# pybedtools Documentation

Release 0.2.3dev

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**CHAPTER** 

**ONE** 

## **OVERVIEW**

pybedtools is a Python wrapper for Aaron Quinlan's BEDtools and is designed to leverage the "genome algebra" power of BEDtools from within Python scripts.

This documentation is written assuming you know how to use BEDTools and Python.

See full online documentation, including installation instructions, at http://daler.github.com/pybedtools.

**Note:** pybedtools is still very much in progress. Please keep that in mind when assesing whether to use this package in production code.

## **CONTENTS:**

## 2.1 Installation

To use pybedtools you'll need the latest version of the package and the latest version of BEDTools.

## 2.1.1 Installing pybedtools

To install the latest version of pybedtools you have 2 options:

#### **Option 1:** install from source

• go to http://github.com/daler/pybedtools



- click the Downloads link (
- choose either a .tar.gz or a .zip file, whatever you're comfortable with
- · unzip into a temporary directory
- from the command line, run:

```
python setup.py install
```

(you may need admin rights to do this)

**Option 2:** use pip to automatically download from the Python Package Index:

```
sudo pip install --upgrade pybedtools
```

## 2.1.2 Installing BEDTools

To install BEDTools,

- follow the instructions at https://github.com/arq5x/bedtools to install
- make sure all its programs are on your path

## 2.2 Three brief examples

Here are three examples to show typical usage of pybedtools. More info can be found in the docstrings of pybedtools methods and in the *Tutorial*. Before running the examples, you need to import pybedtools:

```
>>> from pybedtools import bedtool, cleanup
```

After running the examples, clean up any intermediate temporary files with:

```
>>> cleanup()
```

## 2.2.1 Example 1: Save a BED file of intersections, with track line

This example saves a new BED file of intersections between a . bed and b . bed, adding a track line to the output:

```
>>> a = bedtool('a.bed')
>>> a.intersect('b.bed').saveas('a-and-b.bed', trackline="track name='a and b' color=128,0,0")
```

## 2.2.2 Example 2: Intersections for a 3-way Venn diagram

This example gets values for a 3-way Venn diagram of overlaps. This demonstrates operator overloading of bedtool objects:

```
>>> # set up 3 different bedtools
>>> a = bedtool('a.bed')
>>> b = bedtool('b.bed')
>>> c = bedtool('c.bed')

>>> (a-b-c).count() # unique to a
>>> (a+b-c).count() # in a and b, not c
>>> (a+b+c).count() # common to all
>>> # ... and so on, for all the combinations.
```

## 2.2.3 Example 3: Flanking sequences

This example gets the genomic sequences of the 100 bp on either side of features.

The bedtool.slop() method automatically downloads the chromSizes table from UCSC for the dm3 genome, but you can pass your own file using the standard BEDTools slop argument of g. Note that this example assumes you have a local copy of the entire dm3 genome saved as dm3.fa.

```
>>> # set up bedtool
>>> mybed = bedtool('in.bed')

>>> # add 100 bp of "slop" to either side. genome='dm3' tells
>>> # the slop() method to download the dm3 chromSizes table from
>>> # UCSC.
>>> extended_by_100 = mybed.slop(genome='dm3', l=100, r=100)

>>> # Delete the middle of the now-200-bp-bigger features so
>>> # all we're left with is the flanking region
>>> flanking_features = extended_by_100.subtract('in.bed')
>>> # Assuming you have the dm3 genome on disk as 'dm3.fa', save the
```

```
>>> # sequences as a new file 'flanking.fa'
>>> seqs = flanking_features.sequence(fi='dm3.fa').save_seqs('flanking.fa')
>>> # We could have done this all in one line
>>> # (this demonstrates "chaining" of bedtool objects)
>>> bedtool('in.bed').slop(genome='dm3',l=100,r=100).subtract('in.bed').flanking_features.sequence(fine)
```

For more, continue on to the *Tutorial*.

## 2.3 Tutorial

This tutorial assumes that

- 1. You know how to use BEDTools (if not, check out the BEDTools documentation)
- 2. You know how to use Python (if not, check out some tutorials like Learn Python the Hard Way)

### 2.3.1 A brief note on conventions

Throughout this documentation I've tried to use consistent typography, as follows:

- Python variables and arguments are shown in italics: *s=True*
- Files look like this: filename.bed
- Methods, which are often linked to documentation look like this: bedtool.merge().
- BEDTools programs look like this: intersectBed.
- Arguments that are passed to BEDTools programs, as if you were on the command line, look like this: -d.
- The ">>>" in the examples below indicates a Python interpreter prompt and means to type the code into an interactive Python interpreter like IPython (don't type the >>>)

Onward!

#### 2.3.2 Create a bedtool

First, follow the *Installation* instructions if you haven't already done so to install both BEDTools and pybedtools.

Then import the pybedtools module:

```
>>> import pybedtools
```

Then set up a bedtool instance using a BED format file. This can be a file that you already have, or one of the example files as shown below. Some methods currently only work for BED files – see *Limitations* for more info on this.

For this tutorial, we'll use some example files that come with pybedtools. We can get the filename for the example files using the pybedtools.example\_files() function:

```
>>> # get an example filename to use
>>> bed_filename_a = pybedtools.example_bed_fn('a.bed')
```

The filename will depend on where you have installed pybedtools. Once you have a filename, creating a bedtool object is easy:

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```
>>> # create a new bedtool using that filename
>>> a = pybedtools.bedtool(bed_filename_a)
```

Set up a second one so we can do intersections and subtractions – this time, let's make a new bedtool all in one line:

```
>>> # create another bedtool to play around with
>>> b = pybedtools.bedtool(pybedtools.example_bed_fn('b.bed'))
```

See *Creating a bedtool* for more information, including convenience functions for working with example bed files and making bedtool objects directly from strings.

#### 2.3.3 Intersections

Here's how to intersect a with b:

