

Meraculous Assembler Manual

Lawrence Berkeley National Lab
Genomics Division
DOE Joint Genome Institute
Walnut Creek, CA

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Meraculous is a whole genome assembler for Next Generation Sequencing data geared to eukaryotic genomes. It is a hybrid k-mer/read-based assembler that capitalizes on the high accuracy of Illumina sequence by eschewing an explicit error correction step which we argue to be redundant with the assembly process. Meraculous achieves high performance with large datasets by utilizing lightweight data structures and multi-threaded parallelization, allowing assembling human-sized genomes on commodity clusters in under a day. The process pipeline implements a highly transparent and portable model of job control and monitoring where different assembly stages can be executed and re-executed separately or in unison on a wide variety of architectures

1. BEFORE YOU BEGIN

1.1 Capabilities and limitations

A few warnings up front

- Currently Meraculous works with Illumina data only. It relies on Illumina naming conventions and Phred-like sequence quality scores. Long-read/low-depth sequencing platforms are not supported at this time.
- An overall mean depth of read coverage of at least 30x is strongly recommended. Low-coverage datasets will likely result in a highly fragmented assembly or an aborted process altogether.
- Meraculous deals with genomic diploidy by creating a pseudo-haploid assembly where haplotypes are "squashed", i.e., a contig is formed with a single majority allele. However, the higher the polymorphic rate the less effective this process is. As a result, genomes with polymorphism rates of over 0.05 are better to assemble as haploid, letting Meraculous keep both haplotypes as distinct contigs, in essence imitating a meta-genome.
- Although it is capable of assembling small bacterial genomes, it may not be the most resource-efficient choice for these scenarios.
- Meraculous relies heavily on distributed and threaded computing and will perform best on a multiple-core server or in a cluster environment. For more on this, see sections ['Operating System requirements'](#) and ['Hardware considerations'](#)

Meraculous performs the assembly by first traversing a subgraph of the k-mer (deBruijn) graph of oligonucleotides with unique high quality extensions and building a set of preliminary contigs (called UUtigs from here on) where each k-mer is represented only once and no further unique extension is possible. If running in diploid mode, Meraculous attempts to resolve "bubbles" in the graph caused by diploidy and merges qualifying UUtigs into "diplotigs". Then the order and orientation of the

UUtigs or diplotigs is determined and gaps between them are closed using information derived from mapping paired-end reads to the contigs.

While there are numerous factors that can affect the accuracy and performance of this process, the following are of most importance:

Depth of coverage distribution

Meraculous relies on the coverage profile at several key points in the process. Optimal assembly is possible only when the depth profile of the genome being assembled can be clearly distinguished from any low-frequency noise or contamination. The depth distribution is also used to distinguish haplotypes from repeats (both appear as near-identical copies in a diploid genome), as well as during the order-and-orientation (oNo) stage.

Library composition

The best assembly can be obtained when a paired-end/mate-pair library is included with mean insert size well over the size of the largest repeats. Since the repeat composition is often unknown ahead of time, it is recommended that a balanced mix of several libraries of different insert sizes is used. Artifacts in such libraries (chimeras, insert-less pairs, untrimmed adapter, etc.) or non-Poisson insert size distribution can confuse the assembly algorithms and result in highly fragmented assembly. However, there are options a user can set to mitigate those effects, and it is crucial that those options are set correctly. See section '[Run configuration](#)' for more details.

Repeat content

Like most assemblers, Meraculous attempts to resolve ambiguities caused by repeats using paired-end linkage and distance information. Because repeating elements can take complex shapes and/or can be confounded by variants in diploid genomes, many repeats may remain unresolved as Meraculous will not make ambiguous joins. Identical repeat copies may "collapse" into what then looks like a unique contig that "falls out" of the scaffold, thus adding to the total number of scaffolds and contigs. During gap closure, some of these repeats will be recovered if a repeat-induced gap can be fully traversed by "k-mer-walking".

Read data quality

Meraculous avoids an explicit error correction step instead relying on k-mer coverage and base quality scores (e.g. low quality extensions of k-mers are ignored during the graph building stage). K-mers containing sequencing errors are expected to occur at a much lower frequency than "true" k-mers and can therefore be eliminated using the depth cutoff. High errors rates in the sequence,

however, can still hinder the assembly and clog up the data structures since a greater number of unique k-mers would result from sequencing error.

k-mer size

If the chosen k-mer size is too short for the genome then there will be too many non-unique k-mers. If it's too long then the k-mers will be more likely to include a sequencing error. Both situations hinder the assembly, so it's important to arrive at a "sweet spot" in the middle.

Users have an option to have the k-mer size auto-selected based on the provided genome size, but that is a ballpark approximation. For best results, we advise to start with 3-4 pilot runs with different k-mer sizes, stopping after the `meraculous_mercount` stage and reviewing the k-mer frequency histogram (`mercount.png`). As a rule of thumb, the largest value of `k` that yields a distinct peak at least $\sim 30X$ is a reasonable choice.

Insert size distribution and library bias

Meraculous uses user-defined estimated average insert size and standard deviation values to set various cutoffs during the calculation of the actual assembly-based insert size distribution. Therefore, it's important that these estimates are close to reality.

1.2 Operating system requirements

Meraculous can run on any 64-bit Linux system. This release was developed and tested on Debian 6.0.7 Linux, kernel 2.6.32-5-amd64.

Meraculous can execute distributed/parallel jobs on either a single multi-core system or on a cluster. In the latter case, the cluster array job submission is executed via a wrapper script `cluster_submit.sh`. Depending on your site's cluster configuration, this script may need to be modified by your sysadmin to ensure proper functionality, or even substituted by your own wrapper. This release was tested with a Linux cluster running Univa Grid Engine 8.1.4. At the minimum, the current implementation requires that your cluster scheduler accepts `qsub`, `qstat`, and `qacct` commands for submitting and monitoring of the jobs.

