Assembly Tutorial

Michael Schatz

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CSHL Advanced Sequencing

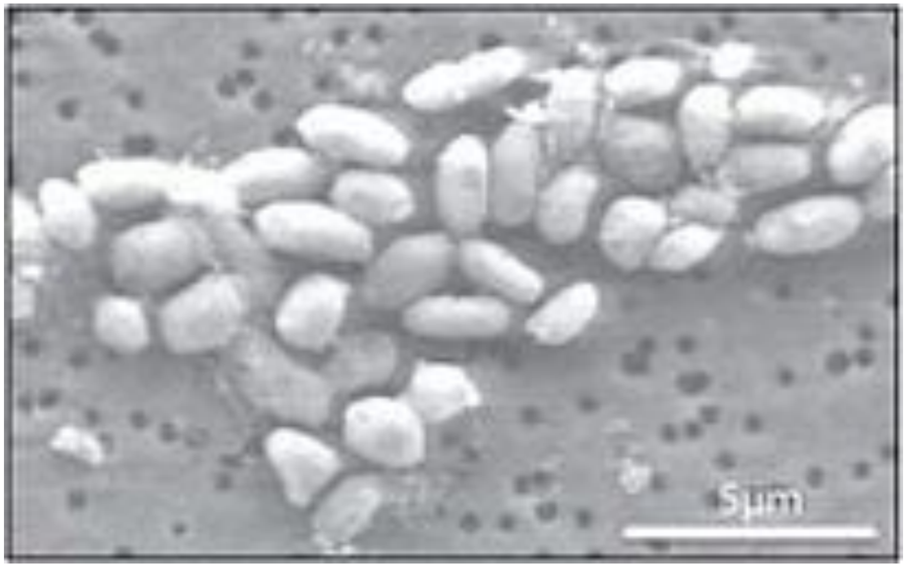
Outline

1. Sample Data for your mission!

2. ALLPATHS-LG

3. MUMmer

Halomonas sp. GFAJ-1



Library 1: Fragment

Avg Read length: 100bp

Insert length: 180bp

Library 2: Short jump

Avg Read length: 50bp

Insert length: 2000bp

A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus
Wolfe-Simon et al (2010) *Science. 332(6034)1163-1166.*

Digital Information Storage

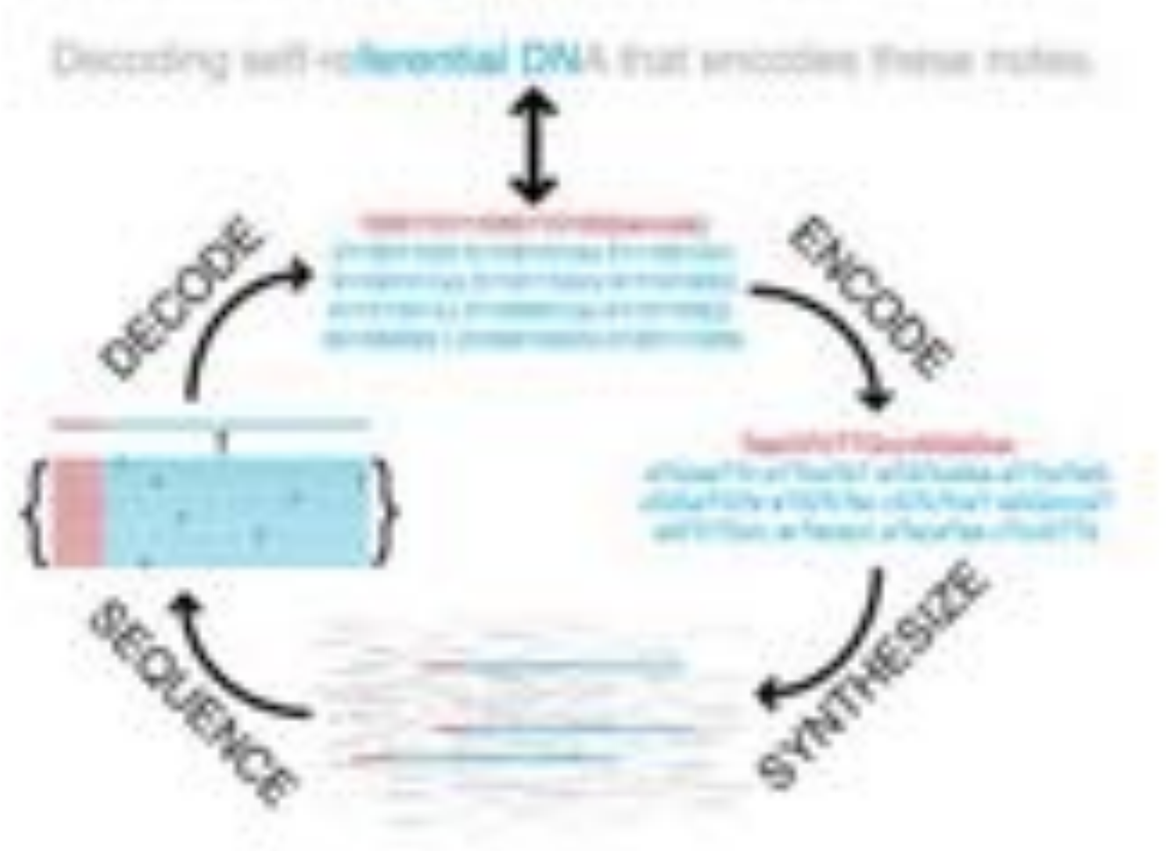


Fig. S1. Schematic of DNA information storage.

