MUMMER 3+ MANU+A+L

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1. Introduction

MUMmer is an open source software package for the rapid alignment of very large DNA and amino acid sequences. The latest version, release 3.0, includes a new suffix tree algorithm that has further improved the efficiency of the package and has been integral to making MUMmer an open source product. If you are familiar with the previous versions of MUMmer, you will find the new version is very similar because most of the changes have been to the implementation and not the interface, however this document assumes no previous experience with MUMmer, so past users may find it desirable to skip or skim through some of the sections.

1.1. Description

MUMmer is a modular and versatile package that relies on a suffix tree data structure for efficient pattern matching. Suffix trees are suited for large data sets because they can be constructed and searched in linear time and space. This allows `mummer` to find all 20 base pair maximal exact matches between two ~5 million base pair bacterial genomes in 20 seconds, using 90 MB of RAM, on a typical 1.7 GHz Linux desktop computer. Using a seed and extend strategy, other parts of the MUMmer pipeline use these exact matches as alignment anchors to generate pair-wise alignments similar to BLAST output. Also included are some utilities to handle the alignment output and a primitive plotting tool (`mummerplot`) that allows the user to convert MUMmer output to `gnuplot` files for dot and percent identity plots. Another graphical utility called MapView is included with the MUMmer distribution and displays sequence alignments to a annotated reference sequence for exon refinement and investigation.

This modular design has an important side effect, it allows for the easy reuse of MUMmer modules in other software. For instance, one can imagine primer design, repeat masking and even comparative annotation tools based on the efficient matching algorithm MUMmer provides. Another advantage of MUMmer is its speed. Its low runtime and memory requirements allow it to be used on most any computer. MUMmer's efficiency also makes it ideal for aligning huge sequences such as completed and draft eukarotic genomes. MUMmer has been successfully used to align the mouse and human genomes, showing it can handle most any input available. In addition, its ability to handle multiple sequences facilitate many vs. many searches, and make the comparison of unfinished draft sequence quite simple. However, because of it's many abilities, inexperienced users may find it difficult to determine the best methods for their application, so please refer to the Running MUMmer and Use cases sections for brief descriptions, use case examples, and tips on making the most of the MUMmer package, or if you want to understand more about a specific utility, refer to Program descriptions section for more detailed information and output formats.

1.2. Comparative genomics

1.2.1. Available sequence

The MUMmer package provides efficient means for comparing an entire genome against another. However, until 1999 there were no two genomes of sufficient similarity to compare. With the publication of the second strain of *Helicobacter pylori* in 1999, following the publication of the first strain in 1997, the scientific world had its first chance to look at two complete bacterial genomes whose DNA sequences were highly similar. The number of pairs of closely-related genomes has exploded in recent years, facilitating many comparative studies. For instance, the published databases include the following genomes for which multiple strains and/or multiple species have been sequenced:

multiple strains of...

- *Agrobacterium tumefaciens*
- *Bacillus anthracis*
- *Brucella melitensis*
- *Buchnera aphidicola*
- *Chlamydophila pneumoniae*

multiple species of...

- *Bacillus*
- *Chlamydia*
- *Clostridium*
- *Corynebacterium*
- *Lactobacillus*

- *Escherichia coli*
- *Helicobacter pylori*
- *Mycobacterium tuberculosis*
- *Neisseria meningitidis*
- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- *Yersinia pestis*
- *Listeria*
- *Methanosarcina*
- *Mycobacterium*
- *Mycoplasma*
- *Plasmodium*
- *Pseudomonas*
- *Pyrococcus*
- *Rickettsia*
- *Saccharomyces*
- *Staphylococcus*
- *Streptococcus*
- *Thermoplasma*
- *Vibrio*
- *Xanthomonas*
- *Xylella*

Most of these genomes can be obtained from the NCBI ftp site: ftp://ftp.ncbi.nlm.nih.gov/genomes/

1.2.2. Human vs. Human

With the capability to align the entire human genome to itself, there is no genome too large for MUMmer. The following table gives run times and space requirements for a cross comparison of all human chromosomes. The 1st column indicates the chromosome number, with "Un" referring to unmapped contigs. Column 2 shows chromosome length and column 4 shows the length of the total genomic DNA searched against the chromosome in column 1. Column 3 shows the time to construct the suffix tree, and column 5 the time to stream the query sequence through it. Column 6 shows the maximum amount of computer memory occupied by the program and data, and column 7 shows memory usage for the suffix tree in bytes per base pair. Each human chromosome was used as a reference, and the rest of the genome was used as a query and streamed against it. To avoid duplication, we only included chromosomes in the query if they had not already been compared; thus we first used chromosome 1 as a reference, and streamed the other 23 chromosomes against it. Then we used chromosome 2 as a reference, and streamed chromosomes 3–22, X, and Y against that, and so on.

Chr	Ref length (Mbp)	Suffix time (min)	Qry length (Mbp)	Query time (min)	Total space (Mb)	Suffix space (bytes/bp)
1	221.8	24.6	2617.1	679.5	3702	15.43
2	237.6	27.4	2379.5	625.8	3908	15.43
3	194.8	21.2	2184.7	565.0	3232	15.43
4	188.4	22.4	1996.3	518.0	3121	15.43
5	177.7	18.6	1818.6	461.4	2952	15.43
6	175.8	17.9	1642.8	407.6	2900	15.43
7	153.8	15.7	1489.0	360.1	2550	15.43
8	142.8	14.4	1346.2	322.3	2378	15.43
9	117.0	10.7	1229.2	303.7	1974	15.43
10	131.1	13.2	1098.1	263.3	2195	15.43
11	133.2	13.1	964.9	225.6	2228	15.43
12	129.4	12.5	835.5	195.9	2168	15.43
13	95.2	8.6	740.3	163.6	1633	15.44
14	88.2	7.5	652.1	141.0	1523	15.44
15	83.6	6.8	568.5	122.1	1451	15.44
16	80.9	6.4	487.6	106.3	1409	15.44

17	80.7	6.6	406.9	91.8	1406	15.44
18	74.6	6.3	332.3	78.8	1311	15.44
19	56.4	3.7	275.8	56.1	1026	15.45
20	59.4	4.6	216.4	45.8	1073	15.45
21	33.9	2.1	182.5	33.7	673	15.48
22	33.8	2.0	148.6	26.4	672	15.48
Un	1.4	0.03	147.3	10.0	164	16.96
X	147.3	14.6		4.8	2327	15.57

The Human Chromosomes can be obtained from the NCBI ftp site:
ftp://ftp.ncbi.nih.gov/genomes/H_sapiens/

1.3. OSI open source

The key difference between version 3.0 and previous versions of MUMmer, is its qualification as an open source project. Previous versions of MUMmer were always free for non-profit, but now MUMmer is free for all organizations, both for- and non-profit. Please refer to the LICENSE file included in the package for a description of the Artistic License, the same OSI certified open source license used by Perl and countless other packages. We encourage you to contact us (though you are not required to) if you wish to contribute to our ongoing improvement and development of the software, and simple suggestions on how to improve MUMmer are always welcome. Enjoy the freedom of open source!

To receive software update notices, please join the MUMmer mailing list. This list will only be used to announce major version releases and help us keep track of MUMmer users.

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2. Installation

MUMmer comes as a source distribution only, and needs to be compiled before use. This sections describes the steps and requirements necessary to compile the package. Installation problems are usually caused by incompatible versions of one or more OS utilities, so if installation fails please check that you have the needed system requirements before alerting us of your problem. The INSTALL file included in the source distribution also contains much of the same information provided in this section.

2.1. System Requirements

MUMmer is mostly written in C and C++. With some technical expertise it could be ported to any system with a C++ compiler, but our distribution was specifically designed to be compiled with the GNU GCC compiler and has been successfully tested on the following three platforms:

- Redhat Linux 6.2 and 7.3 (Pentium 4)
- Compaq Tru64 UNIX 5.1 (alpha)
- SunOS UNIX 5.8 (sparc)
- Mac OS X 10.2.8 (PowerPC G4)

MUMmer also requires some third party software to run successfully. In the absence of one or more of the below utilities, certain MUMmer programs may fail to run correctly. Listed in parenthesis are the versions used to test the MUMmer package. These versions, or subsequent versions should assure the proper execution of the various MUMmer programs. These utilities must be accessible via the system path:

- make (GNU make 3.79.1)

- perl (PERL 5.6.0)
- sh (GNU sh 1.14.7)
- csh (tcsh 6.10.00)
- g++ (GNU gcc 2.95.3)
- sed (GNU sed 3.02)
- awk (GNU awk 3.0.4)
- ar (GNU ar 2.9.5)

For running the MUMmer display programs, these additional system utilities are required:

- fig2dev (fig2dev 3.2.3)
- gnuplot (gnuplot 4.0)
- xfig (xfig 3.2)

Sufficient memory and disk space are also necessary, but required sizes vary considerably with input size, so please be aware of your disk and memory usage, as insufficient capacities will result in incorrect or missing output. In general, 512 MB of RAM and 1 GB of disk space is sufficient for most mid-sized comparisons. For Mac OSX, the Mac development kit must be downloaded and installed. This kit will include `gcc`, `ar`, and `make` which are necessary for building MUMmer. MUMmer is not supported for any Mac operating system other than OSX.

2.2. Obtaining MUMmer

The current MUMmer release can be downloaded from our SourceForge.net project page.

2.3. Compilation and installation

For explanation purposes, let's suppose you just downloaded the `MUMmer3.0.tar.gz` distribution from the SourceForge site. The first step would be to move this file to the desired installation directory and type:

```
tar -xvzf MUMmer3.0.tar.gz
```

to extract the MUMmer source into a `MUMmer3.0` subdirectory. Switch to this newly created subdirectory and execute:

```
make check
```

to assure the makefile can identify the necessary utilities. If no error messages appear, the diagnostics were successful and you may continue. However, if error messages are displayed, the listed programs are not accessible via your system path. Install the utilities if necessary, add them to your system `PATH` variable, and continue with the MUMmer installation by typing:

```
make install
```

This will attempt to compile the MUMmer scripts and executables. If the `make` command issues no errors, the compilation was successful and you are ready to begin using MUMmer. If the command fails, it is likely that `make` was confused by the existence of more than one copy of the same utility, such as two versions of `gcc`. When this happens, it is important to arrange you system `PATH` variable so that the more recent versions are listed first, or to hard code the location of your utility location in the makefile. The same advice goes for your `LD_LIBRARY_PATH` variable if your system is having a difficult time locating the appropriate C or C++ libraries at runtime.

It is important to note that the `make` command dynamically builds the MUMmer scripts to reference the install directory, therefore if the install directory is moved after the `make` command is issued the MUMmer scripts will fail. If you need certain MUMmer executables in a directory other than the install directory, it is recommend to leave the install directory untouched and link the needed executables to the desired destination. An alternative would be to move the install directory and reissue the `make` command at the

new location.

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3. Running MUMmer

The five most commonly used programs in the MUMmer package are `mummer`, `nucmer`, `promer`, `run-mummer1` and `run-mummer3`, so this section covers the basics of executing these tools and what each of them specializes in. To better understand how to view the outputs of these programs, please refer to the use cases section or the MUMmer examples webpage for a brief walk-through of each major module with full input data and expected outputs. For further information, please refer to the Program descriptions section for a detailed explanation of each program and its output.

mummer

`mummer` efficiently locates *maximal unique matches* between two sequences using a suffix tree data structure. This makes `mummer` most suited for generating lists of exact matches that can be displayed as a dot plot, or used as anchors in generating pair-wise alignments.

```
mummer [options] <reference file> <query file1> . . . [query file32]
```

There must be exactly one reference file and at least one query file. Both the reference and query files should be in multi-FastA format and may contain any set of upper and lowercase characters, thus DNA and protein sequences are both allowed and matching is case insensitive. The maximum number of query files is 32, but there is no limit on how many sequences each reference or query file may contain. Output is to `stdout`. Refer to the mummer section for a list of options and output descriptions.

NUCmer

NUCmer is a Perl script pipeline for the alignment of multiple *closely related* nucleotide sequences. It begins by finding maximal exact matches of a given length, it then clusters these matches to form larger inexact alignment regions, and finally, it extends alignments outward from each of the matches to join the clusters into a single high scoring pair-wise alignment. This makes NUCmer most suited for locating and displaying highly conserved regions of DNA sequence. To increase NUCmer's accuracy, it may be desirable to mask the input sequences to avoid the alignment of uninteresting sequence, or to change the uniqueness constraints (see the NUCmer section) to reduce the number of repeat induced alignments.

```
nucmer [options] <reference file> <query file>
```

Both the reference and query files should be in multi-FastA format and may contain any set of upper and lowercase characters, however *only* the DNA characters *a*, *c*, *t* and *g* will be aligned (case insensitive). There is no limit on how many sequences the reference or query files may contain. Output is written to the files `out.cluster` and `out.delta`. Each is an ASCII file, but not formatted for human consumption, so it is necessary to run a utility program to parse the output. The two primary utility programs for viewing the contents of a `.delta` file are `show-aligns`, and `show-coords`. `show-aligns` displays all of the pair-wise alignments between two sequences, while `show-coords` displays a summary of the coordinates, percent identity, etc. of the alignment regions. Refer to the NUCmer section for a list of options and output descriptions.

PROmer

PROmer is a Perl script pipeline for the alignment of multiple *somewhat divergent* nucleotide sequences. It works exactly like NUCmer, but with a small twist. Before any of the exact matching takes place, the input sequences are translated in all six amino acid reading frames. This allows PROmer to identify regions of conserved protein sequences that may not be conserved on the DNA level and thus gives it a higher sensitivity than NUCmer. Note however, this increase in sensitivity will result in huge amounts of output for highly similar sequences, therefore it is recommended that PROmer only be used when the input

sequences are too divergent to produce a reasonable amount of NUCmer output. As with NUCmer, it is recommended to mask the input sequences to avoid the alignment of uninteresting sequence, or to change the uniqueness constraints (see the PROmer section) to reduce the number of repeat induced alignments.

```
promer [options] <reference file> <query file>
```

Both the reference and query files should be in multi-FastA format and may contain any set of upper and lowercase characters, however *only* valid DNA characters will result in correctly translated sequence, all other characters will be translated into masking characters and therefore will not be matched by the BLOSUM scoring matrix. There is no limit on how many sequences the reference or query files may contain. Output is written to the same files as NUCmer and can also be viewed with the same utility programs (see above). Refer to the PROmer section for a list of options and output descriptions.

run-mummer1 and run-mummer3

`run-mummer1` and `run-mummer3` are cshell script pipelines for the general alignment of two sequences. They follow the same three steps of NUCmer and PROmer, in that they match, cluster and extend, however they handle any input sequence, not just nucleotide. This non-discrimination can be useful, however the program interface is not very user friendly and the output can be difficult to parse. In their favor, the `run-mummer*` programs are good at aligning very similar DNA sequences and identifying their differences, this makes them well suited for SNP and error detection. `run-mummer1` is recommended for one vs. one comparisons with no rearrangements, while `run-mummer3` is recommended for one vs. many comparisons that may involved rearrangements. Sequence masking is only recommended if a different character is used to mask the reference and query sequences so that they are not aligned.

```
run-mummer1 <reference file> <query file> <prefix> [-r]
```

or

```
run-mummer3 <reference file> <query file> <prefix>
```

The reference and query files should both be in FastA format and may contain any set of upper and lowercase characters. The reference file *may only contain a single sequence*, and `run-mummer1` only allows a single query sequence, but `run-mummer3` has no limit on the number of query sequences . The `-r` option for `run-mummer1` reverses the query sequence, while `run-mummer3` automatically finds both forward and reverse matches. Output is written to the files `<prefix>.out`, `<prefix>.gaps`, `<prefix>.errorsgaps` and `<prefix>.align`. There are no utilities included to parse these files, so they must be viewed as raw text files. Refer to the run-mummer1 and run-mummer3 sections for info on changing the program parameters and output descriptions.