1.3 Hardware considerations

Disk space and memory requirements depend on many factors, but the following guidelines are a good estimate.

Disk space

This will differ dramatically depending on the cleanup policy chosen. For details on the different cleanup options see section '[Executing the run](#)'.

Total input sequence (Gbp)	cleanup_level 0	cleanup_level 1	cleanup_level 2
450 (Human)	6.9 TB	2.9 TB	39 GB
13 (N.sebacea)	67 GB	49 GB	200 MB
1.2 (E.coli)	13 GB	7.6 GB	42 MB

Memory

Where possible, memory usage is optimized by partitioning the input data into blocks that can fit under smaller memory caps. However, for larger genomes like H.sapiens, the memory-limiting step is often UUtig generation where all the building blocks must be kept in memory at once.

Genome Size (Gbp)	Total sequence earmarked for contiging (Gbp)	Memory required (GB)
3.1 (H.sapiens)	290	109
0.7 (S.bicolor)	63	18
0.05 (N.fluitans)	10	3.7
0.005 (E.coli)	0.7	0.2

Multi-threading

Many Meraculous components run as threads on multiple cores of a single computer, while others are single-threaded. To take advantage of multi-threading users must specify how many cores are available (parameters `local_num_procs` and `cluster_slots_per_task`). Keep in mind that while multi-threading greatly improves run times it has no effect on the memory usage since the size of the input data chunk is independent of the threading level chosen.

1.4 Internal job execution and control

When following the progress of the run or reviewing the log files, it is important to understand how the Meraculous components get executed on the system. Meraculous makes heavy use of distributed task arrays for parallel execution. First, the data is sub-divided into chunks of smaller size. Then a "command set" containing all the chunks is submitted for execution either locally or on the cluster as task arrays. Meraculous waits for the local system or the cluster scheduler software to signal that all the parallel tasks have been completed successfully before continuing. In the event of failure, users can opt to automatically retry the failed tasks. If there are still failed tasks the program terminates. After examining the error logs and making corrections to the data or the settings, user can resume the assembly from the point where it was terminated (see section ' [Working in Stages](#) '). While this will re-form the command set from scratch, only those tasks that haven't successfully completed will actually get re-executed.

1.5 Availability and getting help

Meraculous source code along with a small test dataset is available from <http://sourceforge.net/projects/meraculous20>.

Questions can be addressed to Eugene Goltsman at egoltsman@lbl.gov
If you're running into problems with installation, please include the capture of the screen output of install.sh. If there are errors during Meraculous execution that you don't understand, please send your config file and the log/debug.log file from your run.

2. INSTALLATION

2.1 Software dependencies

At the time of the installation the following software should be available on your system:

cmake >= 2.8

GCC g++ >= 4.4.7

GNU make 3.81

Boost C++ library >= 1.50.0

Perl (>= 5.10)

[Log4perl.pm](#) (>= 1.31)

gnuplot (>= 3.7)

qqacct (optional but highly recommended for Grid Engine cluster environments:
<http://portal.nersc.gov/dna/plant/assembly/meraculous2/extras/qqacct>)

2.2 Installation procedure

After you have downloaded and unpacked the source distribution, build and install the software:

```
install.sh <installation directory>
```

..or build it step by step:

```
mkdir build
```

```
cd build
```

```
cmake -DCMAKE_INSTALL_PREFIX=<installation directory> ..
```

(The installation directory should be different from the build directory)

[Note Omitting the -DCMAKE_INSTALL_PREFIX option will install the package into system default locations (/usr/local/bin, etc..)]

```
make
```

```
make install
```

Note: If you're planning to run Meraculous in a cluster environment, additional configuration may be required. This documentation assumes that an SGE-like

cluster system is in place and can, at the minimum, accept commands `qstat`, `qsub`, and `qacct`, and has the `$SGE_ROOT` variable set to the root directory of the cluster control software. To test if this is the case, type "qstat" on the command line. There should be no errors (the result may be blank, that is ok). Note, however, that even with SGE-like systems, additional changes to the `cluster_submit.sh` wrapper may be needed on the part of the user, which is outside of the scope of this document.

2.3 Test run

The distribution archive contains a small validation dataset to help you confirm that the software has been installed properly. The dataset is meant to be kept its original location, and the validation run should be executed directly from that location. To perform the run, do the following:

```
cd <install_dir>/etc/meraculous/test/pipeline
bash <install_dir>/bin/run_meraculous.sh -c meraculous.config
```

The run should take no more than 5-10 minutes. When the run completes you will see a new run directory named `run_[date]_[time]`. Inside that directory, open the file `log/debug.log`, it should end with the following:

```
meraculous.pl main:: 840> ran to completion successfully
```

If you don't see this then something went wrong. The `debug.log` file will have the most detailed record of everything that happened during the run, and it is the first file you should examine when troubleshooting. See section '[Troubleshooting tips](#)' for more details.

3. RUNNING MERACULOUS

3.1 Workflow steps and stages

The Meraculous pipeline is executed in stages which can be run and re-run individually or all at once. Each stage is followed by a cleanup of intermediate files. The level of aggressiveness of this cleanup is controlled by the `-cleanup_level` command line option (for more on this see section '[Executing the run](#)'). Before starting your first assembly, we strongly recommend to familiarize yourself with what happens during each stage, what the key outputs are, and how to monitor the process, which means reading on beyond this chapter, to the end of the document.