Encoding/decoding algorithm implemented in dna-encode.pl from David Dooling.

Next-generation Digital Information Storage in DNA
Church et al (2010) *Science*. 337(6102)1628

Mission Impossible

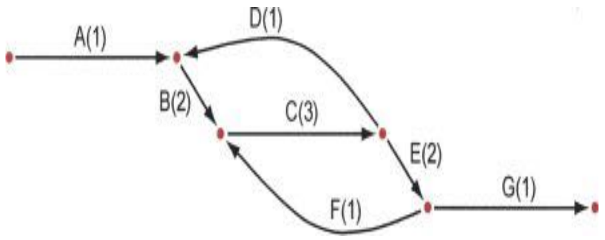
1. ***Setup virtual machine***

   ```
   sudo apt-get install gnuplot-x11
   cd ~/allpathslg-43436
   sudo make install
   ```

2. ***Download Reference Genome***

   ```
   wget http://schatzlab.cshl.edu/asm.tgz
   tar xzvf asm.tgz
   ```

3. ***Decode the secret message***
   1. *Assemble the reads, Align to reference, Extract secret*
   2. *dna-encode.pl -d*



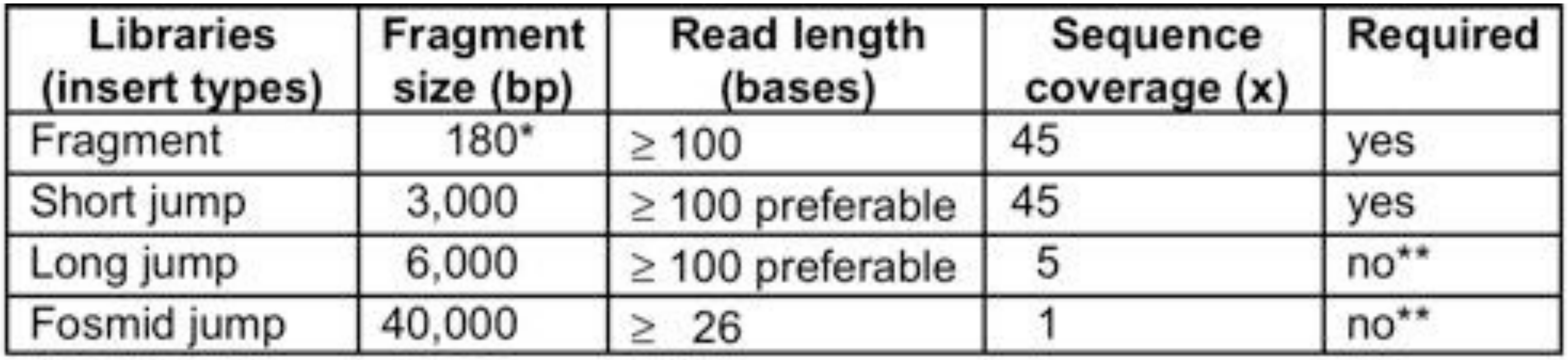
Running ALLPATHS-LG
Iain MacCallum

BROAD INSTITUTE

How to use ALLPATHS-LG

1. **Data requirements (*** most critical thing ***)**
2. Computational requirements & Installation
3. Preparing your data
4. Assembling
5. What is an ALLPATHS-LG assembly?

ALLPATHS-LG sequencing model



Libraries (insert types)	Fragment size (bp)	Read length (bases)	Sequence coverage (x)	Required
Fragment	180*	≥ 100	45	yes
Short jump	3,000	≥ 100 preferable	45	yes
Long jump	6,000	≥ 100 preferable	5	no**
Fosmid jump	40,000	≥ 26	1	no**

*See next slide.