```
>>> a_and_b = a.intersect(b)
```

 $a\_and\_b$  is a new bedtool instance. It now points to a temp file on disk, which is stored in the attribute  $a\_and\_b.fn$ ; this temp file contains the intersection of a and b.

We can either print the new bedtool (which will show ALL features – use with caution if you have huge files!) or use the bedtool.head() method to get the first N lines (10 by default). Here's what a, b, and a\_and\_b look like:

```
>>> a.head()
chr1 1 100 feature1
                           0
chr1
       100 200 feature2
                           0
chr1 150 500 feature3
                           0
       900 950 feature4
>>> b.head()
chr1 155 200 feature5
                           0
chr1
       800 901 feature6
                          0
>>> a_and_b.head()
     155 200 feature2
                           0
chr1
chr1
       155 200 feature3
                           0
       900 901 feature4
```

The bedtool.intersect() method simply wraps the BEDTools program intersectBed. This means that we can pass bedtool.intersect() any arguments that intersectBed accepts. For example, if we want to use the intersectBed switch -u (which acts as a True/False switch to indicate that we want to see the features in a that overlapped something in b), then we can use the keyword argument u=True, like this:

a\_with\_b is another, different temp file whose name is stored in a\_with\_b.fn. You can read more about the use of temp files in *Principle 1: Temporary files are created automatically*. More on arguments that you can pass to bedtool objects in a moment, but first, some info about saving files.

## 2.3.4 Saving the results

If you want to save the results as a meaningful filename for later use, use the bedtool.saveas() method. This also lets you optionally specify a trackline for directly uploading to the UCSC Genome Browser, instead of opening up the files afterward and manually adding a trackline:

```
>>> a_with_b.saveas('intersection-of-a-and-b.bed', trackline='track name="a and b"')
<bedtool (intersection-of-a-and-b.bed)>
```

Note that the bedtool.saveas() method returns a new bedtool object which points to the newly created file on disk. This allows you to insert a bedtool.saveas() call in the middle of a chain of commands (described in another section below).

## 2.3.5 Default arguments

Recall that we passed the u=True argument to a .intersect ():

```
>>> a_with_b = a.intersect(b, u=True)
```

While we're on the subject of arguments, note that we didn't have to specify -a or -b arguments, like you would need if calling intersectBed from the command line. That's because bedtool objects make some assumptions for convenience.

We could have supplied the arguments a=a.fn and b=b.fn. But since we're calling a method on a, pybedtools assumes that the file a points to (specifically, a.fn) is the one we want to use as input. So by default, we don't need to explicitly give the keyword argument a=a.fn because the a.intersect() method does so automatically.

We're also calling a method that takes a second bed file as input — other such methods include bedtool.subtract() and bedtool.closest(). In these cases, pybedtools assumes the first unnamed argument to these methods are the second file you want to operate on (and if you pass a bedtool, it'll automatically use the file in the fn attribute of that bedtool). So a.intersect(b) is just a more convenient form of a.intersect(a=a.fn, b=b.fn), which does the same thing.

OK, enough about arguments for now, but you can read more about them in *Principle 2: Names and arguments are as similar as possible to BEDTools*, *Principle 3: Sensible default args* and *Principal 4: Other arguments have no defaults*.

## 2.3.6 Chaining methods together (pipe)

One useful thing about bedtool methods is that they often return a new bedtool. In practice, this means that we can chain together multiple method calls all in one line, similar to piping on the command line.

```
>>> # Intersect and then merge all on one line, displaying the first
>>> # 10 lines of the results
>>> a.intersect(b, u=True).merge().head()
chr1    100 500
chr1    900 950
```

In general, methods that return bedtool objects have the following text in their docstring to indicate this:

```
.. note::

This method returns a new bedtool instance
```

A rule of thumb is that all methods that wrap BEDTools programs return bedtool objects, so you can chain these together. Other pybedtools-unique methods return bedtool objects too, just check the docs (according to *Principle* 6: *Check the help*). For example, as we saw in one of the examples above, the bedtool.saveas() method returns

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a bedtool object. That means we can sprinkle those commands within the example above to save the intermediate steps as meaningful filenames for later use:

```
>>> a.intersect(b, u=True).saveas('a-with-b.bed').merge().saveas('a-with-b-merged.bed')
<bedtool (a-with-b-merged.bed)>
```

Now we have new files in the current directory called a-with-b.bed and a-with-b-merged.bed.

I found myself doing intersections so much that I thought it would be useful to overload the + and – operators to do intersections. To illustrate, these two example commands do the same thing:

```
>>> result_1 = a.intersect(b, u=True)
>>> result_2 = a+b
>>> # To test equality, convert to strings
>>> str(result_1) == str(result_2)
True
```

And the - operator assumes intersectBed's -v option:

```
>>> result_1 = a.intersect(b, v=True)
>>> result_2 = a-b

>>> # To test equality, convert to strings
>>> str(result_1) == str(result_2)
True
```

If you want to operating on the resulting bedtool that is returned by an addition or subtraction, you'll need to wrap the operation in parentheses:

```
>>> merged = (a+b).merge()
```

You can learn more about chaining in *Principle 5: Chaining together commands*.

## 2.3.7 Methods specific to pybedtools

In no particular order, here are some other useful things that bedtool objects can do.

#### Counting

We can easily count features in bedtool objects:

```
>>> a.count()
4
>>> a.intersect(b, u=True).merge().count()
2
```

#### Lengths of features

We can get the lengths of features, which is useful for things like getting a histogram of binding site sizes after running a peak-calling algorithm on ChIP-seq data:

```
chr1 900 950 feature4 0 + >>> a.lengths()
[99, 100, 350, 50]
```

#### Creating genome files ('chromSizes')

Some BEDTools programs require a "chromSizes" file to prevent out-of-range coordinates. bedtool objects have a convenience method to get this file for you and return the resulting filename:

```
>>> # Download Drosophila melanogaster chromSizes table from UCSC
>>> chromsizes = a.get_genome('hg19', fn='hg19.genome')

>>> # print first few lines of that file
>>> f = open(chromsizes)
>>> for i in range(5):
... print f.readline(),
chr1 249250621
chr2 243199373
chr3 198022430
chr4 191154276
chr5 180915260
```

#### **Feature centers**

We can get the midpoints of features, with bedtool.feature\_centers(), which is useful for things like extracting out the center N bp of features for de novo motif detection.