`meraculous_import`

- Creates links to the original input sequence files and names these links in a standard format.
- Validates the format and the pairing schema (dual-file or interleaved) of the input sequence files and auto-detects the quality encoding offset (Phred+33 or Phred+64). For more on the types of input data supported, see section '[Input Data Preparation](#)'.
- Divides the input into chunks to allow for parallel processing in the future. This takes two forms. One is at the read level, and for that we simply split the sequence files into chunks of ~500 mb of total sequence. (Note that it is these chunks that will be used from here on and **not** the original files you provided in the config file.) The second type is at the k-mer level. Here we use a sampling of the reads to estimate k-mer frequency rates in the dataset, and based on that define *prefix blocks* to group k-mers in a load-balanced fashion. Because processing the total k-mer space of the dataset is memory-intensive, this load balancing is needed to ensure that the data structures for storing the k-mers require similar amounts of memory. The *number* of blocks is set by the user in the config file (for more on this see section '[Input Data Preparation](#)').
- Generates a human-readable summary of the input dataset extrapolated from a sub-sampling of the reads.

meraculous_mercount

- Counts k-mer frequencies across all libraries that are earmarked for contig generation in the config file, producing a set of `.mercount` files.
- Builds the k-mer frequency histogram which is used to determine the minimum depth cutoff. Users should review this histogram (`mercount.png`) as it can be helpful in identifying features and abnormalities in the dataset, such as contamination or low quality data.

meraculous_mergraph

- For each k-mer that satisfies the minimum depth cutoff Meraculous counts all possible single nucleotide extensions of that k-mer (subject to minimum depth and minimum quality cutoffs) and records them in `.mergraph` files which now provide the basis for the initial contig assembly.

meraculous_ufx

- Classifies each k-mer in the graph as either unique (U), fork (F), or terminal (X), based on the possible single-base extensions of the k-mer in either direction. The main output is a `.UFX` file.

meraculous_contigs

- Loads the UFX information into memory-efficient data structures and builds the initial set of contigs known as UUtigs. A UUtig is built from a tiling path of 'U' mers overlapping by k-1 bp, and terminates on either end with either an 'F' mer or an 'X' mer.
- Generates various statistics for the user to review (`UUtigs.fa.stats`). These can be useful to detect problems in contig formation. For example, a lack of long contigs and/or contig sizes failing to add up to the expected genome size may indicate insufficient depth of usable k-mers. If this coincides with a bi-modal k-mer depth distribution (see stage `meraculous_mercount`), then this suggests contamination in the dataset.

[Note: In a diploid genome, the total length of all contigs at this stage should be larger than the expected genome size because haplotype variants have not been merged or filtered out yet]

meraculous_bubble

- Builds depth and size statistics for the UUtigs, which will later be used in selecting contigs for scaffolding.
- For diploid assemblies, interrogates ends of UUtigs to detect *bubbles* in the graph caused by diploidy and then merges qualifying UUtigs into a new type of contigs called "*bubblotigs*". Internally, these bubblotigs are further categorized as either "*diplotigs*" or "*isotigs*". Diplotigs are bubblotigs that represent a single (potentially mosaic) path through both haplotypes, while isotigs are those that represent a path through only a single haplotype. Regions of polymorphism where bubbles couldn't be resolved will manifest themselves as isotigs with roughly half the depth of the rest of the bubblotigs, and based on this will be subject to filtering later on.

Important !!!

Once this stage has completed, we recommend pausing and reviewing the status of the current assembly. Examine the files `mercount.png`, `kha.png`, `UUtigs.fa`, and confirm that the results make sense (see section '[Key output files](#)' on how to interpret these). Based on what you see you may wish to adjust your parameters or even rerun some stages before continuing. For example, seeing an unusually high number of contigs in `UUtigs.fa` and a large low-depth peak in `mercount.png` may mean that your `min_depth_cutoff` parameter should be raised and everything restarted from `meraculous_mergraph`.

For diploid assemblies, you should examine the file `bubblotigs.depth.hist` and verify that there are two distinct peaks in the distribution, one at roughly half depth of the other. These are the

full depth contigs and the half-depth “isotigs” that still contain polymorphisms. At this point you may need to adjust your *bubble_min_depth_cutoff* parameter to a value that corresponds to the local minimum between the two peaks. If you had originally set it to 0 Meraculous will auto-detected this threshold. If you can't clearly identify the two peaks (e.g. low polymorphism), you will be better off keeping all the contigs, i.e. setting *bubble_min_depth_cutoff* to 1.

meraculous_merblast

- Generates depth- and size-filtered contigs to be used from here on. For diploid assemblies, half-depth isotigs are filtered out based on the *bubble_min_depth_cutoff* value (these sequences will be recovered later, during gap closure).
- Maps reads from libraries earmarked for scaffolding (i.e. having the `lib_seq` parameter's `scaffRound` setting set to non-zero) to the contigs, creating `blastMap*.merged` files

meraculous_ono

- Uses read mappings to "splint" gaps, i.e., link two or more contigs into a scaffold if a single read aligns to the contigs' respective 5' and 3' ends.

[Note: Only libraries earmarked for gap closure by the user are used in splinting. To minimize the effect of chimeras and other library prep artifacts, we strongly recommend to use only short insert paired-end Illumina libraries for this purpose.]

- For each library earmarked for oNo, determines the actual insert-size distribution based on read pairs mapping to the same contig. This estimate is then used to determine whether a given pair can be informative in linking contigs. Using this information, for each *set* of libraries (as defined by the user with the `lib_seq` parameter's `scaffRound` setting), linkage between contigs is established and scaffolds are built. This process is then iterated for the next `ono` set, bootstrapping from the scaffolds from the previous round.

[Note: Meraculous builds the scaffolds using a *range* of cutoffs for the minimum number of linking pairs (currently from 3 to 10). At the end of each round the set of scaffolds with the highest N50 is chosen to be used downstream.]

meraculous_gap_closure

- Based on read pairs mapping to neighboring contigs in a scaffold, a gap size model is generated. Then the scaffold and the gap size information is used to locate the reads suitable for "walking" across the gaps. The actual sequence is drawn from the original fastq files in the form of k-mers. Meraculous closes the gaps only if enough consistent and high quality sequence exists to bridge it entirely. Currently, no partial extensions of contigs into the gaps are made.