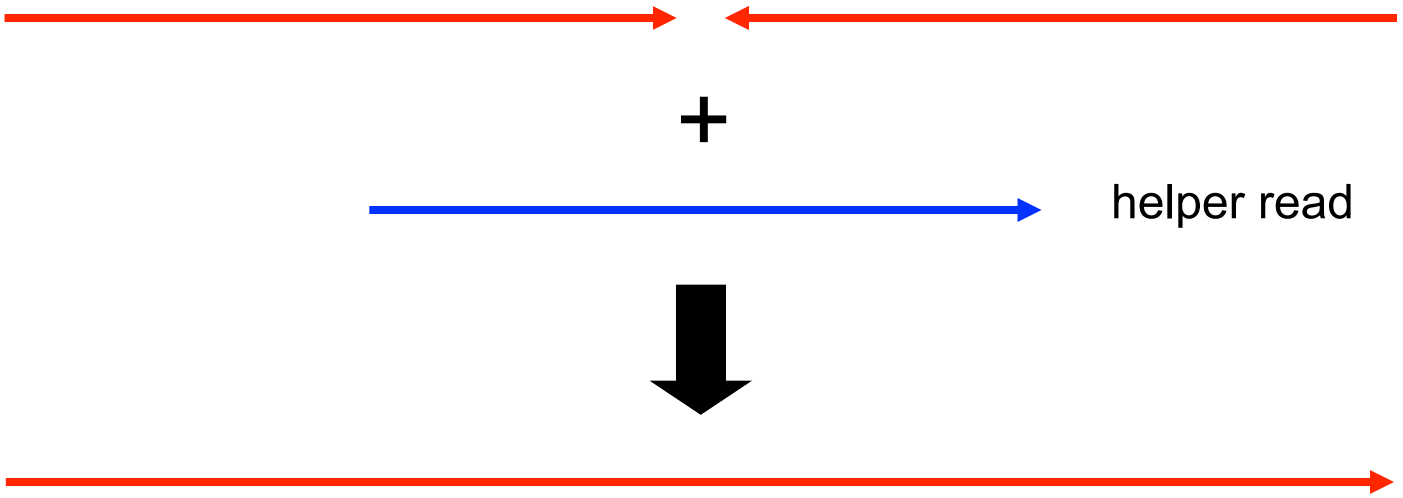
**For best results. Normally not used for small genomes. However essential to assemble long repeats or duplications.

Cutting coverage in half still works, with some reduction in quality of results.

All: protocols are either available, or in progress.

Libraries from 180 bp fragments

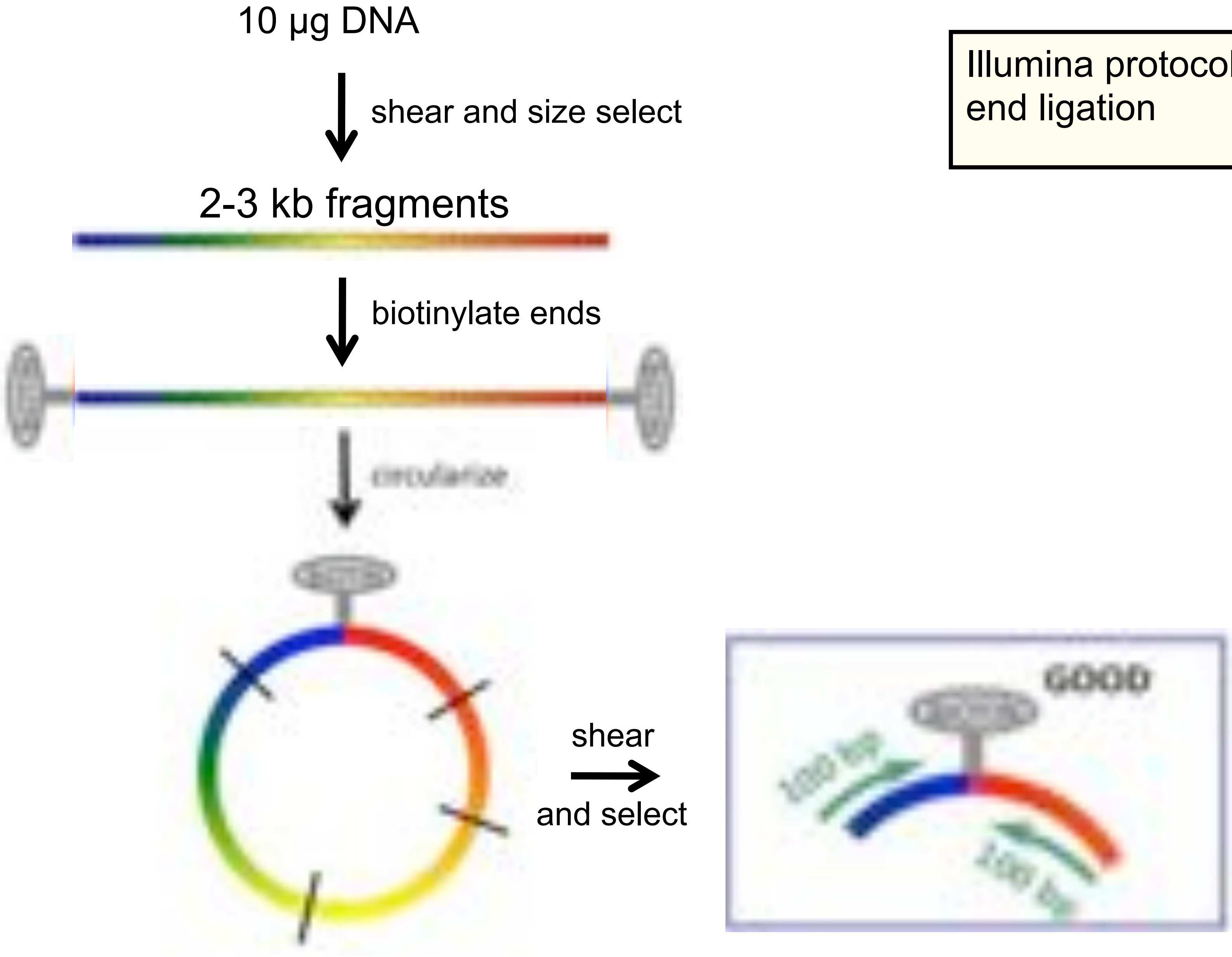
Pairs of 100 base reads from these libraries are merged to create ‘reads’ that are twice as long:



For longer reads, fragment size would be increased proportionally.

Short jumping libraries (2-3 kb)

Illumina protocol, blunt-end ligation



10 µg DNA

shear and size select

2-3 kb fragments

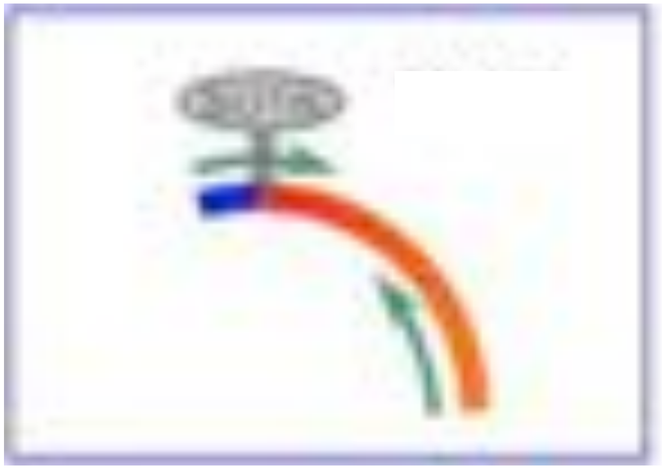
biotinylate ends

shear

and select

Short jumping libraries (2-3 kb)

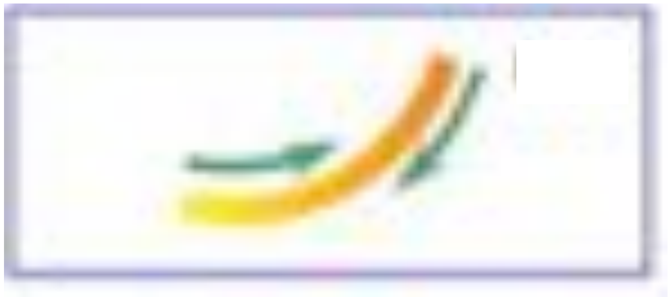
Problem 1. Read passes through circularization junction. This reduces the effective read length (and complicates algorithm).



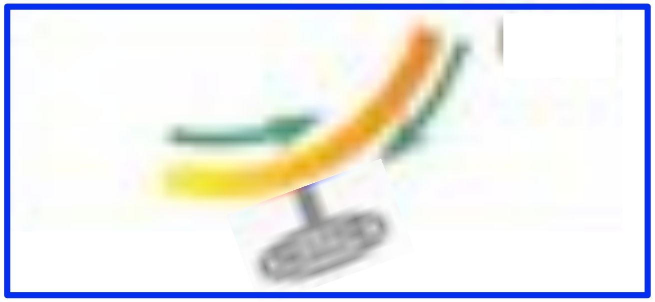
What might be done to reduce incidence of this:
shear circles to larger size and select larger fragments

Short jumping libraries (2-3 kb)

Problem 2. Reads come from nonjumped fragments and are thus in reverse orientation and close together on the genome. This reduces yield (and complicates algorithm).



Putative cause: original DNA is nicked or becomes nicked during process – biotins become ‘ectopically’ attached at these nicks



How to use ALLPATHS-LG

1. Data requirements
2. **Computational requirements & installation**
3. Preparing your data
4. Assembling
5. What is an ALLPATHS-LG assembly?