```
>>> a.feature_centers(n=50).saveas('middle-100-bp.bed')
<bedtool (middle-100-bp.bed)>
```

#### **Filtering**

Only get features of a certain size:

```
>>> # only features that are smaller than 60 bp
>>> a.size_filter(min=0, max=60).head()
chr1 900 950 feature4 0.0 +
```

#### Working with counted intersections

There are several methods that help you deal with counted intersections – the files that are returned when you use the c=True kwarg with bedtool.intersect(). First, do an intersection:

```
>>> print a.intersect(b, c=True)
chr1    1    100 feature1    0    +    0
chr1    100 200 feature2    0    +    1
chr1    150 500 feature3    0    -    1
chr1    900 950 feature4    0    +    1
```

You can retrieve these counts later using the bedtool.counts() method:

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```
>>> result = a.intersect(b, c=True)
>>> print result.counts()
[0, 1, 1, 1]
```

## 2.4 Topical documentation

#### 2.4.1 Release notes

#### 0.2.3dev

- Added history mechanism see *Using the history and tags*.
- · Added tagging mechanism

#### 2.4.2 Future work

The following is a list of items I plan to work on for future releases of pybedtools. Help and feedback are welcome!:

- Finish wrapping the rest of the BEDTools programs
- Better mechanism for handling temp files.
  - bedtool objects could keep track of all their 'parent' tempfiles, and as such would retain the history of their creation. done
  - indexing into the history of a bedtool would give you access to these previous files
  - could have a bedtool.delete\_history(), which would delete all the intermediate tempfiles on disk, cleaning up only for this particular bedtool
- Universal "interval" file support
  - Should have a parent UniversalInterval class and then have GFF, GTF, VCF, etc. etc. all subclassed from that
  - bedtool should auto-detect the interval file format
- Support for "derived" file types
  - currently the handling of output created by a.intersect(b, c=True) is special-cased, and a.intersect(b, wao=True) is unsupported by custom bedtool methods.
  - these should probably just be implemented as subclasses of the as-yet hypothetical UniversalInterval class.
- Support for groupBy
  - it would be nice to have support for filo
- · Randomization methods still under development
- Have wrappers use subprocess.Popen instead of os.system calls for better trapping of errors
- More universal wrapper perhaps as decorator?
- Full unit tests to bolster the tutorial doctests

## 2.4.3 Why use pybedtools?

pybedtools makes working with BEDTools from Python code easy.

Calling BEDTools from Python "by hand" gets awkward for things like geting the number of intersecting features between two bed files, for example:

```
>>> # The annoying way of calling BEDTools from Python...
>>> p1 = subprocess.Popen(['intersectBed','-a','a.bed','-b','b.bed','-u'], stdout=subprocess.PIPE)
>>> # then pipe it to wc -1 to get a line count
>>> p2 = subprocess.Popen(['wc','-1'], stdin=subprocess.PIPE)
>>> # finally, parse the results
>>> results = p2.communicate()[0]
>>> count = int(results.split()[-1])
```

If we wanted to get the number of features unique to a.bed, it would mean another 4 lines of this, with the only difference being the -v argument instead of -u for the intersectBed call.. For me, this got old quickly, hence the creation of pybedtools.

Here's how to do the same thing with pybedtools:

```
>>> from pybedtools import bedtool
>>> a = bedtool('a.bed')
>>> count = a.intersect('b.bed', u=True).count()
```

Behind the scenes, the pybedtools.bedtool class does something very similar to the subprocess example above, but in a more Python-friendly way.

Furthermore, for the specific case of intersections, the + and – operators have been overloaded, making many intersections extremely easy:

```
>>> a = bedtool('a.bed')
>>> b = bedtool('b.bed')
>>> c = bedtool('c.bed')

>>> (a+b).count() # number of features in a and b
>>> (a-b).count() # number of features in a not b
>>> (a+b+c).count() # number of features in a, b and c
```

The other BEDTools programs are wrapped as well, like bedtool.merge(), bedtool.slop(), and others.

In addition to wrapping the BEDtools programs, there are many additional bedtool methods provided in this module that you can use in your Python code.

#### 2.4.4 Limitations

There are some limitations you need to be aware of.

- pybedtools makes heavy use of temporary files. This makes it very convenient to work with, but if you are limited by disk space, you'll have to pay attention to this feature (see temp principle below for more info).
- bedtool methods that wrap BEDTools programs (bedtool.intersect(), bedtool.merge(), bedtool.subtract(), etc) will work on BAM, GFF, VCF, and everything that BEDTools supports. However, many pybedtools-specific methods (for example bedtool.lengths() or bedtool.size\_filter()) currently only work on BED files. I hope to add support for all interval files soon.

• Not all BEDTools programs are wrapped – this package is still a work in progress. I wrapped the ones I use most often and still need to wrap the others. The following table shows what's currently wrapped:

BEDTools program	bedtool method name
intersectBed	<pre>bedtool.intersect()</pre>
subtractBed	<pre>bedtool.subtract()</pre>
fastaFromBed	bedtool.sequence()
slopBed	bedtool.slop()
windowBed	bedtool.window()
closestBed	bedtool.closest()
shuffleBed	bedtool.shuffle()

## 2.4.5 Creating a bedtool

To create a bedtool, first you need to import the pybedtools module. For these examples, I'm assuming you have already done the following:

```
>>> import pybedtools
>>> from pybedtools import bedtool
```

Next, you need a BED file to work with. If you already have one, then great – move on to the next section. If not, pybedtools comes with some example bed files used for testing. You can take a look at the list of example files that ship with pybedtools with the list\_example\_beds() function:

```
>>> # list the example bed files
>>> pybedtools.list_example_beds()
['a.bed', 'b.bed']
```

Once you decide on a file to use, feed the your choice to the example\_bed\_fn() function to get the full path:

```
>>> # get the full path to an example bed file
>>> bedfn = pybedtools.example_bed_fn('a.bed')
```

The full path of *bedfn* will depend on your installation (this is similar to the data() function in R, if you're familiar with that).

