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# khmer Documentation

*Release 2.0+759.ge21b78f*

**2010-2014 Michael R. Crusoe, Greg Edverson, Jordan Fish, Adina**

January 21, 2017



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# What is khmer?

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The khmer software is a set of command-line tools for working with DNA shotgun sequencing data from genomes, transcriptomes, metagenomes, and single cells. khmer can make *de novo* assemblies faster, and sometimes better. khmer can also identify (and fix) problems with shotgun data. You can read more about khmer in [our software paper](#).

khmer is free and open source software.

**To install khmer**, you will need a Linux or Mac computer, together with Python 2.7 or Python 3.x. See [our installation docs](#) for detailed instructions.

**To use khmer**, you will generally need to work at the UNIX command line. See [our command line documentation](#).

We have **additional documentation** in several places, including [protocols for metagenome and mRNAseq assembly](#) and [recipes for several common research tasks](#). You might also be interested in [papers using or citing khmer](#).

**To get help**, please follow [this guide](#).

We welcome contributions to the khmer project! We are friendly and supportive of new contributors, and have a [code of conduct](#). Please see our [docs on getting started on khmer development](#).



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## Details

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**Contact** [khmer-project@idyll.org](mailto:khmer-project@idyll.org)

**GitHub** <https://github.com/dib-lab/khmer>

**Chat** <https://gitter.im/dib-lab/khmer>

**License** BSD

## 2.1 Introduction to khmer

### 2.1.1 Introduction

khmer is a library and toolkit for doing k-mer-based dataset analysis and transformations. Our focus in developing it has been on scaling assembly of metagenomes and mRNA.

khmer can be used for a number of transformations, including inexact transformations (abundance filtering and error trimming) and exact transformations (graph-size filtering, to throw away disconnected reads; and partitioning, to split reads into disjoint sets). Of these, only partitioning is not constant memory. In all cases, the memory required for assembly with Velvet or another de Bruijn graph assembler will be more than the memory required to use our software. Our software will not increase the memory required for Velvet, either, although we may not be able to *decrease* the memory required for assembly for every data set.

Most of khmer relies on an underlying probabilistic data structure known as a [Bloom filter](#) (also see [Count-Min Sketch](#) and [These Are Not The k-mers You’re Looking For](#)), which is essentially a set of hash tables, each of different size, with no collision detection. These hash tables are used to store the presence of specific k-mers and/or their count. The lack of collision detection means that the Bloom filter may report a k-mer as being “present” when it is not, in fact, in the data set; however, it will never incorrectly report a k-mer as being absent when it *is* present. This one-sided error makes the Bloom filter very useful for certain kinds of operations.

khmer is also independent of a specific k-size (K), and currently works for  $K \leq 32$ . We will be integrating code for  $K \leq 64$  soon.

khmer is implemented in C++ with a Python wrapper, which is what all of the scripts use.

Documentation for khmer is provided on the Web sites for [khmer-protocols](#) and [khmer-recipes](#). khmer-protocols provides detailed protocols for using khmer to analyze either a transcriptome or a metagenome. khmer-recipes provides individual recipes for using khmer in a variety of sequence-oriented tasks such as extracting reads by coverage, estimating a genome or metagenome size from unassembled reads, and error-trimming reads via streaming k-mer abundance.

## 2.1.2 Using khmer

khmer comes “out of the box” with a number of scripts that make it immediately useful for a few different operations, including:

- normalizing read coverage (“digital normalization”)
- dividing reads into disjoint sets that do not connect (“partitioning”)
- eliminating reads that will not be used by a de Bruijn graph assembler;
- removing reads with low- or high-abundance k-mers;
- trimming reads of certain kinds of sequencing errors;
- counting k-mers and estimating data set coverage based on k-mer counts;
- running Velvet and calculating assembly statistics;
- optimizing assemblies on various parameters;
- converting FASTQ to FASTA;

and a few other random functions.

## 2.1.3 Practical considerations

The most important thing to think about when using khmer is whether or not the transformation or filter you’re applying is appropriate for the data you’re trying to assemble. Two of the most powerful operations available in khmer, graph-size filtering and graph partitioning, only make sense for assembly datasets with many theoretically unconnected components. This is typical of metagenomic data sets.

The second most important consideration is memory usage. The effectiveness of all of the Bloom filter-based functions (which is everything interesting in khmer!) depends critically on having enough memory to do a good job. See [Setting khmer memory usage](#) for more information.

## 2.1.4 Copyright and license

Portions of khmer are Copyright California Institute of Technology, where the exact counting code was first developed. All other code developed through 2014 is copyright Michigan State University. Portions are copyright Michigan State University and Regents of the University of California. All the code is freely available for use and re-use under the BSD License.

## 2.2 Contributors and Acknowledgements

khmer is a product of the Lab for Data Intensive Biology at the University of California, Davis (the successor to the GED lab at Michigan State University),

<http://ivory.idyll.org/lab/>

—  
C. Titus Brown <[titus@idyll.org](mailto:titus@idyll.org)> wrote the initial ktable and hashtable implementations, as well as hashbits and counting\_hash.

Jason Pell implemented many of the C++ k-mer filtering functions.

Qingpeng contributed code to do unique k-mer counting.

Adina Howe, Rosangela Canino-Koning, and Arend Hintze contributed significantly to discussions of approaches and algorithms; Adina wrote a number of scripts.

Jared T. Simpson (University of Cambridge, Sanger Institute) contributed paired-end support for digital normalization.

Eric McDonald thoroughly revised many aspects of the code base, made much of the codebase thread safe, and otherwise improved performance dramatically.

Michael R. Crusoe took over maintainership June, 2013.

Last updated by MRC on 2015-07-31

## 2.3 Citations

### 2.3.1 Software Citation

If you use the khmer software, you must cite:

Crusoe et al., The khmer software package: enabling efficient nucleotide sequence analysis. 2015.

<http://dx.doi.org/10.12688/f1000research.6924.1>

```
@article{khmer2015,
  author = "Crusoe, Michael R. and Alameldin, Hussien F. and Awad, Sherine
and Bucher, Elmar and Caldwell, Adam and Cartwright, Reed and Charbonneau,
Amanda and Constantinides, Bede and Edverson, Greg and Fay, Scott and Fenton,
Jacob and Fenzl, Thomas and Fish, Jordan and Garcia-Gutierrez, Leonor and
Garland, Phillip and Gluck, Jonathan and Gonz lez, Iv n and Guermond, Sarah
and Guo, Jiarong and Gupta, Aditi and Herr, Joshua R. and Howe, Adina and
Hyer, Alex and H rpfner, Andreas and Irber, Luiz and Kidd, Rhys and Lin, David
and Lippi, Justin and Mansour, Tamer and McA'Nulty, Pamela and McDonald, Eric
and Mizzi, Jessica and Murray, Kevin D. and Nahum, Joshua R. and Nanlohy,
Kaben and Nederbragt, Alexander Johan and Ortiz-Zuazaga, Humberto and Ory,
Jeramia and Pell, Jason and Pepe-Ranne , Charles and Russ, Zachary N and
Schwarz, Erich and Scott, Camille and Seaman, Josiah and Sievert, Scott and
Simpson, Jared and Skennerton, Connor T. and Spencer, James and Srinivasan,
Ramakrishnan and Standage, Daniel and Stapleton, James A. and Stein, Joe and
Steinman, Susan R and Taylor, Benjamin and Trimble, Will and Wiencko, Heather
L. and Wright, Michael and Wyss, Brian and Zhang, Qingpeng and zyme, en and
Brown, C. Titus"
  title = "The khmer software package: enabling efficient nucleotide
sequence analysis",
  year = "2015",
  month = "08",
```

```
publisher = "F1000",
url = "http://dx.doi.org/10.12688/f1000research.6924.1"
}
```

If you use any of our published scientific methods you should *also* cite the relevant paper(s) as directed below. Additionally some scripts use the [SeqAn library](#) for read parsing: the full citation for that library is also included below.

To see a quick summary of papers for a given script just run it without using any command line arguments.

## 2.3.2 Graph partitioning and/or compressible graph representation

The **load-graph.py**, **partition-graph.py**, and **find-knots.py** scripts are part of the compressible graph representation and partitioning algorithms described in:

Pell J, Hintze A, Canino-Koning R, Howe A, Tiedje JM, Brown CT. Scaling metagenome sequence assembly with probabilistic de Bruijn graphs Proc Natl Acad Sci U S A. 2012 Aug 14;109(33):13272-7. <http://dx.doi.org/10.1073/pnas.1121464109>. PMID: 22847406

```
@article{Pell2012,
  author = "Pell, Jason and Hintze, Arend and Canino-Koning, Rosangela and
  Howe, Adina and Tiedje, James M. and Brown, C. Titus",
  title = "Scaling metagenome sequence assembly with probabilistic de Bruijn
  graphs",
  volume = "109",
  number = "33",
  pages = "13272-13277",
  year = "2012",
  doi = "10.1073/pnas.1121464109",
  abstract = "Deep sequencing has enabled the investigation of a wide range of
  environmental microbial ecosystems, but the high memory requirements for de
  novo assembly of short-read shotgun sequencing data from these complex
  populations are an increasingly large practical barrier. Here we introduce a
  memory-efficient graph representation with which we can analyze the k-mer
  connectivity of metagenomic samples. The graph representation is based on a
  probabilistic data structure, a Bloom filter, that allows us to efficiently
  store assembly graphs in as little as 4 bits per k-mer, albeit inexactly. We
  show that this data structure accurately represents DNA assembly graphs in low
  memory. We apply this data structure to the problem of partitioning assembly
  graphs into components as a prelude to assembly, and show that this reduces the
  overall memory requirements for de novo assembly of metagenomes. On one soil
  metagenome assembly, this approach achieves a nearly 40-fold decrease in the
  maximum memory requirements for assembly. This probabilistic graph
  representation is a significant theoretical advance in storing assembly graphs
  and also yields immediate leverage on metagenomic assembly.",
  URL = "http://www.pnas.org/content/109/33/13272.abstract",
  eprint = "http://www.pnas.org/content/109/33/13272.full.pdf+html",
  journal = "Proceedings of the National Academy of Sciences"
}
```

## 2.3.3 Digital normalization

The **normalize-by-median.py** and **count-median.py** scripts are part of the digital normalization algorithm, described in:

A Reference-Free Algorithm for Computational Normalization of Shotgun Sequencing Data Brown CT, Howe AC, Zhang Q, Pyrkosz AB, Brom TH arXiv:1203.4802 [q-bio.GN] <http://arxiv.org/abs/1203.4802>

```
@unpublished{diginorm,
  author = "C. Titus Brown and Adina Howe and Qingpeng Zhang and Alexis B.
Pyrkosz and Timothy H. Brom",
  title = "A Reference-Free Algorithm for Computational Normalization of
Shotgun Sequencing Data",
  year = "2012",
  eprint = "arXiv:1203.4802",
  url = "http://arxiv.org/abs/1203.4802",
}
```

## 2.3.4 Efficient k-mer error trimming

The **script trim-low-abund.py** is described in:

Crossing the streams: a framework for streaming analysis of short DNA sequencing reads Zhang Q, Awad S, Brown CT <https://dx.doi.org/10.7287/peerj.preprints.890v1>

```
@unpublished{semistream,
  author = "Qingpeng Zhang and Sherine Awad and C. Titus Brown",
  title = "Crossing the streams: a framework for streaming analysis of
short DNA sequencing reads",
  year = "2015",
  eprint = "PeerJ Preprints 3:e1100",
  url = "https://dx.doi.org/10.7287/peerj.preprints.890v1"
}
```

## 2.3.5 K-mer counting

The **abundance-dist.py**, **filter-abund.py**, and **load-into-counting.py** scripts implement the probabilistic k-mer counting described in:

These Are Not the K-mers You Are Looking For: Efficient Online K-mer Counting Using a Probabilistic Data Structure Zhang Q, Pell J, Canino-Koning R, Howe AC, Brown CT. <http://dx.doi.org/10.1371/journal.pone.0101271>

```
@article{khmer-counting,
  author = "Zhang, Qingpeng AND Pell, Jason AND Canino-Koning, Rosangela
AND Howe, Adina Chuang AND Brown, C. Titus",
  journal = "PLOS ONE",
  publisher = "Public Library of Science",
  title = "These Are Not the K-mers You Are Looking For: Efficient Online
K-mer Counting Using a Probabilistic Data Structure",
  year = "2014",
  month = "07",
  volume = "9",
  url = "http://dx.doi.org/10.1371/journal.pone.0101271",
  pages = "e101271",
  abstract = "<p>K-mer abundance analysis is widely used for many purposes in
nucleotide sequence analysis, including data preprocessing for de novo
assembly, repeat detection, and sequencing coverage estimation. We present the
khmer software package for fast and memory efficient <italic>online</italic>
counting of k-mers in sequencing data sets. Unlike previous methods based on
data structures such as hash tables, suffix arrays, and trie structures, khmer
relies entirely on a simple probabilistic data structure, a Count-Min Sketch.
The Count-Min Sketch permits online updating and retrieval of k-mer counts in
memory which is necessary to support online k-mer analysis algorithms. On
```

sparse data sets this data structure is considerably more memory efficient than any exact data structure. In exchange, the use of a Count-Min Sketch introduces a systematic overcount for k-mers; moreover, only the counts, and not the k-mers, are stored. Here we analyze the speed, the memory usage, and the miscount rate of khmer for generating k-mer frequency distributions and retrieving k-mer counts for individual k-mers. We also compare the performance of khmer to several other k-mer counting packages, including Tallymer, Jellyfish, BFCOUNTER, DSK, KMC, Turtle and KANalyze. Finally, we examine the effectiveness of profiling sequencing error, k-mer abundance trimming, and digital normalization of reads in the context of high khmer false positive rates. khmer is implemented in C++ wrapped in a Python interface, offers a tested and robust API, and is freely available under the BSD license at [github.com/dib-lab/khmer](https://github.com/dib-lab/khmer).

```
number = "7",
doi = "10.1371/journal.pone.0101271"
}
```

### 2.3.6 FASTA and FASTQ reading

Several scripts use the SeqAn library for FASTQ and FASTA reading as described in:

SeqAn An efficient, generic C++ library for sequence analysis Döring A, Weese D, Rausch T, Reinert K.  
<http://dx.doi.org/10.1186/1471-2105-9-11>

```
@Article{SeqAn,
  AUTHOR = {Döring, Andreas and Weese, David and Rausch, Tobias and Reinert,
    Knut},
  TITLE = {SeqAn An efficient, generic C++ library for sequence analysis},
  JOURNAL = {BMC Bioinformatics},
  VOLUME = {9},
  YEAR = {2008},
  NUMBER = {1},
  PAGES = {11},
  URL = {http://www.biomedcentral.com/1471-2105/9/11},
  DOI = {10.1186/1471-2105-9-11},
  PubMedID = {18184432},
  ISSN = {1471-2105},
  ABSTRACT = {BACKGROUND: The use of novel algorithmic techniques is pivotal
    to many important problems in life science. For example the sequencing of
    the human genome [1] would not have been possible without advanced assembly
    algorithms. However, owing to the high speed of technological progress and
    the urgent need for bioinformatics tools, there is a widening gap between
    state-of-the-art algorithmic techniques and the actual algorithmic
    components of tools that are in widespread use. RESULTS: To remedy this
    trend we propose the use of SeqAn, a library of efficient data types and
    algorithms for sequence analysis in computational biology. SeqAn comprises
    implementations of existing, practical state-of-the-art algorithmic
    components to provide a sound basis for algorithm testing and development.
    In this paper we describe the design and content of SeqAn and demonstrate
    its use by giving two examples. In the first example we show an application
    of SeqAn as an experimental platform by comparing different exact string
    matching algorithms. The second example is a simple version of the well-
    known MUMmer tool rewritten in SeqAn. Results indicate that our
    implementation is very efficient and versatile to use. CONCLUSION: We
    anticipate that SeqAn greatly simplifies the rapid development of new
    bioinformatics tools by providing a collection of readily usable, well-
    designed algorithmic components which are fundamental for the field of
```

```
sequence analysis. This leverages not only the implementation of new
algorithms, but also enables a sound analysis and comparison of existing
algorithms.},
}
```

## 2.4 What's New In khmer 2.0?

### 2.4.1 New behavior

#### Streaming I/O from Unix Pipes

All scripts now accept input from named (like `/dev/stdin`, or that created using `<( list )` process substitution) and unnamed pipes (like output piped in from another program with `|`). The STDIN stream can also be specified using a single dash: `-`.

#### New parameter for memory usage, and/or tablesize/number of table parameters.

There is now a `-M/ --max-memory-usage` parameter that sets the number of tables ( `-N/ --n_tables`) and tablesize ( `-x/ --max-tablesize`) parameters automatically to match the desired memory usage.

#### Digital normalization script now supports mixed paired and unpaired read input

**normalize-by-median.py** now supports mixed paired and unpaired (or “broken-paired”) input. Behavior can be forced to either treat all reads as singletons or to require all reads be properly paired using `--force_single` or `--paired`, respectively. If `--paired` is set, `--unpaired-reads` can be used to include a file of unpaired reads. The unpaired reads will be examined after all of the other sequence files. *normalize-by-median.py* `--quiet` can be used to reduce the amount of diagnostic output.

#### Mixed-pair sequence file format support

*split-paired-reads.py* `--output-orphaned/-0` has been added to allow for orphaned reads and give them a file to be sorted into.

#### Scripts now output columnar data in CSV format by default

All scripts that output any kind of columnar data now do so in CSV format, with headers. Previously this had to be enabled with `--csv`. (Affects **abundance-dist-single.py**, **abundance-dist.py**, **count-median.py**, and **count-overlap.py**.) *normalize-by-median.py* `--report` also now outputs in CSV format.

#### Reservoir sampling script extracts paired reads by default

**sample-reads-randomly.py** now retains pairs in the output, by default. This can be overridden to match previous behavior with `--force_single`.

## 2.4.2 New scripts

### Estimate number of unique kmers

**unique-kmers.py** estimates the k-mer cardinality of a dataset using the HyperLogLog probabilistic data structure. This allows very low memory consumption, which can be configured through an expected error rate. Even with low error rate (and higher memory consumption), it is still much more efficient than exact counting and alternative methods. It supports multicore processing (using OpenMP) and streaming, and so can be used in conjunction with other scripts (like **normalize-by-median.py** and **filter-abund.py**). This is the work of Luiz Irber and it is the subject of a paper in draft.

## 2.4.3 Incompatible changes

### New datastructure and script names

For clarity the Count-Min Sketch based data structure previously known as “counting\_hash” or “counting\_table” and variations of these is now known as `countgraph`. Likewise with the Bloom Filter based data structure previously known as “hashbits”, “presence\_table” and variations of these is now known as `nodegraph`. Many options relating to `table` have been changed to `graph`.

### Binary file formats have changed

All binary khmer formats (presence tables, counting tables, tag sets, stop tags, and partition subsets) have changed. Files are now pre-pended with the string `OxLI` to indicate that they are from this project.

Files of the above types made in previous versions of khmer are not compatible with v2.0; the reverse is also true.

In addition to the `OxLI` string, the `Nodegraph` and `Countgraph` file format now includes the number of occupied bins. See [khmer/Oxli Binary File Formats](#) for details.

### load-graph.py no longer appends .pt to the specified filename

Previously, **load-graph.py** appended a `.pt` extension to the specified output filename and **partition-graph.py** appended a `.pt` to the given input filename. Now, **load-graph.py** writes to the specified output filename and **partition-graph.py** does not append a `.pt` to the given input filename.

### Some reporting options have been turned always on

The total number of unique k-mers will always be reported every time a new `countgraph` is made. The `--report-total-kmers` option has been removed from **abundance-dist-single.py**, **filter-abund-single.py**, and **normalize-by-median.py** to reflect this. Likewise with `write-fp-rate` for **load-into-counting.py** and **load-graph.py**; the false positive rate will always be written to the `.info` files.

### An uncommon error recovery routine was removed

To simplify the codebase `--save-on-failure` and its helper option `--dump-frequency` have been removed from **normalize-by-median.py**.

## Single file output option names have been normalized

`--out` is now `--output` for both `normalize-by-median.py` and `trim-low-abund.py`.

## Miscellaneous changes

The common option `--min-tablesize` was renamed to `--max-tablesize` to reflect this more desirable behavior.

In conjunction with the new `split-paired-reads.py --output-orphaned` option, the option `--force-paired/-p` has been eliminated.

As CSV format is now the default, the `--csv` option has been removed.

## Removed script

`count-overlap.py` has been removed.

## 2.5 Release notes

Contents:

### 2.5.1 khmer v1.0 release notes

582 changed files with 40,527 additions and 31,772 deletions.

The team has been hard at work since v0.8 to refine the codebase into a stable product.

<https://khmer.readthedocs.org/en/latest/>

With the 1.0 release we are making a commitment to using Semantic Versioning[0]: the version number will reflect the impact of the changes between releases. New major versions will likely require you to change how you use the project. Minor versions indicate new functionality that doesn't impact the existing. Patch versions indicate backwards-compatible fixes. Right now we are limiting this promise to the command-line interface. A future release will introduce a stable and mature Python API to the khmer project and at that time we will extend the version system to include that API.

### New items of note:

CITATION: Each script now outputs information on how to cite it. There is a new paper to describes the project overall: MR Crusoe et al., 2014. doi: 10.6084/m9.figshare.979190

The documentation for the scripts has undergone an overhaul. The scripts now output extensive notes and the formal documentation website is generated from the scripts themselves and will never be out of sync.