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4. Use cases and walk-throughs

Because of its breadth, MUMmer can be overwhelming at first, and sometimes the hardest part of using MUMmer is deciding which alignment program to run for a particular application. This section attempts to overview some of the basic MUMmer use cases and propose the best MUMmer alignment routine for each case. This section only gives a set of command line calls to generate alignments for each use case. For further information, please refer to the Program descriptions section for a detailed explanation of each program and its output, and the MUMmer examples webpage for a brief walk-through of each major module with full input data and expected outputs.

4.1. Aligning two finished sequences

The most basic use case is the alignment of two contiguous sequences. For all of the one vs. one use cases the `mummer` program alone, when coupled with `mummerplot`, may be all that is necessary to visualize a global alignment of the two sequences. This process alone can be very helpful in determining the large scale differences between the two sequences. For a single reference sequence `ref.fasta` and a single query

sequence `qry.fasta` in FastA format, type:

```
mummer -mum -b -c ref.fasta qry.fasta > ref_qry.mums

mummerplot --postscript --prefix=ref_qry ref_qry.mums

gnuplot ref_qry.gp
```

Then view or print the postscript plot `ref_qry.ps` in whatever manner you wish.

4.1.1. Highly similar sequences without rearrangements

When comparing two near identical sequences, the object of the alignment is usually SNP and small indel identification. The original MUMmer1.0 pipeline still proves to be a handy tool for this type of analysis, although `run-mummer3` with `combineMUMs -D` can prove to be even handier. Its LIS clustering algorithm and reliance on unique matches give it some reliability advantages over the newer pipelines. For a single reference sequence `ref.fasta` and a single query sequence `qry.fasta` in FastA format, type:

```
run-mummer1 ref.fasta qry.fasta ref_qry
```

or for sequences that match on the reverse strand

```
run-mummer1 ref.fasta qry.fasta ref_qry -r
```

SNP detection and one-to-one global alignment can also be performed by `nucmer` as described in the SNP detection walkthrough. The NUCmer pipeline provides a more user-friendly method for SNP detection while sacrificing a small degree of sensitivity.

4.1.2. Highly similar sequences with rearrangements

Often two sequences are highly similar, but large chunks of the sequence are rearranged, inverted and inserted. In order to align these and produce an output that is similar to the MUMmer1.0 pipeline, use `run-mummer3`. It uses a clustering method that allows for these types of large scale mutations, but retains many of the other features of `run-mummer1`. To hunt for SNPs more accurately, you can edit the script and add the `-D` option to the `combineMUMs` command line, thus producing a concise file of only the difference positions between the two sequences. For a single reference sequence `ref.fasta` and a single query sequence `qry.fasta` in FastA format, type:

```
run-mummer3 ref.fasta qry.fasta ref_qry
```

SNP detection and one-to-one local alignment can also be performed by `nucmer` as described in the SNP detection walkthrough. The NUCmer pipeline provides a more user-friendly method for SNP detection while sacrificing a small degree of sensitivity.

4.1.3. Fairly similar sequences

While `run-mummer1` and `run-mummer3` focus more on what is different between two sequences, `nucmer` focuses on what is the same. It has very few restrictions on what it will align, so rearrangements, inversions and repeats will all be identified by `nucmer`. For a single reference sequence `ref.fasta` and a single query sequence `qry.fasta` in FastA format, type:

```
nucmer --maxgap=500 --mincluster=100 --prefix=ref_qry ref.fasta qry.fasta

show-coords -r ref_qry.delta > ref_qry.coords

show-aligns ref_qry.delta refname qryname > ref_qry.aligns
```

Where `refname` and `qryname` are the FastA IDs of the two sequences. The output of NUCmer can often be voluminous and is best visualized with `mummerplot`. In addition, its output can be filtered in a varity of ways with the `delta-filter` program. For example, to select and display a one-to-one local mapping of reference to query sequences, use:

```
delta-filter -q -r ref_qry.delta > ref_qry.filter

mummerplot ref_qry.filter -R ref.fasta -Q qry.fasta
```

This will first filter the delta file, selecting only those alignments which comprise the one-to-one mapping between reference and query, and then display a dotplot of the selected alignments. Note that NUCmer allows for multiple reference and query sequences, so the above methods will also work for such and input. See the delta-filter and mummerplot sections for more details.

4.1.4. Fairly dissimilar sequences

Sometimes two sequences exhibit poor similarity on the DNA level, but their protein sequences are conserved. In this case, `promer` will be the most useful MUMmer tool, since it translates the DNA input sequences into amino acids before proceeding with the alignment. For a single DNA reference sequence `ref.fasta` and a single DNA query sequence `qry.fasta` in FastA format, type:

```
promer --prefix=ref_qry ref.fasta qry.fasta

show-coords -r ref_qry.delta > ref_qry.coords

show-aligns -r ref_qry.delta refname qryname > ref_qry.aligns
```

Where `refname` and `qryname` are the FastA IDs of the two sequences. Note that the `-k` option can be added to `show-coords` to reduce the amount of output by only displaying the best frame in situations where the same hit is represented in multiple, overlapping frames. The output of PROmer can often be voluminous and is best visualized with `mummerplot`. In addition, its output can be filtered in a varity of ways with the `delta-filter` program. For example, to select and display a one-to-one local mapping of reference to query sequences, use:

```
delta-filter -q -r ref_qry.delta > ref_qry.filter

mummerplot ref_qry.filter -R ref.fasta -Q qry.fasta
```

This will first filter the delta file, selecting only those alignments which comprise the one-to-one mapping between reference and query, and then display a dotplot of the selected alignments. Note that PROmer allows for multiple reference and query sequences, so the above methods will also work for such an input. See the delta-filter and mummerplot sections for more details.

4.2. Aligning two draft sequences

Many times it is necessary to align two genomes that have not yet been completed, or two genomes with multiple chromosomes. This can make things a little more complicated, since a separate alignment would have to be generated for each possible pairing of the sequences. However, both NUCmer and PROmer automate this process and accept multi-FastA inputs, thus simplifying the process of aligning two sets of contigs, scaffolds or chromosomes. Since NUCmer and PROmer have an almost identical user interface, this use case will only be explained using `nucmer`. If the two inputs are too divergent for `nucmer` to align, simply use `promer` instead. For two sets of contigs, `ref.fasta` and `qry.fasta`, type:

```
nucmer --prefix=ref_qry ref.fasta qry.fasta

show-coords -rcl ref_qry.delta > ref_qry.coords

show-aligns ref_qry.delta refname qryname > ref_qry.aligns
```

Where `refname` and `qryname` are the FastA IDs of two contigs. The `show-aligns` step will have to be repeated for every combination of contigs that the user wishes to analyze. Because the output of the all-vs-all comparison described above can be immense, it is often essential to filter the resulting alignment data with the `delta-filter` program. To map each reference to a position in the query, use `delta-filter -r`. To map each query to a position in the reference, use `delta-filter -q`. To determine a one-to-one mapping of each reference and query, combine the options and use `delta-filter -r -q`. Also, the `mummerplot` utility provides a very handy visualization method for viewing contig mappings, type:

```
mummerplot ref_qry.delta -R ref.fasta -Q qry.fasta --filter --layout
```

This will generate a plot displaying the one-to-one mapping between the two contig sets. When plotted to an X11 terminal, the plot is zoom-able and browse-able via the mouse and keyboard commands provided by gnuplot 4.0. See the delta-filter and mummerplot sections for more details.

4.3. Mapping a draft sequence to a finished sequence

There are many benefits of mapping a draft sequence to the finished sequence of a related organism. Determining the location and orientation of each query contig as it maps to the finished reference sequence can significantly speed up the closure process of the draft sequence, and by examining the areas of conservation, the annotation of the draft sequence can be improved and refined. Since NUCmer and PROmer have an almost identical user interface, this use case will only be explained using `nucmer`. If the two inputs are to divergent for `nucmer`, simply use `promer` instead. For a finished reference chromosome(s) `ref.fasta` and a set of near identical contigs `qry.fasta`, type:

```
nucmer --prefix=ref_qry ref.fasta qry.fasta

show-coords -rcl ref_qry.delta > ref_qry.coords

show-aligns ref_qry.delta refname qryname > ref_qry.aligns

show-tiling ref_qry.delta > ref_qry.tiling
```

Where `refname` and `qryname` are the FastA IDs of two sequences. The `show-aligns` step will have to be repeated for every combination of sequences that the user wishes to analyze. If mapping the draft sequences to each of their repeat locations is not required, the `delta-filter` program can quickly select the optimal placement of each draft sequence to the reference using the following:

```
delta-filter -q ref_qry.delta > ref_qry.filter
```

The newly created delta file `ref_qry.filter` can then be substituted for the original in the above procedures in order to generate slimmed down versions of the output.

4.4. SNP detection

Joining a couple of the MUMmer components together can form a quite reliable SNP detection pipeline. MUMmer can perform all steps of this pipeline from aligning the sequences, to selecting the one-to-one mapping, and finally calling the SNP positions. The user can then process these SNP positions to assign quality scores based on the underlying traces and surrounding context. Such methods have been successfully applied to various SNP studies for organisms including *Bacillus anthracis* and *Yersinia pestis*. Of important note, a SNP pipeline built with `nucmer` allows for the identification of SNPs between two genomes with many rearrangements. The *Yersinia pestis* strains, for example, demonstrate significant genome "shuffling", and make SNP detection difficult with global alignment programs such as `run-mummer1`. However, a pipeline built with `nucmer` (like shown below) is capable of finding all of the SNPs between two genomes, regardless of their structural similarity.

To find a reliable set of SNPs between to highly similar multi-FastA sequence sets `ref.fasta` and `qry.fasta`, type:

```
nucmer --prefix=ref_qry ref.fasta qry.fasta

show-snps -Clr ref_qry.delta > ref_qry.snps
```

The `-C` option in `show-snps` assures that only SNPs found in uniquely aligned sequence will be reported, thus excluding SNPs contained in repeats. An alternative method which first attempts to determine the "correct" repeat copy is:

```
nucmer --prefix=ref_qry ref.fasta qry.fasta

delta-filter -r -q ref_qry.delta > ref_qry.filter

show-snps -Clr ref_qry.filter > ref_qry.snps
```

Now, conflicting repeat copies will first be eliminated with `delta-filter` and the SNPs will be re-called in hopes of finding some that were previously masked by another repeat copy.

4.5. Identifying repeats

Although MUMmer was not specifically designed to identify repeats, it does has a few methods of identifying exact and exact tandem repeats. In addition to these methods, the `nucmer` alignment script can be used to align a sequence (or set of sequences) to itself. By ignoring all of the hits that have the same coordinates in both inputs, one can generate a list of inexact repeats. When using this method of repeat detection, be sure to set the `--maxmatch` and `--nosimplify` options to ensure the correct results.

To find large inexact repeats in a set of sequences `seq.fasta`, type the following and ignore all hits with the same start coordinate in each copy of the sequence:

```
nucmer --maxmatch --nosimplify --prefix=seq_seq seq.fasta seq.fasta

show-coords -r seq_seq.delta > seq_seq.coords
```

To find exact repeats of length 50 or greater in a single sequence `seq.fasta`, type:

```
repeat-match -n 50 seq.fasta > seq.repeats
```

To find exact tandem repeats of length 50 or greater in a single sequence `seq.fasta`, type:

```
exact-tandems seq.fasta 50 > seq.tandems
```

---

5. Program descriptions

The most commonly used MUMmer pipelines (`nucmer`, `promer`, `run-mummer1` and `run-mummer3`) are comprised of three main sections. The first section identifies a certain subset of maximal exact matches between the two inputs, the second section clusters these matches into groups that will likely make good alignment anchors, and the third and final section extends alignments between these clustered matches to produce the final gapped alignment. These three sections also outline the primary types of programs included in the MUMmer package - the Maximal exact matching section describes the programs that compute different types maximal exact matches, the Clustering section describes the two different types of clustering algorithms, and Alignment generators describes the scripts that combine matching, clustering and extending in order to produce high scoring pair-wise alignments. Finally, the Utilities section reviews a few of the tools that have been developed for interpreting and displaying the output of the MUMmer alignment routines.

It is noteworthy to point out the simplicity of improving the current MUMmer pipeline. For instance, if a different and/or better clustering algorithm was needed for a certain application, a program could be

written in any language and inserted into the pipeline. So long as the program was able to read the appropriate input and produce output that mimics the existing module, it could be swapped for the existing module with a single edit to the calling script. NUCmer for example is a Perl script that invokes various MUMmer routines. If you were to develop a new clustering algorithm called `mygaps` you could edit the line in NUCmer that defines the location of `mgaps` to instead define the location of `mygaps`. It's that easy, as long as `mygaps` had the same input and output `mgaps` the transition would be seamless.

5.1. Maximal exact matching

The heart of the MUMmer package is its suffix tree based maximal matching routines. These can be used for repeat detection within a single sequence as is done by `repeat-match` and `exact-tandems`, or can be used for the alignment of two or more sequences as is done by `mummer`. Most every other program in the MUMmer packages builds off of the output of the `mummer` maximal exact matcher, so it is of great importance to first understand the workings of this program.