[Note: when running in diploid mode, if a gap represents a polymorphic region that was removed at the beginning of this stage (i.e. a half-depth isotig), Meraculous will attempt to walk across it using reads from the most common haplotype]

- The final Fasta sequence files are built from the scaffolds and the gap closing info, and any scaffolds under 1kb are filtered out. This constitutes the final assembly result.

meraculous_final_results

- Generates a brief summary report on the final assembly. For a more in-depth report users should run the standalone script `evaluate_meraculous_run.sh`

3.2 Input data preparation

Meraculous supports the following types in input data:

- Illumina-style sequence in fastq format is the only fully supported input data type at this moment. Using Perl regular expression notation, the supported fastq header formats are:

Illumina versions pre-1.8:

```
/^@\S+:\d+:\d+:\d+:\d+\#[ACTGN0]*)/[12]\s*\S*$/
```

Example: `@071112_SLXA-EAS1_s_4:1:1:672:654/1`

Illumina versions 1.8 and higher:

```
/^@\S+:\d+:\d+:\d+:\d+\s+[12]\:[YN]\:\d+:\d+:[ACTGN0]*$/
```

Example: @HISEQ03:379:C2WP8ACXX:7:1101:1465:2056 2:N:0:ACTTGA

- For paired libraries, read pairs can be either interleaved within a single file or be split into separate files, e.g. SRA000271.fastq.1 & SRA000271.fastq.2. In the latter case, the reads must be in the same order in both files and in one-to-one correspondence. [**Note:** If your input has been filtered in such a way that some reads have their pairs missing, you will need to edit the files and add dummy reads to take place of the missing pairs]
- All sequence files belonging to a single library should be definable by a single wildcard expression, e.g. SRA0*fastq* (two wildcards are required if reads 1 and 2 are in separate files, e.g. "SRA*fastq1,SRA*fastq2") For more on specifying the inputs see section '[Run configuration](#)'.
- Both Phred+33 and Phred+64 quality encoding schemas are supported. You can have a mix of libraries encoded with either schema, but each individual library must be of one common encoding scheme throughout.
- Both uncompressed and compressed (gzip) fastq files are supported.
- All reads should be at least k-mer size + 1 in length. The upper limit is currently 500 bp
- All reads should be free of adapter or barcode sequence as Meraculous does no explicit trimming or error-correction of the sequence. [**Note:** since low quality data is "naturally" filtered out by Meraculous based on k-mer depth, low complexity sequence, if present in large amounts, will tend to escape this filter]
- Meraculous is optimized for assembly of haploid and diploid genomes only. Running on polyploid or metagenomic datasets and interpreting results can be non-trivial.

3.3 Run configuration

The configuration file contains the parameters guiding the entire assembly process and must be passed to the program with the -c <file> argument.

The format of the configuration file is parameter followed by one or more values. Spaces or tabs can be used as field separators.

[**Note:** an additional optional configuration file named `.meraculous.conf` can be placed in your user home directory and contain default parameters that are not likely to change for your assembly jobs, e.g. `cluster_queue`, `cluster_slots_per_task`, etc. If there is any redundancy in the parameters in the two files, the run-specific config file takes precedence.]

Basic assembly parameters:

The values of these parameters should be set once, at the onset of the run. It's not advisable to change them at subsequent resume/restart attempts.

lib_seq	<p>A multi-argument parameter defining various properties of the input datasets. Normally, for every sequencing library, a separate <code>lib_seq</code> parameter line should be given. <u>The following are the mandatory arguments that must be given as values-only on a single line, in the exact order they're listed here, separated by one or more spaces or tabs. None can be omitted.</u></p> <p>[wildcard] - a bash-style expression (typically a full path) defining the sequence files for a single library. If fwd and rev read pairs are in separate files, then two wildcards should be provided, separated by a comma, without spaces. (See section 'Input data preparation' for more on input data requirements.)</p> <p>[name] - name of the library (caps, numbers)</p> <p>[insertAvg] - estimated average insert size in bp</p> <p>[insertSdev] - estimated std deviation of insert size in bp</p> <p>[avgReadLn] - estimated average read length in bp</p> <p>[hasInnieArtifact] - Whether or not a significant fraction of read pairs is in non-dominant orientation, e.g. "innies" in an "outie" library or vice versa. (0=false, 1=true)</p> <p>[isRevComped] - Whether or not the read pairs are in the "outie" orientation, i.e <-- -->. (0=false, 1=true)</p> <p>[useForContig] - Whether or not to use this libray for initial contig generation. Our recommendation is to use only paired-end libraries for this purpose. (0=false, 1=true)</p>
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	<p>[scaffRound] - Assigns the library to a scaffolding round. Libraries of the same type and similar insert size should be grouped into the same round for the sake of performance. To completely exclude a library from being used in scaffolding, set this to 0. <i>(positive integers, can be non-consecutive)</i></p> <p>[useForGapClosing] - Whether or not to use this library for gap closing. It's best to use the same libraries that were used for contig generation for this purpose. <i>(0=false, 1=true)</i></p> <p>[5p_wiggleRoom] - During linkage analysis and gap closure, allow reads from this library to have an unaligned 5' end up to this many bp. This option is for cases when a library is known to contain untrimmed adapter sequence. <i>(positive integer, 0 for default behavior)</i></p> <p>[3p_wiggleRoom] - During linkage analysis and gap closure, allow reads from this library to have an unaligned 3' end up to this many bp. This option can be used when the library is of poor sequence quality at the 3' end. Beware that allowing reads to map with large unaligned segments can potentially lead to erroneous linkage and other problems in the assembly. Use at your own risk! <i>(positive integer, 0 for default behavior)</i></p> <p>Example 1: two sets of files - one with fwd reads and the other with reverse reads</p> <pre>lib_seq /path/fastq*.0,/path/fastq*.1 ECO1 200 20 36 0 0 1 1 1 0 0</pre> <p>Example 2: one set of files, all with fwd/rev reads interleaved and up to 20 bp of 5' adapter present</p> <pre>lib_seq /path/to/fastq ECO2 200 20 100 0 0 0 1 0 20 0</pre>
genome_size	<p>Approximate genome size in Gb. Used in estimating depth of read coverage. <i>(positive integer or float)</i></p>
mer_size	<p>The k-mer size to use in meraculous. Must be an odd integer less than the size of the smallest read in the dataset. The optimal k-mer size depends on the quality of the sequence data and on the genome's repeat content. Picking a k-mer size that's too small means a given sequence will have a lower likelihood of being unique. Too large, and you're increasing the likelihood that the sequence will contain an error, which will cause it to be thrown out entirely by the low-depth filter. To have Meraculous auto-select one based on genome_size, set this parameter to 0</p> <p>There are a number of 3rd party tools available that help you pick the best k-mer size (e.g. kmergenie), but the following are ballpark guidelines for genomes of</p>