Computational requirements

- 64-bit Linux

- runs multi-threaded on a single machine

- memory requirements
  - about 160 bytes per genome base, implying
    - need 512 GB for mammal (Dell R315, 48 processors, $39,000)
    - need 1 GB for bacterium (theoretically)
  - if coverage different than recommended, adjust...
  - potential for reducing usage

- wall clock time to complete run
  - 5 Mb genome → 1 hour (8 processors)
  - 2500 Mb genome → 500 hours (48 processors)

Installing ALLPATHS-LG

Web page:

http://www.broadinstitute.org/software/allpaths-lg/blog/

General instructions:

http://www.broadinstitute.org/science/programs/genome-biology/computational-rd/general-instructions-building-our-software

Getting the ALLPATHS-LG source

Our current system is to release code daily if it passes a test consisting of several small assemblies:

Download the latest build from:
ftp://ftp.broadinstitute.org/pub/crd/ALLPATHS/Release-LG/

Unpack it:

```
   % tar xzf allpathslg-39099.tar.gz
```

(substitute the latest revision id for 39099)

This creates a source code directory `allpathslg-39099`:

```
   % cd allpathslg-39099
```

Building ALLPATHS-LG

Step one: `./configure`

Options:

`-prefix=<prefix path>`

put binaries in `<prefix path>/bin`, else `./bin`

Step two: `make` **and** `make install`

Options:

`–j<n>`

compile with n parallel threads

Step three: add bin directory to your path

How to use ALLPATHS-LG

1. Data requirements
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5. What is an ALLPATHS-LG assembly?

Preparing data for ALLPATHS-LG

Before assembling, prepare and import your read data.

ALLPATHS-LG expects reads from:

- At least one fragment library.
    - One should come from fragments of size ~180 bp.
    - This isn’t checked but otherwise results will be bad.

- At least one jumping library.

IMPORTANT: use all the reads, including those that fail the Illumina purity filter (PF). These low quality reads may cover ‘difficult’ parts of the genome.

ALLPATHS-LG input format

ALLPATHS-LG can import data from:
BAM, FASTQ, FASTA/QUALA or FASTB/QUALB files.

You must also provide two metadata files to describe them:

`in_libs.csv`	- describes the libraries
`in_groups.csv`	- ties files to libraries

FASTQ format: consists of records of the form
@<read name>
<sequence of bases, multiple lines allowed>
+
<sequence of quality scores, with Qn represented by ASCII code n+33, multiple lines allowed>

Libraries – in_libs.csv (1 of 2)

For fragment libraries only

`frag_size` - estimated mean fragment size

`frag_stddev` - estimated fragment size std dev

For jumping libraries only

`insert_size` - estimated jumping mean insert size

`insert_stddev` - estimated jumping insert size std dev

These values determine how a library is used. If `insert_size` is ≥ 20000, the library is assumed to be a Fosmid jumping library.

`paired` - always 1 (only supports paired reads)

`read_orientation` - `inward` or `outward`.

Paired reads can either point towards each other, or away from each other. Currently fragment reads must be `inward`, jumping reads `outward`, and Fosmid jumping reads `inward.`

Libraries – in_libs.csv (2 of 2)

Reads can be trimmed to remove non-genomic bases produced by the library construction method:

`genomic_start`
`genomic_end` - inclusive zero-based range of read bases to be kept; if blank or 0 keep all bases

Reads are trimmed in their original orientation.

Extra optional fields (descriptive only – ignored by ALLPATHS)

`project_name` - a string naming the project.
`organism_name` - the organism name.
`type` - fragment, jumping, EcoP15I, *etc*.

EXAMPLE

```
library_name,     type, paired, frag_size, frag_stddev, insert_size, insert_stddev, read_orientation, genomic_start, genomic_end
Solexa-11541, fragment,      1,       180,          10,            ,              ,           inward               ,
Solexa-11623,  jumping,      1,          ,            ,        3000,           500,          outward              0,          25
```

Input files – in_groups.csv

Each line in `in_groups.csv` comma separated value file, corresponds to a BAM or FASTQ file you wish to import for assembly.
The library name must match the names in `in_libs.csv`.

`group_name` - a unique nickname for this file

`library_name` - library to which the file belongs

`file_name` - the absolute path to the file
(should end in .bam or .fastq)

(use wildcards ‘?’, ‘*’ for paired fastqs)

Example:

```
group_name, library_name,                         file_name
302GJ,      Solexa-11541, /seq/Solexa-11541/302GJABXX.bam
303GJ,      Solexa-11623, /seq/Solexa-11623/303GJABXX.?.fastq
```

How to import assembly data files

```
PrepareAllPathsInputs.pl
    IN_GROUPS_CSV=<in groups file>
    IN_LIBS_CSV=<in libs file>
    DATA_DIR=<full path of data directory>
    PLOIDY=<ploidy, either 1 or 2>
    PICARD_TOOLS_DIR=<picard tools directory>
```

- `IN_GROUPS_CSV` and `IN_LIBS_CSV`: optional arguments with default values `./in_groups.csv` and `./in_libs.csv`. These arguments determine where the data are found.

- `DATA_DIR`: imported data will be placed here.

- `PLOIDY`: either 1 (for a haploid or inbred organism), or 2 (for a diploid organism) – we have not tried to assemble organisms having higher ploidy!

- `PICARD_TOOLS_DIR`: path to Picard tools, for data conversion from BAM.

Putting it all together

1. Collect the BAM or FASTQ files that you wish to assemble. Create a `in_libs.csv` metadata file to describe your libraries and a `in_groups.csv` metadata file to describe your data files.