Now that you have a filename – either one of the example files or your own, you create a new bedtool simply by pointing it to that filename:

```
>>> # create a new bedtool from the example bed file
>>> mybedtool = bedtool(bedfn)
```

Alternatively, you can construct BED files from scratch by using the from\_string keyword argument. However, all spaces will be converted to tabs using this method, so you'll have to be careful if you add "name" columns. This can be useful if you want to create *de novo* BED files on the fly:

Of course, you'll usually be using your own bed files that have some biological importance for your work that are saved in places convenient for you, for example:

```
>>> a = bedtool('/data/sample1/peaks.bed')
```

## 2.4.6 Design principles

#### Principle 1: Temporary files are created automatically

Let's illustrate some of the design principles behind pybedtools by merging features in a bed that are 100 bp or less apart (d=100) in a strand-specific way (s=True):

```
>>> from pybedtools import bedtool
>>> import pybedtools
>>> a = bedtool(pybedtools.example_bed_fn('a.bed'))
>>> merged_a = a.merge(d=100, s=True)
```

Now *merged\_a* is a bedtool instance that contains the results of the merge.

bedtool objects must always point to a file on disk. So in the example above,  $merged_a$  is a bedtool, but what file does it point to? You can always check the bedtool. fn attribute to find out:

```
>>> # what file does *merged_a* point to?
>>> merged_a.fn
'/tmp/pybedtools.MPPp5f.tmp'
```

Note that the specific filename will be different for you since it is a randomly chosen name (handled by Python's tempfile module). This shows one important aspect of pybedtools: every operation results in a new temporary file. Temporary files are stored in /tmp by default, and have the form /tmp/pybedtools.\*.tmp.

Future work on pybedtools will focus on streamlining the temp files, keeping only those that are needed. For now, when you are done using the pybedtools module, make sure to clean up all the temp files created with:

```
>>> # Deletes all tempfiles created this session.
>>> # Don't do this yet if you're following the tutorial!
>>> pybedtools.cleanup()
```

If you forget to do this, from the command line you can always do a:

```
rm /tmp/pybedtools.*.tmp
```

to clean everything up.

If you need to specify a different directory than that used by default by Python's tempdir module, then you can set it with:

```
>>> pybedtools.set_tempdir('/scratch')
```

You'll need write permissions to this directory, and it needs to already exist.

#### Principle 2: Names and arguments are as similar as possible to BEDTools

Returning again to this example:

```
>>> merged_a = a.merge(d=100, s=True)
```

This demonstrates another pybedtools principle: the bedtool methods that wrap BEDTools programs do the same thing and take the exact same arguments as the BEDTools program. Here we can pass d=100 and s=True only because the underlying BEDTools program, mergeBed, can accept these arguments. Need to know what arguments mergeBed can take? See the docs for bedtool.merge(); for more on this see *Principle 6: Check the help*.

In general, remove the "Bed" from the end of the BEDTools program to get the corresponding bedtool method. So there's a bedtool.subtract() method for subtractBed, a bedtool.intersect() method for intersectBed, and so on.

Since these methods just wrap BEDTools programs, they are as up-to-date as the version of BEDTools you have installed on disk. If you are using a cutting-edge version of BEDTools that has some hypothetical argument -z for intersectBed, then you can use a intersectBed (z=True).

#### Principle 3: Sensible default args

If we were running the mergeBed program from the command line, we would would have to specify the input file with the mergeBed -i option.

pybedtools assumes that if we're calling the merge() method on a, we want to operate on the bed file that a points to.

In general, BEDTools programs that accept a single BED file as input (by convention typically specified with the -i option) the default behavior for pybedtools is to use the bedtool's file (indicated in the bedtool fin attribute) as input.

We can still pass a file using the *i* keyword argument if we wanted to be absolutely explicit. In fact, the following two versions produce the same output:

```
>>> # The default is to use existing file for input -- no need
>>> # to specify "i" . . .
>>> result1 = a.merge(d=100, s=True)
>>> # . . . but you can always be explicit if you'd like
>>> result2 = a.merge(i=a.fn, d=100, s=True)
>>> # Confirm that the output is identical
>>> str(result1) == str(result2)
True
```

Methods that have this type of default behavior are indicated by the following text in their docstring:

```
.. note::

For convenience, the file this bedtool object points to is passed as "-i"
```

There are some BEDTools programs that accept two BED files as input, like intersectBed where the the first file is specified with -a and the second file with -b. The default behavior for pybedtools is to consider the bedtool's file as -a and the first non-keyword argument to the method as -b, like this:

```
>>> result3 = a.intersect(b)
```

This is exactly the same as passing the a and b arguments explicitly:

```
>>> result4 = a.intersect(a=a.fn, b=b.fn)
>>> str(result3) == str(result4)
True
```

Furthermore, the first non-keyword argument used as -b can either be a filename *or* another bedtool object; that is, these commands also do the same thing:

```
>>> result5 = a.intersect(b=b.fn)
>>> result6 = a.intersect(b=b)
>>> str(result5) == str(result6)
True
```

Methods that accept either a filename or another bedtool instance as their first non-keyword argument are indicated by the following text in their docstring:

```
.. note::
    This method accepts either a bedtool or a file name as the first
    unnamed argument
```

#### Principal 4: Other arguments have no defaults

Only the BEDTools arguments that refer to BED (or other interval) files have defaults. In the current version of BEDTools, this means only the -i, -a, and -b arguments have defaults. All others have no defaults specified by pybedtools; they pass the buck to BEDTools programs. This means if you do not specify the d kwarg when calling bedtool.merge(), then it will use whatever the installed version of BEDTools uses for -d (currently, mergeBed's default for -d is 0).

-d is an option to BEDTools mergeBed that accepts a value, while -s is an option that acts as a switch. In pybedtools, simply pass a value (integer, float, whatever) for value-type options like -d, and boolean values (*True* or *False*) for the switch-type options like -s.