	<p>various sizes, assuming good sequence quality:</p> <p style="text-align: center;">Eschirichia coli: 31 Pichia stipitis: 41 Arabidopsis halleri: 51 Homo sapiens: 61</p>																														
is_diploid	Specifies whether to attempt to identify and merge haplotype variants (0=false, 1=true)																														
num_prefix_blocks	<p>Memory usage is optimized by breaking down the DNA search space by prefix, partitioning the k-mers into load-balanced blocks so that each can be processed separately and require similar amount of resources. The greater this number, the less RAM per process. The downside is that greater and greater fraction of run-time will be due to fixed costs like I/O of reading the input, scheduling, etc., so you get less efficiency. Note that only those libraries that will be used in UUtig generation (and therefore turned into k-mer structures) should be considered here.</p> <p>Note: Memory footprint is roughly proportional to the size of the input dataset, i.e., the total sequence used for contigging, but will vary with sequence quality, genome size, and repeat content. The following examples can be used as guidelines for estimating the desired number of blocks:</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th># of blocks</th> <th colspan="4">Peak memory during k-mer counting (GB)*</th> </tr> <tr> <th></th> <th>H.sapiens (290 Gbp)</th> <th>S.bicolor - highX (63 Gbp)</th> <th>S.bicolor-lowX (14 Gbp)</th> <th>N.fluitans (10 Gbp)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>276</td> <td>139</td> <td>23</td> <td>8</td> </tr> <tr> <td>4</td> <td>133</td> <td>42</td> <td>7</td> <td>3</td> </tr> <tr> <td>16</td> <td>24</td> <td>9</td> <td>2</td> <td>0.6</td> </tr> <tr> <td>32</td> <td>12</td> <td>4</td> <td>1</td> <td>0.3</td> </tr> </tbody> </table> <p>* Unlike with k-mer counting, the UUtig assembly stage cannot be partitioned this way and thus has fixed memory requirements which can be the limiting factor for large genomes. For more, see section ‘Hardware considerations’</p>	# of blocks	Peak memory during k-mer counting (GB)*					H.sapiens (290 Gbp)	S.bicolor - highX (63 Gbp)	S.bicolor-lowX (14 Gbp)	N.fluitans (10 Gbp)	1	276	139	23	8	4	133	42	7	3	16	24	9	2	0.6	32	12	4	1	0.3
# of blocks	Peak memory during k-mer counting (GB)*																														
	H.sapiens (290 Gbp)	S.bicolor - highX (63 Gbp)	S.bicolor-lowX (14 Gbp)	N.fluitans (10 Gbp)																											
1	276	139	23	8																											
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16	24	9	2	0.6																											
32	12	4	1	0.3																											

Optional assembly parameters

min_depth_cutoff	<p>K-mers less frequent than this cutoff will get excluded from assembly early on. To help determine the right cutoff, start by running with this set to 0, then, after the stage meraculous_mercount has completed, look at the k-mer frequency histogram file (mercount.png) and look for a high count (y), low frequency (x) peak that's distinct from the main</p>
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	<p>frequency distribution. That peak likely represents erroneous sequence which is best to keep out of the assembly. With the setting left at 0, the program will attempt to auto-detect the best cutoff. (<i>default: auto-detect</i>)</p>
bubble_min_depth_cutoff	<p><i>Valid only when 'is_diploid' is on.</i> After bubble resolution, some fraction of contigs still represents near-identical haplotype copies which couldn't be merged together. In the contig depth distribution at that stage, these contigs (termed isotigs) form a second peak at 1/2 the depth of the main contig set. This cutoff sets the depth threshold to filter these half-depth isotigs out. Meraculous will later attempt to recover them during gap-closure. To keep all haplotypes, set it to 1. To auto-detect, set it to 0 (<i>default: auto-detect</i>)</p>
min_mercount_to_report	<p>Lowest k-mer count to report: counts below this threshold will not be output by the mercounter and won't participate in any further steps. (<i>default = 2</i>)</p>
no_read_validation	<p>Set to 1 to skip validation of input fastq reads' headers, sequence, and q-scores. This will speed up the processing of reads in stage meraculous_import. We recommend using this option only when re-running with a previously validated dataset. (<i>0=false, 1=true</i>)</p>
fallback_on_est_insert_size	<p>If the program can't determine the actual <u>assembly-based</u> insert size average for a library, this option will allow it to continue using the initial estimates provided by user (see lib_seq). (<i>0=false, 1=true</i>)</p>
gap_close_aggressive	<p>Close gaps more aggressively, accepting closures that might violate the estimated gap size. (<i>0=false, 1=true</i>)</p>
gap_close_rpt_depth_ratio	<p>If the average k-mer depth of a given scaffold exceeds the overall modal peak depth for all scaffolds by more than this factor the scaffold is assumed to be a collapsed repeat and is excluded from consideration during meraculous_gap_closure. Raise this cutoff if you know the depth distribution in your dataset to be highly irregular, e.g. it's a metagenome or the like. (<i>default = 2.0</i>)</p>