2. Prepare input files

```
% cd /path/to/data/
% PrepareAllPathsInputs.pl \
    DATA_DIR=`pwd` PLOIDY=1 >& prepare.log
```

How to use ALLPATHS-LG

1. Data requirements
2. Computational requirements & installation
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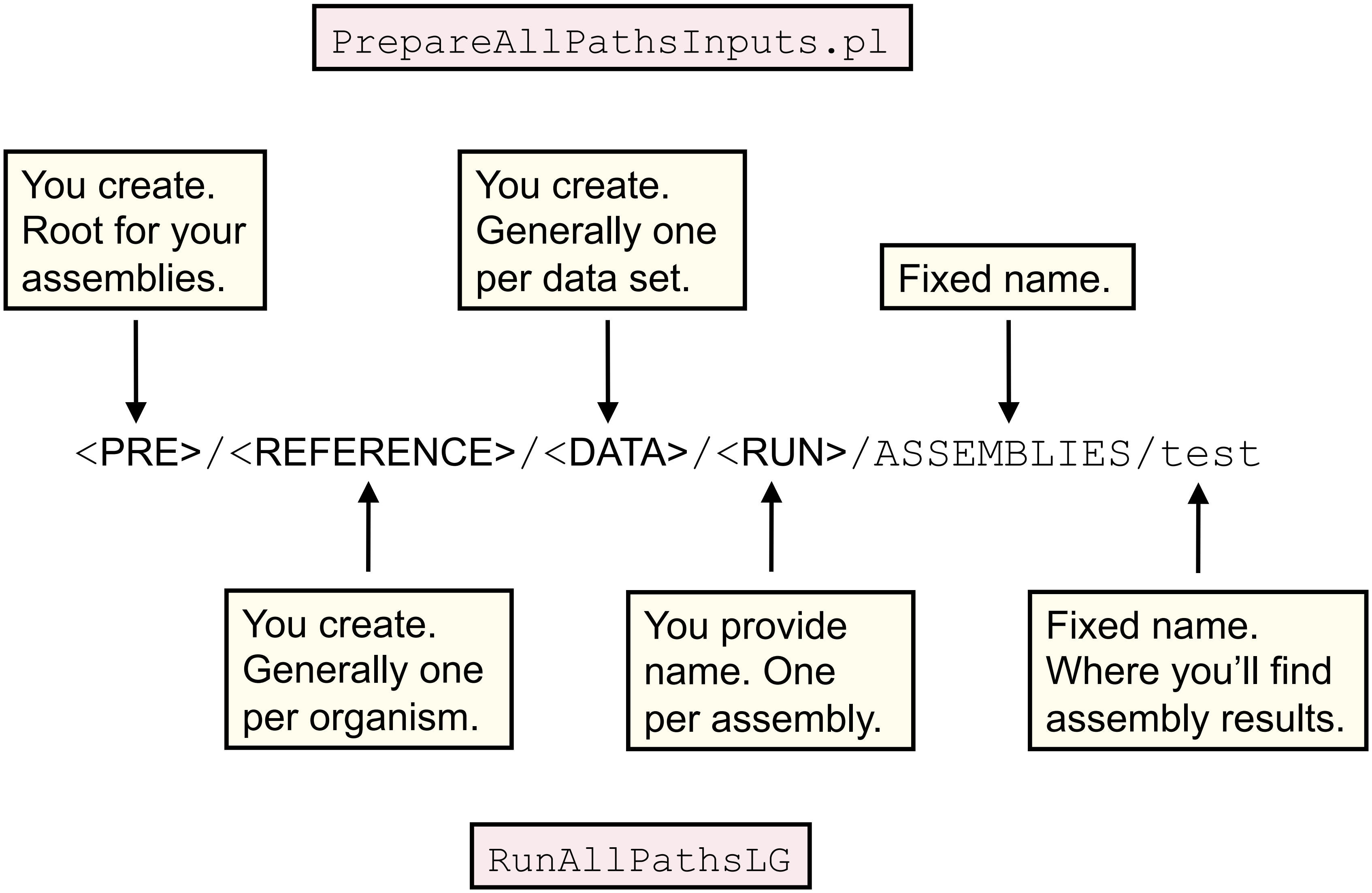
4. **Assembling**
5. What is an ALLPATHS-LG assembly?

ALLPATHS-LG directory structure



PrepareAllPathsInputs.pl

You create. Root for your assemblies.

You create. Generally one per data set.

Fixed name.

<PRE>/<REFERENCE>/<DATA>/<RUN>/ASSEMBLIES/test

You create. Generally one per organism.

You provide name. One per assembly.

Fixed name. Where you'll find assembly results.

RunAllPathsLG

How to assemble

Do this:

```
RunAllPathsLG              \
   PRE=<prefix path>          \
   REFERENCE_NAME=<reference dir>   \
   DATA_SUBDIR=<data dir>        \
   RUN=<run dir>
```

Automatic resumption. If the pipeline crashes, fix the problem, then run the same `RunAllPathsLG` command again. Execution will resume where it left off.