5.1.1. mummer

`mummer` is a suffix tree algorithm designed to find maximal exact matches of some minimum length between two input sequences. MUMmer's namesake program originally stood for Maximal Unique Matcher, however in subsequent versions the meaning of *unique* has been skewed. The original version (1.0) required all maximal matches to be unique in both the reference and the query sequence (MUMs); the second version (2.0) required uniqueness only in the reference sequence (MUM-candidates); and the current version (3.0) can ignore uniqueness completely, however it defaults to finding MUM-candidates and can be switched on the command line. To restate, by default `mummer` will only find maximal matches that are unique in the entire set of reference sequences. The match lists produced by `mummer` can be used alone to generate alignment dot plots, or can be passed on to the clustering algorithms for the identification of longer non-exact regions of conservation. These match lists have great versatility because they contain huge amounts of information and can be passed forward to other interpretation programs for clustering, analysis, searching, etc.

`mummer` achieves its high performance by using a very efficient data structure known as a suffix tree. This data structure can be both constructed and searched in linear time, making it ideal for large scale pattern matching. To save memory, only the reference sequence(s) is used to construct the suffix tree and the query sequences are then streamed through the data structure while all of the maximal exact matches are extracted and displayed to the user. Because only the reference sequence is loaded into memory, the space requirement for any particular `mummer` run is only dependent on the size of the reference sequence. Therefore, if you have a reasonably sized sequence set that you want to match against an enormous set of sequences, it is wise to make the smaller file the reference to assure the process will not exhaust your computer's memory resources. The query files are loaded into memory one at a time, so for an enormous query that will require a significant amount of memory just to load the character string, it is helpful to partition the query into multiple smaller files using the syntax described below.

Command line syntax

```
mummer [options] <reference file> <query file1> . . . [query file32]
```

There must be exactly one reference file and at least one query file. Both the reference and query files should be in multi-FastA format and may contain any set of upper and lowercase characters, thus DNA and protein sequences are both allowed and matching is case insensitive. The maximum number of query files is 32, but there is no limit on how many sequences each reference or query file may contain.

Program options

```
-mum             Compute MUMs, i.e. matches that are unique in both the reference and query
```

-mumreference	Compute MUM-candidates, i.e. matches that are unique in the reference but not necessarily in the query
-maxmatch	Compute all maximal matches regardless of their uniqueness
-n	Only match the characters *a*, *c*, *g*, or *t* (case insensitive)
-l int	Minimum match length (default 20)
-b	Compute both forward and reverse complement matches
-r	Only compute reverse complement matches
-s	Show the matching substring in the output
-c	Report the query position of a reverse complement match relative to the forward strand of the query sequence
-F	Force 4 column output format that prepends every match line with the reference sequence identifier
-L	Show the length of the query sequence on the header line
-help	Show the possible options and exit

Option grouping is not allowed, therefore each option should be separated by a space. The options `-mum`, `-mumreference`, and `-maxmatch` cannot be combined, and if neither is used, then the program will default to `-mumreference`. For a string to be unique in the reference, it must occur only once in the concatenation of *all* the reference superstrings, but for string to be unique in the query it need only be unique in its own superstring. Setting either the `-mum` or `-mumreference` option can significantly cut down on the number of repeat induced matches as opposed to `-maxmatch`, and is recommended for most all applications. Also, setting the `-l` option any lower than around 15 can significantly increase the number of spurious matches and therefore balloon the runtime. When dealing with masked DNA sequence, use the `-n` option to avoid matching the masking characters. Options `-b` and `-r` exclude each other, and if neither is used then only forward matches will be reported. All reverse complementing will affect only the query sequences. Option `-c` can only be used in combination with `-b` or `-r`, as it would have no relevance without these options. The `-F` option is useful for forcing `mummer` to output a consistent format regardless of the number of input sequences.

For those familiar with the previous versions of MUMmer, the `-mum` option mimics the functionality of MUMmer1.0; the `-mumreference` option mimics the functionality of MUMmer2.0; and the `-maxmatch` option mimics the functionality of the `max-match` program included with MUMmer2.0. The default behavior of the current version is `-mumreference` because it is a good balance between finding all matches and only unique matches.

Output format

Output formatting varies depending on the command line parameters used. Program diagnostic information is always output to `stderr` while the match lists are output to `stdout`. This allows for the match output to be redirected into a file, which is quite useful since the output is generally quite large. The standard output format that results from running `mummer` on a single reference sequence with the `-b` option is as follows:

```
> ID1
 4655667         1        31
 4655699        33       319
 4656019       353       520
 4656540       874        20
> ID1 Reverse
  741743        22       872
> ID2
 4655520         1       498
 4656019       500       274
 4656317       798        39
 4656376       855        29
> ID2 Reverse
> ID3
> ID3 Reverse
 4655178        27       840
 4656019       868       171
(output continues ...)
```

For each query sequence, the corresponding ID tag is reported on each line beginning with a `'>'` symbol, even if there are no matches corresponding to this sequence. Reverse complemented matches follow a query header that has the keyword `Reverse` following the sequence tag, thus creating two headers for each query sequence and alternating forward and reverse match lists. For each match, the three columns list the position in the reference sequence, the position in the query sequence, and the length of the match respectively. Reverse complemented query positions are reported relative to the *reverse* of the query sequence unless the `-c` option was used. As was stated above the `-L` option adds the sequence lengths to the header line and the `-s` option adds the match strings to the output, if these options were used the format would be as follows:

```
> ID1  Len = 893
 4655667         1        31
ctgacgacaaccatgcaccacctgtcactct
 4655699        33       319
ctcccgaaggagaagccctatctctagggttgtcagaggatgtcaagacctgg . . .
 4656019       353       520
gttcctccatatctctacgcatttcaccgctacacatggaattccactttcct . . .
 4656540       874        20
tttcgaaccatgcggttcaa
> ID1 Reverse  Len = 893
  741743        22       872
tgaaaggcggcttcggctgtcacttatggatggacccgcgtcgcattagctag . . .
> ID2  Len = 884
 4655520         1       498
tcataaggggcatgatgatttgacgtcatccccaccttcctccggtttgtcac . . .
 4656019       500       274
gttcctccatatctctacgcatttcaccgctacacatggaattccactttcct . . .
 4656317       798        39
aagccttcatcactcacgcggcgttgctccgtcagactt
 4656376       855        29
cctactgctgcctcccgtaggagtctggg
> ID2 Reverse  Len = 884
> ID3  Len = 1039
> ID3 Reverse  Len = 1039
 4655178        27       840
atcaattctccatagaaaggaggtgatccagccgcaccttccgatacggctac . . .
 4656019       868       171
gttcctccatatctctacgcatttcaccgctacacatggaattccactttcct . . .
```

```
(output continues ...)
```

Where the length of each query is noted after the `Len` keyword and the match string is listed on the line after its match coordinates. Note that the ellipsis marks are not part of the actual output, but added to fit the output into the webpage. Finally, when dealing with multiple reference sequences (or the `-F` option), it is necessary to output the ID of the reference sequence. This is placed at the beginning of each match line, creating an four column output format as follows:

```
> ID1
  220594       479         1       728
> ID1 Reverse
  220716      3527         1        20
  220716      3548        22       840
> ID2
> ID2 Reverse
  219093        13       401       484
  220716      3682         2        29
  220716      3731        49        39
  220716      3794       112       693
> ID3
  219093        13       188       721
  220716      3897         2       590
  220716      4488       593       423
> ID3 Reverse
  220594         1        38       509
(output continues ...)
```

5.1.2. repeat-match

`repeat-match` is a suffix tree algorithm designed to find maximal exact repeats within a single input sequence. It uses a similar algorithm to `mummer`, but altered slightly to find maximal exact matches within a single sequence.

Command line syntax

```
repeat-match [options] <sequence file>
```

The sequence file should contain only one sequence in FastA format, however if multiple sequences exist the first one will be used. The sequence may contain any set of upper and lowercase characters, thus DNA and protein sequences are both allowed and matching is case insensitive.

Program options

```
  -f                Use the forward strand only

  -n int            Minimum match length (default 20)

  -t                Only output tandem repeats
```

The program will report both forward and reverse complement repeats by default unless the `-f` option is used. While the `-t` option identifies tandem repeats, the `exact-tandems` script is a wrapper for `repeat-match` and does a more graceful job of reporting the tandem repeats.

Output format

Output formatting varies depending on the command line parameters. Program diagnostic information is always output to `stderr` while the match lists are output to `stdout`. This allows for the match output to be redirected into a file, which is quite useful since the output can be quite large. The standard output format that results from running `repeat-match` with default parameters is as follows:

```
Long Exact Matches:
   Start1     Start2    Length
  4919485    4919506r        22
  4997298    4997319r        22
  4919485    4997298         22
  3461866    3751066         53
   537897    4650529r        76
(output continues ...)
```

The three columns are the first position of the repeat, the second position of the repeat, and the length of the repeat respectively. Reverse complement repeat positions are denoted by an `'r'` following the Start2 position, and are relative to the forward strand of the sequence.

5.1.3. exact-tandems

`exact-tandems` is a wrapper cshell script for the `repeat-match` program. It provides a list of exact tandem repeats within a single input sequence.

Command line syntax

```
exact-tandems <sequence file> <min length>
```

As with `repeat-match` the sequence file should contain only one sequence in FastA format, however if multiple sequences exist the first one will be used. The sequence may contain any set of upper and lowercase characters, thus DNA and protein sequence are both allowed and matching is case insensitive. The minimum match length parameter should be a positive integer, this value will be passed to the `repeat-match` program via the `-n` option.

Output format

Program diagnostic information is always output to `stderr` while the match lists are output to `stdout`. This allows for the match output to be redirected into a file, which is quite useful since the output can be quite large. The output format of `exact-tandems` is as follows:

```
Finding matches
Tandem repeats
   Start   Extent  UnitLen     Copies
  416173      150       45        3.3
  554810      102       42        2.4
  554943      109       42        2.6
  880346      191       63        3.0
  880370       62       21        3.0
(output continues ...)
```

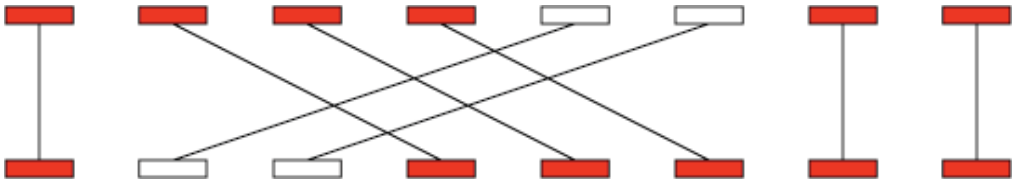
The four columns are the first position of the tandem, the extent of the repeat region, the length of each tandem repeat unit, and the number of repeat units respectively.

5.2. Clustering

MUMmer's clustering algorithms attempt to order small individual matches into larger match clusters in order to make the output of `mummer` more intelligible. A dot plot makes it easy to spot alignment regions from a match list, however when examining the data without graphic aids, it is very difficult to draw any reasonable conclusions from the simple flat file list of matches. Clustering the matches together into larger groups of neighboring matches makes this process much easier by ordering the data and removing spurious matches.

5.2.1. gaps

`gaps` is the primary clustering algorithm for `run-mummer1`, and although classified as a "clustering" step, `gaps` is more of a sorting routine. It implements the LIS (longest increasing subset) algorithm to extract the longest consistent set of matches between two sequences, and generates a single cluster that represents the best "straight-line" arrangement of matches between the sequences. By straight-line, we mean no rearrangements or inversions, just a simple path of agreeing matches between the two sequences. This limits the usability of this program to the alignment of genomes that are very similar and with no large scale mutations. To further illustrate the purpose of this program, consider the following set of MUMs (illustrated as line connecting two rectangles) between two sequences:



The rectangles connected by lines are maximal exact matches between two sequences, however only the red rectangles would be included in the LIS because they form the longest increasing subset of matches, i.e. the longest subset of matches that are consistently ordered in both genomes. Note that the empty rectangles will be discarded, even though they probably represent a major rearrangement between the two sequences. Because of this limitation `gaps` is best suited for the comparison of near identical sequences with the goal of finding minor mutations like SNPs and small indels.

Command line syntax

```
mummer [params] | tail +2 | gaps <reference file> [-r]
```

or

```
gaps <reference file> [-r] < <match list>
```

Because `gaps` receives its input from `stdin`, the input can either be piped directly from filtered `mummer` output, or redirected as input from a file. The strange syntax is a result of a legacy issue described in the Known problems section, and requires the header be stripped from the `mummer` output. In addition, `gaps` is only designed to handle a single reference and a single query sequence, thus the preceding `mummer` run must also follow this constraint. The `-r` is optional and designates the incoming matches as reverse complement matches which must reference the reverse complement of the sequence, therefore forcing `mummer` to be run *without* the `-c` option. Please refer to the `run-mummer1` script for an example of how to use this program in an alignment pipeline. A rewrite of this algorithm to handle multiple reference and/or query sequences may eventually appear, but is not currently in development.

Output format

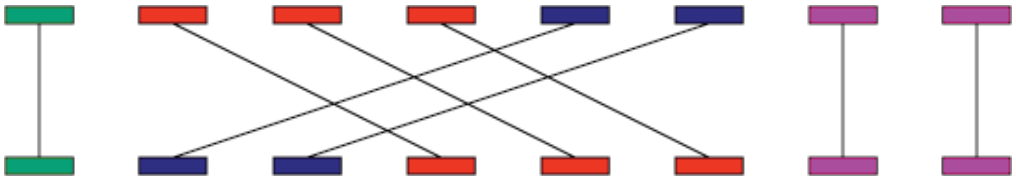
The `stdout` output of `gaps` shares much in common with the standard three column match output, with the addition of three extra columns:

```
> /home/aphillip/data/GHP.1con  Consistent matches
     183       17     22    none      -      -
     238       72    108    none     33     33
     347      181     92    none      1      1
     458      292     50    none     19     19
     705      539     44    none      1      1
     750      584     38    none      1      1
     807      641     23     -16      0      4
(output continues ...)
> Wrap around
  334398   329917     47    none      -    225
  334446   329965     62    none      1      1
  334539   330058     20    none     31     31
  334560   330079     92    none      1      1
  334653   330172     77    none      1      1
  334740   330259     41    none     10     10
(output continues ...)
> /home/aphillip/data/GHP.1con  Other matches
 1317231     4891     21    none      -      -
 1317275     4927     21    none      -      -
 1317804     5399     25    none    508    451
  947580     5436     36    none      -      -
   23406     5518     34    none      -      -
  333079     6592     32    none      -      -
(output continues ...)
```

Where the first line is the location of the reference file, and the first three columns are the same as the three column match format described in the mummer section. The final three columns are the overlap between this match and the previous match, the gap between the start of this match and the end of the previous match in the reference, and the gap between the start of this match and the end of the previous match in the query respectively. A couple suggestions on how to visually scan through this output: a gap size == 1 means a single mismatch between the two sequences, e.g. a SNP, an overlap like seen in the last line of the `Consistent matches` indicates the existence of a tandem repeat, and a `'-'` character means that the gap size could not be calculated. The `Wrap around` list is for circular genomes where the consistent set of matches wraps around the origin of the reference, and the `Other matches` list shows the matches that were not included in the LIS (like the white boxes in the above image). Finally, if the `-r` was passed on the command line the `Consistent matches` and `Other matches` headers would contain the `reverse` keyword after the reference file.