<https://khmer.readthedocs.org/en/latest/scripts.html>

### Notable bugs fixed/issues closed:

git clone of the khmer repo reqs > 0.5 GiB #223 @mr-c new khmer/file module #357 @RamRS Floating point exception in count-overlap.py #282 @qingpeng add documentation for sample-reads-randomly #192 @mr-c only build zlib and bzip2 when needed #168 @mr-c

## Minor updates

khmer tools should output intelligent error messages when fed empty files #135 @RamRS set `IParser::ParserState::ParserState::fill_id` to zero at initialization #356 @mr-c demote nose & sphinx to extra dependencies. #351 @mr-c CID 1054792 (Medium) Uninitialized scalar field (UNINIT\_CTOR) #179 @mr-c CID 1077117 (Medium): Division or modulo by zero (DIVIDE\_BY\_ZERO) #182 @mr-c if `--savehash` is specified then don't continue if there is not enough free disk space #245 @RamRS finish fixing implicit downcasts #330 @mr-c Clean up compile warnings in `subset.cc` #172 @mr-c all scripts need to output their version #236 @mr-c environmental variables need documenting #303 @mr-c C++ code should be consistently formatted #261 @mr-c Clean up ancillary files #146 @mr-c squash option not implemented in `abundance-dist-single.py` #271 @RamRS Add documentation on how to tie into a particular tagged version #29 @mr-c `pip install -e` fails with compile error #352 @mr-c remove the unused `KTable` object #337 @luizirber `zlib 1.2.3 -> zlib 1.2.8` #336 @mr-c CID 1173035: Uninitialized scalar field (UNINIT\_CTOR) #311 @mr-c CID 1153101: Resource leak in object (CTOR\_DTOR\_LEAK) #309 @mr-c remove `khmer::read_parsers::IParser::ParserState::thread_id` #323 @mr-c several modifications about `count-overlap.py` script #324 @qingpeng fixed `runscript` to handle `SystemExit` #332 @ctb CID 1063852: Uninitialized scalar field (UNINIT\_CTOR) #313 @mr-c [infrastructure] update to new Doxyfile format, make version number autoupdate #315 @mr-c Removed an extraneous using namespace `khmer;` in `kmer.hh`, #276 @fishjord Minimum and recommended python version #94 @mr-c `KmerCount` class appears to be unused #302 @mr-c If `loadhash` is specified in e.g. `normalize-by-median`, don't complain about default `hashsize` parameters #117 @RamRS

## Known Issues

All of these are pre-existing.

Some users have reported that `normalize-by-median.py` will utilize more memory than it was configured for. This is being investigated in <https://github.com/dib-lab/khmer/issues/266>

Some FASTQ files confuse our parser when running with more than one thread. For example, while using `load-into-counting.py`. If you experience this then add `--threads=1` to your command line. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/249>

If your k-mer table (hashfile) gets truncated, perhaps from a full filesystem, then our tools currently will get stuck. This is being tracked in <https://github.com/dib-lab/khmer/issues/247> and <https://github.com/dib-lab/khmer/issues/96> and <https://github.com/dib-lab/khmer/issues/246>

Paired-end reads from Casava 1.8 currently require renaming for use in `normalize-by-median` and `abund-filter` when used in paired mode. The integration of a fix for this is being tracked in <https://github.com/dib-lab/khmer/issues/23>

`annotate-partitions.py` only outputs FASTA even if given a FASTQ file. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/46>

A user reported that `abundance-dist-single.py` fails with small files and many threads. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/75>

## Contributors

@camillescott, @mr-c, @ctb, @luizirber, @RamRS, @qingpeng

[0] <http://semver.org/>

## 2.5.2 khmer v1.0.1 release notes

This is bugfix release. Note: the installation instructions have been slightly simplified.

<https://khmer.readthedocs.org/en/v1.0.1/>

### New items of note:

This release successfully installs and passes its unit tests on Debian 6.0 “Squeeze”, Debian 7.0 “Wheezy”, Fedora 19, OS X 7 “Lion”, OS X 8 “Mountain Lion”, Red Hat Enterprise Linux 6, Scientific Linux 6, Ubuntu 10.04 LTS, and Ubuntu 12.04 LTS. Thanks to the [UW-Madison Build and Test Lab](#) for their testing infrastructure.

### Notable bugs fixed/issues closed:

fixed thread hanging issue #406 @ctb Explicit python2 invocation #404 @mr-c MANIFEST.in, setup.py: fix to correct zlib packaging #365 @mr-c fixed check\_space\_for\_hashtable to use args.n\_tables #382 @ctb Bug fix: make-initial-stoptags.py error on missing .ht input file, actual input file is .pt #391 @mr-c

### Minor updates

include calc-best-assembly.py in v1.0.1 #409 @ctb updated normalize-by-median documentation for loadtable #378 @ctb updated diginorm for new FP rate info; corrected spelling error #398 @ctb Add spellcheck to code review checklist. #397 @ctb

### Known Issues

All of these are pre-existing.

Some users have reported that normalize-by-median.py will utilize more memory than it was configured for. This is being investigated in <https://github.com/dib-lab/khmer/issues/266>

Some FASTQ files confuse our parser when running with more than one thread. For example, while using load-into-counting.py. If you experience this then add “--threads=1” to your command line. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/249>

If your k-mer table (hashfile) gets truncated, perhaps from a full filesystem, then our tools currently will get stuck. This is being tracked in <https://github.com/dib-lab/khmer/issues/247> and <https://github.com/dib-lab/khmer/issues/246>

Paired-end reads from Casava 1.8 currently require renaming for use in normalize-by-median and abund-filter when used in paired mode. The integration of a fix for this is being tracked in <https://github.com/dib-lab/khmer/issues/23>

annotate-partitions.py only outputs FASTA even if given a FASTQ file. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/46>

A user reported that abundance-dist-single.py fails with small files and many threads. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/75>

### Contributors

@mr-c, @ctb, @luizirber, @RamRS, @ctSkenerton

## 2.5.3 khmer v1.1 release notes

This is v1.1, a minor version release; this version adds several new scripts.

Docs at: <https://khmer.readthedocs.org/en/v1.1/>

Release notes w/links: <https://github.com/dib-lab/khmer/releases/tag/v1.1>

### New items of note:

- removed unnecessary files from PyPI package; distribution is now under 2 MB (#419) @mr-c
- tests are now distributed with package and can be run after ‘pip install’ (#451) @mr-c
- complain properly on file read failures (#333) @ctb
- Sequence loading scripts will now report total numbers of k-mers if given `--report_total_kmers` (#491/#429) @mr-c
- added metagenome protocol to acceptance testing (#472) @SherineAwad @ctb

### Notable bugs fixed/issues closed:

- removed `sandbox/load-into-hashbits.py` (superseded by `scripts/load-graph.py --no-tagset`) (#480, @wrightmhw)
- promoted `extract-long-sequences.py` to `scripts` (#461, @wrightmhw)
- promoted `fastq-to-fasta.py` to `scripts` (#436, @wrightmhw)
- remove incorrect filesystem space check from `abundance-dist.py` (#452, @chuckpr)
- when counting hash writes fail, produce error message (#411, @znruss)
- removed a number of memory leaks found by Coverity and valgrind (#451, @mr-c)
- updated reservoir sampling to produce multiple subsamples with `-S` (#197, @ctb)
- fixed pip2, python2 issues (#428 and #485, @accaldwell @mr-c)
- removed untested/unused code and scripts (#438, @mr-c)

### Known issues:

All of these are pre-existing.

Some users have reported that `normalize-by-median.py` will utilize more memory than it was configured for. This is being investigated in <https://github.com/dib-lab/khmer/issues/266>

Some FASTQ files confuse our parser when running with more than one thread. For example, while using `load-into-counting.py`. If you experience this then add “`--threads=1`” to your command line. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/249>

If your k-mer table is truncated on write, an error may not be reported; this is being tracked in <https://github.com/dib-lab/khmer/issues/443>. However, khmer will now (correctly) fail when trying to read a truncated file (See #333).

Paired-end reads from Casava 1.8 currently require renaming for use in `normalize-by-median` and `abund-filter` when used in paired mode. The integration of a fix for this is being tracked in <https://github.com/dib-lab/khmer/issues/23>

Some scripts only output FASTA even if given a FASTQ file. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/46>

A user reported that `abundance-dist-single.py` fails with small files and many threads. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/75>

### Contributors

@mr-c, @ctb, @camillescott, @wrightmhw, @chuckpr, @luizirber, @accaldwell, @znruss

## 2.5.4 khmer v1.2 release notes

This is the v1.2 release of khmer: minor new features and bug fixes. The start of this release cycle coincided with the Mozilla Science Lab Global Sprint 2014. We honor and thank the 19 new contributors (including four Michigan State University undergraduates) who volunteered their time to contribute!

Docs at: <https://khmer.readthedocs.org/en/v1.2/>

### New items of note:

@mr-c and @ctb are proud to announce khmer's code of conduct [http://khmer.readthedocs.org/en/v1.2/dev/CODE\\_OF\\_CONDUCT.html](http://khmer.readthedocs.org/en/v1.2/dev/CODE_OF_CONDUCT.html) #664 All scripts list which files have been created during their execution #477 @bocajnotnef All scripts now only output status messages to STDERR instead of STDOUT #626 @b-wyss docs/ a fairly major re-organization and brand new developer docs @ctb @mr-c load-into-counting.py: --summary-info: machine readable summary in JSON or TSV format #649 @kdmurray91 scripts/extract-partitions.py: added documentation for .dist columns #516 @chuckpr Makefile: a new target `make install-dependencies` is useful for developers #539 @mr-c Sandbox scripts have been cleaned up, or removed (see the sandbox/README.rst for details) #589 @ctb

### Notable bugs fixed/issues closed:

do-partition.py's excessive spawning of threads fixed. #637 @camillescott Fixed unique k-mer count reporting in load-graph, load-into-counting, and normalize-by-median. #562 @mr-c Clarified and test the requirement for a 64-bit operating system #529 @Echelon9 Removed some of the broken multi-threading options #511 @majoras-masque Fix `table.get("wrong_length_string")` gives core dump #585 @Echelon9 filter-abund lists parameters that it doesn't use #524 @jstapleton Reduction of memory required to run the test suite #542 @leogargu BibTeX included in CITATIONS #541 @HLWiencko

### Additional fixes/features

delete `ScoringMatrix::assign` as it is unused #502 @RodPic Root all of our C++ exceptions to a common base exception #508 @iglpdc deleted `KhmerError` #503 @drlabratory normalize-by-median reporting output after main loop exits, in case it hadn't been triggered #586 @ctb Many issues discovered by `cppcheck` cleaned up #506 @brtaylor92 Developers have a new Makefile target to autofix formatting: `make format` #612 @brtaylor92 normalize-by-median.py test coverage increased #361 @SherineAwad Several unused functions were removed #599 @brtaylor92 Developer docs now link to the `stdc++` docs as appropriate #629 @mr-c Added tests for non-sequential access to input files #644 @bocajnotnef Removed `khmer/theadng_args.py` #653 @bocajnotnef Improved test for maximum k value #658 @pgarland `ReadParser` no longer crashes if `n_threads = 0` #86 @jjarong

### Known issues:

All of these are pre-existing.

Multithreaded reading will drop reads. This major issue has been present for several khmer releases and was only found via a much larger test case that we had been previously using. Credit to @camillescott. Workaround: disable threading. The next release will fix this and the other FAST[AQ] parsing issues. <https://github.com/dib-lab/khmer/issues/681>

Some users have reported that `normalize-by-median.py` will utilize more memory than it was configured for. This is being investigated in <https://github.com/dib-lab/khmer/issues/266>

Some FASTQ files confuse our parser when running with more than one thread. For example, while using `load-into-counting.py`. If you experience this then add `"-threads=1"` to your command line. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/249>

If your k-mer table is truncated on write, an error may not be reported; this is being tracked in <https://github.com/dib-lab/khmer/issues/443>. However, khmer will now (correctly) fail when trying to read a truncated file (See #333).

Paired-end reads from Casava 1.8 currently require renaming for use in normalize-by-median and abund-filter when used in paired mode. The integration of a fix for this is being tracked in <https://github.com/dib-lab/khmer/issues/23>

Some scripts only output FASTA even if given a FASTQ file. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/46>

A user reported that abundance-dist-single.py fails with small files and many threads. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/75>

## Contributors

@mr-c, @ctb, \*@bocajnotnef, \*@Echelon9, \*@jlippi, \*@kdmurray91, @qingpeng, \*@leogargu, \*@jiarong, \*@brtaylor92, \*@iglpdc, @camillescott, \*@HLWiencko, \*@cowguru2000, \*@drlaboratory, \*@jstapleton, \*@b-wyss, \*@jgluck, @fishjord, \*@SherineAwad, \*@pgarland, \*@majoras-masque, @chuckpr, \*@RodPic, @luizirber, \*@jrher

\* Denotes new contributor

## 2.5.5 khmer v1.3 release notes

This is the v1.3 release of khmer featuring a new FAST[AQ] parser from the SeqAn project.

Docs at: <https://khmer.readthedocs.org/en/v1.3/>

### New items of note:

Fixes the two multithreaded reading of sequence files issues: FASTQ parsing and the recently found read dropping issue. Several khmer scripts now support reading from non-seekable plain and gzipped FAST[AQ] files (a.k.a pipe or streaming support). @mr-c #642

### Notable bugs fixed/issues closed:

restore threading to load-graph.py #699 @mr-c

### Additional fixes/features

increase filter\_abund.py coverage #568 @wrightmhw Provide scripts/ testing coverage for check\_space\_for\_hashtable #386 #678 #718 @b-wyss Use absolute URI in CODE\_OF\_CONDUCT #684 @jsspencer give SeqAn credit #712 @mr-c Added testing to make sure all sandbox scripts are import-able and execfile-able. #709 @ctb reduce memory requirements to run tests #701 @ctb Two minor bug fixes to sandbox scripts #706 @ctb Upgrade of trim-low-abund for better, more profitable streaming. #601 @ctb Add -force or -expert or -ignore flag to all khmer scripts that do sanity checking #399 #647 @jessicamizzi Add XDECREF for returned read tuple in ReadParser.read\_pair\_iterator() #693 @mr-c @camillescott

### Known issues:

All of these are pre-existing.

Some users have reported that normalize-by-median.py will utilize more memory than it was configured for. This is being investigated in #266

If your k-mer table is truncated on write, an error may not be reported; this is being tracked in <https://github.com/dib-lab/khmer/issues/443>. However, khmer will now (correctly) fail when trying to read a truncated file (See #333).

Paired-end reads from Casava 1.8 currently require renaming for use in `normalize-by-median` and `abund-filter` when used in paired mode. The integration of a fix for this is being tracked in #23

Some scripts only output FASTA even if given a FASTQ file. This issue is being tracked in #46

A user reported that `abundance-dist-single.py` fails with small files and many threads. This issue is being tracked in #75

## Contributors

@mr-c, @ctb, @camillescott, @b-wyss, @wrightmhw, @jsspencer

## 2.5.6 khmer v1.4 release notes

This is the v1.4 release of khmer featuring the results of our March and April (PyCon) coding sprints and the 16 new contributors; the use of the new v0.8 release of `screed` (the library we use for pure Python reading of nucleotide sequence files); and the addition of @luizirber's HyperLogLog counter for quick cardinality estimation.

Documentation is at <https://khmer.readthedocs.org/en/v1.4/>

### New items of note:

Casava 1.8 read naming is now fully supported and in general the scripts no longer mangle read names. Side benefits: `split-paired-reads.py` will no longer drop reads with 'bad' names; `count-median.py` can generate output in CSV format. #759 #818 @ctb #873 @ahaerpfer

Most scripts now support a "broken" interleaved paired-read format for FASTA/ FASTQ nucleotide sequence files. `'trim-low-abund.py <http://khmer.readthedocs.org/en/v1.4/user/scripts.html#trim-low-abund-py>'` has been promoted from the sandbox as well (with streaming support). #759 @ctb #963 @sguermond #933 @standage

The script to transform an interleaved paired-read nucleotide sequence file into two files now allows one to name the output files which can be useful in combination with named pipes for streaming processing #762 @ctb

Streaming everywhere: thanks to `screed` v0.8 we now support streaming of almost all inputs and outputs. #830 @aditi9783 #812 @mr-c #917 @bocajnotnef #882 @standage

Need a quick way to count total number of unique k-mers in very low memory? the `unique-kmers.py` script in the sandbox uses a HyperLogLog counter to quickly (and with little memory) provide an estimate with a controllable error rate. #257 #738 #895 #902 @luizirber

`normalize-by-median.py` can now process both a paired interleaved sequence file and a file of unpaired reads in the same invocation thus removing the need to write the counting table to disk as required in the workaround. #957 @sustinmotion

### Notable bugs fixed/issues closed:

Paired-end reads from Casava 1.8 no longer require renaming for use in `normalize-by-median.py` and `abund-filter.py` when used in paired mode #818 @ctb

Python version support clarified. We do not (yet) support Python 3.x #741 @mr-c

If a single output file mode is chosen for `normalize-by-median.py` we now default to overwriting the output. Appending the output is available by using the append redirection operator from the shell. #843 @drtamermansour

Scripts that consume sequence data using C++ will now properly throw an error on truncated files. #897 @kdmurray91  
And while writing to disk we properly check for errors #856 #962 @mr-c  
`abundance-dist-single.py` no longer fails with small files and many threads. #900 @mr-c

## Additional fixes/features

### Of interest to users:

Many documentation updates #753 @PamelaM, #782 @bocajnotnef, #845 @alameldin, #804 @ctb, #870 @SchwarzEM, #953 #942 @safay, #929, @davelin1, #687 #912 #926 @mr-c

Installation instructions for Conda, Arch Linux, and Mac Ports have been added #723 @reedacartwright #952 @elm-beech #930 @ahaerper

The example script for the STAMPS database has been fixed to run correctly #781 @drtamermansour

`split-paired-reads.py`: added `-o` option to allow specification of an output directory #752 @bede

Fixed a string formatting and a boundary error in `sample-reads-randomly.py` #773 @qingpeng #995 @ctb

CSV output added to `abundance-dist.py`, `abundance-dist-single.py`, and `count-overlap.py`, and `readstats.py` #831 #854 #855 @drtamermansour #959 @anotherthomas

TSV/JSON output of `load-into-counting.py` enhanced with the total number of reads processed #996 @kdmurray91  
Output files are now also checked to be writable *before* loading the input files #672 @pgarland @bocajnotnef  
`interleave-reads.py` now prints the output filename nicely #827 @kdmurray91

Cleaned up error for input file not existing #772 @jessicamizzi #851 @ctb

Fixed error in `find-knots.py` #860 @TheOneHyer

The help text for `load-into-counting.py` for the `--no-bigcounts/-b` flag has been clarified #857 @kdmurray91

@lexnederbragt confirmed an old bug has been fixed with his test for whitespace in sequence identifiers interacting with the `extract-partitions.py` script #979

Now safe to copy-and-paste from the user documentation as the smart quotes have been turned off. #967 @ahaerper

The script `make-coverage.py` has been restored to the sandbox. #920 @SherineAwad

`normalize-by-median.py` will warn if two of the input files have the same name #932 @elmbeech

### Of interest to developers:

Switched away from using `--user` install for developers #740 @mr-c @drtamermansour & #883 @standage

Developers can now see a summary of important Makefile targets via `make help` #783 @standage

The unused `khmer.load_pe` module has been removed #828 @kdmurray91

Versioneer bug due to new screed release was squashed #835 @mr-c

A Python 2.6 and 2.7.2 specific bug was worked around #869 @kdmurray91 @ctb

Added functions `hash_find_all_tags_list` and `hash_get_tags_and_positions` to CountingHash objects #749 #765 @ctb

The `make diff-cover` and ChangeLog formatting requirements have been added to checklist #766 @mr-c

A useful message is now presented if large tables fail to allocate enough memory #704 @mr-c

A checklist for developers adding new CPython types was added #727 @mr-c

The sandbox graduation checklist has been updated to include streaming support #951 @sguermond

Specific policies for sandbox/ and scripts/ content, and a process for adding new command line scripts into scripts/ have been added to the developer documentation #799 @ctb

Sandbox scripts update: corrected #! Python invocation #815 @Echelon9, executable bits, copyright headers, no underscores in filenames #823 #826 #850 @alameldin several scripts deleted, docs + requirements updated #852 @ctb

Avoid running big-memory tests on OS X #819 @ctb

Unused callback code was removed #698 @mr-c

The CPython code was updated to use the new checklist and follow additional best practices #785 #842 @luizirber

Added a read-only view of the raw counting tables #671 @camillescott #869 @kdmurray91

Added a Python method for quickly getting the number of underlying tables in a counting or presence table #879 #880 @kdmurray91

The C++ library can now be built separately for the brave and curious developer #788 @kdmurray91

The ReadParser object now keeps track of the number of reads processed #877 @kdmurray91

Documentation is now reproducible #886 @mr-c

Python future proofing: specify floor division #863 @mr-c

Miscellaneous spelling fixes; thanks codespell! #867 @mr-c

Debian package list update #984 @mr-c

`khmer.kfile.check_file_status()` has been renamed to `check_input_files()` #941 @proteasome  
`filter-abund.py` now uses it to check the input counting table #931 @safay

`normalize-by-median.py` was refactored to not pass the `ArgParse` object around #965 @susinmotion

Developer communication has been clarified #969 @sguermond

Tests using the `'fail_okay=true'` parameter to `runscript` have been updated to confirm the correct error occurred. 3 faulty tests were fixed and the docs were clarified #968 #971 @susinmotion

FASTA test added for `extract-long-sequences.py` #901 @jessicamizzi

'added silly test for empty file warning' #557 @wltrimbl @bocajnotnef

A couple tests were made more resilient and some extra error checking added in CPython land #889 @mr-c

Copyright added to pull request checklist #940 @sguermond

`khmer_exceptions` are now based on `std::strings` which plugs a memory leak #938 @anotherthomas

Python docstrings were made PEP257 compliant #936 @ahaerpfer

Some C++ comments were converted to be Doxygen compliant #950 @josiahseaman

The counting and presence table warning logic was refactored and centralized #944 @susinmotion

The release checklist was updated to better run the post-install tests #911 @mr-c

The unused method `find_all_tags_truncate_on_abundance` was removed from the CPython API #924 @anotherthomas

OS X warnings quieted #887 @mr-c

## Known issues:

All of these are pre-existing.

