **start**, **end**, **name**, and **score**.

   For example: `chr1 11873 14409 uc001aaa.3 0`
4. **BED6**: A BED file where each feature is described by **chrom**, **start**, **end**, **name**, **score**, and **strand**.

   For example: `chr1 11873 14409 uc001aaa.3 0 +`
5. **BED12**: A BED file where each feature is described by all twelve columns listed above.

   For example: `chr1 11873 14409 uc001aaa.3 0 + 11873 11873 0 3 354,109,1189, 0, 739,1347,`

BEDPE format

We have defined a new file format, the browser extensible data paired-end (BEDPE) format, in order to concisely describe disjoint genome features, such as structural variations or paired-end sequence alignments. We chose to define a new format because the existing "blocked" BED format (a.k.a. BED12) does not allow inter-chromosomal feature definitions. In addition, BED12 only has one strand field, which is insufficient for paired-end sequence alignments, especially when studying structural variation.

The BEDPE format is described below. The description is modified from: http://genome.ucsc.edu/FAQ/FAQformat#format1.

1. **chrom1** - The name of the chromosome on which the **first** end of the feature exists.

- *Any string can be used*. For example, "chr1", "III", "myChrom", "contig1112.23".
- *This column is required*.
- *Use "." for unknown*.

2. **start1** - The zero-based starting position of the **first** end of the feature on **chrom1**.
   - *The first base in a chromosome is numbered 0*.
   - *As with BED format, the start position in each BEDPE feature is therefore interpreted to be 1 greater than the start position listed in the feature. This column is required*.
   - *Use -1 for unknown*.
3. **end1** - The one-based ending position of the first end of the feature on **chrom1**.
   - *The end position in each BEDPE feature is one-based*.
   - *This column is required*.
   - *Use -1 for unknown*.
4. **chrom2** - The name of the chromosome on which the **second** end of the feature exists.
   - *Any string can be used*. For example, "chr1", "III", "myChrom", "contig1112.23".
   - *This column is required*.
   - *Use "." for unknown*.
5. **start2** - The zero-based starting position of the **second** end of the feature on **chrom2**.
   - *The first base in a chromosome is numbered 0*.
   - *As with BED format, the start position in each BEDPE feature is therefore interpreted to be 1 greater than the start position listed in the feature. This column is required*.
   - *Use -1 for unknown*.
6. **end2** - The one-based ending position of the **second** end of the feature on **chrom2**.
   - *The end position in each BEDPE feature is one-based*.
   - *This column is required*.
   - *Use -1 for unknown*.
7. **name** - Defines the name of the BEDPE feature.
   - *Any string can be used*. For example, "LINE", "Exon3", "HWIEAS_0001:3:1:0:266#0/1", or "my_Feature".
   - *This column is optional*.
8. **score** - The UCSC definition requires that a BED score range from 0 to 1000, inclusive. *However, bedtools allows any string to be stored in this field in order to allow greater flexibility in annotation features*. For example, strings allow scientific notation for p-values, mean enrichment values, etc. It should be noted that this flexibility could prevent such annotations from being correctly displayed on the UCSC browser.
   - *Any string can be used*. For example, 7.31E-05 (p-value), 0.33456 (mean enrichment value), "up", "down", etc.
   - *This column is optional*.
9. **strand1** - Defines the strand for the first end of the feature. Either '+' or '-'.
   - *This column is optional*.
   - *Use "." for unknown*.
10. **strand2** - Defines the strand for the second end of the feature. Either '+' or '-'.

- *This column is optional.*
- *Use "." for unknown.*

11. **Any number of additional, user-defined fields** - bedtools allows one to add as many additional fields to the normal, 10-column BEDPE format as necessary. These columns are merely "passed through" **pairToBed** and **pairToPair** and are not part of any analysis. One would use these additional columns to add extra information (e.g., edit distance for each end of an alignment, or "deletion", "inversion", etc.) to each BEDPE feature.

- *These additional columns are optional.*

Entries from an typical BEDPE file:

```
chr1  100   200   chr5  5000  5100  bedpe_example1  30   +  -
chr9  1000  5000  chr9  3000  3800  bedpe_example2  100  +  -
```

Entries from a BEDPE file with two custom fields added to each record:

```
chr1  10    20    chr5  50    60    a1     30       +    -  0  1
chr9  30    40    chr9  80    90    a2     100      +    -  2  1
```

GFF format

The GFF format is described on the Sanger Institute's website (http://www.sanger.ac.uk/resources/software/gff/spec.html). The GFF description below is modified from the definition at this URL. All nine columns in the GFF format description are required by bedtools.

1. **seqname** - The name of the sequence (e.g. chromosome) on which the feature exists.

- *Any string can be used.* For example, "chr1", "III", "myChrom", "contig1112.23".
- *This column is required.*

2. **source** - The source of this feature. This field will normally be used to indicate the program making the prediction, or if it comes from public database annotation, or is experimentally verified, etc.

- *This column is required.*

3. **feature** - The feature type name. Equivalent to BED's **name** field.

- *Any string can be used.* For example, "exon", etc.
- *This column is required.*

4. **start** - The one-based starting position of feature on **seqname**.

- *This column is required.*
- *bedtools accounts for the fact the GFF uses a one-based position and BED uses a zero-based start position.*

5. **end** - The one-based ending position of feature on **seqname**.

- *This column is required.*

6. **score** - A score assigned to the GFF feature. Like BED format, bedtools allows any string to be stored in this field in order to allow greater flexibility in annotation features. We note that this differs from the GFF definition in the interest of flexibility.

- *This column is required.*

7. **strand** - Defines the strand. Use '+', '-' or '.'

- *This column is required.*

8. **frame** - The frame of the coding sequence. Use '0', '1', '2', or '.'.

- *This column is required.*

9. **attribute** - Taken from http://www.sanger.ac.uk/resources/software/gff/spec.html: From version 2 onwards, the attribute field must have an tag value structure following the syntax used within objects in a .ace file, flattened onto one line by semicolon separators. Free text values must be quoted with double quotes. *Note: all non-printing characters in such free text value strings (e.g. newlines, tabs, control characters, etc) must be explicitly represented by their C (UNIX) style backslash-escaped representation (e.g. newlines as 'n', tabs as 't').* As in ACEDB, multiple values can follow a specific tag. The aim is to establish consistent use of particular tags, corresponding to an underlying implied ACEDB model if you want to think that way (but acedb is not required).

- *This column is required.*

An entry from an example GFF file :

```
seq1 BLASTX similarity 101 235 87.1 + 0 Target "HBA_HUMAN" 11 55 ;
E_value 0.0003 dJ102G20 GD_mRNA coding_exon 7105 7201 . - 2 Sequence
"dJ102G20.C1.1"
```

Genome file format

Some of the bedtools (e.g., genomeCoverageBed, complementBed, slopBed) need to know the size of the chromosomes for the organism for which your BED files are based. When using the UCSC Genome Browser, Ensemble, or Galaxy, you typically indicate which which species/genome build you are working. The way you do this for bedtools is to create a "genome" file, which simply lists the names of the chromosomes (or scaffolds, etc.) and their size (in basepairs).

Genome files must be **tab-delimited** and are structured as follows (this is an example for *C. elegans*):

```
chrI  15072421
chrII 15279323
...
chrX  17718854
chrM  13794
```

bedtools includes pre-defined genome files for human and mouse in the **/genomes** directory included in the bedtools distribution.

One can also create a suitable genome file by running *samtools faidx* on the appropriate FASTA reference genome. Then use the resulting .fai file as a genome file, as bedtools will only care about the first two columns, which define the chromosome name and length. For example:

```
# download GRCh38
wget ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/GRCh38_reference_
↪genome/GRCh38_full_analysis_set_plus_decoy_hla.fa
# create an index of it
samtools faidx GRCh38_full_analysis_set_plus_decoy_hla.fa
# use the .fai index as a genome file with bedtools
bedtools complement my.grch38.bed -g GRCh38_full_analysis_set_plus_decoy_hla.fa.fai
```

SAM/BAM format

The SAM / BAM format is a powerful and widely-used format for storing sequence alignment data (see http://samtools.sourceforge.net/ for more details). It has quickly become the standard format to which most DNA sequence alignment programs write their output. Currently, the following bedtools support input in BAM format: `intersect`, `window`,

`coverage`, `genomecov`, `pairtobed`, `bamtobed`. Support for the BAM format in bedtools allows one to (to name a few): compare sequence alignments to annotations, refine alignment datasets, screen for potential mutations and compute aligned sequence coverage.

VCF format

The Variant Call Format (VCF) was conceived as part of the 1000 Genomes Project as a standardized means to report genetic variation calls from SNP, INDEL and structural variant detection programs (see http://www.1000genomes.org/wiki/doku.php?id=1000_genomes:analysis:vcf4.0 for details). bedtools now supports the latest version of this format (i.e, Version 4.0). As a result, bedtools can be used to compare genetic variation calls with other genomic features.

4.5 Release History

4.5.1 Version 2.30.0 (23-Dec-2021)

1. Thanks to Hao Hou (github: @38), we have substantial improvements in the speed associated with parsing input files and in printing results. It turns our that these tasks consume a large proportion of run time, especially as input files increase in size. These changes result in a 2-3X improvement in speed, depending on input types, options, etc.
2. Thanks to John Marshall (github: @jmarshall), who improved the stability and cleanliness of the code used for random number generation. These changes also squash a bug that arises on Debian systems.
3. John Marshall cleaned up some lingering data type problems in the *slop* tool.
4. Thanks to @gringer for adding teh *-ignoreD* option to the *genomecov* tool, which allows *D* CIGAR operations to be ignored when calculating coverage. This is useful for long-read technologies with high INDEL error rates.
5. Added a fix for a bug that did not properly handle the splitting of intervals in BED12 records with one block.
6. Thanks to John Marshall (github: @jmarshall), we have addressed numerical instability issues in the *fisher* tool.
7. Thanks to Hao Hou (github: @38), reference genomes can be read as an environment variable (*CRAM_REFERENCE*) when using CRAM input files.
8. Added a *-rna* option to the *getfasta* tool to allow support for RNA genomes.
9. Thanks to Hao Hou (github: @38), we fixed input file format detection bugs arising in ZSH.
10. Thanks to Josh Shapiro (github:@jashapiro) for clarifying a confusing inconcistency in the documentation for the *coverage* tool.
11. Thanks to Hao Hou (github: @38), we suppressed unnecessary warnings when reading GZIPP'ed files.
12. Thanks to Hao Hou (github: @38), we fixed an overflow bug in the *shuffle* tool.
13. Thanks to Hao Hou (github: @38), we fixed an data type bug in the *shift* tool.
14. Thanks to John Marshall (github: @jmarshall) and Hao Hou (github: @38), we have cleaned up the internal support for htslib.

4.5.2 Version 2.29.2 (17-Dec-2019)

1. Fixed a bug that mistakenly removed a BAM/CRAM header line (sorting criteria).

4.5.3 Version 2.29.1 (9-Dec-2019)

1. Fixed a bug that now allows blocked intersection to be counted based on unique base pairs of overlap. The resolution for issue 750 in version 2.29.0 mistakenly allowed for fractional overlap to be counted based upon redundant overlap.
2. Moved to Github Continuous Integration for automatic testing.
3. Fixed a bug that injected erroneous quality values with BAM records had no valid quality values.
4. Fixed a bug that destroyed backwards compatibility in the *getfasta* tool. Thanks to Torsten Seeman for reporting this.
5. Fixed a corner case bug in the *reldist* tool.
6. Fixed a bug in the *bedtobam* tool that caused the last character in read names to be removed.
7. Fixed a bug causing a segfault in the *jaccard* tool.
8. Fixed a bug causing a corner case issue in the way coordinates are reported in the *flank* tool.

4.5.4 Version 2.29.0 (3-Sep-2019)

1. Added a new *-C* option to the *intersect* tool that separately reports the count of intersections observed for each database (*-b*) file given. Formerly, the *-c* option reported to sum of all intersections observed across all database files.
2. Fixed an important bug in *intersect* that prevented some split reads from being counted properly with using the *-split* option with the *-f* option.
3. Fixed a bug in *shuffle* such that shuffled regions should have the same strand as the chose *-incl* region.
4. Added a new *-L* option to *L'imit the output of the 'complement* tool to solely the chromosomes that are represented in the *-i* file.
5. Fixed a regression in the *multicov* tool introduced in 2.28 that caused incorrect counts.
6. Added support for multi-mapping reads in the *bamtofastq* tool.
7. Fixed a bug that prevented the "window" tool from properly adding interval "slop" to BAM records.
8. Fixed a bug that caused the *slop* tool to not truncate an interval's end coordinate when it overlapped the end of a chromosome.
9. Added support for the "=" and "X" CIGAR operations to *bamtobed*.
10. Various other minor bug fixes and improvements to documentation.

4.5.5 Version 2.28.0 (23-Mar-2019)

1. Included support for htslib to enable CRAM support and long-term stability (Thanks to Hao Hou!)
2. Included support for genomes with large chromosomes by moving to 64-bit integeres throughout the code base. Thanks to Brent Pedersen and John Marshall!
3. We now provide a statically-linked binary for LINUX (not OSX) systems.
4. Various minor bug fixes.

4.5.6 Version 2.27.0 (6-Dec-2017)

1. Fixed a big memory leak and algorithmic flaw in the *split* option. Thanks to Neil Kindlon!
2. Resolved compilation errors on OSX High Sierra. Many thanks to @jonchang!
3. Fixed a bug in the *shift* tool that caused some intervals to exceed the end of the chromosome. Thanks to @wlholtz
4. Fixed major bug in *groupby* that prevented proper functionality.
5. Speed improvements to the *shuffle* tool.
6. Bug fixes to the p-value calculation in the *fisher* tool. Thanks to Brent Pedersen.
7. Allow BED headers to start with chrom or chr
8. Fixes to the "k-closest" functionality in the *closest* tool. Thanks to Neil Kindlon.
9. Fixes to the output of the freqasc, freqdesc, distinct_sort_num and distinct_sort, and num_desc operations in the *groupby* tool. Thanks to @ghuls.
10. Many minor bug fixes and compilation improvements from Luke Goodsell.
11. Added the *-fullHeader* option to the *maskfasta* tool. Thanks to @ghuls.
12. Many bug fixes and performance improvements from John Marshall.
13. Fixed bug in the *-N/-f* behavior in *subtract*.
14. Full support for .fai files as genome (*-g*) files.
15. Many other minor bug fixes and functionality improvements.

4.5.7 Version 2.26.0 (7-July-2016)

1. Fixed a major memory leak when using `-sorted`. Thanks to Emily Tsang and Stephen Montgomery.
2. Fixed a bug for BED files containing a single record with no newline. Thanks to @jmarshall.
3. Fixed a bug in the contigency table values for thr `fisher` tool.
4. The `getfasta` tool includes name, chromosome and position in fasta headers when the `-name` option is used. Thanks to @rishavray.
5. Fixed a bug that now forces the `coverage` tool to process every record in the `-a` file.
6. Fixed a bug preventing proper processing of BED files with consecutive tabs.
7. VCF files containing structural variants now infer SV length from either the SVLEN or END INFO fields. Thanks to Zev Kronenberg.
8. Resolve off by one bugs when intersecting GFF or VCF files with BED files.
9. The `shuffle` tool now uses roulette wheel sampling to shuffle to `-incl` regions based upon the size of the interval. Thanks to Zev Kronenberg and Michael Imbeault.
10. Fixed a bug in `coverage` that prevented correct calculation of depth when using the `-split` option.
11. The `shuffle` tool warns when an interval exceeds the maximum chromosome length.
12. The `complement` tool better checks intervals against the chromosome lengths.
13. Fixes for `stddev`, `min`, and `max` operations. Thanks to @jmarshall.
14. Enabled `stdev`, `sstdev`, `freqasc`, and `freqdesc` options for `groupby`.

15. Allow `-s` and `-w` to be used in any order for `makewindows`.
16. Added new `-bedOut` option to `getfasta`.
17. The `-r` option forces the `-F` value for `intersect`.
18. Add `-pc` option to the `genomecov` tool, allowing coverage to be calculated based upon paired-end fragments.

4.5.8 Version 2.25.0 (3-Sept-2015)

1. Added new *-F* option that allows one to set the minimum fraction of overlap required for the B interval. This complements the functionality of the *-f* option.Available for *intersect*, *coverage*, *map*, *subtract*, and *jaccard*.
2. Added new *-e* option that allows one to require that the minimum fraction overlap is achieved in either A _OR_ B, not A _AND_ B which is the behavior of the *-r* option. Available for *intersect*, *coverage*, *map*, *subtract*, and *jaccard*.
3. Fixed a longstanding bug that prevented *genomecov* from reporting chromosomes that lack a single interval.
4. Modified a src directory called "aux" to "driver" to prevent compilation errors on Windows machines. Thanks very much to John Marshall.
5. Fixed a regression that caused the *coverage* tool to complain if BED files had less than 5 columns.
6. Fixed a variable overload bug that prevented compilation on Debian machines.
7. Speedups to the *groupby* tool.
8. New *-delim* option for the *groupby* tool.
9. Fixed a bug in *map* that prevented strand-specifc overlaps from being reported when using certain BEDPLUS formats.
10. Prevented excessive memory usage when not using pre-sorted input.

4.5.9 Version 2.24.0 (27-May-2015)

1. The *coverage* tool now takes advantage of pre-sorted intervals via the *-sorted* option. This allows the *coverage* tool to be much faster, use far less memory, and report coverage for intervals in their original order in the input file.
2. We have changed the behavior of the *coverage* tool such that it is consistent with the other tools. Specifically, coverage is now computed for the intervals in the A file based on the overlaps with the B file, rather than vice versa.
3. The `subtract` tool now supports pre-sorted data via the `-sorted` option and is therefore much faster and scalable.
4. The `-nonamecheck` option provides greater tolerance for chromosome labeling when using the `-sorted` option.
5. Support for multiple SVLEN tags in VCF format, and fixed a bug that failed to process SVLEN tags coming at the end of a VCF INFO field.
6. Support for reverse complementing IUPAC codes in the `getfasta` tool.
7. Provided greater flexibility for "BED+" files, where the first 3 columns are chrom, start, and end, and the remaining columns are free-form.
8. We now detect stale FAI files and recreate an index thanks to a fix from @gtamazian.

9. New feature from Pierre Lindenbaum allowing the `sort` tool to sort files based on the chromosome order in a `faidx` file.
10. Eliminated multiple compilation warnings thanks to John Marshall.
11. Fixed bug in handling INS variants in VCF files.

4.5.10 Version 2.23.0 (22-Feb-2015)

1. Added `-k` option to the closest tool to report the k-closest features in one or more -b files.
2. Added `-fd` option to the closest tool to for the reporting of downstream features in one or more -b files. Requires -D to dictate how "downstream" should be defined.
3. Added `-fu` option to the closest tool to for the reporting of downstream features in one or more -b files. Requires -D to dictate how "downstream" should be defined.
4. Pierre Lindenbaum added a new split tool that will split an input file into multiple sub files. Unlike UNIX split, it can balance the chunking of the sub files not just by number of lines, but also by total number of base pairs in each sub file.
5. Added a new spacing tool that reports the distances between features in a file.
6. Jay Hesselberth added a `-reverse` option to the makewindows tool that reverses the order of the assigned window numbers.
7. Fixed a bug that caused incorrect reporting of overlap for zero-length BED records. Thanks to @roryk.
8. Fixed a bug that caused the map tool to not allow `-b` to be specified before `-a`. Thanks to @semenko.
9. Fixed a bug in `makewindows` that mistakenly required `-s` with `-n`.

4.5.11 Version 2.22.1 (01-Jan-2015)

1. When using -sorted with intersect, map, and closest, bedtools can now detect and warn you when your input datasets employ different chromosome sorting orders.
2. Fixed multiple bugs in the new, faster closest tool. Specifically, the -iu, -id, and -D options were not behaving properly with the new "sweeping" algorithm that was implemented for the 2.22.0 release. Many thanks to Sol Katzman for reporting these issues and for providing a detailed analysis and example files.
3. We FINALLY wrote proper documentation for the closest tool (http://bedtools.readthedocs.org/en/latest/content/tools/closest.html)
4. Fixed bug in the tag tool when using -intervals, -names, or -scores. Thanks to Yarden Katz for reporting this.
5. Fixed issues with chromosome boundaries in the slop tool when using negative distances. Thanks to @acdaugherty!
6. Multiple improvements to the fisher tool. Added a -m option to the fisher tool to merge overlapping intervals prior to comparing overlaps between two input files. Thanks to@brentp
7. Fixed a bug in makewindows tool requiring the use of -b with -s.
8. Fixed a bug in intersect that prevented -split from detecting complete overlaps with -f 1. Thanks to @tleonardi .
9. Restored the default decimal precision to the groupby tool.
10. Added the -prec option to the merge and map tools to specific the decimal precision of the output.

4.5.12 Version 2.22.0 (12-Nov-2014)

1. The "closest" tool now requires sorted files, but this requirement now enables it to simultaneously find the closest intervals from many (not just one) files.
2. We now have proper support for "imprecise" SVs in VCF format. This addresses a long standing (sorry) limitation in the way bedtools handles VCF files.

4.5.13 Version 2.21.0 (18-Sep-2014)

1. Added ability to intersect against multiple *-b* files in the *intersect* tool.
2. Fixed a bug causing slowdowns in the *-sorted* option when using *-split* with very large split alignments.
3. Added a new *fisher* tool to report a P-value associated with the significance of the overlaps between two interval sets. Thanks to @brentp!
4. Added a "genome" file for GRCh38. Thanks @martijnvermaat!
5. Fixed a bug in the *-pct* option of the *slop* tool. Thanks to @brentp!
6. Tweak to the Makefile to accomodate Intel compilers. Thanks to @jmarshall.
7. Many updates to the docs from the community. Thank you!

4.5.14 Version 2.20.1 (23-May-2014)

1. Fixed a float rounding bug causing occassional off-by-one issues in the slop added by the `slop` tool. Thanks to @slw287r.
2. Fixed a bug injected in 2.19 arising when files have a single line not ending in a newline. Thanks to @cwarden45.

4.5.15 Version 2.20.0 (22-May-2014)

1. The `merge` tool now supports BAM input.

- The `-n`, `-nms`, and `-scores` options are deprecated in favor of the new, substantially more flexible, `-c` and `-o` options. See the docs.
- It now supports the `-header` option.
- It now supports the `-S` option.

2. The `map` tool now supports BAM input.
3. The `jaccard` tool is now ~3 times faster.

- It now supports the `-split` option.
- It now supports the `-s` option.
- It now supports the `-S` option.

4. We have fixed several CLANG compiler issues/ Thanks to John Marshall for the thorough report.
5. We added support for "X" and "=" CIGAR operators. Thanks to Pierre Lindenbaum.
6. Fixed bugs for empty files.
7. Improved the `-incl` option in the `shuffle` tool such that the distibution is much more random.
8. Fixed a bug in `slop` when very large slop values are used.

4.5.16 Version 2.19.1 (6-Mar-2014)

1. Bug fix to intersect causing BAM footers to be erroneously written when -b is BAM
2. Speedup for the map tool. - http://bedtools.readthedocs.org/en/latest/_images/map-speed-comparo.png
3. Map tool now allows multiple columns and operations in a single run. - http://bedtools.readthedocs.org/en/latest/content/tools/map.html#multiple-operations-and-columns-at-the-same-time

4.5.17 Version 2.19.0 (8-Feb-2014)

Bug Fixes

1. Fixed a long standing bug in which the number of base pairs of overlap was incorrectly calculated when using the -wo option with the -split option. Thanks to many for reporting this.
2. Fixed a bug in which certain flavors of unmapped BAM alignments were incorrectly rejected in the latest 2.18.* series. Thanks very much to Gabriel Pratt.

Enhancements

1. Substantially reduced memory usage, especially when dealing with unsorted data. Memory usage ballooned in the 2.18.* series owing to default buffer sizes we were using in a custom string class. We have adjusted this and the memory usage has returned to 2.17.* levels while maintaining speed increases. Thanks so much to Ian Sudberry rightfully complaining about this!

New features

1. The latest version of the "map" function is ~3X faster than the one available in version 2.17 and 2.18
2. The map function now supports the "-split" option, as well as "absmin" and "absmax" operations.
3. In addition, it supports multiple chromosome sorting criterion by supplying a genome file that defines the expected chromosome order. Here is an example of how to run map with datasets having chromosomes sorted in "version" order, as opposed to the lexicographical chrom order that is the norm.

4.5.18 Version 2.18.2 (8-Jan-2014)

bedtools

The changes to bedtools reflect fixes to compilation errors, performance enhancements for smaller files, and a bug fix for BAM files that lack a formal header. Our current focus for the 2.19.* release is is on addressing some standing bug/enhancements and also in updating some of the other more widely used tools (e.g., coverage, map, and substract) to use the new API. We will also continue to look into ways to improve performance while hopefully reducing memory usage for algorithms that work with unsorted data (thanks to Ian Sudberry for the ping!).

pybedtools

Ryan Dale has updated pybedtools to accomodate bedtools 2.18.*, added unit tests, and provided new functionality and bug fixes. The details for this release are here: http://pythonhosted.org/pybedtools/changes.html

4.5.19 Version 2.18.1 (16-Dec-2013)

Fixes that address compilation errors with CLANG and force compilation of custom BamTools library.

4.5.20 Version 2.18.0 (13-Dec-2013)

The Google Code site is deprecated

It looks like the Google Code service is going the way of the venerable Google Reader. As such, we are moving the repository and all formal release tarballs to Github. We have started a new repository prosaically named "bedtools2". The original bedtools repository will remain for historical purposes, but we created a new repository to distinguish the two code bases as they will become rather different over time.

[https://github.com/arq5x/bedtools2{]}(https://github.com/arq5x/bedtools2)

We gutted the core API and algorithms

Much of Neil's hard work has been devoted to completely rewriting the core file/stream writing API to be much more flexible in the adoption of new formats. In addition, he has substantially improved many of the core algorithms for detecting interval intersections.

Improved performance

The 2.18.0 release leverages these improvements in the "intersect" tool. Forthcoming releases will see the new API applied to other tools, but we started with intersect as it is the most widely used tool in the suite.

Performance with sorted datasets. The "chromsweep" algorithm we use for detecting intersections is now **60 times faster** than when it was first release in version 2.16.2, and is 15 times than the 2.17 release. This makes the algorithm slightly faster that the algorithm used in the bedops `bedmap` tool. As an example, the following [figure](https://dl.dropboxusercontent.com/u/515640/bedtools-intersect-sorteddata.png) demonstrates the speed when intersecting GENCODE exons against 1, 10, and 100 million BAM alignments from an exome capture experiment. Whereas in version 2.16.2 this wuld have taken 80 minutes, **it now takes 80 seconds**.

Greater flexibility. In addition, BAM, BED, GFF/GTF, or VCF files are now automatically detected whether they are a file, stream, or FIFO in either compressed or uncompressed form. As such, one now longer has specify *-abam* when using BAM input as the "A" file with `intersect`. Moreover, any file type can be used for either the A or the B file.

Better support for different chromosome sorting criteria

Genomic analysis is plagued by different chromosome naming and sorting conventions. Prior to this release, the `-sorted` option in the `intersect` tool required that the chromosomes were sorted in alphanumeric order (e.g. chr1, chr10, etc. or 1, 10, etc.). Starting with this release, we now simply require by default that the records are **GROUPED** by chromosome and that within each chromosome group, the records are sorted by chromosome position. This will allow greater flexibility.

One problem that can arise however, is if two different files are each grouped by chromosome, yet the two files follow a different chromosome order. In order to detect and enforce the same order, one can explicitly state the expected chromosome order through the use of a genome (aka chrom-sizes) file. Please see the documentation [here](http://bedtools.readthedocs.org/en/latest/content/tools/intersect.html#sorted-invoke-a-memory-efficient-algorithm-for-very-large-files) and [here](http://bedtools.readthedocs.org/en/latest/content/tools/intersect.html#g-define-an-alternate-chromosome-sort-order-via-a-genome-file) for examples.

New tools

1. The `jaccard` tool. While not exactly new, there have been improvements to the tool and there is finally documentation. Read more here: http://bedtools.readthedocs.org/en/latest/content/tools/jaccard.html

2. The `reldist` tool. Details here: http://bedtools.readthedocs.org/en/latest/content/tools/reldist.html

3. The `sample` tool. Uses reservoir sampling to randomly sample a specified number of records from BAM, BED, VCF, and GFF/GTF files.

Enhancements

1. Improvements in the consistency of the output of the `merge` tool. Thanks to @kcha.

2. A new `-allowBeyondChromEnd` option in the `shuffle` tool. Thanks to @stephenturner. [docs](http://bedtools.readthedocs.org/en/latest/content/tools/shuffle.html#allowbeyondchromend-allow-records-to-extend-beyond-the-chrom-length)

3. A new `-noOverlapping` option that prevents shuffled intervals from overlapping one another. Thanks to @brentp. [docs](http://bedtools.readthedocs.org/en/latest/content/tools/shuffle.html#nooverlapping-prevent-shuffled-intervals-from-overlapping)

4. Allow the user to specify the maximum number of shuffling attempts via the `-maxTries` option in the `shuffle` tool.

5. Various improvements to the documentation provided by manu different users. Thanks to all.

6. Added the number of intersections (`n_intersections`) to the Jaccard output. Thanks to @brentp.

7. Various improvements to the `tag` tool.

8. Added the `-N` (remove any) option to the `subtract` tool.

4.5.21 Version 2.17.0 (3-Nov-2012)

New tools

We have added a new tool (bedtools "jaccard") for measuring the Jaccard statistic between two interval files. The Jaccard stat measures the ratio of the length of the intersection over the length of the union of the two sets. In this case, the union is measured as the sum of the lengths of the intervals in each set minus the length of the intersecting intervals. As such, the Jaccard statistic provides a "distance" measure between 0 (no intersections) and 1 (self intersection). The higher the score, the more the two sets of intervals overlap one another. This tool was motivated by Favorov et al, 2012. For more details, see see PMID: 22693437.

We anticipate releasing other statistical measures in forthcoming releases.

New Features & enhancements

1. The genome file drives the BAM header in "bedtools bedtobam"

2. Substantially improvement the performance of the -sorted option in "bedtools intersect" and "bedtools map". For many applications, bedtools is now nearly as fast as the BEDOPS suite when intersecting pre-sorted data. This improvement is thanks to Neil Kindlon, a staff scientist in the Quinlan lab.

3. Tightened the logic for handling split (blocked) BAM and BED records

4. **Added ranged column selection to "bedtools groupby". Thanks to Brent Pedersen"**

- e.g., formerly “bedtools groupby -g 1,2,3,4,5”; now “-g 1-5”

5. “bedtools getfasta” now properly extracts sequences based on blocked (BED12) records (e.g., exons from genes in BED12 format).
6. “bedtools groupby” now allows a header line in the input.
7. With -N, the user can now force the closest interval to have a different name field in “bedtools closest”
8. With -A, the user can now force the subtraction of entire interval when any overlap exists in “bedtools subtract”.
9. “bedtools shuffle” can now shuffle BEDPE records.
10. Improved random number generation.
11. Added -split, -s, -S, -f, -r options to “bedtools multicov”
12. Improvements to the regression testing framework.
13. Standardized the tag reporting logic in “bedtools bamtobed”
14. Improved the auto-detection of VCF format. Thanks to Michael James Clark.

Bug fixes

1. Fixed a bug in bedtobam’s -bed12 mode.
2. Properly include unaligned BAM alignments with “bedtools intersect“‘s -v option.
3. Fixed off by one error in “bedtools closest“‘s -d option

4.”bedtools bamtobed” fails properly for non-existent file.

5. Corrected missing tab in “bedtools annotate“‘s header.
6. Allow int or uint tags in “bedtools bamtobed”
7. “bedtools flank” no longer attempts to take flanks prior to the start of a chromosome.
8. Eliminated an extraneous tab from “bedtools window” -c.
9. Fixed a corner case in the -sorted algorithm.

10.Prevent numeric overflow in “bedtools coverage -hist”

4.5.22 Version 2.14.1-3 (2-Nov-2011)

Bug Fixes

1. Corrected the help for closestBed. It now correctly reads -io instead of -no.
2. Fixed regression in closestBed injected in version 2.13.4 whereby B features to the right of an A feature were missed.

New tool

1. Added the multiIntersectBed tool for reporting common intervals among multiple **sorted** BED/GFF/VCF files.

4.5.23 Version 2.13.4 (26-Oct-2011)

Bug Fixes

1. The -sorted option (chromsweep) in intersectBed now obeys -s and -S. I had neglected to implement that. Thanks to Paul Ryvkin for pointing this out.
2. The -split option was mistakenly splitting of D CIGAR ops.
3. The Makefile was not including zlib properly for newer versions of GCC. Thanks to Istvan Albert for pointing this out and providing the solution.

Improvements

1. Thanks to Jacob Biesinger for a new option (-D) in closestBed that will report _signed_ distances. Moreover, the new option allows fine control over whether the distances are reported based on the reference genome or based on the strand of the A or B feature. Many thanks to Jacob.
2. Thanks to some nice analysis from Paul Ryvkin, I realized that the -sorted option was using way too much memory in certain cases where there is a chromosome change in a sorted BED file. This has been corrected.

4.5.24 Version 2.13.3 (30-Sept-2011)

Bug Fixes

1. intersectBed detected, but did not report overlaps when using BAM input and -bed.

Other

1. Warning that -sorted trusts, but does not enforce that data is actually sorted.

4.5.25 Version 2.13.2 (23-Sept-2011)

New algorithm

1. Preliminary release of the chrom_sweep algorithm.

New options

1. genomeCoverageBed no longer requires a genome file when working with BAM input. It instead uses the BAM header.
2. tagBam now has a -score option for annotating alignments with the BED "scores" field in annotation files. This overrides the default behavior, which is to use the -labels associated with the annotation files passed in on the command line.

Bug fixes

1. Correct a bug that prevented proper BAM support in intersectBed.
2. Improved detection of GFF features with negative coordinates.

4.5.26 Version 2.13.1 (6-Sept-2011)

New options

1. tagBam now has -s and -S options for only annotating alignments with features on the same and opposite strand, respectively.
2. tagBam now has a -names option for annotating alignments with the "name" field in annotation files. This overrides the default behavior, which is to use the -labels associated with the annotation files passed in on the command line. Currently, this works well with BED files, but given the limited metadata support for GFF files, annotating with -names and GFF files may not work as well as wished, depending on the type of GFF file used.

4.5.27 Version 2.13.0 (1-Sept-2011)

New tools

1. tagBam. This tool annotates a BAM file with custom tag fields based on overlaps with BED/GFF/VCF files. For example:

```
$ tagBam -i aln.bam -files exons.bed introns.bed cpg.bed utrs.bed \
                    -tags exonic intonic cpg utr \
                    > aln.tagged.bam
```

For alignments that have overlaps, you should see new BAM tags like "YB:Z:exonic", "YB:Z:cpg;utr" 2. multiBamCov. The new tool counts sequence coverage for multiple bams at specific loci defined in a BED/GFF/VCF file. For example:

> $ multiBamCov -bams aln.1.bam aln.2.bam aln3.bam -bed exons.bed chr1 861306 861409 SAMD11 1 + 181 280 236 chr1 865533 865718 SAMD11 2 + 249 365 374 chr1 866393 866496 SAMD11 3 + 162 298 322

where the last 3 columns represent the number of alignments overlapping each interval from the three BAM file.

The following options are available to control which types of alignments are are counted.

-q	Minimum mapping quality allowed. Default is 0.
-D	Include duplicate-marked reads. Default is to count non-duplicates only
-F	Include failed-QC reads. Default is to count pass-QC reads only
-p	Only count proper pairs. Default is to count all alignments with MAPQ greater than the -q argument, regardless of the BAM FLAG field.

3. **nucBed. This new tool profiles the nucleotide content of intervals in a fasta file. The following information will be reported**

    1) %AT content
    2) %GC content
    3) Number of As observed
    4) Number of Cs observed
    5) Number of Gs observed
    6) Number of Ts observed
    7) Number of Ns observed
    8) Number of other bases observed

9) The length of the explored sequence/interval.

10) The sequence extracted from the FASTA file. (optional, if -seq is used)

11) The number of times a user defined pattern was observed. (optional, if -pattern is used.)

For example: $ nucBed -fi ~/data/genomes/hg18/hg18.fa -bed simrep.bed | head -3 #1_usercol 2_usercol 3_usercol 4_usercol 5_usercol 6_usercol 7_pct_at 8_pct_gc 9_num_A 10_num_C 11_num_G 12_num_T 13_num_N 14_num_oth 15_seq_len chr1 10000 10468 trf 789 + 0.540598 0.459402 155 96 119 98 0 0 468 chr1 10627 10800 trf 346 + 0.445087 0.554913 54 55 41 23 0 0 173

One can also report the sequence itself: $ nucBed -fi ~/data/genomes/hg18/hg18.fa -bed simrep.bed -seq | head -3 #1_usercol 2_usercol 3_usercol 4_usercol 5_usercol 6_usercol 7_pct_at 8_pct_gc 9_num_A 10_num_C 11_num_G 12_num_T 13_num_N 14_num_oth 15_seq_len 16_seq chr1 10000 10468 trf 789 + 0.540598 0.459402 155 96 119 98 0 0 468 ccagggg... chr1 10627 10800 trf 346 + 0.445087 0.554913 54 55 41 23 0 0 173 TCTTTCA...

Or, one can count the number of times that a specific pattern occur in the intervals (reported as the last column): $ nucBed -fi ~/data/genomes/hg18/hg18.fa -bed simrep.bed -pattern CGTT | head #1_usercol 2_usercol 3_usercol 4_usercol 5_usercol 6_usercol 7_pct_at 8_pct_gc 9_num_A 10_num_C 11_num_G 12_num_T 13_num_N 14_num_oth 15_seq_len 16_user_patt_count chr1 10000 10468 trf 789 + 0.540598 0.459402 155 96 119 98 0 0 468 0 chr1 10627 10800 trf 346 + 0.445087 0.554913 54 55 41 23 0 0 173 0 chr1 10757 10997 trf 434 + 0.370833 0.629167 49 70 81 40 0 0 240 0 chr1 11225 11447 trf 273 + 0.463964 0.536036 44 86 33 59 0 0 222 0 chr1 11271 11448 trf 187 + 0.463277 0.536723 37 69 26 45 0 0 177 0 chr1 11283 11448 trf 199 + 0.466667 0.533333 37 64 24 40 0 0 165 0 chr1 19305 19443 trf 242 + 0.282609 0.717391 17 57 42 22 0 0 138 1 chr1 20828 20863 trf 70 + 0.428571 0.571429 10 7 13 5 0 0 35 0 chr1 30862 30959 trf 79 + 0.556701 0.443299 35 22 21 19 0 0 97 0

New options

1. Support for named pipes and FIFOs.
2. "-" is now allowable to indicate that data is being sent via stdin.
3. Multiple tools. Added new -S option to annotateBed, closestBed, coverageBed, intersectBed, pairToBed, subtractBed, and windowBed (-Sm). This new option does the opposite of the -s option: that is, overlaps are only processed if they are on _opposite_ strands. Thanks to Sol Katzman for the great suggestion. Very useful for certain RNA-seq analyses.
4. coverageBed. Added a new -counts option to coverageBed that only reports the count of overlaps, instead of also computing fractions, etc. This is much faster and uses much less memory.
5. fastaFromBed. Added a new -full option that uses the full BED entry when naming each output sequence. Also removed the -fo option such that all output is now written to stdout.
6. **genomeCoverageBed.**
   - Added new -scale option that allows the coverage values to be scaled by a constant. Useful for normalizing coverage with RPM, RPKM, etc. Thanks to Ryan Dale for the useful suggestion.
   - **Added new -5, -3, -trackline, -trackopts, and -dz options. Many thanks to Assaf Gordon for these improvements.** -5: Calculate coverage of 5" positions (instead of entire interval) -3: Calculate coverage of 3" positions (instead of entire interval). -trackline: Adds a UCSC/Genome-Browser track line definition in the first line of the output. -trackopts: rites additional track line definition parameters in the first line. -dz: Report the depth at each genome position with zero-based coordinates, instead of zero-based.
7. **closestBed. See below, thanks to Brent Pedersen, Assaf Gordon, Ryan Layer and Dan Webster for the helpful discussions.**

- closestBed now reports _all_ features in B that overlap A by default. This allows folks to decide which is the "best" overlapping feature on their own. closestBed now has a "-io" option that ignores overlapping features. In other words, it will only report the closest, non-overlapping feature.

An example:

$ cat a.bed chr1 10 20

$ cat b.bed chr1 15 16 chr1 16 40 chr1 100 1000 chr1 200 1000

$ bin/closestBed -a a.bed -b b.bed chr1 10 20 chr1 15 16 chr1 10 20 chr1 16 40

$ bin/closestBed -a a.bed -b b.bed -io chr1 10 20 chr1 100 1000

Updates

1. Updated to the latest version of BamTools. This allows greater functionality and will facilitate new options and tools in the future.

Bug Fixes

1. GFF files cannot have zero-length features.
2. Corrected an erroneous check on the start coordinates in VCF files. Thanks to Jan Vogel for the correction.
3. mergeBed now always reports output in BED format.
4. Updated the text file Tokenizer function to yield 15% speed improvement.
5. Various tweaks and improvements.