5.2.2. mgaps

`mgaps` was introduced into the MUMmer pipeline in an effort to better handle large-scale rearrangements and duplications. Unlike `gaps`, `mgaps` is a full clustering algorithm that is capable of generating multiple groups of consistently ordered matches. Clustering is controlled by a set of command-line parameters that adjust the minimum cluster size, maximum gap between matches, etc. Only matches that were included in clusters will appear in the output, so by adjusting the command-line parameters it is possible to filter out many of the spurious matches, thus leaving only the larger areas of conservation between the input sequences. The major advantage of mgaps is its ability to identify these "islands" of conservation. This frees the user from the single LIS restraints of the `gaps` program and allows for the identification of large-scale rearrangements, duplications, gene families and so on. To further illustrate the purpose of this program, consider once again the following set of MUMs (illustrated as line connecting two rectangles) between two sequences:



Just like before the rectangles connected by lines are maximal exact matches between two sequences, with each distinct cluster having its own unique color. In the previous demonstration using this MUM set, `gaps` failed to identify the blue cluster because it was not consistent with the LIS. However, by using `mgaps`, all regions of conservation have now been identified. The only fallback being the increased complexity of the output, where you once had only one cluster for the whole comparison, you now have four. Because of this, it can sometimes be difficult separating the repetitive clusters from "correct" clusters, making `mgaps` more suited for global alignments instead of localized error detection.

Command line syntax

```
mummer [params] | mgaps [options]
```

or

```
mgaps < <match list>
```

Because `gaps` receives its input from `stdin`, the input can either be piped directly from raw `mummer` output, or redirected as input from a `mummer` output file. `mgaps` is only designed to handle a single reference and one or more query sequences, thus the preceding `mummer` run must also follow this constraint. Please refer to the `run-mummer3` script for an example of how to use this program in an alignment pipeline. Note that in order to cluster reverse complement matches, the reverse complement matches must reference the reverse complement strand of the query sequence, therefore forcing `mummer` to be run *without* the `-c` option. A rewrite of this algorithm to handle multiple reference sequences and a better coordinate system (forward coordinates for reverse complement matches) is doubtful but may eventually appear.

Program options

```
-C          Check that input header labels alternately have the "Reverse" keyword

-d int      Maximum fixed diagonal difference (default 5)

-e          Use extent of cluster (end - start) rather than the sum of the match lengths to
            determine cluster length

-f float    Maximum fraction of separation for diagonal difference (default 0.05)

-l int      Minimum cluster length (default 200)

-s int      Maximum separation between adjacent matches in a cluster (default 1000)
```

The `-d` option can be interpreted as the number of insertions allowed between two matches in the same cluster, while the `-f` option is a fraction equal to (diagonal difference / match separation) where a higher value will increase the indel tolerance. Minimum cluster length is the sum of the contained matches unless the `-e` option is used. The best way to get a feel for what each parameter controls is to cluster the same data set numerous times with different values and observe the resulting differences. It can also be helpful to set these parameters to the size of the element you wish to capture, i.e. set the minimum cluster size to say the smallest exon you expect and set the max gap to the smallest intron you expect to obtain clusters

that could represent single exons (depending of course of the similarity of the two sequences).

Output format

The `stdout` output of `mgaps` shares much in common with the output of `mummer` and `gaps`, with a slightly different header formatting than `gaps` to allow for multiple query sequences and multiple clusters. The output of `mgaps` run on both forward and reverse complement matches is as follows:

```
> ID41
> ID41 Reverse
 5177399        1    232    none      -      -
 5177632      234   6794    none      1      1
 5184433     7035     24    none      7      7
 5184468     7069     23    none     11     10
> ID42
   10181       43   1521    none      -      -
> ID42 Reverse
 4654536       17     36    none      -      -
 4654578       57    298    none      6      4
 4654877      356    226    none      1      1
#
 4655139      845     28    none      -      -
 4655178      884    694    none     11     11
 4655873     1579     20    none      1      1
#
 4850044       17   1492    none      -      -
 4851537     1510    711    none      1      1
 4852249     2222     42    none      1      1
(output continues ...)
```

Headers containing the ID for each query sequence are listed after the `'>'` characters, and a following `Reverse` keyword identifies the reverse matches for that query sequence. Individual clusters for each sequence are separated by a `'#'` character, and the six columns are exactly the same as the `gaps` output (see the gaps section for more details).

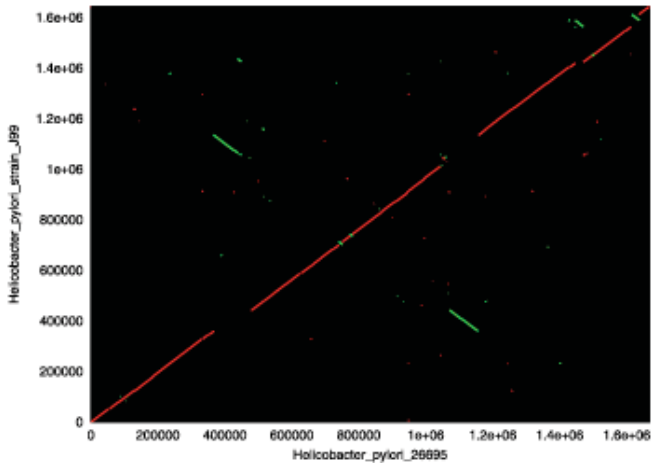
5.3. Alignment generators

The alignment scripts described in this section build upon the data generated by the previous two sections, maximal exact matching and clustering. Each of these scripts independently runs the matching and clustering steps, and then generates pair-wise alignments for each of the clusters. This translates to a basic seed and extend method of alignment. The individual matches within each cluster are used as alignment anchors and only the mismatching sequence between the matches is processed by the Smith-Waterman dynamic programming routine. This reduces both the time and memory necessary to align large sequences, while still producing accurate alignments.

5.3.1. NUCmer

NUCmer (NUCleotide MUMmer) is the most user-friendly alignment script for standard DNA sequence alignment. It is a robust pipeline that allows for multiple reference and multiple query sequences to be aligned in a many vs. many fashion. For instance, a very common use for `nucmer` is to determine the position and orientation of a set of sequence contigs in relation to a finished sequence, however it can be just as effective in comparing two finished sequences to one another. Like all of the other alignment scripts, it is a three step process - maximal exact matching, match clustering, and alignment extension. It begins by using `mummer` to find all of the maximal unique matches of a given length between the two input sequences. Following the matching phase, individual matches are clustered into closely grouped sets with `mgaps`. Finally, the non-exact sequence between matches is aligned via a modified Smith-Waterman

algorithm, and the clusters themselves are extended outwards in order to increase the overall coverage of the alignments. nucmer uses the mgaps clustering routine which allows for rearrangements, duplications and inversions; as a consequence, nucmer is best suited for large-scale global alignments, as is shown in the following plot:



This dot plot represents a nucmer alignment of two different strains of *Helicobacter pylori* (26695 on the x-axis and J99 on the y-axis). Forward matches are shown in red, while reverse matches are shown in green. This alignment, which took only 12 seconds to compute, clearly shows a major inversion event centered around the origin of replication, and demonstrates NUCmer's ability to handle large scale rearrangements between sequences of high nucleotide similarity.

Command line syntax

```
nucmer [options] <reference file> <query file>
```

The reference and query files should both be in multi-FastA format and have no limit on the number of sequences they man contain. However, because nucmer uses mummer for its maximal exact matching, the memory usage will be dependent on the size of the reference file, so it may be advisable to make the smaller of the input files the reference to assure the program does not exhaust your computer's memory resources. In addition, masking the uninteresting regions of the input with any character other than *a*, *c*, *g*, or *t* will both speed up nucmer by reducing the number of possible matches and also cut down on the number of alignments induced by repetitive sequence.

Program options

```
--mum             Use anchor matches that are unique in both the reference and query

--mumreference    Use anchor matches that are unique in the reference but not necessarily unique
                  in the query (default behavior)

--maxmatch        Use all anchor matches regardless of their uniqueness

-b int            Distance an alignment extension will attempt to extend poor scoring regions
--breaklen        before giving up (default 200)

-c int            Minimum cluster length (default 65)
--mincluster
```

```
--[no]delta      Toggle the creation of the delta file. Setting --nodelta prevents the
                 alignment extension step and only outputs the match clusters (default --delta)

--depend         Print the dependency information and exit

-d float         Maximum diagonal difference factor for clustering, i.e. diagonal difference /
--diagfactor     match separation (default 0.12)

--[no]extend     Toggle the outward extension of alignments from their anchoring clusters.
                 Setting --noextend will prevent alignment extensions but still align the DNA
                 between clustered matches and create the .delta file (default --extend)

-f               Align only the forward strands of each sequence
--forward

-g int           Maximum gap between two adjacent matches in a cluster (default 90)
--maxgap

-h               Print the help information and exit
--help

-l int           Minimum length of an maximal exact match (default 20)
--minmatch

-o               Automatically generate the <prefix>.coords file using the 'show-coords'
--coords         program with the -r option

--[no]optimize   Toggle alignment score optimization. Setting --nooptimize will prevent
                 alignment score optimization and result in sometimes longer, but lower scoring
                 alignments (default --optimize)

-p string        Set the output file prefix (default out)
--prefix

-r               Align only the reverse strand of the query sequence to the forward strand of
--reverse        the reference

--[no]simplify   Simplify alignments by removing shadowed clusters. Turn this option off if
                 aligning a sequence to itself to look for repeats (default --simplify)

-V               Print the version information and exit
--version
```

All values are measured in DNA bases unless otherwise noted. Using either the `-mum` or `-mumreference` options (along with masking the input sequences) can help reduce the number of repeat induced alignments, and is suggested for most applications. If no uniqueness options are set, the program will default to `-mumreference`. Decreasing the values of the `-mincluster` and `--minmatch` options will increase the sensitivity of the alignment but may produce less reliable alignments. In addition, significantly raising the value of the `--maxgap` value (say to 1000) can be crucial in producing alignments for more divergent genomes. Setting `--noextend` speeds up the process by preventing alignment extensions outward from each cluster, while `--nodelta` takes this a step further and doesn't even align the sequence between the

matches in a cluster, however both of these reduce the amount of information contained in the output. See `mgaps` description for hints on setting the clustering parameters `--mincluster`, `--diagdiff` and `--maxgap`. The `--coords` option exists only for NUCmer1.0 compatibility; instead, it is recommended to run `show-coords` afterwards with more specific options. The `--nooptimize` option will force alignments within `--breaklen` bases of the sequence end to extend all the way to the sequence end, regardless of the resulting alignment score. The `--prefix` string should be unique in the output directory to prevent overwriting pre-existing data. Finally, by default `nucmer` matches the forward and reverse strands of the query sequences to the forward strand of the reference sequence unless the `--forward` or `--reverse` options were used, and all output coordinates always reference the forward strand of their respective sequence. Only use the `--nosimplify` option when aligning a sequence to itself in order to find inexact repeats.

Output format

Because `nucmer` and `promer` produce the same output files, this section will serve to explain the `<prefix>.cluster` and `<prefix>.delta` for both programs (that we will refer to as the cluster and delta files respectively). The cluster file contains a list of all the clustered maximal exact matches identified in the "seed" phase of the pipeline, and the delta file contains an encoded representation of all the alignments generated in the "extend" phase of the pipeline. The cluster file is a derivative of both the `gaps` and `mgaps` output, while the delta file is a unique format for concise, machine representation of the pair-wise alignments. Several tools described in the Utilities section were designed to interpret these files and extract useful, human-readable information from them, however the full format description for both the cluster and delta files is described below to aid developers.

The "cluster" file format

The "cluster" file contains a list of the match clusters that were generated during the clustering phase of the NUCmer or PROmer pipeline. It is primarily a five column match list, with the exception of the headers to be described later. For example, a few "match" rows could read:

```
168        2     30     -      -
288      122     51    90     90
354      188     84    15     15
```

Where the columns are the start of the match in the reference, the start of the match in the query, the length of the match, the gap between this match and the previous match in the reference, and the gap between this match and the previous match in the query respectively. All coordinates reference the forward strand of each sequence, regardless of match direction, and are *always* measured in DNA bases regardless of alignment type (DNA or amino acid). Therefore, when running PROmer, all the numbers in the length and gaps columns must be multiples of three. In addition, because the matches reference the forward DNA strand forward match clusters will be sorted in ascending fashion while reverse match clusters will be sorted in descending fashion.

Each individual cluster has a header formed from two digits in the set `[-1, -2, -3, 1, 2, 3]`. These two digits represent the direction of the cluster (negative for reverse and positive for forward) and the frame of the cluster in the reference and query sequences respectively (note that matches within the same cluster must have a consistent reading frame). For NUCmer output, the header will always show a `"1"` for the reference and a `"-1"` or `"1"` for the query, since only the query can be reverse complemented. However, for PROmer output all 36 combinations of these two digits are valid headers. For instance, `" -2 3"` would represent a cluster on the 2nd reading frame of the reversed reference sequence and on the 3rd reading frame of the forward query sequence.