Some users have reported that `normalize-by-median.py` will utilize more memory than it was configured for. This is being investigated in <https://github.com/dib-lab/khmer/issues/266>

Some scripts only output FASTA even if given a FASTQ file. This issue is being tracked in <https://github.com/dib-lab/khmer/issues/46>

## Contributors

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\* Indicates new contributors

## Issue reporters

@moorepants, @teshomem, @macmanes, @lexnederbragt, @r-gaia-cs, @magentashades

## 2.5.7 khmer v2.0 release notes

This is the v2.0 release of khmer and the first from our new lab at the University of California, Davis. It features Python 3 compatibility, streaming I/O from Unix Pipes, mixed-pair sequence file format support, and a new parameter to simplify memory usage. We also have a software paper in-prepress describing the project and the citation reminders have been updated to reflect that.

Overall there are an additional 2,380 lines of Python code (mostly tests) and 283 less lines of C++ (despite adding features). This release is the product of over 1,000 commits to the codebase since v1.4.

Documentation is at <https://khmer.readthedocs.org/en/v2.0/>

## New items of note:

### New behavior

#### Streaming I/O from Unix Pipes

All scripts now accept input from named (like `/dev/stdin`, or that created using `<( list )` process substitution) and unnamed pipes (like output piped in from another program with `|`). The STDIN stream can also be specified using a single dash: `-`. #1186 @mr-c #1042 #763 @SherineAwad #1085 @ctb

#### New parameter for memory usage, and/or tablesize/number of table parameters.

There is now a `-M/--max-memory-usage` parameter that sets the number of tables (`-N/--n\_tables`) and `tablesize` (`-x/--max-tablesize`) parameters automatically to match the desired memory usage. #1106 #621 #1126 #390 #1117 #1055 #1050 #1214 #1179 #1133 #1145 @ctb @qingpeng @bocajnotnef

### Digital normalization script now supports mixed paired and unpaired read input

`normalize-by-median.py` now supports mixed paired and unpaired (or “broken-paired”) input. Behavior can be forced to either treat all reads as singletons or to require all reads be properly paired using `--force\_single` or `--paired`, respectively. If `--paired` is set, `--unpaired-reads` can be used to include a file of unpaired reads. The unpaired reads will be examined after all of the other sequence files. `normalize-by-median.py` now has a `--quiet` option to reduce the amount of output. #1200 @bocajnotnef

### Mixed-pair sequence file format support

`split-paired-reads.py --output-orphaned/-0` has been added to allow for orphaned reads and give them a file to be sorted into. #847 #1164 @ctb

### Scripts now output columnar data in CSV format by default

All scripts that output any kind of columnar data now do so in CSV format, with headers. Previously this had to be enabled with `--csv`. (Affects `abundance-dist-single.py`, `abundance-dist.py`, `count-median.py`, and `count-overlap.py`.) `normalize-by-median.py --report` also now outputs in CSV format. #1011 #1180 @ctb

### Reservoir sampling script extracts paired reads by default

`sample-reads-randomly.py` now retains pairs in the output, by default. This can be overridden to match previous behavior with `--force\_single`.

### Most input and output files can be compressed

We support gzip and bzip2 input and output file compression everywhere that it makes sense #505 #747 @bocajnotnef

## New scripts

### Estimate number of unique kmers

`unique-kmers.py` estimates the k-mer cardinality of a dataset using the HyperLogLog probabilistic data structure. This allows very low memory consumption, which can be configured through an expected error rate. Even with low error rate (and higher memory consumption), it is still much more efficient than exact counting and alternative methods. It supports multicore processing (using OpenMP) and streaming, and so can be used in conjunction with other scripts (like `normalize-by-median.py` and `filter-abund.py`). This script is the work of @luizirber and the subject of a paper in draft. #390 #1239 #1252 #1053 #1072 #1145 #1176 #1207 #1204 #1245

## Incompatible changes

### New datastructure and script names

For clarity the Count-Min Sketch based data structure previously known as “counting\_hash” or “counting\_table” and variations of these is now known as `countgraph`. Likewise with the Bloom Filter based data structure previously known as “hashbits”, “presence\_table” and variations of these is now known as `nodegraph`. Many options relating to `table` have been changed to `graph`. #1112 #1209 @mr-c

### Binary file formats have changed

All binary khmer formats (presence tables, counting tables, tag sets, stop tags, and partition subsets) have changed. Files are now pre-pended with the string `OXLI` to indicate that they are from this project. #519 #1031 @mr-c #1159 @luizirber

Files of the above types made in previous versions of khmer are not compatible with v2.0; the reverse is also true.

In addition to the `OXLI` string, the Nodegraph and Countgraph file format now includes the number of occupied bins. See <http://khmer.readthedocs.org/en/v2.0/dev/binary-file-formats> for details. #1093 @ctb @mr-c #1101 #1103 @kdmurray91

### load-graph.py no longer appends .pt to the specified filename

Previously, `load-graph.py` appended a `.pt` extension to the specified output filename and `partition-graph.py` appended a `.pt` to the given input filename. Now, `load-graph.py` writes to the specified output filename and `partition-graph.py` does not append a `.pt` to the given input filename. #1189 #747 @bocajnotnef

### Some reporting options have been turned always on

The total number of unique k-mers will always be reported every time a new countgraph is made. The `--report-total-kmers` option has been removed from `abundance-dist-single.py`, `filter-abund-single.py`, and `normalize-by-median.py` to reflect this. Likewise with `--write-fp-rate` for `load-into-counting.py` and `load-graph.py`; the false positive rate will always be written to the `.info` files. #1097 #1180 @ctb

### An uncommon error recovery routine was removed

To simplify the codebase `--save-on-failure` and its helper option `--dump-frequency` have been removed from `normalize-by-median.py`.

### Single file output option names have been normalized

`--out` is now `--output` for both `normalize-by-median.py` and `trim-low-abund.py`. #1188 #1164 @ctb

### Miscellaneous changes

The common option `--min-tablesize` was renamed to `--max-tablesize` to reflect this more desirable behavior.

In conjunction with the new `split-paired-reads.py --output-orphaned` option, the option `--force-paired/-p` has been eliminated.

As CSV format is now the default, the `--csv` option has been removed.

### Removed script

`count-overlap.py` has been removed.

## Notable bugs fixed/issues closed:

When `normalize-by-median.py` decides to keep both parts of a pair of reads it was only adding the k-mers & counts from one to the countgraph. #1000 #1010 @drtamermansour @bocajnotnef

The partition map file format was not robust to truncation and would hang waiting for more data. #437 #1037 #1048 @ctb

`extract-paired-reads.py` and `split-paired-reads.py` no longer create default files when the user supplies filename(s). #1005 #1132 @kdmurray91

## Additional fixes/features

`find-knots.py` was missing a `--force` option and unit tests. #358 #1078 @ctb The check for excessively high false-positive rate has also received a `--force` option #1168 @bocajnotnef

A bug leading to an infinite loop with large gzipped countgraphs was found #1038 #1043 @kdmurray91

All scripts that create nodegraphs or countgraphs report the total number of unique k-mers. #491 #609 #429 @mr-c

Read pairs from SRA are fully supported. Reported by @macmanes in #1027, fixed by @kdmurray91 @SherineAwad in #1173 #1088

## Of interest to users:

Added `Hashtable::get_kmers()`, `get_kmer_hashes()`, and `get_kmer_counts()` with corresponding CPython functions. #1047 #1049 @ctb

The `DEFAULT_DESIRED_COVERAGE` for `normalize-by-median.py` is now 20. #1073 #1081 @ctb

FIFOs are no longer seen as empty. #1147 #1163 @bocajnotnef

When the k-size is requested to be larger than 32 (which is unsupported) a helpful error message is reported. #1094 #1050 @ctb

We try to report more helpfully during errors, such as suggesting the `--force` option when outputs files already exist. #1162 #1170 @bocajnotnef

There is a paper related to `trim-low-abund.py`: “Crossing the streams: a framework for streaming analysis of short DNA sequencing reads” and it has been added to the CITATION file and program output. #1180 #1130 @ctb

We have dropped support for Python 2.6 #1009 #1180 @ctb

Our user documentation got a bit out of date and has been updated. #1156 #1247 @bocajnotnef @mr-c #1104 @kdmurray91 #1267 @ctb Links to lists of publications that use khmer have been added #1063 #1222 @mr-c The help text from the scripts has also had a thorough cleanup for formatting. #1268 @mr-c

`fastq-to-fastq.py`'s `--n_keep` option has incorrect help text. We now point out that all reads with Ns will be dropped by default unless this option is supplied. #657 #814 #1208 @ACharbonneau @bocajnotnef

We've updated the URL to the '88m-reads.fa.gz' file. #1242 #1269 @mr-c

@camillescott designed and implemented an optimization for `normalize-by-median.py` #862

`abundance-dist.py` can now be used without counts over 255 with `--no-bigcount`. #1067 #909 @drtamermansour @bocajnotnef Its input file requirement can no longer be overridden #1201 #1202 @bocajnotnef

khmer v2.0 will be released as a package for the Debian GNU/Linux operating system. Big thanks to @kdmurray91 for his assistance. #1148 #1240 The C++ library, now named liboxli, will have its own package as well.

`sandbox/multi-rename.py` now wraps long FASTA sequences at 80 columns. #450 #1136 @SherineAwad

**Of interest to developers:**

The khmer project is now a Python 3 codebase with backwards compatibility to Python 2.7. Huge credit to @luizirber #978 #922 #1045 #1066 #1089 #1157 #1191 #1108 Many developer impacting changes including the file `khmer/\_khmermodule.cc` is now `khmer/\_khmer.cc`. #169 #904

@camillescott did an extensive refactor of the C++ graph traversal code which removed a considerable amount of redundant code and will be very useful for future work. #1231 #1080

We now use some and allow all C++11 features in the codebase. #598 #1122 @mr-c

`normalize-by-median.py` was extensively refactored. #1006 #1010 #1057 #1039 #1135 #1182 @bocajnotnef @ctb @camillescott

The CPython glue was refactored so that CountingHash and Hashbits inherit from Hashtable. #1044 @ctb

The tests no longer stop on the first failed test. #1124 #1134 @ctb and some noisy tests were silenced #1125 #1137 @bocajnotnef

The `check_space()` calls were cleaned up. #1167 #1166 #1170 #993

Developer docs have been expanded #737 #1184 @bocajnotnef #1083 #1282 @ctb @mr-c #1269

A lot of code was deleted: TRACE related code in #274 #1180 @ctb `hashtable_collect_high_abundance_kmers` in #1142 #1044 @ctb `lib/ht-diff.cc`, `lib/test-HashTables.cc`, `lib/test-Parser.cc` #1144, @mr-c `bink.ipynb`, `lib/graphtest.cc`, `lib/primes.hh` #1289 @mr-c

@bocajnotnef deleted more unused code and added new tests elsewhere to increase testing coverage in #1236. @mr-c had his own go in #1279

cppcheck installation for OSX has been documented #777 #952 #945 @elmbeech

ccache and git-merge-changelog has been documented for Linux users #610 #1122 #614 @mr-c

The graphalign parameters can be saved/loaded from disk. In addition the `align_forward` method has been introduced. #755 #750 @mr-c @ctb

`labelhash` is now known as `graphlabels` #1032 #1209 @mr-c It is also now a ‘friend’ of Hashtable and one can make either a nodegraph or countgraph version. These graphlabels can now be saved & loaded from disk. #1021 @ctb

Spelling is hard; we’ve added instructions on how to run codespell to the developer docs. #890 #1203 @bocajnotnef

A redundant and contradictory named test has been removed. Reported by @jgluck in #662 fixed by @bocajnotnef in #1220 @SherineAwad contributed some additional tests #809 #615.

The new oxli command, while disabled in the v2.0 release, has been added to all the QA makefile targets as we continue to refactor the codebase. #1199 #1218 @bocajnotnef

The CPython code was audited to ensure that all possible C++ exceptions were caught and dealt with. The exception hierarchy was also simplified #1016 #1015 #1017 #1151 @kdmurray91 @mr-c

`get_kadian_count` has been removed. #1034 #1194 @ctb

We use argparse’s `metavars` to aid with autogenerated documentation for the scripts. This has been documented in the dev docs. #620 #1222 @mr-c

Sometimes one makes a lot of commits while refining a feature or pull request. We’ve documented a field-tested way to turn a pile of commits into a single commit without the pain of `git rebase`. #1013 #660 #1222 @mr-c

We use Coverity to test for various issues with our C++ code. The Makefile target has been updated for changes on their side. #1007 #1222 @mr-c

There is a new `update()` function to merge two nodegraphs of the same size and ksize. #1051 @ctb

Despite the checklist, formatting errors still occur. We must be vigilant! #1075 @luizirber

There is a new `filter_on_median` function. #862 #1077 @camillescott

There are new scripts in the `sandbox/` which output k-mer counts: `sandbox/{count-kmers.py,count-kmers-single.py}`. #983 @ctb

A large effort to make the codebase 'pylint clean' has begun with #1175 @bocajnotnef Likewise the cpychecker tool was re-run on the CPython code and issues found there were addressed #1196 @mr-c

As repeatedly promised, we've updated our list of contributors to include everyone with a commit in git. #1023 @mr-c  
`thread_utils.is_pair()` has been dropped in favor of `utils.check_is_pair()` #1284 @mr-c

The Doxygen produced documentation is improving. The location of included headers is now autodetected for Doxygen and cppcheck.

### Known issues:

`load-graph.py` in multithreaded mode will find slightly different number of unique kmers. This is being investigated in #1248

### Contributors

@ctb, @bocajnotnef, @mr-c, @luizirber, @kdmurray91, @SherineAwad, @camillescott, \*@ACharbonneau

\* Indicates new contributors

### Issue reporters

@jgluck, @ACharbonneau, @macmanes

## 2.6 The khmer user documentation

Contents:

### 2.6.1 Installing and running khmer

You'll need a 64-bit operating system, internet access, and Python 2.7.x OR Python 3.3 or greater.

#### Build requirements

##### OS X

1. From a terminal download the virtualenv package and create a virtual environment with it:

```
curl -O https://pypi.python.org/packages/source/v/virtualenv/virtualenv-1.11.6.tar.gz
tar xzf virtualenv*
cd virtualenv-*; python2.7 virtualenv.py ../khmerEnv; cd ..
source khmerEnv/bin/activate
```

## Linux

1. Install the python development environment, virtualenv, pip, gcc, and g++.

- On recent Debian and Ubuntu this can be done with:

```
sudo apt-get install python2.7-dev python-virtualenv python-pip gcc \
g++
```

- For RHEL6:

```
sudo yum install -y python-devel python-pip git gcc gcc-c++ make
sudo pip install virtualenv
```

2. Create a virtualenv and activate it:

```
cd a/writable/directory/
python2.7 -m virtualenv khmerEnv
source khmerEnv/bin/activate
```

Linux users without root access can try the OS X instructions above.

## Installing khmer inside the virtualenv

1. Use pip to download, build, and install khmer and its dependencies:

```
pip2 install khmer
```

2. The scripts are now in the env/bin directory and ready for your use. You can directly use them by name, see [khmer's command-line interface](#).
3. When returning to khmer after installing it you will need to reactivate the virtualenv first:

```
source khmerEnv/bin/activate
```

## 2.6.2 A few examples

See the [examples](#) directory for complete examples.

### STAMPS data set

The 'stamps' data set is a fake metagenome-like data set containing two species, mixed at a 10:1 ratio. The source genomes are in [data/stamps-genomes.fa](#). The reads file is in [data/stamps-reads.fa.gz](#), and consists of 100-base reads with a 1% error rate.

The example shows how to construct k-mer abundance histograms, as well as the effect of digital normalization and partitioning on the k-mer abundance distribution.

See the [script for running everything](#) and the [IPython Notebook](#).

For an overall discussion and some slides to explain what's going on, visit the [Web site for a 2013 HMP metagenome assembly webinar](#) that Titus Brown gave.

### 2.6.3 An assembly handbook for khmer - rough draft

**date** 2012-11-2

An increasing number of people are asking about using our assembly approaches for things that we haven't yet written (or posted) papers about. Moreover, our assembly strategies themselves are also under constant evolution as we do more research and find ever-wider applicability of our approaches.

Note, this is modified from [Titus' blog post, here](#) – go check the bottom of that for comments.

#### Authors

This handbook distills the cumulative expertise of Adina Howe, Titus Brown, Erich Schwarz, Jason Pell, Camille Scott, Elijah Lowe, Kanchan Pavangadkar, Likit Preeyanon, and others.

#### Introduction

khmer is really focused on short read data, and, more specifically, Illumina, because that's where we have a too-much-data problem. However, a lot of the prescriptions below can be adapted to longer read technologies such as 454 and Ion Torrent without much effort.

Don't try to use our k-mer approaches with PacBio – the error rate is too high.

There are many blog posts about this stuff on [Titus Brown's blog](#). We will try to link them in where appropriate.

#### Preparing your sequences

Do all the quality filtering, trimming, etc. that you think you should do.

The khmer tools work “out of the box” on interleaved paired-end data.

All of our scripts will take in .fq or .fastq files as FASTQ, and all other files as FASTA. gzip files are always accepted. Let us know if not; that's a bug!

#### Genome assembly, including MDA samples and highly polymorphic genomes

1. Apply digital normalization as follows.

Broadly, normalize each insert library separately, in the following way:

For high-coverage libraries (> ~50x), do three-pass digital normalization: run **normalize-by-median.py** with `--cutoff=20` and then run **filter-abund.py** with `--cutoff=2`. Now split out the remaining paired-end/interleaved and single-end reads using **extract-paired-reads.py**, and run **normalize-by-median.py** on the paired-end and single-end files (using `--unpaired-reads`) with `--cutoff=5`.

For low-coverage libraries (< 50x) do single-pass digital normalization: run **normalize-by-median.py** to `--cutoff=10`.

2. Extract any remaining paired-end reads and lump remaining orphan reads into singletons using **extract-paired-reads.py**
3. Then assemble as normal, with appropriate insert size specs etc. for the paired end reads.

You can read about this process in the [digital normalization paper](#).

## mRNAseq assembly

1. Apply single-pass digital normalization. Run **normalize-by-median.py** with `--cutoff=20`.
2. Extract any remaining paired-end reads and lump remaining orphan reads into singletons using **extract-paired-reads.py**
3. Then assemble as normal, with appropriate insert size specs etc. for the paired end reads.

You can read about this process in the [digital normalization paper](#).

## Metagenome assembly

1. Apply single-pass digital normalization. Run **normalize-by-median.py** with `--cutoff=20` (we've also found `--cutoff=10` works fine).
2. Run `sandbox/filter-below-abund.py` with `--cutoff=50` (if you ran **normalize-by-median.py** with `--cutoff=10`) or with `--cutoff=100` if you ran **normalize-by-median.py** with `--cutoff=20`)
3. Partition reads with **load-graph.py**, etc. etc.
4. Assemble groups as normal, extracting paired-end reads and lumping remaining orphan reads into singletons using **extract-paired-reads.py**.

(We actually use Velvet at this point, but there should be no harm in using a metagenome assembler such as MetaVelvet or MetaIDBA or SOAPdenovo.)

Read more about this in the [partitioning paper](#). We have some upcoming papers on partitioning and metagenome assembly, too; we'll link those in when we can.

## Metatranscriptome assembly

(Not tested by us!)

1. Apply single-pass digital normalization by running **normalize-by-median.py** with `--cutoff=20`.
2. Extract any remaining paired-end reads and lump remaining orphan reads into singletons using **extract-paired-reads.py**.
3. Then assemble with a genome or metagenome assembler, *not* an mRNAseq assembler. Use appropriate insert size specs etc. for the paired end reads.

## Preprocessing Illumina for other applications

(Not tested by us!)

Others have told us that you can apply digital normalization to Illumina data prior to using Illumina for [RNA scaffolding](#) or [error correcting PacBio reads](#).

Our suggestion for this, based on no evidence whatsoever, is to run **normalize-by-median.py** with `--cutoff=20` on the Illumina data.

## Quantifying mRNAseq or metagenomes assembled with digital normalization

For now, khmer only deals with assembly! So: assemble. Then, go back to your original, unnormalized reads, and map those to your assembly with e.g. bowtie. Then count as you normally would).

## Philosophy of digital normalization

The basic philosophy of digital normalization is “load your most valuable reads first.” Diginorm gets rid of redundancy iteratively, so you are more likely to retain the first reads fed in; this means you should load in paired end reads, or longer reads, first.