4.5.28 Version 2.12.0 (April-3-2011)

New Tool

1. Added new tool called "flankBed", which allows one to extract solely the flanking regions that are upstream and downstream of a given feature. Unlike slopBed, flankBed does not include the original feature itself. A new feature is created for each flabking region. For example, imagine the following feature:

chr1 100 200

The following would create features for solely the 10 bp regions flanking this feature. $ bin/flankBed -i a.bed -b 10 -g genomes/human.hg18.genome chr1 90 100 chr1 200 210

In contrast, slopBed would return: bin/slopBed -i a.bed -b 10 -g genomes/human.hg18.genome chr1 90 210

FlankBed has all of the same features as slopBed.

New Features

1. Added new "-scores" feature to mergeBed. This allows one to take the sum, min, max, mean, median, mode, or antimode of merged feature scores. In addition, one can use the "collapse" operation to get a comma-separated list of the merged scores. 2. mergeBed now tolerates multiple features in a merged block to have the same feature name. 3. Thanks to Erik Garrison's "fastahack" library, fastaFromBed now reports its output in the order of the input file. 4. Added a "-n" option to bed12ToBed6, which forces the score field to be the 1-based block number from the original BED12 feature. This is useful for tracking exon numbers, for example. 5. Thanks to Can Alkan, added a new "-mc" option to maskFastaFromBed that allows one to define a custom mask character, such as "X" (-n X).

Bug Fixes

1. Thanks to Davide Cittaro, intersectBed and windowBed now properly capture unmapped BAM alignments when using the "-v" option.
2. ClosestBed now properly handles cases where b.end == a.start
3. Thanks to John Marshall, the default constructors are much safer and less buggy.
4. Fixed bug in shuffleBed that complained about a lack of -incl and -excl.
5. Fixed bug in shuffleBed for features that would go beyond the end of a chromosome.
6. Tweaked bedToIgv to make it more Windows friendly.

4.5.29 Version 2.11.2 (January-31-2010)

Fixed a coordinate reporting bug in coverageBed. Added "max distance (-d)" argument back to the new implementation of mergeBed.

4.5.30 Version 2.11.0 (January-21-2010)

Enhancements:

1. Support for zero length features (i.e., start = end) - For example, this allows overlaps to be detected with insertions in the reference genome, as reported by dbSNP.
2. Both 8 and 9 column GFF files are now supported.
3. slopBed can now extend the size of features by a percentage of it's size (-pct) instead of just a fixed number of bases.
4. Two improvements to shuffleBed: 3a. A -f (overlapFraction) parameter that defines the maximum overlap that a randomized feature can have with an -excl feature. That is, if a chosen locus has more than -f overlap with an -excl feature, a new locus is sought. 3b. A new -incl option (thanks to Michael Hoffman and Davide Cittaro) that, defines intervals in which the randomized features should be placed. This is used instead of placing the features randomly in the genome. Note that a genome file is still required so that a randomized feature does not go beyond the end of a chromosome.
5. bamToBed can now optionally report the CIGAR string as an additional field.
6. pairToPair can now report the entire paired feature from the B file when overlaps are found.
7. complementBed now reports all chromosomes, not just those with features in the BED file.
8. Improved randomization seeding in shuffleBed. This prevents identical output for runs of shuffleBed that occur in the same second (often the case).

Bug Fixes:

1. Fixed the "BamAlignmentSupportData is private" compilation issue.
2. Fixed a bug in windowBed that caused positions to run off the end of a chromosome.

Major Changes:

1. The groupBy command is now part of the filo package (https://github.com/arq5x/filo) and will no longer be distributed with BEDTools.

4.5.31 Version 2.10.0 (September-21-2010)

New tools

1. annotateBed. Annotates one BED/VCF/GFF file with the coverage and number of overlaps observed from multiple other BED/VCF/GFF files. In this way, it allows one to ask to what degree one feature coincides with multiple other feature types with a single command. For example, the following will annotate the fraction of the variants in variants.bed that are covered by genes, conservaed regions and know variation, respectively. $ annotateBed -i variants.bed -files genes.bed conserv.bed known_var.bed

This tool was suggested by Can Alkan and was motivated by the example source code that he kindly provided.

New features

1. New frequency operations (freqasc and freqdesc) added to groupBy. These operations report a histogram of the frequency that each value is observed in a given column.
2. Support for writing uncompressed bam with the -ubam option.
3. Shorthand arguments for groupBy (-g eq. -grp, -c eq. -opCols, -o eq. -opCols).
4. In addition, all BEDTools that require only one main input file (the -i file) will assume that input is coming from standard input if the -i parameter is ignored.

Bug fixes

1. Increased the precision of the output from groupBy.

4.5.32 Version 2.9.0 (August-16-2010)

New tools

1. unionBedGraphs. This is a very powerful new tool contributed by Assaf Gordon from CSHL. It will combine/merge multiple BEDGRAPH files into a single file, thus allowing comparisons of coverage (or any text-value) across multiple samples.

New features

1. New "distance feature" (-d) added to closestBed by Erik Arner. In addition to finding the closest feature to each feature in A, the -d option will report the distance to the closest feature in B. Overlapping features have a distance of 0.
2. New "per base depth feature" (-d) added to coverageBed. This reports the per base coverage (1-based) of each feature in file B based on the coverage of features found in file A. For example, this could report the per-base depth of sequencing reads (-a) across each capture target (-b).

Bug Fixes

1. Fixed bug in closestBed preventing closest features from being found for A features with start coordinates < 2048000. Thanks to Erik Arner for pointing this out.
2. Fixed minor reporting annoyances in closestBed. Thanks to Erik Arner.
3. Fixed typo/bug in genomeCoverageBed that reported negative coverage owing to numeric overflow. Thanks to Alexander Dobin for the detailed bug report.
4. Fixed other minor parsing and reporting bugs/annoyances.

4.5.33 Version 2.8.3 (July-25-2010)

1. Fixed bug that caused some GFF files to be misinterpreted as VCF. This prevented the detection of overlaps.
2. Added a new "-tag" option in bamToBed that allows one to choose the _numeric_ tag that will be used to populate the score field. For example, one could populate the score field with the alignment score with "-tag AS".
3. Updated the BamTools API.

4.5.34 Version 2.8.2 (July-18-2010)

1. Fixed a bug in bedFile.h preventing GFF strands from being read properly.
2. Fixed a bug in intersectBed that occasionally caused spurious overlaps between BAM alignments and BED features.
3. Fixed bug in intersectBed causing -r to not report the same result when files are swapped.
4. Added checks to groupBy to prevent the selection of improper opCols and groups.
5. Fixed various compilation issues, esp. for groupBy, bedToBam, and bedToIgv.
6. Updated the usage statements to reflect bed/gff/vcf support.
7. Added new fileType functions for auto-detecting gzipped or regular files. Thanks to Assaf Gordon.

4.5.35 Version 2.8.1 (July-05-2010)

1. Added bedToIgv.

4.5.36 Version 2.8.0 (July-04-2010)

1. Proper support for "split" BAM alignments and "blocked" BED (aka BED12) features. By using the "-split" option, intersectBed, coverageBed, genomeCoverageBed, and bamToBed will now correctly compute overlaps/coverage solely for the "split" portions of BAM alignments or the "blocks" of BED12 features such as genes.
2. Added native support for the 1000 Genome Variant Calling Format (VCF) version 4.0.
3. New bed12ToBed6 tool. This tool will convert each block of a BED12 feature into discrete BED6 features.

4. Useful new groupBy tool. This is a very useful new tool that mimics the "groupBy" clause in SQL. Given a file or stream that is sorted by the appropriate "grouping columns", groupBy will compute summary statistics on another column in the file or stream. This will work with output from all BEDTools as well as any other tab-delimited file or stream. Example summary operations include: sum, mean, stdev, min, max, etc. Please see the help for the tools for examples. The functionality in groupBy was motivated by helpful discussions with Erik Arner at Riken.
5. Improvements to genomeCoverageBed. Applied several code improvements provided by Gordon Assaf at CSHL. Most notably, beyond the several efficiency and organizational changes he made, he include a "-strand" option which allows one to specify that coverage should only be computed on either the "+" or the "-" strand.
6. Fixed a bug in closestBed found by Erik Arner (Riken) which incorrectly reported "null" overlaps for features that did not have a closest feature in the B file.
7. Fixed a careless bug in slopBed also found by Erik Arner (Riken) that caused an infinite loop when the "-excl" option was used.
8. Reduced memory consumption by ca. 15% and run time by ca. 10% for most tools.
9. Several code-cleanliness updates such as templated functions and common tyedefs.
10. Tweaked the genome binning approach such that 16kb bins are the most granular.

4.5.37 Version 2.7.1 (May-06-2010)

Fixed a typo that caused some compilers to fail on closestBed.

4.5.38 Version 2.7.0 (May-05-2010)

General: 1. "Gzipped" BED and GFF files are now supported as input by all BEDTools. Such files must end in ".gz". 2. Tools that process BAM alignments now uniformly compute an ungapped alignment end position based on the BAM CIGAR string. Specifically, "M", "D" and "N" operations are observed when computing the end position. 3. bamToBed requires the BAM file to be sorted/grouped by read id when creating BEDPE output. This allows the alignments end coordinate for each end of the pair to be properly computed based on its CIGAR string. The same requirement applies to pairToBed. 4. Updated manual. 5. Many silent modifications to the code that improve clarity and sanity-checking and facilitate future additions/modifications.

New Tools: 1. bedToBam. This utility will convert BED files to BAM format. Both "blocked" (aka BED12) and "unblocked" (e.g. BED6) formats are acceptable. This allows one to, for example, compress large BED files such as dbSNP into BAM format for efficient visualization.

Changes to existing tools:

intersectBed

1. Added -wao option to report 0 overlap for features in A that do not intersect any features in B. This is an extension of the -wo option.

bamToBed

1. Requires that BAM input be sorted/grouped by read name.

pairToBed

1. Requires that BAM input be sorted/grouped by read name.
2. Allows use of minimum mapping quality or total edit distance for score field.

windowBed

1. Now supports BAM input.

genomeCoverageBed

1. -bga option. Thanks to Gordon Assaf for the suggestion.
2. Eliminated potential seg fault.

Acknowledgements:

1. Gordon Assaf: for suggesting the -bga option in genomeCoverageBed and for testing the new bedToBam utility.
2. Ivan Gregoretti: for helping to expedite the inclusion of gzip support.
3. Can Alkan: for suggesting the addition of the -wao option to intersectBed.
4. James Ward: for pointing out that bedToBam did not need to create "dummy" seq and qual entries.

4.5.39 Version 2.6.1 (Mar-29-2010)

1. Fixed a careless command line parsing bug in coverageBed.

4.5.40 Version 2.6.0 (Mar-23-2010)

Specific improvements / additions to tools

1. intersectBed. Added an option (-wo) that reports the number of overlapping bases for each intersection b/w A and B files. Not sure why this wasn't added sooner; it's obvious.

2. coverageBed - native BAM support - can now report a histogram (-hist) of coverage for each feature in B. Useful for exome sequencing projects, for example. Thanks for the excellent suggestion from Jose Bras - faster

3. genomeCoverageBed - native BAM support - can now report coverage in BEDGRAPH format (-bg). Thanks for the code and great suggestion from Gordon Assaf, CSHL.

4. bamToBed - support for "blocked" BED (aka BED12) format. This facilitates the creation of BED entries for "split" alignments (e.g. RNAseq or SV). Thanks to Ann Loraine, UNCC for test data to support this addition.

5. fastaFromBed - added the ability to extract sequences from a FASTA file according to the strand in the BED file. That is, when "-" the extracted sequence is reverse complemented. Thanks to Thomas Doktor, U. of Southern Denmark for the code and suggestion.

6. ***NEW*** overlap - newly added tool for computing the overlap/distance between features on the same line.For example:

```
$ cat test.out
chr1    10      20      A       chr1    15      25      B
chr1    10      20      C       chr1    25      35      D

$ cat test.out | overlaps -i stdin -cols 2,3,6,7
chr1    10      20      A       chr1    15      25      B       5
chr1    10      20      C       chr1    25      35      D       -5
```

Bug fixes

1. Fixed a bug in pairToBed when comparing paired-end BAM alignments to BED annotations and using the "notboth" option.
2. Fixed an idiotic bug in intersectBed that occasionally caused segfaults when blank lines existed in BED files.

3. Fixed a minor bug in mergeBed when using the -nms option.

General changes

1. Added a proper class for genomeFiles. The code is much cleaner and the tools are less sensitive to minor problems with the formatting of genome files. Per Gordon Assaf's wise suggestion, the tools now support "chromInfo" files directly downloaded from UCSC. Thanks Gordon—I disagreed at first, but you were right.
2. Cleaned up some of the code and made the API a bit more streamlined. Will facilitate future tool development, etc.

4.5.41 Version 2.5.4 (Mar-3-2010)

1. Fixed an insidious bug that caused malform BAM output from intersectBed and pairToBed. The previous BAM files worked fine with samtools as BAM input, but when piped in as SAM, there was an extra tab that thwarted conversion from SAM back to BAM. Many thanks to Ivan Gregoretti for reporting this bug. I had never used the BAM output in this way and thus never caught the bug!

4.5.42 Version 2.5.3 (Feb-19-2010)

1. Fixed bug to "re-allow" track and "browser" lines.
2. Fixed bug in reporting BEDPE overlaps.
3. Fixed bug when using type "notboth" with BAM files in pairToBed.
4. When comparing BAM files to BED/GFF annotations with intersectBed or pairToBed, the __aligned__ sequence is used, rather than the __original__ sequence.
5. Greatly increased the speed of pairToBed when using BAM alignments.
6. Fixed a bug in bamToBed when reporting edit distance from certain aligners.

4.5.43 Version 2.5.2 (Feb-2-2010)

1. The start and end coordinates for BED and BEDPE entries created by bamToBed are now based on the __aligned__ sequence, rather than the original sequence. It's obvious, but I missed it originally. . . sorry.
2. Added an error message to mergeBed preventing one from using "-n" and "-nms" together.
3. Fixed a bug in pairToBed that caused neither -type "notispan" nor "notospan" to behave as described.

4.5.44 Version 2.5.1 (Jan-28-2010)

1. Fixed a bug in the new GFF/BED determinator that caused a segfault when start = 0.

4.5.45 Version 2.5.0 (Jan-27-2010)

1. Added support for custom BED fields after the 6th column.
2. Fixed a command line parsing bug in pairToBed.
3. Improved sanity checking.

4.5.46 Version 2.4.2 (Jan-23-2010)

1. Fixed a minor bug in mergeBed when -nms and -s were used together.
2. Improved the command line parsing to prevent the occasional segfault.

4.5.47 Version 2.4.1 (Jan-12-2010)

1. Updated BamTools libraries to remove some compilation issues on some systems/compilers.

4.5.48 Version 2.4.0 (Jan-11-2010)

1. Added BAM support to intersectBed and pairToBed
2. New bamToBed feature.
3. Added support for GFF features
4. Added support for "blocked" BED format (BED12)
5. Wrote complete manual and included it in distribution.
6. Fixed several minor bugs.
7. Cleaned up code and improved documentation.

4.5.49 Version 2.3.3 (12/17/2009)

Rewrote complementBed to use a slower but much simpler approach. This resolves several bugs with the previous logic.

4.5.50 Version 2.3.2 (11/25/2009)

Fixed a bug in subtractBed that prevent a file from subtracting itself when the following is used: $ subtractBed -a test.bed -b test.bed

4.5.51 Version 2.3.1 (11/19/2009)

Fixed a typo in closestBed that caused all nearby features to be returned instead of just the closest one.

4.5.52 Version 2.3.0 (11/18/2009)

1. **Added four new tools:**
    - shuffleBed. Randomly permutes the locations of a BED file among a genome. Useful for testing for significant overlap enrichments.
    - slopBed. Adds a requested number of base pairs to each end of a BED feature. Constrained by the size of each chromosome.
    - maskFastaFromBed. Masks a FASTA file based on BED coordinates. Useful making custom genome files from targeted capture experiment, etc.

- pairToPair. Returns overlaps between two paired-end BED files. This is great for finding structural variants that are private or shared among samples.

2. Increased the speed of intersectBed by nearly 50%.
3. Improved corrected some of the help messages.
4. Improved sanity checking for BED entries.

4.5.53 Version 2.2.4 (10/27/2009)

1. Updated the mergeBed documentation to describe the -names option which allows one to report the names of the features that were merged (separated by semicolons).

4.5.54 Version 2.2.3 (10/23/2009)

1. Changed windowBed to optionally define "left" and "right" windows based on strand. For example by default, -l 100 and -r 500 will add 100 bases to the left (lower coordinates) of a feature in A when scanning for hits in B and 500 bases to the right (higher coordinates).

However if one chooses the -sw option (windows bases on strandedness), the behavior changes. Assume the above example except that a feature in A is on the negative strand ("-"). In this case, -l 100, -r 500 and -sw will add 100 bases to the right (higher coordinates) and 500 bases to the left (lower coordinates).

In addition, there is a separate option (-sm) that can optionally force hits in B to only be tracked if they are on the same strand as A.

NOTE: This replaces the previous -s option and may affect existing pipelines.

4.5.55 Version 2.2.2 (10/20/2009)

1. Improved the speed of genomeCoverageBed by roughly 100 fold. The memory usage is now less than 2.0 Gb.

4.5.56 Version 2.2.1

1. Fixed a very obvious bug in subtractBed that caused improper behavior when a feature in A was overlapped by more than one feature in B. Many thanks to folks in the Hannon lab at CSHL for pointing this out.

4.5.57 Version 2.2.0

Notable changes in this release

1. **coverageBed will optionally only count features in BED file A (e.g. sequencing reads) that overlap with** the intervals/windows in BED file B on the same strand. This has been requested several times recently and facilitates CHiP-Seq and RNA-Seq experiments.
2. **intersectBed can now require a minimum __reciprocal__ overlap between intervals in BED A and BED B. For example,** previously, if one used -f 0.90, it required that a feature in B overlap 90% of the feature in A for the "hit" to be reported. If one adds the -r (reciprocal) option, the hit must also cover 90% of the feature in B. This helps to exclude overlaps between say small features in A and large features in B:

   A ========== B **********

   -f 0.50 (Reported), whereas -f 0.50 -r (Not reported)

3. **The score field has been changed to be a string. While this deviates from the UCSC definition, it allows one to track** much more meaningful information about a feature/interval. For example, score could now be:

   7.31E-05 (a p-value) 0.334577 (mean enrichment) 2:2.2:40:2 (several values encoded in a string)

4. **closestBed now, by default, reports __all__ intervals in B that overlap equally with an interval in A. Previously, it** merely reported the first such feature that appeared in B. Here's a cartoon explaining the difference.

5. Several other minor changes to the algorithms have been made to increase speed a bit.

4.5.58 Version 2.1.2

1. Fixed yet another bug in the parsing of "track" or "browser" lines. Sigh...
2. Change the "score" column (i.e. column 5) to b stored as a string. While this deviates from the UCSC convention, it allows significantly more information to be packed into the column.

4.5.59 Version 2.1.1

1. Added limits.h to bedFile.h to fix compilation issues on some systems.
2. Fixed bug in testing for "track" or "browser" lines.

4.5.60 Version 2.1.0

1. Fixed a bug in peIntersectBed that prevented -a from being correctly handled when passed via stdin.
2. Added new functionality to coverageBed that calculates the density of coverage.
3. Fixed bug in geneomCoverageBed.

4.5.61 Version 2.0.1

1. Added the ability to retain UCSC browser track/browser headers in BED files.

4.5.62 Version 2.0

1. Sped up the file parsing. ~10-20% increase in speed.
2. Created reportBed() as a common method in the bedFile class. Cleans up the code quite nicely.
3. Added the ability to compare BED files accounting for strandedness.
4. Paired-end intersect.
5. Fixed bug that prevented overlaps from being reported when the overlap fraction requested is 1.0

4.5.63 Version 1.2, 04/27/2009.

1. **Added subtractBed.**
   A. Fixed bug that prevented "split" overlaps from being reported.
   B. Prevented A from being reported if >=1 feature in B completely spans it.
2. Added linksBed.

3. Added the ability to define separate windows for upstream and downstream to windowBed.

4.5.64 Version 1.1, 04/23/2009.

Initial release.

4.6 The BEDTools suite

bedtools consists of a suite of sub-commands that are invoked as follows:

```
bedtools [sub-command] [options]
```

For example, to intersect two BED files, one would invoke the following:

```
bedtools intersect -a a.bed -b b.bed
```

4.6.1 The full list of *bedtools* sub-commands.

annotate

`bedtools annotate`, well, annotates one BED/VCF/GFF file with the coverage and number of overlaps observed from multiple other BED/VCF/GFF files. In this way, it allows one to ask to what degree one feature coincides with multiple other feature types with a single command.

Usage and option summary

Usage:

```
bedtools annotate [OPTIONS] -i <BED/GFF/VCF> -files FILE1 FILE2 FILE3 ... FILEn
```

(or):

```
annotateBed [OPTIONS] -i <BED/GFF/VCF> -files FILE1 FILE2 FILE3 ... FILEn
```

Option	Description
-names	A list of names (one per file) to describe each file in -i. These names will be printed as a header line.
-counts	Report the count of features in each file that overlap -i. Default behavior is to report the fraction of -i covered by each file.
-both	Report the count of features followed by the % coverage for each annotation file. Default is to report solely the fraction of -i covered by each file.
-s	Force strandedness. That is, only include hits in A that overlap B on the same strand. By default, hits are included without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.

Default behavior - annotate one file with coverage from others.

By default, the fraction of each feature covered by each annotation file is reported after the complete feature in the file to be annotated.

```
$ cat variants.bed
chr1 100  200   nasty 1  -
chr2 500  1000  ugly  2  +
chr3 1000 5000  big   3  -

$ cat genes.bed
chr1 150  200   geneA 1  +
chr1 175  250   geneB 2  +
chr3 0    10000 geneC 3  -

$ cat conserve.bed
chr1 0    10000 cons1 1  +
chr2 700  10000 cons2 2  -
chr3 4000 10000 cons3 3  +

$ cat known_var.bed
chr1 0    120   known1   -
chr1 150  160   known2   -
chr2 0    10000 known3   +

$ bedtools annotate -i variants.bed -files genes.bed conserve.bed known_var.bed
chr1  100     200     nasty   1       -       0.500000        1.000000        0.300000
chr2  500     1000    ugly    2       +       0.000000        0.600000        1.000000
chr3  1000    5000    big     3       -       1.000000        0.250000        0.000000
```

`-count` Report the count of hits from the annotation files

```
$ bedtools annotate -counts -i variants.bed -files genes.bed conserve.bed known_var.
↪bed
chr1  100     200     nasty   1       -       2       1       2
chr2  500     1000    ugly    2       +       0       1       1
chr3  1000    5000    big     3       -       1       1       0
```

`-both` Report both the count of hits and the fraction covered from the annotation files

```
$ bedtools annotate -both -i variants.bed -files genes.bed conserve.bed known_var.bed
#chr  start   end     name    score   +/-     cnt1    pct1    cnt2    pct2    cnt3
↪pct3
chr1  100     200     nasty   1       -       2       0.500000        1       1.
↪000000         2       0.300000
chr2  500     1000    ugly    2       +       0       0.000000        1       0.
↪600000         1       1.000000
chr3  1000    5000    big     3       -       1       1.000000        1       0.
↪250000         0       0.000000
```

-s Restrict the reporting to overlaps on the same strand.

```
$ bedtools annotate -s -i variants.bed -files genes.bed conserve.bed known_var.bed
chr1  100     200     nasty   1       -       0.000000        0.000000        0.000000
chr2  500     1000    ugly    2       +       0.000000        0.000000        0.000000
chr3  1000    5000    big     3       -       1.000000        0.000000        0.000000
```

-S Restrict the reporting to overlaps on the opposite strand.

```
$ bedtools annotate -S -i variants.bed -files genes.bed conserve.bed known_var.bed
chr1  100     200     nasty   1       -       0.500000        1.000000        0.300000
chr2  500     1000    ugly    2       +       0.000000        0.600000        1.000000
chr3  1000    5000    big     3       -       0.000000        0.250000        0.000000
```

bamtobed

`bedtools bamtobed` is a conversion utility that converts sequence alignments in BAM format into BED, BED12, and/or BEDPE records.

Usage and option summary

Usage:

```
bedtools bamtobed [OPTIONS] -i <BAM>
```

(or):

```
bamToBed [OPTIONS] -i <BAM>
```

Option	Description
-bedpe	Write BAM alignments in BEDPE format. Only one alignment from paired-end reads will be reported. Specifically, if each mate is aligned to the same chromosome, the BAM alignment reported will be the one where the BAM insert size is greater than zero. When the mate alignments are interchromosomal, the lexicographically lower chromosome will be reported first. Lastly, when an end is unmapped, the chromosome and strand will be set to "." and the start and end coordinates will be set to -1. *By default, this is disabled and the output will be reported in BED format*.
-mate1	When writing BEDPE (-bedpe) format, always report mate one as the first BEDPE "block".
-bed12	Write "blocked" BED (a.k.a. BED12) format. This will convert "spliced" BAM alignments (denoted by the "N" CIGAR operation) to BED12. *Forces -split*.
-split	Report each portion of a "split" BAM (i.e., having an "N" CIGAR operation) alignment as a distinct BED intervals.
-splitD	Report each portion of a "split" BAM while obeying both "N" CIGAR and "D" operation. Forces *-split*.
-ed	Use the "edit distance" tag (NM) for the BED score field. Default for BED is to use mapping quality. Default for BEDPE is to use the *minimum* of the two mapping qualities for the pair. When -ed is used with -bedpe, the total edit distance from the two mates is reported.
-tag	Use other *numeric* BAM alignment tag for BED score. Default for BED is to use mapping quality. Disallowed with BEDPE output.
-color	An R,G,B string for the color used with BED12 format. Default is (255,0,0).
-cigar	Add the CIGAR string to the BED entry as a 7th column.

Default behavior

By default, each alignment in the BAM file is converted to a 6 column BED. The BED "name" field is comprised of the RNAME field in the BAM alignment. If mate information is available, the mate (e.g., "/1" or "/2") field will be appended to the name.

```
$ bedtools bamtobed -i reads.bam | head -3
chr7    118970079    118970129    TUPAC_0001:3:1:0:1452#0/1    37    -
chr7    118965072    118965122    TUPAC_0001:3:1:0:1452#0/2    37    +
chr11   46769934     46769984     TUPAC_0001:3:1:0:1472#0/1    37    -
```

`-tag` Set the score field based on BAM tags

One can override the choice of the BAM *MAPQ* as the result BED record's *score* field by using the `-tag` option. In the example below, we use the `-tag` option to select the BAM edit distance (the *NM* tag) as the score column in the resulting BED records.

```
$ bedtools bamtobed -i reads.bam -tag NM | head -3
chr7    118970079    118970129    TUPAC_0001:3:1:0:1452#0/1    1    -
chr7    118965072    118965122    TUPAC_0001:3:1:0:1452#0/2    3    +
chr11   46769934     46769984     TUPAC_0001:3:1:0:1472#0/1    1    -
```

-bedpe Set the score field based on BAM tags

The -bedpe option converts BAM alignments to BEDPE format, thus allowing the two ends of a paired-end alignment to be reported on a single text line. Specifically, if each mate is aligned to the same chromosome, the BAM alignment reported will be the one where the BAM insert size is greater than zero. When the mate alignments are interchromosomal, the lexicographically lower chromosome will be reported first. Lastly, when an end is unmapped, the chromosome and strand will be set to "." and the start and end coordinates will be set to -1.

Note: When using this option, it is required that the BAM file is sorted/grouped by the read name. This allows bamToBed to extract correct alignment coordinates for each end based on their respective CIGAR strings. It also assumes that the alignments for a given pair come in groups of twos. There is not yet a standard method for reporting multiple alignments using BAM. bamToBed will fail if an aligner does not report alignments in pairs.

```
$ bedtools bamtobed -i reads.ba -bedpe | head -3
chr7    118965072    118965122    chr7    118970079    118970129 TUPAC_0001:3:1:0:1452#0␣
↪37     +     -
chr11   46765606     46765656     chr11   46769934     46769984 TUPAC_0001:3:1:0:1472#0 37␣
↪    +     -
chr20   54704674     54704724     chr20   54708987     54709037 TUPAC_0001:3:1:1:1833#0 37␣
↪    +
```

One can easily use samtools and bamToBed together as part of a UNIX pipe. In this example, we will only convert properly-paired (FLAG == 0x2) reads to BED format.

```
$ samtools view -bf 0x2 reads.bam | bedtools bamtobed -i stdin | head
chr7    118970079    118970129    TUPAC_0001:3:1:0:1452#0/1    37    -
chr7    118965072    118965122    TUPAC_0001:3:1:0:1452#0/2    37    +
chr11   46769934     46769984     TUPAC_0001:3:1:0:1472#0/1    37    -
chr11   46765606     46765656     TUPAC_0001:3:1:0:1472#0/2    37    +
chr20   54704674     54704724     TUPAC_0001:3:1:1:1833#0/1    37    +
chr20   54708987     54709037     TUPAC_0001:3:1:1:1833#0/2    37    -
chrX    9380413      9380463      TUPAC_0001:3:1:1:285#0/1     0     -
chrX    9375861      9375911      TUPAC_0001:3:1:1:285#0/2     0     +
chrX    131756978    131757028    TUPAC_0001:3:1:2:523#0/1     37    +
chrX    131761790    131761840    TUPAC_0001:3:1:2:523#0/2     37    -
```

-split Creating BED12 features from "spliced" BAM entries.

bedtools bamtobed will, by default, create a BED6 feature that represents the entire span of a spliced/split BAM alignment. However, when using the -split command, a BED12 feature is reported where BED blocks will be created for each aligned portion of the sequencing read.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Exons       ***************                                      **********

```

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```
BED/BAM A      ^^^^^^^^^^^^.................................^^^^

Result      ===============                                   ====
```

bamtofastq

`bedtools bamtofastq` is a conversion utility for extracting FASTQ records from sequence alignments in BAM format.

Note: If you are using CRAM as input, you will need to specify the *full path* describing the location of the relevant reference genome in FASTA format via the CRAM_REFERENCE environment variable. For example:

> *export CRAM_REFERENCE=/path/to/ref/g1k_v37_decoy.fa*

Usage and option summary

Usage:

```
bedtools bamtofastq [OPTIONS] -i <BAM> -fq <FASTQ>
```

(or):

```
bamToFastq [OPTIONS] -i <BAM> -fq <FASTQ>
```

Option	Description
-fq2	FASTQ for second end. Used if BAM contains paired-end data. BAM should be sorted by query name (`samtools sort -n -o aln.qsort.bam aln.bam`) if creating paired FASTQ with this option.
-tags	Create FASTQ based on the mate info in the BAM R2 and Q2 tags.

Default behavior

By default, each alignment in the BAM file is converted to a FASTQ record in the `-fq` file. The order of the records in the resulting FASTQ exactly follows the order of the records in the BAM input file.

```
$ bedtools bamtofastq -i NA18152.bam -fq NA18152.fq

$ head -8 NA18152.fq
@NA18152-SRR007381.35051
GGAGACATATCATATAAGTAATGCTAGGGTGAGTGGTAGGAAGTTTTTTCATAGGAGGTGTATGAGTTGGTCGTAGCGGAATCGGGGGTATGCTGTTCGAA
+
<<<;;<;<;;<;;;;;;;;;;;<<<:;;;;;;;;;;;;;;;;;;::::::;;;;<<;;;;;;;;;;;;;;;;;;;;;;;;;;;;<<<
↪<<;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;<<;;;;;:;
↪;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;<<<;;;;;;;;;;;;;<<<<<<<<;;;;;;;;;:;;;;;;;;;;;;;;;;;
↪:;;;;8;;8888;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;8966689666666299866669:899
@NA18152-SRR007381.637219
AATGCTAGGGTGAGTGGTAGGAAGTTTTTTCATAGGAGGTGTATGAGTTGGTCGTAGCGGAATCGGGGGTATGCTGTTCGAATTCATAAGAACAGGGAGG
```

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```
+
<<<<<<<<<<;;<;<;;;;<<;<888888899<;;;;;;<;;;;;;;;;;;;;;;;;;;;;;;;;;<<<<<;;;;;;;;;;<;<<<<<;
↪;;;;;;;;;;;;;<<<<;;;;;;;;::;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;<<<<;;;;;;;;;;;;;;;;;;;;;
↪;<;;;;;;;;;;;;;;;;;;;;;;;;;;<888<;<<;;;;<<<<<<;;;;;<<<<<<<<<<;;;;;;;;;;;;;;888888899:::;;
↪8;;;;;;;;;;;;;;;;;;;;99;;9966689666696666660 0;96666669966
```

`-fq2` Creating two FASTQ files for paired-end sequences.

If your BAM alignments are from paired-end sequence data, one can use the `-fq2` option to create two distinct FASTQ output files — one for end 1 and one for end 2.

Note: When using this option, it is required that the BAM file is sorted/grouped by the read name. This keeps the resulting records in the two output FASTQ files in the same order. One can sort the BAM file by query name with `samtools sort -n -o aln.qsort.bam aln.bam`.

```
$ samtools sort -n -o aln.qsort.bam aln.bam

$ bedtools bamtofastq -i aln.qsort.bam \
                      -fq aln.end1.fq \
                      -fq2 aln.end2.fq

$ head -8 aln.end1.fq
@SRR069529.2276/1
CAGGGAGAAGGAGGTAGGAAAGAGAAAGGACCAGGGAGGGGCGCATACACAGGACGCTCCGTGCGGTGATAGCAGCACCACACTGTGTTCAGTCGTCTGGC
+
=;@>==#########################################################################################
↪################
@SRR069529.2406/1
GCTGGGAAAAGGATTCAGGATGTTGGTTTCTATCTTTGAGTTGCTGCTGTGCGGCTGTCCCTACACTCGCAGTACCCCTCGGACACCGTCTACTGTGGAGC
+
=5@><<:?<?

$ head -8 aln.end2.fq
@SRR069529.2276/2
AGACCCAGAGAGGGACAGGATCTGTCCCAGATCATAAAATAGGGGGAGTGCTCCGTAGAGGCGTGCGCGGTGGCACCGTGCAGTAGTACGGGTGAGCGGGC
+
#########################################################################################
↪################
@SRR069529.2406/2
TTCCCTACCCCTGGGGTCAGGGACTACAGCCAAGGGGAGAACTTTAGCAAGTAGACGTTAGTTATTTTGATTCCAGTGGGGACGCGCGTGTAGCGAGTTGT
+
@>=AABB?AAACABBA>@?AAAA>B@@AB@AA:B@AA@??################################################
↪################
```

bed12tobed6

bed12ToBed6 is a convenience tool that converts BED features in BED12 (a.k.a. "blocked" BED features such as genes) to discrete BED6 features. For example, in the case of a gene with six exons, bed12ToBed6 would create six separate BED6 features (i.e., one for each exon).

Usage and option summary

Usage:

```
bed12ToBed6 [OPTIONS] -i <BED12>
```

Option	Description
-i	The BED12 file that should be split into discrete BED6 features. *Use "stdin" when using piped input.*

Default behavior

Figure:

```
head data/knownGene.hg18.chr21.bed | tail -n 3
chr21 10079666  10120808   uc002yiv.1  0  -  10081686  1 0 1 2 0 6 0 8  0     4   528,
↪91,101,215, 0,1930,39750,40927,
chr21 10080031  10081687   uc002yiw.1  0  -  10080031  1 0 0 8 0 0 3 1  0     2   200,
↪91,    0,1565,
chr21 10081660  10120796   uc002yix.2  0  -  10081660  1 0 0 8 1 6 6 0  0     3   27,
↪101,223,0,37756,38913,

head data/knownGene.hg18.chr21.bed | tail -n 3 | bed12ToBed6 -i stdin
chr21 10079666  10080194  uc002yiv.1 0  -
chr21 10081596  10081687  uc002yiv.1 0  -
chr21 10119416  10119517  uc002yiv.1 0  -
chr21 10120593  10120808  uc002yiv.1 0  -
chr21 10080031  10080231  uc002yiw.1 0  -
chr21 10081596  10081687  uc002yiw.1 0  -
chr21 10081660  10081687  uc002yix.2 0  -
chr21 10119416  10119517  uc002yix.2 0  -
chr21 10120573  10120796  uc002yix.2 0  -
```

bedpetobam

bedtobam

bedToBam converts features in a feature file to BAM format. This is useful as an efficient means of storing large genome annotations in a compact, indexed format for visualization purposes.

Usage and option summary

Usage:

```
bedToBam [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> > <BAM>
```

Option	Description
-mapq	Set a mapping quality (SAM MAPQ field) value for all BED entries. *Default: 255*
-ubam	Write uncompressed BAM output. The default is write compressed BAM output.
-bed12	Indicate that the input BED file is in BED12 (a.k.a "blocked" BED) format. In this case, bedToBam will convert blocked BED features (e.g., gene annotations) into "spliced" BAM alignments by creating an appropriate CIGAR string.

Default behavior

The default behavior is to assume that the input file is in unblocked format. For example:

```
head -5 rmsk.hg18.chr21.bed
chr21 9719768  9721892  ALR/Alpha  1004  +
chr21 9721905  9725582  ALR/Alpha  1010  +
chr21 9725582  9725977  L1PA3 3288 +
chr21 9726021  9729309  ALR/Alpha  1051  +
chr21 9729320  9729809  L1PA3 3897 -

bedToBam -i rmsk.hg18.chr21.bed -g human.hg18.genome > rmsk.hg18.chr21.bam

samtools view rmsk.hg18.chr21.bam | head -5
ALR/Alpha  0   chr21 9719769  255  2124M * 0  0  *  *
ALR/Alpha  0   chr21 9721906  255  3677M * 0  0  *  *
L1PA3      0   chr21 9725583  255  395M  * 0  0  *  *
ALR/Alpha  0   chr21 9726022  255  3288M * 0  0  *  *
L1PA3      16  chr21 9729321  255  489M  * 0  0  *  *
```

Creating "spliced" BAM entries from "blocked" BED features

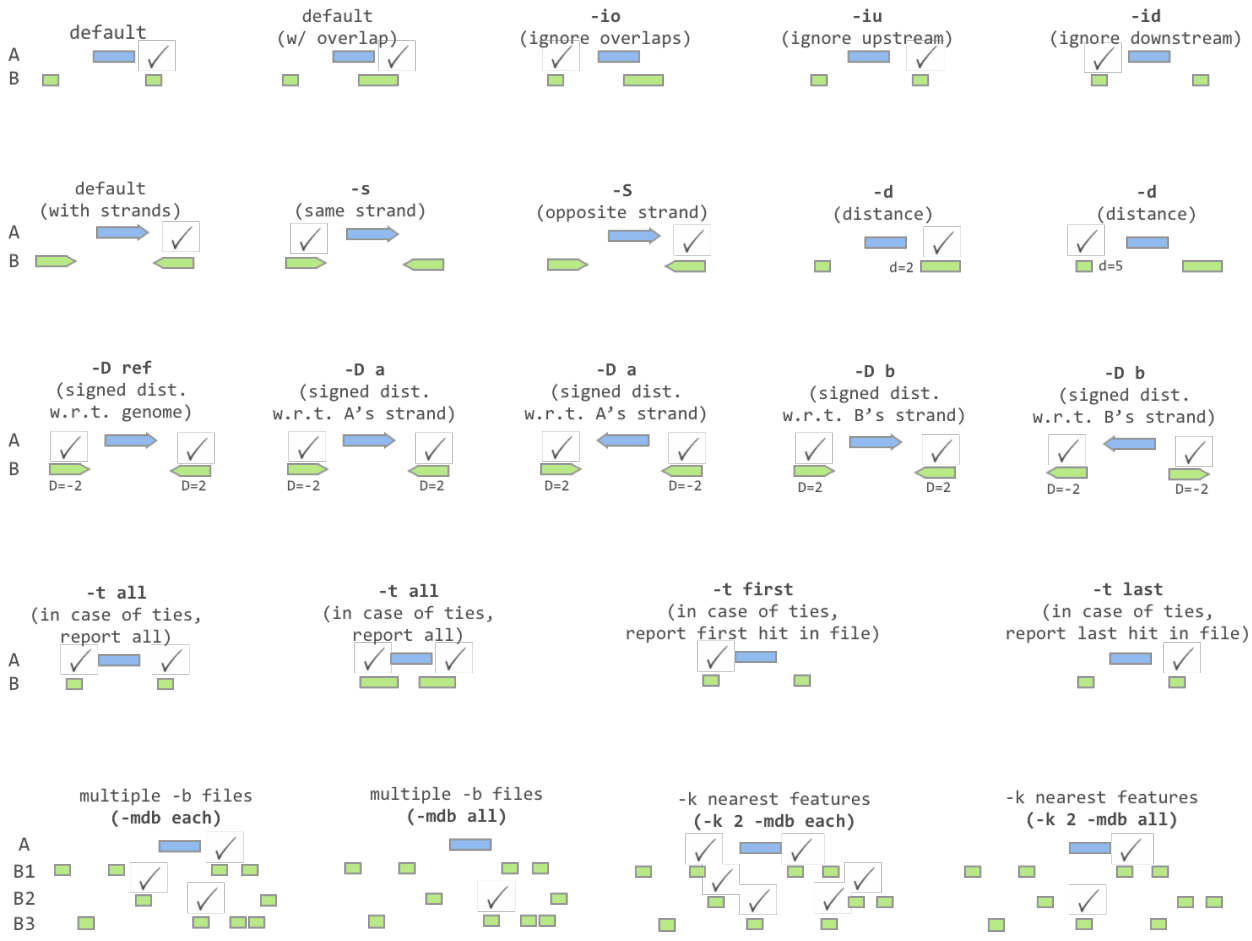
Optionally, **bedToBam** will create spliced BAM entries from "blocked" BED features by using the -bed12 option. This will create CIGAR strings in the BAM output that will be displayed as "spliced" alignments. The image illustrates this behavior, as the top track is a BAM representation (using bedToBam) of a BED file of UCSC genes.

For example:

```
bedToBam -i knownGene.hg18.chr21.bed -g human.hg18.genome -bed12 > knownGene.bam

samtools view knownGene.bam | head -2
uc002yip.1  16   chr21 9928614   2                      5                      5

298M1784N71M1411N93M3963N80M1927N106M3608N81M1769N62M11856N89M98N82M816N61M6910N65M
738N64M146N100M1647N120M6478N162M1485N51M6777N60M9274N54M880N54M1229N54M2377N54M112
68N58M2666N109M2885N158M     *   0  0  *  *
uc002yiq.1  16   chr21 9928614   2                      5                      5

298M1784N71M1411N93M3963N80M1927N106M3608N81M1769N62M11856N89M98N82M816N61M6910N65M
738N64M146N100M1647N120M6478N162M1485N51M6777N60M10208N54M1229N54M2377N54M11268N58M
2666N109M2885N158M       *   0   0  *  *
```

closest

Similar to *intersect*, *closest* searches for overlapping features in A and B. In the event that no feature in B overlaps the current feature in A, *closest* will report the nearest (that is, least genomic distance from the start or end of A) feature in B. For example, one might want to find which is the closest gene to a significant GWAS polymorphism. Note that *closest* will report an overlapping feature as the closest—that is, it does not restrict to closest *non-overlapping* feature. The following iconic "cheatsheet" summarizes the functionality available through the various options provided by the *closest* tool.



Note: `bedtools closest` requires that all input files are presorted data by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files).

Note: Reports "none" for chrom and "-1" for all other fields when a feature is not found in B on the same chromosome

as the feature in A. E.g. *none -1 -1*

Important: As of version 2.22.0, the *closest* tool can accept multiple files for the *-b* option. This allows one to identify the closest intervals between a single query (*-a*) file and multiple database files (*-b*) at once! This functionality now requires that all input files be sorted by chromosome and start coordinate in an identical manner (e.g., *sort -k1,1 -k2,2n*).

Usage and option summary

Usage:

```
bedtools closest [OPTIONS] -a <FILE> \
                           -b <FILE1, FILE2, ..., FILEN>
```

(or):

```
closestBed [OPTIONS] -a <FILE> \
                     -b <FILE1, FILE2, ..., FILEN>
```

Option	Description
-s	Require same strandedness. That is, find the closest feature in B that overlaps A on the _same_ strand. By default, overlaps are reported without respect to strand.
-S	Require opposite strandedness. That is, find the closest feature in B that overlaps A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-d	In addition to the closest feature in B, report its distance to A as an extra column. The reported distance for overlapping features will be 0.
-D	Like *-d*, report the closest feature in B, and its distance to A as an extra column. However unlike *-d*, use negative distances to report upstream features. The options for defining which orientation is "upstream" are: - *ref* Report distance with respect to the reference genome. B features with a lower (start, stop) are upstream - *a* Report distance with respect to A. When A is on the - strand, "upstream" means B has a higher (start,stop). - *b* Report distance with respect to B. When B is on the - strand, "upstream" means A has a higher (start,stop).
-io	Ignore features in B that overlap A. That is, we want close, yet not touching features only.
-iu	Ignore features in B that are upstream of features in A. This option requires -D and follows its orientation rules for determining what is "upstream".
-id	Ignore features in B that are downstream of features in A. This option requires -D and follows its orientation rules for determining what is "downstream".
-fu	Choose first from features in B that are upstream of features in A. This option requires -D and follows its orientation rules for determining what is "upstream".
-fd	Choose first from features in B that are downstream of features in A. This option requires -D and follows its orientation rules for determining what is "downstream".
-t	Specify how ties for closest feature should be handled. This occurs when two features in B have exactly the same "closeness" with A. By default, all such features in B are reported. Here are all the options: - *all* Report all ties (default). - *first* Report the first tie that occurred in the B file. - *last* Report the last tie that occurred in the B file.
-mdb	Specify how multiple databases should be resolved.

Default behavior

The *closest* tool first searches for features in B that overlap a feature in A. If overlaps are found, each feature in B that overlaps A is reported. If no overlaps are found, *closestBed* looks for the feature in B that is *closest* (that is, least genomic distance to the start or end of A) to A. For example,

For example, consider the case where one of the intervals in B overlaps the interval in B, yet another does not:

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  15  25  b2  2 +

$ bedtools closest -a a.bed -b b.bed
chr1  10  20  a1  1 - chr1  15  25  b2  2 +
```

Now compare what happens when neither interval in B overlaps the record in A, yet one is closer than the other.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  30  40  b2  2 +

$ bedtools closest -a a.bed -b b.bed
chr1  10  20  a1  1 - chr1  7 8 b1  1
```

But what if each interval in B is equally close to the interval in A? In this case, the default behavior is to report all intervals in B that are tied for proximity. Check out the *-t* option to adjust this behaviour.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  22  23  b2  2 +

$ bedtools closest -a a.bed -b b.bed
chr1  10  20  a1  1 - chr1  7   8   b1  1 -
chr1  10  20  a1  1 - chr1  22  23  b2  2 +
```

Using multiple *-b* files.