Results. The assembly files are:

`final.contigs.fasta`	- fasta contigs
`final.contigs.efasta`	- efasta contigs
`final.assembly.fasta`	- scaffolded fasta
`final.assembly.efasta`	- scaffolded efasta

Putting it all together

1. Collect the BAM or FASTQ files that you wish to assemble. Create a `in_libs.csv` metadata file to describe your libraries and a `in_groups.csv` metadata file to describe your data files.

2. Prepare input files

```
% PrepareAllPathsInputs.pl \
   DATA_DIR=`pwd` PLOIDY=1 >& prepare.log
```

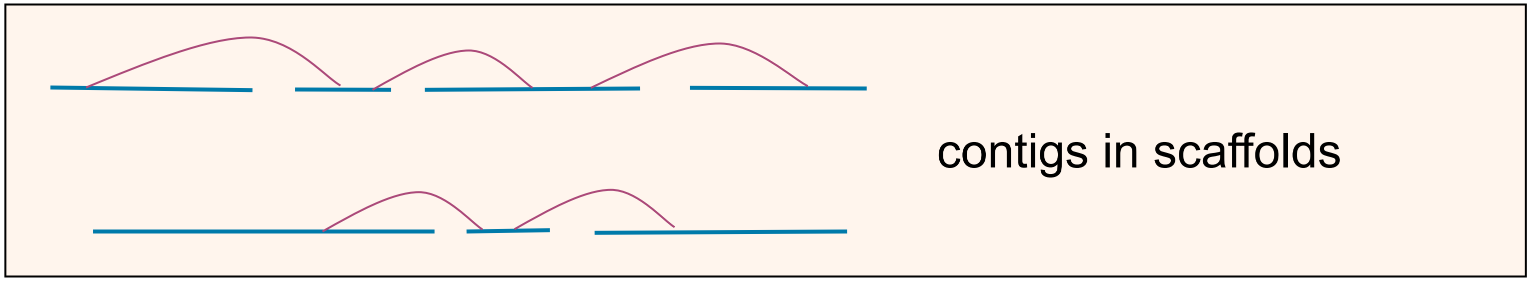
3. Assemble.

```
% RunAllPathsLG \
   PRE=. REFERENCE_NAME=. \
   DATA_SUBDIR=. RUN=default THREADS=4 >& run.log
```

How to use ALLPATHS-LG

1. Data requirements
2. Computational requirements & installation
3. Preparing your data
4. Assembling
5. **What is an ALLPATHS-LG assembly?**

1. Linear assemblies



contigs in scaffolds

contig: a contiguous sequence of bases….

```
CTGCCCCCTGTGCCAATGGGTTTGAGGCTCTTCCCACTTCCTTTTCTATTAGATTCAATGTATCTGGTTTTATGTTGAGG
TCCTAGATCCACTTGGACTTGAGCTTTGTACAAGATGACATATATAGGTCTGTTTTTATTCTTCTACATACAGACAGCCA
GTTATACCAGCACCATTTATTGAAGACACTTTCTTTATTCCATTGTATATTTTTTTACTTCCTTGTCAAAAATCAAGTGA
CCATGAGTATGTGGTTTCATTTCTGGGTCTTCAATTGTATTCCATTAGTCAACATATCTGTCTCTGTACCAATACCATGC
```

scaffold: a sequence of contigs, separated by gaps….

```
TCCTAGATCCACTTGGACTTGAGCTTTGTACAAGATGACATATATAGGTCTGTTTTTATTCTTCTACATACAGACAGCCA
GTTATACCAGCACCATTTATTGAAGACACTTTCTTTATTCCATTGTATATTTTTTTACTTCCTTGTCAAAAATCAAGTGA
CCATGAGTATGTGGTTTCATTTCTGGGTCTTCAATTGTATTCCATTAGTCAACATATCTGTCTCTGTACCAATACCATGC
NNNNNNNN
AGTTTTTACCACAATTGCTCTATAGTAAAGCTTGAGGTCAGGGTTGGTGATCCCTCCAGCCATTCTTTCATTATTAAGAA
TTGTTTTCCCTAGTCTGGGTTTTTTGCTTTTCCAGGCGAATTTGAGAATTGCTCTTTCCATGTCTTTGAAGAATTGTGTT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GGGATTTTGATGGGGTTTGCATTGAATCTGTAGATTGTCTTTGGTAAGATGGTTAGTTTTACTATGTTAATTCTGCCAAT
CCACAAGCATGGGAGCGCTCTCCATTTTCTGAGATCTTCTTCAATTTCTTTCTTGAGAAACTTGAAGTTATTGTCATACA
```

Number of Ns = predicted gap size,
with error bars (can’t be displayed in fasta format)