## Iterative and independent normalization

You can use `--loadgraph` and `--savegraph` to do iterative normalizations on multiple files in multiple steps. For example, break

```
normalize-by-median.py [ ... ] file1.fa file2.fa file3.fa
```

into multiple steps like so:

```
normalize-by-median.py [ ... ] --savegraph file1.ct file1.fa
normalize-by-median.py [ ... ] --loadgraph file1.ct --savegraph file2.ct file2.fa
normalize-by-median.py [ ... ] --loadgraph file2.ct --savegraph file3.ct file3.fa
```

The results should be identical!

If you want to independently normalize multiple files for speed reasons, go ahead. Just remember to do a combined normalization at the end. For example, instead of

```
normalize-by-median.py [ ... ] file1.fa file2.fa file3.fa
```

you could do

```
normalize-by-median.py [ ... ] file1.fa
normalize-by-median.py [ ... ] file2.fa
normalize-by-median.py [ ... ] file3.fa
```

and then do a final

```
normalize-by-median.py [ ... ] file1.fa.keep file2.fa.keep file3.fa.keep
```

The results will not be identical, but should not differ significantly. The multipass approach will take more total time but may end up being faster walltime because you can execute the independent normalizations on multiple computers.

For a cleverer approach that we will someday implement, read [the Beachcomber’s Dilemma](#).

## 2.6.4 khmer’s command-line interface

The simplest way to use khmer’s functionality is through the command line scripts, located in the `scripts/` directory of the khmer distribution. Below is our documentation for these scripts. Note that all scripts can be given `-h/--help` which will print out a list of arguments taken by that script.

Scripts that use k-mer counting tables or k-mer graphs take an `-M` parameter, which sets the maximum memory usage in bytes. This should generally be set as high as possible; see [Setting khmer memory usage](#) for more information.

1. *k-mer counting and abundance filtering*
2. *Partitioning*
3. *Digital normalization*
4. *Read handling: interleaving, splitting, etc.*

**Note:** Almost all scripts take in either FASTA and FASTQ format, and output the same.

Gzip and bzip2 compressed files are detected automatically.

---

## k-mer counting and abundance filtering

### load-into-counting.py

Build a k-mer countgraph from the given sequences.

usage: load-into-counting.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [--small-count] [--threads THREADS] [-b] [--summary-info FORMAT] [-f] [-q] output\_countgraph\_filename input\_sequence\_filename [input\_sequence\_filename ...]

**output\_countgraph\_filename**

The name of the file to write the k-mer countgraph to.

**input\_sequence\_filename**

The names of one or more FAST[AQ] input sequence files.

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**--ksize <ksize>, -k <ksize>**

k-mer size to use

**--n\_tables <n\_tables>, -N <n\_tables>**

number of tables to use in k-mer countgraph

**-U <unique\_kmers>, --unique-kmers <unique\_kmers>**

approximate number of unique kmers in the input set

**--fp-rate <fp\_rate>**

Override the automatic FP rate setting for the current script

**--max-tablesize <max\_tablesize>, -x <max\_tablesize>**

upper bound on tablesizes to use; overrides --max-memory-usage/-M

**-M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>**

maximum amount of memory to use for data structure

**--small-count**

Reduce memory usage by using a smaller counter for individual kmers.

**--threads <threads>, -T <threads>**

Number of simultaneous threads to execute

**-b, --no-bigcount**

The default behaviour is to count past 255 using bigcount. This flag turns bigcount off, limiting counts to 255.

**--summary-info {json,tsv}, -s {json,tsv}**

What format should the machine readable run summary be in? (*json* or *tsv*, disabled by default)

**-f, --force**

Overwrite output file if it exists

**-q, --quiet**

Note: with `-b/--no-bigcount` the output will be the exact size of the k-mer countgraph and this script will use a constant amount of memory. In exchange k-mer counts will stop at 255. The memory usage of this script with `-b` will be about 1.15x the product of the `-x` and `-N` numbers.

Example:

```
load-into-counting.py -k 20 -x 5e7 out data/100k-filtered.fa
```

Multiple threads can be used to accelerate the process, if you have extra cores to spare.

Example:

```
load-into-counting.py -k 20 -x 5e7 -T 4 out data/100k-filtered.fa
```

### abundance-dist.py

Calculate abundance distribution of the k-mers in the sequence file using a pre-made k-mer countgraph.

usage: abundance-dist.py [-version] [-info] [-h] [-z] [-s] [-b] [-f] [-q] input\_count\_graph\_filename input\_sequence\_filename output\_histogram\_filename

**input\_count\_graph\_filename**

The name of the input k-mer countgraph file.

**input\_sequence\_filename**

The name of the input FAST[AQ] sequence file.

**output\_histogram\_filename**

The columns are: (1) k-mer abundance, (2) k-mer count, (3) cumulative count, (4) fraction of total distinct k-mers.

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**-z, --no-zero**

Do not output zero-count bins

**-s, --squash**

Overwrite existing output\_histogram\_filename

**-b, --no-bigcount**

Do not count k-mers past 255

**-f, --force**

Continue even if specified input files do not exist or are empty.

**-q, --quiet**

Example:

```
load-into-counting.py -x 1e7 -N 2 -k 17 counts \
    tests/test-data/test-abund-read-2.fa
abundance-dist.py counts tests/test-data/test-abund-read-2.fa test-dist
```

## abundance-dist-single.py

Calculate the abundance distribution of k-mers from a single sequence file.

usage: abundance-dist-single.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-small-count] [-threads THREADS] [-z] [-b] [-s] [-savegraph filename] [-f] [-q] input\_sequence\_filename output\_histogram\_filename

### input\_sequence\_filename

The name of the input FAST[AQ] sequence file.

### output\_histogram\_filename

The name of the output histogram file. The columns are: (1) k-mer abundance, (2) k-mer count, (3) cumulative count, (4) fraction of total distinct k-mers.

### --version

show program's version number and exit

### --info

### -h, --help

show this help message and exit

### --ksize <ksize>, -k <ksize>

k-mer size to use

### --n\_tables <n\_tables>, -N <n\_tables>

number of tables to use in k-mer countgraph

### -U <unique\_kmers>, --unique-kmers <unique\_kmers>

approximate number of unique kmers in the input set

### --fp-rate <fp\_rate>

Override the automatic FP rate setting for the current script

### --max-tablesize <max\_tablesize>, -x <max\_tablesize>

upper bound on tablesizes to use; overrides -max-memory-usage/-M

### -M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>

maximum amount of memory to use for data structure

### --small-count

Reduce memory usage by using a smaller counter for individual kmers.

### --threads <threads>, -T <threads>

Number of simultaneous threads to execute

### -z, --no-zero

Do not output zero-count bins

### -b, --no-bigcount

Do not count k-mers past 255

### -s, --squash

Overwrite output file if it exists

### --savegraph <filename>

Save the k-mer countgraph to the specified filename.

### -f, --force

Overwrite output file if it exists

### -q, --quiet

Note that with `-b/--no-bigcount` this script is constant memory; in exchange, k-mer counts will stop at 255. The memory usage of this script with `-b` will be about 1.15x the product of the `-x` and `-N` numbers.

To count k-mers in multiple files use `load_into_counting.py` and `abundance_dist.py`.

Example:

```
abundance-dist-single.py -x 1e7 -N 2 -k 17 \
    tests/test-data/test-abund-read-2.fa test-dist
```

## filter-abund.py

Trim sequences at a minimum k-mer abundance.

usage: filter-abund.py [-version] [-info] [-h] [-threads THREADS] [-cutoff CUTOFF] [-variable-coverage] [-normalize-to NORMALIZE\_TO] [-o optional\_output\_filename] [-f] [-q] [-gzip | -bzip] input\_count\_graph\_filename input\_sequence\_filename [input\_sequence\_filename ...]

**input\_count\_graph\_filename**

The input k-mer countgraph filename

**input\_sequence\_filename**

Input FAST[AQ] sequence filename

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**--threads <threads>, -T <threads>**

Number of simultaneous threads to execute

**--cutoff <cutoff>, -C <cutoff>**

Trim at k-mers below this abundance.

**--variable-coverage, -V**

Only trim low-abundance k-mers from sequences that have high coverage.

**--normalize-to <normalize\_to>, -Z <normalize\_to>**

Base the variable-coverage cutoff on this median k-mer abundance.

**-o <optional\_output\_filename>, --output <optional\_output\_filename>**

Output the trimmed sequences into a single file with the given filename instead of creating a new file for each input file.

**-f, --force**

Overwrite output file if it exists

**-q, --quiet**

**--gzip**

Compress output using gzip

**--bzip**

Compress output using bzip2

Trimmed sequences will be placed in `${input_sequence_filename}.abundfilt` for each input sequence file. If the input sequences are from RNAseq or metagenome sequencing then `--variable-coverage` should be used.

Example:

```
load-into-counting.py -k 20 -x 5e7 countgraph data/100k-filtered.fa
filter-abund.py -C 2 countgraph data/100k-filtered.fa
```

### filter-abund-single.py

Trims sequences at a minimum k-mer abundance (in memory version).

usage: filter-abund-single.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [--small-count] [--threads THREADS] [--cutoff CUTOFF] [--variable-coverage] [--normalize-to NORMALIZE\_TO] [--savegraph filename] [-o optional\_output\_filename] [-f] [-q] [--gzip | -bzip] input\_sequence\_filename

#### **input\_sequence\_filename**

FAST[AQ] sequence file to trim

#### **--version**

show program's version number and exit

#### **--info**

#### **-h, --help**

show this help message and exit

#### **--ksize <ksize>, -k <ksize>**

k-mer size to use

#### **--n\_tables <n\_tables>, -N <n\_tables>**

number of tables to use in k-mer countgraph

#### **-U <unique\_kmers>, --unique-kmers <unique\_kmers>**

approximate number of unique kmers in the input set

#### **--fp-rate <fp\_rate>**

Override the automatic FP rate setting for the current script

#### **--max-tablesize <max\_tablesize>, -x <max\_tablesize>**

upper bound on tablesizes to use; overrides -max-memory-usage/-M

#### **-M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>**

maximum amount of memory to use for data structure

#### **--small-count**

Reduce memory usage by using a smaller counter for individual kmers.

#### **--threads <threads>, -T <threads>**

Number of simultaneous threads to execute

#### **--cutoff <cutoff>, -C <cutoff>**

Trim at k-mers below this abundance.

#### **--variable-coverage, -V**

Only trim low-abundance k-mers from sequences that have high coverage.

#### **--normalize-to <normalize\_to>, -Z <normalize\_to>**

Base the variable-coverage cutoff on this median k-mer abundance.

#### **--savegraph <filename>**

If present, the name of the file to save the k-mer countgraph to

**-o** <optional\_output\_filename>, **--outfile** <optional\_output\_filename>  
 Override default output filename and output trimmed sequences into a file with the given filename.

**-f**, **--force**  
 Overwrite output file if it exists

**-q**, **--quiet**

**--gzip**  
 Compress output using gzip

**--bzip**  
 Compress output using bzip2

Trimmed sequences will be placed in `${input_sequence_filename}.abundfilt`.

This script is constant memory.

To trim reads based on k-mer abundance across multiple files, use **load-into-counting.py** and **filter-abund.py**.

Example:

```
filter-abund-single.py -k 20 -x 5e7 -C 2 data/100k-filtered.fa
```

### trim-low-abund.py

Trim low-abundance k-mers using a streaming algorithm.

usage: trim-low-abund.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-small-count] [-cutoff CUTOFF] [-trim-at-coverage TRIM\_AT\_COVERAGE] [-o output\_filename] [-variable-coverage] [-l filename] [-s filename] [-q] [-force] [-ignore-pairs] [-tempdir TEMPDIR] [-gzip | -bzip] [-diginorm] [-diginorm-coverage DIGI-NORM\_COVERAGE] [-single-pass] input\_filenames [input\_filenames ...]

**input\_filenames**

**--version**  
 show program's version number and exit

**--info**

**-h**, **--help**  
 show this help message and exit

**--ksize** <ksize>, **-k** <ksize>  
 k-mer size to use

**--n\_tables** <n\_tables>, **-N** <n\_tables>  
 number of tables to use in k-mer countgraph

**-U** <unique\_kmers>, **--unique-kmers** <unique\_kmers>  
 approximate number of unique kmers in the input set

**--fp-rate** <fp\_rate>  
 Override the automatic FP rate setting for the current script

**--max-tablesize** <max\_tablesize>, **-x** <max\_tablesize>  
 upper bound on tablesize to use; overrides `-max-memory-usage/-M`

**-M** <max\_memory\_usage>, **--max-memory-usage** <max\_memory\_usage>  
 maximum amount of memory to use for data structure

**--small-count**  
Reduce memory usage by using a smaller counter for individual kmers.

**--cutoff** <cutoff>, **-C** <cutoff>  
remove k-mers below this abundance

**--trim-at-coverage** <trim\_at\_coverage>, **-Z** <trim\_at\_coverage>, **--normalize-to** <trim\_at\_coverage>  
trim reads when entire read above this coverage

**-o** <output\_filename>, **--output** <output\_filename>  
only output a single file with the specified filename; use a single dash “-” to specify that output should go to STDOUT (the terminal)

**--variable-coverage**, **-V**  
Only trim low-abundance k-mers from sequences that have high coverage.

**-l** <filename>, **--loadgraph** <filename>  
load a precomputed k-mer graph from disk

**-s** <filename>, **--savegraph** <filename>  
save the k-mer countgraph to disk after allreads are loaded.

**-q**, **--quiet**

**--force**

**--ignore-pairs**

**--tempdir** <tempdir>, **-T** <tempdir>  
Set location of temporary directory for second pass

**--gzip**  
Compress output using gzip

**--bzip**  
Compress output using bzip2

**--diginorm**  
Eliminate high-coverage reads altogether (digital normalization).

**--diginorm-coverage** <diginorm\_coverage>  
Coverage threshold for **-diginorm**

**--single-pass**  
Do not do a second pass across the low coverage data

The output is one file for each input file, <input file>.abundtrim, placed in the current directory. This output contains the input sequences trimmed at low-abundance k-mers.

The **-V/--variable-coverage** parameter will, if specified, prevent elimination of low-abundance reads by only trimming low-abundance k-mers from high-abundance reads; use this for non-genomic data sets that may have variable coverage.

Note that the output reads will not necessarily be in the same order as the reads in the input files; if this is an important consideration, use **load-into-counting.py** and **filter-abund.py**. However, read pairs will be kept together, in “broken-paired” format; you can use **extract-paired-reads.py** to extract read pairs and orphans.

Example:

```
trim-low-abund.py -x 5e7 -k 20 -C 2 data/100k-filtered.fa
```

### count-median.py

Count k-mers summary stats for sequences

usage: count-median.py [-version] [-info] [-h] [-f] input\_count\_graph\_filename input\_sequence\_filename output\_summary\_filename

**input\_count\_graph\_filename**  
input k-mer countgraph filename

**input\_sequence\_filename**  
input FAST[AQ] sequence filename

**output\_summary\_filename**  
output summary filename

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**-f, --force**  
Overwrite output file if it exists

Count the median/avg k-mer abundance for each sequence in the input file, based on the k-mer counts in the given k-mer countgraph. Can be used to estimate expression levels (mRNAseq) or coverage (genomic/metagenomic).

The output file contains sequence id, median, average, stddev, and seq length, in comma-separated value (CSV) format.

Example:

```
load-into-counting.py counts tests/test-data/test-reads.fq.gz
count-median.py counts tests/test-data/test-reads.fq.gz medians.txt
```

NOTE: All 'N's in the input sequences are converted to 'A's.

### unique-kmers.py

Estimate number of unique k-mers, with precision  $\leq$  ERROR\_RATE.

usage: unique-kmers.py [-version] [-info] [-h] [-q] [-ksize KSIZE] [-error-rate ERROR\_RATE] [-report filename] [-stream-records] [-diagnostics] input\_sequence\_filename [input\_sequence\_filename ...]

**input\_sequence\_filename**  
Input FAST[AQ] sequence filename(s).

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**-q, --quiet**

**--ksize <ksize>, -k <ksize>**  
k-mer size to use

**--error-rate** <error\_rate>, **-e** <error\_rate>

Acceptable error rate

**--report** <filename>, **-R** <filename>

generate informational report and write to filename

**--stream-records**, **-S**

write input sequences to STDOUT

**--diagnostics**

print out recommended tablesize arguments and restrictions

A HyperLogLog counter is used to do cardinality estimation. Since this counter is based on a tradeoff between precision and memory consumption, the `-e/--error-rate` can be used to control how much memory will be used. In practice the memory footprint is small even at low error rates (< 0.01).

`-k/--ksize` should be set to the desired k-mer size.

Informational output is sent to STDERR, but a report file can be generated with `-R/--report`.

`--stream-records` will write the sequences taken in to STDOUT. This is useful for workflows: count unique kmers in a stream, then do digital normalization.

`--diagnostics` will provide detailed options for tablesize and memory limitations for various false positive rates. This is useful for configuring other khmer scripts. This will be written to STDERR.

Example:

```
unique-kmers.py -k 17 tests/test-data/test-abund-read{-2,-3}.fa
```

Example:

```
unique-kmers.py -k 17 --diagnostics tests/test-data/test-abund-read.fa
```

Example:

```
unique-kmers.py --stream-records -k 17 tests/test-data/test-reads.fa | \  
normalize-by-median.py -k 17 -o normalized /dev/stdin
```

Example:

```
unique-kmers.py -R unique_count -k 30 \  
tests/test-data/test-abund-read-paired.fa
```

## Partitioning

### do-partition.py

Load, partition, and annotate FAST[AQ] sequences

usage: do-partition.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-threads THREADS] [-subset-size SUBSET\_SIZE] [-no-big-traverse] [-keep-subsets] [-f] graphbase input\_sequence\_filename [input\_sequence\_filename ...]

**graphbase**

base name for output files

**input\_sequence\_filename**

input FAST[AQ] sequence filenames

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--ksize <ksize>, -k <ksize>**  
k-mer size to use

**--n\_tables <n\_tables>, -N <n\_tables>**  
number of tables to use in k-mer countgraph

**-U <unique\_kmers>, --unique-kmers <unique\_kmers>**  
approximate number of unique kmers in the input set

**--fp-rate <fp\_rate>**  
Override the automatic FP rate setting for the current script

**--max-tablesize <max\_tablesize>, -x <max\_tablesize>**  
upper bound on tablesize to use; overrides `--max-memory-usage/-M`

**-M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>**  
maximum amount of memory to use for data structure

**--threads <threads>, -T <threads>**  
Number of simultaneous threads to execute

**--subset-size <subset\_size>, -s <subset\_size>**  
Set subset size (usually 1e5-1e6 is good)

**--no-big-traverse**  
Truncate graph joins at big traversals

**--keep-subsets**  
Keep individual subsets (default: False)

**-f, --force**  
Overwrite output file if it exists

Load in a set of sequences, partition them, merge the partitions, and annotate the original sequences files with the partition information.

This script combines the functionality of `load-graph.py`, `partition-graph.py`, `merge-partitions.py`, and `annotate-partitions.py` into one script. This is convenient but should probably not be used for large data sets, because `do-partition.py` doesn't provide save/resume functionality.

Example:

```
do-partition.py -k 20 example tests/test-data/random-20-a.fa
```

## load-graph.py

Load sequences into the compressible graph format plus optional tagset.

usage: `load-graph.py` [`--version`] [`--info`] [`-h`] [`--ksize KSIZE`] [`--n_tables N_TABLES`] [`-U UNIQUE_KMERS`] [`--fp-rate FP_RATE`] [`--max-tablesize MAX_TABLESIZE` | `-M MAX_MEMORY_USAGE`] [`--threads THREADS`] [`--no-build-tagset`] [`-f`] `output_nodigraph_filename` `input_sequence_filename` [`input_sequence_filename ...`]

**output\_nodigraph\_filename**  
output k-mer nodegraph filename.

**input\_sequence\_filename**  
input FAST[AQ] sequence filename

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--ksize <ksize>, -k <ksize>**  
k-mer size to use

**--n\_tables <n\_tables>, -N <n\_tables>**  
number of tables to use in k-mer countgraph

**-U <unique\_kmers>, --unique-kmers <unique\_kmers>**  
approximate number of unique kmers in the input set

**--fp-rate <fp\_rate>**  
Override the automatic FP rate setting for the current script

**--max-tablesize <max\_tablesize>, -x <max\_tablesize>**  
upper bound on tablesize to use; overrides **--max-memory-usage/-M**

**-M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>**  
maximum amount of memory to use for data structure

**--threads <threads>, -T <threads>**  
Number of simultaneous threads to execute

**--no-build-tagset, -n**  
Do NOT construct tagset while loading sequences

**-f, --force**  
Overwrite output file if it exists

See **extract-partitions.py** for a complete workflow.

## partition-graph.py

Partition a sequence graph based upon waypoint connectivity

usage: partition-graph.py [--version] [--info] [-h] [--stoptags filename] [--subset-size SUBSET\_SIZE] [--no-big-traverse] [-f] [--threads THREADS] basename

**basename**  
basename of the input k-mer nodegraph + tagset files

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--stoptags <filename>, -S <filename>**  
Use stoptags in this file during partitioning

**--subset-size <subset\_size>, -s <subset\_size>**  
Set subset size (usually 1e5-1e6 is good)

**--no-big-traverse**  
Truncate graph joins at big traversals

**-f, --force**  
Overwrite output file if it exists

**--threads <threads>, -T <threads>**  
Number of simultaneous threads to execute

The resulting partition maps are saved as `${basename}.subset.#.pmap` files.