As of version, 2.22.0, the *closest* tool allows one to find the closest intervals in multiple *-b* files. Consider the following examples.

Note: When using multiple *-b* files, an additional column describing the file number from which the closest B interval came will be added between the columns representing the full A interval and the columns representing the full A interval. This file number will refer to the order in which the files were provided on the command line.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b1.bed
chr1  5   6   b1.1  1 -
chr1  30  40  b1.2  2 +

$ cat b2.bed
chr1  0   1   b2.1  1 -
chr1  21  22  b2.2  2 +

# In this example, the 7th column reflects the file number from
# which the closest interval came.

$ bedtools closest -a a.bed -b b1.bed b2.bed
chr1  10  20  a1  1 - 1 chr1  5   6   b1.1  1 -
chr1  10  20  a1  1 - 2 chr1  21  22  b2.2  2 +
```

Instead of using file numbers, you can also provide more informative labels via the *-names* option.

```
$ bedtools closest -a a.bed -b b1.bed b2.bed -names b1 b2
chr1  10  20  a1  1 - b1  chr1  5   6   b1.1  1 -
chr1  10  20  a1  1 - b2  chr1  21  22  b2.2  2 +
```

Or, you can use the full original filename via the *-filenames* option.

```
$ bedtools closest -a a.bed -b b1.bed b2.bed -filenames
chr1  10  20  a1  1 - b1.bed  chr1  5   6   b1.1  1 -
chr1  10  20  a1  1 - b2.bed  chr1  21  22  b2.2  2 +
```

`-mdb` Find the closest interval in each* or among **all *-b* files.

By default, the closest interval from **each** file is reported when using multiple *-b* files.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b1.bed
chr1  5   6   b1.1  1 -
chr1  30  40  b1.2  2 +

$ cat b2.bed
chr1  0   1   b2.1  1 -
chr1  21  22  b2.2  2 +

$ bedtools closest -a a.bed -b b1.bed b2.bed -d
chr1  10  20  a1  1 - 1 chr1  5   6   b1.1  1 - 5
chr1  10  20  a1  1 - 2 chr1  21  22  b2.2  2 + 2

$ bedtools closest -a a.bed -b b1.bed b2.bed -mdb each -d
chr1  10  20  a1  1 - 1 chr1  5   6   b1.1  1 - 5
chr1  10  20  a1  1 - 2 chr1  21  22  b2.2  2 + 2
```

However, one can optionally choose to report only the closest interval(s) observed among **all** of the *-b* files. In this example, the second interval from b2.bed is only 2 base pairs away from the interval in A, whereas the first interval in b1.bed is 5 base pairs away. Therefore, when using *mdb all*, the the second interval from b2.bed wins.

```
$ bedtools closest -a a.bed -b b1.bed b2.bed -mdb all -d
chr1  10  20  a1  1 - 2 chr1  21  22  b2.2  2 + 2
```

-io Ignoring overlapping intervals

This option prevents intervals in B that overlap the interval in A from being reported as "closest".

Without *-ip* the second record in B will be reported as closest.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  15  25  b2  2 +

$ bedtools closest -a a.bed -b b.bed
chr1  10  20  a1  1 - chr1  15  25  b2  2 +
```

Yet with *-io*, the overlapping interval is ignored in favor of the closest, non-overlapping interval.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  15  25  b2  2 +

$ bedtools closest -a a.bed -b b.bed -io
chr1  10  20  a1  1 - chr1  7 8 b1  1 -
```

-s Requiring closest intervals to have the *same* strand

The *-s* option finds the closest interval that is also on the same strand as the interval in A.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  2   3   b1  1 -
chr1  21  22  b2  2 +

$ bedtools closest -a a.bed -b b.bed -s
chr1  10  20  a1  1 - chr1  2 3 b1  1 -
```

-S Requiring closest intervals to have the *opposite* strand

The *-s* option finds the closest interval that is also on the same strand as the interval in A.

```
$ cat a.bed
chr1  10  20  a1  1 -
```

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```
$ cat b.bed
chr1  15  16   b1  1 -
chr1  21  22  b2  2 +

$ bedtools closest -a a.bed -b b.bed -S
chr1  10  20  a1  1 - chr1  21  22  b2  2 +
```

-t Controlling how ties for "closest" are broken

When there are two or more features in B are tied for proximity to the interval in A, *closest* will, by default, report all such intervals in B. As shown in the examples below, this behavior can be changed via the *-t* option:

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  30  40   b1  1 -
chr1  30  40   b2  2 +

# default
$ bedtools closest -a a.bed -b b.bed
chr1  10  20  a1  1 - chr1  30  40  b1  1 -
chr1  10  20  a1  1 - chr1  30  40  b2  2 +

# -t all (default)
$ bedtools closest -a a.bed -b b.bed -t all
chr1  10  20  a1  1 - chr1  30  40  b1  1 -
chr1  10  20  a1  1 - chr1  30  40  b2  2 +

# -t first
$ bedtools closest -a a.bed -b b.bed -t first
chr1  10  20  a1  1 - chr1  30  40  b1  1 -

# -t last
$ bedtools closest -a a.bed -b b.bed -t last
chr1  10  20  a1  1 - chr1  30  40  b2  1 +
```

-d Reporting the distance to the closest feature in base pairs

One often wants to also know the distance in base pairs between the interval in A and the closest interval(s) in B. *closest* will optionally report the distance to the closest feature in the B file using the *-d* option. The distance (in base pairs) will be reported as the last column in the output.

When a feature in B overlaps a feature in A, a distance of 0 is reported.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ cat b.bed
chr1  7   8   b1  1 -
chr1  22  23  b2  2 +

$ bedtools closest -a a.bed -b b.bed
```

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```
chr1  10  20  a1  1 - chr1  7   8   b1  1 - 3
chr1  10  20  a1  1 - chr1  22  23  b2  2 + 3
```

–D Reporting signed distances to the closest feature in base pairs

Whereas the *-d* option always reports distances as positive integers, the *-D* option will use negative integers to report distances to "upstream" features. There are three options for dictating how "upstream" should be defined.

1. *-D ref*: Report distance with respect to the reference genome. That is, B features with lower start/stop coordinates are considered to be upstream.
2. *-D a*: Report distance with respect to the orientation of the interval in A. That is, when A is on the - strand, "upstream" means B has higher start/stop coordinates. When A is on the + strand, "upstream" means B has lower start/stop coordinates.
3. *-D b*: Report distance with respect to the orientation of the interval in B. That is, when B is on the - strand, "upstream" means A has higher start/stop coordinates. When B is on the + strand, "upstream" means A has lower start/stop coordinates.

This is best demonstrated through multiple examples.

```
$ cat a.bed
chr1  10  20  a1  1 +

$ cat b.bed
chr1  7   8   b1  1 +
chr1  22  23  b2  2 -

$ bedtools closest -a a.bed -b b.bed -D ref
chr1  10  20  a1  1 + chr1  7   8   b1  1 + -3
chr1  10  20  a1  1 + chr1  22  23  b2  2 - 3
```

Since the A record is on the "+" strand in this example, *-D ref* and *-D a* have the same effect.

```
$ bedtools closest -a a.bed -b b.bed -D a
chr1  10  20  a1  1 + chr1  7   8   b1  1 + -3
chr1  10  20  a1  1 + chr1  22  23  b2  2 - 3
```

However, the signs of the distances change if the A interval is on the "-" strand.

```
$ cat a.bed
chr1  10  20  a1  1 -

$ bedtools closest -a a.bed -b b.bed -D a
chr1  10  20  a1  1 - chr1  7   8   b1  1 + 3
chr1  10  20  a1  1 - chr1  22  23  b2  2 - -3
```

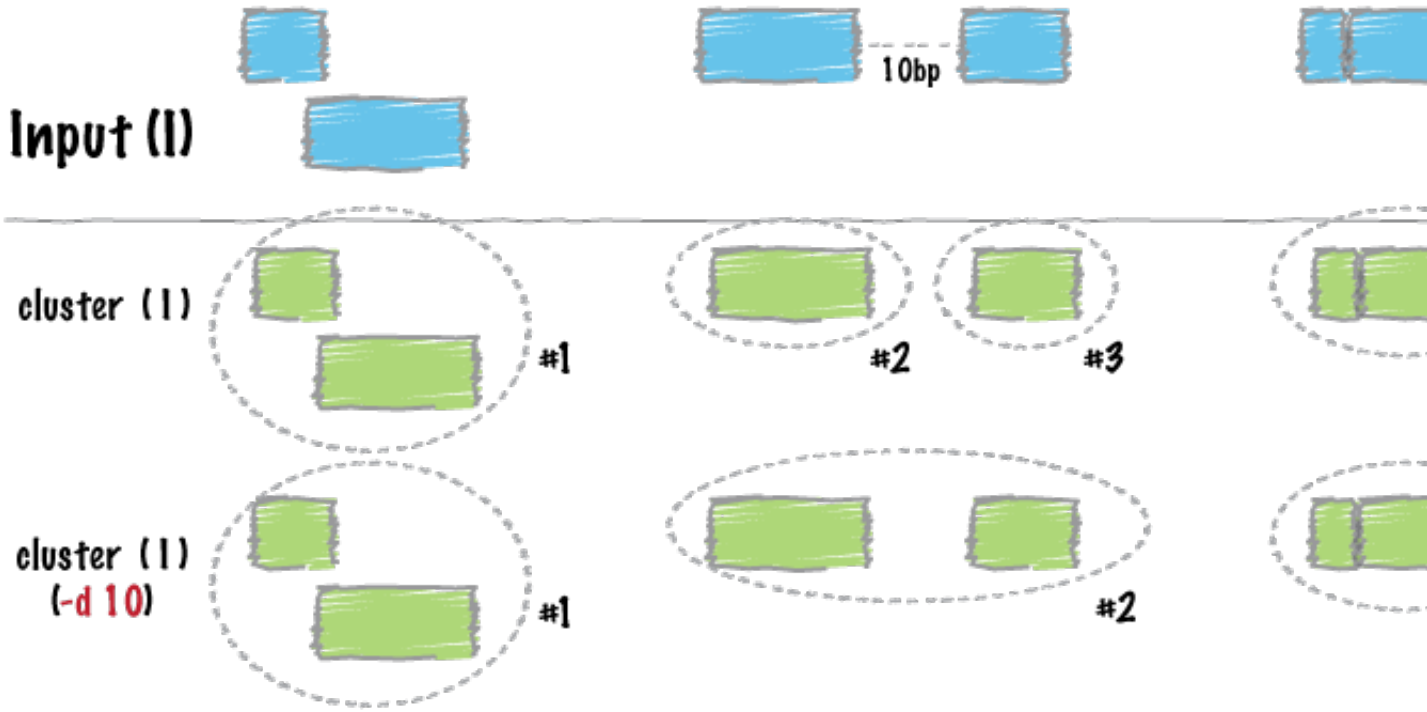
Let's switch the A interval back to the "+" strand and now report distances with respect to the orientation of the closest B records.

```
$ cat a.bed
chr1  10  20  a1  1 +

$ bedtools closest -a a.bed -b b.bed -D b
chr1  10  20  a1  1 + chr1  7   8   b1  1 + 3
chr1  10  20  a1  1 + chr1  22  23  b2  2 - 3
```

Let's flip the stand of the two B records and compare.

```
$ cat b.bed
chr1  7   8   b1  1 -
chr1  22  23  b2  2 +

$ bedtools closest -a a.bed -b b.bed -D b
chr1  10  20  a1  1 + chr1  7   8   b1  1 - -3
chr1  10  20  a1  1 + chr1  22  23  b2  2 + -3
```

cluster



Similar to *merge*, `cluster` report each set of overlapping or "book-ended" features in an interval file. In contrast to `merge`, `cluster` does not flatten the cluster of intervals into a new meta-interval; instead, it assigns an unique cluster ID to each record in each cluster. This is useful for having fine control over how sets of overlapping intervals in a single interval file are combined.

Note: `bedtools cluster` requires that you presort your data by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files).

See also:

merge

Usage and option summary

Usage:

```
bedtools cluster [OPTIONS] -i <BED/GFF/VCF>
```

(or):

```
clusterBed [OPTIONS] -i <BED/GFF/VCF>
```

Option	Description
-s	Force strandedness. That is, only cluster features that are the same strand. *By default, this is disabled.*
-d	Maximum distance between features allowed for features to be clustered. *Default is 0. That is, overlapping and/or book-ended features are clustered.*

Default behavior

By default, `bedtools cluster` collects overlapping (by at least 1 bp) and/or bookended intervals into distinct clusters. In the example below, the 4th column is the cluster ID.

```
$ cat A.bed
chr1  100  200
chr1  180  250
chr1  250  500
chr1  501  1000

$ bedtools cluster -i A.bed
chr1  100     200     1
chr1  180     250     1
chr1  250     500     1
chr1  501     1000    2
```

`-s` Enforcing "strandedness"

The `-s` option will only cluster intervals that are overlapping/bookended *and* are on the same strand.

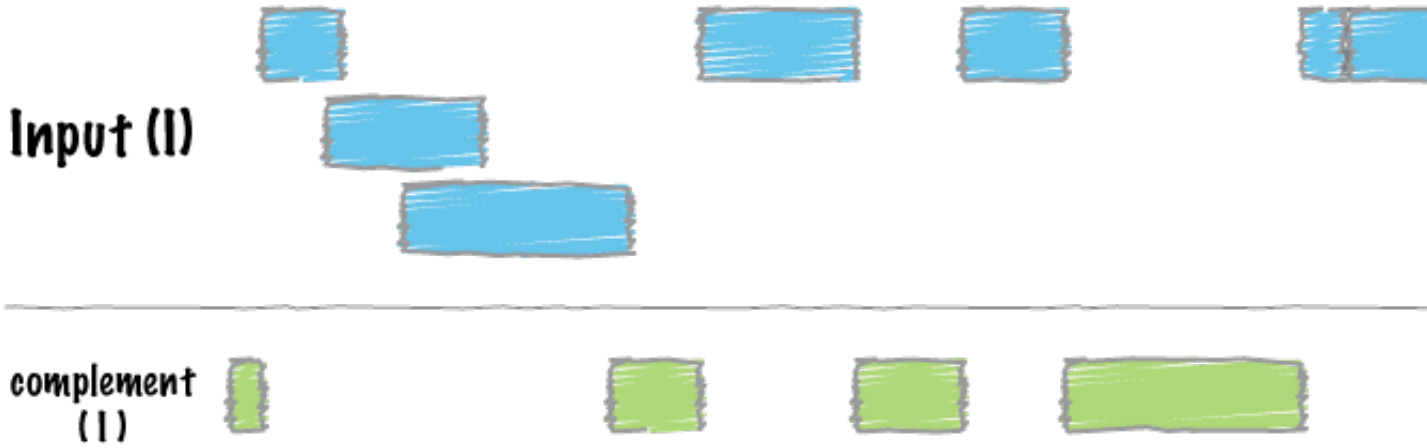
```
$ cat A.bed
chr1  100  200   a1  1 +
chr1  180  250   a2  2 +
chr1  250  500   a3  3 -
chr1  501  1000  a4  4 +

$ bedtools cluster -i A.bed -s
chr1  100     200     a1      1       +       1
chr1  180     250     a2      2       +       1
chr1  501     1000    a4      4       +       2
chr1  250     500     a3      3       -       3
```

-d Controlling how close two features must be in order to cluster

By default, only overlapping or book-ended features are combined into a new feature. However, one can force `cluster` to combine more distant features with the `-d` option. For example, were one to set `-d` to 1000, any features that overlap or are within 1000 base pairs of one another will be clustered.

```
$ cat A.bed
chr1  100  200
chr1  501  1000

$ bedtools cluster -i A.bed
chr1  100  200    1
chr1  501  1000   2

$ bedtools cluster -i A.bed -d 1000
chr1  100  200    1
chr1  501  1000   1
```

complement



`bedtools complement` returns all intervals in a genome that are **not** covered by at least one interval in the input BED/GFF/VCF file.

See also:

merge

Usage and option summary

Usage:

```
bedtools complement -i <BED/GFF/VCF> -g <GENOME>
```

(or):

```
complementBed -i <BED/GFF/VCF> -g <GENOME>
```

Default behavior

By default, `bedtools complement` returns all genomic intervals that are not covered by at least one record from the input file.

```
$ cat A.bed
chr1  100  200
chr1  400  500
chr1  500  800

$ cat my.genome
chr1  1000
chr2  800

$ bedtools complement -i A.bed -g my.genome
chr1  0   100
chr1  200 400
chr1  800 1000
chr2  0   800
```

`-L` Only report chromosomes that are in the *-i* file

Use the "**-L**" option to *L'imit the output to solely the chromosomes that are represented in the '-i* file. Chromosomes that are in *-g* but not *-i* will be suppressed

For example (note the difference in coverage with and without **-s**:

```
$ cat A.bed
chr1  100  200
chr1  400  500
chr1  500  800

$ cat my.genome
chr1  1000
chr2  800

$ bedtools complement -i A.bed -g my.genome
chr1  0   100
chr1  200 400
chr1  800 1000
```

coverage

The `bedtools coverage` tool computes both the *depth* and *breadth* of coverage of features in file B on the features in file A. For example, `bedtools coverage` can compute the coverage of sequence alignments (file B) across 1 kilobase (arbitrary) windows (file A) tiling a genome of interest. One advantage that `bedtools coverage` offers is that it not only *counts* the number of features that overlap an interval in file A, it also computes the fraction of bases in the interval in A that were overlapped by one or more features. Thus, `bedtools coverage` also computes the *breadth* of coverage observed for each interval in A.

Note: If you are trying to compute coverage for very large files and are having trouble with excessive memory usage, please presort your data by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files) and then use the `-sorted` option. This invokes a memory-efficient algorithm designed for large files.

Important: As of version 2.24.0, the *coverage* tool has changed such that the coverage is computed for the A file, not the B file. This changes the command line interface to be consistent with the other tools. Also, the *coverage* tool can accept multiple files for the *-b* option. This allows one to measure coverage between a single query (*-a*) file and multiple database files (*-b*) at once!

See also:

intersect genomecov

Usage and option summary

Usage:

```
bedtools coverage [OPTIONS] -a <FILE> \
                            -b <FILE1, FILE2, ..., FILEN>
```

(or):

```
coverageBed [OPTIONS] -a <FILE> \
                      -b <FILE1, FILE2, ..., FILEN>
```

Option	Description
-a	BAM/BED/GFF/VCF file "A". Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	One or more BAM/BED/GFF/VCF file(s) "B". Use "stdin" if passing B with a UNIX pipe. **NEW!!!**: -b may be followed with multiple databases and/or wildcard (*) character(s).
-abam	BAM file A. Each BAM alignment in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe: For example: samtools view -b <BAM> | bedtools intersect -abam stdin -b genes.bed. **Note**: no longer necessary after version 2.19.0
-hist	Report a histogram of coverage for each feature in A as well as a summary histogram for _all_ features in A. Output (tab delimited) after each feature in A: 1) depth 2) # bases at depth 3) size of A 4) % of A at depth
-d	Report the depth at each position in each A feature. Positions reported are one based. Each position and depth follow the complete A feature.
-counts	Only report the count of overlaps, don't compute fraction, etc. Restricted by -f and -r.
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-F	Minimum overlap required as a fraction of B. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-e	Require that the minimum fraction be satisfied for A _OR_ B. In other words, if -e is used with -f 0.90 and -F 0.10 this requires that either 90% of A is covered OR 10% of B is covered. Without -e, both fractions would have to be satisfied.
-s	Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-split	Treat "split" BAM (i.e., having an "N" CIGAR operation) or BED12 entries as distinct BED intervals.
-sorted	For very large B files, invoke a "sweeping" algorithm that requires position-sorted (e.g., `sort -k1, 1 -k2,2n` for BED files) input. When using -sorted, memory usage remains low even for very large files.
-g	Specify a genome file the defines the expected chromosome order in the input files for use with the `-sorted` option.
-header	Print the header from the A file prior to results.
-sortout	When using *multiple databases* (*-b*), sort the output DB hits for each record.

Default behavior

After each interval in A, `bedtools coverage` will report:

1) The number of features in B that overlapped (by at least one base pair) the A interval.
2) The number of bases in A that had non-zero coverage from features in B.
3) The length of the entry in A.
4) The fraction of bases in A that had non-zero coverage from features in B.

Below are the number of features in B (N=...) overlapping A and fraction of bases in A with coverage.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

BED FILE A  ***************     ***************     ******    **************

BED File B  ^^^^ ^^^^               ^^              ^^^^^^^^^     ^^^ ^^ ^^^^
              ^^^^^^^^                                          ^^^^^ ^^^^^ ^^


Result      [  N=3, 10/15 ]     [  N=1, 2/15 ]     [N=1,6/6]    [N=6, 12/14 ]
```

For example:

```
$ cat A.bed
chr1  0   100
chr1  100 200
chr2  0   100

$ cat B.bed
chr1  10  20
chr1  20  30
chr1  30  40
chr1  100 200

$ bedtools coverage -a A.bed -b B.bed
chr1  0   100  3  30  100 0.3000000
chr1  100 200  1  100 100 1.0000000
chr2  0   100  0  0   100 0.0000000
```

`-s` Calculating coverage by strand

Use the "**-s**" option if one wants to only count coverage if features in A are on the same strand as the feature / window in A. This is especially useful for RNA-seq experiments.

For example (note the difference in coverage with and without **-s**:

```
$ cat A.bed
chr1  0   100 b1  1  +
chr1  100 200 b2  1  -
chr2  0   100 b3  1  +

$ cat B.bed
chr1  10  20  a1  1  -
chr1  20  30  a2  1  -
chr1  30  40  a3  1  -
chr1  100 200 a4  1  +
```

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```
$ bedtools coverage -a A.bed -b B.bed
chr1  0   100 b1  1  +  3  30  100  0.3000000
chr1  100 200 b2  1  -  1  100 100  1.0000000
chr2  0   100 b3  1  +  0  0   100  0.0000000

$ bedtools coverage -a A.bed -b B.bed -s
chr1  0   100 b1  1  +  0  0   100  0.0000000
chr1  100 200 b2  1  -  0  0   100  0.0000000
chr2  0   100 b3  1  +  0  0   100  0.0000000
```

`-hist` Creating a histogram of coverage for each feature in the A file

One should use the "**-hist**" option to create, for each interval in A, a histogram of coverage of the features in B across A.

In this case, each entire feature in A will be reported, followed by the depth of coverage, the number of bases at that depth, the size of the feature, and the fraction covered. After all of the features in A have been reported, a histogram summarizing the coverage among all features in A will be reported.

```
$ cat A.bed
chr1  0   100 b1  1  +
chr1  100 200 b2  1  -
chr2  0   100 b3  1  +

$ cat B.bed
chr1  10  20  a1  1  -
chr1  20  30  a2  1  -
chr1  30  40  a3  1  -
chr1  100 200 a4  1  +

$ bedtools coverage  -a A.bed -b B.bed -hist
chr1  0   100 b1  1  +  0  70  100  0.7000000
chr1  0   100 b1  1  +  1  30  100  0.3000000
chr1  100 200 b2  1  -  1  100 100  1.0000000
chr2  0   100 b3  1  +  0  100 100  1.0000000
all   0   170 300 0.5666667
all   1   130 300 0.4333333
```

`-d` Reporting the per-base of coverage for each feature in the A file

One should use the "**-d**" option to create, for each interval in A, a detailed list of coverage at each of the positions across each A interval.

The output will consist of a line for each one-based position in each A feature, followed by the coverage detected at that position.

```
$ cat A.bed
chr1  0  10

$ cat B.bed
chr1  0  5
chr1  3  8
```

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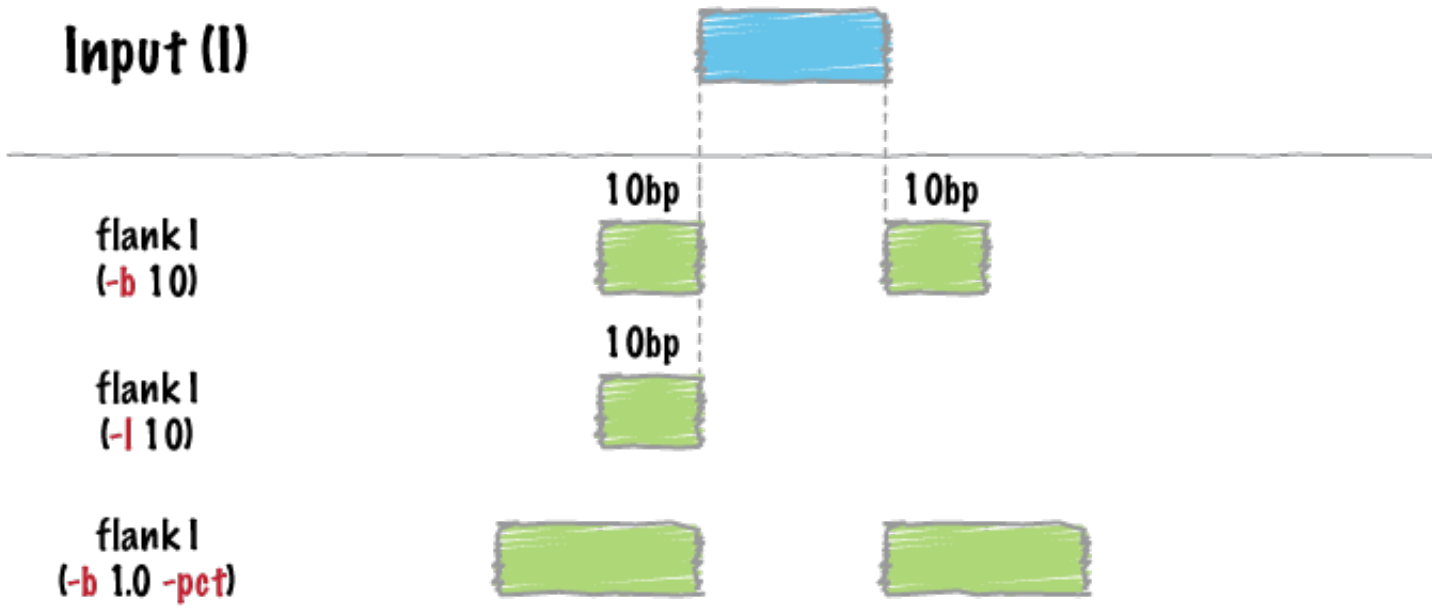
```
chr1  4  8
chr1  5  9

$ bedtools coverage -a A.bed -b B.bed -d
chr1  0  10  B  1  1
chr1  0  10  B  2  1
chr1  0  10  B  3  1
chr1  0  10  B  4  2
chr1  0  10  B  5  3
chr1  0  10  B  6  3
chr1  0  10  B  7  3
chr1  0  10  B  8  3
chr1  0  10  B  9  1
chr1  0  10  B  10 0
```

expand

flank



`bedtools flank` will create two new flanking intervals for each interval in a BED/GFF/VCF file. Note that `flank` will restrict the created flanking intervals to the size of the chromosome (i.e. no start < 0 and no end > chromosome

size).

Note: In order to prevent creating intervals that violate chromosome boundaries, `bedtools flank` requires a *genome* file defining the length of each chromosome or contig.

See also:

slop

Usage and option summary

Usage:

```
bedtools flank [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-b or (-l and -r)]
```

(or):

```
flankBed [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-b or (-l and -r)]
```

Option	Description
-b	Increase the BED/GFF/VCF entry by the same number base pairs in each direction. *Integer*.
-l	The number of base pairs to subtract from the start coordinate. *Integer*.
-r	The number of base pairs to add to the end coordinate. *Integer*.
-s	Define -l and -r based on strand. For example. if used, -l 500 for a negative-stranded feature, it will add 500 bp to the *end* coordinate.
-pct	Define -l and -r as a fraction of the feature's length. E.g. if used on a 1000bp feature, -l 0.50, will add 500 bp "upstream". Default = false.

Default behavior

By default, `bedtools flank` will either add a fixed number of bases in each direction (`-b`) or an asymmetric number of bases in each direction with `-l` and `-r`.

```
$ cat A.bed
chr1 100 200
chr1 500 600

$ cat my.genome
chr1 1000

$ bedtools flank -i A.bed -g my.genome -b 5
chr1  95      100
chr1  200     205
chr1  495     500
chr1  600     605

$ bedtools flank -i A.bed -g my.genome -l 2 -r 3
chr1  98      100
chr1  200     203
chr1  498     500
chr1  600     603
```

However, if the requested number of bases exceeds the boundaries of the chromosome, `bedtools flank` will "clip" the feature accordingly.

```
$ cat A.bed
chr1 100 200
chr1 500 600

$ cat my.genome
chr1 1000

$ bedtools flank -i A.bed -g my.genome -b 800
chr1  0       100
chr1  200     1000
chr1  0       500
chr1  600     1000
```

`-pct` Resizing features by a given fraction

`bedtools flank` will optionally create flanking intervals whose size is user-specified fraction of the original interval.

For example:

```
$ cat A.bed
chr1 100 200
chr1 500 700

################################################################
# note the flanking intervals from the second record in A.bed
# are 20bp whereas the flanking intervals from the first record
# are only 10bp
################################################################
$ bedtools flank -i A.bed -g my.genome -b 0.1 -pct
chr1  90      100
chr1  200     210
chr1  480     500
chr1  700     720
```

fisher

Perform fisher's exact test on the number of overlaps/unique intervals between 2 files.

Traditionally, in order to test whether 2 sets of intervals are related spatially, we resort to shuffling the genome and checking the simulated (shuffled) versus the observed. We can do the same analytically for many scenarios using Fisher's Exact Test .

This implementation can calculate the number of overlaps and the number of intervals unique to each file and it infers (or accepts) the number that are not present in each file.

Given a pair of input files *-a* and *-b* in the usual BedTools parlance:

```
$ cat a.bed
chr1  10  20
chr1  30  40
chr1  51      52

$ cat b.bed
chr1  15   25
chr1  51      52
```

And a genome of 500 bases:

```
$ echo -e "chr1\t500" > t.genome
```

We may wish to know **if the amount of overlap between the 2 sets of intervals is more than we would expect given their coverage and the size of the genome**. We can do this with `fisher` as:

```
$ bedtools fisher -a a.bed -b b.bed -g t.genome
# Number of query intervals: 3
# Number of db intervals: 2
# Number of overlaps: 2
# Number of possible intervals (estimated): 37
# phyper(2 - 1, 3, 37 - 3, 2, lower.tail=F)
# Contingency Table Of Counts
#_____________________________________________
#           |  in -b       | not in -b    |
#     in -a | 2            | 1            |
# not in -a | 0            | 34           |
#_____________________________________________
# p-values for fisher's exact test
left    right   two-tail    ratio
1   0.0045045   0.0045045   inf
```

Where we can see the constructed contingency table and the pvalues for left, right and two-tail tests. From here, we can say that given **500 bases** of genome, it is unlikely that we would see **as many** overlaps as we do if the intervals from *a* and *b* were not related.

Note: the total number of **possible** intervals in the above example was estimated to be 37. This is based on a heuristic that uses the mean sizes of intervals in the *a* and *b* sets and the size of the genome. The reported p-value will depend greatly on this. Below, we show how well the reported value matches with simulations.

The above had a fairly low p-value (0.0045), but if our genome were only **60 bases**:

```
$ echo -e "chr1\t60" > t.genome
$ bedtools fisher -a a.bed -b b.bed -g t.genome
# Number of query intervals: 3
# Number of db intervals: 2
# Number of overlaps: 2
# Number of possible intervals (estimated): 4
# phyper(2 - 1, 3, 4 - 3, 2, lower.tail=F)
# Contingency Table Of Counts
#_____________________________________________
#           |  in -b       | not in -b    |
#     in -a | 2            | 1            |
# not in -a | 0            | 1            |
#_____________________________________________
```

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```
# p-values for fisher's exact test
left    right   two-tail    ratio
1   0.5 1   inf
```

We can see that neither tail is significant. Intuitively, this makes sense; if we randomly place 3 intervals (from *-a*), and 2 (from *-b*) intervals from within 60 bases, it doesn't seem unlikely that we'd see 2 overlaps.

Note also that since the genome size is much smaller, the number of possible intervals is also decreased.

Evaluation

The p-value depends on knowing or inferring the total number of possible intervals (to fill in the lower right corner of the contingency table). This inference is not straightforward since we will most likely have variable sized intervals within and between files. Below, we show the correspondence of the p-value reported by *fisher* and one from simulated data.

Note: The `fisher` tool requires that your data is pre-sorted by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files).

This uses Heng Li's implementation of Fisher's exact test in kfunc.c.