Optional resource utilization parameters

<code>local_num_procs</code>	<i>Valid only when 'use_cluster' is off.</i> Number of processors to occupy simultaneously when running jobs locally. This should normally equal the number of available cpus. For non-threaded processes this sets the maximum number of processes executed in parallel. For threaded processes this sets the number of threads. <i>(default = 1)</i>
<code>num_procs_<stage></code>	Can be used to override 'local_num_procs' on a per-stage basis
<code>local_max_memory</code>	Set a memory limit (GB) for local processes. Any process that exceeds this limit will be terminated.
<code>local_max_retries</code>	Number of retries before failure for local jobs
<code>use_cluster</code>	Specifies whether to use a cluster for job submissions. Requires an SGE-like cluster system to be configured and ready to accept scheduling and monitoring commands like qsub, qacct, and qstat.
<code>cluster_slots_per_task</code>	Number of slots to allocate to each cluster task. Threaded tasks will spin this many threads each when run on the cluster. For non-threaded tasks this option is ignored. Typically, you would set this to the number of cpus on the smallest available node. If the nodes are shared and are heavily used, you may want to refrain from occupying all the CPUs on them.
<code>cluster_ram_request</code>	Amount of memory (GB) to request per task on the cluster If you request too much your process will have few available nodes to use, too little and your process will get killed by the cluster scheduler if it exceeds this limit.
<code>cluster_ram_<stage></code>	Can be used to override the above cluster_ram_request on a per-stage basis.
<code>cluster_walltime</code>	Walltime limit for cluster tasks. Must be specified as hh:mm:ss
<code>cluster_walltime_<stage></code>	Can be used to override the above cluster_walltime limit on a per-stage basis
<code>cluster_max_retries</code>	Number of retries before failure for cluster jobs
<code>cluster_project</code>	Name of project to which cluster jobs will be assigned. (This is needed only when your cluster uses project-based allocation schema)

<code>cluster_queue</code>	Name of queue to which cluster jobs will be assigned.
----------------------------	---

3.4 Executing the run

An assembly run that includes all stages, with intermediate data cleaned up at the end, is executed as follows:

```
run_meraculous.sh -c <config file>
```

This will generate a run directory named `run_<date>_<time>`. Inside the run directory, files `log/info.log`, `log/error.log`, and `log/debug.log` contain the detailed record of the assembly process. One way to monitor a running assembly is to watch the `info.log` or `debug.log` files "live" with `'tail -f'`. See section [‘Logs’](#) for more on these files.

Other command line options:

<code>-dir <></code> <code>-label <></code>	For new assembly runs, <code>-dir</code> lets you name your output run folder, while <code>-label</code> will attach the specified string as a prefix to the default name. For resuming or restarting an existing run, you're required to provide the run folder with the <code>-dir</code> option.
<code>-archive</code>	When restarting a stage from the beginning, save any existing stage directories under the subdirectory <code>old/</code>
<code>-debug</code>	Record all commands and additional troubleshooting information in the file <code>logs/debug.log</code> (Currently on by default)
<code>-cleanup_level <0 1 2></code>	Determines how aggressively the pipeline should clean up intermediate data after each stage. The possible arguments are: 0 - Do not delete any intermediate outputs. This will provide the ability to go back and examine full stage outputs or restart from any stage at any point in the future. The flip side is that with this option, the

	<p>disk space footprint may be several times the size of the input dataset.</p> <p>1 (default) - Delete files that are not used in any of the subsequent stages and that are generally not informative to the user. You will still be able to rerun any stage individually.</p> <p>2 - Delete as much as possible, as soon as possible. With this option you will not be able to rerun the stages individually once they have completed. The use of this option should be reserved to cases where disk space is tight and when you're confident that parts of the assembly will not need to be rerun.</p>
<code>-restart</code>	Restart a previously failed run from the last successful stage
<code>-resume</code>	Similar to <code>-restart</code> , but but preserves any partial results from successfully completed processes within the stage
<code>-step</code>	Execute one stage and stop
<code>-start <stage></code>	Re-run starting with this stage (requires <code>-resume</code> or <code>-restart</code>)
<code>-stop <stage></code>	Stop after this stage
<p>Invalid combinations:</p> <p><code>-restart</code> with <code>-resume</code></p> <p><code>-start</code> without <code>-restart/resume</code></p> <p><code>-archive</code> without <code>-restart</code></p> <p>See more about <code>-restart</code> and <code>-resume</code> options in section 'Working in stages'.</p>	

3.5 Working in stages

A Meraculous run consists of stages that can be executed and re-executed separately or as a set. Each stage reloads the parameters from the main user-specified config file. It also loads parameters from local parameter files created by preceding stages and writes local parameters for subsequent stages to use.

Note: Users are free to change the parameters in the config file between restart/resume attempts, however, the following parameters are considered "set in stone" and cannot be changed, removed, or added after the onset on the run:

```
mer_size
is_diploid
num_prefix_blocks
local_num_procs
cluster_slots_per_task
```

Upon successful completion a checkpoint file is created inside the checkpoints/ directory, which signals to Meraculous.pl that the stage has been completed, and the next time the pipeline is executed that stage will be skipped.

If for some reason the run exits in the middle of a stage, the user has an option to resume from where the previous attempt left off (presumably after making necessary corrections). Various milestones in the stage's progress are marked by "resume checkpoints" (not to be confused with checkpoint files described above). Once the pipeline determines the stage it needs to execute, it will jump to the specified resume checkpoint inside that stage, if one exists. Use the `-resume` option to enable this behavior.

If, instead of resuming, you wish to rerun an entire stage from the beginning or rerun starting with a certain stage, use the `-restart` option combined with `-start [stage]` and `-stop [stage]` to specify which stage to start and end the run with. The `-restart` option will cause the deletion of all previously completed stages from the '-start' stage and onward. If you wish to save those, use the `-archive` option, and they will be moved to a newly created `old/` subdirectory in your run.