See ‘Artifact removal’ to understand the stoptags argument.

### merge-partition.py

Merge partition map ‘.pmap’ files.

usage: merge-partition.py [-version] [-info] [-h] [-ksize KSIZE] [-keep-subsets] [-f] graphbase

**graphbase**  
basename for input and output files

**--version**  
show program’s version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--ksize <ksize>, -k <ksize>**  
k-mer size (default: 32)

**--keep-subsets**  
Keep individual subsets (default: False)

**-f, --force**  
Overwrite output file if it exists

Take the `${graphbase}.subset.#.pmap` files and merge them all into a single `${graphbase}.pmap.merged` file for **annotate-partitions.py** to use.

### annotate-partitions.py

Annotate sequences with partition IDs.

usage: annotate-partitions.py [-version] [-info] [-h] [-ksize KSIZE] [-f] graphbase input\_sequence\_filename [input\_sequence\_filename ...]

**graphbase**  
basename for input and output files

**input\_sequence\_filename**  
input FAST[AQ] sequences to annotate.

**--version**  
show program’s version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--ksize <ksize>, -k <ksize>**  
k-mer size (default: 32)

**-f, --force**  
Overwrite output file if it exists

Load in a partitionmap (generally produced by **partition-graph.py** or **merge-partitions.py**) and annotate the sequences in the given files with their partition IDs. Use **extract-partitions.py** to extract sequences into separate group files.

Example (results will be in random-20-a.fa.part):

```
load-graph.py -k 20 example tests/test-data/random-20-a.fa
partition-graph.py example
merge-partitions.py -k 20 example
annotate-partitions.py -k 20 example tests/test-data/random-20-a.fa
```

### **extract-partitions.py**

Separate sequences that are annotated with partitions into grouped files.

usage: extract-partitions.py [-version] [-info] [-h] [-max-size MAX\_SIZE] [-min-partition-size MIN\_PART\_SIZE] [-no-output-groups] [-output-unassigned] [-f] [-gzip | -bzip] output\_filename\_prefix input\_partition\_filename [input\_partition\_filename ...]

**output\_filename\_prefix**

**input\_partition\_filename**

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**--max-size <max\_size>, -X <max\_size>**  
Max group size (n sequences)

**--min-partition-size <min\_part\_size>, -m <min\_part\_size>**  
Minimum partition size worth keeping

**--no-output-groups, -n**  
Do not actually output groups files.

**--output-unassigned, -U**  
Output unassigned sequences, too

**-f, --force**  
Overwrite output file if it exists

**--gzip**  
Compress output using gzip

**--bzip**  
Compress output using bzip2

Example (results will be in example.group0000.fa):

```
load-graph.py -k 20 example tests/test-data/random-20-a.fa
partition-graph.py example
merge-partitions.py -k 20 example
annotate-partitions.py -k 20 example tests/test-data/random-20-a.fa
extract-partitions.py example random-20-a.fa.part
```

(**extract-partitions.py** will produce a partition size distribution in <base>.dist. The columns are: (1) number of reads, (2) count of partitions with n reads, (3) cumulative sum of partitions, (4) cumulative sum of reads.)

## Artifact removal

The following scripts are specialized scripts for finding and removing highly-connected k-mers (HCKs). See [Partitioning large data sets \(50m+ reads\)](#).

**make-initial-stoptags.py** Find an initial set of highly connected k-mers.

usage: make-initial-stoptags.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-small-count] [-subset-size SUBSET\_SIZE] [-stoptags filename] [-f] graphbase

### graphbase

basename for input and output filenames

### --version

show program's version number and exit

### --info

### -h, --help

show this help message and exit

### --ksize <ksize>, -k <ksize>

k-mer size to use

### --n\_tables <n\_tables>, -N <n\_tables>

number of tables to use in k-mer countgraph

### -U <unique\_kmers>, --unique-kmers <unique\_kmers>

approximate number of unique kmers in the input set

### --fp-rate <fp\_rate>

Override the automatic FP rate setting for the current script

### --max-tablesize <max\_tablesize>, -x <max\_tablesize>

upper bound on tablesizes to use; overrides -max-memory-usage/-M

### -M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>

maximum amount of memory to use for data structure

### --small-count

Reduce memory usage by using a smaller counter for individual kmers.

### --subset-size <subset\_size>, -s <subset\_size>

Set subset size (default 1e4 is prob ok)

### --stoptags <filename>, -S <filename>

Use stoptags in this file during partitioning

### -f, --force

Overwrite output file if it exists

Loads a k-mer nodegraph/tagset pair created by **load-graph.py**, and does a small set of traversals from graph waypoints; on these traversals, looks for k-mers that are repeatedly traversed in high-density regions of the graph, i.e. are highly connected. Outputs those k-mers as an initial set of stoptags, which can be fed into **partition-graph.py**, **find-knots.py**, and **filter-stoptags.py**.

The k-mer countgraph size options parameters are for a k-mer countgraph to keep track of repeatedly-traversed k-mers. The subset size option specifies the number of waypoints from which to traverse; for highly connected data sets, the default (1000) is probably ok.

**find-knots.py** Find all highly connected k-mers.

usage: find-knots.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-small-count] [-f] graphbase

#### **graphbase**

Basename for the input and output files.

#### **--version**

show program's version number and exit

#### **--info**

#### **-h, --help**

show this help message and exit

#### **--ksize <ksize>, -k <ksize>**

k-mer size to use

#### **--n\_tables <n\_tables>, -N <n\_tables>**

number of tables to use in k-mer countgraph

#### **-U <unique\_kmers>, --unique-kmers <unique\_kmers>**

approximate number of unique kmers in the input set

#### **--fp-rate <fp\_rate>**

Override the automatic FP rate setting for the current script

#### **--max-tablesize <max\_tablesize>, -x <max\_tablesize>**

upper bound on tablesize to use; overrides -max-memory-usage/-M

#### **-M <max\_memory\_usage>, --max-memory-usage <max\_memory\_usage>**

maximum amount of memory to use for data structure

#### **--small-count**

Reduce memory usage by using a smaller counter for individual kmers.

#### **-f, --force**

Continue past warnings

Load an k-mer nodegraph/tagset pair created by **load-graph.py**, and a set of pmap files created by **partition-graph.py**. Go through each pmap file, select the largest partition in each, and do the same kind of traversal as in **make-initial-stoptags.py** from each of the waypoints in that partition; this should identify all of the Highly Connected Kmers in that partition. These HCKs are output to **<graphbase>.stoptags** after each pmap file.

Parameter choice is reasonably important. See the pipeline in [Partitioning large data sets \(50m+ reads\)](#) for an example run.

This script is not very scalable and may blow up memory and die horribly. You should be able to use the intermediate stoptags to restart the process, and if you eliminate the already-processed pmap files, you can continue where you left off.

**filter-stoptags.py** Trim sequences at stoptags.

usage: filter-stoptags.py [-version] [-info] [-h] [-ksize KSIZE] [-f] input\_stoptags\_filename input\_sequence\_filename [input\_sequence\_filename ...]

**input\_stoptags\_filename**

**input\_sequence\_filename**

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**--ksize <ksize>, -k <ksize>**

k-mer size

**-f, --force**

Overwrite output file if it exists

Load stoptags in from the given *.stoptags* file and use them to trim or remove the sequences in *<file1-N>*. Trimmed sequences will be placed in *<fileN>.stopfilt*.

## Digital normalization

### normalize-by-median.py

Do digital normalization (remove mostly redundant sequences)

usage: normalize-by-median.py [-version] [-info] [-h] [-ksize KSIZE] [-n\_tables N\_TABLES] [-U UNIQUE\_KMERS] [-fp-rate FP\_RATE] [-max-tablesize MAX\_TABLESIZE | -M MAX\_MEMORY\_USAGE] [-small-count] [-q] [-C CUTOFF] [-p] [-force\_single] [-u unpaired\_reads\_filename] [-s filename] [-R report\_filename] [-report-frequency report\_frequency] [-f] [-o filename] [-l filename] [-gzip | -bzip] input\_sequence\_filename [input\_sequence\_filename ...]

**input\_sequence\_filename**

Input FAST[AQ] sequence filename.

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**--ksize <ksize>, -k <ksize>**

k-mer size to use

**--n\_tables <n\_tables>, -N <n\_tables>**

number of tables to use in k-mer countgraph

**-U <unique\_kmers>, --unique-kmers <unique\_kmers>**

approximate number of unique kmers in the input set

**--fp-rate <fp\_rate>**

Override the automatic FP rate setting for the current script

```

--max-tablesize <max_tablesize>, -x <max_tablesize>
    upper bound on tablesize to use; overrides --max-memory-usage/-M
-M <max_memory_usage>, --max-memory-usage <max_memory_usage>
    maximum amount of memory to use for data structure
--small-count
    Reduce memory usage by using a smaller counter for individual kmers.
-q, --quiet
-C <cutoff>, --cutoff <cutoff>
    when the median k-mer coverage level is above this number the read is not kept.
-p, --paired
    require that all sequences be properly paired
--force_single
    treat all sequences as single-ended/unpaired
-u <unpaired_reads_filename>, --unpaired-reads <unpaired_reads_filename>
    include a file of unpaired reads to which -p/--paired does not apply.
-s <filename>, --savegraph <filename>
    save the k-mer countgraph to disk after all reads are loaded.
-R <report_filename>, --report <report_filename>
--report-frequency <report_frequency>
-f, --force
    continue past file reading errors
-o <filename>, --output <filename>
    only output a single file with the specified filename; use a single dash "-" to specify that output should go to
    STDOUT (the terminal)
-l <filename>, --loadgraph <filename>
    load a precomputed k-mer graph from disk
--gzip
    Compress output using gzip
--bzip
    Compress output using bzip2

```

Discard sequences based on whether or not their median k-mer abundance lies above a specified cutoff. Kept sequences will be placed in <fileN>.keep.

By default, paired end reads will be considered together; if either read should be kept, both will be kept. (This keeps both reads from a fragment, and helps with retention of repeats.) Unpaired reads are treated individually.

If `-p/--paired` is set, then proper pairing is required and the script will exit on unpaired reads, although `--unpaired-reads` can be used to supply a file of orphan reads to be read after the paired reads.

`--force_single` will ignore all pairing information and treat reads individually.

With `-s/--savegraph`, the k-mer countgraph will be saved to the specified file after all sequences have been processed. `-l/--loadgraph` will load the specified k-mer countgraph before processing the specified files. Note that these graphs are in the same format as those produced by `load-into-counting.py` and consumed by `abundance-dist.py`.

To append reads to an output file (rather than overwriting it), send output to STDOUT with `-output -` and use UNIX file redirection syntax (`>>`) to append to the file.

Example:

```
normalize-by-median.py -k 17 tests/test-data/test-abund-read-2.fa
```

Example:

```
normalize-by-median.py -p -k 17 \
tests/test-data/test-abund-read-paired.fa
```

Example:

```
normalize-by-median.py -p -k 17 -o - tests/test-data/paired.fq \
>> appended-output.fq
```

Example:

```
normalize-by-median.py -k 17 -f tests/test-data/test-error-reads.fq \
tests/test-data/test-fastq-reads.fq
```

Example:

```
normalize-by-median.py -k 17 -s test.ct \
tests/test-data/test-abund-read-2.fa \
tests/test-data/test-fastq-reads.fq
```

## Read handling: interleaving, splitting, etc.

### extract-long-sequences.py

Extract FASTQ or FASTA sequences longer than specified length (default: 200 bp).

usage: extract-long-sequences.py [--version] [--info] [-h] [-o output] [-l LENGTH] [--gzip | -bzip] input\_filenames [input\_filenames ...]

#### input\_filenames

Input FAST[AQ] sequence filename.

#### --version

show program's version number and exit

#### --info

#### -h, --help

show this help message and exit

#### -o <output>, --output <output>

The name of the output sequence file.

#### -l <length>, --length <length>

The minimum length of the sequence file.

#### --gzip

Compress output using gzip

#### --bzip

Compress output using bzip2

Example:

```
extract-long-sequences.py --length 10 tests/test-data/paired-mixed.fa
```

### extract-paired-reads.py

Take a mixture of reads and split into pairs and orphans.

usage: extract-paired-reads.py [-version] [-info] [-h] [-d OUTPUT\_DIR] [-output-paired filename] [-output-single filename] [-f] [-gzip | -bzip] [infile]

**infile**

**--version**

show program's version number and exit

**--info**

**-h, --help**

show this help message and exit

**-d <output\_dir>, --output-dir <output\_dir>**

Output split reads to specified directory. Creates directory if necessary

**--output-paired <filename>, -p <filename>**

Output paired reads to this file

**--output-single <filename>, -s <filename>**

Output orphaned reads to this file

**-f, --force**

Overwrite output file if it exists

**--gzip**

Compress output using gzip

**--bzip**

Compress output using bzip2

Many read-handling programs (assemblers, mappers, etc.) require that you give them either perfectly interleaved files, or files containing only single reads. This script takes files that were originally interleaved but where reads may have been orphaned (via error filtering, application of abundance filtering, digital normalization in non-paired mode, or partitioning) and separates the interleaved reads from the orphaned reads.

The default output is two files, *<input file>.pe* and *<input file>.se*, placed in the current directory. The *.pe* file contains interleaved and properly paired sequences, while the *.se* file contains orphan sequences.

The directory into which the interleaved and orphaned reads are output may be specified using *-d/--output-dir*. This directory will be created if it does not already exist.

Alternatively, you can specify the filenames directly with *-p/--output-paired* and *-s/--output-single*, which will override the *-d/--output-dir* option.

Example:

```
extract-paired-reads.py tests/test-data/paired.fq
```

### fastq-to-fasta.py

Converts FASTQ format (.fq) files to FASTA format (.fa).

usage: fastq-to-fasta.py [-version] [-info] [-h] [-o filename] [-n] [-gzip | -bzip] input\_sequence

**input\_sequence**

The name of the input FASTQ sequence file.

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**-o <filename>, --output <filename>**  
The name of the output FASTA sequence file.

**-n, --n\_keep**  
Option to keep reads containing 'N's in input\_sequence file. Default is to drop reads

**--gzip**  
Compress output using gzip

**--bzip**  
Compress output using bzip2

### interleave-reads.py

Produce interleaved files from R1/R2 paired files

usage: interleave-reads.py [-version] [-info] [-h] [-o filename] [-no-reformat] [-f] [-gzip | -bzip] left right

**left**

**right**

**--version**  
show program's version number and exit

**--info**

**-h, --help**  
show this help message and exit

**-o <filename>, --output <filename>**

**--no-reformat**  
Do not reformat read names or enforce consistency

**-f, --force**  
Overwrite output file if it exists

**--gzip**  
Compress output using gzip

**--bzip**  
Compress output using bzip2

The output is an interleaved set of reads, with each read in <R1> paired with a read in <R2>. By default, the output goes to stdout unless *-o/--output* is specified.

As a “bonus”, this file ensures that if read names are not already formatted properly, they are reformatted consistently, such that they look like the pre-1.8 Casava format (*@name/1*, *@name/2*). This reformatting can be switched off with the *--no-reformat* flag.

Example:

```
interleave-reads.py tests/test-data/paired.fq.1 \
    tests/test-data/paired.fq.2 -o paired.fq
```

### readstats.py

Display summary statistics for one or more FASTA/FASTQ files.

usage: readstats.py [-version] [-info] [-h] [-o filename] [-csv] filenames [filenames ...]

#### filenames

#### --version

show program's version number and exit

#### --info

#### -h, --help

show this help message and exit

#### -o <filename>, --output <filename>

output file for statistics; defaults to stdout.

#### --csv

Use the CSV format for the statistics, including column headers.

Report number of bases, number of sequences, and average sequence length for one or more FASTA/FASTQ files; and report aggregate statistics at end.

With `-o/--output`, the output will be saved to the specified file.

Example:

```
readstats.py tests/test-data/test-abund-read-2.fa
```

### sample-reads-randomly.py

Uniformly subsample sequences from a collection of files

usage: sample-reads-randomly.py [-version] [-info] [-h] [-N NUM\_READS] [-M MAX\_READS] [-S NUM\_SAMPLES] [-R RANDOM\_SEED] [-force\_single] [-o filename] [-f] [-gzip | -bzip] filenames [filenames ...]

#### filenames

#### --version

show program's version number and exit

#### --info

#### -h, --help

show this help message and exit

#### -N <num\_reads>, --num\_reads <num\_reads>

#### -M <max\_reads>, --max\_reads <max\_reads>

#### -S <num\_samples>, --samples <num\_samples>

#### -R <random\_seed>, --random-seed <random\_seed>

Provide a random seed for the generator

#### --force\_single

Ignore read pair information if present

#### -o <filename>, --output <filename>

#### -f, --force

Overwrite output file if it exists

**--gzip**

Compress output using gzip

**--bzip**

Compress output using bzip2

Take a list of files containing sequences, and subsample 100,000 sequences (*-N/--num\_reads*) uniformly, using reservoir sampling. Stop after first 100m sequences (*-M/--max\_reads*). By default take one subsample, but take *-S/--samples* samples if specified.

The output is placed in *-o/--output <file>* (for a single sample) or in *<file>.subset.0* to *<file>.subset.S-1* (for more than one sample).

This script uses the [reservoir sampling](#) algorithm.

**split-paired-reads.py**

Split interleaved reads into two files, left and right.

usage: split-paired-reads.py [-version] [-info] [-h] [-d output\_directory] [-0 output\_orphaned] [-1 output\_first] [-2 output\_second] [-f] [-gzip | -bzip] [infile]

**infile****--version**

show program's version number and exit

**--info****-h, --help**

show this help message and exit

**-d <output\_directory>, --output-dir <output\_directory>**

Output split reads to specified directory. Creates directory if necessary

**-0 <output\_orphaned>, --output-orphaned <output\_orphaned>**

Allow "orphaned" reads and extract them to this file

**-1 <output\_first>, --output-first <output\_first>**

Output "left" reads to this file

**-2 <output\_second>, --output-second <output\_second>**

Output "right" reads to this file

**-f, --force**

Overwrite output file if it exists

**--gzip**

Compress output using gzip

**--bzip**

Compress output using bzip2

Some programs want paired-end read input in the One True Format, which is interleaved; other programs want input in the Insanely Bad Format, with left- and right- reads separated. This reformats the former to the latter.

The directory into which the left- and right- reads are output may be specified using *-d/--output-dir*. This directory will be created if it does not already exist.

Alternatively, you can specify the filenames directly with *-1/--output-first* and *-2/--output-second*, which will override the *-d/--output-dir* setting on a file-specific basis.

`-O:option:’-output-orphans’` will allow broken-paired format, and orphaned reads will be saved separately, to the specified file.

Example:

```
split-paired-reads.py tests/test-data/paired.fq
```

Example:

```
split-paired-reads.py -O reads-output-file tests/test-data/paired.fq
```

Example:

```
split-paired-reads.py -1 reads.1 -2 reads.2 tests/test-data/paired.fq
```

## 2.6.5 Blog posts and additional documentation

### Hashtable and filtering

The basic inexact-matching approach used by the hashtable code is described in this blog post:

<http://ivory.idyll.org/blog/jul-10/kmer-filtering>

A test data set (soil metagenomics, 88m reads, 10gb) is here:

<http://ci.oxli.org/userContent/88m-reads.fa.gz>

### Illumina read abundance profiles

khmer can be used to look at systematic variations in k-mer statistics across Illumina reads; see, for example, this blog post:

<http://ivory.idyll.org/blog/jul-10/illumina-read-phenomenology>

The `fasta-to-abundance-hist` and `abundance-hist-by-position` scripts can be used to generate the k-mer abundance profile data, after loading all the k-mer counts into a `.ct` file:

```
# first, load all the k-mer counts:
load-into-counting.py -k 20 -x 1e7 25k.ct data/25k.fq.gz

# then, build the '.freq' file that contains all of the counts by position
python sandbox/fast-to-abundance-hist.py 25k.ct data/25k.fq.gz

# sum across positions.
python sandbox/abundance-hist-by-position.py data/25k.fq.gz.freq > out.dist
```

The hashtable method `‘dump_kmers_by_abundance’` can be used to dump high abundance k-mers, but we don’t have a script handy to do that yet.

You can assess high/low abundance k-mer distributions with the `hi-lo-abundance-by-position` script:

```
load-into-counting.py -k 20 25k.ct data/25k.fq.gz
python sandbox/hi-lo-abundance-by-position.py 25k.ct data/25k.fq.gz
```

This will produce two output files, `<filename>.pos.abund=1` and `<filename>.pos.abund=255`.

## Finding valleys/minima in $k$ -mer abundance profiles

Using  $k$ -mer abundance profiles to dynamically calculate the abundance threshold separating erroneous  $k$ -mers from real  $k$ -mers is described in this blog post:

<https://bitsandbugs.org/2016/07/29/mash-and-khmer-abundance/>

## 2.6.6 Setting khmer memory usage

If you look at the documentation for the scripts ([khmer's command-line interface](#)) you'll see a `-M` parameter that sets the maximum memory usage for any script that uses  $k$ -mer counting tables or  $k$ -mer graphs. What is this?

khmer uses a special data structure that lets it store counting tables and  $k$ -mer graphs in very low memory; the trick is that you must fix the amount of memory khmer can use before running it. (See [Pell et al., 2012](#) and [Zhang et al., 2014](#) for the details.) This is what the `-M` parameter does.