See also:

jaccard reldist intersect

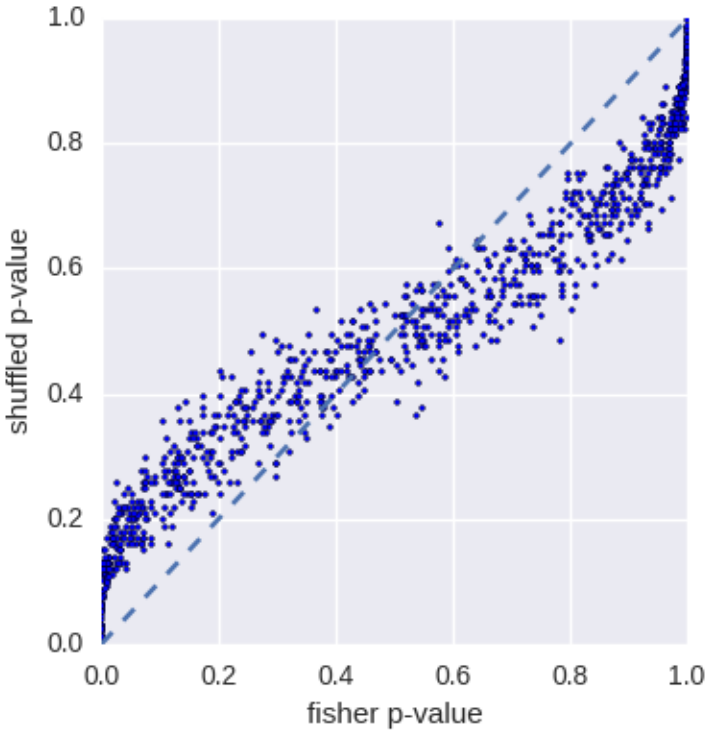
Usage and option summary

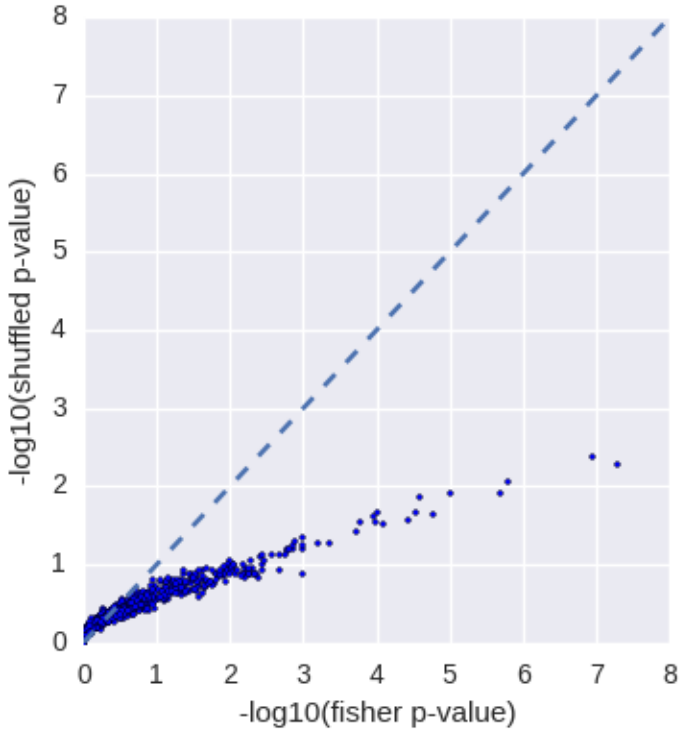
Usage:

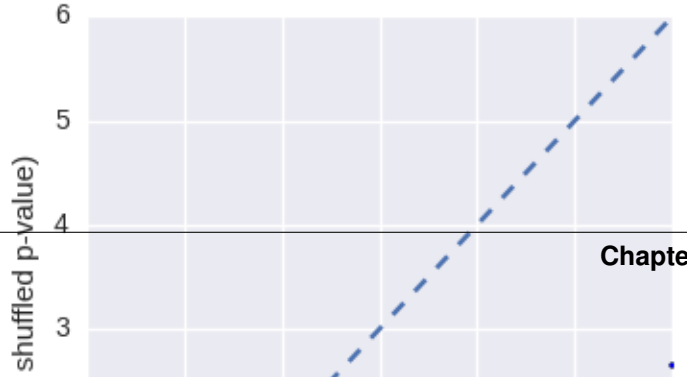
```
bedtools fisher [OPTIONS] -a <BED/GFF/VCF> -b <BED/GFF/VCF> -g <genome>
```

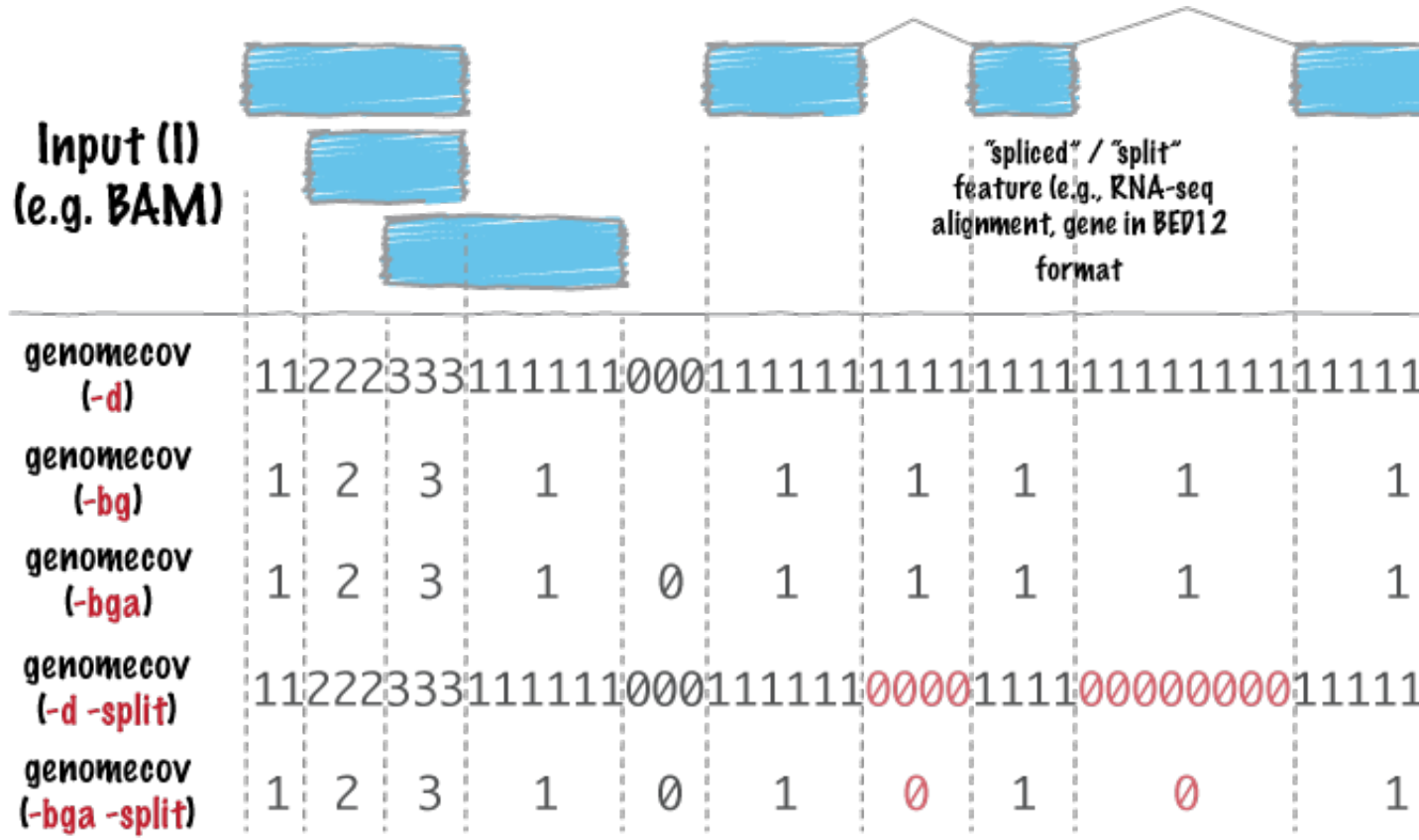
Option	Description
-a	BED/GFF/VCF file A. Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	BED/GFF/VCF file B. Use "stdin" if passing B with a UNIX pipe.
-g	genome file listing chromosome size.
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-s	Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-split	Treat "split" BAM (i.e., having an "N" CIGAR operation) or BED12 entries as distinct BED intervals.

genomecov


1.0
0.8
0.6
0.4
0.2
0.0
shuffled p-value
0.0
0.2
0.4
0.6
0.8
1.0
fisher p-value


8
7
6
5
4
3
2
1
0
-log10(shuffled p-value)
0
1
2
3
4
5
6
7
8
-log10(fisher p-value)


6
5
4
3
shuffled p-value)



`bedtools genomecov` computes histograms (default), per-base reports (`-d`) and BEDGRAPH (`-bg`) summaries of feature coverage (e.g., aligned sequences) for a given genome.

Note: 1. If using BED/GFF/VCF, the input (`-i`) file must be grouped by chromosome. A simple `sort -k 1,1 in.bed > in.sorted.bed` will suffice. Also, if using BED/GFF/VCF, one must provide a genome file via the `-g` argument.

2. If the input is in BAM (-ibam) format, the BAM file must be sorted by position. Using `samtools sort aln.bam aln.sorted` will suffice.

Usage and option summary

Usage:

```
bedtools genomecov [OPTIONS] [-i|-ibam] -g (iff. -i and not -ibam)
```

(or):

```
genomeCoverageBed [OPTIONS] [-i|-ibam] -g (iff. -i and not -ibam)
```

Option	Description
-ibam	BAM file as input for coverage. Each BAM alignment in A added to the total coverage for the genome. Use "stdin" or simply "-" if passing it with a UNIX pipe: For example: `samtools view -b <BAM> | genomeCoverageBed -ibam stdin`
-g	Provide a genome file to define chromosome lengths. Required when not using -ibam option.
-d	Report the depth at each genome position with 1-based coordinates.
-dz	Report the depth at each genome position with 0-based coordinates. Unlike, *-d*, this reports only non-zero positions.
-bg	Report depth in BedGraph format. For details, see: http://genome.ucsc.edu/goldenPath/help/bedgraph.html
-bga	Report depth in BedGraph format, as above (i.e., -bg). However with this option, regions with zero coverage are also reported. This allows one to quickly extract all regions of a genome with 0 coverage by applying: "grep -w 0$" to the output.
-split	Treat "split" BAM or BED12 entries as distinct BED intervals when computing coverage. For BAM files, this uses the CIGAR "N" and "D" operations to infer the blocks for computing coverage. For BED12 files, this uses the BlockCount, BlockStarts, and BlockEnds fields (i.e., columns 10,11,12).
-strand	Calculate coverage of intervals from a specific strand. With BED files, requires at least 6 columns (strand is column 6).
-5	Calculate coverage of 5' positions (instead of entire interval).
-3	Calculate coverage of 3' positions (instead of entire interval).
-max	Combine all positions with a depth >= max into a single bin in the histogram.
-scale	Scale the coverage by a constant factor. Each coverage value is multiplied by this factor before being reported. Useful for normalizing coverage by, e.g., reads per million (RPM). `Default is 1.0; i.e., unscaled.`
-trackline	Adds a UCSC/Genome-Browser track line definition in the first line of the output. See here for more details about track line definition:
-trackopts	Writes additional track line definition parameters in the first line.
-pc	Calculates coverage of intervals from left point of a pair reads to the right point.

Default behavior

By default, `bedtools genomecov` will compute a histogram of coverage for the genome file provided. The default output format is as follows:

1. chromosome (or entire genome)
2. depth of coverage from features in input file
3. number of bases on chromosome (or genome) with depth equal to column 2.
4. size of chromosome (or entire genome) in base pairs
5. fraction of bases on chromosome (or entire genome) with depth equal to column 2.

For example:

```
$ cat A.bed
chr1  10  20
chr1  20  30
chr2  0   500

$ cat my.genome
chr1  1000
chr2  500

$ bedtools genomecov -i A.bed -g my.genome
chr1   0  980  1000  0.98
chr1   1  20   1000  0.02
chr2   1  500  500   1
genome 0  980  1500  0.653333
genome 1  520  1500  0.346667
```

`-max` Controlling the histogram's maximum depth

Using the `-max` option, `bedtools genomecov` will "lump" all positions in the genome having feature coverage greater than or equal to `-max` into the `-max` histogram bin. For example, if one sets `-max` equal to 50, the max depth reported in the output will be 50 and all positions with a depth >= 50 will be represented in bin 50.

`-d` Reporting "per-base" genome coverage

Using the `-d` option, `bedtools genomecov` will compute the depth of feature coverage for each base on each chromosome in genome file provided.

The "per-base" output format is as follows:

1. chromosome
2. chromosome position
3. depth (number) of features overlapping this chromosome position.

For example:

```
$ cat A.bed
chr1  10  20
chr1  20  30
chr2  0   500
```

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```
$ cat my.genome
chr1  1000
chr2  500

$ bedtools genomecov -i A.bed -g my.genome -d | \
      head -15 | \
      tail -n 10
chr1  6  0
chr1  7  0
chr1  8  0
chr1  9  0
chr1  10 0
chr1  11 1
chr1  12 1
chr1  13 1
chr1  14 1
chr1  15 1
```

`-bg` Reporting genome coverage in BEDGRAPH format.

Whereas the `-d` option reports an output line describing the observed coverage at each and every position in the genome, the `-bg` option instead produces genome-wide coverage output in BEDGRAPH format. This is a much more concise representation since consecutive positions with the same coverage are reported as a single output line describing the start and end coordinate of the interval having the coverage level, followed by the coverage level itself.

For example, below is a snippet of BEDGRAPH output of the coverage from a 1000 Genome Project BAM file:

```
$ bedtools genomecov -ibam NA18152.bam -bg | head
chr1  554304  554309  5
chr1  554309  554313  6
chr1  554313  554314  1
chr1  554315  554316  6
chr1  554316  554317  5
chr1  554317  554318  1
chr1  554318  554319  2
chr1  554319  554321  6
chr1  554321  554323  1
chr1  554323  554334  7
```

Using this format, one can quickly identify regions of the genome with sufficient coverage (in this case, 10 or more reads) by piping the output to an `awk` filter.

```
$ bedtools genomecov -ibam NA18152.bam -bg | \
    awk '$4 > 9' | \
    head
chr1  554377  554381  11
chr1  554381  554385  12
chr1  554385  554392  16
chr1  554392  554408  17
chr1  554408  554410  19
chr1  554410  554422  20
chr1  554422  554423  19
chr1  554423  554430  22
```

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```
chr1  554430  554440  24
chr1  554440  554443  25
```

`-bga` Reporting genome coverage for *all* positions in BEDGRAPH format.

The `-bg` option reports coverage in BEDGRAPH format only for those regions of the genome that actually have coverage. But what about the uncovered portion of the genome? By using the `-bga` option, one receives a complete report including the regions with zero coverage.

For example, compare the output from `-bg`:

```
$ bedtools genomecov -ibam NA18152.bam -bg | head
chr1  554304  554309  5
chr1  554309  554313  6
chr1  554313  554314  1
chr1  554315  554316  6
chr1  554316  554317  5
chr1  554317  554318  1
chr1  554318  554319  2
chr1  554319  554321  6
chr1  554321  554323  1
chr1  554323  554334  7
```

to the output from `-bga`:

```
# Note the first record reports that the first 554304
# base pairs of chr1 had zero coverage
$ bedtools genomecov -ibam NA18152.bam -bga | head
chr1  0       554304  0
chr1  554304  554309  5
chr1  554309  554313  6
chr1  554313  554314  1
chr1  554314  554315  0
chr1  554315  554316  6
chr1  554316  554317  5
chr1  554317  554318  1
chr1  554318  554319  2
chr1  554319  554321  6
```

`-strand` Reporting genome coverage for a specific strand.

Whereas the default is to count coverage regardless of strand, the `-strand` option allows one to report the coverage observed for a specific strand.

Compare:

```
$ bedtools genomecov -ibam NA18152.bam -bg | head
chr1  554304  554309  5
chr1  554309  554313  6
chr1  554313  554314  1
chr1  554315  554316  6
chr1  554316  554317  5
chr1  554317  554318  1
```

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```
chr1  554318  554319  2
chr1  554319  554321  6
chr1  554321  554323  1
chr1  554323  554334  7
```

to

```
$ bedtools genomecov -ibam NA18152.bam -bg -strand + | head
chr1  554385  554392  4
chr1  554392  554408  5
chr1  554408  554430  6
chr1  554430  554451  7
chr1  554451  554455  8
chr1  554455  554490  9
chr1  554490  554495  10
chr1  554495  554496  9
chr1  554496  554574  10
chr1  554574  554579  11
```

`-scale` Scaling coverage by a constant factor.

The `-scale` option allows one to scale the coverage observed in an interval file by a constant factor. Each coverage value is multiplied by this factor before being reported. This can be useful for normalizing coverage by, e.g., metrics such as reads per million (RPM).

Compare:

```
$ bedtools genomecov -ibam NA18152.bam -bg | head
chr1  554304  554309  5
chr1  554309  554313  6
chr1  554313  554314  1
chr1  554315  554316  6
chr1  554316  554317  5
chr1  554317  554318  1
chr1  554318  554319  2
chr1  554319  554321  6
chr1  554321  554323  1
chr1  554323  554334  7
```

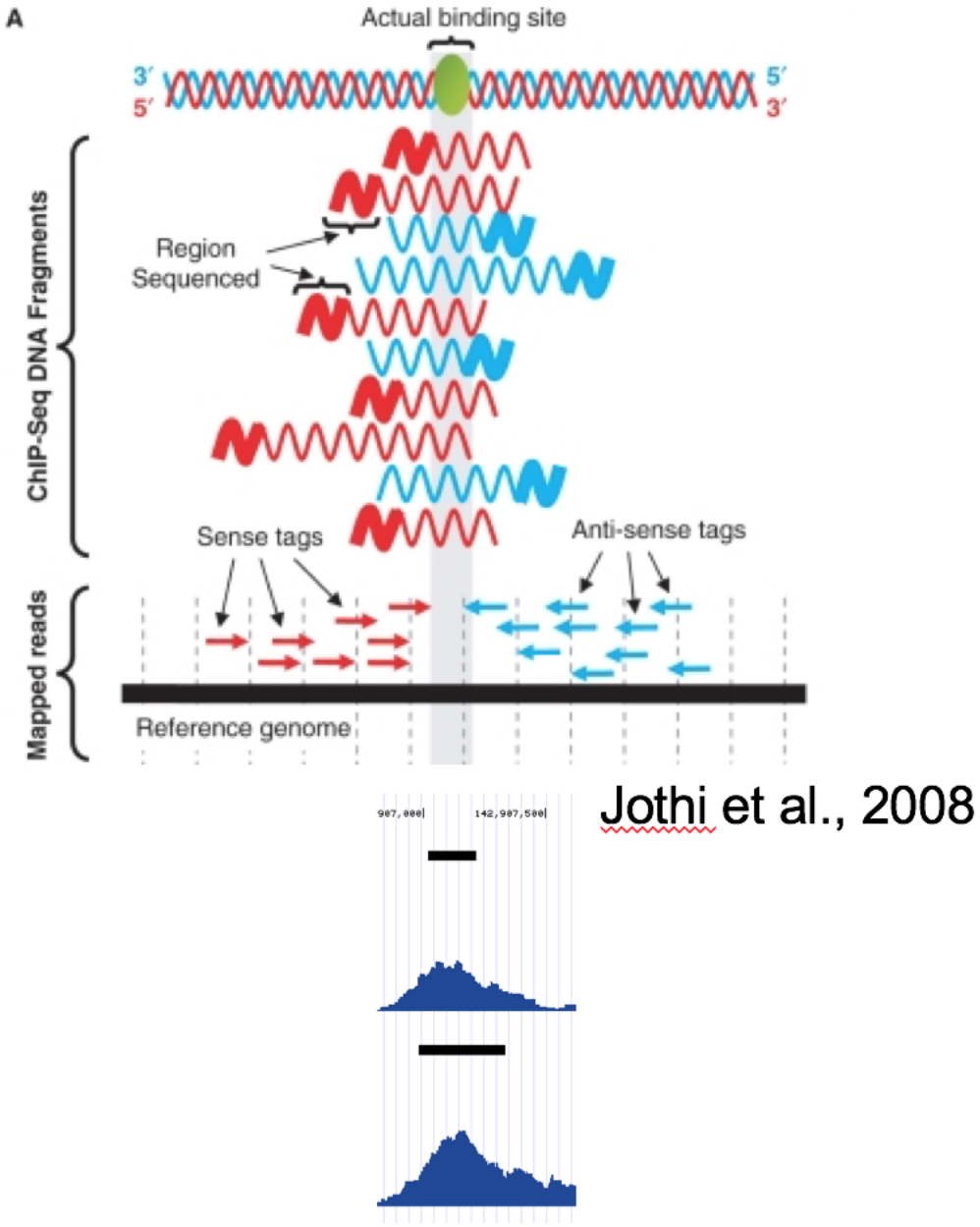
to

```
$ bedtools genomecov -ibam NA18152.bam -bg -scale 10.0 | head
chr1  554304  554309  50
chr1  554309  554313  60
chr1  554313  554314  10
chr1  554315  554316  60
chr1  554316  554317  50
chr1  554317  554318  10
chr1  554318  554319  20
chr1  554319  554321  60
chr1  554321  554323  10
chr1  554323  554334  70
```

`-split` Reporting coverage with spliced alignments or blocked BED features

`bedtools genomecov` will, by default, screen for overlaps against the entire span of a spliced/split BAM alignment or blocked BED12 feature. When dealing with RNA-seq reads, for example, one typically wants to only screen for overlaps for the portions of the reads that come from exons (and ignore the interstitial intron sequence). The `-split` command allows for such overlaps to be performed.

Coverage by fragment



In ChiP-Seq the binding site is usually not at the coordinate where reads map, but in the middle of the fragment. For this reason we often try to estimate average fragment size for single-read experiment and extend the reads in the 5'-3' direction up to the estimated fragment length. The coverage "by estimated fragments" or by actual pair-end fragments graph is expected to peak at the actual binding site.

`-fs` Forces to use provided fragment size.

`-pc` Calculates coverage for paired-end reads, coverage is calculated as the number of fragments covering each base pair

getfasta

`bedtools getfasta` extracts sequences from a FASTA file for each of the intervals defined in a BED/GFF/VCF file.

Tip: 1. The headers in the input FASTA file must *exactly* match the chromosome column in the BED file.

2. You can use the UNIX `fold` command to set the line width of the FASTA output. For example, `fold -w 60` will make each line of the FASTA file have at most 60 nucleotides for easy viewing.

3. BED files containing a single region require a newline character at the end of the line, otherwise a blank output file is produced.

See also:

maskfasta

Usage and option summary

Usage

```
$ bedtools getfasta [OPTIONS] -fi <input FASTA> -bed <BED/GFF/VCF>
```

(or):

```
$ getFastaFromBed [OPTIONS] -fi <input FASTA> -bed <BED/GFF/VCF>
```

Option	Description
-fo	Specify an output file name. By default, output goes to stdout.
-name	Use the name field and coordinates for the FASTA header
-name+	(deprecated) Use the name field and coordinates for the FASTA header
-nameOnly	Use the name field for the FASTA header
-tab	Report extract sequences in a tab-delimited format instead of in FASTA format.
-bedOut	Report extract sequences in a tab-delimited BED format instead of in FASTA format.
-s	Force strandedness. If the feature occupies the antisense strand, the sequence will be reverse complemented. *Default: strand information is ignored.*
-split	Given BED12 input, extract and concatenate the sequences from the BED "blocks" (e.g., exons)
-fullHeader	Use full fasta header. By default, only the word before the first space or tab is used.
-rna	The FASTA is RNA not DNA. Reverse complementation handled accordingly.

Default behavior

`bedtools getfasta` will extract the sequence defined by the coordinates in a BED interval and create a new FASTA entry in the output file for each extracted sequence. By default, the FASTA header for each extracted sequence will be formatted as follows: "<chrom>:<start>-<end>".

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 5 10

$ bedtools getfasta -fi test.fa -bed test.bed
>chr1:5-10
AAACC

# optionally write to an output file
$ bedtools getfasta -fi test.fa -bed test.bed -fo test.fa.out

$ cat test.fa.out
>chr1:5-10
AAACC
```

`-name` Using the BED "name" column as a FASTA header.

Using the `-name` option, one can set the FASTA header for each extracted sequence to be the "name" columns from the BED feature.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG
```

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```
$ cat test.bed
chr1 5 10 myseq

$ bedtools getfasta -fi test.fa -bed test.bed -name
>myseq
AAACC
```

-tab Creating a tab-delimited output file in lieu of FASTA output.

Using the `-tab` option, the `-fo` output file will be tab-delimited instead of in FASTA format.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 5 10 myseq

$ bedtools getfasta -fi test.fa -bed test.bed -name -tab
myseq AAACC
```

-bedOut Creating a tab-delimited BED file in lieu of FASTA output.

Using the `-tab` option, the `-fo` output file will be tab-delimited instead of in FASTA format.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 5 10 myseq

$ bedtools getfasta -fi test.fa -bed test.bed -tab
chr1 5 10 AAACC
```

-s Forcing the extracted sequence to reflect the requested strand

`bedtools getfasta` will extract the sequence in the orientation defined in the strand column when the "-s" option is used.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 20 25 forward 1 +
chr1 20 25 reverse 1 -

$ bedtools getfasta -fi test.fa -bed test.bed -s -name
>forward
CGCTA
```

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```
>reverse
TAGCG
```

-split Extracting BED "blocks".

One can optionally request that FASTA records be extracting and concatenating each block in a BED12 record. For example, consider a BED12 record describing a transcript. By default, `getfasta` will extract the sequence representing the entire transcript (introns, exons, UTRs). Using the -split option, `getfasta` will instead produce separate a FASTA record representing a transcript that splices together each BED12 block (e.g., exons and UTRs in the case of genes described with BED12).

```
$ cat genes.bed12
chr1  164404  173864  ENST00000466557.1       0       -       173864  173864  0
↪6       387,59,66,216,132,112,  0,1479,3695,4644,8152,9348,
chr1  235855  267253  ENST00000424587.1       0       -       267253  267253  0
↪4       2100,150,105,158,       0,2562,23161,31240,
chr1  317810  328455  ENST00000426316.1       0       +       328455  328455  0
↪2       323,145,        0,10500,

$ bedtools getfasta -fi chr1.fa -bed genes.bed12 -split -name
>ENST00000466557.1
gaggcgggaagatcacttgatatcaggagtcgaggcgggaagatcacttgacgtcaggagttcgagactggcccggccaacatggtgaaaccgcatctcca
>ENST00000424587.1
ccaggaagtgaaaatgacactttactgttttaatttgcatttctctgcttacaagtggattacacacattttcgtgtgctgttggctacttatTCATTCAG
>ENST00000426316.1
AATGATCAAATTATGTTTCCCATGCATCAGGTGCAATGGGAAGCTCTTctggagagtgagagaagcttccagttaaggtgacattgaagccaagtcctgaa

# use the UNIX fold command to wrap the FASTA sequence such that each line
# has at most 60 characters
$ bedtools getfasta -fi chr1.fa -bed genes.bed12 -split -name | \
      fold -w 60
>ENST00000466557.1
gaggcgggaagatcacttgatatcaggagtcgaggcgggaagatcacttgacgtcaggag
ttcgagactggcccggccaacatggtgaaaccgcatctccactaaaaatacaaaaattag
cctggtatggtggtgggcacctgtaatcccagtgacttgggaggctaaggcaggagaatt
tcttgaacccaggaggcagaggttgcagtgaccagcaaggttgcgccattgcaccccagc
ctgggcgataagagtgaaaactccatctcaaaaaaaaaaaaaaaaaaaaaTTCCTTTGG
GAAGGCCTTCTACATAAAAATCTTCAACATGAGACTGGAAAAAAGGGTATGGGATCATCA
CCGGACCTTTGGCTTTTACAGCTCGAGCTGACAAAGTTGATTTATCAAGTTGTAAATCTT
CACCTGTTGAATTCATAAGTTCATGTCATATTTTCTTTCAGACAATTCTTCAGTTTGTTT
ACGTAGATCAGCGATACGATGATTCCATTTCTtcggatccttgtaagagcagagcaggtg
atggagagggtgggaggtgtagtgacagaagcaggaaactccagtcattcgagacgggca
gcacaagctgcggagtgcaggccacctctacggccaggaaacggattctcccgcagagcc
tcggaagctaccgaccctgctcccaccttgactcagtaggacttactgtagaattctggc
cttcagacCTGAGCCTGGCAGCTCTCTCCAACTTTGGAAGCCCAGGGGCATGGCCCCTGT
CCACAGATGCACCTGGCATGAGGCGTGCCCAGAGGGACAGAGGCAGATGAGTttcgtctc
ctccactggattgtgagggcCAGAGTTGAACTCCCTCATTTTCCGTTCCCCAGCATTGGC
AGGTTCTGGGACTGGTGGCTGTGGTGGCTCGTTGGTCTTTGTCTCTTAGAAGGTGGGGAA
TAATCATCATCT
>ENST00000424587.1
ccaggaagtgaaaatgacactttactgttttaatttgcatttctctgcttacaagtggat
tacacacattttcgtgtgctgttggctacttatTCATTCAGAAAACATACTAAGTGCTGG
CTCTTTTTCATGTCCTTTATCAAGTTTGGATCATGTCATTTGCTATTTTCTTTCTGATGT
AAACTCTCAAAGTCTGAAGTGTATTGTCTTTTCCTGACACATATGTTGTAAATAATTTTC
```

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```
TGGCTTACATTTTGACTTTTAATTTCATTCACGATGTTTTTAATGAATAATTTTAATTTT
TATGAATGCAAGTTAAAATAATTCTTTCATTGTGGTCTCTGACATGTCATGCCAATAAGG
GTCTTCTCCTCCAAGAGCACAGAAATATTTGCCAATACTGTCCTTAAAATCGGTCACAGT
TTCATTTTTTATATATGCATTTTACTTCAATTGGGGCTTCATTTTACTGAATGCCCTATT
TGAAGCAAGTTTCTCAGTTAATTCTTTTCTCAAAGGGCTAAGTATGGTAGATTGCAAACA
TAAGTGGCCACATAATGCTCTCACCTCctttgcctcctctcccaggaggagatagcgtcc
atctttccactccttaatctgggcttggccgtgtgacttgcactggccaatgggatatta
acaagtctgatgtgcacagaggctgtagaatgtgcacgggggcttggtctctcttgctgc
cctggagaccagctgccCCACGAAGGAACCAGAGCCAACCTGCTGCTTCCTGGAGGAAGA
CAGTCCCTCTGTCCCTCTGTCTCTGCCAACCAGTTAACCTGCTGCTTCCTGGAGGGAGAC
AGTCCCTCAGTCCCTCTGTCTCTGCCAACCAGTTAACCTGCTGCTTCCTGGAGGAAGACA
GTCACTCTGTCTCTGccaacccagttgaccgcagacatgcaggtctgctcaggtaagacc
agcacagtccctgccctgtgagccaaaccaaatggtccagccacagaatcgtgagcaaat
aagtgatgcttaagtcactaagatttgggCAAAAGCTGAGCATTTATCCCAATCCCAATA
CTGTTTGTCCTTCTGTTTATCTGTCTGTCCTTCCCTGCTCATTTAAAATGCCCCCACTGC
ATCTAGTACATTTTTATAGGATCAGGGATCTGCTCTTGGATTAATGTTGTGTTCCCACCT
CGAGGCAGCTTTGTAAGCTTCTGAGCACTTCCCAATTCCGGGTGACTTCAGGCACTGGGA
GGCCTGTGCATCAGCTGCTGCTGTCTGTAGCTGACTTCCTTCACCCCTCTGCTGTCCTCA
GCTCCTTCACCCCTGGGCCTCAGGAAATCAATGTCATGCTGACATCACTCTAGATCTAAA
AGTTGGGTTCTTGgaccaggcgtggtggctcacacctgtaatcccagcactttgggaggc
cgaggcgggtggatcacaaggtcaggagatcaagacgattctggctaacacggtgaaacc
ccgtctctactaaaaatacaaaaaaattagccgggtgtggtggcaggtgcctgtagcccc
agctacttgggaggctgaggcaggagaatggcttgaacctgggaggtggagcttgcagtg
agccaagatcacgccactgcactccagaatgggagagagagcgagactttctcaaaaaaa
aaaaaaaaaCTTAGGTTCTTGGATGTTCGGGAAAGGGGGTTATTATCTAGGATCCTTGAA
GCACCCCCAAGGGCATCTTCTCAAAGTTGGATGTGTGCATTTTCCTGAGAGGAAAGCTTT
CCCACATTATACAGCTTCTGAAAGGGTTGCTTGACCCACAGATGTGAAGCTGAGGCTGAA
GGAGACTGATGTGGTTTCTCCTCAGTTTCTCTGTGCAGCACCAGGTGGCAGCAGAGGTCA
GCAAGGCAAACCCGAGCCCGGGGATGCGGAGTGGGGGCAGCTACGTCCTCTCTTGAGCTA
CAGCAGATTCACTCTGTTCTGTTTCATTGTTGTTTAGTTTGCGTTGTGTTTCTCCAACTT
TGTGCCTCATCAGGAAAAGCTTTGGATCACAATTCCCAGtgctgaagaaaaggccaaact
cttggttgtgttctttgattAGTgcctgtgacgcagcttcaggaggtcctgagaacgtgt
gcacagtttagtcggcagaaacttagggaaatgtaagaccaccatcagcacataggagtt
ctgcattggtttggtctgcattggtttggtCTTTTCCTGGATACAGGTCTTGATAGGTCT
CTTGATGTCATTTCACTTCAGATTCTTCTTTAGAAAACTTGGACAATAGCATTTGCTGTC
TTGTCCAAATTGTTACTTCAAGTTTGCTCTTAGCAAGTAATTGTTTCAGTATCTATATCA
AAAATGGCTTAAGCCTGCAACATGTTTCTGAATGATTAACAAGGTGATAGTCAGTTCTTC
ATTGAATCCTGGATGCTTTATTTTTCTTAATAAGAGGAATTCATATGGATCAG
>ENST00000426316.1
AATGATCAAATTATGTTTCCCATGCATCAGGTGCAATGGGAAGCTCTTctggagagtgag
agaagcttccagttaaggtgacattgaagccaagtcctgaaagatgaggaagagttgtat
gagagtggggagggaaggggggaggtggagggaTGGGGAATGGGCCGGGATGGGATAGCGC
AAACTGCCCGGGAAGGGAAACCAGCACTGTACAGACCTGAACAACGAAGATGGCATATTT
TGTTCAGGGAATGGTGAATTAAGTGTGGCAGGAATGCTTTGTAGACACAGTAATTTGCTT
GTATGGAATTTTGCCTGAGAGACCTCATTCCTCACGTCGGCCATTCCAGGCCCCGTTTTT
CCCTTCCGGCAGCCTCTTGGCCTCTAATTTGTTTATCTTTTGTGTATAAATCCCAAAATA
TTGAATTTTGGAATATTTCCACCATTATGTAAATATTTTGATAGGTAA
```

groupby

`bedtools groupby` is a useful tool that mimics the "group by" clause in database systems. Given a file or stream that is sorted by the appropriate "grouping columns" (`-g`), `groupby` will compute summary statistics on another column (`-c`) in the file or stream. This will work with output from all BEDTools as well as any other tab-delimited file or stream. As such, this is a generally useful tool for all command-line analyses, not just genomics related research.

Note: When using `bedtools groupby`, the input data must be ordered by the same columns as specified with the `-grp` argument, which establish which columns should be used to define a group of similar data. For example, if `-grp` is 1,2,3, the data should be pre-grouped accordingly. When `bedtools groupby` detects changes in the group columns it then summarizes all lines with that group. For example, `sort -k1,1 -k2,2 -k3,3 data.txt | bedtools groupby -g 1,2,3 -c 4 -o mean`.

Usage and option summary

Usage

```
bedtools groupby [OPTIONS] -i <input> -g <group columns> -c <op. column> -o
↪<operation>
```

or:

```
groupBy [OPTIONS] -i <input> -g <group columns> -c <op. column> -o <operation>
```

Option	Description
-i	The input file that should be grouped and summarized. *Use "stdin" when using piped input*. **Note: if -i is omitted, input is assumed to come from standard input (stdin)**
-g (-grp)	Specifies which column(s) (1-based) should be used to group the input. Columns may be comma-separated with each column must be explicitly listed. Or, ranges (e.g. 1-4) are also allowed. *Default: 1,2,3*
-c (-opCol)	Specify the column (1-based) that should be summarized. *Required*.
-o (-op)	Specify the operation that should be applied to **opCol**. Valid operations: **sum** - *numeric only* **count** - *numeric or text* **count_distinct** - *numeric or text* **min** - *numeric only* **max** - *numeric only* **mean** - *numeric only* **median** - *numeric only* **mode** - *numeric or text* **antimode** - *numeric or text* **stdev** - *numeric only* **sstdev** - *numeric only* **collapse** (i.e., print a comma separated list) - *numeric or text* **distinct** (i.e., print a comma separated list) - *numeric or text* **concat** (i.e., print a comma separated list) - *numeric or text* **freqasc** - *print a comma separated list of values observed and the number of times they were observed.* Reported in ascending order of frequency* **freqdesc** - *print a comma separated list of values observed and the number of times they were observed.* Reported in descending order of frequency* **first** - *numeric or text* **last** - *numeric or text* *Default: sum*

Default behavior.

Let's imagine we have three incredibly interesting genetic variants that we are studying and we are interested in what annotated repeats these variants overlap.

```
$ cat variants.bed
chr21  9719758 9729320 variant1
chr21  9729310 9757478 variant2
chr21  9795588 9796685 variant3

$ bedtools intersect -a variants.bed -b repeats.bed -wa -wb > variantsToRepeats.bed
$ cat variantsToRepeats.bed
chr21  9719758 9729320 variant1   chr21  9719768 9721892 ALR/Alpha   1004  +
chr21  9719758 9729320 variant1   chr21  9721905 9725582 ALR/Alpha   1010  +
chr21  9719758 9729320 variant1   chr21  9725582 9725977 L1PA3       3288  +
chr21  9719758 9729320 variant1   chr21  9726021 9729309 ALR/Alpha   1051  +
chr21  9729310 9757478 variant2   chr21  9729320 9729809 L1PA3       3897  -
chr21  9729310 9757478 variant2   chr21  9729809 9730866 L1P1        8367  +
chr21  9729310 9757478 variant2   chr21  9730866 9734026 ALR/Alpha   1036  -
chr21  9729310 9757478 variant2   chr21  9734037 9757471 ALR/Alpha   1182  -
chr21  9795588 9796685 variant3   chr21  9795589 9795713 (GAATG)n    308   +
chr21  9795588 9796685 variant3   chr21  9795736 9795894 (GAATG)n    683   +
chr21  9795588 9796685 variant3   chr21  9795911 9796007 (GAATG)n    345   +
chr21  9795588 9796685 variant3   chr21  9796028 9796187 (GAATG)n    756   +
chr21  9795588 9796685 variant3   chr21  9796202 9796615 (GAATG)n    891   +
chr21  9795588 9796685 variant3   chr21  9796637 9796824 (GAATG)n    621   +
```

We can see that variant1 overlaps with 3 repeats, variant2 with 4 and variant3 with 6. We can use bedtools groupby to summarize the hits for each variant in several useful ways. The default behavior is to compute the *sum* of the opCol.

```
$ bedtools groupby -i variantsToRepeats.bed -g 1,2,3 -c 9
chr21 9719758 9729320 6353
chr21 9729310 9757478 14482
chr21 9795588 9796685 3604
```

Computing the min and max.

Now let's find the *min* and *max* repeat score for each variant. We do this by "grouping" on the variant coordinate columns (i.e. cols. 1,2 and 3) and ask for the min and max of the repeat score column (i.e. col. 9).

```
$ bedtools groupby -i variantsToRepeats.bed -g 1,2,3 -c 9 -o min
chr21 9719758 9729320 1004
chr21 9729310 9757478 1036
chr21 9795588 9796685 308
```

We can also group on just the *name* column with similar effect.

```
$ bedtools groupby -i variantsToRepeats.bed -g 4 -c 9 -o min
variant1 1004
variant2 1036
variant3 308
```

What about the *max* score? Let's keep the coordinates and the name of the variants so that we stay in BED format.

```
$ bedtools groupby -i variantsToRepeats.bed -grp 1-4 -c 9 -o max
chr21 9719758 9729320 variant1 3288
chr21 9729310 9757478 variant2 8367
chr21 9795588 9796685 variant3 891
```

Computing the mean and median.

Now let's find the *mean* and *median* repeat score for each variant.

```
$ cat variantsToRepeats.bed | bedtools groupby -g 1-4 -c 9 -o mean
chr21 9719758 9729320 variant1 1588.25
chr21 9729310 9757478 variant2 3620.5
chr21 9795588 9796685 variant3 600.6667

$ bedtools groupby -i variantsToRepeats.bed -g 1-4 -c 9 -o median
chr21 9719758 9729320 variant1 1030.5
chr21 9729310 9757478 variant2 2539.5
chr21 9795588 9796685 variant3 652
```

Computing the mode and "antimode".

Now let's find the *mode* and *antimode* (i.e., the least frequent) repeat score for each variant (in this case they are identical).

```
$ bedtools groupby -i variantsToRepeats.bed -g 1-4 -c 9 -o mode
chr21 9719758 9729320 variant1 1004
chr21 9729310 9757478 variant2 1036
chr21 9795588 9796685 variant3 308

$ bedtools groupby -i variantsToRepeats.bed -g 1-4 -c 9 -o antimode
chr21 9719758 9729320 variant1 1004
chr21 9729310 9757478 variant2 1036
chr21 9795588 9796685 variant3 308
```

Computing the count of lines for a given group.

Figure:

```
$ bedtools groupby -i variantsToRepeats.bed -g 1-4 -c 9 -o count
chr21 9719758 9729320 variant1 4
chr21 9729310 9757478 variant2 4
chr21 9795588 9796685 variant3 6
```

Collapsing: listing all of the values in the opCol for a given group.

Now for something different. What if we wanted all of the names of the repeats listed on the same line as the variants? Use the collapse option. This "denormalizes" things. Now you have a list of all the repeats on a single line.

```
$ bedtools groupby -i variantsToRepeats.bed -grp 1-4 -c 9 -o collapse
chr21 9719758 9729320 variant1 ALR/Alpha,ALR/Alpha,L1PA3,ALR/Alpha,
chr21 9729310 9757478 variant2 L1PA3,L1P1,ALR/Alpha,ALR/Alpha,
chr21 9795588 9796685 variant3 (GAATG)n,(GAATG)n,(GAATG)n,(GAATG)n,(GAATG)n,(GAATG)n,
```

Computing frequencies: freqasc and freqdesc.

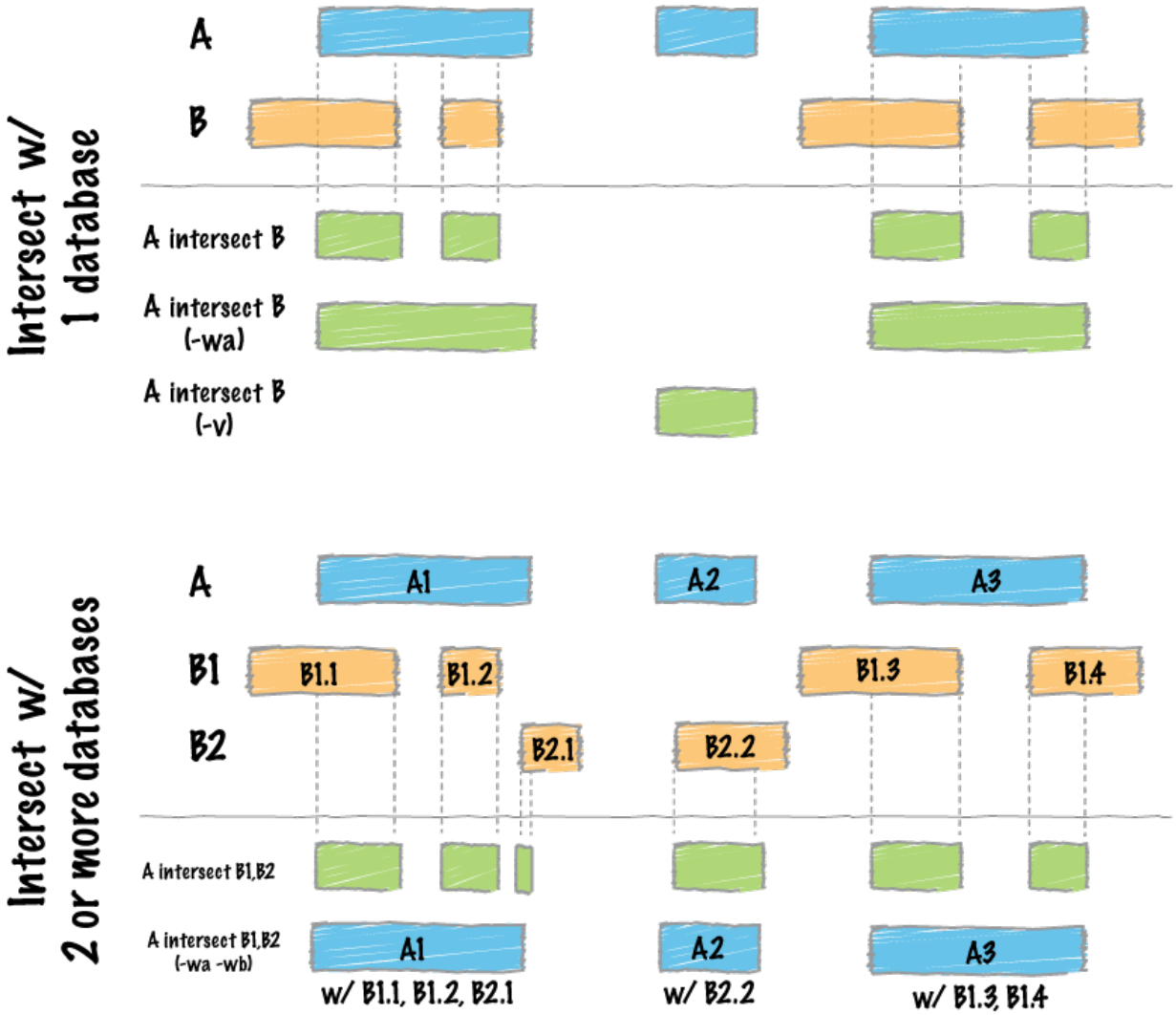
What if we want to report each distinct value along with its number of occurrence (much like uniq -c)? The `freqasc` and freqdesc‘‘ operations handle this.