There are also three other types of headers. The first line of each cluster file lists the two original input files separated by a space, while the second line of each cluster file lists the type of alignment data - either `"NUCMER"` or `"PROMER"`. Finally the third type of header lists two sequences and their sequence lengths following a `'>'` character and separated by whitespace. Each of these sequence headers is unique, so all clusters/matches between any two sequences will appear under a single header identifying those two

sequences. These sequence headers will only be present if there is at least one cluster between the two sequences, otherwise the sequence header will be omitted from the file. Below is a short example of what a cluster file might look like:

```
/home/username/reference.fasta /home/username/query.fasta
PROMER
>tagA1 tagB1 1000 2000000
 1  3
     184       18     21     -      -
     223       57    123    18     18
 3  2
     168        2     30     -      -
     288      122     51    90     90
     354      188     84    15     15
     483      317     24    45     45
     558      392     81    51     51
     642      476    144     3      3
>tagA2 tagB1 2000000 2000000
-3 -2
 1665663  1641799     18     -      -
 1665585  1641712     21    60     69
 1665546  1641673     39    18     18
(output continues ...)
```

The "delta" file format

The "delta" file is an encoded representation of the all-vs-all alignment between the input sequences to either the NUCmer or PROmer pipeline. It is the primary output of these alignment scripts and there are various utilities described in section 5.4. that are designed to take the delta file as input, and output some human-readable information to the user. Also, the delta-filter utility is designed to manipulate these files and select desired alignments. The primary function of the delta file is to catalog the coordinates of each alignment and note the distance between insertions and deletions contained in these alignments. By only storing the location of each indel as an offset, disk space is efficiently utilized, and a potentially enormous alignment can be stored in a relatively small space. The first two lines of the file are identical to the cluster file. The first line lists the two original input files separated by a space, while the second line specifies the alignment data type, either `"NUCMER"` or `"PROMER"`. Every grouping of alignments have a unique header specifying the two aligning sequences. Only sequences with shared alignments will have a header, therefore, as in the cluster file, there can be no empty headers (i.e. those that have no alignments following them). An example header might look like

```
>tagA1 tagB1 500 20000000
```

Following this sequence header is the alignment data. Each alignment following also has a header that describes the coordinates of the alignment and some error information. These coordinates are inclusive and reference the forward strand of the DNA sequence, regardless of the alignment type (DNA or amino acid). Thus, if the start coordinate is greater than the end coordinate, the alignment is on the reverse strand. The four coordinates are the start and end in the reference and the start and end in the query respectively. The three digits following the location coordinates are the number of errors (non-identities + indels), similarity errors (non-positive match scores), and stop codons (does not apply to DNA alignments, will be `"0"`). An example header might look like:

```
2631 3401 2464 3234 15 15 2
```

Notice that the start coordinate points to the first base in the first codon, and the end coordinate points to the last base in the last codon. Therefore making `(end - start + 1) % 3 = 0`. This makes determining the frame of the amino acid alignment a simple matter of determining the reading frame of the start

coordinate for the reference and query. Obviously, these calculations are not necessary when dealing with vanilla DNA alignments.

Each of these alignment headers is followed by a string of signed digits, one per line, with the final line before the next header equaling 0 (zero). Each digit represents the distance to the next insertion in the reference (positive int) or deletion in the reference (negative int), as measured in DNA bases OR amino acids depending on the alignment data type. For example, with the `PROMER` data type, the delta sequence `(1, -3, 4, 0)` would represent an insertion at positions 1 and 7 in the translated reference sequence and an insertion at position 3 in the translated query sequence. Or with letters:

```
A = ABCDACBDCAC$
B = BCCDACDCAC$
Delta = (1, -3, 4, 0)
A = ABC.DACBDCAC$
B = .BCCDAC.DCAC$
```

Using this delta information, it is possible to re-generate the alignments calculated by `nucmer` or `promer` as is done in the `show-coords` program. This allows various utilities to be crafted to process and analyze the alignment data using a universal format. This also means the delta only needs to be created once, yet it can be analyzed numerous times without ever having to rerun the costly alignment algorithm. Below is an example of what a delta file might look like:

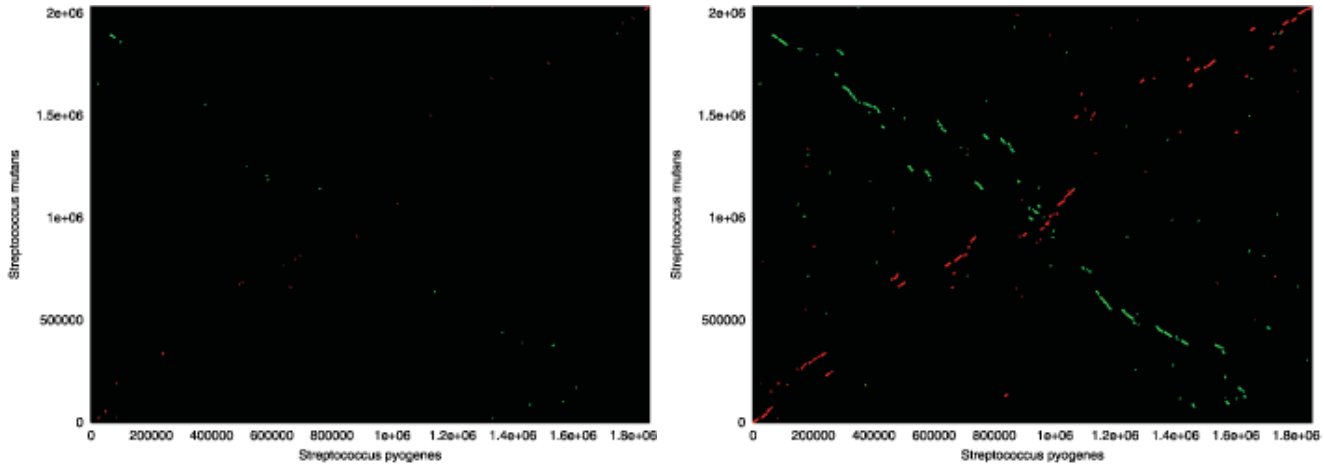
```
/home/username/reference.fasta /home/username/query.fasta
PROMER
>tagA1 tagB1 3000000 2000000
1667803 1667078 1641506 1640769 14 7 2
-145
-3
-1
-40
0
1667804 1667079 1641507 1640770 10 5 3
-146
-1
-1
-34
0
>tagA2 tagB4 4000 3000
2631 3401 2464 3234 4 0 0
0
2608 3402 2456 3235 10 5 0
7
1
1
1
1
0
(output continues ...)
```

5.3.2. PROmer

PROmer (PROtein MUMmer) is a close relative to the NUCmer script. It follows the exact same steps as NUCmer and even uses most of the same programs in its pipeline, with one exception - all matching and alignment routines are performed on the six frame amino acid translation of the DNA input sequence. This provides `promer` with a much higher sensitivity than `nucmer` because protein sequences tends to diverge much slower than their underlying DNA sequence. Therefore, on the same input sequences, `promer` may find many conserved regions that `nucmer` will not, simply because the DNA sequence is not as

highly conserved as the amino acid translation.

All of this is performed behind the scenes, as the input is still the raw DNA sequence and output coordinates are still reported in reference to the DNA, so the two programs (`nucmer` and `promer`) exhibit little difference in their interfaces and usability. Because of its greatly increased sensitivity, it is usually best to use `promer` on those sequences that cannot be adequately compared by `nucmer`, because if run on very similar sequences the `promer` output can be quite voluminous. This is because `promer` makes no effort to distinguish between proteins and junk amino acid translations, therefore a single highly conserved gene may have up to *six* alignments in `promer` output, one for each of the six amino acid reading frames, when only the correct reading frame would be sufficient. This makes `promer` ideally suited for highly divergent sequences that show little DNA sequence conservation, as is shown in the following two plots:



These dot plots represent two comparisons of *Streptococcus pyogenes* (x-axis) and *Streptococcus mutans* (y-axis), with forward matches colored red and reverse matches colored green. The graph generated with `nucmer` output is on the left, while the graph generated with `promer` output is on the right (both run with default parameters). It is clearly visible that `promer` has aligned the two genomes with a much greater sensitivity, thus demonstrating the effectiveness of comparing two divergent genomes on the amino acid level.

Command line syntax

```
promer [options] <reference file> <query file>
```

The reference and query files should both be in multi-FastA format and have no limit on the number of sequences they man contain. However, because `promer` uses `mummer` for its maximal exact matching, the memory usage will be dependent on the size of the reference file, so it may be advisable to make the smaller of the input files the reference to assure the program does not exhaust your computer's memory resources. In addition, masking the uninteresting regions of the input with *n* or *x* will both speed up `promer` by reducing the number of possible matches and also cut down on the number of alignments induced by repetitive sequence.

Program options

```
--mum            Use anchor matches that are unique in both the reference and query

--mumreference   Use anchor matches that are unique in the reference but not necessarily unique
                 in the query (default behavior)
```

```
--maxmatch       Use all anchor matches regardless of their uniqueness

-b int           Distance an alignment extension will attempt to extend poor scoring regions
--breaklen       before giving up (default 60)

-c int           Minimum cluster length (default 20)
--mincluster

--[no]delta      Toggle the creation of the delta file. Setting --nodelta prevents the
                 alignment extension step and only outputs the match clusters (default --delta)

--depend         Print the dependency information and exit

-d float         Maximum diagonal difference factor for clustering, i.e. diagonal difference /
--diagfactor     match separation (default 0.11)

--[no]extend     Toggle the outward extension of alignments from their anchoring clusters.
                 Setting --noextend will prevent alignment extensions but still align the DNA
                 between clustered matches and create the .delta file (default --extend)

-g int           Maximum gap between two adjacent matches in a cluster (default 30)
--maxgap

-h               Print the help information and exit
--help

-l int           Minimum length of an maximal exact match (default 6)
--minmatch

-m int           Maximum stop codon bookend masking length (default 8)
--masklen

-o               Automatically generate the <prefix>.coords file using the 'show-coords'
--coords         program with the -r option

--[no]optimize   Toggle alignment score optimization. Setting --nooptimize will prevent
                 alignment score optimization and result in sometimes longer, but lower scoring
                 alignments (default --optimize)

-p string        Set the output file prefix (default out)
--prefix

-V               Print the version information and exit
--version

-x type          The alignment matrix type, 1 [BLOSUM 45], 2 [BLOSUM 62] or 3 [BLOSUM 80]
--matrix         (default 2)
```

All values are measured in amino acids unless otherwise noted. Refer to the NUCmer Program options section for more information regarding their shared options. The `--masklen` value determines the number of amino acids between stop codons that will be automatically masked by `promer`, e.g. if an amino acid

sequence were `...AAA*AAAA*AAA...` and the `--masklen` value were greater than or equal to 4, the sequence would be masked to read `...AAA*XXXX*AAA...` for the duration of the script. The `--matrix` option sets the BLOSUM matrix for scoring mismatches in the amino acid sequence, where options `1` assumes greater diversity between the two sequences and `3` assumes greater similarity between the two sequences.

Output format

Output files follow the same format as described in the NUCmer Output format section.

5.3.3. run-mummer1

`run-mummer1` is a legacy script from the original MUMmer1.0 release. It has been updated to utilize the new suffix tree code of version 3.0, however all other programs called from this script are identical to the original MUMmer release back in 1999. Even though it is an outdated program, it still has some advantages over the newer alignment scripts (`nucmer`, `promer`, `run-mummer3`). Like all of the alignment scripts, `run-mummer1` is a three step process - matching, clustering and extension. However, unlike the newer alignment scripts, `run-mummer1` uses the `gaps` program for its clustering step. The `gaps` program does not allow for rearrangements like `mgaps`, instead if finds the single longest increasing subset of matches across the full length of both sequences. This makes it well suited for SNP and small indel identification between small (< 10 Mbp), very similar sequences with few to no rearrangements.

Command line syntax

```
run-mummer1 <reference file> <query file> <prefix> [-r]
```

The reference and query files must both be in FastA format and contain *only* one sequence. Memory usage will be dependent on the size of the reference sequence, so it may be advisable to make the smaller of the input files the reference to assure the program does not exhaust your computer's memory resources. `run-mummer1` uses a simplified scoring function that does not recognize masking characters, so it is not recommended to perform any masking on the input sequences. The `<prefix>` value will be prefixed to the names of the resulting output files. The `-r` is optional and tells the script to reverse complement the query input sequence, thus all output coordinates will reference the reverse complement of the query. If the `-r` option is omitted, all matching will be limited to the forward strand of each sequence; if it is included, all matching will be limited to the forward strand of the reference and the reverse strand of the query.

Program options

There are no available command line options for `run-mummer1`. Instead, the user must directly edit the `csh` script to alter the command line values passed to the individual pipeline programs. The only available tweak is changing the minimum match length value for `mummer`, set with the `-l` option within the script. Decreasing this value may increase the sensitivity of the script, but may drastically increase the resulting runtime.

Output format

There are four output files generated with each call of `run-mummer1`, and each of these files is prefixed with the `<prefix>` value set on the command line. Each of these files will be referred to by its file extension (out, gaps, errorsgaps, align), and are described below.

The "out" file format

The standard output of the `mummer` program with it's header information stripped, see the mummer output section for more information. Just a simple three column list, noting the position and length of every maximal exact match. Note that for reverse complement matches (produced with the `-r` option), the query

start positions will reference the reverse complement of the query input sequence.

The "gaps" file format

The standard output of the `gaps` program, see the gaps output section for more information.

The "errorsgaps" file format

An annotated version of the gaps format, with an extra column listing the number of errors counted in each gap. This is perhaps the most useful output file produced by `run-mummer1` as it is easy to parse and identify SNPs, which appear as a `'1'` in the final column. A `'-'` character in the final column means the alignment was too large to compute. Example slice from an errorsgaps file:

```
  403382   356512     77    none      1      1       -
  403466   356595     56    none      7      6       4
  403542   356670     81    none     20     19       2
  403626   356756     75    none      3      5       4
```

The "align" file format

The align file is difficult to parse, but contains some useful visual information. It intersperses the gaps output file with the actual pair-wise alignment of each gap. Each alignment follows the listing of the two involved matches and uses a `'^'` character to identify the non-identities. If an alignment was too large to process in memory a tag reading `"*** Too long ***"` will be listed in its place. Example align file:

```
> /home/aphillip/data/mgen.seq reverse Consistent matches
  170273   729167    158    none      8      8
  170433   729327     34    none      2      2
    Errors = 2
T:  gaaggtctttttgattgtaaag
S:  gaaggtctttaagattgtaaag
              ^^
  170501   729395    155    none     34     34
    Errors = 4
T:  aagaatgactctagcaggcaatggctggagtttgactgtaccactttgaataag
S:  aagaatgactttagcaggtaatggctagagtttgactgtaccattttgaataag
             ^       ^       ^                 ^
  170659   729553    187    none      3      3
    Errors = 2
T:  tggaaactatcagtctagagtgt
S:  tggaaactattaatctagagtgt
             ^ ^
  170856   729750    281    none     10     10
    Errors = 2
T:  tagctgtcggagcgatcccttcggtagtga
S:  tagctgtcggggcgatcccctcggtagtga
             ^        ^
(output continues ...)
```

Each alignment region is padded with 10bp of the exact match surrounding it on either side.

5.3.4. run-mummer3

`run-mummer3` is the simplest pipeline of the latest MUMmer3.0 programs. It runs the same matching and

clustering algorithm as `nucmer` and `promer`, however it uses a different extension technique and does not perform the important pre- and post-processing steps of NUC/PROmer. Because of its simplistic form, `run-mummer3` can only handle a single reference sequence, but like `run-mummer1` its error-focused output makes it a handy tool for detecting SNPs and other small errors. The only major difference between `run-mummer3` and `run-mummer1` is the new version's ability to handle multiple query sequences and its tolerance of large rearrangements. This makes `run-mummer3` well suited for error detection between highly similar sequences that may have large rearrangements, inversions etc. Edit the script by adding the `-D` option to the `combineMUMs` command line to output a format designed for SNP identification. Still, `run-mummer3` provides few advantages of the more user friendly `nucmer` program, and should be avoided where possible.