If you set it too low, khmer will warn you to set it higher at the end. See below for some good choices for various kinds of data.

**Note for khmer 1.x users:** As of khmer 2.0, the `-M` parameter sets the `-N/--n_tables` and `-x/--max-tablesize` parameters automatically. You can still set these parameters directly if you wish.

### The really short version

There is no way (except for experience, rules of thumb, and intuition) to know what this parameter should be up front. So, use the maximum available memory:

```
-M 16e9
```

for a machine with 16 GB of free memory, for example.

### The short version

This parameter specifies the maximum memory usage of the primary data structure in khmer, which is basically  $N$  big hash tables of size  $x$ . The **product** of the number of hash tables and the size of the hash tables specifies the total amount of memory used, which is what the `-M` parameter sets.

These tables are used to track  $k$ -mers. If they are too small, khmer will fail in various ways (and will complain), but there is no harm in making it too large. So, **the absolute safest thing to do is to specify as much memory as is available**. Most scripts will inform you of the total memory usage, and (at the end) will complain if it's too small.

Life is a bit more complicated than this, however, because some scripts – `load-into-counting.py` and `load-graph.py` – keep ancillary information that will consume memory beyond this table data structure. So if you run out of memory, decrease the table size.

Also see the rules of thumb, below.

### The long version

khmer's scripts, at their heart, represents  $k$ -mers in a very memory efficient way by taking advantage of two data structures, [Bloom filters](#) and [Count-Min Sketches](#), that are both *probabilistic* and *constant memory*. The “probabilistic” part means that there are false positives: the less memory you use, the more likely it is that khmer will think that  $k$ -mers are present when they are not, in fact, present.

Digital normalization (`normalize-by-median.py` and `filter-abund.py`) uses the Count-Min Sketch data structure.

Graph partitioning (`load-graph.py` etc.) uses the Bloom filter data structure.

The practical ramifications of this are pretty cool. For example, your digital normalization is guaranteed not to increase in memory utilization, and graph partitioning is estimated to be 10-20x more memory efficient than any other de Bruijn graph representation. And hash tables (which is what Bloom filters and Count-Min Sketches use) are really fast and efficient. Moreover, the optimal memory size for these primary data structures is dependent on the number of k-mers, but not explicitly on the size of k itself, which is very unusual.

In exchange for this memory efficiency, however, you gain a certain type of parameter complexity. Unlike your more typical k-mer package (like the Velvet assembler, or Jellyfish or Meryl or Tallymer), you are either guaranteed not to run out of memory (for digital normalization) or much less likely to do so (for partitioning).

The biggest problem with khmer is that there is a minimum hash number and size that you need to specify for a given number of k-mers, and you cannot confidently predict what it is before actually loading in the data. This, by the way, is also true for de Bruijn graph assemblers and all the other k-mer-based software – the final memory usage depends on the total number of k-mers, which in turn depends on the true size of your underlying genomic variation (e.g. genome or transcriptome size), the number of errors, and the k-mer size you choose (the k parameter) [ see Conway & Bromage, 2011 ]. **The number of reads or the size of your data set is only somewhat correlated with the total number of k-mers.** Trimming protocols, sequencing depth, and polymorphism rates are all important factors that affect k-mer count.

The bad news is that we don't have good ways to estimate total k-mer count a priori, although we can give you some rules of thumb, below. In fact, counting the total number of distinct k-mers is a somewhat annoying challenge. Frankly, we recommend *just guessing* instead of trying to be all scientific about it.

The good news is that you can never give khmer too much memory! k-mer counting and set membership simply gets more and more accurate as you feed it more memory. (Although there may be performance hits from memory I/O, e.g. see the NUMA architecture.) The other good news is that khmer can measure the false positive rate and detect dangerously low memory conditions. For partitioning, we actually *know* what a too-high false positive rate is – our [k-mer percolation paper](#) lays out the math. For digital normalization, we assume that a false positive rate of 10% is bad. In both cases the data-loading scripts will exit with an error-code.

### Rules of thumb

For digital normalization, we recommend:

- -M 8e9 for any amount of sequencing for a single microbial genome, MDA-amplified or single colony.
- -M 16e9 for up to a billion mRNAseq reads from any organism. Past that, increase it.
- -M 32e9 for most eukaryotic genome samples.
- -M 32e9 will also handle most “simple” metagenomic samples (HMP on down)
- For metagenomic samples that are more complex, such as soil or marine, start as high as possible. For example, we are using -M 256e9 for ~300 Gbp of soil reads.

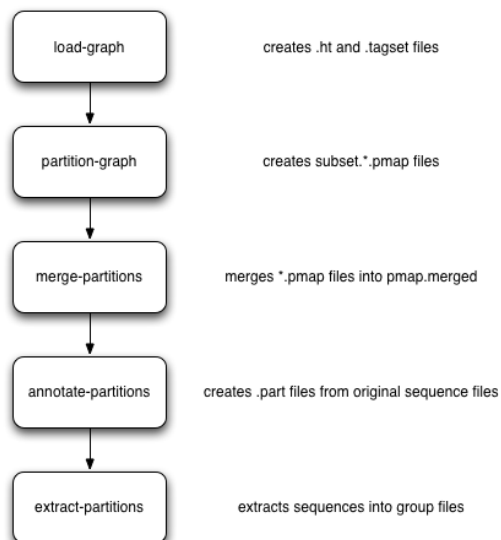
For partitioning of complex metagenome samples, we recommend starting as high as you can – something like half your system memory. So if you have 256 GB of RAM, use -M 128e9 which will use 128 GB of RAM for the basic graph storage, leaving other memory for the ancillary data structures.

### 2.6.7 Partitioning large data sets (50m+ reads)

“Partitioning” is what khmer calls the process of separating reads that do not connect to each other into different logical bins. The goal of partitioning is to apply divide & conquer to the process of metagenomic assembly.

## Basic partitioning

The basic workflow for partitioning is in the figure below:

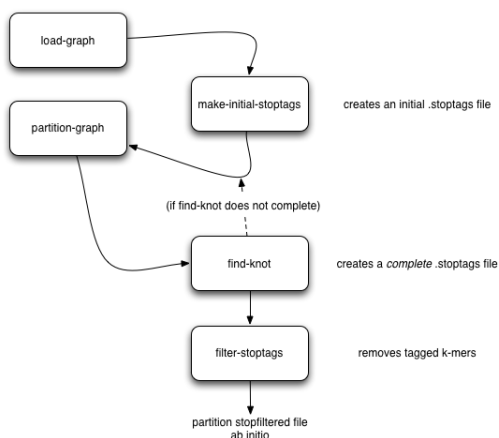


Briefly, you load everything into khmer’s probabilistic graph representation; exhaustively explore the graph to find all disconnected sequences; merge the results of the (parallelized) graph exploration; annotate sequences with their partition; and then extract the different partitions into files grouped by partition size. These groups can then be assembled individually.

## Artifact removal

As part of our partitioning research, we discovered that large Illumina data sets tend to contain a single large, connected component. This connected component seems to stem from sequencing artifacts that causes knots in the assembly graph. We have developed tools to forcibly remove the knot at the heart of the graph.

Here’s the workflow:



## Running on an example data set

Here is a set of commands for running both basic partitioning and artifact removal on a small soil metagenomics data set that we’ve made available for this purpose.

The data set is about 1.1G and you can download it from here:

<https://s3.amazonaws.com/public.ged.msu.edu/khmer/iowa-corn-50m.fa.gz>

```
cd /path/to/data

# the next command will create a '50m.ct' and a '50m.tagset',
# representing the de Bruijn graph
load-graph.py -k 32 -N 4 -x 16e9 50m iowa-corn-50m.fa.gz

# this will then partition that graph. should take a while.
# update threads to something higher if you have more cores.
# this creates a bunch of files, 50m.subset.*.pmap
partition-graph.py --threads 4 -s 1e5 50m

# now, merge the pmap files into one big pmap file, 50m.pmap.merged
merge-partitions.py 50m

# next, annotate the original sequences with their partition numbers.
# this will create iowa-corn-50m.fa.gz.part
annotate-partitions.py 50m iowa-corn-50m.fa.gz

# now, extract the partitions in groups into 'iowa-corn-50m.groupNNNN.fa'
extract-partitions.py iowa-corn-50m iowa-corn-50m.fa.gz.part

# at this point, you can assemble the group files individually. Note,
# however, that the last one them is quite big? this is because it's
# the lump! yay!

# if you want to break up the lump, go through the partitioning bit
# on the group file, but this time with a twist:
mv iowa-corn-50m.group0005.fa corn-50m.lump.fa

# create graph,
load-graph.py -x 8e9 lump corn-50m.lump.fa

# create an initial set of stoptags to help in knot-traversal; otherwise,
# partitioning and knot-traversal (which is systematic) is really expensive.
make-initial-stoptags.py lump

# now partition the graph, using the stoptags file
partition-graph.py --stoptags lump.stoptags lump

# use the partitioned subsets to find the k-mers that nucleate the lump
find-knots.py -x 2e8 -N 4 lump

# remove those k-mers from the fasta files
filter-stoptags.py *.stoptags corn-50m.lump.fa

# now, reload the filtered data set in and partition again.
# NOTE: 'load-graph.py' uses the file extension to determine
# if the file is formatted as FASTA or FASTQ. The default is
# fasta, therefore if your files are fastq formatted you need
# to append 'fastq' to the name so that 'load-graph.py'
# will parse the file correctly
load-graph.py -x 8e9 lumpfilt corn-50m.lump.fa.stopfilt
partition-graph.py -T 4 lumpfilt
merge-partitions.py lumpfilt
annotate-partitions.py lumpfilt corn-50m.lump.fa.stopfilt
```

```
extract-partitions.py corn-50m-lump corn-50m.lump.fa.stopfilt.part

# and voila, after all that, you should now have your de-knotted lump in
# corn-50m-lump.group*.fa. The *.group???.fa files can now be
# assembled individually by your favorite assembler.
```

## Post-partitioning assembly

The ‘extract-partitions’ script takes reads belonging to each partition and aggregates them into ‘group’ files; each group file contains at least one entire partition (and generally a lot more). Note, you can control the number of reads in each file (equiv, the size of these files) with some of the arguments that ‘extract-partitions’ takes.

Now that you have these files... what do you do with them? The short answer is: assemble them! Each of these group files contains reads that do not connect to reads in other files, so the files can be assembled individually (which is the whole point of partitioning).

If you’re using Velvet, checkout the `sandbox/velvet-assemble.sh` script, which you can run like this:

```
bash /path/to/khmer/sandbox/velvet-assemble.sh <groupfile> <k>
```

This script does three things:

- first, it breaks the reads up into paired reads and single reads, and puts them in separate files (.pe and .se);
- second, it strips off the partition information from the reads, which confuses Velvet;
- and third, it runs `velveth` and `velvetg` to actually assemble.

You can implement your own approach, of course, but this is an example of what we do ourselves.

## 2.6.8 Example API Usage

Examples of how to run khmer command-line scripts are included in the main khmer documentation, as well as the [khmer protocols collection](#). This document is intended for programmers and developers who want to use khmer’s Python or C++ API.

**Note:** The khmer command-line scripts are under [semantic versioning](#), but (as of khmer v2) neither the Python API nor the C++ API are covered under semantic versioning. Consequently, updates to the khmer code base may require changes to the examples below at any time. We expect to have the Python API under semantic versioning by version 5.0.

### Python API

The following example demonstrates the basics of counting k-mers with khmer.

```
>>> import khmer
>>>
>>> ksize = 11
>>>
>>> # exact counting with and odd k requires a single table with 4^k bytes
>>> # even k will collapse k-mers with their reverse complement, and requires 4^(k-1) + k bytes
>>> cg = khmer.Countgraph(ksize, 4**ksize, 1)
>>>
>>> # load all k-mers from the given string (returns the number of k-mers processed)
>>> cg.consume('ATGGCGATGGCAAGTAGGACCCAGATGGACCAAAG')
25
>>>
```

```
>>> # what is the count of the k-mer "ATGGCGATGGC"?
>>> cg.get('ATGGCGATGGC')
1
>>> # increase the count of the k-mer by 1
>>> cg.add('ATGGCGATGGC')
1
>>> # what is the k-mer's count now?
>>> cg.get('ATGGCGATGGC')
2
>>>
>>> # what is the count of this bogus k-mer?
>>> cg.get('GTGGCGATGGC')
0
>>>
```

## C++ API

The *count-stuff.cpp* file in the *examples/c++-api/* directory contains source code that calls khmer’s C++ API directly. To run this example, issue the following commands from your terminal. The *Makefile* in *examples/c++-api/* shows how to compile and link against the khmer library.

```
cd examples/c++-api/
make
./count-stuff
```

## 2.6.9 Known Issues

*load-graph.py* in multithreaded mode will find slightly different number of unique kmers. This is being investigated in <https://github.com/dib-lab/khmer/issues/1248>

### 2.6.10 Deploying the khmer project tools on Galaxy

This document is for people interested in deploying the khmer tools on the Galaxy platform.

---

We are developing the support for running all the khmer scripts in [Galaxy](#).

#### Install the tools & tool description

In the administrative interface select “Search and browse tool sheds” under the heading “Tool sheds”. Click on “Galaxy test tool shed” and search for khmer. Click on the “khmer” button and choose “Preview and install”. Click the “Install to Galaxy” button at the top. At the bottom of the next page click the “Install” button.

#### Single Output Usage

For one or more files into a single file:

1. Choose ‘Normalize By Median’ from the ‘khmer protocols’ section of the ‘Tools’ menu.
2. Compatible files already uploaded to your Galaxy instance should be listed. If not then you may need to [set their datatype manually](#).

3. After selecting the input files specify if they are paired-interleaved or not.
4. Specify the sample type or show the advanced parameters to set the tablesize yourself. Consult [Setting khmer memory usage](#) for assistance.

### 2.6.11 Papers using khmer

khmer was first published in [Scaling metagenome sequence assembly with probabilistic de Bruijn graphs](#), Pell et al., 2012.

Since then, we have [published several additional papers](#).

Please also see [the instructions for citing our papers and software](#).

#### Biological uses outside of the group

See [this list of papers](#) for publications using khmer to analyze their sequencing data.

#### Tools building on khmer concepts

Several [research papers](#) have built off of concepts introduced in or elaborated on khmer.

### 2.6.12 How to get help

First, be sure that you:

1. Read the documentation (this site)
2. Google search for the error output and/or keywords related to your problem. Here you can search results from the mailing list, where others may have discussed solutions to the same issue.

#### Mailing List

The primary way to get help is through the khmer discussion list: <http://lists.idyll.org/listinfo/khmer>, though we are also available for closer-to-realtime support via [Gitter](#).

#### Asking a question

1. Include your:
  - OS version (Mac OS X or Linux): `uname -mrs`
  - Python version: `python --version`
  - and khmer version: `pip freeze | grep khmer`
2. Precisely describe what you are trying to do. Reread it from the perspective of someone else trying to reproduce your task.
3. Copy-and-paste the exact command that is causing the problem. Include the steps you performed leading up to the issue.
4. Include the complete error message; if it is large, include a link to a file.

## GitHub

You are also welcome to report an issue you are having using GitHub: <https://github.com/dib-lab/khmer/issues/new>

## 2.7 The khmer developer documentation

This section of the documentation is for people who are contributing (or would like to contribute to) the khmer project codebase, either by contributing code or by helping improve the documentation.

Please note that this project is released with a [Contributor Code of Conduct](#). By participating in the development of this project you agree to abide by its terms.

Contents:

### 2.7.1 Contributor Code of Conduct

As contributors and maintainers of this project, we pledge to respect all people who contribute through reporting issues, posting feature requests, updating documentation, submitting pull requests or patches, and other activities.

We are committed to making participation in this project a harassment-free experience for everyone, regardless of level of experience, gender, gender identity and expression, sexual orientation, disability, personal appearance, body size, race, age, or religion.

Examples of unacceptable behavior by participants include the use of sexual language or imagery, derogatory comments or personal attacks, trolling, public or private harassment, insults, or other unprofessional conduct.

Project maintainers have the right and responsibility to remove, edit, or reject comments, commits, code, wiki edits, issues, and other contributions that are not aligned to this Code of Conduct. Project maintainers or contributors who do not follow the Code of Conduct may be removed from the project team.

Instances of abusive, harassing, or otherwise unacceptable behavior may be reported by emailing [khmer-project@idyll.org](mailto:khmer-project@idyll.org) which only goes to C. Titus Brown and Michael R. Crusoe. To report an issue involving either of them please email [Judi Brown Clarke, Ph.D.](#), the Diversity Director at the BEACON Center for the Study of Evolution in Action, an NSF Center for Science and Technology.

This Code of Conduct is adapted from the [Contributor Covenant](#), version 1.0.0, available at <http://contributor-covenant.org/version/1/0/0/>

### 2.7.2 Getting started with khmer development

#### Contents

- *Getting started with khmer development*
  - *One-time Preparation*
  - *Building khmer and running the tests*
  - *Claiming an issue and starting to develop*
  - *After your first issue is successfully merged...*
  - *Your second contribution...*
  - *Advanced merging with git-merge-changelog*
  - *Pull request cleanup (commit squashing)*

This document is for people who would like to contribute to khmer. It walks first-time contributors through making their own copy of khmer, building it, and submitting changes for review and merge into the master copy of khmer.

---

Start by making your own copy of khmer and setting yourself up for development; then, build khmer and run the tests; and finally, claim an issue and start developing!

If you're unfamiliar with git and branching in particular, check out the [git-scm book](#).

We've provided a quick guide to the khmer code base here: [A quick guide to the khmer codebase](#).

## One-time Preparation

1. Install the dependencies.

OS X users

- (a) Install Xcode from the [Mac App Store](#) (requires root).
- (b) [Register as an Apple Developer](#).
- (c) Install the Xcode command-line tools: Xcode -> preferences -> Downloads -> Command Line Tools (requires root).

Linux users

- (a) Install the python development environment, virtualenv, pip, gcc, and g++.

On recent Debian and Ubuntu this can be done with:

```
sudo apt-get install python2.7-dev python-virtualenv python-pip gcc \
g++ git astyle gcovr cppcheck enchant
```

For RHEL6:

```
sudo yum install -y python-devel python-pip git gcc gcc-c++ make enchant
sudo pip install virtualenv
```

2. Get a [GitHub](#) account.

(We use GitHub to manage khmer contributions.)

3. Fork [github.com/dib-lab/khmer](#).

Visit that page, and then click on the 'fork' button (upper right).

(This makes a copy of the khmer source code in your own GitHub account.)

4. Clone your copy of khmer to your local development environment.

Your clone URL should look something like this:

```
https://github.com/empty-titus/khmer.git
```

and the UNIX shell command should be:

```
git clone https://github.com/empty-titus/khmer.git
```

(This makes a local copy of khmer on your development machine.)

5. Add a git reference to the khmer dib-lab repository:

```
cd khmer
git remote add dib https://github.com/dib-lab/khmer.git
cd ../
```

(This makes it easy for you to pull down the latest changes in the main repository.)

6. Create a virtual Python environment within which to work with [virtualenv](#):

```
python2.7 -m virtualenv env
```

This gives you a place to install packages necessary for running khmer.

OS X users and others may need to download virtualenv first:

```
curl -O https://pypi.python.org/packages/source/v/virtualenv/virtualenv-1.11.6.tar.gz
tar xzf virtualenv*
cd virtualenv-*; python2.7 virtualenv.py ../env; cd ..
```

[Mac ports](#) users on the OS X platform can install pip by execution from the command line:

```
sudo port install py27-pip
```

[Homebrew](#) users on the OS X platform will have pip already installed

[Conda](#) users on any platform should instead create a separate Conda environment:

```
conda create -n khmer anaconda
```

7. Activate the virtualenv and install a few packages:

```
source env/bin/activate
cd khmer
make install-dependencies
```

(This installs [Sphinx](#) and [pytest](#), packages we use for building the documentation and running the tests.)

In Conda to activate the previously created environment and install dependencies:

```
source activate khmer
cd khmer
make install-dependencies
```

8. Cppcheck installation:

[Debian](#) and [Ubuntu](#) Linux distro users can install cppcheck by executing from the command line:

```
sudo apt-get install cppcheck
```

[Mac ports](#) users on the OS X platform can install cppcheck by executing from the command line:

```
sudo port install cppcheck
```

[Homebrew](#) users on the OS X platform can install cppcheck by executing from the command line:

```
sudo brew install cppcheck
```

9. ccache installation:

Debian and Ubuntu Linux distro users can install ccache to speed up their compile times:

```
sudo apt-get install ccache
echo 'export PATH="/usr/lib/ccache:$PATH" # enable ccache' >> ~/.bashrc
export PATH="/usr/lib/ccache:$PATH"
```

## Building khmer and running the tests

1. Activate (or re-activate) the virtualenv:

```
source ../env/bin/activate
```

... or for Conda users:

```
source activate khmer
```

You can run this many times without any ill effects.

(This puts you in the development environment.)

2. Build khmer:

```
make
```

If this fails, we apologize – please [go create a new issue](#), paste in the failure message, and we’ll try to help you work through it!

(This takes the C++ source code and compiles it into something that Python can run.)

3. Run the tests:

```
make test
```

You should see lots of output, with something like:

```
===== 658 passed, 22 deselected in 40.93 seconds =====
```

at the end.

(This will run all of the Python tests in the tests/ directory.)

Congratulations! You’re ready to develop!