```
$ cat variantsToRepeats.bed | bedtools groupby -g 1 -c 8 -o freqdesc
chr21 (GAATG)n:6,ALR/Alpha:5,L1PA3:2,L1P1:1,

$ cat variantsToRepeats.bed | bedtools groupby -g 1 -c 8 -o freqasc
chr21 L1P1:1,L1PA3:2,ALR/Alpha:5,(GAATG)n:6,
```

igv

intersect



By far, the most common question asked of two sets of genomic features is whether or not any of the features in the two sets "overlap" with one another. This is known as feature intersection. `bedtools intersect` allows one to screen for overlaps between two sets of genomic features. Moreover, it allows one to have fine control as to how the intersections are reported. `bedtools intersect` works with both BED/GFF/VCF and BAM files as input.

Note: If you are trying to intersect very large files and are having trouble with excessive memory usage, please presort your data by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files) and then use the `-sorted` option. This invokes a memory-efficient algorithm designed for large files. This algorithm has been *substantially* improved in recent (>=2.18.0) releases.

Important: As of version 2.21.0, the *intersect* tool can accept multiple files for the *-b* option. This allows one to

identify overlaps between a single query (*-a*) file and multiple database files (*-b*) at once!

See also:

subtract map window

Usage and option summary

Usage:

```
bedtools intersect [OPTIONS] -a <FILE> \
                             -b <FILE1, FILE2, ..., FILEN>
```

(or):

```
intersectBed [OPTIONS] -a <FILE> \
                       -b <FILE1, FILE2, ..., FILEN>
```

Option	Description
-a	BAM/BED/GFF/VCF file "A". Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	One or more BAM/BED/GFF/VCF file(s) "B". Use "stdin" if passing B with a UNIX pipe. **NEW!!!**: -b may be followed with multiple databases and/or wildcard (*) character(s).
-abam	BAM file A. Each BAM alignment in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe: For example: samtools view -b <BAM> | bedtools intersect -abam stdin -b genes.bed. **Note**: no longer necessary after version 2.19.0
-ubam	Write uncompressed BAM output. The default is write compressed BAM output.
-bed	When using BAM input (-abam), write output as BED. The default is to write output in BAM when using -abam. For example: `bedtools intersect –abam reads.bam –b genes.bed –bed`
-wa	Write the original entry in A for each overlap.
-wb	Write the original entry in B for each overlap. Useful for knowing what A overlaps. Restricted by -f and -r.
-loj	Perform a "left outer join". That is, for each feature in A report each overlap with B. If no overlaps are found, report a NULL feature for B.
-wo	Write the original A and B entries plus the number of base pairs of overlap between the two features. Only A features with overlap are reported. Restricted by -f and -r.
-wao	Write the original A and B entries plus the number of base pairs of overlap between the two features. However, A features w/o overlap are also reported with a NULL B feature and overlap = 0. Restricted by -f and -r.
-u	Write original A entry once if any overlaps found in B. In other words, just report the fact at least one overlap was found in B. Restricted by -f and -r.
-c	For each entry in A, report the number of hits in B while restricting to -f. Reports 0 for A entries that have no overlap with B. Restricted -f, -F, -r, and -s.
-C	For each entry in A, separately report the number of overlaps with each B file on a distinct line. Reports 0 for A entries that have no overlap with B. Overlaps restricted by -f, -F, -r, and -s.
-v	Only report those entries in A that have no overlap in B. Restricted by -f and -r.
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-F	Minimum overlap required as a fraction of B. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-e	Require that the minimum fraction be satisfied for A _OR_ B. In other words, if -e is used with -f 0.90 and -F 0.10 this requires that either 90% of A is covered OR

Default behavior

By default, if an overlap is found, `bedtools intersect` reports the shared interval between the two overlapping features.

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed
chr1  15   20
```

Intersecting against MULTIPLE -b files.

As of version 2.21.0, the *intersect* tool can detect overlaps between a single *-a* file and multiple *-b* files (instead of just one previously). One simply provides multiple *-b* files on the command line.

For example, consider the following query (*-a*) file and three distinct (*-b*) files:

```
$ cat query.bed
chr1  1   20
chr1  40  45
chr1  70  90
chr1  105 120
chr2  1   20
chr2  40  45
chr2  70  90
chr2  105 120
chr3  1   20
chr3  40  45
chr3  70  90
chr3  105 120
chr3  150 200
chr4  10  20

$ cat d1.bed
chr1  5   25
chr1  65  75
chr1  95  100
chr2  5   25
chr2  65  75
chr2  95  100
chr3  5   25
chr3  65  75
chr3  95  100

$ cat d2.bed
chr1  40  50
chr1  110 125
chr2  40  50
chr2  110 125
chr3  40  50
chr3  110 125
```

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```
$ cat d3.bed
chr1  85  115
chr2  85  115
chr3  85  115
```

We can now compare query.bed to all three database files at once.:

```
$ bedtools intersect -a query.bed \
    -b d1.bed d2.bed d3.bed
chr1  5   20
chr1  40  45
chr1  70  75
chr1  85  90
chr1  110 120
chr1  105 115
chr2  5   20
chr2  40  45
chr2  70  75
chr2  85  90
chr2  110 120
chr2  105 115
chr3  5   20
chr3  40  45
chr3  70  75
chr3  85  90
chr3  110 120
chr3  105 115
```

Clearly this is not completely informative because we cannot tell from which file each intersection came. However, if we use *-wa* and *-wb*, this becomes abundantly clear. When these options are used, the first column after the complete *-a* record lists the file number from which the overlap came. The number corresponds to the order in which the files were given on the command line.

```
$ bedtools intersect -wa -wb \
    -a query.bed \
    -b d1.bed d2.bed d3.bed \
    -sorted
chr1  1   20  1 chr1  5   25
chr1  40  45  2 chr1  40  50
chr1  70  90  1 chr1  65  75
chr1  70  90  3 chr1  85  115
chr1  105 120 2 chr1  110 125
chr1  105 120 3 chr1  85  115
chr2  1   20  1 chr2  5   25
chr2  40  45  2 chr2  40  50
chr2  70  90  1 chr2  65  75
chr2  70  90  3 chr2  85  115
chr2  105 120 2 chr2  110 125
chr2  105 120 3 chr2  85  115
chr3  1   20  1 chr3  5   25
chr3  40  45  2 chr3  40  50
chr3  70  90  1 chr3  65  75
chr3  70  90  3 chr3  85  115
chr3  105 120 2 chr3  110 125
chr3  105 120 3 chr3  85  115
```

In many cases, it may be more useful to report an informative "label" for each file instead of a file number. One can do this with the *-names* option.

```
$ bedtools intersect -wa -wb \
    -a query.bed \
    -b d1.bed d2.bed d3.bed \
    -names d1 d2 d3 \
    -sorted
chr1  1   20  d1  chr1  5   25
chr1  40  45  d2  chr1  40  50
chr1  70  90  d1  chr1  65  75
chr1  70  90  d3  chr1  85  115
chr1  105 120 d2  chr1  110 125
chr1  105 120 d3  chr1  85  115
chr2  1   20  d1  chr2  5   25
chr2  40  45  d2  chr2  40  50
chr2  70  90  d1  chr2  65  75
chr2  70  90  d3  chr2  85  115
chr2  105 120 d2  chr2  110 125
chr2  105 120 d3  chr2  85  115
chr3  1   20  d1  chr3  5   25
chr3  40  45  d2  chr3  40  50
chr3  70  90  d1  chr3  65  75
chr3  70  90  d3  chr3  85  115
chr3  105 120 d2  chr3  110 125
chr3  105 120 d3  chr3  85  115
```

Or perhaps it may be more useful to report the file name. One can do this with the *-filenames* option.

```
$ bedtools intersect -wa -wb \
    -a query.bed \
    -b d1.bed d2.bed d3.bed \
    -sorted \
    -filenames
chr1  1   20  d1.bed  chr1  5   25
chr1  40  45  d2.bed  chr1  40  50
chr1  70  90  d1.bed  chr1  65  75
chr1  70  90  d3.bed  chr1  85  115
chr1  105 120 d2.bed  chr1  110 125
chr1  105 120 d3.bed  chr1  85  115
chr2  1   20  d1.bed  chr2  5   25
chr2  40  45  d2.bed  chr2  40  50
chr2  70  90  d1.bed  chr2  65  75
chr2  70  90  d3.bed  chr2  85  115
chr2  105 120 d2.bed  chr2  110 125
chr2  105 120 d3.bed  chr2  85  115
chr3  1   20  d1.bed  chr3  5   25
chr3  40  45  d2.bed  chr3  40  50
chr3  70  90  d1.bed  chr3  65  75
chr3  70  90  d3.bed  chr3  85  115
chr3  105 120 d2.bed  chr3  110 125
chr3  105 120 d3.bed  chr3  85  115
```

Other options to *intersect* can be used as well. For example, let's use *-v* to report those intervals in query.bed that do not overlap any of the intervals in the three database files:

```
$ bedtools intersect -wa -wb \
    -a query.bed \
```

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```
    -b d1.bed d2.bed d3.bed \
    -sorted \
    -v
chr3  150 200
chr4  10  20
```

Or, let's report only those intersections where 100% of the query record is overlapped by a database record:

```
$ bedtools intersect -wa -wb \
    -a query.bed \
    -b d1.bed d2.bed d3.bed \
    -sorted \
    -names d1 d2 d3
    -f 1.0
chr1  40  45  d2  chr1  40  50
chr2  40  45  d2  chr2  40  50
chr3  40  45  d2  chr3  40  50
```

`-wa` Reporting the original A feature

Instead, one can force `bedtools intersect` to report the *original* **"A"** feature when an overlap is found. As shown below, the entire "A" feature is reported, not just the portion that overlaps with the "B" feature.

For example:

```
$ cat A.bed
chr1  10  20
chr1  30   40

$ cat B.bed
chr1  15  20

$ bedtools intersect -a A.bed -b B.bed -wa
chr1  10   20
```

`-wb` Reporting the original B feature

Similarly, one can force `bedtools intersect` to report the *original* **"B"** feature when an overlap is found. If just -wb is used, the overlapping portion of A will be reported followed by the *original* **"B"**. If both -wa and -wb are used, the *originals* of both **"A"** and **"B"** will be reported.

For example (-wb alone):

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed -wb
chr1  15  20  chr1 15  20
```

Now -wa and -wb:

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed -wa -wb
chr1  10  20  chr1 15  20
```

`-loj` Left outer join. Report features in A with and without overlaps

By default, `bedtools intersect` will only report features in A that have an overlap in B. The `-loj` option will report every A feature no matter what. When there is an overlap (or more than 1), it will report A with its overlaps. Yet when there are no overlaps, an A feature will be reported with a NULL B feature to indicate that there were no overlaps

For example (*without* `-loj`):

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed
chr1  10  20  chr1    15  20
```

Now *with* `-loj`:

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed -loj
chr1  10  20  chr1 15  20
chr1  30  40  . -1  -1
```

`-wo` Write the *amount* of overlap between intersecting features

The `-wo` option reports a column after each combination of intersecting "A" and "B" features indicating the *amount* of overlap in bases pairs that is observed between the two features.

Note: When an interval in A does not intersect an interval in B, it will not be reported. If you would like to report such intervals with an overlap equal to 0, see the `-wao` option.

```
$ cat A.bed
chr1    10    20
```

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```
chr1    30    40

$ cat B.bed
chr1    15  20
chr1    18  25

$ bedtools intersect -a A.bed -b B.bed -wo
chr1    10    20    chr1    15  20  5
chr1    10    20    chr1    18  25  2
```

`-wao` Write *amounts* of overlap for all features.

The `-wao` option extends upon the `-wo` option in that, unlike `-wo`, it reports an overlap of 0 for features in A that do not have an intersection in B.

```
$ cat A.bed
chr1    10    20
chr1    30    40

$ cat B.bed
chr1    15  20
chr1    18  25

$ bedtools intersect -a A.bed -b B.bed -wao
chr1    10    20    chr1    15  20  5
chr1    10    20    chr1    18  25  2
chr1    30    40    .       -1  -1  0
```

`-u` (unique) Reporting the mere presence of *any* overlapping features

Often you'd like to simply know a feature in "A" overlaps one or more features in B without reporting each and every intersection. The `-u` option will do exactly this: if an one or more overlaps exists, the A feature is reported. Otherwise, nothing is reported.

For example, without `-u`:

```
$ cat A.bed
chr1  10  20

$ cat B.bed
chr1  15  20
chr1  17  22

$ bedtools intersect -a A.bed -b B.bed
chr1  15   20
chr1  17   20
```

Now with `-u`:

```
$ cat A.bed
chr1  10  20

$ cat B.bed
```

(continues on next page)

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```
chr1  15  20
chr1  17  22

$ bedtools intersect -a A.bed -b B.bed -u
chr1  10   20
```

-c Reporting the number of overlapping features

The -c option reports a column after each "A" feature indicating the *number* (0 or more) of overlapping features found in "B". Therefore, *each feature in A is reported once*.

```
$ cat A.bed
chr1    10    20
chr1    30    40

$ cat B.bed
chr1    15  20
chr1    18  25

$ bedtools intersect -a A.bed -b B.bed -c
chr1    10    20    2
chr1    30    40    0
```

The *-c* option can also work with multiple -B files. In this case, the reported count will reflect the total number of intersections observed across _all_ *-B* files. It will not report a separate count for each database file. This can be achieved with the *-C* option.

```
$ cat A.bed
chr1    10    20
chr1    30    40

$ cat B.bed
chr1    15  20
chr1    18  25

$ cat C.bed
chr1    16  21
chr1    19  26

$ bedtools intersect -a A.bed -b B.bed -c
chr1    10    20    4
chr1    30    40    0
```

-C Reporting the number of overlapping features for each database file

Unlike the the *-c* option, in the case of multiple *-B* files, the *-C* option will report a separate count for each database file.

```
$ cat A.bed
chr1    10    20
chr1    30    40
```

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(continued from previous page)

```
$ cat B.bed
chr1    15  20
chr1    18  25

$ cat C.bed
chr1    16  21
chr1    19  26

$ bedtools intersect -a A.bed -b B.bed -C
chr1    10    20    1 2
chr1    10    20    2 2
chr1    30    40    1 0
chr1    30    40    1 0
```

If you would like to see more useful information than the file _number_ from which the counts came, one can use the *-filenames* or *-names* options.

```
$ bedtools intersect -a A.bed -b B.bed -C -filenames
chr1    10    20    A.bed 2
chr1    10    20    B.bed 2
chr1    30    40    A.bed 0
chr1    30    40    B.bed 0

$ bedtools intersect -a A.bed -b B.bed -C -names a b
chr1    10    20    a 2
chr1    10    20    b 2
chr1    30    40    a 0
chr1    30    40    b 0
```

`-v` Reporting the absence of any overlapping features

There will likely be cases where you'd like to know which "A" features do not overlap with any of the "B" features. Perhaps you'd like to know which SNPs don't overlap with any gene annotations. The `-v` (an homage to "grep -v") option will only report those "A" features that have no overlaps in "B".

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15  20

$ bedtools intersect -a A.bed -b B.bed -v
chr1  30  40
```

`-f` Requiring a minimal overlap fraction

By default, `bedtools intersect` will report an overlap between A and B so long as there is at least one base pair is overlapping. Yet sometimes you may want to restrict reported overlaps between A and B to cases where the feature in B overlaps at least X% (e.g. 50%) of the A feature. The -f option does exactly this.

For example (note that the second B entry is not reported):

```
$ cat A.bed
chr1 100 200

$ cat B.bed
chr1 130 201
chr1 180 220

$ bedtools intersect -a A.bed -b B.bed -f 0.50 -wa -wb
chr1 100 200 chr1 130 201
```

`-r, and -f` Requiring reciprocal minimal overlap fraction

Similarly, you may want to require that a minimal fraction of both the A and the B features is overlapped. For example, if feature A is 1kb and feature B is 1Mb, you might not want to report the overlap as feature A can overlap at most 1% of feature B. If one set -f to say, 0.02, and one also enable the -r (reciprocal overlap fraction required), this overlap would not be reported.

For example (note that the second B entry is not reported):

```
$ cat A.bed
chr1 100 200

$ cat B.bed
chr1 130 201
chr1 130 200000

$ bedtools intersect -a A.bed -b B.bed -f 0.50 -r -wa -wb
chr1 100 200 chr1 130 201
```

`-s` Enforcing *same* strandedness

By default, `bedtools intersect` will report overlaps between features even if the features are on opposite strands. However, if strand information is present in both BED files and the "-s" option is used, overlaps will only be reported when features are on the same strand.

For example (note that the first B entry is not reported):

```
$ cat A.bed
chr1 100 200 a1 100 +

$ cat B.bed
chr1 130 201 b1 100 -
chr1 132 203 b2 100 +

$ bedtools intersect -a A.bed -b B.bed -wa -wb -s
chr1 100 200 a1 100 + chr1 132 203 b2 100 +
```

`-S` Enforcing *opposite* "strandedness"

The `-s` option enforces that overlaps be on the *same* strand. In some cases, you may want to enforce that overlaps be found on *opposite* strands. In this, case use the `-S` option.

For example:

```
$ cat A.bed
chr1 100 200 a1 100 +

$ cat B.bed
chr1 130 201 b1 100 -
chr1 132 203 b2 100 +

$ bedtools intersect -a A.bed -b B.bed -wa -wb -S
chr1 100 200 a1 100 + chr1 130 201 b1 100 -
```

-abam Default behavior when using BAM input (deprecated since 2.18.0)

When comparing alignments in BAM format (**-abam**) to features in BED format (**-b**), `bedtools intersect` will, **by default**, write the output in BAM format. That is, each alignment in the BAM file that meets the user's criteria will be written (to standard output) in BAM format. This serves as a mechanism to create subsets of BAM alignments are of biological interest, etc. Note that only the mate in the BAM alignment is compared to the BED file. Thus, if only one end of a paired-end sequence overlaps with a feature in B, then that end will be written to the BAM output. By contrast, the other mate for the pair will not be written. One should use **pairToBed(Section 5.2)** if one wants each BAM alignment for a pair to be written to BAM output.

```
$ bedtools intersect -abam reads.unsorted.bam -b simreps.bed | \
       samtools view - | \
           head -3

BERTHA_0001:3:1:15:1362#0 99 chr4 9236904 0 50M = 9242033 5 1 7 9
AGACGTTAACTTTACACACCTCTGCCAAGGTCCTCATCCTTGTATTGAAG W c T U ] b \ g c e g X g f c b f␣
↪c c b d d g g V Y P W W _
\c`dcdabdfW^a^gggfgd XT:A:R NM:i:0 SM:i:0 AM:i:0 X0:i:19 X1:i:2 XM:i:0 XO:i:0 XG:i:0␣
↪MD:Z:50
BERTHA _0001:3:1:16:994#0 83 chr6 114221672 37 25S6M1I11M7S =
114216196 -5493 G A A A G G C C A G A G T A T A G A A T A A A C A C A A C A A T G T C␣
↪C A A G G T A C A C T G T T A
gffeaaddddgggggggedgcgeggdeggggggffcgggggggegdfggfgf XT:A:M NM:i:3 SM:i:37 AM:i:37␣
↪XM:i:2 X O : i :
1 XG:i:1 MD:Z:6A6T3
BERTHA _0001:3:1:16:594#0 147 chr8 43835330 0 50M =
43830893 -4487 CTTTGGGAGGGCTTTGTAGCCTATCTGGAAAAAGGAAATATCTTCCCATG U
\e^bgeTdg_Kgcg`ggeggg_ggggggggggddgdggVg\gWdfgfgff XT:A:R NM:i:2 SM:i:0 AM:i:0 X0:i:10␣
↪X1:i:7 X M : i :
2 XO:i:0 XG:i:0 MD:Z:1A2T45
```

Note: As of version 2.18.0, it is no longer necessary to specify a BAM input file via `-abam`. Bedtools now autodetects this when `-a` is used.

-ubam Default behavior when using BAM input

The `-ubam` option writes *uncompressed* BAM output to stdout. This is useful for increasing the speed of pipelines that accept the output of `bedtools` intersect as input, since the receiving tool does not need to uncompress the data.

`-bed` Output BED format when using BAM input

When comparing alignments in BAM format (**-abam**) to features in BED format (**-b**), `bedtools intersect` will **optionally** write the output in BED format. That is, each alignment in the BAM file is converted to a 6 column BED feature and if overlaps are found (or not) based on the user's criteria, the BAM alignment will be reported in BED format. The BED "name" field is comprised of the RNAME field in the BAM alignment. If mate information is available, the mate (e.g., "/1" or "/2") field will be appended to the name. The "score" field is the mapping quality score from the BAM alignment.

```
$ bedtools intersect -abam reads.unsorted.bam -b simreps.bed -bed | head -20

chr4  9236903   9236953   BERTHA_0001:3:1:15:1362#0/1  0   +
chr6  114221671 114221721 BERTHA_0001:3:1:16:994#0/1   37  -
chr8  43835329  43835379  BERTHA_0001:3:1:16:594#0/2   0   -
chr4  49110668  49110718  BERTHA_0001:3:1:31:487#0/1   23  +
chr19 27732052  27732102  BERTHA_0001:3:1:32:890#0/2   46  +
chr19 27732012  27732062  BERTHA_0001:3:1:45:1135#0/1  37  +
chr10 117494252 117494302 BERTHA_0001:3:1:68:627#0/1   37  -
chr19 27731966  27732016  BERTHA_0001:3:1:83:931#0/2   9   +
chr8  48660075  48660125  BERTHA_0001:3:1:86:608#0/2   37  -
chr9  34986400  34986450  BERTHA_0001:3:1:113:183#0/2  37  -
chr10 42372771  42372821  BERTHA_0001:3:1:128:1932#0/1 3   -
chr19 27731954  27732004  BERTHA_0001:3:1:130:1402#0/2 0   +
chr10 42357337  42357387  BERTHA_0001:3:1:137:868#0/2  9   +
chr1  159720631 159720681 BERTHA_0001:3:1:147:380#0/2  37  -
chrX  58230155  58230205  BERTHA_0001:3:1:151:656#0/2  37  -
chr5  142612746 142612796 BERTHA_0001:3:1:152:1893#0/1 37  -
chr9  71795659  71795709  BERTHA_0001:3:1:177:387#0/1  37  +
chr1  106240854 106240904 BERTHA_0001:3:1:194:928#0/1  37  -
chr4  74128456  74128506  BERTHA_0001:3:1:221:724#0/1  37  -
chr8  42606164  42606214  BERTHA_0001:3:1:244:962#0/1  37  +
```

`-split` Reporting overlaps with spliced alignments or blocked BED features

As described in section 1.3.19, bedtools intersect will, by default, screen for overlaps against the entire span of a spliced/split BAM alignment or blocked BED12 feature. When dealing with RNA-seq reads, for example, one typically wants to only screen for overlaps for the portions of the reads that come from exons (and ignore the interstitial intron sequence). The **-split** command allows for such overlaps to be performed.

For example, the diagram below illustrates the *default* behavior. The blue dots represent the "split/ spliced" portion of the alignment (i.e., CIGAR "N" operation). In this case, the two exon annotations are reported as overlapping with the "split" BAM alignment, but in addition, a third feature that overlaps the "split" portion of the alignment is also reported.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Exons       ---------------                                      ----------

BED/BAM  A     ***********.........................................****

BED File B  ^^^^^^^^^^^^^^                       ^^^^^^^^         ^^^^^^^^^^

Result      ===============                      ========         ==========
```

In contrast, when using the **-split** option, only the exon overlaps are reported.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Exons       ---------------                                        ----------
BED/BAM  A     ***********.........................................****
BED File B  ^^^^^^^^^^^^^^^                        ^^^^^^^^          ^^^^^^^^^^
Result      ===============                                        ==========
```

`-sorted` Invoke a memory-efficient algorithm for very large files.

The default algorithm for detecting overlaps loads the B file into an R-tree structure in memory. While fast, it can consume substantial memory for large files. For these reason, we provide an alternative, memory efficient algorithm that depends upon inout files that have been sorted by chromosome and then by start position. When both input files are position-sorted, the algorithm can "sweep" through the data and detect overlaps on the fly in a manner much like the way database systems join two tables. This option is invoked with the `-sorted` option.

Note: By default, the `-sorted` option requires that the records are **GROUPED** by chromosome and that within each chromosome group, the records are sorted by chromosome position. One way to achieve this (for BED files for example) is use the UNIX sort utility to sort both files by chromosome and then by position. That is, `sort -k1,1 -k2,2n in.bed > in.sorted.bed`. However, since we merely require that the chromsomes are grouped (that is, all records for a given chromosome come in a single block in the file), sorting criteria other than the alphanumeric criteria that is used by the `sort` utility are fine. For example, you could use the "version sort" (`-V`) option in newer versions of GNU sort to make the chromosomes come in this (chr1, chr2, chr3) order instead of this (chr1, chr10, chr11) order.

For example:

```
$ bedtools intersect -a big.sorted.bed -b huge.sorted.bed -sorted
```

`-g` Define an alternate chromosome sort order via a genome file.

As described above, the `-sorted` option expects that the input files are grouped by chromosome. However, there arise cases where ones input files are sorted by a different criteria and it is to computationally onerous to resort the files alphanumerically. For example, the GATK expects that BAM files are sorted in a very specific manner. The `-g` option allows one to specify an exact ording that should be expected in the input (e.g., BAM, BED, etc.) files. All you need to do is re-order you genome file to specify the order. Also, the use of a genome file to specify the expected order allows the `intersect` tool to detect when two files are internally grouped but each file actually follows a different order. This will cause incorrect results and the `-g` file will alert you to such problems.

For example, an alphanumerically ordered genome file would look like the following:

```
$ cat hg19.genome
chr1  249250621
chr10 135534747
chr11 135006516
chr12 133851895
chr13 115169878
chr14 107349540
chr15 102531392
```

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```
chr16 90354753
chr17 81195210
chr18 78077248
chr19 59128983
chr2  243199373
chr20 63025520
chr21 48129895
chr22 51304566
chr3  198022430
chr4  191154276
chr5  180915260
chr6  171115067
chr7  159138663
chr8  146364022
chr9  141213431
chrM  16571
chrX  155270560
chrY  59373566
```

However, if your input BAM or BED files are ordered such as `chr1, chr2, chr3`, etc., one need to simply reorder the genome file accordingly:

```
$ sort -k1,1V hg19.genome > hg19.versionsorted.genome
$ cat hg19.versionsorted.genome
chr1  249250621
chr2  243199373
chr3  198022430
chr4  191154276
chr5  180915260
chr6  171115067
chr7  159138663
chr8  146364022
chr9  141213431
chr10 135534747
chr11 135006516
chr12 133851895
chr13 115169878
chr14 107349540
chr15 102531392
chr16 90354753
chr17 81195210
chr18 78077248
chr19 59128983
chr20 63025520
chr21 48129895
chr22 51304566
chrM  16571
chrX  155270560
chrY  59373566
```

At this point, one can now use the `-sorted` option along with the genome file in order to properly process the input files that abide by something other than an alphanumeric sorting order.

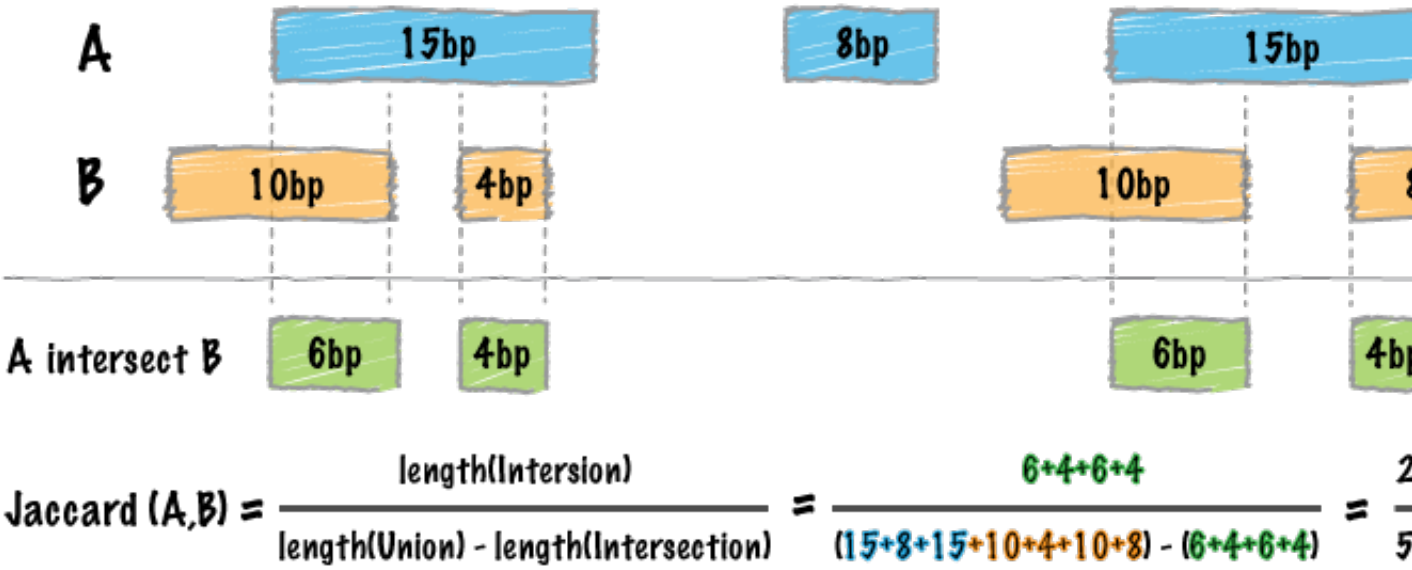
```
$ bedtools intersect -a a.versionsorted.bam -b b.versionsorted.bed \
    -sorted \
    -g hg19.versionsorted.genome
```

Et voila.

`-header` Print the header for the A file before reporting results.

By default, if your A file has a header, it is ignored when reporting results. This option will instead tell bedtools to first print the header for the A file prior to reporting results.

jaccard



Whereas the bedtools `intersect` tool enumerates each an every intersection between two sets of genomic intervals, one often needs a single statistic reflecting the *similarity* of the two sets based on the intersections between them. The Jaccard statistic is used in set theory to represent the ratio of the intersection of two sets to the union of the two sets. Similarly, Favorov et al [1] reported the use of the Jaccard statistic for genome intervals: specifically, it measures the ratio of the number of intersecting base pairs between two sets to the number of base pairs in the union of the two sets. The bedtools jaccard tool implements this statistic, yet modifies the statistic such that the length of the intersection is subtracted from the length of the union. As a result, the final statistic ranges from 0.0 to 1.0, where 0.0 represents no overlap and 1.0 represent complete overlap.

```
[1] Exploring Massive, Genome Scale Datasets with the GenometriCorr Package.
Favorov A, Mularoni L, Cope LM, Medvedeva Y, Mironov AA, et al. (2012)
PLoS Comput Biol 8(5): e1002529. doi:10.1371/journal.pcbi.1002529
```

Note: The `jaccard` tool requires that your data is pre-sorted by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files).

See also:

reldist intersect

Usage and option summary

Usage:

```
bedtools jaccard [OPTIONS] -a <BED/GFF/VCF> -b <BED/GFF/VCF>
```

Option	Description
-a	BED/GFF/VCF file A. Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	BED/GFF/VCF file B. Use "stdin" if passing B with a UNIX pipe.
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-F	Minimum overlap required as a fraction of B. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-e	Require that the minimum fraction be satisfied for A _OR_ B. In other words, if -e is used with -f 0.90 and -F 0.10 this requires that either 90% of A is covered OR 10% of B is covered. Without -e, both fractions would have to be satisfied.**-s** Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-split	Treat "split" BAM (i.e., having an "N" CIGAR operation) or BED12 entries as distinct BED intervals.

Default behavior

By default, `bedtools jaccard` reports the length of the intersection, the length of the union (minus the intersection), the final Jaccard statistic reflecting the similarity of the two sets, as well as the number of intersections.

```
$ cat a.bed
chr1  10  20
chr1  30  40

$ cat b.bed
chr1  15   20

$ bedtools jaccard -a a.bed -b b.bed
intersection  union   jaccard n_intersections
5     20      0.25    1
```

Controlling which intersections are included

One can also control which intersections are included in the statistic by requiring a certain fraction of overlap with respect to the features in A (via the `-f` parameter) or also by requiring that the fraction of overlap is reciprocal (`-r`) in A and B.

```
$ cat a.bed
chr1  10  20
chr1  30  40

$ cat b.bed
chr1  15   20
```

Require 10% overlap with respect to the intervals in A:

```
$ bedtools jaccard -a a.bed -b b.bed -f 0.1
intersection  union   jaccard n_intersections
5 20  0.25    1
```

Require 60% overlap with respect to the intervals in A:

```
$ bedtools jaccard -a a.bed -b b.bed -f 0.6
intersection  union   jaccard n_intersections
0 25  0.25    0
```

links

Creates an HTML file with links to an instance of the UCSC Genome Browser for all features / intervals in a file. This is useful for cases when one wants to manually inspect through a large set of annotations or features.

Usage and option summary

Usage:

```
linksBed [OPTIONS] -i <BED/GFF/VCF> > <HTML file>
```

Option	Description
-base	The "basename" for the UCSC browser. *Default: http://genome.ucsc.edu*
-org	The organism (e.g. mouse, human). *Default: human*
-db	The genome build. *Default: hg18*

Default behavior

By default, **linksBed** creates links to the public UCSC Genome Browser.

For example:

```
head genes.bed
chr21 9928613  10012791  uc002yip.1 0  -
chr21 9928613  10012791  uc002yiq.1 0  -
chr21 9928613  10012791  uc002yir.1 0  -
chr21 9928613  10012791  uc010gkv.1 0  -
chr21 9928613  10061300  uc002yis.1 0  -
chr21 10042683 10120796  uc002yit.1 0  -
chr21 10042683 10120808  uc002yiu.1 0  -
chr21 10079666 10120808  uc002yiv.1 0  -
chr21 10080031 10081687  uc002yiw.1 0  -
chr21 10081660 10120796  uc002yix.2 0  -
```

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```
linksBed -i genes.bed > genes.html
```

When genes.html is opened in a web browser, one should see something like the following, where each link on the page is built from the features in genes.bed:

Creating HTML links to a local UCSC Browser installation

Optionally, **linksBed** will create links to a local copy of the UCSC Genome Browser.

For example:

```
head -3 genes.bed
chr21 9928613 10012791 uc002yip.1 0 -
chr21 9928613 10012791 uc002yiq.1 0 -

linksBed -i genes.bed -base http://mirror.uni.edu > genes.html
```

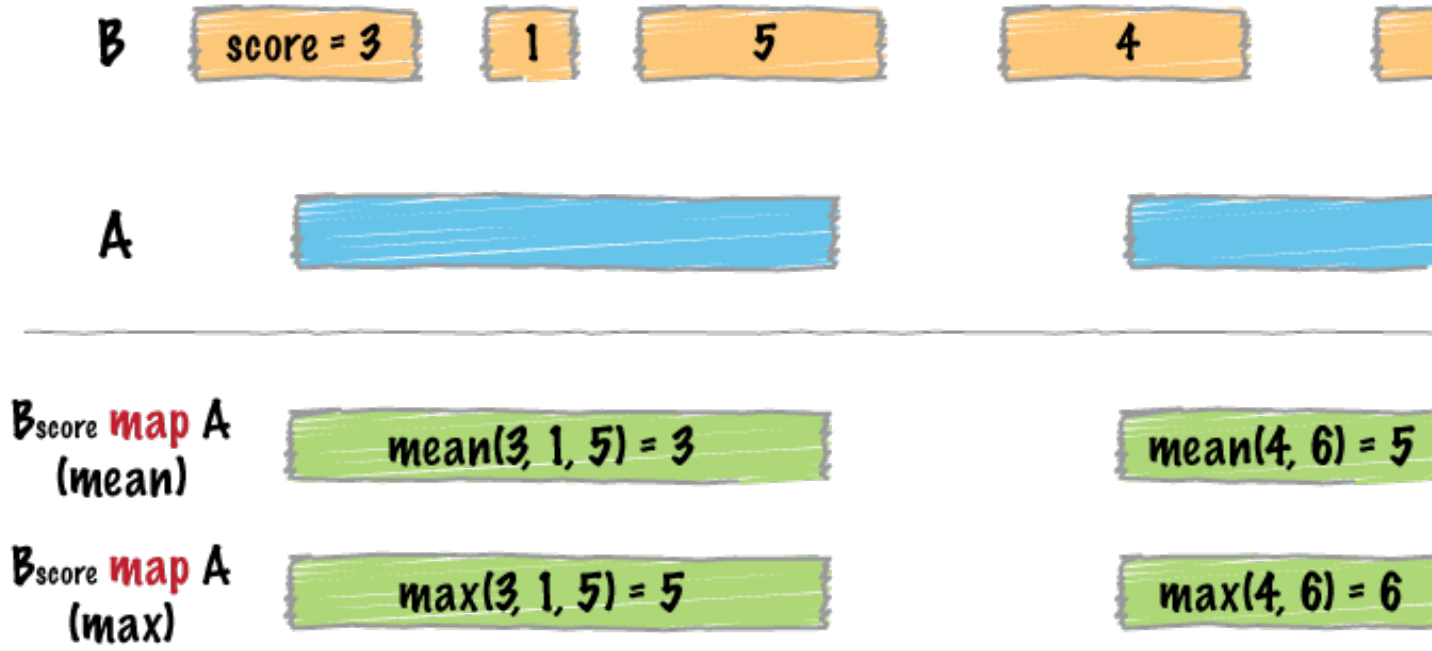
One can point the links to the appropriate organism and genome build as well:

```
head -3 genes.bed
chr21 9928613 10012791 uc002yip.1 0 -
chr21 9928613 10012791 uc002yiq.1 0 -

linksBed -i genes.bed -base http://mirror.uni.edu -org mouse -db mm9 > genes.html
```

makewindows

map



`bedtools map` allows one to map overlapping features in a B file onto features in an A file and apply statistics and/or summary operations on those features.

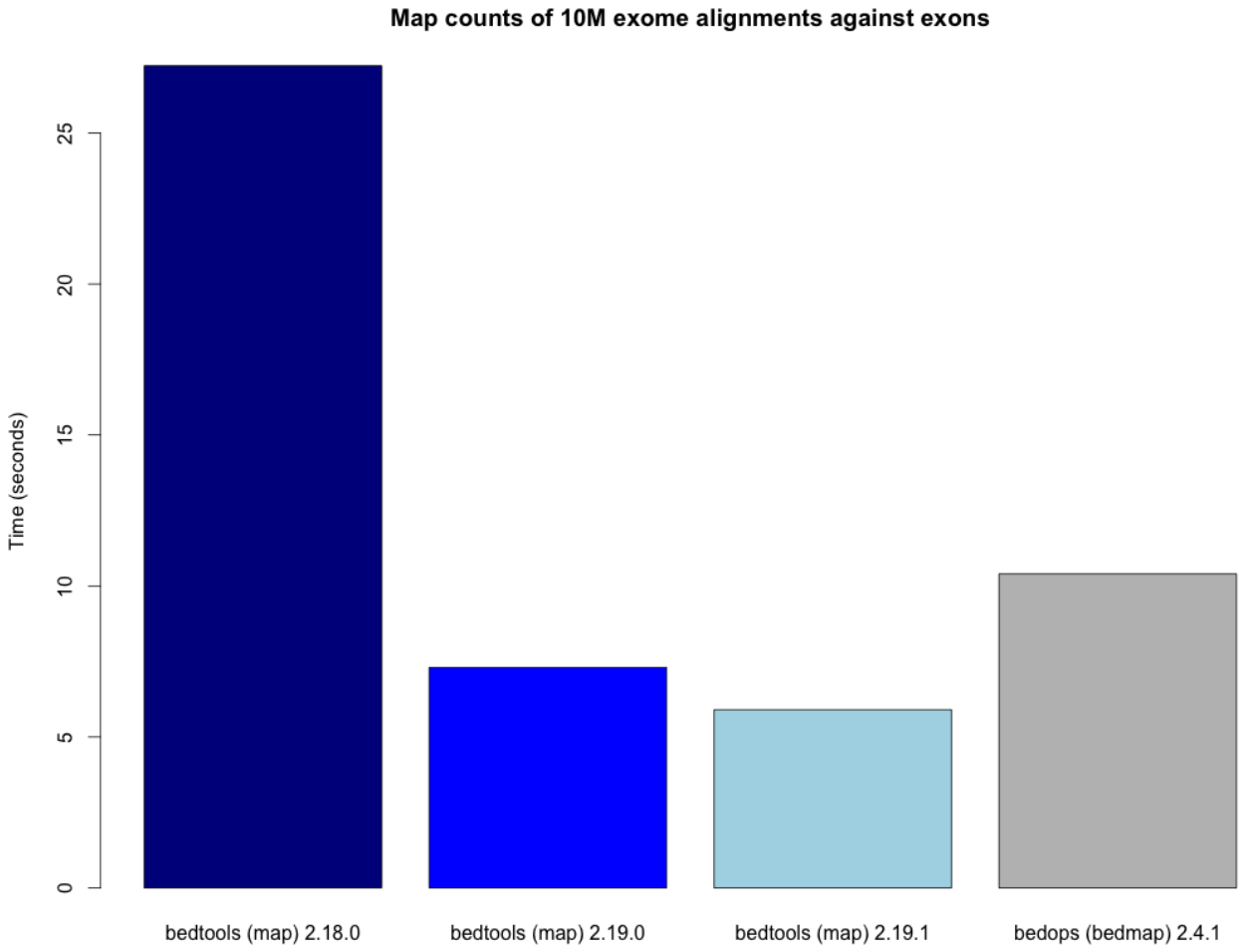
For example, one could use `bedtools map` to compute the average score of BEDGRAPH records that overlap genes. Since the fourth column in BEDGRAPH is the score, the following command illustrates how this would be done:

```
$ bedtools map -a genes.bed -b peaks.bedgraph -c 4 -o mean
```

Another example is discussed in this Biostars post.

Note: `bedtools map` requires each input file to be sorted by genome coordinate. For BED files, this can be done with `sort -k1,1 -k2,2n`. Other sorting criteria are allowed if a genome file (*-g*) is provides that specifies the expected chromosome order.

Note: The `map` tool is substantially faster in versions 2.19.0 and later. The plot below demonstrates the increased speed when, for example, counting the number of exome alignments that align to each exon. The bedtools times are compared to the bedops *bedmap* utility as a point of reference.



Usage and option summary

Usage:

```
bedtools map [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>
```

(or):

```
mapBed [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>
```

Option	Description
-c	Specify the column from the B file to map onto intervals in A. `Default: 5`
-o	Specify the operation that should be applied to `-c`. Valid operations: **sum** - *numeric only* **count** - *numeric or text* **count_distinct** - *numeric or text* **min** - *numeric only* **max** - *numeric only* **absmin** - *numeric only* **absmax** - *numeric only* **mean** - *numeric only* **median** - *numeric only* **antimode** - *numeric or text* **collapse** (i.e., print a comma separated list) - *numeric or text* **distinct** (i.e., print a comma separated list) - *numeric or text* **concat** (i.e., print a comma separated list) - *numeric or text*
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-F	Minimum overlap required as a fraction of B. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-e	Require that the minimum fraction be satisfied for A _OR_ B. In other words, if -e is used with -f 0.90 and -F 0.10 this requires that either 90% of A is covered OR 10% of B is covered. Without -e, both fractions would have to be satisfied.
-s	Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-null	The value to print if no overlaps are found for an A interval. `Default: "."`
-header	Print the header from the A file prior to results.
-split	Treat "split" BAM (i.e., having an "N" CIGAR operation) or BED12 entries as distinct BED intervals. When using -sorted, memory usage remains low even for very large files.

Default behavior - compute the `sum` of the `score` column for all overlaps.

By default, `map` computes the sum of the 5th column (the `score` field for BED format) for all intervals in B that overlap each interval in A.

Tip: Records in A that have no overlap will, by default, return `.` for the computed value from B. This can be changed with the `-null` option.