Here's another example using both types of keyword arguments; the bedtool object *b* (or it could be a string filename too) is implicitly passed to intersectBed as -b (see *Principle 3: Sensible default args* above):

```
>>> a.intersect(b, v=True, f=0.5)
```

Again, any option that can be passed to a BEDTools program can be passed to the corresonding bedtool method.

### **Principle 5: Chaining together commands**

Most methods return new bedtool objects, allowing you to chain things together just like piping commands together on the command line. To give you a flavor of this, here is how you would get the merged regions of features shared between a . bed (as referred to by the bedtool a we made previously) and b . bed: (as referred to by the bedtool b):

```
>>> a.intersect(b).merge().saveas('shared_merged.bed')
<br/>bedtool (shared_merged.bed)>
```

This is equivalent to the following BEDTools commands:

```
intersectBed -a a.bed -b b.bed | merge -i stdin > shared_merged.bed
```

Methods that return a new bedtool instance are indicated with the following text in their docstring:

```
.. note::
    This method returns a new bedtool instance
```

#### Principle 6: Check the help

If you're unsure of whether a method uses a default, or if you want to read about what options an underlying BED-Tools program accepts, check the help. Each pybedtool method that wraps a BEDTools program also wraps the BEDTools program help string. There are often examples of how to use a method in the docstring as well.

## 2.4.7 Using the history and tags

BEDTools makes it very easy to do rather complex genomic algebra. Sometimes when you're doing some exploratory work, you'd like to rewind back to a previous step.

To assist this sort of workflow, bedtool instances keep track of their history, in the bedtool.history attribute. Let's make an example bedtool, c, that has some history:

```
>>> a = pybedtools.example_bedtool('a.bed')
>>> b = pybedtools.example_bedtool('b.bed')
>>> c = a.intersect(b, u=True)
```

c now has a history which tells you all sorts of useful things (described in more detail below):

```
>>> print c.history
[<HistoryStep> bedtool("/home/ryan/pybedtools/pybedtools/test/a.bed").intersect("/home/ryan/pybedtools/
```

First, the history describes the full commands, including all the names of the temp files and all the arguments that you would need to run in order to re-create it. Note that since bedtool objects are fundamentally file-based, the command refers to the underlying filenames (i.e., a.bed and b.bed) instead of the bedtool instances (i.e., a and b). A simple copy-paste of the command should do the trick. Be aware that if you run the command again, you'll get another temp file that has the same contents as c's temp file.

To avoid such cluttering of your temp dir, the history also reports **tags**. bedtool objects, when created, get a random tag assigned to them. You can get get the bedtool associated with tag with the pybedtools.find\_tagged() function. These tags are used to keep track of instances during this session.

So in this case, we could get a reference to the a instance with:

```
>>> should_be_a = pybedtools.find_tagged('klkreuay')
```

Here's confirmation that the parent of the first step of c's history is a (note that HistoryStep objects have a HistoryStep.parent\_tag and HistoryStep.result\_tag):

```
>>> pybedtools.find_tagged(c.history[0].parent_tag) == a
True
```

Let's make something with a more complicated history:

```
>>> a = pybedtools.example_bedtool('a.bed')
>>> b = pybedtools.example_bedtool('b.bed')
>>> c = a.intersect(b)
>>> d = c.slop(genome='hg19', b=1)
>>> e = d.merge()
>>> # this step adds complexity!
>>> f = e.subtract(b)
```

Let's see what the history of f (the last bedtool created) looks like . . . note that here I'm formatting the results to make it easier to see:

Those first three history steps correspond to c, d, and e respectively, as we can see by comparing the code snippet above with the commands in each history step. In other words, e can be described by the sequence of 3 commands in the first three history steps. In fact, if we checked e. history, we'd see exactly those same 3 steps.

When f was created above, it operated both on e, which had its own history, as well as b – note the nesting of the list. You can do arbitrarily complex "genome algebra" operations, and the history of the bedtools will keep track of this. It may not be useful in every situation, but the ability to backtrack and have a record of what you've done can sometimes be helpful.

## 2.4.8 Example: Flanking segs

import pybedtools

The bedtool.slop() method (which calls slopBed) needs a chromosome size file. If you specify a genome name to the bedtool.slop() method, it will retrieve this file for you automatically from the UCSC Genome Browser MySQL database.

```
a = pybedtools.bedtool('in.bed')
extended = a.slop(genome='dm3',l=100,r=100)
flanking = extended.subtract(a).saveas('flanking.bed')
flanking.sequence(fi='dm3.fa')
flanking.save_seqs('flanking.fa')

Or, as a one-liner:
pybedtools.bedtool('in.bed').slop(genome='dm3',l=100,r=100).subtract(a).sequence(fi='dm3.fa').save_set
Don't forget to clean up!:
```

## 2.4.9 Example: Region centers that are fully intergenic

Useful for, e.g., motif searching:

pybedtools.cleanup()

```
a = pybedtools.bedtool('in.bed')
# Sort by score
a = a.sorted(col=5,reverse=True)
# Exclude some regions
a = a.subtract('regions-to-exclude.bed')
```

```
# Get 100 bp on either side of center
a = a.peak_centers(100).saveas('200-bp-peak-centers.bed')
```

## 2.4.10 Example: Histogram of feature lengths

Note that you need matplotlib installed to plot the histogram.

```
import pylab as p
a = pybedtools.bedtool('in.bed')
p.hist(a.lengths(),bins=50)
p.show()
```

## 2.5 Module documentation

## 2.5.1 pybedtools module-level functions

```
pybedtools.data_dir()
```

Returns the data directory that contains example files for tests and documentation.

```
pybedtools.example_bed_fn(bed)
```

Return a bed file from the pybedtools examples directory. Use <code>list\_example\_beds()</code> to see a list of files that are included.

```
pybedtools.example_bedtool(fn)
```

Return a bedtool using a bed file from the pybedtools examples directory. Use <code>list\_example\_beds()</code> to see a list of files that are included.

```
pybedtools.list_example_beds()
```

Returns a list of bed files in the examples dir. Choose one and pass it to example\_bed() to get the full path to an example BED file.