If you wish to step through the stages, stopping after each one, use `-step` option

Note: The ability to resume the assembly process from a middle point is the main reason why Meraculous writes a large amount of intermediate data to disk. Setting `-cleanup_level` to 0 will cause many of these files to be deleted as soon as they're used and thus prohibits the 'restart' behavior while 'resume' should still be possible.

4. EVALUATING RESULTS & TROUBLESHOOTING

4.1 Key output files

- `meraculous_final_results/SUMMARY.txt`: Brief summary of assembly inputs and results (for creating a more detailed report see [Run evaluation script](#))
- `meraculous_final_results/final.scaffolds.fa`: The final set of scaffolds over 1 kb in total length in Fasta format. Gaps between contigs are filled with stretches of Ns whose number corresponds to the estimated gap size.
- `meraculous_merblast/contigs.fa`: The initial set of UUtigs (or bubbletigs if diploid) that were used as input to the scaffolding stages. The total size of the contigs in this file should be fairly close to the estimated genome size, with the remainder assumed to be in gaps that are to be spanned and filled in later stages. The file is in Fasta format; run the included `fasta_stats` program on this file to get a breakdown on contig size distribution.

```
Main genome contig total: 3190345
Main genome contig sequence total: 2691.5 MB (-> 0.0% gap)
Main genome contig N/L50: 284358/2.5 KB
```

Minimum Scaffold Length	Number of Scaffolds	Number of Contigs	Total Scaffold Length	Total Contig Length	Scaffold Contig Coverage
All	3,190,345	3,190,345	2,691,458,526	2,691,458,526	100.00%
1 kb	713,602	713,602	2,037,767,767	2,037,767,767	100.00%
2.5 kb	290,691	290,691	1,361,689,624	1,361,689,624	100.00%
5 kb	89,217	89,217	661,837,734	661,837,734	100.00%
10 kb	11,367	11,367	143,937,099	143,937,099	100.00%
25 kb	47	47	1,298,589	1,298,589	100.00%
50 kb	0	0	0	0	0.00%

- `meraculous_mercount/mercount.png`: Histogram of k-mer frequencies across the entire dataset used for contig generation. This can be useful for identifying various anomalies and trends with the dataset. Normally you should be able to identify the single peak corresponding to unique genomic kmers (two peaks if diploid), a low-depth peak for a low-depth/erroneous kmer population, and additional high-depth peaks for repeat k-mers.

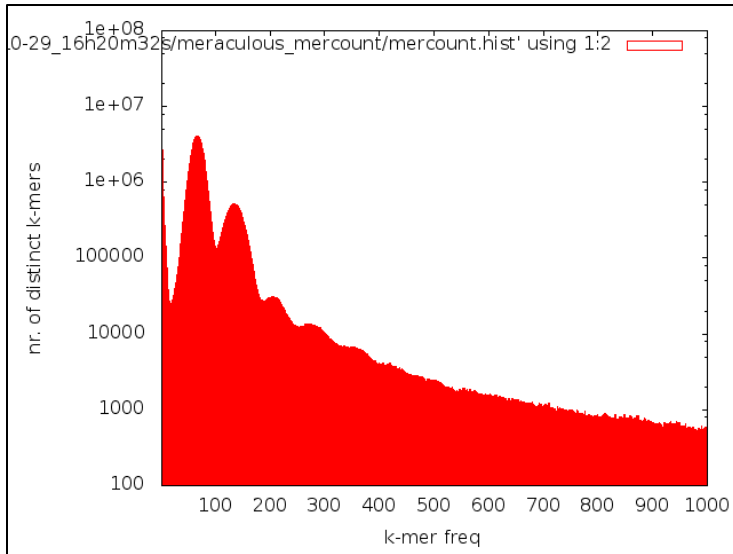


Figure 1: mercount.png – k-mer abundance histogram

- meraculous_mercount/kha.png: This plot shows the cumulative fraction of all k-mers in the dataset as a function of k-mer depth, which can be useful in identifying distinct k-mer “populations”, e.g. repeats, polymorphic regions, contamination.

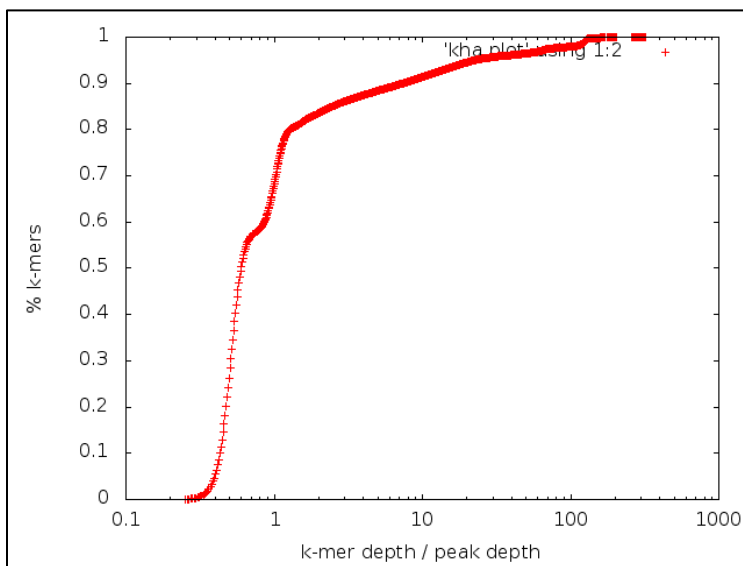


Figure 2: kha.png - Cumulative depth plot

- meraculous_ono/*.srf: Scaffold reference files from each scaffolding round. The last digit in the file name specifies the scaffolding round; the first digit specifies the minimum

number of links cutoff used to form the scaffolds. These files allow mapping of the original contigs to the current scaffolds.