```
$ cat a.bed
chr1        10      20      a1      1       +
chr1        50      60      a2      2       -
chr1        80      90      a3      3       -

$ cat b.bed
chr1        12      14      b1      2       +
chr1        13      15      b2      5       -
chr1        16      18      b3      5       +
chr1        82      85      b4      2       -
chr1        85      87      b5      3       +

$ bedtools map -a a.bed -b b.bed
chr1        10      20      a1      1       +       12
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       5
```

`mean` Compute the mean of a column from overlapping intervals

```
$ cat a.bed
chr1        10      20      a1      1       +
chr1        50      60      a2      2       -
chr1        80      90      a3      3       -

$ cat b.bed
chr1        12      14      b1      2       +
chr1        13      15      b2      5       -
chr1        16      18      b3      5       +
chr1        82      85      b4      2       -
chr1        85      87      b5      3       +

$ bedtools map -a a.bed -b b.bed -c 5 -o mean
chr1        10      20      a1      1       +       4
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       2.5
```

`collapse` List each value of a column from overlapping intervals

```
$ bedtools map -a a.bed -b b.bed -c 5 -o collapse
chr1        10      20      a1      1       +       2,5,5
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       2,3
```

`distinct` List each *unique* value of a column from overlapping intervals

```
$ bedtools map -a a.bed -b b.bed -c 5 -o distinct
chr1        10      20      a1      1       +       2,5
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       2,3
```

`-s` Only include intervals that overlap on the *same* strand.

```
$ bedtools map -a a.bed -b b.bed -c 5 -o collapse -s
chr1        10      20      a1      1       +       2,5
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       2
```

`-S` Only include intervals that overlap on the *opposite* strand.

```
$ bedtools map -a a.bed -b b.bed -c 5 -o collapse -S
chr1        10      20      a1      1       +       5
chr1        50      60      a2      2       -       .
chr1        80      90      a3      3       -       3
```

Multiple operations and columns at the same time.

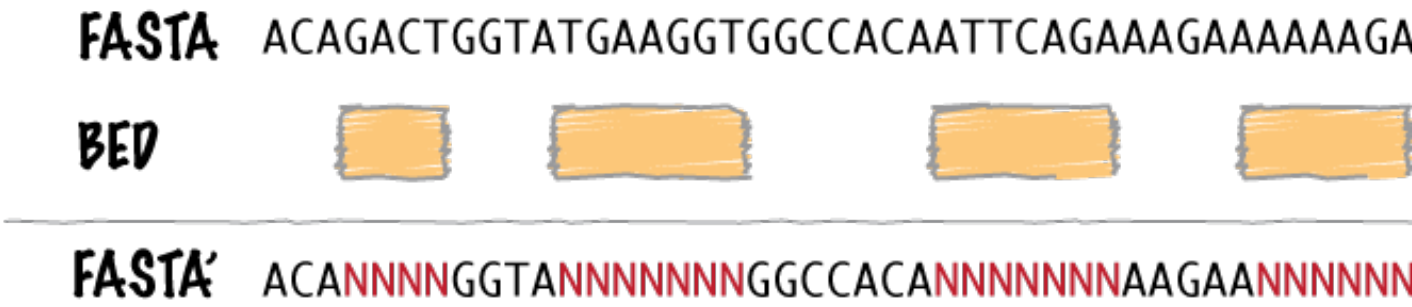
As of version 2.19.1, multiple columns and operations are allowed at the same time in a single run. This greatly expedites analyses by preventing one from having to process the same file over an over for each column/operation.

```
$ bedtools map -a a.bed -b b.bed -c 5,5,5,5 -o min,max,median,collapse
```

Or, apply the same function to multiple columns:

```
$ bedtools map -a a.bed -b b.bed -c 3,4,5,6 -o mean
```

maskfasta



`bedtools maskfasta` masks sequences in a FASTA file based on intervals defined in a feature file. The headers in the input FASTA file must exactly match the chromosome column in the feature file. This may be useful for creating your own masked genome file based on custom annotations or for masking all but your target regions when aligning sequence data from a targeted capture experiment.

Usage and option summary

Usage

```
$ bedtools maskfasta [OPTIONS] -fi <input FASTA> -bed <BED/GFF/VCF> -fo <output FASTA>
```

(or):

```
$ maskFastaFromBed [OPTIONS] -fi <input FASTA> -bed <BED/GFF/VCF> -fo <output FASTA>
```

Note: The input (`-fi`) and output (`-fo`) FASTA files must be different.

See also:

getfasta

Option	Description
-soft	Soft-mask (that is, convert to lower-case bases) the FASTA sequence. *By default, hard-masking (that is, conversion to Ns) is performed.*
-mc	Replace masking character. That is, instead of masking with Ns, use another character.

Default behavior

bedtools maskfasta will mask a FASTA file based on the intervals in a BED file. The newly masked FASTA file is written to the output FASTA file.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGG

$ cat test.bed
```

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```
chr1 5 10

$ bedtools maskfasta -fi test.fa -bed test.bed -fo test.fa.out

$ cat test.fa.out
>chr1
AAAAANNNNNCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGGG
```

`-soft` Soft-masking the FASTA file.

Using the **-soft** option, one can optionally "soft-mask" the FASTA file.

```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 5 10

$ bedtools maskfasta -fi test.fa -bed test.bed -fo test.fa.out -soft

$ cat test.fa.out
>chr1
AAAAAaaaccCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGGG
```

`-mc` Specify a masking character.

Using the **-mc** option, one can optionally choose a masking character to each base that will be masked by the BED file.

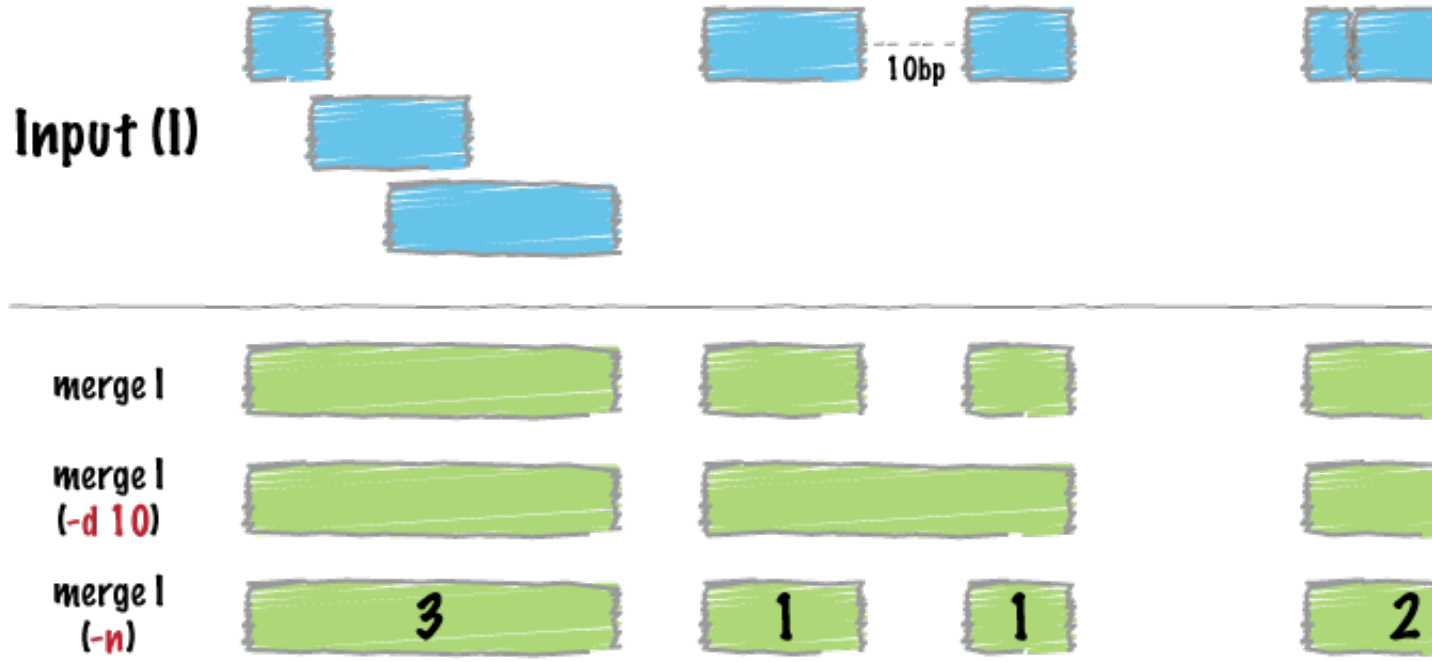
```
$ cat test.fa
>chr1
AAAAAAAACCCCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGGG

$ cat test.bed
chr1 5 10

$ bedtools maskfasta -fi test.fa -bed test.bed -fo test.fa.out -mc X

$ cat test.fa.out
>chr1
AAAAAXXXXXCCCCCCCCCCGCTACTGGGGGGGGGGGGGGGGGG
```

merge



`bedtools merge` combines overlapping or "book-ended" features in an interval file into a single feature which spans all of the combined features.

Note: `bedtools merge` requires that you presort your data by chromosome and then by start position (e.g., `sort -k1,1 -k2,2n in.bed > in.sorted.bed` for BED files).

See also:

cluster complement

Usage and option summary

Usage:

```
bedtools merge [OPTIONS] -i <BED/GFF/VCF/BAM>
```

(or):

```
mergeBed [OPTIONS] -i <BED/GFF/VCF/BAM>
```

Option	Description
-s	Force strandedness. That is, only merge features that are the same strand. *By default, this is disabled*.
-S	Force merge for one specific strand only. Follow with + or - to force merge from only the forward or reverse strand, respectively. *By default, merging is done without respect to strand*.
-d	Maximum distance between features allowed for features to be merged. *Default is 0. That is, overlapping and/or book-ended features are merged.*
-c	Specify columns from the input file to operate upon (see -o option, below). Multiple columns can be specified in a comma-delimited list.
-o	Specify the operation that should be applied to `-c`. Valid operations: sum, min, max, absmin, absmax, mean, median, collapse (i.e., print a delimited list (duplicates allowed)), distinct (i.e., print a delimited list (NO duplicates allowed)), count count_distinct (i.e., a count of the unique values in the column), **Default:** sum Multiple operations can be specified in a comma-delimited list. If there is only column, but multiple operations, all operations will be applied on that column. Likewise, if there is only one operation, but multiple columns, that operation will be applied to all columns. Otherwise, the number of columns must match the the number of operations, and will be applied in respective order. E.g., `-c 5,4,6 -o sum,mean,count` will give the sum of column 5, the mean of column 4, and the count of column 6. The order of output columns will match the ordering given in the command.
-header	Print the header from the A file prior to results.
-delim	Specify a custom delimiter for the -nms and -scores concat options Example: `-delim "|"` `Default: ";"`

Default behavior

By default, `bedtools merge` combines overlapping (by at least 1 bp) and/or bookended intervals into a single, "flattened" or "merged" interval.

```
$ cat A.bed
chr1  100  200
chr1  180  250
chr1  250  500
chr1  501  1000

$ bedtools merge -i A.bed
chr1  100  500
chr1  501  1000
```

-s Enforcing "strandedness"

The `-s` option will only merge intervals that are overlapping/bookended *and* are on the same strand.

```
$ cat A.bed
chr1  100  200   a1  1 +
chr1  180  250   a2  2 +
chr1  250  500   a3  3 -
chr1  501  1000  a4  4 +

$ bedtools merge -i A.bed -s
chr1  100  250
chr1  501  1000
chr1  250  500
```

To also report the strand, you could use the `-c` and `-o` operators (see below for more details):

```
$ bedtools merge -i A.bed -s -c 6 -o distinct
chr1  100 250 +
chr1  501 1000  +
```

-S Reporting merged intervals on a specific strand.

The `-S` option will only merge intervals for a specific strand. For example, to only report merged intervals on the "+" strand:

```
$ cat A.bed
chr1  100  200   a1  1 +
chr1  180  250   a2  2 +
chr1  250  500   a3  3 -
chr1  501  1000  a4  4 +

$ bedtools merge -i A.bed -S +
chr1  100 250
chr1  501 1000
```

To also report the strand, you could use the `-c` and `-o` operators (see below for more details):

```
$ bedtools merge -i A.bed -S + -c 6 -o distinct
chr1  100 250 +
chr1  501 1000  +
```

-d Controlling how close two features must be in order to merge

By default, only overlapping or book-ended features are combined into a new feature. However, one can force `merge` to combine more distant features with the `-d` option. For example, were one to set `-d` to 1000, any features that overlap or are within 1000 base pairs of one another will be combined.

```
$ cat A.bed
chr1  100  200
chr1  501  1000

$ bedtools merge -i A.bed
chr1  100  200
chr1  501  1000

$ bedtools merge -i A.bed -d 1000
chr1  100  200  1000
```

-c and -o Applying operations to columns from merged intervals.

When merging intervals, we often want to summarize or keep track of the values observed in specific columns (e.g., the feature name or score) from the original, unmerged intervals. When used together, the `-c` and `-o` options allow one to select specific columns (`-c`) and apply operation (`-o`) to each column. The result will be appended to the default, merged interval output. For example, one could use the following to report the count of intervals that we merged in each resulting interval (this replaces the `-n` option that existed prior to version `2.20.0`).

```
$ cat A.bed
chr1  100  200
chr1  180  250
chr1  250  500
chr1  501  1000

$ bedtools merge -i A.bed -c 1 -o count
chr1  100  500  3
chr1  501  1000 1
```

We could also use these options to report the mean of the score (#5) field:

```
$ cat A.bed
chr1  100  200   a1  1 +
chr1  180  250   a2  2 +
chr1  250  500   a3  3 -
chr1  501  1000  a4  4 +

$ bedtools merge -i A.bed -c 5 -o mean
chr1  100 500 2
chr1  501 1000  4
```

Let's get fancy and report the mean, min, and max of the score column:

```
$ bedtools merge -i A.bed -c 5 -o mean,min,max
chr1  100 500 2 1 3
chr1  501 1000  4 4 4
```

Let's also report a comma-separated list of the strands:

```
$ bedtools merge -i A.bed -c 5,5,5,6 -o mean,min,max,collapse
chr1  100 500 2 1 3 +,+,-
chr1  501 1000  4 4 4 +
```

Hopefully this provides a clear picture of what can be done.

`-n` Reporting the number of features that were merged

Deprecated since version 2.20.0.

See the `-c` and `-o` operators.

`-nms` Reporting the names of the features that were merged

Deprecated since version 2.20.0.

See the `-c` and `-o` operators.

`-scores` Reporting the scores of the features that were merged

Deprecated since version 2.20.0.

See the `-c` and `-o` operators.

`-delim` Change the delimiter for `-c` and `-o`

One can override the use of a comma as the delimiter for the `-c` and `-o collapse|distinct` options via the use of the `-delim` option.

```
$ cat A.bed
chr1  100  200  A1
chr1  150  300  A2
chr1  250  500  A3
```

Compare:

```
$ bedtools merge -i A.bed -c 4 -o collapse
chr1  100  500  A1,A2,A3
```

to:

```
$ bedtools merge -i A.bed -c 4 -o collapse -delim "|"
chr1  100  500  A1|A2|A3
```

multicov

`bedtools multicov`, reports the count of alignments from multiple position-sorted and indexed BAM files that overlap intervals in a BED file. Specifically, for each BED interval provided, it reports a separate count of overlapping alignments from each BAM file.

Note: `bedtools multicov` depends upon index BAM files in order to count the number of overlaps in each BAM file. As such, each BAM file should be position sorted (`samtool sort aln.bam aln.sort`) and indexed (`samtools index aln.sort.bam`) with either samtools or bamtools.

Usage and option summary

Usage:

```
bedtools multicov [OPTIONS] -bams BAM1 BAM2 BAM3 ... BAMn -bed  <BED/GFF/VCF>
```

(or):

```
multiBamCov [OPTIONS] -bams BAM1 BAM2 BAM3 ... BAMn -bed  <BED/GFF/VCF>
```

Option	Description
-split	Treat "split" BAM or BED12 entries as distinct BED intervals.
-s	Require same strandedness. That is, only report hits in B that overlap A on the _same_ strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-f	Minimum overlap required as a fraction of each -bed record. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction overlap is reciprocal for -bed and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap 90% of the -bed record and the -bed record _also_ overlaps 90% of B.
-q	Minimum mapping quality (MAPQ) allowed. Default is 0.
-D	Include duplicate reads. Default counts non-duplicates only
-F	Include failed-QC reads. Default counts pass-QC reads only
-p	Only count proper pairs. Default counts all alignments with `MAPQ > -q` argument, regardless of the BAM FLAG field.

Default behavior.

By default, `multicov` will report the count of alignments in each input BAM file that overlap.

```
$ cat ivls-of-interest.bed
chr1 0   10000   ivl1
chr1 10000   20000   ivl2
chr1 20000   30000   ivl3
chr1 30000   40000   ivl4

$ bedtools multicov -bams aln1.bam aln2.bam aln3.bam -bed ivls-of-interest.bed
chr1 0       10000   ivl1    100 2234    0
chr1 10000   20000   ivl2    123 3245    1000
```

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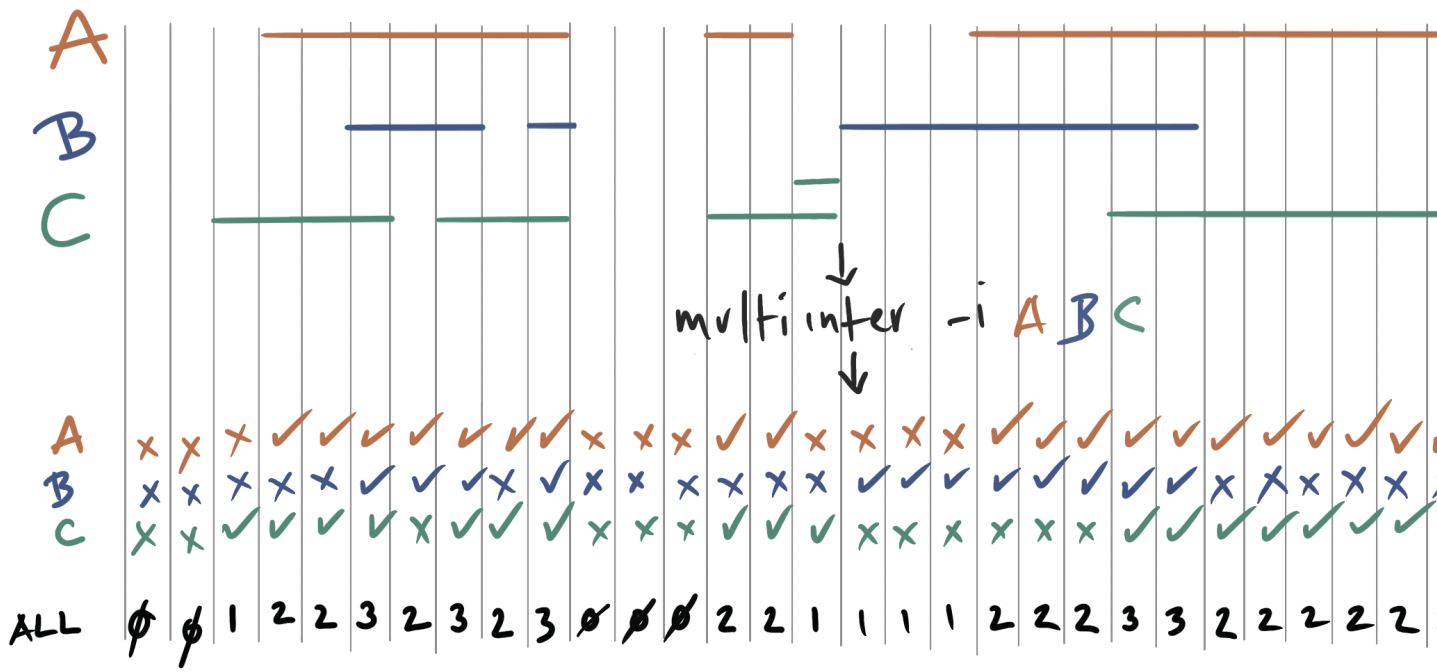
```
chr1 20000   30000   ivl3    213 2332    2034
chr1 30000   40000   ivl4    335 7654    0
```

The output of `multicov` reflects a distinct report of the overlapping alignments for each record in the `-bed` file. In the example above, each line of the output reflects **a)** the original line from the `-bed` file followed by **b)** the count of alignments that overlap the `-bed` interval from each input `-bam` file. In the example above, the output consists of 7 columns: the first four of which are the columns from the `-bed` file and the last 3 are the count of overlapping alignments from the 3 input `-bam` files. The order of the counts reflects the order of the files given on the command line.

Note: `bedtools multicov` will work with a single BAM as well.

```
$ bedtools multicov -bams aln1.bam -bed ivls-of-interest.bed
chr1 0       10000   ivl1    100
chr1 10000   20000   ivl2    123
chr1 20000   30000   ivl3    213
chr1 30000   40000   ivl4    335
```

multiinter



`bedtools multiinter` identifies common intervals among multiple (and subsets thereof) sorted BED/GFF/VCF files.

Note:

1. All files must be sorted in the same mmanner (e.g., `sort -k 1,1 -k2,2n in.bed > in.sorted.bed`)

Usage and option summary

Usage:

```
bedtools multiinter [OPTIONS] -i FILE1 FILE2 .. FILEn
```

(or):

```
multiIntersect [OPTIONS]  -i FILE1 FILE2 .. FILEn
```

Option	Description
-header	Print a header line (chrom/start/end + names of each file).
-names	**A list of names (one/file) to describe each file in -i.** These names will be printed in the header line.
-g	Use genome file to calculate empty regions.
-empty	Report empty regions (i.e., start/end intervals w/o | values in all files). Requires the '-g FILE' parameter.
-filler TEXT	Use TEXT when representing intervals having no value. | Default is '0', but you can use 'N/A' or any text.
-examples	Show usage examples on the command line.

Default behavior

By default, `bedtools multiinter` will inspect all of the intervals in each input file and report the sub-intervals that are overlapped by 0, 1, 2, ... N files. The default output format is as follows:

1. chromosome (or entire genome)
2. 0-based start coordinate of the sub-interval.
3. 1-based end coordinate of the sub-interval.
4. The number of files whose intervals overlap this sub interval at least once.
5. The list of file numbers (by order on the command line) whose intervals overlap this sub interval at least once.
6. Columns reflecting whether each file had (1) or did not have (0) 1 or more intervals overlapping this sub interval.

For example:

```
$ cat a.bed
chr1  6   12
chr1  10  20
chr1  22  27
chr1  24  30

cat b.bed
chr1  12  32
chr1  14  30

$ cat c.bed
chr1  8   15
chr1  10  14
chr1  32  34

$ cat sizes.txt
chr1  5000

$ bedtools multiinter -i a.bed b.bed c.bed
chr1        6       8       1       1       1       0       0
chr1        8       12      2       1,3     1       0       1
chr1        12      15      3       1,2,3   1       1       1
chr1        15      20      2       1,2     1       1       0
chr1        20      22      1       2       0       1       0
chr1        22      30      2       1,2     1       1       0
chr1        30      32      1       2       0       1       0
chr1        32      34      1       3       0       0       1
```

-header Add a header with columns names

For example:

```
$ bedtools multiinter -header -i a.bed b.bed c.bed
chrom       start   end     num     list    a.bed   b.bed   c.bed
chr1        6       8       1       1       1       0       0
chr1        8       12      2       1,3     1       0       1
chr1        12      15      3       1,2,3   1       1       1
chr1        15      20      2       1,2     1       1       0
chr1        20      22      1       2       0       1       0
chr1        22      30      2       1,2     1       1       0
chr1        30      32      1       2       0       1       0
chr1        32      34      1       3       0       0       1
```

-names Add custom labels for each file in the header

For example:

```
$ bedtools multiinter -header -names A B C -i a.bed b.bed c.bed
chrom       start   end     num     list    A       B       C
chr1        6       8       1       1       1       0       0
chr1        8       12      2       1,3     1       0       1
chr1        12      15      3       1,2,3   1       1       1
```

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```
chr1        15      20      2       1,2     1       1       0
chr1        20      22      1       2       0       1       0
chr1        22      30      2       1,2     1       1       0
chr1        30      32      1       2       0       1       0
chr1        32      34      1       3       0       0       1
```

`-empty` Report the sub intervals not covered by any file

Note that this option requires a `-g` file so that it knows the full range of each chromosome or contig.

For example:

```
 $ bedtools multiinter -header -names A B C -i a.bed b.bed c.bed -empty -g sizes.txt
chrom   start   end     num     list    A       B       C
chr1    0       6       0       none    0       0       0
chr1    6       8       1       A       1       0       0
chr1    8       12      2       A,C     1       0       1
chr1    12      15      3       A,B,C   1       1       1
chr1    15      20      2       A,B     1       1       0
chr1    20      22      1       B       0       1       0
chr1    22      30      2       A,B     1       1       0
chr1    30      32      1       B       0       1       0
chr1    32      34      1       C       0       0       1
chr1    34      5000    0       none    0       0       0
```

nuc

overlap

overlap computes the amount of overlap (in the case of positive values) or distance (in the case of negative values) between feature coordinates occurring on the same input line and reports the result at the end of the same line. In this way, it is a useful method for computing custom overlap scores from the output of other BEDTools.

Usage and option summary

Usage:

```
overlap [OPTIONS] -i <input> -cols s1,e1,s2,e2
```

Option	Description
-i	Input file. Use "stdin" for pipes.
-cols	Specify the columns (1-based) for the starts and ends of the features for which you'd like to compute the overlap/distance. The columns must be listed in the following order: *start1,end1,start2,end2*

Default behavior

The default behavior is to compute the amount of overlap between the features you specify based on the start and end coordinates. For example:

```
bedtools window -a A.bed -b B.bed -w 10
chr1  10  20  A  chr1  15  25  B
chr1  10  20  C  chr1  25  35  D
```

Now let's say we want to compute the number of base pairs of overlap # between the overlapping features from the output of windowBed.

```
bedtools windo -a A.bed -b B.bed -w 10 | overlap -i stdin -cols 2,3,6,7
chr1  10  20  A  chr1  15  25  B  5
chr1  10  20  C  chr1  25  35  D  -5
```

pairtobed

pairtopair

pairToPair compares two BEDPE files in search of overlaps where each end of a BEDPE feature in A overlaps with the ends of a feature in B. For example, using pairToPair, one could screen for the exact same discordant paired-end alignment in two files. This could suggest (among other things) that the discordant pair suggests the same structural variation in each file/sample.

Usage and option summary

Usage:

```
pairToPair [OPTIONS] -a <BEDPE> -b <BEDPE>
```

Option	Description
-a	BEDPE file A. Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	BEDPE file B. Use "stdin" if passing B with a UNIX pipe.
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-is	Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-type	Approach to reporting overlaps between BEDPE and BED. **either** Report overlaps if either ends of A overlap B. **neither** Report A if neither end of A overlaps B. **both** Report overlaps if both ends of A overlap B. *-Default behavior.*

Default behavior

By default, a BEDPE feature from A will be reported if *both* ends overlap a feature in the BEDPE B file. If strand information is present for the two BEDPE files, it will be further required that the overlaps on each end be on the same strand. This way, an otherwise overlapping (in terms of genomic locations) F/R alignment will not be matched with a R/R alignment.

Default: Report A if *both* ends overlaps B.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

BEDPE/BAM A         *****.................................*****

BED File B         ^^^^^^^^                                         ^^^^^^

Result              =====.................................=====
```

Default when strand information is present in both BEDPE files: Report A if *both* ends overlaps B *on the same strands*.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

BEDPE A         >>>>>.................................>>>>>

BEDPE B            <<<<<.............................>>>>>

Result



BEDPE A         >>>>>.................................>>>>>

BEDPE B            >>>>>.............................>>>>>

Result          >>>>>.................................>>>>>
```

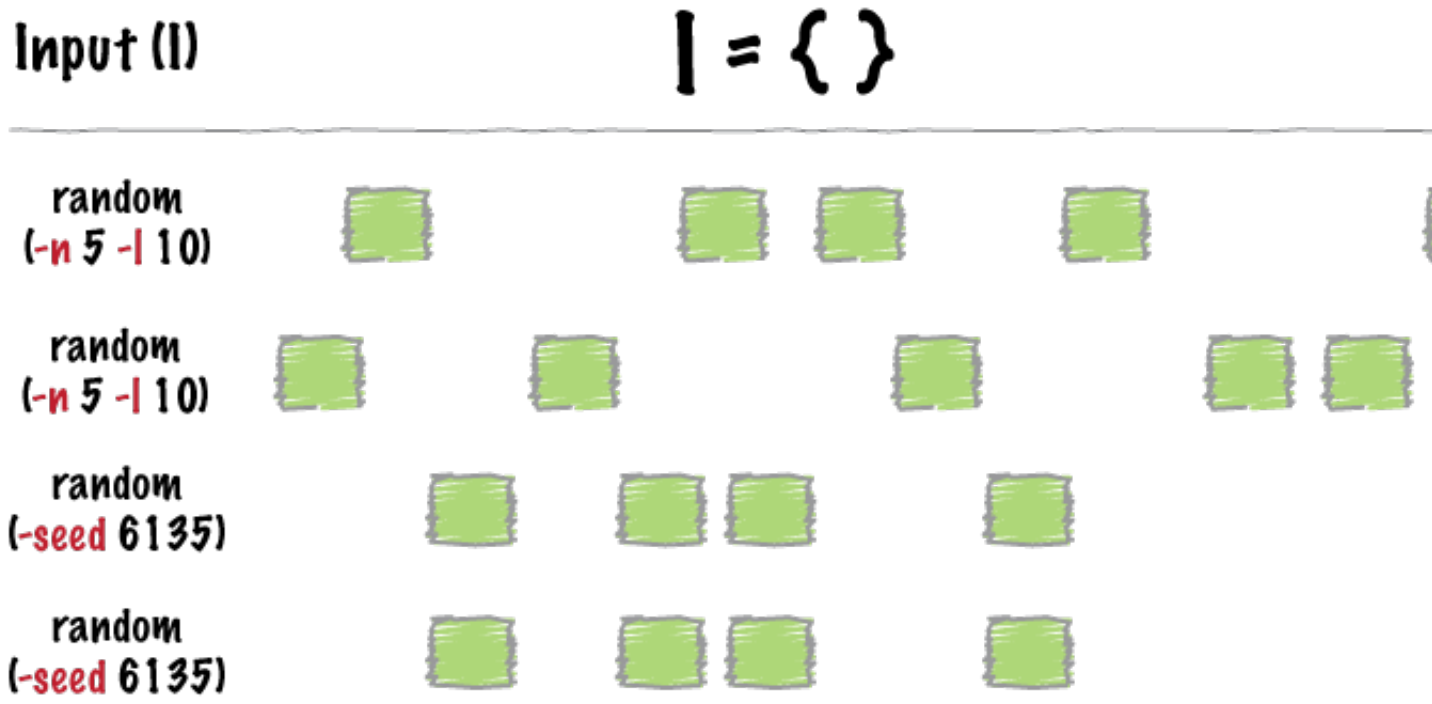
`-type neither` Optional overlap requirements

Using then **-type neither, pairToPair** will only report A if *neither* end overlaps with a BEDPE feature in B.

-type neither: Report A only if *neither* end overlaps B.

```
Chromosome  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

BEDPE/BAM A         *****.................................*****

BED File B         ^^^^^^^^......................................^^^^^^

Result



BEDPE/BAM A         *****.................................*****

BED File B    ^^^^................................................^^^^^^

Result              =====.................................=====
```

random



bedtools random will generate a random set of intervals in BED6 format. One can specify both the number (`-n`) and the size (`-l`) of the intervals that should be generated.

See also:

shuffle jaccard

Usage and option summary

Usage:

```
bedtools random [OPTIONS] -g <GENOME>
```

(or): : :

randomBed [OPTIONS] -g <GENOME>

Option	Description
-l	The length of the intervals to generate. `Default = 100`
-n	The number of intervals to generate. `Default = 1,000,000`
-seed	Supply an integer seed for the shuffling. This will allow feature shuffling experiments to be recreated exactly as the seed for the pseudo-random number generation will be constant. *By default, the seed is chosen automatically*.

Default behavior

By default, *bedtools random* generate 1 million intervals of length 100 placed randomly in the genome specificed with `-g`.

```
$ bedtools random -g hg19.genome
chr2  87536758        87536858        1       100     -
chrX  46051735        46051835        2       100     +
chr18 5237041 5237141 3       100     -
chr12 45809998        45810098        4       100     +
chrX  42034890        42034990        5       100     -
chr10 77510935        77511035        6       100     -
chr3  39844278        39844378        7       100     -
chr6  101012700       101012800       8       100     +
chr12 38123482        38123582        9       100     +
chr7  88508598        88508698        10      100     -

$ bedtools random -g hg19.genome
chr3  141987850       141987950       1       100     +
chr5  137643331       137643431       2       100     +
chr2  155523858       155523958       3       100     -
chr5  147874094       147874194       4       100     +
chr1  71838335        71838435        5       100     -
chr8  71154323        71154423        6       100     -
chr2  133240474       133240574       7       100     +
chr9  131495427       131495527       8       100     +
chrX  125952943       125953043       9       100     +
chr3  59685545        59685645        10      100     +
```

`-n` Specify the *number* of intervals to generate.

The *-n* option allows one to override the default of generating 1 million intervals.

```
$ bedtools random -g hg19.genome -n 3
chr20 47975280        47975380        1       100     -
```

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```
chr16 23381222        23381322        2       100     +
chr3  104913816       104913916       3       100     -
```

-l Specify the *length* of intervals to generate.

The *-l* option allows one to override the default interval length of 100bp.