Example usage:

```
>>> choices = list_example_beds()
>>> bedfn = example_bed(choices[0])
>>> mybedtool = bedtool(bedfn)
```

### 2.5.2 bedtool methods that wrap BEDTools programs

The following methods wrap **BEDTools**\_ programs. This package is still in development; the goal is to eventually support all **BEDTools**\_ programs.

```
bedtool.intersect
```

```
bedtool.intersect(*args, **kwargs)
    pybedtools help:
```

Intersect with another BED file. If you want to use BAM as input, you need to specify *abam='filename.bam'*. Returns a new bedtool object.

```
# create new bedtool object
a = bedtool('in.bed')

# get overlaps with "other.bed"
overlaps = a.intersect('other.bed')

# use v=True to get the inverse, or those unique to in.bed
unique_to_a = a.intersect('other.bed', v=True)

# features unique to "other.bed"
unique_to_other = bedtool('other.bed').intersect(a, v=True)
```

**Note:** This method returns a new bedtool instance

Note: For convenience, the file this bedtool object points to is passed as "-a"

Note: This method accepts either a bedtool or a file name as the first unnamed argument

#### Original BEDtools program help:

-abam

Program: intersectBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Report overlaps between two feature files.

The A input file is in BAM format. Output will be BAM as

Usage: intersectBed [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>

#### **Options:**

ubum	well.
-ubam	Write uncompressed BAM output. Default is to write compressed BAM.
-bed	When using BAM input (-abam), write output as BED. The default is to write output in BAM when using -abam.
-wa	Write the original entry in A for each overlap.
-wb	Write the original entry in B for each overlap Useful for knowing <b>what</b> A overlaps. Restricted by -f and -r.
-wo	Write the original A and B entries plus the number of base pairs of overlap between the two features Overlaps restricted by -f and -r.
	Only A features with overlap are reported.
-wao	Write the original A and B entries plus the number of base pairs of overlap between the two features Overlapping features restricted by -f and -r.
	However, A features w/o overlap are also reported with a NULL B feature and overlap = $0$ .
-u	Write the original A entry <b>once</b> if <b>any</b> overlaps found in B.  - In other words, just report the fact >=1 hit was found.  - Overlaps restricted by -f and -r.

-c	For each entry in A, report the number of overlaps with B Reports 0 for A entries that have no overlap with B Overlaps restricted by -f and -r.
-v	Only report those entries in A that have <b>no overlaps</b> with B Similar to "grep -v" (an homage).
-f	Minimum overlap required as a fraction of A Default is 1E-9 (i.e., 1bp) FLOAT (e.g. 0.50)
-r	Require that the fraction overlap be reciprocal for A and B In other words, if -f is 0.90 and -r is used, this requires
	that B overlap 90% of A and A <b>also</b> overlaps 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.
-split	Treat "split" BAM or BED12 entries as distinct BED intervals.

## bedtool.merge

```
bedtool.merge (*args, **kwargs)
    pybedtools help:
```

Merge overlapping features together. Returns a new bedtool object.

#### Example usage:

```
a = bedtool('in.bed')
# allow merging of features 100 bp apart
b = a.merge(d=100)
```

**Note:** This method returns a new bedtool instance

**Note:** For convenience, the file this bedtool object points to is passed as "-i"

#### Original BEDtools program help:

Program: mergeBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Merges overlapping BED/GFF/VCF entries into a single interval.

Usage: mergeBed [OPTIONS] -i <bed/gff/vcf>

#### **Options:**

-S	Force strandedness. That is, only merge features that are
	the same strand By default, merging is done without re-
	spect to strand.

**-n** Report the number of BED entries that were merged. - Note: "1" is reported if no merging occurred.

-d Maximum distance between features allowed for features

to be merged. - Def. 0. That is, overlapping & book-ended

features are merged. - (INTEGER)

**-nms** Report the names of the merged features separated by semi-

colons.

#### bedtool.subtract

```
bedtool.subtract(*args, **kwargs)
    pybedtools help:
```

Subtracts from another BED file and returns a new bedtool object.

Example usage:

```
a = bedtool('in.bed')
# do a "stranded" subtraction
b = a.subtract('other.bed', s=True)
# Require 50% of features in a to overlap
c = a.subtract('other.bed', s=0.5)
```

Note: This method returns a new bedtool instance

Note: This method accepts either a bedtool or a file name as the first unnamed argument

Original BEDtools program help:

Program: subtractBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Removes the portion(s) of an interval that is overlapped

by another feature(s).

Usage: subtractBed [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>

#### **Options:**

-f Minimum overlap required as a fraction of A. - Default is

1E-9 (i.e., 1bp). - (FLOAT) (e.g. 0.50)

-s Force strandedness. That is, only report hits in B that overlap A on the same strand. - By default, overlaps are re-

ported without respect to strand.

### bedtool.sequence

```
bedtool.sequence(**kwargs)
    pybedtools help:
```

Wraps fastaFromBed. *fi* is passed in by the user; *bed* is automatically passed in as the bedfile of this object; *fo* by default is a temp file. Use save\_seqs() to save as a file.

```
a = bedtool('in.bed')
a.sequence(fi='genome.fa')
a.print_sequence()
```

**Note:** This method returns a new bedtool instance

#### Original BEDtools program help:

Program: fastaFromBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Extract DNA sequences into a fasta file based on feature coordinates.

Usage: fastaFromBed [OPTIONS] -fi <fasta> -bed <bed/gff/vcf> -fo <fasta>

## **Options:**

-fi	Input FASTA file
-bed	BED/GFF/VCF file of ranges to extract from -fi
-fo	Output file (can be FASTA or TAB-delimited)
-name	Use the name field for the FASTA header
-tab	Write output in TAB delimited format Default is FASTA format.
-s	Force strandedness. If the feature occupies the antisense strand, the sequence will be reverse complemented By default, strand information is ignored.

#### bedtool.closest

```
bedtool.closest (*args, **kwargs)
    pybedtools help:
```

Return a new bedtool object containing closest features in *other*. Note that the resulting file is no longer a valid BED format; use the special "\_closest" methods to work with the resulting file.