4.2 Logs

The log files are critical for both monitoring the run progress and troubleshooting the results. You will find yourself looking at these files a lot, so it pays to get familiar with them early.

`info.log` - This is a concise record of the run, designed to inform a non-expert user of the key events during the run and raise a basic "Errors encountered" flag in case of a run failure. During a run, a good practice is watch this file in real time (e.g. `'tail -f run_abc/log/info.log'`)

`debug.log` - This is the most complete and verbose record of the process, designed for troubleshooting a failed run and is generally geared to a more expert user. In addition to the general messages from `info.log`, it records all commands the pipeline executes as local system calls, parallel job sets, resource usage and timing statistics. It will also capture all errors thrown by the Meraculous pipeline with information on which component threw the error.

`*.err` files - Errors from standalone programs executed by the pipeline will normally be captured in that program's standard error file. These files are always named and placed according to the program's intended output file and are specified as part of the command record in the `debug.log` file. For example, if Meraculous starts a `oNo4.pl` process which is meant to output a file `p3.2.srf`, then the standard error will be captured in the file `p3.2.srf.err` in the same directory. See more on this in section '[Troubleshooting tips](#)'.

4.3 Run evaluation script

The bash script `evaluate_meraculous_run.sh` collects various stats from the current run including any restart/resume attempts, and could be very useful in troubleshooting and understanding what happened at the different assembly stages (see below). We encourage users to run this script after the entire run is finished. It does not get executed automatically.

4.4 Troubleshooting tips

Errors and abnormal termination

When a run aborts prematurely, the error that caused the exit appears at the end of the debug.log and error.log files, e.g.

```
2014/03/18 13:31:12 meraculous.pl main::run_meraculous_gap_closure 2705> No fastq files
found for library ECO
2014/03/18 13:31:12 meraculous.pl main:: 711> Stage meraculous_gap_closure failed (0.001786
seconds)
2014/03/18 13:31:12 meraculous.pl main:: 771> ERRORS ENCOUNTERED!
2014/03/18 13:31:12 meraculous.pl main:: 775> Total run time: 0.832589 seconds.
```

Often, in order not to overload the logs with repetitive error messages, the core executables write errors and other troubleshooting info to stderr which gets captured by the pipeline into corresponding .err files inside the stage directories. If debug.log doesn't provide enough information about the root cause of the early exit, look for the last command that was attempted and see where the stderr was redirected to. Then review the messages in that file. For example, debug.log may have the following entry:

```
2014/03/26 12:08:49 M_Job_Set.pm M_Job_Set::run_job_set_local 765> Local command returned a
non-zero exit status! Check stderr outputs for more clues!
Return value (65280) Command ( perl gapPlacer.pl -b
../meraculous_merblast/blastMap.ECO.f0.merged -m 19 -i 215:10 -s
../meraculous_ono/ROUND_1/p7.1.srf
-f ../meraculous_merblast/ECO.fastq.info.0 -c ../meraculous_merblast/contigs.fa -F 10 >
gapData.ECO.0 2> gapData.ECO.0.err)
```

The file gapData.ECO.0.err will contain more info on what went wrong:

```
$ tail gapData.ECO.0.err
.
.
.
Total reads placed in gaps = 487780 (aligned) + 1090614 (projected)
Reading sequence file ../meraculous_import/ECO.fastq.0_00001...
Couldn't open ../meraculous_import/ECO.fastq.0_00001
```

When running in the *cluster* mode, the actual submission commands and any submission-related errors returned are captured in the files `linkedScript.template.submit.<date>.err` inside the stage directories. These can be useful in troubleshooting cluster compatibility issues. As the submission commands are formed by the `cluster_submit.sh` wrapper - that is typically the script to edit if one needs to modify the syntax of the submission (this is different from the job monitoring commands which are issued and logged directly by the pipeline)

```
$ cat linkedScript.template.submit.20140630-122647.err

qsub -v MERACULOUS_ROOT -cwd -r n -b n -S /bin/bash -w e -j y -N gapClosure -o run_2014-06-27_15h58m30s/meraculous_gap_closure/JOB_SET_LOG.gapClosure
-P plant-assembly.p -l h_rt=00:30:00 -l ram.c=1G -l h_vmem=1G -t 1-1 run_2014-06-27_15h58m30s/meraculous_gap_closure/linkedScript.template
```

Problems with assembly results

The prime causes of poor assemblies are usually sequencing library quality and/or settings, depth of coverage, and sequencing artifacts (e.g. untrimmed adapter). The output of `evaluate_meraculous_run.sh` can give clues about the input data. The section **MERCOUNTS** of the report, for instance, gives two different depth of coverage based on the k-mer count. If either of the estimates is too low or too high it may be a sign of a sub-optimal depth distribution in the dataset.

```
Total mers counted: 12787940
Average 19-mer depth (based on total count and genome size estimate): 55.22065
Weighted average 19-mer depth (based on observed frequencies) : 55.0550390445998
```

You should also refer to `mercount.png` and `kha.png` files for the actual distribution plots.

If the coverage is sufficient, check how well each library did during scaffolding. The section **LIBRARY MAPPING ANALYSIS** can point to a library whose data is getting rejected for a specific reason.

library	total_mapped	hits_omitted(rate)	total_spans
ECO1	6383564	6035869 (.915)	74585
ECO2	20522013	734836 (.035)	317968

Read mappings omitted for following reasons:

library	truncated_align	singleton	minlen
ECO1	4036475	499394	0
ECO2	510942	223894	0

6. CITING AND FEEDBACK

If you use Meraculous in your research, please cite [Chapman et al., 2014](#)
We would also like to reference back to your publications on our site. Please email the reference, the name of your lab, department and institution to egoltsman@lbl.gov.

We welcome your comments and bug reports. Please send them along with the debug.log file to egoltsman@lbl.gov.