```
$ bedtools random -g hg19.genome -l 5
chr9  54133731        54133736        1       5       +
chr1  235288830       235288835       2       5       -
chr8  26744718        26744723        3       5       +
chr3  187313616       187313621       4       5       -
chr11 88996846        88996851        5       5       -
chr13 84714855        84714860        6       5       -
chr13 10759738        10759743        7       5       -
chr6  122569739       122569744       8       5       +
chr17 50884025        50884030        9       5       -
chr11 38576901        38576906        10      5       +
```

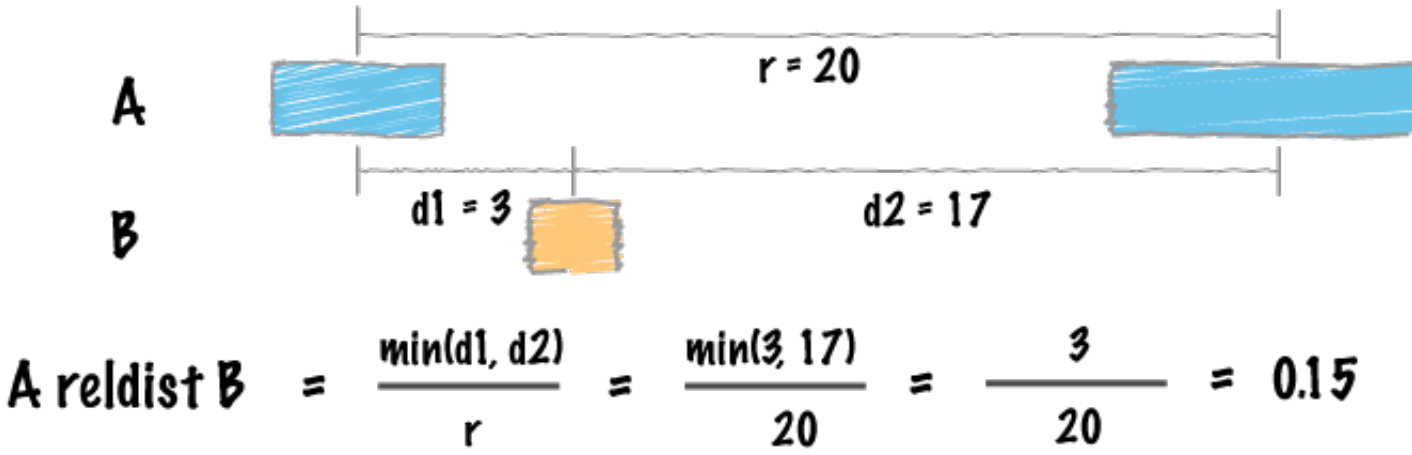
-seed Defining a "seed" for the random interval creation.

bedtools random uses a pseudo-random number generator to permute the locations of BED features. Therefore, each run should produce a different result. This can be problematic if one wants to exactly recreate an experiment. By using the *seed* option, one can supply a custom integer seed for *bedtools random*. In turn, each execution of *bedtools random* with the same seed and input files should produce identical results.

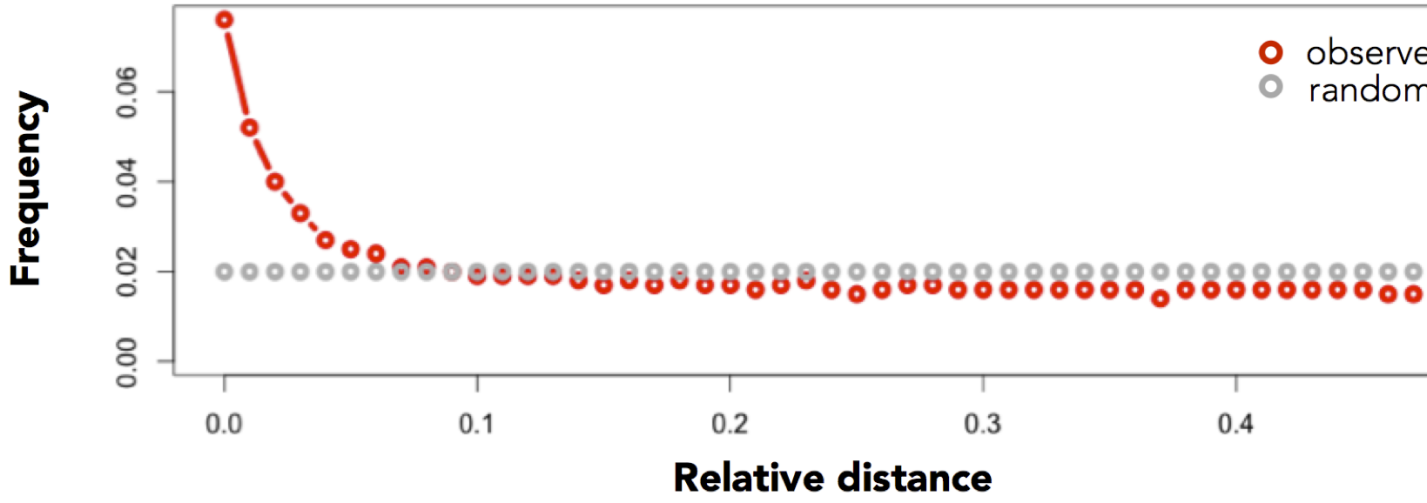
```
$ bedtools random -g hg19.genome -seed 71346
chrY  23380696        23380796        1       100     -
chr14 94368315        94368415        2       100     +
chr14 45353323        45353423        3       100     -
chr14 100546766       100546866       4       100     -
chr12 43294368        43294468        5       100     -
chr1  141470585       141470685       6       100     -
chr10 31273665        31273765        7       100     +
chr5  19102979        19103079        8       100     +
chr3  116730634       116730734       9       100     -
chr3  101222965       101223065       10      100     -

# (same seed, thus same as above)
$ bedtools random -g hg19.genome -seed 71346
chrY  23380696        23380796        1       100     -
chr14 94368315        94368415        2       100     +
chr14 45353323        45353423        3       100     -
chr14 100546766       100546866       4       100     -
chr12 43294368        43294468        5       100     -
chr1  141470585       141470685       6       100     -
chr10 31273665        31273765        7       100     +
chr5  19102979        19103079        8       100     +
chr3  116730634       116730734       9       100     -
chr3  101222965       101223065       10      100     -
```

reldist



Traditional approaches to summarizing the similarity between two sets of genomic intervals are based upon the number or proportion of *intersecting* intervals. However, such measures are largely blind to spatial correlations between the two sets where, despite consistent spacing or proximity, intersections are rare (for example, enhancers and transcription start sites rarely overlap, yet they are much closer to one another than two sets of random intervals). Favorov et al [1] proposed a *relative distance* metric that describes distribution of relative distances between each interval in one set and the two closest intervals in another set (see figure above). If there is no spatial correlation between the two sets, one would expect the relative distances to be uniformly distributed among the relative distances ranging from 0 to 0.5. If, however, the intervals tend to be much closer than expected by chance, the distribution of observed relative distances would be shifted towards low relative distance values (e.g., the figure below).



[1] Exploring Massive, Genome Scale Datasets with the GenometriCorr Package.
Favorov A, Mularoni L, Cope LM, Medvedeva Y, Mironov AA, et al. (2012)
PLoS Comput Biol 8(5): e1002529. doi:10.1371/journal.pcbi.1002529

See also:

jaccard closest

Usage and option summary

Usage:

```
bedtools reldist [OPTIONS] -a <BED/GFF/VCF> -b <BED/GFF/VCF>
```

Option	Description
-a	BED/GFF/VCF file A. Each feature in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe.
-b	BED/GFF/VCF file B. Use "stdin" if passing B with a UNIX pipe.
-detail	Instead of a summary, report the relative distance for each interval in A

Default behavior

By default, `bedtools reldist` reports the distribution of relative distances between two sets of intervals. The output reports the frequency of each relative distance (ranging from 0.0 to 0.5). If the two sets of intervals are randomly distributed with respect to one another, each relative distance "bin" with be roughly equally represented (i.e., a uniform distribution). For example, consider the relative distance distance distribution for exons and AluY elements:

```
$ bedtools reldist \
    -a data/refseq.chr1.exons.bed.gz \
    -b data/
    aluY.chr1.bed.gz
0.00  164 43408 0.004
0.01  551 43408 0.013
0.02  598 43408 0.014
0.03  637 43408 0.015
0.04  793 43408 0.018
0.05  688 43408 0.016
0.06  874 43408 0.020
0.07  765 43408 0.018
0.08  685 43408 0.016
0.09  929 43408 0.021
0.10  876 43408 0.020
0.11  959 43408 0.022
0.12  860 43408 0.020
0.13  851 43408 0.020
0.14  903 43408 0.021
0.15  893 43408 0.021
0.16  883 43408 0.020
0.17  828 43408 0.019
0.18  917 43408 0.021
0.19  875 43408 0.020
0.20  897 43408 0.021
0.21  986 43408 0.023
0.22  903 43408 0.021
0.23  944 43408 0.022
0.24  904 43408 0.021
0.25  867 43408 0.020
0.26  943 43408 0.022
0.27  933 43408 0.021
0.28  1132  43408 0.026
```

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```
0.29  881 43408 0.020
0.30  851 43408 0.020
0.31  963 43408 0.022
0.32  950 43408 0.022
0.33  965 43408 0.022
0.34  907 43408 0.021
0.35  884 43408 0.020
0.36  965 43408 0.022
0.37  944 43408 0.022
0.38  911 43408 0.021
0.39  939 43408 0.022
0.40  921 43408 0.021
0.41  950 43408 0.022
0.42  935 43408 0.022
0.43  919 43408 0.021
0.44  915 43408 0.021
0.45  934 43408 0.022
0.46  843 43408 0.019
0.47  850 43408 0.020
0.48  1006  43408 0.023
0.49  937 43408 0.022
```

In contrast, consider the relative distance distribution observed between exons and conserved elements:

```
$ bedtools reldist \
   -a data/refseq.chr1.exons.bed.gz \
   -b data/gerp.chr1.bed.gz
reldist  count total fraction
0.00  20629 43422 0.475
0.01  2629  43422 0.061
0.02  1427  43422 0.033
0.03  985 43422 0.023
0.04  897 43422 0.021
0.05  756 43422 0.017
0.06  667 43422 0.015
0.07  557 43422 0.013
0.08  603 43422 0.014
0.09  487 43422 0.011
0.10  461 43422 0.011
0.11  423 43422 0.010
0.12  427 43422 0.010
0.13  435 43422 0.010
0.14  375 43422 0.009
0.15  367 43422 0.008
0.16  379 43422 0.009
0.17  371 43422 0.009
0.18  346 43422 0.008
0.19  389 43422 0.009
0.20  377 43422 0.009
0.21  411 43422 0.009
0.22  377 43422 0.009
0.23  352 43422 0.008
0.24  334 43422 0.008
0.25  315 43422 0.007
0.26  370 43422 0.009
0.27  330 43422 0.008
0.28  330 43422 0.008
```

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```
0.29  280 43422 0.006
0.30  309 43422 0.007
0.31  326 43422 0.008
0.32  287 43422 0.007
0.33  294 43422 0.007
0.34  306 43422 0.007
0.35  307 43422 0.007
0.36  309 43422 0.007
0.37  271 43422 0.006
0.38  293 43422 0.007
0.39  311 43422 0.007
0.40  331 43422 0.008
0.41  320 43422 0.007
0.42  299 43422 0.007
0.43  327 43422 0.008
0.44  321 43422 0.007
0.45  326 43422 0.008
0.46  306 43422 0.007
0.47  354 43422 0.008
0.48  365 43422 0.008
0.49  336 43422 0.008
0.50  38  43422 0.001
```

Moreover, if one compares the relative distances for one set against itself, every interval should be expected to overlap an interval in the other set (itself). As such, the relative distances will all be 0.0:

```
$ bedtools reldist \
    -a data/refseq.chr1.exons.bed.gz \
    -b data/refseq.chr1.exons.bed.gz
reldist  count total fraction
0.00  43424 43424 1.000
```

shift

content/tools/../images/tool-glyphs/shift-glyph.png

`bedtools shift` will move each feature in a feature file by a user-defined number of bases. While something like this could be done with an `awk '{OFS="\t" print $1,$2+<shift>,$3+<shift>}'`, `bedtools shift` will restrict the resizing to the size of the chromosome (i.e. no features before 0 or past the chromosome end).

Note: In order to prevent the extension of intervals beyond chromosome boundaries, `bedtools shift` requires a *genome* file defining the length of each chromosome or contig.

See also:

slop

Usage and option summary

Usage:

```
bedtools shift [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-s or (-m and -p)]
```

(or):

```
shiftBed [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-s or (-m and -p)]
```

Option	Description
-s	Shift the BED/GFF/VCF entry -s base pairs. *Integer* or *Float* (e.g. 0.1) if used with -pct
-m	Shift entries on the - strand -m base pairs. *Integer* or *Float* (e.g. 0.1) if used with -pct
-p	Shift entries on the + strand -p base pairs. *Integer* or *Float* (e.g. 0.1) if used with -pct
-pct	Define -s, -m and -p as a fraction of the feature's length. E.g. if used on a 1000bp feature, -s 0.50, will shift the feature 500 bp "upstream". Default = false.
-header	Print the header from the input file prior to results.

Default behavior

By default, `bedtools shift` will shift features a fixed number of bases. This shift can either be applied to all features (`-s`), or seperately to features on the plus (`-p`) and minus (`-m`) strands. Shifts can either be positive or negative.

```
$ cat A.bed
chr1 5 100 +
chr1 800 980 -

$ cat my.genome
chr1 1000

$ bedtools shift -i A.bed -g my.genome -s 5
chr1 10 105 +
chr1 805 985 -

$ bedtools shift -i A.bed -g my.genome -p 2 -m -3
chr1 7 102 +
chr1 797 977 -
```

However, if the requested number of bases exceeds the boundaries of the chromosome, `bedtools shift` will "clip" the feature accordingly.

```
$ cat A.bed
chr1  5   100 +
chr1  800 980 +

$ cat my.genome
chr1  1000

$ bedtools shift -i A.bed -g my.genome -s 5000
chr1  999   1000 +
chr1  999   1000 +
```

`-pct` Shifting features by a given fraction

`bedtools shift` will shift the feature by a user-specific fraction of the feature length. Hence, 0.5 will add half the length of the feature to the start and end coordinates.

For example:

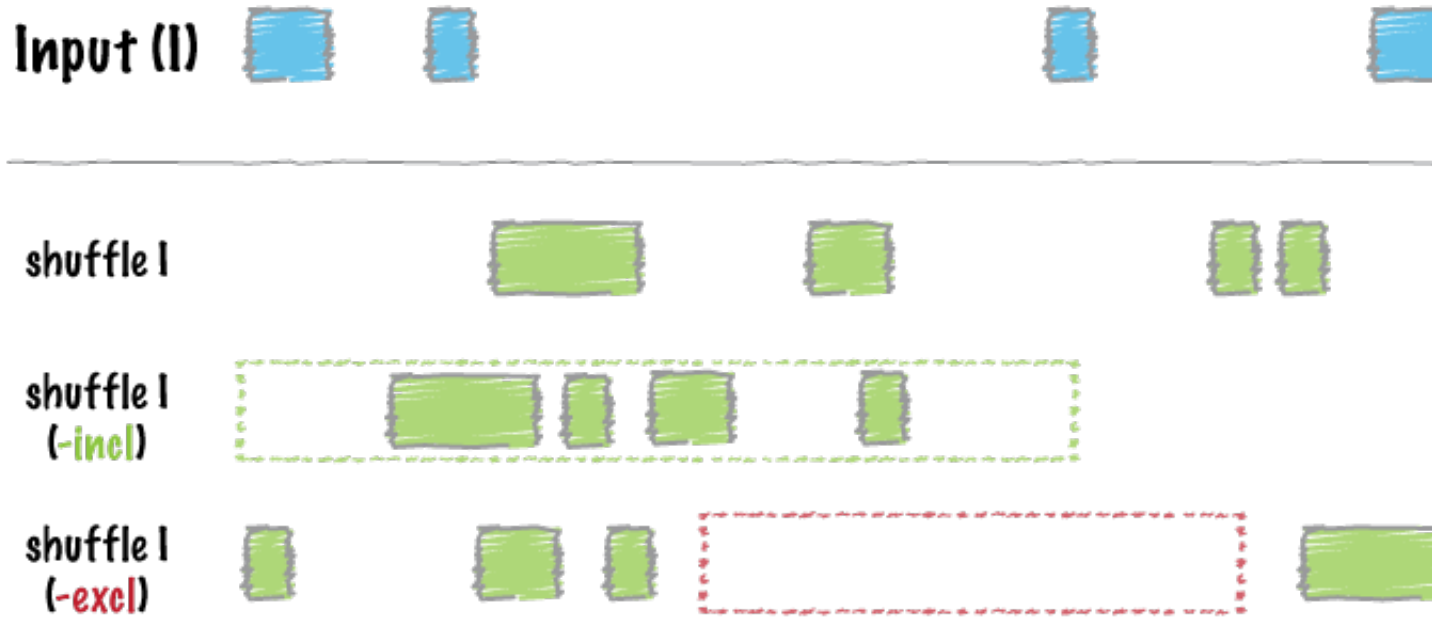
```
$ cat A.bed
chr1 100 200 a1 1 +

$ bedtools shift -i A.bed -g my.genome -s 0.5 -pct
chr1 150  250 a1 1 +
```

`-header` Print the header for the A file before reporting results.

By default, if your A file has a header, it is ignored when reporting results. This option will instead tell bedtools to first print the header for the A file prior to reporting results.

shuffle



bedtools shuffle will randomly permute the genomic locations of a feature file among a genome defined in a genome file. One can also provide an "exclusions" BED/GFF/VCF file that lists regions where you do not want the permuted features to be placed. For example, one might want to prevent features from being placed in known genome gaps. *shuffle* is useful as a *null* basis against which to test the significance of associations of one feature with another.

See also:

random jaccard

Usage and option summary

Usage:

```
bedtools shuffle [OPTIONS] -i <BED/GFF/VCF> -g <GENOME>
```

(or):

```
shuffleBed [OPTIONS] -i <BED/GFF/VCF> -g <GENOME>
```

Option	Description
-excl	A BED file of coordinates in which features from -i should *not* be placed (e.g., genome gaps).
-incl	A BED file of coordinates in which features from -i *should* be placed.
-chrom	Keep features in -i on the same chromosome. Solely permute their location on the chromosome. *By default, both the chromosome and position are randomly chosen*.
-seed	Supply an integer seed for the shuffling. This will allow feature shuffling experiments to be recreated exactly as the seed for the pseudo-random number generation will be constant. *By default, the seed is chosen automatically*.
-f	Maximum overlap (as a fraction of the `-i` feature) with an `-excl` feature that is tolerated before searching for a new, randomized locus.
-chromFirst	Instead of choosing a position randomly among the entire genome (the default), first choose a chrom randomly, and then choose a random start coordinate on that chrom. This leads to features being ~uniformly distributed among the chroms, as opposed to features being distribute as a function of chrom size.
-bedpe	Indicate that the A file is in BEDPE format.
-maxTries	Max. number of attempts to find a home for a shuffled interval in the presence of -incl or -excl. *Default = 1000.*
-noOverlapping	Don't allow shuffled intervals to overlap.
-allowBeyondChromEnd	Allow the original the length of the original records to extend beyond the length of the chromosome.

Default behavior

By default, *bedtools shuffle* will reposition each feature in the input BED file on a random chromosome at a random position. The size and strand of each feature are preserved.

For example:

```
$ cat A.bed
chr1  0  100  a1  1  +
chr1  0  1000 a2  2  -

$ cat my.genome
chr1  10000
chr2  8000
chr3  5000
chr4  2000

$ bedtools shuffle -i A.bed -g my.genome
chr4  1498  1598  a1  1  +
chr3  2156  3156  a2  2  -
```

`-chrom` Requiring that features be shuffled on the same chromosome

The *-chrom* option behaves the same as the default behavior except that features are randomly placed on the same chromosome as defined in the BED file.

```
$ cat A.bed
chr1  0  100  a1  1  +
chr1  0  1000 a2  2  -
```

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```
$ cat my.genome
chr1  10000
chr2  8000
chr3  5000
chr4  2000

$ bedtools shuffle -i A.bed -g my.genome -chrom
chr1  9560  9660  a1  1  +
chr1  7258  8258  a2  2  -
```

`-excl` Excluding certain genome regions from `bedtools shuffle`

One may want to prevent BED features from being placed in certain regions of the genome. For example, one may want to exclude genome gaps from permutation experiment. The *excl* option defines a BED file of regions that should be excluded. `bedtools shuffle` will attempt to permute the locations of all features while adhering to the exclusion rules. However it will stop looking for an appropriate location if it cannot find a valid spot for a feature after 1,000,000 tries.

For example (*note that the exclude file excludes all but 100 base pairs of the chromosome*):

```
$ cat A.bed
chr1  0  100   a1  1  +
chr1  0  1000  a2  2  -

$ cat my.genome
chr1  10000

$ cat exclude.bed
chr1  100  10000

$ bedtools shuffle -i A.bed -g my.genome -excl exclude.bed
chr1  0  100  a1  1  +
Error, line 2: tried 1000000 potential loci for entry, but could not avoid excluded
regions. Ignoring entry and moving on.
```

For example (*now the exclusion file only excludes the first 100 bases of the chromosome*):

```
$ cat A.bed
chr1  0  100  a1  1  +
chr1  0  1000 a2  2  -

$ cat my.genome
chr1  10000

$ cat exclude.bed
chr1  0  100

$ bedtools shuffle -i A.bed -g my.genome -excl exclude.bed
chr1  147  247  a1  1  +
chr1  2441 3441 a2  2  -
```

`-seed` Defining a "seed" for the random replacement.

bedtools shuffle uses a pseudo-random number generator to permute the locations of BED features. Therefore, each run should produce a different result. This can be problematic if one wants to exactly recreate an experiment. By using the *seed* option, one can supply a custom integer seed for *bedtools shuffle*. In turn, each execution of *bedtools shuffle* with the same seed and input files should produce identical results.

For example (*note that the exclude file below excludes all but 100 base pairs of the chromosome*):

```
$ cat A.bed
chr1 0 100 a1 1 +
chr1 0 1000 a2 2 -

$ cat my.genome
chr1 10000

$ bedtools shuffle -i A.bed -g my.genome -seed 927442958
chr1 6177 6277 a1 1 +
chr1 8119 9119 a2 2 -

$ bedtools shuffle -i A.bed -g my.genome -seed 927442958
chr1 6177 6277 a1 1 +
chr1 8119 9119 a2 2 -

. . .

$ bedtools shuffle -i A.bed -g my.genome -seed 927442958
chr1 6177 6277 a1 1 +
chr1 8119 9119 a2 2 -
```

`-noOverlapping` Prevent shuffled intervals from overlapping.

There often arise cases where one wants to shuffle intervals throughout the genome, yet one wants to prevent the intervals from occupying a single common base pair. The `-noOverlapping` option allows one to enforce no such overlaps.

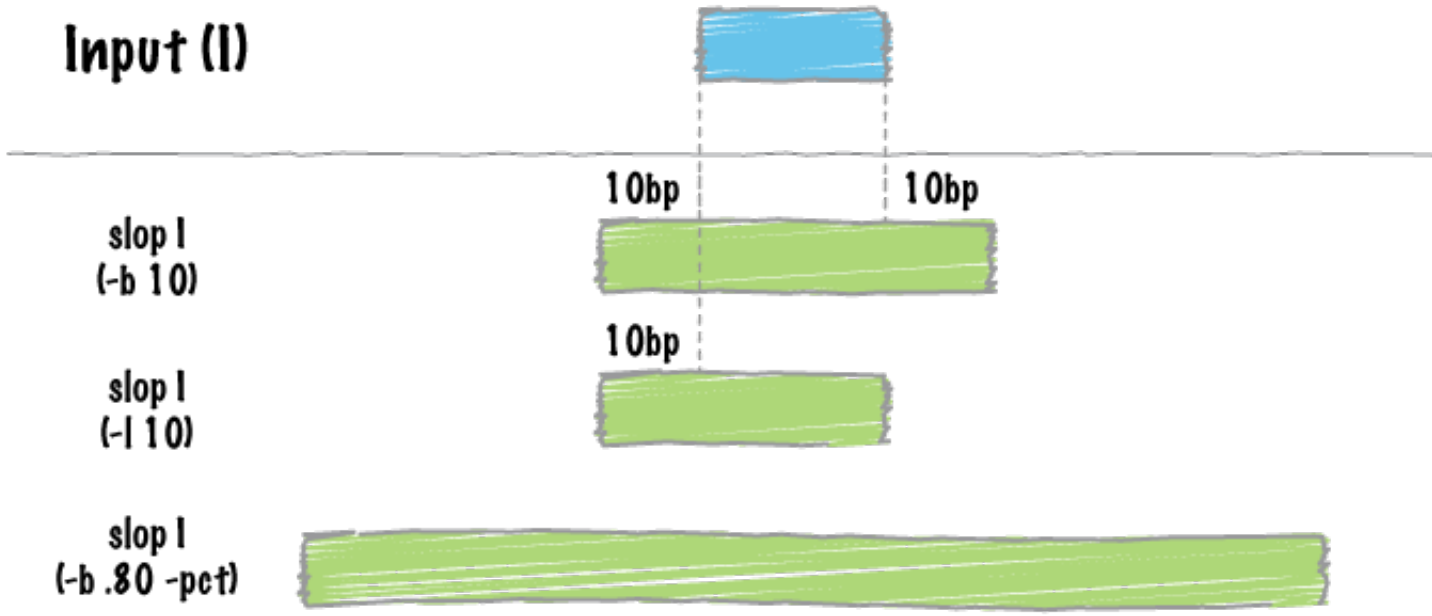
```
$ bedtools shuffle -i A.bed -g my.genome -noOverlapping
```

`-allowBeyondChromEnd` Allow records to extend beyond the chrom. length.

By default, `shuffle` requires that an interval's original length must be fully-contained within the chromosome. Yet there are cases where you might want to allow shuffled intervals to be relocated to a position in which the entire original interval cannot fit w/o exceeding the end of the chromosome. By using the `-noOverlapping` option, `shuffle` will allow intervals to be shuffled to locations that are so close to the chromosome end that the full length of the original record cannot be contained within the chromosome length. In such cases, the end coordinate for the shuffled interval will be set (i.e., truncated) to the chromosome's length.

```
$ bedtools shuffle -i A.bed -g my.genome -allowBeyondChromEnd
```

slop



`bedtools slop` will increase the size of each feature in a feature file by a user-defined number of bases. While something like this could be done with an `awk '{OFS="\t" print $1,$2-<slop>,$3+<slop>}'`, `bedtools slop` will restrict the resizing to the size of the chromosome (i.e. no start < 0 and no end > chromosome size).

Note: In order to prevent the extension of intervals beyond chromosome boundaries, `bedtools slop` requires a *genome* file defining the length of each chromosome or contig.

See also:

flank

Usage and option summary

Usage:

```
bedtools slop [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-b or (-l and -r)]
```

(or):

```
slopBed [OPTIONS] -i <BED/GFF/VCF> -g <GENOME> [-b or (-l and -r)]
```

Option	Description
-b	Increase the BED/GFF/VCF entry by the same number base pairs in each direction. *Integer*.
-l	The number of base pairs to subtract from the start coordinate. *Integer*.
-r	The number of base pairs to add to the end coordinate. *Integer*.
-s	Define -l and -r based on strand. For example. if used, -l 500 for a negative-stranded feature, it will add 500 bp to the *end* coordinate.
-pct	Define -l and -r as a fraction of the feature's length. E.g. if used on a 1000bp feature, -l 0.50, will add 500 bp "upstream". Default = false.
-header	Print the header from the input file prior to results.

Default behavior

By default, `bedtools slop` will either add a fixed number of bases in each direction (`-b`) or an asymmetric number of bases in each direction with `-l` and `-r`.

```
$ cat A.bed
chr1 5 100
chr1 800 980

$ cat my.genome
chr1 1000

$ bedtools slop -i A.bed -g my.genome -b 5
chr1 0 105
chr1 795 985

$ bedtools slop -i A.bed -g my.genome -l 2 -r 3
chr1 3 103
chr1 798 983
```

However, if the requested number of bases exceeds the boundaries of the chromosome, `bedtools slop` will "clip" the feature accordingly.

```
$ cat A.bed
chr1  5   100
chr1  800 980

$ cat my.genome
chr1  1000

$ bedtools slop -i A.bed -g my.genome -b 5000
chr1  0   1000
chr1  0   1000
```

`-s` Resizing features according to strand

`bedtools slop` will optionally increase the size of a feature based on strand.

For example:

```
$ cat A.bed
chr1 100 200 a1 1 +
chr1 100 200 a2 2 -

$ cat my.genome
chr1 1000

$ bedtools slop  -i A.bed -g my.genome -l 50 -r 80 -s
chr1 50  280 a1 1 +
chr1 20  250 a2 2 -
```

-pct Resizing features by a given fraction

`bedtools slop` will optionally increase the size of a feature by a user-specific fraction.

For example:

```
$ cat A.bed
chr1 100 200 a1 1 +

$ bedtools slop -i A.bed -g my.genome -b 0.5 -pct
chr1 50  250 a1 1 +

$ bedtools slop -i a.bed -l 0.5 -r 0.0 -pct -g my.genome
chr1  50      200     a1      1       +
```

-header Print the header for the A file before reporting results.

By default, if your A file has a header, it is ignored when reporting results. This option will instead tell bedtools to first print the header for the A file prior to reporting results.

sort

The `bedtools sort` tool sorts a feature file by chromosome and other criteria.

Usage and option summary

Usage:

```
bedtools sort [OPTIONS] -i <BED/GFF/VCF>
```

(or):

```
sortBed [OPTIONS] -i <BED/GFF/VCF>
```

Option	Description
-sizeA	Sort by feature size in ascending order.
-sizeD	Sort by feature size in descending order.
-chrThenSizeA	Sort by chromosome (asc), then by feature size (asc).
-chrThenSizeD	Sort by chromosome (asc), then by feature size (desc).
-chrThenScoreA	Sort by chromosome (asc), then by score (asc).
-chrThenScoreD	Sort by chromosome (asc), then by score (desc).
-g	Define sort order by order of tab-delimited file with chromosome names in the first column.
-faidx	Define sort order by order of tab-delimited file with chromosome names in the first column. Sort by specified chromosome order.

Default behavior

By default, `bedtools sort` sorts a BED file by chromosome and then by start position in ascending order.

For example:

```
cat A.bed
chr1 800 1000
chr1 80  180
chr1 1   10
chr1 750 10000

sortBed -i A.bed
chr1 1   10
chr1 80  180
chr1 750 10000
chr1 800 1000
```

Optional sorting behavior

`bedtools sort` will also sort a BED file by chromosome and then by other criteria.

For example, to sort by chromosome and then by feature size (in descending order):

```
cat A.bed
chr1 800 1000
chr1 80  180
chr1 1   10
chr1 750 10000

sortBed -i A.bed -sizeD
chr1 750 10000
chr1 800 1000
chr1 80  180
chr1 1   10
```

Disclaimer: it should be noted that `bedtools sort` is merely a convenience utility, as the UNIX sort utility will sort BED files more quickly while using less memory. For example, UNIX sort will sort a BED file by chromosome then by start position in the following manner:

```
sort -k 1,1 -k2,2n a.bed
chr1 1   10
chr1 80  180
chr1 750 10000
chr1 800 1000
```

subtract

`bedtools subtract` bedtools' subtract searches for features in B that overlap A by at least the number of base pairs given by the -f option. If an overlapping feature is found in B, the overlapping portion is removed from A and the remaining portion of A is reported. If a feature in B overlaps all of a feature in A, the A feature will not be reported. If a feature in B does not overlap a feature in A by at least the -f amount, the A feature will be reported in its entirety.

Usage and option summary

Usage:

```
bedtools subtract [OPTIONS] -a <BED/GFF/VCF> -b <BED/GFF/VCF>
```

(or):

```
subtractBed [OPTIONS] -a <BED/GFF/VCF> -b <BED/GFF/VCF>
```

Option	Description
-f	Minimum overlap required as a fraction of A. Default is 1E-9 (i.e. 1bp).
-F	Minimum overlap required as a fraction of B. Default is 1E-9 (i.e., 1bp).
-r	Require that the fraction of overlap be reciprocal for A and B. In other words, if -f is 0.90 and -r is used, this requires that B overlap at least 90% of A and that A also overlaps at least 90% of B.
-e	Require that the minimum fraction be satisfied for A _OR_ B. In other words, if -e is used with -f 0.90 and -F 0.10 this requires that either 90% of A is covered OR 10% of B is covered. Without -e, both fractions would have to be satisfied.
-s	Force "strandedness". That is, only report hits in B that overlap A on the same strand. By default, overlaps are reported without respect to strand.
-S	Require different strandedness. That is, only report hits in B that overlap A on the _opposite_ strand. By default, overlaps are reported without respect to strand.
-A	Remove entire feature if any overlap. That is, by default, only subtract the portion of A that overlaps B. Here, if any overlap is found (or `-f` amount), the entire feature is removed.
-N	Same as -A except when used with -f, the amount is the sum of all features (not any single feature).

Default behavior

By default, `bedtools subtracts` removes each overlapping interval in B from A. If a feature in B *completely* overlaps a feature in A, the A feature is removed.

```
$ cat A.bed
chr1  10   20
chr1  100  200

$ cat B.bed
chr1  0    30
chr1  180  300

$ bedtools subtract -a A.bed -b B.bed
chr1  100  180
```

`-f` Requiring a minimal overlap fraction before subtracting

This option behaves the same as the `-f` option for `bedtools intersect`. In this case, `subtract` will only subtract an overlap with B if it covers at least the fraction of A defined by `-f`. If an overlap is found, but it does not meet the overlap fraction, the original A feature is reported without subtraction.

```
$ cat A.bed
chr1  100  200

$ cat B.bed
chr1  180  300

$ bedtools subtract -a A.bed -b B.bed -f 0.10
chr1  100  180
```

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```
$ bedtools subtract -a A.bed -b B.bed -f 0.80
chr1  100  200
```

-s Enforcing same "strandedness"

This option behaves the same as the `-s` option for `bedtools intersect` while scanning for features in B that should be subtracted from A.

```
$ cat A.bed
chr1  100  200    a1  1   +

$ cat B.bed
chr1  80   120    b1  1   +
chr1  180  300    b2  1   -

$ bedtools subtract -a A.bed -b B.bed -s
chr1  120  200    a1  1   +
```

-S Enforcing opposite "strandedness"

This option behaves the same as the `-s` option for `bedtools intersect` while scanning for features in B that should be subtracted from A.

```
$ cat A.bed
chr1  100  200    a1  1   +

$ cat B.bed
chr1  80   120    b1  1   +
chr1  180  300    b2  1   -

$ bedtools subtract -a A.bed -b B.bed -S
chr1  100  180    a1  1   +
```

-A Remove features with any overlap

Unlike the default behavior, the `-A` option will completely remove a feature from A if it has even 1bp of overlap with a feature in B.

```
$ cat A.bed
chr1  100  200

$ cat B.bed
chr1  180  300

$ bedtools subtract -a A.bed -b B.bed
chr1  100  180

$ bedtools subtract -a A.bed -b B.bed -A
```

summary

Genomics experiments have numerous sources of both technical and biological variation that can confound analysis and interpretation. Therefore, one of the most important steps in genomics data analysis is generating high-level summary stats of one's data to ask if the basic observations are in line with the expectation. Doing such quality control as early as possible in the analysis workflow helps to head off unnecessary time spent chasing technical artifacts that masquerade as biological signal. **This quality control is the motivation behind the** `bedtools summary` **command.**

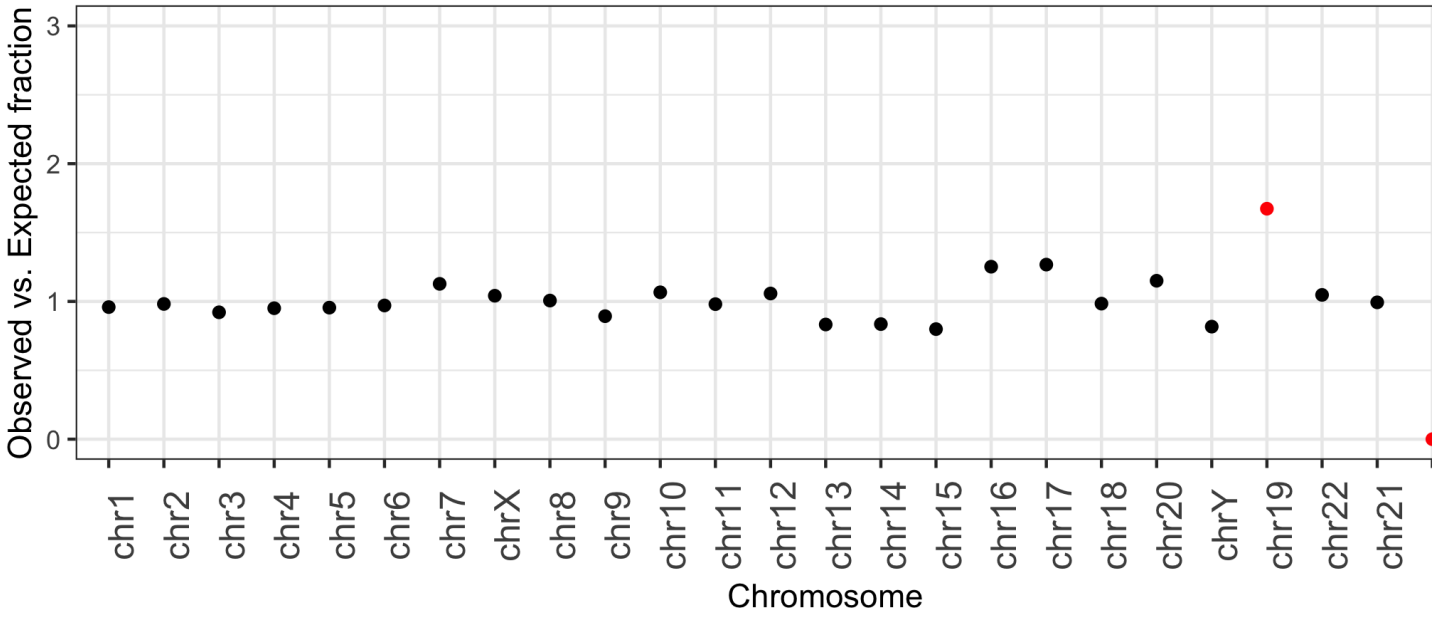
Given an input interval file in standard formats, as well as a genome file defining the chromosome names and lengths relevant to your data, `bedtools summary` will compute a number of summary statistics detailing, for each chromosome, the number of intervals, the total number of base pairs, and the fraction of intervals and base pairs observed in your input file. From these summary measures, one can get a quick sense of questions like:

- Are all chromosomes represented in my data or annotation file?
- Do all chromosomes have the expected number of intervals or fraction of base pairs represented?
- Which chromosomes are outliers?

For example, the following plot was generated directly from the output of `bedtools summary`. It depicts, for each chromosome, the the fraction of all intervals in the RepeatMasker track from UCSC **observed** on each chromosome versus the fraction of RepeatMasker intervals that are **expected** for each chromosome, based on the fraction of the genome that each chromosome represents.

This plot highlights that `chr19`, `chrM`, and `chrMT` (the different mitochindrial reference genomes) are outliers. Chromosome 19 has more than 1.5 times the intervals that are expected based upon the length of the chromosome. ChrM has no intervals, making the observed to expected ratio be 0. This former is because the repeat content of chromosome 19 "is approximately 55%, more than 10% higher than the genome-wide average" (`Grimwood J, et al. Nature. 2004;428:529-35`). The latter because are indeed no repeat annotations provided by UCSC for the mitochondrial genome.

In this case, the extremes in obersved versus expected ratios make sense. However, **this tool provides the ability to detect cases that do not and are either artifacts or biological signal**.



Usage and option summary

Usage:

```
bedtools summary -i <BED/GFF/VCF> -g <GENOME>
```

Default behavior

`bedtools summary` scans the input interval file and the chromosome lengths provided in the "genome" file and reports the following columns for each chromosome.

1. **chrom** (chromosome name)
2. **chrom_length** (the length of the chromosome in bp)
3. **num_ivls** (the total number of intervals observed for the chromosome)
4. **total_ivl_bp** (the total number of bp observed in the intervals for the chromosome)
5. **chrom_frac_genome** (the fraction of the genome represented by the chromosome)
6. **frac_all_ivls** (the fraction of all intervals observed the chromosome)
7. **frac_all_bp** (the fraction of all bp observed the chromosome)
8. **min** (the smallest interval observed on that chromosome)
9. **max** (the largest interval observed on that chromosome)
10. **mean** (the mean interval length observed on that chromosome)

In addition, `bedtools summary` reports a final line with the chromosome name "all", which depicts the metrics above but tabulated across all chromosomes in the input file.

As an example, let's recreate the figure above by running `bedtools summary` on the simple repeats track from UCSC. We'll begin by downloading the data in track the track table and converting it to BED format.