#### Example usage:

```
a = bedtool('in.bed')
# get the closest feature in 'other.bed' on the same strand
b = a.closest('other.bed', s=True)
```

**Note:** This method returns a new bedtool instance

**Note:** For convenience, the file this bedtool object points to is passed as "-a"

Note: This method accepts either a bedtool or a file name as the first unnamed argument

Original BEDtools program help:

Program: closestBed (v2.11.2) Authors: Aaron Quinlan (aaronquinlan@gmail.com)

Erik Arner, Riken

-t

**Summary: For each feature in A, finds the closest** feature (upstream or downstream) in B.

Usage: closestBed [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>

#### **Options:**

-s	Force 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.

-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.

How ties for closest feature are handled. This occurs when two features in B have exactly the same overlap 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.

**Notes:** 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

#### bedtool.window

```
bedtool.window(*args, **kwargs)
    pybedtools help:
```

Intersect with a window.

Example usage:

```
a = bedtool('in.bed')
# Consider features up to 500 bp away as overlaps
b = a.window(w=500)
```

**Note:** For convenience, the file this bedtool object points to is passed as "-a"

Note: This method accepts either a bedtool or a file name as the first unnamed argument

Original BEDtools program help:

Program: windowBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Examines a "window" around each feature in A and

reports all features in B that overlap the window. For each overlap the entire entry in A and B are reported.

Usage: windowBed [OPTIONS] -a <bed/gff/vcf> -b <bed/gff/vcf>

#### **Options:**

-abam	The A input file is in BAM format. Output will be BAM as well.
-ubam	Write uncompressed BAM output. Default is to write compressed BAM.
-bed	When using BAM input (-abam), write output as BED. The default is to write output in BAM when using -abam.
-W	Base pairs added upstream and downstream of each entry in A when searching for overlaps in B Creates symterical "windows" around A Default is 1000 bp (INTEGER)
-1	Base pairs added upstream (left of) of each entry in A when searching for overlaps in B Allows one to define assymterical "windows" Default is 1000 bp (INTEGER)
-r	Base pairs added downstream (right of) of each entry in A when searching for overlaps in B Allows one to define assymterical "windows" Default is 1000 bp (INTEGER)
-SW	Define -l and -r based on strand. For example if used, -l 500 for a negative-stranded feature will add 500 bp down-stream Default = disabled.
-sm	Only report hits in B that overlap A on the same strand By default, overlaps are reported without respect to strand.
-u	Write the original A entry <b>once</b> if <b>any</b> overlaps found in B In other words, just report the fact >=1 hit was found.
-c	For each entry in A, report the number of overlaps with B Reports 0 for A entries that have no overlap with B Overlaps restricted by -f.
-v	Only report those entries in A that have <b>no overlaps</b> with B Similar to "grep -v."

#### bedtool.sort

bedtool.sort(\*args, \*\*kwargs)
 pybedtools help:

Note: For convenience, the file this bedtool object points to is passed as "-i"

#### Original BEDtools program help:

Program: sortBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Sorts a feature file in various and useful ways.

Usage: sortBed [OPTIONS] -i <bed/gff/vcf>

## **Options:**

-sizeA Sort by feature size in ascending order.
 -sizeD Sort by feature size in descending order.
 -chrThenSizeA Sort by chrom (asc), then feature size (asc).

-chrThenSizeD Sort by chrom (asc), then feature size (desc).
 -chrThenScoreA Sort by chrom (asc), then score (asc).
 -chrThenScoreD Sort by chrom (asc), then score (desc).

#### bedtool.slop

```
bedtool.slop(*args, **kwargs)
    pybedtools help:
```

Wraps slopBed, which adds bp to each feature. Returns a new bedtool object.

If *genome* is specified with a genome name, the genome file will be automatically retrieved from UCSC Genome Browser.

#### Example usage:

```
a = bedtool('in.bed')
# increase the size of features by 100 bp in either direction
b = a.slop(genome='dm3', b=100)
# grow features by 10 bp upstream and 500 bp downstream,
# using a genome file you already have constructed called
# dm3.genome.
c = a.slop(g='dm3.genome', l=10, r=500, s=True)
```

Note: This method returns a new bedtool instance

**Note:** For convenience, the file this bedtool object points to is passed as "-i"

#### Original BEDtools program help:

Program: slopBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Add requested base pairs of "slop" to each feature.

Usage: slopBed [OPTIONS] -i <bed/gff/vcf> -g <genome> [-b <int> or (-l and -r)]

#### **Options:**

-b	Increase the BED/GFF/VCF entry by -b base pairs in each direction (Integer) or (Float, e.g. $0.1$ ) if used with -pct.
-1	The number of base pairs to subtract from the start coordinate (Integer) or (Float, e.g. 0.1) if used with -pct.
-r	The number of base pairs to add to the end coordinate (Integer) or (Float, e.g. 0.1) if used with -pct.
-s	Define -l and -r based on strand. E.g. if used, -l 500 for a negative-stranded feature, it will add 500 bp downstream. Default = false.
-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.

**Notes:** 

- 1. Starts will be set to 0 if options would force it below 0.
- (2) Ends will be set to the chromosome length if requested slop would force it above the max chrom length. (3) The genome file should tab delimited and structured as follows:

<chromName><TAB><chromSize>

For example, Human (hg19): chr1 249250621 chr2 243199373 ... chr18\*\*gl000207\*\*random 4262

**Tips:** One can use the UCSC Genome Browser's MySQL database to extract chromosome sizes. For example, H. sapiens:

mysql -user=genome -host=genome-mysql.cse.ucsc.edu -A -e / "select chrom, size from hg19.chromInfo" > hg19.genome

#### bedtool.shuffle

bedtool.shuffle(\*args, \*\*kwargs)
 pybedtools help:

Note: For convenience, the file this bedtool object points to is passed as "-i"

Original BEDtools program help:

Program: shuffleBed (v2.11.2) Author: Aaron Quinlan (aaronquinlan@gmail.com) Summary: Randomly permute the locations of a feature file among a genome.