Command line syntax

```
run-mummer3 <reference file> <query file> <prefix>
```

The reference and query files should both be FastA format. The reference file may *only* have a single sequence, but there is no limit on the number of sequences the query file may contain. It is *very* important that the reference file only contain one sequence, because the script will give you no indication something went wrong and there will just be empty output files. `run-mummer3` uses a simplified scoring function that does not recognize masking characters, so it is not recommended to perform any masking on the input sequences. The `<prefix>` value will be prefixed to the names of the resulting output files. Both forward and reverse complement matches will be found by default; to change this behavior or change any parameters, requires requires hand editing the script.

Program options

There are no available command line options for `run-mummer3`. Instead, the user must directly edit the `csh` script to alter the command line values passed to the individual pipeline programs. Altering these parameters is suggested for most applications, as the default values may not always produce the best output. Parameter values may be added or changed for `mummer`, `mgaps` and `combineMUMs`. Run these programs with the `-help` option for a list of available options, or refer to this manual for more information on `mummer` or `mgaps`. Note that the `-c` option cannot be used for `mummer` in this script, or `mgaps` will fail to cluster the reverse complement matches.

Output format

Like `run-mummer1`, `run-mummer3` produces four output files prefixed with the value set on the command line. Each of these files will be referred to by its file extension (out, gaps, errorsgaps, align), and are described below.

The "out" file format

Pure, unadulterated `mummer` output. See the mummer output section for more information. Just a simple three column list, noting the position and length of every maximal exact match. Note that for reverse complement matches, the query start positions will reference the reverse complement of the query input sequence.

The "gaps" file format

The standard output of the `mgaps` program, see the mgaps output section for more information.

The "errorsgaps" file format

An annotated version of the gaps format, with an extra column listing the number of errors counted in

each gap. This is perhaps the most useful output file produced by `run-mummer1` as it is easy to parse and identify SNPs, which appear as a `'1'` in the final column. A `'-'` character in the final column means the alignment was too large to compute. Example slice from an errorsgaps file:

```
  403382   356512     77    none      1      1       -
  403466   356595     56    none      7      6       4
  403542   356670     81    none     20     19       2
  403626   356756     75    none      3      5       4
```

The "align" file format

The align file is difficult to parse, but contains some useful visual information. It intersperses the `mgaps` output file with the actual pair-wise alignment of each gap. Each alignment follows the listing of the two involved matches and uses a `'^'` character to identify the non-identities and a `'='` character to identify the MUM portion. The gap alignment is also padded with 10bp of the exact match surrounding it on either side. Example align file:

```
(... output continues)
> ID21
 3944620       24    983    none      -      -
 3945604     1008     22    none      1      1
     Errors = 1
A: agactctttctttggttgatt
B: agactctttccttggttgatt
   ==========^==========
 3945655     1059     26    none     29     29
     Errors = 3
A: cttgcgattgtctttgcatttgtctttgtttctttttcttcatgctgct
B: cttgcgattggctttgcatttggctttgtttctttttcctcatgctgct
   ==========^           ^               ^==========
 3945684     1088     29    none      3      3
     Errors = 2
A: ttacttttttctc-cattatagta
B: ttacttttttt-tctcattatagta
   ==========^  ^==========
Region:    3944620 .. 3945743          24 .. 1146            8 / 1124        0.71%
> ID21 Reverse
> ID22
> ID22 Reverse
 5183942        8     31    none      -      -
 5183980       47   4221    none      7      8
     Errors = 3
A: cccagaaaac-accacctccggccagta
B: cccagaaaaccaccactcccggccagta
   ==========^     ^^==========
 5188202     4269    314    none      1      1
     Errors = 1
A: tgcaccagaacgtaataatcc
B: tgcaccagaaagtaataatcc
   ==========^==========
Region:    5183942 .. 5188515         4578 .. 4               4 / 4575        0.09%
(output continues ...)
```

After each cluster, the align file prints a line beginning with the `Region` keyword that shows the start and stop of the alignment in the reference and the start and stop of the alignment in the query respectively. The query coordinates in the region line will reference the forward strand of the query, while the lines taken from the gaps file will still reference the reverse strand of the query. The region line also shows and

error ratio and the error percentage.

5.4. Utilities

MUMmer includes a few utility programs intended to parse the delta encoded alignment files and output their contents to the user. The majority of these programs will only operate on the delta file output of NUCmer or PROmer, however the generalized visualization tool, `mummerplot`, will function on a variety of input.

5.4.1. delta-filter

`delta-filter` is a utility program for the manipulation of the delta encoded alignment files output by the NUCmer and PROmer pipelines. It takes a delta file as input and filters the information based on the various command line switches, outputting only the desired alignments to stdout. Options to filter by alignment length, identity, uniqueness and consistency are provided. Certain combinations of these options can greatly reduce the number of unwanted alignments in the delta file, thus making the output of programs such as `show-coords` more comprehendible.

Command line syntax

```
delta-filter [options] <delta file> > <filtered delta file>
```

The `<delta file>` may represent either NUCmer of PROmer data. The `<filtered delta file>` will be the filtered down version of the input. Output will be to stdout. `delta-filter` run with no options is the identity function.

Program options

```
-g          Global alignment using length*identity weighted LIS (longest increasing subset). For
            every reference-query pair, leave only the alignments which form the longest
            mutually consistent set

-h          Print the help information and exit

-i float    Set the minimum alignment identity [0, 100], (default 0)

-l int      Set the minimum alignment length (default 0)

-q          Query alignment using length*identity weighted LIS. For each query, leave only the
            alignments which form the longest consistent set for the query

-r          Reference alignment using length*identity weighted LIS. For each reference, leave
            only the alignments which form the longest consistent set for the reference.

-u float    Set the minimum alignment uniqueness, i.e. percent of the alignment matching to
            unique reference AND query sequence [0, 100], (default 0)

-o float    Set the maximum alignment overlap for -r and -q options as a percent of the
            alignment length [0, 100], (default 75)
```

The `-g` option simulates the behavior of MUMmer1 by performing a similar algorithm to determine the

longest mutually consistent set of matches, while the `-r` and `-q` option only require the match set to be consistent with respect to either the reference or query respectively. The difference being, the `-g` option does not allow for inversions, translocations, etc. while the `-r` and `-q` options do. However, none of these options (`-g -r -q`) allow for the inclusion of multiple repeat copies. Use `-g` when aligning two sequences which are globally consistent, use `-r` for determining the best mapping of a reference to a query (one-to-many), use `-q` for determining the best mapping of a query to a reference (many-to-one), and use `-r` and `-q` in conjunction for a one-to-one mapping of reference to query. The `-u` option is handy for keeping only those alignments which are anchored in unique sequence. The `-o` option sets the alignment overlap tolerance for the `-r` and `-q` options, i.e. the amount two adjacent alignments included by `-r` or `-q` are allowed to overlap.

Output format

Output format is the same as the input format. See the NUCmer Output format section for more details.

5.4.2. mapview

`mapview` is a utility script for displaying sequence alignments as provided by NUCmer or PROmer. It takes the output from `show-coords` or `mgaps` and converts it to a FIG, PDF or PS image file. By default, it produces FIG files which can be viewed with the common system utility `xfig` or converted to PDF or PS with the `fig2dev` utility (neither programs are included with MUMmer). `mapview` is useful for mapping multiple query contigs (e.g. from a draft sequencing project) against an annotated reference sequence. Exons and other features can also be plotted with the NUCmer or PROmer alignments, aiding in exon refinement and analysis. Individual MUMmer hits are plotted according to their percent identity, making regions of high or low similarity easily distinguishable.

Command line syntax

```
mapview [options] <coords file> [UTR coords] [CDS coords]
```

The `<coords file>` must be produced with the `show-coords` program run with the `-r -l` options (see show-coords section), or the `mgaps` program. This coords file may represent either NUCmer or PROmer data, and it is recommended that it be generated with the `-k` option (or run on a filtered delta file) to reduce redundancy in the PROmer output, however this option does not always select the proper reading frame. The optional UTR and CDS coordinate files which refer to the reference sequence, should be in GFF format. These contain the coordinates of coding sequences and untranslated regions for genes on the reference genome and will be displayed graphically if provided.

Program options

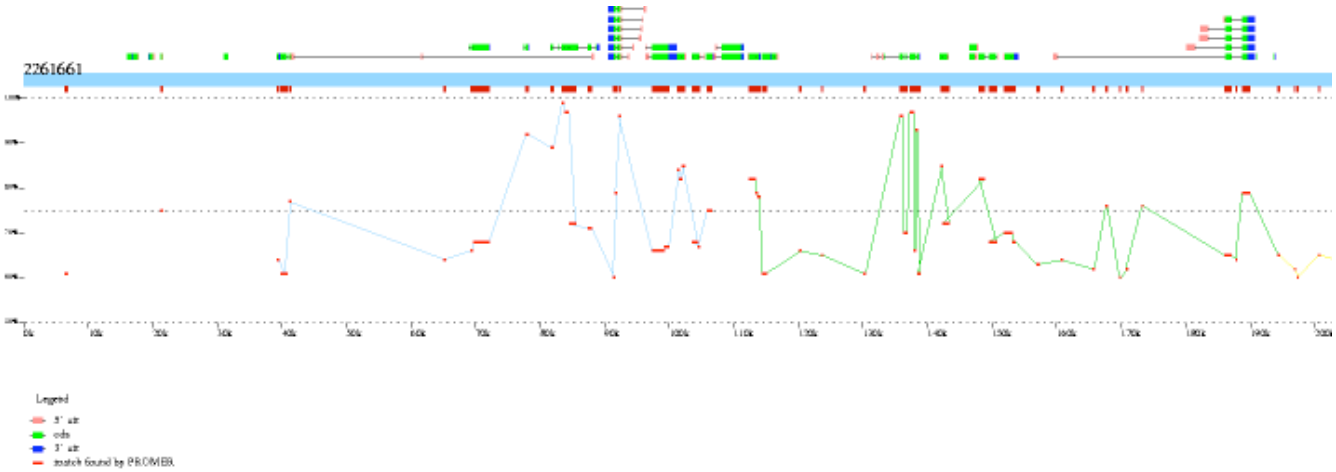
```
-d int        Set the maximum distance, in base-pairs, between graphically linked matches
--maxdist     (default 50000)

-f string     Set the output file format to 'fig', 'pdf' or 'ps' (default 'fig')
--format

-h            Print help information and exit
--help

-m float      Set the magnification at which the figure is rendered, this option will be used
--mag         when generating PDF or PS files (default 1.0)
```

```
-n int          Set the number of output files used to partition the output, this is to avoid
--num           generating files that are too large to display (default 10)

-p string       Set the output file prefix (default PROMER_graph or NUCMER_graph)
--prefix

-v              Verbose logging of the processed files
--verbose

-V              Display the version information and exit
--version

-x1 int         Set the lower coordinate bound of the display window

-x2 int         Set the upper coordinate bound of the display window

-g|ref          If the input file is provided by 'mgaps', set the reference sequence ID (as it
                appears in the first column of the UTR/CDS coords file)

-I              Display the name of the query sequences

-Ir             Display the name of the reference genes
```

All matches from the same contig are linked by drawing lines between each successive pair of matches, if the matches occur too far apart, then this can get a little messy. The `-d` option can help clean up the plots by limiting the distance a link can span. The `-n` value can be increased or decreased if the resulting FIG files are either too big or too small respectively.

Output format

The `mapview` script produces FIG output files (or PDF or PS if requested) that graphically represent the alignment described in the input coords file. An example of the resulting figures can be seen below.



The above MapView FIG shows a 220 kbp slice of *D. melanogaster* chromosome 2L and its alignment to *D. pseudoobscura*. The alignment, generated by PROmer, shows all regions of conserved amino acid sequence. The blue rectangle spanning the figure represents the reference (*D. melanogaster*), with

annotated genes shown above it and the PROmer alignments shown below it. Alternative splice variants of the same gene are stacked vertically. Exons are shown as boxes, with intervening introns connecting them. The 5' and 3' UTRs are colored pink and blue to indicate the gene's direction of translation. PROmer matches are shown twice, once just below the reference genome, where all matches are collapsed into red boxes, and in a larger display showing the separate matches within each contig, where the contigs are colored differently to indicate contig boundaries. The vertical position of the matches indicates their percent identity, ranging from 50% at the bottom of the display to 100% just below the red rectangles. Percent identity is of the amino acid translations used by PROmer. Matches from the same query sequence are connected by lines of the same color.

5.4.3. mummerplot

`mummerplot` is a script utility that takes output from `mummer`, `nucmer`, `promer` or `show-tiling`, and converts it to a format suitable for plotting with `gnuplot`. The primary plot type is an alignment dotplot where a sequence is laid out on each axis and a point is plotted at every position where the two sequences show similarity. As an extension to this plot style, `mummerplot` is also able to offset multiple 1-vs-1 dotplots to form a multiplot where multiple sequences can be laid out on each axis. This plot style is especially handy for browsing an alignment of two contig sets. Identity plots are also possible by coloring each data point with a color gradient representing identity, or by collapsing the y-axis data onto a single line and then vertically offsetting the data points by their identities. In addition to producing the plot data, `mummerplot` also generates a `gnuplot` script that will be evaluated in order to generate the graph. Since `mummerplot` simply generates `gnuplot` input, `gnuplot` must also be installed and accessible from the system path. Information about the free `gnuplot` software is currently available at www.gnuplot.info.

Command line syntax

```
mummerplot [options] <match file>
```

The `<match file>` can either be a three column match list from `mummer` (either 3 or 4 column format), the delta file from `nucmer` or `promer`, the cluster file from `nucmer` or `promer`, or the default output from `show-tiling`. `mummerplot` will automatically detect the type of input file it is given, regardless of its file extension, or it will fail if the input file is of an unrecognized type. If the X11 terminal is selected for output (default behavior), an X11 window will be spawned and the plot will be drawn to the screen. If a terminal other than X11 is selected, an extra file will be output containing the plot graphic. The leftover `<prefix>.gp` script contains the commands necessary for generating the plot, and may be edited afterwards and rerun with gnuplot to change line thickness, labels, colors, etc.