```
curl -s http://hgdownload.soe.ucsc.edu/goldenPath/hg38/database/simpleRepeat.txt.gz  \
    | gzcat \
    | cut -f 2-5 \
    | grep -v -E 'Un|fix|random|alt|hap' \
    > simrep.grch38.bed

head simrep.grch38.bed
chr1        10000   10468   trf
chr1        10627   10800   trf
chr1        10757   10997   trf
chr1        11225   11447   trf
chr1        11271   11448   trf
chr1        11283   11448   trf
chr1        19305   19443   trf
chr1        20828   20863   trf
chr1        30862   30959   trf
chr1        44835   44876   trf
```

Now, let's make a "genome" file for GRCh38 from the *chromInfo* table at UCSC

```
curl -s http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/chromInfo.txt.gz \
    | gzcat \
    | cut -f 1-2 \
```

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```
    | grep -v -E 'Un|fix|random|alt|hap' \
    > grch38.genome.txt

head grch38.genome.txt
chr1        249250621
chr2        243199373
chr3        198022430
chr4        191154276
chr5        180915260
chr6        171115067
chr7        159138663
chrX        155270560
chr8        146364022
chr9        141213431
```

Now, let's run `bedtools summary`.

```
bedtools summary -i simrep.grch38.bed -g grch38.genome.txt | column -t
chrom  chrom_length  num_ivls  total_ivl_bp  chrom_frac_genome  frac_all_ivls  frac_
↪all_bp  min  max     mean
chr1   249250621     74548     15557884      0.080514834        0.077210928    0.
↪048725518  25   124438  208.696195740
chr2   243199373     74474     14493548      0.078560114        0.077134284    0.
↪045392139  25   336509  194.612186803
chr3   198022430     56894     13946854      0.063966714        0.058926309    0.
↪043679955  25   500000  245.137518895
chr4   191154276     56685     10160257      0.061748110        0.058709844    0.
↪031820766  25   136950  179.240663315
chr5   180915260     53887     16801740      0.058440625        0.055811896    0.
↪052621133  25   500000  311.795794904
chr6   171115067     51802     11222841      0.055274892        0.053652418    0.
↪035148658  25   500000  216.648797344
chr7   159138663     55972     20054618      0.051406183        0.057971375    0.
↪062808775  25   150228  358.297327235
chrX   155270560     50432     27398336      0.050156679        0.052233481    0.
↪085808462  25   500000  543.272842640
chr8   146364022     45937     15650021      0.047279621        0.047577915    0.
↪049014080  25   500000  340.684437382
chr9   141213431     39329     10932158      0.045615838        0.040733870    0.
↪034238272  25   159861  277.966843805
chr10  135534747     45074     11407694      0.043781466        0.046684087    0.
↪035727596  25   110000  253.088121755
chr11  135006516     41279     14127024      0.043610833        0.042753526    0.
↪044244227  25   500000  342.232709126
chr12  133851895     44151     13878240      0.043237859        0.045728117    0.
↪043465064  25   356015  314.335802134
chr13  115169878     29907     9423815       0.037203051        0.030975307    0.
↪029514313  25   110000  315.103989033
chr14  107349540     27973     9245970       0.034676866        0.028972223    0.
↪028957323  25   173523  330.531941515
chr15  102531392     25557     9565023       0.033120471        0.026469921    0.
↪029956561  25   110000  374.262354736
chr16  90354753      35288     11959674      0.029187080        0.036548522    0.
↪037456334  25   138208  338.916175470
chr17  81195210      32093     16264416      0.026228295        0.033239393    0.
↪050938295  25   132210  506.790141152
chr18  78077248      23966     18684937      0.025221107        0.024822089    0.
↪058519091  25   500000  779.643536677
```

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```
chr20  63025520      22608     12620160      0.020358983        0.023415580    0.
↪039524901  25   500000  558.216560510
chrY   59373566      15130     4564760       0.019179301        0.015670458    0.
↪014296307  25   227093  301.702577660
chr19  59128983      30854     11391752      0.019100294        0.031956135    0.
↪035677667  25   396802  369.214753355
chr22  51304566      16760     10691540      0.016572792        0.017358684    0.
↪033484683  25   498537  637.920047733
chr21  48129895      14911     9253172       0.015547285        0.015443636    0.
↪028979879  25   499939  620.560123399
chrM   16571         0         0             0.000005353        0.000000000    0.
↪000000000  -1   -1      -1
chrMT  16569         0         0             0.000005352        0.000000000    0.
↪000000000  -1   -1      -1
all    3095710552    965511    319296434     1.0                1.0            1.0
↪      25   500000  330.702015824
```

Notice the following:

1. There are 0 intervals reported for *chrM* or *chrMT*; therefore, the min, max, and mean are all "-1".
2. The last line in the output is has an "genome" chromosome, meaning it is a summary of all of the chromosomes.

Using this report, there are many high-level sanity checks one can explore. For example, we can create the plot described above by saving the output to a file.

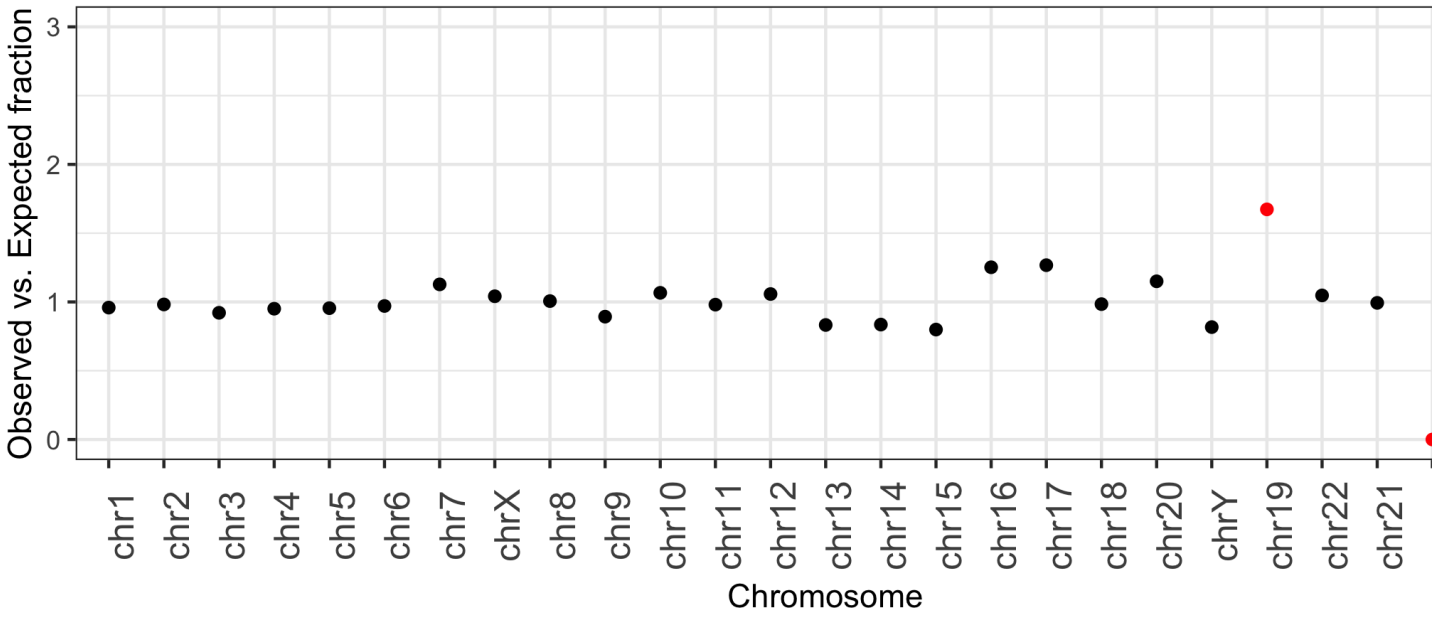
```
bedtools summary -i simrep.grch38.bed -g grch38.genome.txt > ~/simrep.summary.tsv
```

Now run following R code. (Sorry, I am not an R expert)

```
if (!require("dplyr")) install.packages("dplyr")
if (!require("ggplot2")) install.packages("ggplot2")
library(dplyr)
library(ggplot2)

x = read_tsv('~/simrep.summary.tsv')
x = x %>% mutate(obs_v_exp = frac_all_ivls/chrom_frac_genome)

p = ggplot(x) +
ylim(0,3) +
ylab("Observed vs. Expected fraction") +
xlab("Chromosome") +
geom_point(aes(x=factor(chrom, level=chrom),
               y=obs_v_exp,
               color=ifelse(obs_v_exp>1.5 | obs_v_exp<0.5, 'red', 'black'))) +
scale_color_identity() +
theme_bw()
p + theme(axis.text.x = element_text(size = 12, angle = 90))
```



tag

unionbedg

bedtools unionbedg combines multiple BEDGRAPH files into a single file such that one can directly compare coverage (and other text-values such as genotypes) across multiple sample

Usage and option summary

Usage:

```
bedtools unionbedg [OPTIONS] -i FILE1 FILE2 FILE3 ... FILEn
```

Option	Description
-header	Print a header line, consisting of chrom, start, end followed by the names of each input BEDGRAPH file.
-names	A list of names (one per file) to describe each file in -i. These names will be printed in the header line.
-empty	Report empty regions (i.e., start/end intervals w/o values in all files). *Requires the '-g FILE' parameter (see below).*
-g	The genome file to be used to calculate empty regions.
-filler TEXT	Use TEXT when representing intervals having no value. Default is '0', but you can use 'N/A' or any other text.
-examples	Show detailed usage examples.

Default behavior

```
cat 1.bg
chr1 1000 1500 10
chr1 2000 2100 20

cat 2.bg
chr1 900 1600 60
chr1 1700 2050 50

cat 3.bg
chr1 1980 2070 80
chr1 2090 2100 20

cat sizes.txt
chr1 5000

bedtools unionbedg -i 1.bg 2.bg 3.bg
chr1 900  1000 0  60 0
chr1 1000 1500 10 60 0
chr1 1500 1600 0  60 0
chr1 1700 1980 0  50 0
chr1 1980 2000 0  50 80
chr1 2000 2050 20 50 80
chr1 2050 2070 20 0  80
chr1 2070 2090 20 0  0
chr1 2090 2100 20 0  20
```

`-header` Add a header line to the output

```
bedtools unionbedg -i 1.bg 2.bg 3.bg -header
chrom  start  end  1  2  3
chr1   900    1000 0  60 0
chr1   1000   1500 10 60 0
chr1   1500   1600 0  60 0
chr1   1700   1980 0  50 0
chr1   1980   2000 0  50 80
chr1   2000   2050 20 50 80
chr1   2050   2070 20 0  80
chr1   2070   2090 20 0  0
chr1   2090   2100 20 0  20
```

`-names` Add a header line with custom file names to the output

```
bedtools unionbedg -i 1.bg 2.bg 3.bg -header -names WT-1 WT-2 KO-1
chrom  start  end   WT-1  WT-2  KO-1
chr1   900    1000  0     60    0
chr1   1000   1500  10    60    0
chr1   1500   1600  0     60    0
chr1   1700   1980  0     50    0
chr1   1980   2000  0     50    80
chr1   2000   2050  20    50    80
chr1   2050   2070  20    0     80
chr1   2070   2090  20    0     0
chr1   2090   2100  20    0     20
```

`-empty` Include regions that have zero coverage in all BEDGRAPH files.

```
bedtools unionbedg -i 1.bg 2.bg 3.bg -empty -g sizes.txt -header
chrom  start  end  WT-1  WT-2  KO-1
chrom  start  end  1     2     3
chr1   0      900  0     0     0
chr1   900    1000 0     60    0
chr1   1000   1500 10    60    0
chr1   1500   1600 0     60    0
chr1   1600   1700 0     0     0
chr1   1700   1980 0     50    0
chr1   1980   2000 0     50    80
chr1   2000   2050 20    50    80
chr1   2050   2070 20    0     80
chr1   2070   2090 20    0     0
chr1   2090   2100 20    0     20
chr1   2100   5000 0     0     0
```

`-filler` Use a custom value for missing values.

```
bedtools unionbedg -i 1.bg 2.bg 3.bg -empty -g sizes.txt -header -filler N/A
chrom start end  WT-1  WT-2  KO-1
chrom start end  1     2     3
chr1  0     900  N/A   N/A   N/A
chr1  900   1000 N/A   60    N/A
chr1  1000  1500 10    60    N/A
chr1  1500  1600 N/A   60    N/A
chr1  1600  1700 N/A   N/A   N/A
chr1  1700  1980 N/A   50    N/A
chr1  1980  2000 N/A   50    80
chr1  2000  2050 20    50    80
chr1  2050  2070 20    N/A   80
chr1  2070  2090 20    N/A   N/A
chr1  2090  2100 20    N/A   20
chr1  2100  5000 N/A   N/A   N/A
```

Use BEDGRAPH files with non-numeric values.

```
cat 1.snp.bg
chr1 0 1 A/G
chr1 5 6 C/T

cat 2.snp.bg
chr1 0 1 C/C
chr1 7 8 T/T

cat 3.snp.bg
chr1 0 1 A/G
chr1 5 6 C/T

bedtools unionbedg -i 1.snp.bg 2.snp.bg 3.snp.bg -filler -/-
chr1 0 1 A/G C/C A/G
```

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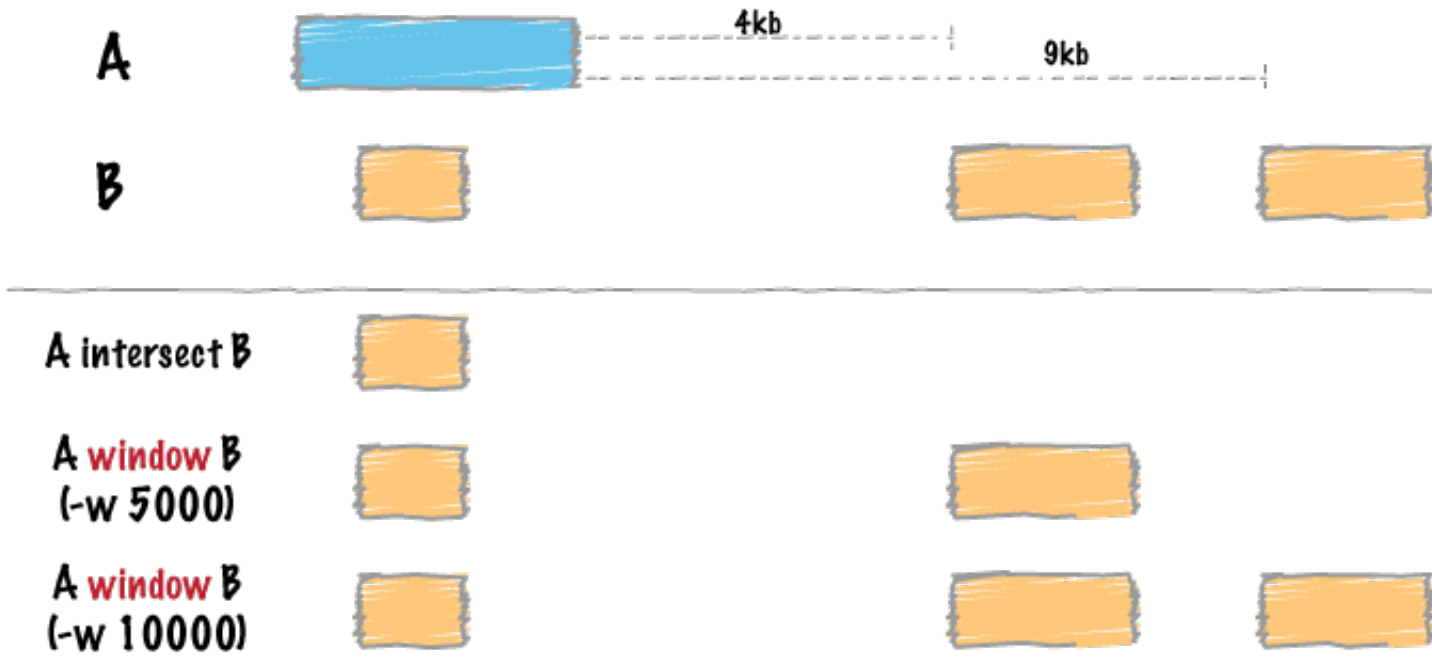
```
chr1 5 6 C/T -/- C/T
chr1 7 8 -/- T/T -/-
```

window



Similar to `bedtools intersect`, `window` searches for overlapping features in A and B. However, `window` adds a specified number (1000, by default) of base pairs upstream and downstream of each feature in A. In effect, this allows features in B that are "near" features in A to be detected.

Usage and option summary

Usage:

```
bedtools window [OPTIONS] [-a|-abam] -b <BED/GFF/VCF>
```

(or):

```
bedtools window [OPTIONS] [-a|-abam] -b <BED/GFF/VCF>
```

Option	Description
-abam	BAM file A. Each BAM alignment in A is compared to B in search of overlaps. Use "stdin" if passing A with a UNIX pipe: For example: samtools view -b <BAM> | bedtools window -abam stdin -b genes.bed
-ubam	Write uncompressed BAM output. The default is write compressed BAM output.
-bed	When using BAM input (-abam), write output as BED. The default is to write output in BAM when using -abam. For example: bedtools window -abam reads.bam -b genes.bed -bed
-w	Base pairs added upstream and downstream of each entry in A when searching for overlaps in B. *Default is 1000 bp*.
-l	Base pairs added upstream (left of) of each entry in A when searching for overlaps in B. *Allows one to create asymmetrical "windows". Default is 1000bp*.
-r	Base pairs added downstream (right of) of each entry in A when searching for overlaps in B. *Allows one to create asymmetrical "windows". Default is 1000bp*.
-sw	Define -l and -r based on strand. For example if used, -l 500 for a negative-stranded feature will add 500 bp downstream. *By default, this is disabled*.
-sm	Only report hits in B that overlap A on the same strand. *By default, overlaps are reported without respect to strand*.
-Sm	Only report hits in B that overlap A on the opposite strand. *By default, overlaps are reported without respect to strand*.
-u	Write original A entry once if any overlaps found in B. In other words, just report the fact at least one overlap was found in B.
-c	For each entry in A, report the number of hits in B while restricting to -w, -l, and -r. Reports 0 for A entries that have no overlap with B.
-v	Only report those entries in A that have *no overlaps* with B.
-header	Print the header from the A file prior to results.

Default behavior

By default, `bedtools window` adds 1000 bp upstream and downstream of each A feature and searches for features in B that overlap this "window". If an overlap is found in B, both the *original* A feature and the *original* B feature are reported.

```
$ cat A.bed
chr1  100  200

$ cat B.bed
chr1  500  1000
chr1  1300 2000

$ bedtools window -a A.bed -b B.bed
chr1  100  200  chr1  500  1000
```

`-w` Defining a custom window size

Instead of using the default window size of 1000bp, one can define a custom, *symmetric* window around each feature in A using the **-w** option. One should specify the window size in base pairs. For example, a window of 5kb should be defined as `-w 5000`.

For example (note that in contrast to the default behavior, the second B entry is reported):

```
$ cat A.bed
chr1  100  200

$ cat B.bed
chr1  500  1000
chr1  1300 2000

$ bedtools window -a A.bed -b B.bed -w 5000
chr1  100  200  chr1  500   1000
chr1  100  200  chr1  1300  2000
```

`-l and -r` Defining *asymmetric* windows

One can also define asymmetric windows where a differing number of bases are added upstream and downstream of each feature using the `-l` (upstream) and `-r` (downstream)** options.

Note: By default, the `-l` and `-r` options ignore strand. If you want to define *upstream* and *downstream* based on strand, use the `-sw` option (below) with the `-l` and `-r` options.

For example (note the difference between -l 200 and -l 300):

```
$ cat A.bed
chr1  1000  2000

$ cat B.bed
chr1  500   800
chr1  10000 20000

$ bedtools window -a A.bed -b B.bed -l 200 -r 20000
chr1  1000   2000  chr1  10000  20000

$ bedtools window -a A.bed -b B.bed -l 300 -r 20000
chr1  1000   2000  chr1  500    800
chr1  1000   2000  chr1  10000  20000
```

`-sw` Defining asymmetric windows based on strand

Especially when dealing with gene annotations or RNA-seq experiments, you may want to define asymmetric windows based on "strand". For example, you may want to screen for overlaps that occur within 5000 bp upstream of a gene (e.g. a promoter region) while screening only 1000 bp downstream of the gene. By enabling the `-sw` ("stranded" windows) option, the windows are added upstream or downstream according to strand. For example, imagine one specifies `-l 5000`, `-r 1000` as well as the `-sw` option. In this case, forward stranded ("+") features will screen 5000 bp to the *left* (that is, *lower* genomic coordinates) and 1000 bp to the *right* (that is, *higher* genomic coordinates). By contrast, reverse stranded ("-") features will screen 5000 bp to the *right* (that is, *higher* genomic coordinates) and 1000 bp to the *left* (that is, *lower* genomic coordinates).

For example (note the difference between `-l 200` and `-l 300`):

```
$ cat A.bed
chr1  10000  20000  A.forward  1  +
chr1  10000  20000  A.reverse  1  -
```

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```
$ cat B.bed
chr1  1000   8000   B1
chr1  24000  32000  B2

$ bedtools window -a A.bed -b B.bed -l 5000 -r 1000 -sw
chr1  10000  20000  A.forward  1  +  chr1  1000   8000   B1
chr1  10000  20000  A.reverse  1  -  chr1  24000  32000  B2
```

`-sm` Enforcing matches with the *same* "strandedness"

This option behaves the same as the `-s` option for `bedtools intersect` while scanning for overlaps within the "window" surrounding A. That is, overlaps in B will only be included if the B interval is on the *same* strand as the A interval.

`-Sm` Enforcing matches with the *opposite* "strandedness"

This option behaves the same as the `-S` option for `bedtools intersect` while scanning for overlaps within the "window" surrounding A. That is, overlaps in B will only be included if the B interval is on the *opposite* strand as the A interval.

`-u` Reporting the presence/absence of at least one overlapping feature

This option behaves the same as for `bedtools intersect`. That is, even if multiple overlaps exist, each A interval will only be reported once.

`-c` Reporting the number of overlapping features

This option behaves the same as for `bedtools intersect`. That is, it will report the *count* of intervals in B that overlap each A interval.

`-v` Reporting the absence of any overlapping features

This option behaves the same as for `bedtools intersect`. That is, it will only report those intervals in A that have have *zero* overlaps in B.

`-header` Print the header for the A file before reporting results.

By default, if your A file has a header, it is ignored when reporting results. This option will instead tell bedtools to first print the header for the A file prior to reporting results.

4.7 Example usage

Below are several examples of basic bedtools usage. Example BED files are provided in the /data directory of the bedtools distribution.

4.7.1 bedtools intersect

Report the base-pair overlap between sequence alignments and genes.

```
bedtools intersect -a reads.bed -b genes.bed
```

Report whether each alignment overlaps one or more genes. If not, the alignment is not reported.

```
bedtools intersect -a reads.bed -b genes.bed -u
```

Report those alignments that overlap NO genes. Like "grep -v"

```
bedtools intersect -a reads.bed -b genes.bed -v
```

Report the number of genes that each alignment overlaps.

```
bedtools intersect -a reads.bed -b genes.bed -c
```

Report the entire, original alignment entry for each overlap with a gene.

```
bedtools intersect -a reads.bed -b genes.bed -wa
```

Report the entire, original gene entry for each overlap with a gene.

```
bedtools intersect -a reads.bed -b genes.bed -wb
```

Report the entire, original alignment and gene entries for each overlap.

```
bedtools intersect -a reads.bed -b genes.bed -wa -wb
```

Only report an overlap with a repeat if it spans at least 50% of the exon.

```
bedtools intersect -a exons.bed -b repeatMasker.bed -f 0.50
```

Only report an overlap if comprises 50% of the structural variant and 50% of the segmental duplication. Thus, it is reciprocally at least a 50% overlap.

```
bedtools intersect -a SV.bed -b segmentalDups.bed -f 0.50 -r
```

Read BED A from stdin. For example, find genes that overlap LINEs but not SINEs.

```
bedtools intersect -a genes.bed -b LINES.bed | intersectBed -a stdin -b SINEs.bed -v
```

Retain only single-end BAM alignments that overlap exons.

```
bedtools intersect -abam reads.bam -b exons.bed > reads.touchingExons.bam
```

Retain only single-end BAM alignments that do not overlap simple sequence repeats.

```
bedtools intersect -abam reads.bam -b SSRs.bed -v > reads.noSSRs.bam
```

4.7.2 bedtools bamtobed

Convert BAM alignments to BED format.

```
bedtools bamtobed -i reads.bam > reads.bed
```

Convert BAM alignments to BED format using the BAM edit distance (NM) as the BED "score".

```
bedtools bamtobed -i reads.bam -ed > reads.bed
```

Convert BAM alignments to BEDPE format.

```
bedtools bamtobed -i reads.bam -bedpe > reads.bedpe
```

4.7.3 bedtools window

Report all genes that are within 10000 bp upstream or downstream of CNVs.

```
bedtools window -a CNVs.bed -b genes.bed -w 10000
```

Report all genes that are within 10000 bp upstream or 5000 bp downstream of CNVs.

```
bedtools window -a CNVs.bed -b genes.bed -l 10000 -r 5000
```

Report all SNPs that are within 5000 bp upstream or 1000 bp downstream of genes. Define upstream and downstream based on strand.

```
bedtools window -a genes.bed -b snps.bed -l 5000 -r 1000 -sw
```

4.7.4 bedtools closest

Note: By default, if there is a tie for closest, all ties will be reported. **closestBed** allows overlapping features to be the closest.

Find the closest ALU to each gene.

```
bedtools closest -a genes.bed -b ALUs.bed
```

Find the closest ALU to each gene, choosing the first ALU in the file if there is a tie.

```
bedtools closest -a genes.bed -b ALUs.bed -t first
```

Find the closest ALU to each gene, choosing the last ALU in the file if there is a tie.

```
bedtools closest -a genes.bed -b ALUs.bed -t last
```

4.7.5 bedtools subtract

Note: If a feature in A is entirely "spanned" by any feature in B, it will not be reported.

Remove introns from gene features. Exons will (should) be reported.

```
bedtools subtract -a genes.bed -b introns.bed
```

4.7.6 bedtools merge

Note: `merge` requires that the input is sorted by chromosome and then by start coordinate. For example, for BED files, one would first sort the input as follows: `sort -k1,1 -k2,2n input.bed > input.sorted.bed`

Merge overlapping repetitive elements into a single entry.

```
bedtools merge -i repeatMasker.bed
```

Merge overlapping repetitive elements into a single entry, returning the number of entries merged.

```
bedtools merge -i repeatMasker.bed -n
```

Merge nearby (within 1000 bp) repetitive elements into a single entry.

```
bedtools merge -i repeatMasker.bed -d 1000
```

4.7.7 bedtools coverage

Compute the coverage of aligned sequences on 10 kilobase "windows" spanning the genome.

```
bedtools coverage -a reads.bed -b windows10kb.bed | head
chr1 0     10000 0  10000 0.00
chr1 10001 20000 33 10000 0.21
chr1 20001 30000 42 10000 0.29
chr1 30001 40000 71 10000 0.36
```

Compute the coverage of aligned sequences on 10 kilobase "windows" spanning the genome and created a BEDGRAPH of the number of aligned reads in each window for display on the UCSC browser.

```
bedtools coverage -a reads.bed -b windows10kb.bed | cut -f 1-4 > windows10kb.cov.bedg
```

Compute the coverage of aligned sequences on 10 kilobase "windows" spanning the genome and created a BEDGRAPH of the fraction of each window covered by at least one aligned read for display on the UCSC browser.

```
bedtools coverage -a reads.bed -b windows10kb.bed | \
   awk '{OFS="\t"; print $1,$2,$3,$6}' \
   > windows10kb.pctcov.bedg
```

4.7.8 bedtools complement

Report all intervals in the human genome that are not covered by repetitive elements.

```
bedtools complement -i repeatMasker.bed -g hg18.genome
```

4.7.9 bedtools shuffle

Randomly place all discovered variants in the genome. However, prevent them from being placed in know genome gaps.

```
bedtools shuffle -i variants.bed -g hg18.genome -excl genome_gaps.bed
```

Randomly place all discovered variants in the genome. However, prevent them from being placed in know genome gaps and require that the variants be randomly placed on the same chromosome.

```
bedtools shuffle -i variants.bed -g hg18.genome -excl genome_gaps.bed -chrom
```

4.8 Advanced usage

4.8.1 Mask all regions in a genome except for targeted capture regions.

Step 1. Add 500 bp up and downstream of each probe

```
bedtools slop -i probes.bed -g hg18.genome -b 500 > probes.500bp.bed
```

NB genome is two column chromosome size list - i.e. https://genome.ucsc.edu/goldenpath/help/hg18.chrom.sizes

Step 2. Get a BED file of all regions not covered by the probes (+500 bp up/down)

```
bedtools complement -i probes.500bp.bed -g hg18.genome > probes.500bp.complement.bed
```

Step 3. Create a masked genome where all bases are masked except for the probes +500bp

```
bedtools maskfasta -fi hg18.fa -bed probes.500bp.complement.bed \
-fo hg18.probecomplement.masked.fa
```

4.8.2 Screening for novel SNPs.

Find all SNPs that are not in dbSnp and not in the latest 1000 genomes calls

```
bedtools intersect -a snp.calls.bed -b dbSnp.bed -v | \
bedtools intersect -a - -b 1KG.bed -v | \
> snp.calls.novel.bed
```

4.8.3 Computing the coverage of features that align entirely within an interval.

By default, bedtools `coverage` counts any feature in A that overlaps B by >= 1 bp. If you want to require that a feature align entirely within B for it to be counted, you can first use intersectBed with the "-f 1.0" option.

```
bedtools intersect -a features.bed -b windows.bed -f 1.0 | \
bedtools coverage -a windows.bed -b - \
> windows.bed.coverage
```

4.8.4 Computing the coverage of BAM alignments on exons.

One can combine `samtools` with `bedtools` to compute coverage directly from the BAM data by using `bamtobed`.

```
bedtools bamtobed -i reads.bam | \
bedtools coverage -a exons.bed -b - \
> exons.bed.coverage
```

Take it a step further and require that coverage be from properly-paired reads.

```
samtools view -uf 0x2 reads.bam | \
coverageBed -abam - -b exons.bed \
> exons.bed.proper.coverage
```

4.8.5 Computing coverage separately for each strand.

Use grep to only look at forward strand features (i.e. those that end in "+").

```
bedtools bamtobed -i reads.bam | \
grep \+$  | \
bedtools coverage -a - -b genes.bed \
> genes.bed.forward.coverage
```

Use grep to only look at reverse strand features (i.e. those that end in "-").

```
bedtools bamtobed -i reads.bam | \
grep \-$ | \
bedtools coverage -a - -b genes.bed \
> genes.bed.reverse.coverage
```

4.9 Tips and Tricks

4.9.1 The `-sorted` option

4.10 FAQ

4.10.1 Installation issues

Why am I getting all of these zlib errors?

On certain operating systems (especially free Linux distributions) the complete zlib libraries are not installed. Bedtools depends upon zlib in order to decompress gzipped files.

```
- Building main bedtools binary.
obj/gzstream.o: In function gzstreambuf::open(char const*, int):
gzstream.C:(.text+0x2a5): undefined reference to gzopen64'
collect2: ld returned 1 exit status
make: *** [all] Error 1
```

If you see an error such as the above, it suggests you need to install the `zlib` and `zlib1g-dev` libraries. This is typically straightforward using package managers. For example, on Debian/Ubuntu this would be:

```
apt-get install zlib
apt-get install zlib1g-dev
```

and on Fedora/Centos this would be:

```
yum install zlib
yum install zlib1g-dev
```

Compiling with a specific zlib library

If you need to override the location of the zlib library used to compile bedtools, you can run *make* and specify the *LIBS* argument. For example:

```
make LIBS='/PATH/TO/ZLIB/lib/libz.a'
```

4.10.2 General questions

How do I know what version of bedtools I am using?

Use the –version option.

```
$ bedtools --version
bedtools v2.17.0
```

How do I bring up the help/usage menu?

To receive a high level list of available tools in bedtools, use `-h:

```
$ bedtools -h
bedtools: flexible tools for genome arithmetic and DNA sequence analysis.
usage:    bedtools <subcommand> [options]

The bedtools sub-commands include:

[ Genome arithmetic ]
    intersect     Find overlapping intervals in various ways.
    window        Find overlapping intervals within a window around an interval.
    closest       Find the closest, potentially non-overlapping interval.
    coverage      Compute the coverage over defined intervals.
    map           Apply a function to a column for each overlapping interval.
    genomecov     Compute the coverage over an entire genome.
    merge         Combine overlapping/nearby intervals into a single interval.
    cluster       Cluster (but don't merge) overlapping/nearby intervals.
    complement    Extract intervals _not_ represented by an interval file.
...
```

To display the help for a specific tool (e.g., `bedtools shuffle`), use:

```
$ bedtools merge -h

Tool:    bedtools merge (aka mergeBed)
Version: v2.17.0
Summary: Merges overlapping BED/GFF/VCF entries into a single interval.

Usage:   bedtools merge [OPTIONS] -i <bed/gff/vcf>

```

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```
Options:
    -s      Force strandedness.  That is, only merge features
            that are the same strand.
            - By default, merging is done without respect to strand.

    -n      Report the number of BED entries that were merged.
            - Note: "1" is reported if no merging occurred.
```

4.10.3 Issues with output

I *know* there are overlaps, but none are reported. What might be wrong?

There are two common causes of this problem. The first cause is non-obvious differences in the way chromosomes are named in files being compared. For example, "1" is not the same as "chr1" just as " chr1" is not the same as "chr1". Secondly, users often copy files from a Windows machine to a UNIX machine. This causes issues because Windows uses two bytes to represent the end of a line (`\r\n`) whereas the UNIX convention uses a single byte (`\n`). If your files don't conform to the UNIX convention, you will have problems. One can convert files from Windows to UNIX with the following command:

```
perl -i -p -e 's/\r\n/\n/g;' file.windows > file.unix
```

4.10.4 Installation issues

Bedtools compilation fails with errors related to zlib. How do I fix this?

Some systems, especially Ubuntu, do not come pre-installed with up to date versions of the zlib compression utilities that tools such as *bedtools* and *samtools* depend upon. This can cause compilation errors when you try to compile *bedtools*. Errors include:

```
../utils//gzstream/gzstream.h:50: error: 'gzFile' does not name a type
```

or

```
fatal error: zlib.h: No such file or directory
```

This indicates that you need to install the zlib libraries on your system, which turns out to not be too difficult through the use of package installers. For example, on Ubuntu, you'd want to run:

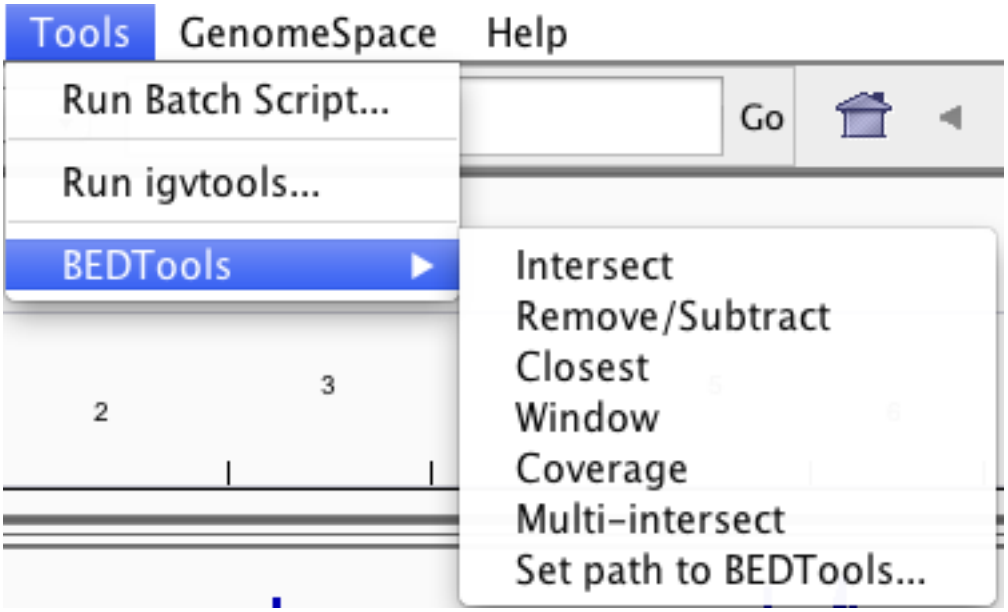
```
sudo apt-get install zlib1g-dev
sudo apt-get install zlib
```

4.11 Related software

Bedtools has been used as an engine behind other genomics software and has been integrated into widely used tools such as Galaxy and IGV. Below is a likely incomplete list. If you know of others, please let us know, or better yet, edit the document on GitHub and send us a pull request. You can do this by clicking on the "Edit and improve this document" link in the lower lefthand corner.

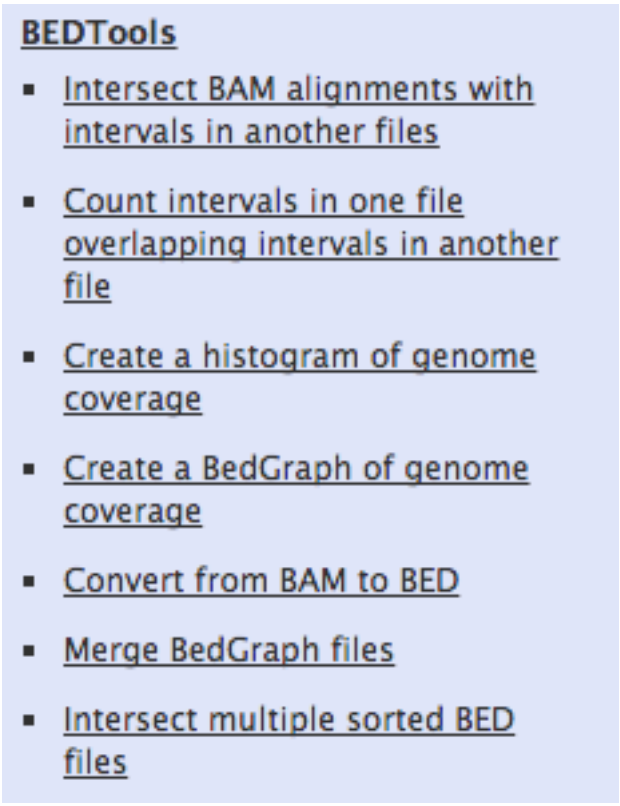
4.11.1 IGV

Bedtools is now integrated into the IGV genome viewer as of IGV version 2.2. We are actively working withe IGV development team to improve and expand this integration. See here and here for details.



4.11.2 Galaxy

Galaxy has its own tools for working with genomic intervals under the "Operate on Genomic Intervals" section. A subset of complementary Bedtools utilities have also been made available on Galaxy in an effort to provide functionality that isn't available with the native Galaxy tools.



4.11.3 Pybedtools

Pybedtools is a really fantastic Python library that wraps (and extends upon) the bedtools utilities and exposes them for easy use and new tool development using Python. Pybedtools is actively maintained by Ryan Dale.

4.11.4 MISO

MISO is "a probabilistic framework that quantitates the expression level of alternatively spliced genes from RNA-Seq data, and identifies differentially regulated isoforms or exons across samples." A subset of the functionality in MISO depends upon `bedtools`. MISO is developed by Yarden Katz.

4.11.5 RetroSeq

RetroSeq is "a tool for discovery and genotyping of transposable element variants (TEVs) (also known as mobile element insertions) from next-gen sequencing reads aligned to a reference genome in BAM format". RetroSeq is developed by Thomas Keane. Source code can be obtained on GitHub.

4.11.6 Intersphinx documentation

BEDTools documentation pages are available via Intersphinx (http://sphinx-doc.org/ext/intersphinx.html). To enable this, add the following to conf.py in a Sphinx project:

intersphinx_mapping = {'bedtools': ('http://bedtools.readthedocs.org/en/latest/', None)}

BEDtools documentation links can then be generated with e.g.:

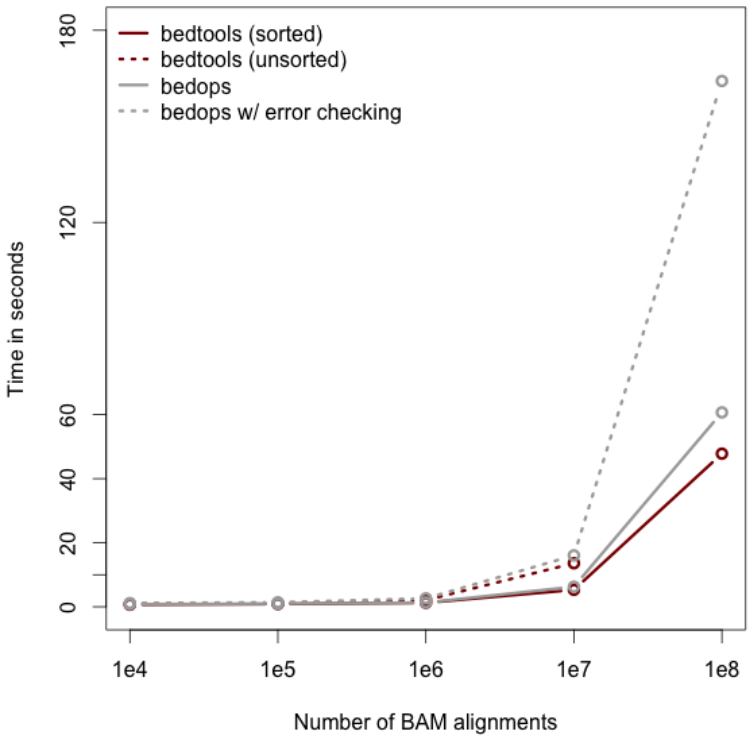
BEDTools:map

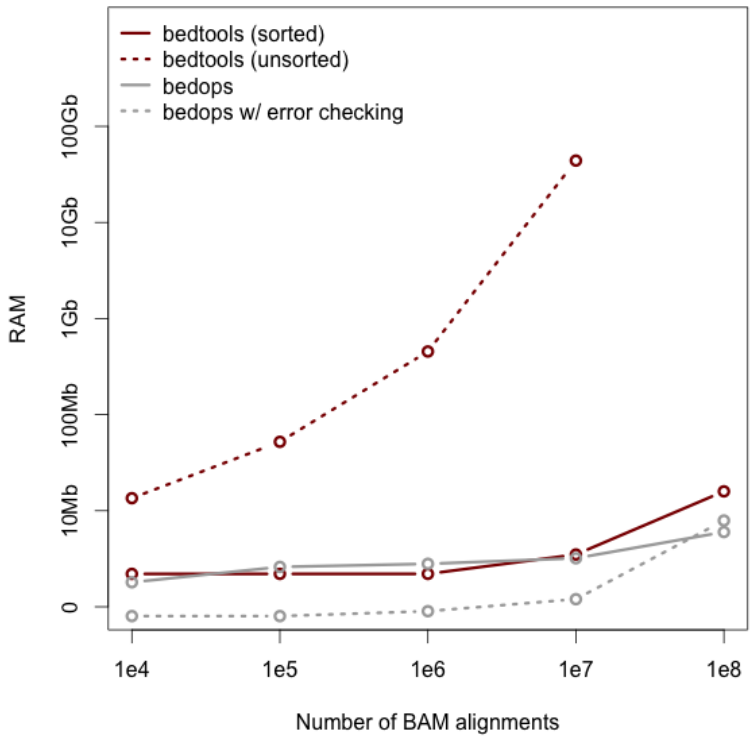
CHAPTER 5

Performance

As of version 2.18, `bedtools` is substantially more scalable thanks to improvements we have made in the algorithm used to process datasets that are pre-sorted by chromosome and start position. As you can see in the plots below, the speed and memory consumption scale nicely with sorted data as compared to the poor scaling for unsorted data. The current version of bedtools intersect is as fast as (or slightly faster) than the `bedops` package's `bedmap` which uses a similar algorithm for sorted data. The plots below represent counting the number of intersecting alignments from exome capture BAM files against CCDS exons. The alignments have been converted to BED to facilitate comparisons to `bedops`. We compare to the bedmap `--ec` option because similar error checking is enforced by `bedtools`.

Note: bedtools could not complete when using 100 million alignments and the R-Tree algorithm used for unsorted data owing to a lack of memory.


bedtools (sorted)
bedtools (unsorted)
bedops
bedops w/ error checking
Time in seconds
0
20
40
60
120
180
1e4
1e5
1e6
1e7
1e8
Number of BAM alignments



Commands used:

```
# bedtools sorted
$ bedtools intersect \
            -a ccds.exons.bed -b aln.bam.bed \
            -c \
            -sorted

# bedtools unsorted
$ bedtools intersect \
            -a ccds.exons.bed -b aln.bam.bed \
            -c

# bedmap (without error checking)
$ bedmap --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed

# bedmap (no error checking)
$ bedmap --ec --echo --count --bp-ovr 1 \
         ccds.exons.bed aln.bam.bed
```

CHAPTER 6

Brief example

Let's imagine you have a BED file of ChiP-seq peaks from two different experiments. You want to identify peaks that were observed in *both* experiments (requiring 50% reciprocal overlap) and for those peaks, you want to find to find the closest, non-overlapping gene. Such an analysis could be conducted with two, relatively simple bedtools commands.

```
# intersect the peaks from both experiments.
# -f 0.50 combined with -r requires 50% reciprocal overlap between the
# peaks from each experiment.
$ bedtools intersect -a exp1.bed -b exp2.bed -f 0.50 -r > both.bed

# find the closest, non-overlapping gene for each interval where
# both experiments had a peak
# -io ignores overlapping intervals and returns only the closest,
# non-overlapping interval (in this case, genes)
$ bedtools closest -a both.bed -b genes.bed -io > both.nearest.genes.txt
```

CHAPTER 7

License

bedtools is freely available under a GNU Public License (Version 2).

CHAPTER 8

Acknowledgments

To do.

CHAPTER 9

Mailing list

If you have questions, requests, or bugs to report, please email the bedtools mailing list