Usage: shuffleBed [OPTIONS] -i <bed/gff/vcf> -g <genome>

#### **Options:**

-excl	A BED/GFF/VCF file of coordinates in which features in -i should not be placed (e.g. gaps.bed).
-incl	Instead of randomly placing features in a genome, the incl options defines a BED/GFF/VCF file of coordinates in which features in -i should be randomly placed (e.g. genes.bed).
-chrom	Keep features in -i on the same chromosome By default, the chrom and position are randomly chosen.
-seed	Supply an integer seed for the shuffling By default, the seed is chosen automatically (INTEGER) $$
-f	Maximum overlap (as a fraction of the -i feature) with an -excl feature that is tolerated before searching for a new, randomized locus. For example, -f 0.10 allows up to 10% of a randomized feature to overlap with a given feature in the -excl file. <b>Cannot be used with -incl file.</b> - Default is 1E-9 (i.e., 1bp) FLOAT (e.g. 0.50)

#### **Notes:**

 The genome file should tab delimited and structured as follows: <chrom-Name><TAB><chromSize>

For example, Human (hg19): chr1 249250621 chr2 243199373 ... chr18\*\*gl000207\*\*random 4262

**Tips:** One can use the UCSC Genome Browser's MySQL database to extract chromosome sizes. For example, H. sapiens:

 $\label{eq:mysql} $$ -user=genome -host=genome-mysql.cse.ucsc.edu -A -e / "select chrom, size from hg19.chromInfo" > hg19.genome$ 

## 2.5.3 bedtool methods unique to pybedtools

The following methods are currently only supported for use with BED format files; support for other file types is under development.

#### bedtool.count

```
bedtool.count()
```

Number of features in BED file. Does the same thing as len(self), which actually just calls this method.

Only counts the actual features. Ignores any track lines, browser lines, lines starting with a "#", or blank lines.

Example usage:

```
a = bedtool('in.bed')
a.count()
```

#### bedtool.saveas

bedtool.saveas(fn, trackline=None)

Save BED file as a new file, adding the optional trackline to the beginning.

Returns a new bedtool for the newly saved file.

A newline is automatically added to the trackline if it does not already have one.

Example usage:

```
a = bedtool('in.bed')
b = a.random_subset(5)
b.saveas('random-5.bed',trackline='track name="random subset" color=128,128,255')
```

**Note:** This method returns a new bedtool instance

#### bedtool.get\_genome

```
bedtool.get_genome(genome, fn=None)
```

Download chrom size info for *genome* from UCSC, removes the header line, and saves in a temp file. Could be useful for end users, but mostly called internally by bedtool.slop() and other methods that need the genome file.

If fn is None, then saves as a temp file.

Returns the filename.

```
a = bedtool('in.bed')
fn = a.get_genome('dm3', 'dm3.genome')
```

```
bedtool.size filter
Returns a new bedtool object containing only those features that are > min and < max.
     Example usage:
     a = bedtool('in.bed')
     # Only return features that are over 10 bp.
     b = a.size_filter(min=10)
bedtool.lengths
bedtool.lengths()
     Returns a list of feature lengths.
     Example usage:
     a = bedtool('in.bed')
     lengths = a.lengths()
     # if you have pylab installed, plot a histogram
     import pylab
     pylab.hist(lengths)
     pylab.show()
bedtool.features
bedtool.features()
     Returns an iterator of bedfeature objects.
bedtool.print_sequence
bedtool.print_sequence()
     Print the sequence that was retrieved by the bedtool.sequence() method.
     See usage example in bedtool.sequence().
bedtool.save_seqs
bedtool.save_seqs(fn)
     Save sequences of features in this bedtool object as a fasta file fn.
     In order to use this function, you need to have called the bedtool.sequence() method.
     A new bedtool object is returned which references the newly saved file.
     Example usage:
     a = bedtool('in.bed')
     # specify the filename of the genome in fasta format
     a.sequence('data/genomes/genome.fa')
```

```
# use this method to save the seqs that correspond to the features
# in "a"
a.save_seqs('seqs.fa')
```

#### bedtool.cat

```
bedtool.cat (other, postmerge=True, **kwargs)
```

Concatenates two bedtools objects (or an object and a file) and does an optional post-merge of the features.

Use *postmerge=False* if you want to keep features separate.

TODO:

currently truncates at BED3 format!

kwargs are sent to bedtool.merge().

Example usage:

```
a = bedtool('in.bed')
# concatenate and merge features together if they overlap and are
# on the same strand
b = a.cat('other.bed', s=True)
```

**Note:** This method returns a new bedtool instance

Note: This method accepts either a bedtool or a file name as the first unnamed argument

#### bedtool.tostring

```
bedtool.tostring()
```

Returns the BED file as a string. You can also print the bedtool object to view its contents.

Example usage:

```
a = bedtool('in.bed')
# this is one looong string which contains the entire file
long_string = a.tostring()
```

#### bedtool.sequence\_coverage

```
bedtool.sequence_coverage()
```

Returns the number of bases covered by this BED file. Does a self.merge() first to remove potentially multiple-counting bases.

```
a = bedtool('in.bed')
# total bp in genome covered by 'in.bed'
total_bp = a.sequence_coverage()
```

#### bedtool.counts

```
bedtool.counts()
```

After running bedtool.intersect() with the kwarg c=True, use this method to return a list of the count of features in "b" that intersected each feature in "a".

#### Example usage:

```
a = bedtool('in.bed')
b = a.intersect('other.bed', c=True)
counts = b.counts()

# assuming you have matplotlib installed, plot a histogram
import pylab
pylab.hist(counts)
pylab.show()
```

#### bedtool.normalized counts

```
bedtool.normalized_counts()
```

After running bedtool.intersect() with the kwarg c=True, use this method to return a list of the density of features in "b" that intersected each feature in "a".

This takes the counts in each feature and divides by the bp in that feature.

```
a = bedtool('in.bed')
b = a.intersect('other.bed', c=True)
counts = b.normalized_counts()

# assuming you have matplotlib installed, plot a histogram
import pylab
pylab.hist(counts)
pylab.show()
```

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