Program options

```
-b int          Highlight alignments with a breakpoint further than the given distance from the
--breaklen      nearest sequence end

--[no]color     Color plot lines with a percent similarity gradient or turn off all color
                (default color by match direction)

-c              Generate a reference coverage plot, also known as a percent identity plot
--coverage      (default behavior for show-tiling input)

--depend        Print dependency information and exit

-f              Only display alignments which represent the "best" one-to-one mapping of
--filter        reference and query subsequences (requires delta formatted input)
```

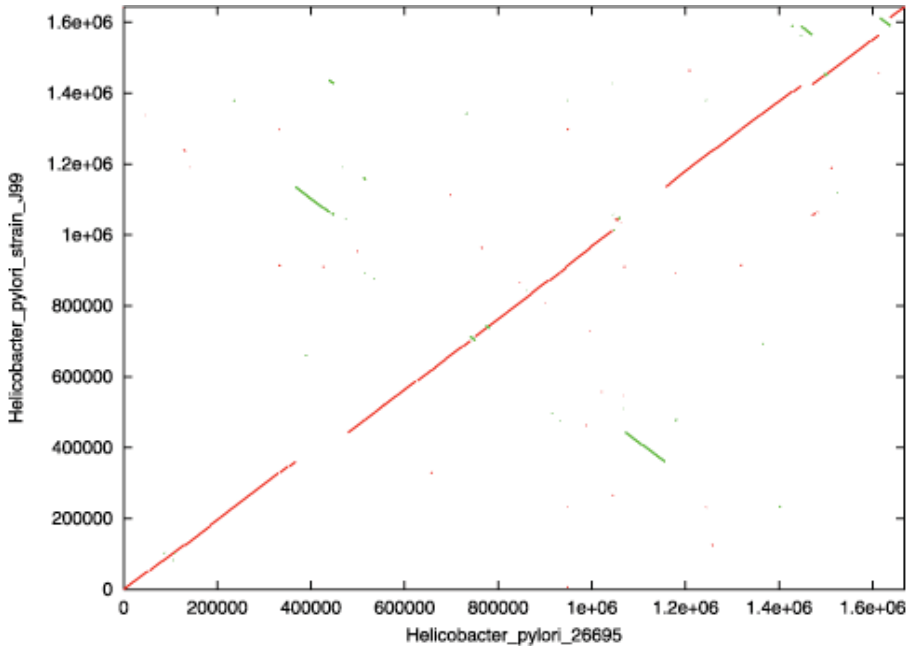
```
-h              Print help information and exit
--help

-l              Layout a multiplot by ordering and orienting sequences such that the largest hits
--layout        cluster near the main diagonal (requires delta formatted input)

-p string       Set the output file prefix (default 'out')
--prefix

--rv            Reverse video, swap the foreground and background colors for x11 plots (requires
                x11 terminal)

-r string       Select a specific reference sequence for the x-axis
--IdR

-q string       Select a specific query sequence for the y-axis
--IdQ

-R string       Generate a multiplot by using the order and length information contained in this
--Rfile         file, either a FastA file of the desired reference sequences or a tab-delimited
                list of sequence IDs, lengths and orientations [ +-]

-Q string       Generate a multiplot by using the order and length information contained in this
--Qfile         file, either a FastA file of the desired query sequences or a tab-delimited list
                of sequence IDs, lengths and orientations [ +-]

-s string       Set the output size to small, medium or large
--size          --small --medium --large (default 'small')

-S              Highlight SNP locations in the alignment
--SNP

-t string       Set the output terminal to x11, postscript or png
--terminal      --x11 --postscript --png

-x range        Set the x-range for the plot in the form "[min,max]"
--xrange

-y range        Set the y-range for the plot in the form "[min,max]"
--yrange

-V              Display version information and exit
--version
```

The `--breaklen` option is only useful for highlighting discrepancies between two near identical sequence sets. The `--color` option looks best when plotted to a postscript terminal and looks worst when plotted to a png terminal. If the alignment is very sparse, many of the alignments will "disappear" because they are too small to be rendered. If this happens, try editing the gnuplot script to plot with "linespoints" instead of "lines". The `--coverage` option is sometimes the only sensible way to plot one vs. many comparisons if "many" is very large, and it is also a useful plot for finding gaps in the reference (e.g. physical gaps in a contig set). The `--filter` option will throw away sometimes valuable repeat information, but is

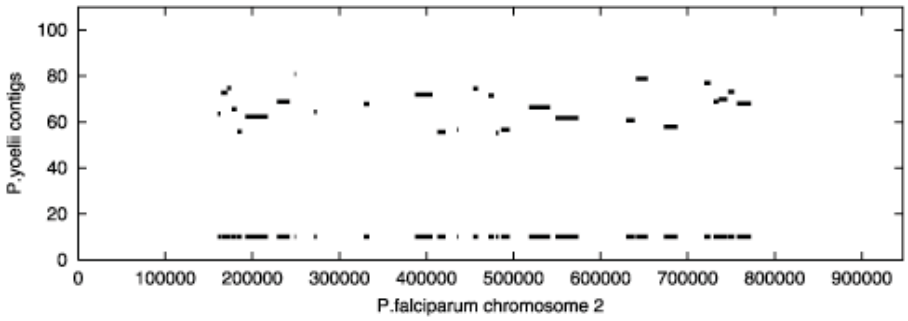
nonetheless very helpful in cleaning up an otherwise noisy plot. The `--layout` feature is only meant to be used for multiplots where the two sequence sets are near identical, and even when this is true, the layout algorithm isn't perfect. The `-R -Q` options are necessary for any multiplot, otherwise the script won't know how long the sequences are. The sequences will be laid out in the order found in these files and every sequence in `--Rfile` and `--Qfile` will be plotted even if no alignments exist. The `--SNP` or `--breaklen` options will change the plot colors so that green is normal and red is highlighted.

Output format

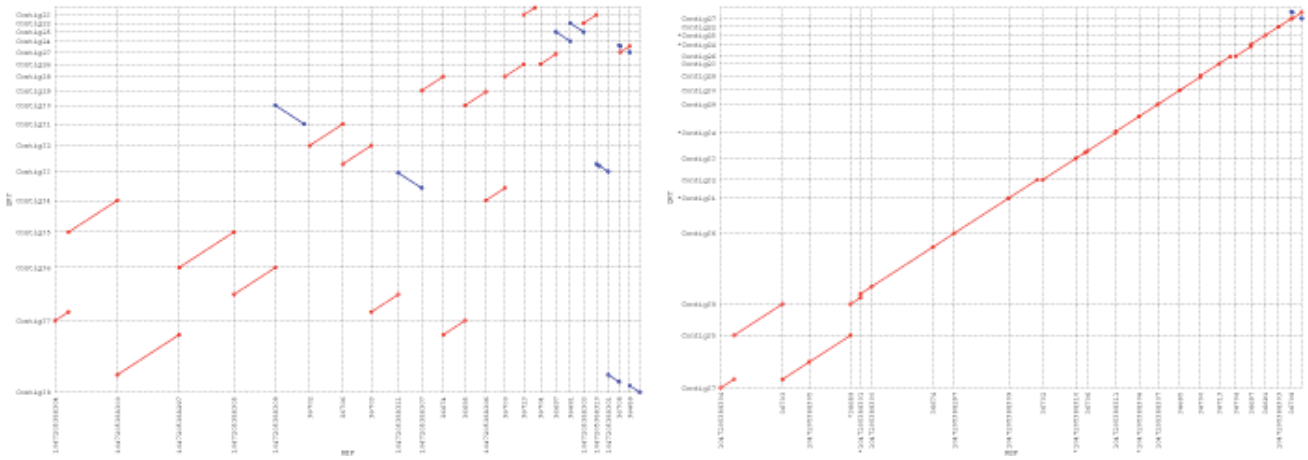
The `mummerplot` script outputs three files, `<prefix>.gp <prefix>.fplot <prefix>.rplot`, when run with standard parameters. The first of which is the gnuplot script. This script contains the commands necessary to generate the plot, and refers to the two data files which contain the forward and reverse matches respectively. If the `--filter` or `--layout` option are specified, an additional `<prefix>.filter` file will be generated containing the filtered delta information. If the `--breaklen` or `--SNP` are included, an additional data file `<prefix>.hplot` will be created containing the highlight information. Finally, if a terminal other than X11 is specified, the plot graphic will saved to the file `<prefix>.ps` or `<prefix>.png` if the terminal is postscript of PNG respectively. Line thickness, color, and many other options can be added or removed from the plot by hand editing the gnuplot script. Examples of the two types of plots are displayed below, the dot plot first, followed by the coverage plot, and finnaly a couple multiplots.



For a dot plot, the reference sequence is laid across the x-axis, while the query sequence is on the y-axis. Wherever the two sequences agree, a colored line or dot is plotted. The forward matches are displayed in red, while the reverse matches are displayed in green. If the two sequences were perfectly identical, a single red line would go from the bottom left to the top right. However, two sequences rarely exhibit this behavior, and in the above plot, multiple gaps and inversions can be identified between these two strains of *Helicobacter pylori*. This plot was generated from `nucmer` output, however running `mummerplot` on a simple match list from `mummer` would produce similar results, but with more "noise". In the newer versions, `mummerplot` plots points at the beginning and end of each line to avoid pixel resolution issues and also uses different plotting colors. Therefore, the output may look slightly different than displayed on these pages.



When there are many query sequences mapping to a single reference sequence, it is often helpful to use a coverage or percent identity plot. This type of plot lays out each of the alignment regions (or for `show-tiling`, the full contigs) according to their percent similarity and mapping location to the reference. For easier visualization of gaps, all of the alignments are also re-plotted at 10% similarity to normalize the y coordinates and produce a secondary 1D plot. Note that since `mummer` produces nothing but exact matches, only the normalized 1D plot will appear in the figure.



A multiplot is a plot for multiple reference and query sequences where each reference/query pair is given its own grid box and their dotplot is drawn within the constraints of that box. Thus, every grid line represents the end of one sequence and the beginning of the next. This allows us to draw every dotplot for the two sequence sets at once, as displayed by the two contig sets in the above left image. With a little shuffling of the order and orientation of the sequences, a more pleasing layout can be obtained as show in the above right image. This is the same contig set as on the left, however the contigs have been reordered and oriented so that the major alignments cluster around the main diagonal of the plot. This allows for easier browsing of the plot by centralizing the important information, and also highlights contigs that have disagreeing sequences by breaking the diagonal. Currently a greedy approach is used to perform the layout, and while good at bringing alignments to the diagonal, it does not always produce the optimal ordering. Therefore, a break in the diagonal does not always signal a disagreement between the two sequence sets (see the `mummerplot --breaklen` option for an easy way to highlight assembly discrepancies).

A quick reference guide for interpretting the dot plot is available here.

5.4.4. show-aligns

`show-aligns` parses the delta encoded alignment output of NUCmer and PROmer, and displays the pair-wise alignments from the two sequences specified on the command line. It is handy for identifying the exact location of errors and looking for SNPs between two sequences.

Command line syntax

```
show-aligns [options] <delta file> <IdR> <IdQ>
```

The <delta file> is the delta output file of either nucmer or promer. <IdR> is the FastA header tag of the desired reference sequence, and <IdQ> is the FastA header tag of the desired query sequence. All alignments between these two sequences will be displayed. Output will be to stdout.

Program options

```
  -h       Print help information and exit

  -q       Sort alignments by the query start coordinate

  -r       Sort alignments by the reference start coordinate

  -w int   Set the screen width of the output (default 60)

  -x int   The alignment matrix type, 1 [BLOSUM 45], 2 [BLOSUM 62] or 3 [BLOSUM 80] (default 2)
```

The -x option applies to amino acid alignments (promer output) and will only affect the error notations, not the alignment.

Output format

Output is to stdout and is slightly different depending on the type of alignment, i.e. nucleotide or amino acid. Each alignment is preceded with a header containing the BEGIN keyword, the frame/direction information and the start and end in the reference and query respectively. Each individual line of the alignment is prefixed with the position of the first base on that line, these positions reference the forward strand of the DNA sequence regardless of alignment type. Errors in nucleotide alignments are marked with a '^' character below the two mismatching sequence bases. Errors in protein alignments are noted with a whitespace in between the two mismatching acids, while similarities (positive alignment scores) are marked with a '+' and identities are noted with a copy of the matching acid. Each alignment is followed by a footer containing the END keyword, the frame/direction information and the start and end in the reference and query respectively. Perhaps the best way to explain this format is by example, so snippets of the two types of alignments are given below.

Nucleotide alignment output

```
/home/aphillip/data/GHP.1con /home/aphillip/data/GHPJ9.1con

=============================================================
-- Alignments between Helicobacter_pylori_26695 and Helicobacter_pylori_strain_J99


-- BEGIN alignment [ +1 4262 - 4316 | +1 4469 - 4522 ]


4262       gatttgaacttccgtttccaccgtgaaagggtggtatccttggccacta
4469       gatttgaacccctgtaaccaccgtgaaagggtggtatcc.taaccacta
                     ^^ ^  ^^                      ^ ^^


4311       gatgaa
4517       gatgaa
```

```
--   END alignment [ +1 4262 - 4316 | +1 4469 - 4522 ]
-- BEGIN alignment [ +1 5198 - 22885 | +1 5389 - 23089 ]
(output continues ...)
```

Amino acid alignment output

```
/home/aphillip/data/mgen.seq /home/aphillip/data/ecoliO157.seq

============================================================
-- Alignments between mgen.seq and Escherichia_coli_O157:H7


-- BEGIN alignment [ +1 31690 - 31995 | +3 3336375 - 3336680 ]


31690      VSFSFYLVPNKRSPASPRPGIMYLLSFNFSSIAARNIST*GCIFSTLLI
           + F  Y VP   SPASPRPGIMY  SF+  SI A   ST GC FS+  I
3336375    IIFILYFVPKILSPASPRPGIMYPCSFSP*SIDAVYSSTSGCAFSSAAI

31837      PSGAATIAITLILIGLSSLIDLIAVNNVVPVASIGSRIITCESEMFSGI
           PSGAAT   TL+L+  +     +      PVASIGS I    S M
3336522    PSGAATSTRTLMLLQPAFFSRSMVAITEPPVASIGSTISAIRSSMLETS

31984      FL*Y
           F  Y
3336669    FWKY


--   END alignment [ +1 31690 - 31995 | +3 3336375 - 3336680 ]
-- BEGIN alignment [ +2 50819 - 51220 | -1 3263900 - 3263499 ]
(output continues ...)
```

5.4.5. show-coords

`show-coords` parses the delta alignment output of NUCmer and PROmer, and displays summary information such as position, percent identity and so on, of each alignment. It is the most commonly used tool for analyzing the delta files.

Command line syntax

```
show-coords [options] <delta file>
```

The `<delta file>` is the delta output file of either `nucmer` or `promer`.