## Claiming an issue and starting to develop

1. Find an open issue and claim it.

Go to [the list of open khmer issues](#) and find one you like; we suggest starting with [the low-hanging fruit issues](#)).

Once you’ve found an issue you like, make sure that no one has been assigned to it (see “assignee”, bottom right near “notifications”). Then, add a comment “I am working on this issue.” You’ve staked your claim!

(We’re trying to avoid having multiple people working on the same issue.)

2. In your local copy of the source code, update your master branch from the main khmer master branch:

```
git checkout master
git pull dib master
```

(This pulls in all of the latest changes from whatever we’ve been doing on dib-lab.)

It is possible that when you do a *git pull* you will get a “merge conflict” – This is what happens when something changed in the branch you’re pulling in in the same place you made a change in your local copy. This frequently happens in the *ChangeLog* file.

Git will complain loudly about merges and tell you specifically in which files they occurred. If you open the file, you’ll see something vaguely like this in the place where the merge occurred:

```
<<<<<< HEAD
Changes made on the branch that is being merged into. In most cases,
this is the branch that you have currently checked out
=====
Changes made on the branch that is being merged in, almost certainly
master.
>>>>>> abcde1234
```

Though there are a variety of tools to assist with resolving merge conflicts they can be quite complicated at first glance and it is usually easy enough to manually resolve the conflict.

To resolve the conflict you simply have to manually ‘meld’ the changes together and remove the merge markers.

After this you’ll have to add and commit the merge just like any other set of changes. It’s also recommended that you run tests.

### 3. Create a new branch and link it to your fork on GitHub:

```
git checkout -b fix/brief_issue_description
git push -u origin fix/brief_issue_description
```

where you replace “brief\_issue\_description” with 2-3 words, separated by underscores, describing the issue.

(This is the set of changes you’re going to ask to be merged into khmer.)

### 4. Make some changes and commit them.

Though this will largely be issue-dependent the basics of committing are simple. After you’ve made a cohesive set of changes, run the command *git status*. This will display a list of all the files git has noticed you changed. A file in the ‘untracked’ section are files that haven’t existed previously in the repository but git has noticed.

To commit changes you have to ‘stage’ them—this is done by issuing the following command:

```
git add path/to/file
```

If you have a large quantity of changes and you don’t want to add each file manually you can do `git add --patch` which will display each set of changes to you before staging them for commit.

Once you have staged your changes, it’s time to make a commit:

```
git commit
```

Git will then open your default console text editor to write a commit message – this is a short (typically 1-3 sentence) description of the changes you’ve made. Please make your commit message informative but concise – these messages become part of the ‘official’ history of the project.

Once your changes have been committed, push them up to the remote branch:

```
git push
```

If this is your first commit on a new branch git will error out, telling you the remote branch doesn’t exist – This is fine, as it will also provide the command to create the branch. Copy/paste/run and you should be set.

You should also visit and read [Coding guidelines and code review checklist](#).

### 5. Periodically update your branch from the main khmer master branch:

```
git pull dib master
```

(This pulls in all of the latest changes from whatever we’ve been doing on dib-lab - important especially during periods of fast change or for long-running pull requests.

### 6. Run the tests and/or build the docs *before* pushing to GitHub:

```
make doc test pep8 diff-cover
```

Make sure they all pass!

7. Push your branch to your own GitHub fork:

```
git push origin
```

(This pushes all of your changes to your own fork.)

8. Repeat until you're ready to merge your changes into "official" khmer.
9. Set up a Pull Request asking to merge things into the central khmer repository.

In a Web browser, go to your GitHub fork of khmer, e.g.:

```
https://github.com/empty-titus/khmer
```

and you will see a list of "recently pushed branches" just above the source code listing. On the right side of that should be a "Compare & pull request" green button. Click on it!

Now:

- add a descriptive title ("updated tests for XXX")
- put the issue number in the comment ("fixes issue #532")

then click "Create pull request."

(This creates a new issue where we can all discuss your proposed changes; the khmer team will be automatically notified and you will receive e-mail notifications as we add comments. See [GitHub flow](#) for more info.)

10. Paste in the committer checklist from [Coding guidelines and code review checklist](#) and, after its pasted in, check off as many of the boxes as you can.
11. As you add new commits to address bugs or formatting issues, you can keep pushing your changes to the pull request by doing:

```
git push origin
```

12. When you are ready to have the pull request reviewed, please mention @luizirber, @camillescott, @mr-c, or @ctb with a comment 'Ready for review!'
13. The khmer team will now review your pull request and communicate with you through the pull request page. Please feel free to add 'ping!' and an @ in the comments if you are looking for feedback – this will alert us that you are still on the line – but we will automatically get notified of your pull request and any new comments, so use sparingly.  
  
If this is still your first issue, please *don't* take another issue until we've merged your first one - thanks!
14. If we request changes, return to the step "Make some changes and commit them" and go from there. Any additional commits you make and push to your branch will automatically be added to the pull request (which is pretty dang cool.)

### After your first issue is successfully merged...

You're now an experienced GitHub user! Go ahead and take some more tasks; you can broaden out beyond the low hanging fruit if you like.

Here are a few suggestions:

- If you're knowledgeable in C++ and/or Python and/or documentation and/or biology, we'd love to attract further contributions to khmer. Please visit the issues list and browse about and find something interesting looking.

- One general thing we'd like to do is increase our test coverage. You can go find test coverage information on [our continuous integration server](#) by clicking down to individual files; or, ask us on [khmer-project@idyll.org](mailto:khmer-project@idyll.org) for suggestions.
- Ask us! Ask [khmer-project@idyll.org](mailto:khmer-project@idyll.org) for suggestions on what to do next. We can suggest particularly ripe low-hanging fruit, or find some other issues that suit your interests and background.
- You can also help other people out by watching for new issues or looking at pull requests. Remember to be nice and polite!

## Your second contribution...

Here are a few pointers on getting started on your second (or third, or fourth, or nth contribution).

So, assuming you've found an issue you'd like to work on there are a couple things to do to make sure your local copy of the repository is ready for a new issue—specifically, we need to make sure it's in sync with the remote repository so you aren't working on a old copy. So:

```
git checkout master
git fetch --all
git pull
```

This puts you on the latest master branch and pulls down updates from GitHub with any changes that may have been made since your last contribution (usually including the merge of your last contribution). Then we merge those changes into your local copy of the master branch.

Now, you can go back to *Claiming an issue and starting to develop*.

## Advanced merging with git-merge-changelog

Often one can get a merge conflict due to updates in the ChangeLog. To teach Git how to handle these on its own you can install a special merge driver.

On Debian & Ubuntu systems you'll need the *git-merge-changelog* package:

```
sudo apt-get install git-merge-changelog
```

Ubuntu 14.04 LTS users will need to add an external repository that contains a backport of the package first before installing:

```
sudo apt-add-repository ppa:misterc/gedlab
sudo apt-get update
sudo apt-get install git-merge-changelog
```

Everyone should then update their *~/.gitconfig* file with the following:

```
[merge "merge-changelog"]
    name = GNU-style ChangeLog merge driver
    driver = /usr/bin/git-merge-changelog %O %A %B
```

## Pull request cleanup (commit squashing)

Submitters are invited to reduce the numbers of commits in their pull requests either via *git rebase -i* *dib/master* or this recipe:

```
git pull # make sure the local is up to date
git pull dib master # get up to date
# fix any merge conflicts
git status # sanity check
git diff dib/master # does the diff look correct? (no merge markers)
git reset --soft dib/master # un-commit the differences from dib/master
git status # sanity check
git commit --all # package all differences in one commit
git status # sanity check
git push # should fail
git push --force # override what's in GitHub's copy of the branch/pull request
```

## 2.7.3 A quick guide to testing (for khmer)

This document is for contributors new to automated testing, and explains some of the motivation and logic behind the khmer project’s testing approach.

One of our most important “secret sauces” for khmer development is that we do a fair bit of testing to make sure our code works and keeps working!

- We maintain fairly complete test coverage of our code. What this means is that we have automated tests that, when run, execute most of the lines of Python and C++ code in our lib/, khmer/ and scripts/ directories. This doesn’t *guarantee* things are correct, but it does mean that at least most of the code works at some basic level.
- we have other tests that we run periodically (for example, before each release) – see [Releasing a new version of khmer](#) for details. These tests check that our code works on multiple systems and with other people’s software.

CTB and others have written a great deal about testing, and testing in Python in particular. Here’s an [introductory guide](#) CTB wrote a long time ago. You might also be interested in reading [this description of the different kinds of tests](#).

For the more general motivation, see [the Lack of Testing Death Spiral](#).

But... how do you do testing??

First, let’s talk about specific goals for testing. What should you be aiming for tests to do? You can always add more testing code, but that might not be useful if they are redundant or over-complicated.

An overall rule is to “keep it simple” – keep things as simple as possible, testing as few things as possible in each test.

We suggest the following approach to writing tests for **new code**:

1. Write a test that just *runs* the new code, generally by copying existing test code to a new test and changing it. Don’t do anything clever for the first test – just run something straightforward, and try to use existing data.
2. Decide which use cases should be tested. This is necessarily code specific but our main advice is “don’t be clever” – write some tests to make sure that the code basically works.
3. Add in tests for edge cases. By this we mean look for special cases in your code – if statements, fence-post bound errors, etc. – and write tests that exercise those bits of code specifically.
4. Make sure tests that expect a function call to fail (esp. with fail\_ok=True) are failing for the expected reason. Run the code from the command line and see what the behavior is. For troubleshooting tests, catch the error with try: ... except: or print err.

For adding tests to **old code**, we recommend a mix of two approaches:

1. use “[stupidity driven testing](#)” and write tests that recapitulate bugs before we fix those bugs.

2. look at test coverage (see [khmer's cobertura test coverage, here](#)) and identify lines of C++ or Python code that are not being executed by the current tests. Then write new tests targeting the new code.

---

Next, to add a test, you have two options: either write a new one from scratch, or copy an existing one. (We recommend the latter.)

To write a new one, you'll need to know how to write tests. For getting an idea of the syntax, read this [introductory guide](#) and the [writing tests documentation from Astropy](#). Then find the right file in `tests/*.py` and add your test!

A better approach is, frankly, to go into the existing test code, find a test that does something similar to what you want to do, copy it, rename it, and then modify it to do the new test.

---

Finally, *where* do you add new tests and how do you run just *your* test?

Put new tests somewhere in `tests/*.py`. If you have trouble figuring out what file to add them to, just put them in *some* file and we'll help you figure out where to move them when we do code review.

To run one specific test rather than all of them, you can do:

```
./setup.py test --addopts "tests/test_scripts.py::test_load_into_counting"
```

Here, you're running just one test – the test function named `test_load_into_counting` in the file `test_scripts.py`.

You can also use `py.test` directly, it is a bit less verbose:

```
py.test tests/test_scripts.py::test_load_into_counting
```

## 2.7.4 A quick guide to the khmer codebase

This document describes the khmer project's directory layout.

---

The `ChangeLog` file lists changes to the codebase, most recent first.

The `lib/` directory contains all of the C++ code.

The `khmer/` directory contains the *khmer* package (`khmer/__init__.py`, etc) and the C++-to-Python bridge (`khmer/_khmer.cc`).

The `scripts/` and `sandbox/` directory contain Python command-line scripts.

The `tests/` directory contains all of the tests. Each test is a function in one of the `tests/test*.py` files.

## 2.7.5 Coding guidelines and code review checklist

This document is for anyone who want to contribute code to the khmer project, and describes our coding standards and code review checklist.

### C++ standards

Any feature in C++11 is fine to use. Specifically we support features found in GCC 4.8.2. See <https://github.com/dib-lab/khmer/issues/598> for an in-depth discussion.

## Coding standards

All plain-text files should have line widths of 80 characters or less unless that is not supported for the particular file format.

For C++, we use [Todd Hoff's coding standard](#), and `astyle -A10` / “One True Brace Style” indentation and bracing. Note: @CTB needs Emacs settings that work for this.

Vim users may want to set the `ARTISTIC_STYLE_OPTIONS` shell variable to “-A10 -max-code-length=80” and run `:%!astyle` to reformat. The four space indentation can be set with:

```
set expandtab
set shiftwidth=4
set softtabstop=4
```

For Python, [PEP 8](#) is our standard. The `pep8` and `autopep8` Makefile targets are helpful.

Code, scripts, and documentation must have their spelling checked.

Python-based *codespell* can be applied to multiple files easily. *codespell* can be installed via the following:

```
mkdir ~/bin
git clone git@github.com:lucasdemarchi/codespell.git
cd codespell
make prefix=${HOME} install
export PATH=$PATH:~/bin/
```

Note, if you want codespell to always be available you will need to add the `export` line to your `$(HOME).bashrc` or equivalent.

To run codespell over only what has been changed on the branch *my-branch*:

```
git diff master..my-branch > diff_file
codespell diff_file
```

To run codespell over a single file:

```
codespell path/to/file
```

To make codespell fix the issues it finds automatically:

```
codespell -w path/to/file
```

Please note that as *codespell* works off of a listing of possible misspellings it may not catch all errors. If you find a spelling error that is not caught by *codespell* feel free to open a pull request at the [project page](#) to add it to the dictionary.

Vim users can run:

```
:setlocal spell spelllang=en_us
```

Use `/s` and `[s` to navigate between misspellings and `z=` to suggest a correctly spelled word. `zg` will add a word as a good word.

GNU *aspell* can also be used to check the spelling in a single file:

```
aspell check --mode ccpp $filename
```

## Code Review

Please read [11 Best Practices for Peer Code Review](#).

See also [Code reviews: the lab meeting for code](#) and [the PyCogent coding guidelines](#).

## Checklist

Each pull request should be automatically populated with the following checklist:

- [ ] Is it mergeable?
- [ ] ``make test`` Did it pass the tests?
- [ ] ``make clean diff-cover`` If it introduces new functionality in ``scripts/`` is it tested?
- [ ] ``make format diff_pylint_report cppcheck doc pydocstyle`` Is it well formatted?
- [ ] Did it change the command-line interface? Only backwards-compatible additions are allowed without a major version increment. Changing file formats also requires a major version number increment.
- [ ] For substantial changes or changes to the command-line interface, is it documented in ``CHANGELOG.md``? See [\[keepachangelog\] \(http://keepachangelog.com/\)](http://keepachangelog.com/) for more details.
- [ ] Was a spellchecker run on the source code and documentation after changes were made?
- [ ] Do the changes respect streaming IO? (Are they tested for streaming IO?)

**Note** that after you submit the pull request you can check and uncheck the individual boxes on the formatted comment; no need to put x or y in the middle.

## CPython Checklist

Here's a checklist for new CPython types with future-proofing for Python 3:

- [ ] the CPython object name is of the form ``khmer_${OBJECTNAME}_Object``
- [ ] Named struct with ``PyObject_HEAD`` macro
- [ ] ``static PyObject khmer_${OBJECTNAME}_Type`` with the following entries
  - [ ] ``PyVarObject_HEAD_INIT(NULL, 0)`` as the object init (this includes the ``ob_size`` field).
  - [ ] all fields should have their name in a comment for readability
  - [ ] The ``tp_name`` field is a dotted name with both the module name and the name of the type within the module. Example: ``khmer.ReadAligner``
  - [ ] Deallocator defined and cast to ``(destructor)`` in ``tp_dealloc``
    - [ ] The object's deallocator must be ``Py_TYPE(obj)->tp_free((PyObject*)obj);``
  - [ ] Do ``_not_`` define a ``tp_getattr``
  - [ ] BONUS: write methods to present the state of the object via ``tp_str`` & ``tp_repr``
  - [ ] ``_Do_`` pass in the array of methods in ``tp_methods``
  - [ ] ``_Do_`` define a new method in ``tp_new``
- [ ] ``PyMethodDef`` arrays contain doc strings
  - [ ] Methods are cast to ``PyCFunctions``s
- [ ] Type methods use their type Object in the method signature.
- [ ] Type creation method decrements the reference to self (``Py_DECREF(self);``) before each error-path exit (``return NULL;``)
- [ ] No factory methods. Example: ``khmer_new_readaligner``
- [ ] Type object is passed to ``PyType_Ready`` and its return code is checked in ``MOD_INIT()``
- [ ] The reference count for the type object is incremented before adding it to the module: ``Py_INCREF(&khmer_${OBJECTNAME}_Type);``.

## 2.7.6 Command line scripts, `scripts/`, and `sandbox/`

---

**Note:** This document applies through khmer/oxli 2.0/3.0 (see [Roadmap to v3.0, v4.0, v5.0](#)) - we will revisit when the Python API falls under semantic versioning for oxli 4.0.

---

khmer has two conflicting goals: first, we want to provide a reliable piece of software to our users; and second, we want to be flexible and enable exploration of new algorithms and programs. To this end, we've split our command line scripts across two directories, `scripts/` and `sandbox/`. The former is the staid, boring, reliable code; the latter is a place for exploration.

As a result, we are committed to high test coverage, stringent code review, and [Semantic Versioning](#) for files in `scripts/`, but explicitly *not* committed to this for files and functionality implemented in `sandbox/`. So, putting a file into `scripts/` is a big deal, especially since it increases our maintenance burden for the indefinite future.

We've roughed out the following process for moving scripts into `scripts/`:

- Command line scripts start in `sandbox/`;
- Once their utility is proven (in a paper, for example), we can propose to move them into `scripts/`;
- There's a procedure for moving scripts from `sandbox/` into `scripts/`.

Read on!

### Sandbox script requirements and suggestions

All scripts in `sandbox/` must:

- be importable (enforced by `test_import_all` in `test_sandbox_scripts.py`)
- be mentioned in `sandbox/README.rst`
- have a hash-bang line (`#!/usr/bin/env python`) at the top
- be command-line executable (`chmod a+x`)
- have a Copyright message (see below)
- have lowercase names
- use '-' as a word separator, rather than '\_' or CamelCase

All *new* scripts being added to `sandbox/` should:

- have decent automated tests
- be used in a protocol (see `khmer-protocols`) or a recipe (see `khmer-recipes`)
- be pep8 clean and pylint clean-ish (see `make pep8` and `make_diff_pylint`).

### Standard and reserved command line options for `scripts/`

The following options are reserved, and the short option flag cannot be redefined in any official script.

- `-h|--help` - this must always print out a descriptive usage statement
- `-v|--version` - this must always print out the khmer version number
- `-x|--max-tablesize` - this must always specify the approximate table size for storing sketches of k-mer hashes

- `-N|--n_tables` - this must always specify the number of tables for storing sketches of k-mer hashes
- `-M|--max-memory-usage` - this must always specify the maximum amount of memory to be consumed for storing sketches of k-mer hashes
- `-U|--unique-kmers` - this must always specify the approximate number of unique k-mers in the data set
- `-k|--ksize` - this must always specify the k-mer size to use
- `-q|--quiet` - this must always indicate that the script’s diagnostic output should be minimized or altogether eliminated

Additionally, all scripts in `scripts/` should have the following options.

- `-h|--help`
- `-v|--version`
- `-f|--force` - if applicable, override any sanity checks that may prevent the script from running

If an option is of type `type=argparse.FileType('w')` then you need to also specify a metavar for the documentation and help formatting. Example:

```
parser.add_argument('-R', '--report', metavar='report_filename',
                    type=argparse.FileType('w'))
```

## Copyright message

The copyright message should be of the form:

```
#
# This file is part of khmer, https://github.com/dib-lab/khmer/, and is
# Copyright (C) ___WHO___, ___YEAR(s)___. It is licensed under
# the three-clause BSD license; see doc/LICENSE.txt.
# Contact: khmer-project@idyll.org
#
```

Where `___WHO___` is replaced with one or more of “Michigan State University” or “The Regents of the University of California” and `___YEAR(s)___` is replaced with the year or years the file was created or modified. The copyright statement for new files should only refer to “The Regents of the University of California”.

## Upgrading a script from ‘sandbox’ to ‘scripts’

First, everything needed (all library support code) should be already committed to khmer master after the usual review process; the relevant script(s) should be in `sandbox/`.

Second, an issue should be started explicitly to discuss whether the script(s) should be moved from `sandbox/` into `scripts/`. This issue should discuss the general need for this script, outside of a particular paper pipeline. (Note that there is no imperative to move a script out of `sandbox/`; if we think it’s useful code to have around and want to keep it functioning, we should just add in automated tests and otherwise level it up.)

Third, assuming we reach general agreement about moving the script(s) into `scripts/`, start a pull request to do so, referencing the issue and containing the following checklist. The PR should start by moving the script from `sandbox/` into `scripts/`, and moving the tests out of the `test_sandbox_scripts.py` file.

Last but not least, intensive code review may raise more general issues that could apply to the entire code base; if contentious or needing discussion, these issues may be punted to general issues so as to not block a merge.

## A checklist for moving a script into the scripts/ directory from sandbox/

Copy or paste this checklist into the PR, in addition to the normal development/PR checklist:

```
- [ ] most or all lines of code are covered by automated tests (see output of ``make diff-cover``)
- [ ] ``make diff_pylint`` is clean
- [ ] the script has been updated with a ``get_parser()`` and added to doc/user/scripts.txt
- [ ] argparse help text exists, with an epilog docstring, with examples and options
- [ ] standard command line options are implemented
- [ ] version and citation information is output to STDERR (`khmer_args.info(...)`)
- [ ] support '-' (STDIN) as an input file, if appropriate
- [ ] support designation of an output file (including STDOUT), if appropriate
- [ ] script reads and writes sequences in compressed format
- [ ] runtime diagnostic information (progress, etc.) is output to STDERR
- [ ] script has been removed from sandbox/README.rst
```

## 2.7.7 A guide for khmer committers

This document is for people with commit rights to [github.com/dib-lab/khmer](https://github.com/dib-lab/khmer).