Program options

```
  -b         Brief output that only displays the non-redundant locations of aligning regions

  -B         Switch output to btab format
```

```
-c          Include percent coverage columns in the output

-d          Include the alignment direction/reading frame in the output (default for promer)

-g          Only display alignments included in the Longest Ascending Subset, i.e. the global
            alignment. Recommened to be used in conjunction with the -r or -q options. Does not
            support circular sequences

-h          Print help information and exit

-H          Omit the output header

-I float    Set minimum percent identity to display

-k          *PROMER ONLY* Knockout (do not display) alignments that overlap another alignment in
            a better reading frame

-l          Include sequence length columns in the output

-L int      Set minimum alignment length to display

-o          Annotate maximal alignments between two sequences, i.e. overlaps between reference
            and query sequences

-q          Sort output lines by query

-r          Sort output lines by reference

-T          Switch output to tab-delimited format
```

The `-b` option alters the output table to only display the location of the aligning regions, not their identity, direction, frame, etc. Also, for protein data, the `-b` option will collapse all overlapping frames, and list a single encompassing region. `-B` switches the output format to "btab" (Blast tablature) which is a tab-delimited table with a different layout than the standard `show-coords` format. The coverage information added with the `-c` option is equal to the length of the alignment divided by the length of the sequence. The `-k` option will select the "best" reading frame by choosing the alignment that is longest, or has the highest percent identity and is within 75% of the length of the longest alignment; only alignments that overlap each other by greater than 50% of their length will be considered for knockout. The `-T` option is different than the `-B` option because it retain the normal ordering of output columns. The output of the `-d` option for NUCmer data will appear under the `[FRM]` column, just like the reading frame info from PROmer data. The `-o` annotations will appear in the final column of the output. The descriptions reference the reference sequence, *e.g.* `[END]` means the overlap is on the end of the reference sequence and `[CONTAINED]` means the reference sequence is contained by the query sequence.

The `-c` and `-l` options are useful when comparing two sets of assembly contigs, in that these options help determine if an alignment spans an entire contig, or is just a partial hit to a different sequence. The `-b` option is useful when the user wishes to identify syntenic regions between two genomes, but is not particularly interested in the actual alignment similarity or appearance. This option also disregards match orientation, so should not be used if this information is needed. The `-g` option comes in handy when comparing sequences that share a linear alignment relationship, that is there are no rearrangements. Large nsertions, deletions and gaps can then be identified by the break between two adjacent alignments in the

output. If there are more than one global alignment that share the same score, then one of them is picked at random to display. This is useful when mapping repetitive reads to a finished sequence.

Output format

Output is to `stdout` and is slightly different depending on the type of alignment, i.e. nucleotide or amino acid. Some of the described columns, such as percent similarity, will not appear for nucleotide comparisons. When run without the `-H` or `-B` options, `show-coords` prints a header tag for each column; the descriptions of each tag follows. `[S1]` start of the alignment region in the reference sequence `[E1]` end of the alignment region in the reference sequence `[S2]` start of the alignment region in the query sequence `[E2]` end of the alignment region in the query sequence `[LEN 1]` length of the alignment region in the reference sequence `[LEN 2]` length of the alignment region in the query sequence `[% IDY]` percent identity of the alignment `[% SIM]` percent similarity of the alignment (as determined by the BLOSUM scoring matrix) `[% STP]` percent of stop codons in the alignment `[LEN R]` length of the reference sequence `[LEN Q]` length of the query sequence `[COV R]` percent alignment coverage in the reference sequence `[COV Q]` percent alignment coverage in the query sequence `[FRM]` reading frame for the reference and query sequence alignments respectively `[TAGS]` the reference and query FastA IDs respectively. All output coordinates and lengths are relative to the forward strand of the reference DNA sequence.

When run with the `-B` option, output format will consist of 21 tab-delimited columns. These are as follows: `[1]` query sequence ID `[2]` date of alignment `[3]` length of query sequence `[4]` alignment type `[5]` reference file `[6]` reference sequence ID `[7]` start of alignment in the query `[8]` end of alignment in the query `[9]` start of alignment in the reference `[10]` end of alignment in the reference `[11]` percent identity `[12]` percent similarity `[13]` length of alignment in the query `[14]` 0 for compatibility `[15]` 0 for compatibility `[16]` NULL for compatibility `[17]` 0 for compatibility `[18]` strand of the query `[19]` length of the reference sequence `[20]` 0 for compatibility `[21]` and 0 for compatibility.

5.4.6. show-snps

`show-snps` is a utility program for reporting polymorphisms contained in a delta encoded alignment file output by NUCmer or PROmer. It catalogs all of the single nucleotide polymorphisms (SNPs) and insertions/deletions within the delta file alignments. Polymorphisms are reported one per line, in a delimited fashion similar to `show-coords`. Pairing this program with the appropriate MUMmer tools can create an easy to use SNP pipeline for the rapid identification of putative SNPs between any two sequence sets, as demonstrated in SNP detection section.

Command line syntax

```
show-snps [options] <delta file>
```

The `<delta file>` is the delta output of either `nucmer` or `promer`. Output will be to stdout.

Program options

```
-C      Do not report SNPs from alignments with an ambiguous mapping, i.e. only report SNPs
        where the [R] and [Q] columns equal 0 and do not output these columns

-h      Print help information and exit

-H      Do not print the output header

-I      Do not report indels
```

```
-l       Include sequence length information in the output

-q       Sort output lines by query IDs and SNP positions

-r       Sort output lines by reference IDs and SNP positions

-S       Specify which alignments to report by passing 'show-coords' lines to stdin

-T       Switch to tab-delimited format

-x int   Include x characters of surrounding SNP context in the output (default 0)
```

The `-C` option is a little confusing, but in simple terms it avoids calling SNPs from repetitive regions. "ambiguous mapping" refers to a position on the reference or query that is covered by more than one alignment. This can be caused by simple repeats, or overlapping alignments caused by tandem repeats that exist in different copy numbers. Either way, calling SNPs from these regions is questionable, and therefore the `-C` option should be invoked in most instances. To generate output suitable for further parsing, use the `-H -T` options. The `[BUFF]` output column will refer to the sequence positions requested by the `-r -q` options, so these options affect more than the order of the output. The `-S` option will accept all forms of `show-coords` output, so output can be piped into `show-snps` or a simple cut/paste from one xterm to another should get the job done. This option is helpful when the user has a specific alignment they would like to see SNPs from. `-x` does nothing other than print out the characters on either side of the listed position for both the reference and query. The `'.'` character is used to represent indels, while `'-'` represents end-of-sequence.

Output format

Output is to stdout and is slightly different depending on which command switches are set. For instance, by default the output is arranged in a table style, however if the `-T` option is active, the output will be tab-delimited. Also, the sequence files, alignment type and column headers are output by default, however if the `-H` option is active, the headers will be stripped from the output. Other options like `-l -C -x` will add or remove columns from the output. So, for description purposes, all possible column headers will be given and it is up to the user to pair the column header with the column number. The descriptions for each header tag follows. `[P1]` position of the SNP in the reference sequence. For indels, this position refers to the 1-based position of the first character before the indel, e.g. for an indel at the very beginning of a sequence this would report 0. For indels on the reverse strand, this position refers to the forward-strand position of the first character before indel on the reverse-strand, e.g. for an indel at the very end of a reverse complemented sequence this would report 1. `[SUB]` character or gap at this position in the reference `[SUB]` character or gap at this position in the query `[P2]` position of the SNP in the query sequence `[BUFF]` distance from this SNP to the nearest mismatch (end of alignment, indel, SNP, etc) in the same alignment `[DIST]` distance from this SNP to the nearest sequence end `[R]` number of repeat alignments which cover this reference position `[Q]` number of repeat alignments which cover this query position `[LEN R]` length of the reference sequence `[LEN Q]` length of the query sequence `[CTX R]` surrounding reference context `[CTX Q]` surrounding query context `[FRM]` sequence direction (NUCmer) or reading frame (PROmer) `[TAGS]` the reference and query FastA IDs respectively. All positions are relative to the forward strand of the DNA input sequence, while the `[BUFF]` distance is relative to the sorted sequence.

5.4.7. show-tiling

`show-tiling` attempts to construct a tiling path out of the query contigs as mapped to the reference sequences. Given the delta alignment information of a few long reference sequences and many small query contigs, `show-tiling` will determine the best mapped location of each query contig. Note that each

contig may only be tiled once, so repetitive regions may cause this program some difficulty. This program is useful for aiding in the scaffolding and closure of an unfinished set of contigs, if a suitable, high similarity reference genome is available. Or, if using PROmer, `show-tiling` will help in the identification of syntenic regions and their contig's mapping to the references.

This program is not suitable for "many vs. many" assembly comparisons, however a new tool based on the concepts of `show-tiling` should be available in the near future that will facilitate the mapping of assembly contigs.

Command line syntax

```
show-tiling [options] <delta file>
```

The `<delta file>` is the delta output file of either `nucmer` or `promer`. Primary output will be to stdout.

Program options

```
-a              Describe the tiling path by printing the tab-delimited alignment regions

-c              Assume the reference sequences are circular, and allow tiled contigs to span the
                origin

-h              Print help information and exit

-g int          Maximum gap between clustered alignments, where -1 will represent infinity
                (nucmer default 1000, promer default -1)

-i float        Minimum percent identity (nucmer default 90.0, promer default 55.0)

-l int          Minimum contig length (default 1)

-p filename     Output a pseudo molecule of the query contigs to file

-R              Deal with repetitive contigs by randomly placing them in one of their copy
                locations (implies -V 0)

-t filename     Output a TIGR assembler style contig list of EVERY mapping contig to file

-u filename     Output the tab-delimited alignment regions of the unusable contigs to file

-v float        Minimum contig alignment coverage (nucmer default 95.0, promer default 50.0)

-V float        Minimum contig coverage difference (nucmer default 10.0, promer default 30.0)

-x              Describe the tiling path by printing the XML contig linking information
```

The `-i` and `-l` options filter out all contigs below these cutoffs. The `-p` option creates a pseudo molecule from the query sequence, and arranges them as the map to the reference. The `-v` option sets the minimum percent of the query contig that must be covered by aligning bases, while the `-V` option sets the difference in percent coverage to determine one mapping is better than another. To include the most possible contigs

in the tiling, set the `-V` option to zero and lower the `-i` and `-v` options to reasonable values. For NUCmer data, percent coverage is the non-redundant number of aligning bases divided by the length of the query sequence, while for PROmer data, percent coverage is the extent of the syntenic region divided by the length of the query sequence. The difference being, `show-tiling` does not penalize a PROmer mapping for having big gaps and small alignments. The `-x` option output can be used as input to the TIGR scaffolder "Bambus", for use as contig linking information. With the exception of the output generated by the `-t` option, all tiling paths include the minimal number of contigs needed to generate the maximum reference coverage. This means that there may be other, smaller contigs that map to the reference, but because they are shadowed by larger contigs, they are not reported. The `-R` option is very useful for maintaining uniform, 'random' coverage of reads when mapping to a reference.

Output format

Output is to `stdout` and differs depending on the command line options. Standard output has an 8 column list per mapped contig, separated by the FastA headers of each reference sequence. These columns are as follows: `[1]` start in the reference `[2]` end in the reference `[3]` gap between this contig and the next `[4]` length of this contig `[5]` alignment coverage of this contig `[6]` average percent identity of this contig `[7]` contig orientation `[8]` contig ID. Output of the `-a` and `-u` options have the same columns as `show-coords` run with the `-THcl` options. Output of the `-x` option follows standard XML format. An example of the standard output of `show-tiling` follows:

```
>gba:6615 5227293 bases
-10807  20017   105     30825   100.00  99.99   +       253
20123   21388   42      1266    100.00  100.00  -       121
21431   93545   37      72115   100.00  100.00  +       272
93583   96184   -15     2602    100.00  100.00  +       51
96170   98575   161     2406    100.00  99.96   -       93
98737   100543  1072    1807    100.00  99.83   -       94
101616  103405  3121    1790    100.00  99.89   +       107
5215716 5216412 73      697     100.00  100.00  -       92
(output continues ...)
>gbx:17223 181677 bases
-12269  43162   -258    55432   100.00  100.00  -       9
42905   49553   -106    6649    100.00  100.00  +       7
49448   112332  -659    62885   100.00  100.00  -       21
111674  112935  -519    1262    100.00  100.00  +       22
112417  116940  -201    4524    100.00  100.00  +       23
116740  160401  -27     43662   100.00  100.00  +       10
160375  167673  1734    7299    100.00  100.00  -       159
>gbx:17224 94829 bases
-89937  5606    54601   95544   100.00  99.99   -       168
60208   61126   -56235  919     100.00  99.24   -       43
```

The negative start positions indicate contigs that are wrapping around the origin, since this output was generated with the `-c` option.

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5. Known problems

MUMmer's modular design is very beneficial, however it has created a small set of inconveniences. Some modules like `mummer` have been updated in the recent 3.0 release, while others like `mgaps` have not. Since it is not always possible to update all modules at once, some legacy issues appear. For example, because `mgaps` was originally written to cluster the output of a matching algorithm that could only handle one reference sequence, its input and output is constrained to handle only a single reference sequence. When `mummer` was updated in the 3.0 release, it was modified to handle multiple reference sequences, but this causes a slight incompatibility as its output can no longer be fed into `mgaps` when it contains multiple

reference sequences. The same type of annoyance occurs between `mummer` and `gaps`, as `gaps` was originally designed to handle only one reference *and* only one query sequence. Such incompatibilities can be inconvenient, but workarounds with stream editors and conversion scripts are common practice by those familiar with MUMmer. Learning more about the output of each program can lead to a better understanding of how the modules communicate with one another and make it possible to format the output of one module so that it can be understood by a legacy module.

`nucmer`, `promer` and `run-mummer3` all have a difficult time with tandem repeats. If the two sequences contain a different number of copies of the same tandem repeat, these alignment routines will sometimes generate a cluster on either side of the tandem and extend alignments past one another, failing to join them into a single alignment region. This generates two overlapping alignments and makes it difficult to determine what caused this erratic behavior. In addition, the %identity for this region may appear artificially low as the alignment extension attempted to align sequence that was offset by the difference in length of the tandem repeats, instead of identifying the single large insertion. Any difference in the tandem between the reference and query can be calculated as the difference of the alignment overlap in each sequence. This bug is more of a nuisance than a critical problem, so a fix is being considered but no timeline has been set for its implementation.

The MUMmer programs do not perform validity checking on their inputs. If any part of the package appears to malfunction, please check that the input files are within the constraints of each program (i.e. number of sequences allowed, FastA format, memory usage, etc.).

This document will be under constant edit, so if you notice any errors please contact us.

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7. Contact information

Please address questions and bug reports via Email to:

mummer-help@lists.sourceforge.net

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