If you have commit privileges to the dib-lab/khmer repository, here are a few useful tips.

First, never merge something unless it's been through a review! This rule can be broken under specific conditions when doing a release; see [Releasing a new version of khmer](#).

Second, need to force another continuous integration run? Put “test this please” in a comment. This is used to ask our continuous integration system to run on someone else’s pull request – by default, it only runs on commits from people who have write privileges to khmer, so you may need to do this if you’re reviewing someone else’s pull request.

Third, we ask that all contributors set up standing Pull Requests while they are working something. (This is a **requirement** if you’re in the DIB lab.) This lets us track what’s going on. On the flip side, please do not review pull requests until they are indicated as “ready for review”.

## 2.7.8 Releasing a new version of khmer

This document is for khmer release managers, and details the process for making a new release of the khmer project.

### How to make a khmer release candidate

Michael R. Crusoe, Luiz Irber, and C. Titus Brown have all been release makers, following this checklist by MRC.

1. The below should be done in a clean checkout:

```
cd `mktemp -d`
git clone git@github.com:dib-lab/khmer.git
cd khmer
```

2. (Optional) Check for updates to versioneer:

```
pip install --upgrade versioneer
versioneer-installer

git diff

./setup.py versioneer
```

```
git diff
git commit -m -a "new version of versioneer.py"
# or
git checkout -- versioneer.py khmer/_version.py khmer/__init__.py MANIFEST.in
```

3. Review the git logs since the last release and diffs (if needed) and ensure that the ChangeLog is up to date:

```
git log --minimal --patch `git describe --tags --always --abbrev=0`..HEAD
```

4. Review the issue list for any new bugs that will not be fixed in this release. Add them to doc/known-issues.txt
5. Check for new authors. Update .mailmap to normalize their email address and name spelling. If they want to opt out update the list-\* Makefile targets to exclude them. Run make list-citation and adapt the output to the relevant parts of CITATION, setup.py, doc/index.rst.
6. Verify that the build is clean: <http://ci.oxli.org/job/khmer-master/>
7. Run through the API examples to verify they are still valid.
8. Submit a build to Coverity Scan if it hasn't been done recently. You can get the token from <https://gitlab.msu.edu/ged-lab/ged-internal-docs/wikis/coverity-scan> or [https://scan.coverity.com/projects/621?tab=project\\_settings](https://scan.coverity.com/projects/621?tab=project_settings)

```
virtualenv coverityenv
source coverityenv/bin/activate
make install-dependencies
make clean
cov_analysis_dir=~/.src/coverity/cov-analysis-linux64-7.5.0/ make coverity-build
COVERITY_TOKEN=${COVERITY_TOKEN} make coverity-upload
```

9. Set your new version number and release candidate:

```
new_version=1.3
rc=rc1
```

and then tag the release candidate with the new version number prefixed by the letter 'v':

```
git tag v${new_version}-${rc}
git push --tags git@github.com:dib-lab/khmer.git
```

10. Test the release candidate. Bonus: repeat on Mac OS X:

```
cd ..
virtualenv testenv1
virtualenv testenv2
virtualenv testenv3
virtualenv testenv4
# First we test the tag

cd testenv1
source bin/activate
git clone --depth 1 --branch v${new_version}-${rc} https://github.com/dib-lab/khmer.git
cd khmer
make install-dependencies
make test
normalize-by-median.py --version 2>&1 | grep khmer\ ${new_version}-${rc} && \
    echo 1st manual version check passed
pip uninstall -y khmer; pip uninstall -y khmer; make install
mkdir ../not-khmer # if there is a subdir named 'khmer' nosetest will execute tests
# there instead of the installed khmer module's tests
```

```

pushd ../not-khmer; nosetests khmer --attr '!known_failing'; popd

# Secondly we test via pip

cd ../../testenv2
source bin/activate
pip install -U setuptools==3.4.1
pip install -e git+https://github.com/dib-lab/khmer.git@v${new_version}-${rc}#egg=khmer
cd src/khmer
make install-dependencies
make dist
make test
cp dist/khmer*tar.gz ../../../../testenv3/
pip uninstall -y khmer; pip uninstall -y khmer; make install
cd ../.. # no subdir named khmer here, safe for nosetesting installed khmer module
normalize-by-median.py --version 2>&1 | grep khmer\ ${new_version}-${rc} && \
    echo 2nd manual version check passed
nosetests khmer --attr '!known_failing'

# Is the distribution in testenv2 complete enough to build another
# functional distribution?

cd ../testenv3/
source bin/activate
pip install -U setuptools==3.4.1
pip install khmer*tar.gz
pip install nose
tar xzf khmer*tar.gz
cd khmer*
make dist
make test
pip uninstall -y khmer; pip uninstall -y khmer; make install
mkdir ../not-khmer
pushd ../not-khmer ; nosetests khmer --attr '!known_failing' ; popd

```

11. Publish the new release on the testing PyPI server. You will need to change your PyPI credentials as documented here: <https://wiki.python.org/moin/TestPyPI>. You may need to re-register:

```
python setup.py register --repository test
```

Now, upload the new release:

```
python setup.py sdist upload -r test
```

Test the PyPI release in a new virtualenv:

```

cd ../../testenv4
source bin/activate
pip install -U setuptools==3.4.1
pip install screed nose
pip install -i https://testpypi.python.org/pypi --pre --no-clean khmer
nosetests khmer --attr '!known_failing'
normalize-by-median.py --version 2>&1 | grep khmer\ ${new_version}-${rc} && \
    echo 3rd manual version check passed
cd build/khmer
make test

```

12. Do any final testing (BaTLab and/or acceptance tests).

13. Make sure any release notes are merged into doc/release-notes/.

## How to make a final release

When you've got a thoroughly tested release candidate, cut a release like so:

1. Create the final tag and publish the new release on PyPI (requires an authorized account).:

```
cd ../../../khmer
git tag v${new_version}
python setup.py register sdist upload
```

2. Delete the release candidate tag and push the tag updates to GitHub.:

```
git tag -d v${new_version}-${rc}
git push git@github.com:dib-lab/khmer.git
git push --tags git@github.com:dib-lab/khmer.git
```

3. Add the release on GitHub, using the tag you just pushed. Name it 'version X.Y.Z', and copy and paste in the release notes.
4. Make a binary wheel on OS X.:

```
virtualenv build
cd build
source bin/activate
pip install -U setuptools==3.4.1 wheel
pip install --no-clean khmer==${new_version}
cd build/khmer
./setup.py bdist_wheel upload
```

5. Update Read the Docs to point to the new version. Visit <https://readthedocs.io/builds/khmer/> and 'Build Version: master' to pick up the new tag. Once that build has finished check the "Activate" box next to the new version at <https://readthedocs.io/dashboard/khmer/versions/> under "Choose Active Versions". Finally change the default version at <https://readthedocs.io/dashboard/khmer/advanced/> to the new version.
6. Delete any RC tags created:

```
git tag -d ${new_version}-${rc}
git push origin :refs/tags/${new_version}-${rc}
```

7. Tweet about the new release.
8. Send email including the release notes to [khmer@lists.idyll.org](mailto:khmer@lists.idyll.org) and [khmer-announce@lists.idyll.org](mailto:khmer-announce@lists.idyll.org)

## BaTLab testing

The UW-Madison Build and Test Lab provides the khmer project with a free cross-platform testing environment.

1. Connect to their head node:

```
ssh mcrusoe@submit-1.batlab.org
```

2. Move into the khmer directory and download a release from PyPI's main server or the test PyPI server:

```
cd khmer/
wget https://testpypi.python.org/packages/source/k/khmer/khmer-1.0.1-rc3.tar.gz
vim khmer-v1.0.inputs # change the 'scp_file' to point to the release
vim khmer-v1.0.run-spec # change 'project_version' at bottom
nmi_submit khmer-v1.0.run-spec
```

## Setuptools Bootstrap

`ez_setup.py` is from <https://bitbucket.org/pypa/setuptools/raw/bootstrap/>

Before major releases it should be examined to see if there are new versions available and if the change would be useful

## Versioning Explanation

Versioneer, from <https://github.com/warner/python-versioneer>, is used to determine the version number and is called by Setuptools and Sphinx. See the files `versioneer.py`, the top of `khmer/__init__.py`, `khmer/_version.py`, `setup.py`, and `doc/conf.py` for the implementation.

The version number is determined through several methods: see <https://github.com/warner/python-versioneer#version-identifiers>

If the source tree is from a git checkout then the version number is derived by `git describe --tags --dirty --always`. This will be in the format `${tagVersion}-${commits_ahead}-${revision_id}-${isDirty}`. Example: `v0.6.1-18-g8a9e430-dirty`

If from an unpacked tarball then the name of the directory is queried.

Lacking either of the two git-archive will record the version number at the top of `khmer/_version.py` via the `$Format:%d$` and `$Format:%H$` placeholders enabled by the “export-subst” entry in `.gitattributes`.

Non source distributions will have a customized `khmer/_version.py` that contains hard-coded version strings. (see `build/*/khmer/_version.py` after a `python setup.py build` for an example)

`ez_setup.py` bootstraps Setuptools (if needed) by downloading and installing an appropriate version

## 2.7.9 Miscellaneous implementation details

Partition IDs are “stored” in FASTA files as an integer in the last tab-separated field. Yeah, dumb, huh?

## 2.7.10 Development miscellany

### Third-party use

We ask that third parties who build upon the codebase to do so from a versioned release. This will help them determine when bug fixes apply and generally make it easier to collaborate. If more intensive modifications happen then we request that the repository is forked, again preferably from a version tag.

### Build framework

`make` should build everything, including tests and “development” code.

### git and GitHub strategies

Still in the works, but read [this](#).

Make a branch on `dib-lab` (preferred so others can contribute) or fork the repository and make a branch there.

Each piece or fix you are working on should have its own branch; make a pull- request to dib-lab/master to aid in code review, testing, and feedback.

If you want your code integrated then it needs to be mergable

Example pull request update using the command line:

1. **Clone the source of the pull request (if needed)** `git clone git@github.com:mr-c/khmer.git`
2. **Checkout the source branch of the pull request** `git checkout my-pull-request`
3. **Pull in the destination of the pull request and resolve any conflicts** `git pull git@github.com:dib-lab/khmer.git master`
4. Push your update to the source of the pull request `git push`
5. Jenkins will automatically attempt to build and test your pull requests.

## Code coverage

Jenkins calculates code coverage for every build. Navigate to the results from the master node first to view the coverage information.

Code coverage should never go down and new functionality needs to be tested.

## Pipelines

All khmer scripts used by a published recommended analysis pipeline must be included in `scripts/` and meet the standards therein implied.

## Command line scripts

Python command-line scripts should use `'-'` instead of `'_'` in the name. (Only filenames containing code for import should use `_`.)

Please follow the command-line conventions used under `scripts/`. This includes most especially standardization of `'-x'` to be hash table size, `'-N'` to be number of hash tables, and `'-k'` to always refer to the k-mer size.

Command line thoughts:

If a filename is required, typically UNIX commands don't use a flag to specify it.

Also, positional arguments typically aren't used with multiple files.

CTB's overall philosophy is that new files, with new names, should be created as the result of filtering etc.; this allows easy chaining of commands. We're thinking about how best to allow override of this, e.g.

```
filter-abund.py <ct file> <filename> [ -o <filename.keep> ]
```

All code in `scripts/` must have automated tests; see `tests/test_scripts.py`. Otherwise it belongs in `sandbox/`.

When files are overwritten, they should only be opened to be overwritten after the input files have been shown to exist. That prevents stupid command line mistakes from trashing important files.

A general error should be signaled by exit code `1` and success by `0`. Linux supports exit codes from `0` to `255` where the value `1` means a general error. An exit code of `-1` will get converted to `255`.

CLI reading:

<http://stackoverflow.com/questions/1183876/what-are-the-best-practices-for-implementing-a-cli-tool-in-perl>  
<http://catb.org/esr/writings/taoup/html/ch11s06.html>  
[http://figshare.com/articles/tutorial\\_pdf/643388](http://figshare.com/articles/tutorial_pdf/643388)

## Python / C integration

The Python extension that wraps the C++ core of khmer lives in `khmer/_khmer.cc`

This wrapper code is tedious and annoying so we use a static analysis tool to check for correctness.

<https://gcc-python-plugin.readthedocs.io/en/latest/cpychecker.html>

Developers using Ubuntu Precise will want to install the `gcc-4.6-plugin-dev` package

Example usage:

```
CC="/home/mcrusoe/src/gcc-plugin-python/gcc-python-plugin/gcc-with-cpychecker
--maxtrans=512" python setup.py build_ext 2>&1 | less
```

False positives abound: ignore errors about the C++ standard library. This tool is primarily useful for reference count checking, error-handling checking, and format string checking.

Errors to ignore: “Unhandled Python exception raised calling ‘execute’ method”, “AttributeError: ‘NoneType’ object has no attribute ‘file’”

Warnings to address:

```
khmer/_khmer.cc:3109:1: note: this function is too complicated
for the reference-count checker to fully analyze: not all paths were
analyzed
```

Adjust `--maxtrans` and re-run.

```
khmer/_khmer.cc:2191:61: warning: Mismatching type in call to
Py_BuildValue with format code "i" [enabled by default]
    argument 2 ("D.68937") had type
        "long long unsigned int"
    but was expecting
        "int"
    for format code "i"
```

See below for a format string cheat sheet One also benefits by matching C type with the function signature used later.

“T” for unsigned int “K” for unsigned long long a.k.a `khmer::HashIntType`.

## Read handling

Several bugs have gone unnoticed due to inconsistencies in read handling. On the C++ side, there are an abundance of consume functions for loading Fasta/Fastq sequences. On the Python side, read handling is sometimes delegated to the C++ library, and sometimes handled in Python using `screed`.

In an attempt to normalize read handling in Python, the functions in `khmer/utils.py` should be used whenever possible. Here, `broken_paired_reader` in `khmer/utils.py` should be used to do all paired-end sequence handling, and sequence loading should go through `khmer.utils.clean_input_reads(iter)`; this is a generator that wraps the iterator produced by `screed.open`, and it adds a `cleaned_seq` attribute to `screed.Record` objects. This attribute should be used for any k-mer or graph operations, while the normal `sequence` attribute is

what should be written out. `write_record` and `write_record_pair` should be used to output records. All of these functions are aware of FASTA and FASTQ records, too.

For applying operations to collections of reads, the `ReadBundle` class is available. This is used to wrap a collection of reads for examination and processing in situations where (for example) something should be done to either both reads in a pair, or neither.

Some basic rules of sequence handling in khmer are:

- consume and produce “broken paired” format, such that pairs of sequences always stay together; see `khmer.utils.broken_paired_reader`.
- when looking at the coverage of reads (for trimming or digital normalization) always consider pairs; see `khmer.utils.ReadBundle(...)`.
- only apply graph or k-mer operations to sequences consisting only of ATCG; typically this will be `record.cleaned_seq`. See `khmer.utils.clean_input_read(...)`.

## 2.7.11 Crazy ideas

1. A JavaScript preprocessor to do things like count k-mers (HLL), and do diginorm on data as uploaded to server. Inspired by a paper that Titus reviewed for PLoS One; not yet published.

## 2.7.12 khmer/Oxli Binary File Formats

- C++ macro definitions are given in parenthesis.
- C++ types are given in square brackets.
- `Len` is the field’s size, in bytes, and `Off` is the field’s zero-based byte offset in the file/section.

### khmer v1.4 and previous

#### CountingHash

The header is in the format below, in file offset order. There is no magic string.

| Field            | Length | Value                             |
|------------------|--------|-----------------------------------|
| Version          | 1      | 0x04 (SAVED_FORMAT_VERSION)       |
| File Type        | 1      | 0x01 (SAVED_COUNTING_HT)          |
| Use Bigcount     | 1      | 1 if bigcounts is used, else 0    |
| K-size           | 1      | k-mer length, $1 \leq k \leq 32$  |
| Number of Tables | 1      | Number of Count-min Sketch tables |

### khmer v2.0 formats

#### Magic string

All formats shall have the “magic string” OXLI as their first bytes, after any external compression/encoding (e.g. gzip encapsulation) is removed. Note that this makes them incompatible with older versions of khmer.

## Countgraph

(a.k.a CountingHash, a Count-min Sketch)

The header is in the format below, again in the order of file offset.

| Field            | Len | Off | Value                                                      |
|------------------|-----|-----|------------------------------------------------------------|
| Magic string     | 4   | 0   | OXLI (SAVED_SIGNATURE)                                     |
| Version          | 1   | 4   | 0x04 (SAVED_FORMAT_VERSION)                                |
| File Type        | 1   | 5   | 0x01 (SAVED_COUNTING_HT)                                   |
| Use Bigcount     | 1   | 6   | 0x01 if bigcounts is used, else 0x00                       |
| K-size           | 4   | 7   | k-mer length, ht._ksize. [uint32_t]                        |
| Number of Tables | 1   | 11  | Number of Count-min Sketch tables, ht._n_tables. [uint8_t] |
| Occupied Bins    | 8   | 12  | Number of occupied bins                                    |

Then follows the Countgraph's tables. For each table:

| Field      | Len | Off | Value                                                        |
|------------|-----|-----|--------------------------------------------------------------|
| Table size | 8   | 0   | Length of this table, ht._tablesizes[i]. [uint64_t]          |
| Bins       | N   | 8   | This table's bins, length given by previous field. [uint8_t] |

Then follows a single value, the [uint64\_t] number of kmer: count pairs. Then follows the Bigcount map, if this number is greater than zero. For each kmer:

| Field | Len | Off | Value                                |
|-------|-----|-----|--------------------------------------|
| Kmer  | 8   | 0   | Kmer's hash [HashIntoType/uint64_t]. |
| Count | 2   | 8   | Kmer's count [uint16_t].             |

## Nodegraph

(a.k.a HashBits, a Bloom Filter)

The header is in the format below, again in the order of file offset. Value macro definitions are given in parenthesis

| Field            | Len | Off | Value                                               |
|------------------|-----|-----|-----------------------------------------------------|
| Magic string     | 4   | 0   | OXLI (SAVED_SIGNATURE)                              |
| Version          | 1   | 4   | 0x04 (SAVED_FORMAT_VERSION)                         |
| File Type        | 1   | 5   | 0x02 (SAVED_HASHBITS)                               |
| K-size           | 4   | 6   | k-mer length, ht._ksize. [unsigned int]             |
| Number of Tables | 1   | 10  | Number of Nodegraph tables. ht._n_tables. [uint8_t] |
| Occupied Bins    | 8   | 11  | Number of occupied bins                             |

Then follows the Nodegraph's tables. For each table:

| Field      | Len   | Off | Value                                                                               |
|------------|-------|-----|-------------------------------------------------------------------------------------|
| Table size | 8     | 0   | Length of table, <b>in bits</b> (uint64_t).                                         |
| Bins       | N/8+1 | 8   | This table's bytes, length given by previous field, divided by 8, plus 1 (uint8_t). |

## Todo

Document Tags, Stoptags, Subset, Labelset

## 2.8 Roadmap to v3.0, v4.0, v5.0

### 2.8.1 Background

To make the khmer project easier to use and easier to build upon several fundamental changes need to happen. This document outlines our plan to do so while minimizing the impact of these changes on our existing users. The version numbers are approximate; there may be additional major number releases to support needed changes to the API along the way. This document has been updated for v2.0 onwards from the [original roadmap](#).

The discussion that lead to this document can be read at <https://github.com/dib-lab/khmer/issues/389>

### 2.8.2 Remainder of v2.x series

Continue transition to a single entrypoint named `oxli`. This will be exempt from the project's semantic versioning and will not be advertised as it is experimental and unstable. (For the 2.0 version we removed the `oxli` script just prior to release and restored it afterwards to the development tree.)

The Python script functionality will continue to migrate to a Python module named `oxli`. As the code moves over there will be no change to external script functionality or their command line interfaces (except for new features).

### 2.8.3 v3.x series

The `oxli` command is now under semantic versioning. Scripts are still the advertised and preferred entry point for users. Developers and workflow systems can start to trial `oxli` but need not switch until 4.0. New functionality is added to both the scripts and the `oxli` command.

### 2.8.4 v4.0 and project renaming

Project renamed to 'oxli'; all references to 'khmer' removed from the code and documentation except for a single note in the docs. All scripts dropped as their functionality has been moved to the `oxli` command. Websites that we maintain that have 'khmer' in the URL will have redirects installed.

Refinement of the Python API continues, however it is not part of the semantic versioning of the project.

### 2.8.5 v5.0

The semantic versioning now extends to the Python API.

### 2.8.6 Python API wishlist

API for multiple container types and implementation of the same.

Cleanup of Python/C++ class hierarchy to cut down on boilerplate glue code.

Switch to new-style Python objects (see LabelHash & Hashbits)

## 2.9 License

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There are two mailing lists dedicated to khmer, an announcements-only list and a discussion list. To search their archives and sign-up for them, please visit the following URLs:

- Discussion: <http://lists.idyll.org/listinfo/khmer>
- Announcements: <http://lists.idyll.org/listinfo/khmer-announce>

The archives for the khmer mailing list are available at: <http://lists.idyll.org/pipermail/khmer/>

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## Symbols

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