1. Linear assemblies

Example of an assembly in fasta format

```
>scaffold_1
TCCTAGATCCACTTGGACTTGAGCTTTGTACAAGATGACATATATAGGTCTGTTTTTATTCTTCTACATACAGACAGCCA
GTTATACCAGCACCATTTATTGAAGACACTTTCTTTATTCCATTGTATATTTTTTTACTTCCTTGTCAAAAATCAAGTGA
CCATGAGTATGTGGTTTCATTTCTGGGTCTTCAATTGTATTCCATTAGTCAACATATCTGTCTCTGTACCAATACCATGC
NNNNNNNN
AGTTTTTACCACAATTGCTCTATAGTAAAGCTTGAGGTCAGGGTTGGTGATCCCTCCAGCCATTCTTTCATTATTAAGAA
TTGTTTTCCCTAGTCTGGGTTTTTTGCTTTTCCAGGCGAATTTGAGAATTGCTCTTTCCATGTCTTTGAAGAATTGTGTT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GGGATTTTGATGGGGTTTGCATTGAATCTGTAGATTGTCTTTGGTAAGATGGTTAGTTTTACTATGTTAATTCTGCCAAT
CCACAAGCATGGGAGCGCTCTCCATTTTCTGAGATCTTCTTCAATTTCTTTCTTGAGAAACTTGAAGTTATTGTCATACA
>scaffold_2
CTGAAGTTGTTTATCAGCTGGAGAAGTTCTCAGGTAGAATTTTTGGGATTGCTTATGTATGCTATCATATCACTTGCAAA
TAGTGATACCTTGATTTCTTTTTTACCAATATGTATCCCATTGATCTCTTTCTGTTGTCTTATTGTTCTAGCTAACACTT
CAAGTACTATATTGAATAGATATGGGGAGAGTGGGAATCCTTGTCTTGTCTCCGATTTCAGTGGGATTGCTTCAAGTATG
```

3. Linearized graph assemblies

Efasta

...ACTGTTT{A,C}GAAAT... A or C at site

...CGCGTTTTTTTTTT{,T,TT}CAT... 0 or 1 or 2 Ts at site

Example of an assembly in efasta format

```
>scaffold_1
TCCTAGATCCACTTGGACTTGAGCTTTGTATATATATATATATATATATA{,TA}CAAGATGACATATATAGGAGACAGCCA
GTTATACCAGCACCATTTATTGAAGACACTTTCTTTATTCCATTGTATATTTTTTTACTTCCTTGTCAAAAATCAAGTGA
CCATGAGTATGTGGTTTCATTTCTGGGTCTTCAATTGTATTCCATTAGTCAACATATCTGTCTCTGTACCAATACCATGC
NNNNNNNN
AGTTTTTACCACAATTGCTCTATAGTAAAGCTTGAGGTCAGGGTTGGTGATCCCTCCAGCCATTCTTTCATTATTAAGAA
TTGTTTTCCCTAGTCTGGGTTTTTTGCTTTTCCAGGCGAATTTGAGAATTGCTCTTTCCATGTCTTTGAAGAATTGTGTT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GGGATTTTGATGGGGTTTGCATTGAATCTGTAGATTGTCTTTGGTAAGATGGTTAGTTTTACTATGTTAATTCTGCCAAT
CCACAAGCATGGGAGCGCTCTCCATTTTCTGAGATCTTCTTCAATTTCTTTCTTGAGAAACTTGAAGTTATTGTCATACA
>scaffold_2
CTGAAGTTGTTTATCAGCTGGAGAAGTTCTCAGGTAGAATTTTTGGGATT{A,C,G}GCTTATGTATGCTATCTTGCAAA
TAGTGATACCTTGATTTCTTTTTTACCAATATGTATCCCATTGATCTCTTTCTGTTGTCTTATTGTTCTAGCTAACACTT
CAAGTACTATATTGAATAGATATGGGGAGAGTGGAATCCTTGTCTTGTCTCCGATTTCAGTGGGATTGCTTCAAGTATG
```

Putting it all together

1. Collect the BAM or FASTQ files that you wish to assemble. Create a `in_libs.csv` metadata file to describe your libraries and a `in_groups.csv` metadata file to describe your data files.

2. Prepare input files

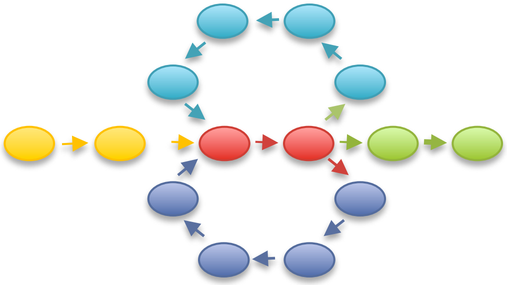
```
% PrepareAllPathsInputs.pl \
   DATA_DIR=`pwd` PLOIDY=1 >& prepare.log
```

3. Assemble.

```
% RunAllPathsLG \
   PRE=. REFERENCE_NAME=. \
   DATA_SUBDIR=. RUN=default THREADS=4 >& run.log
```

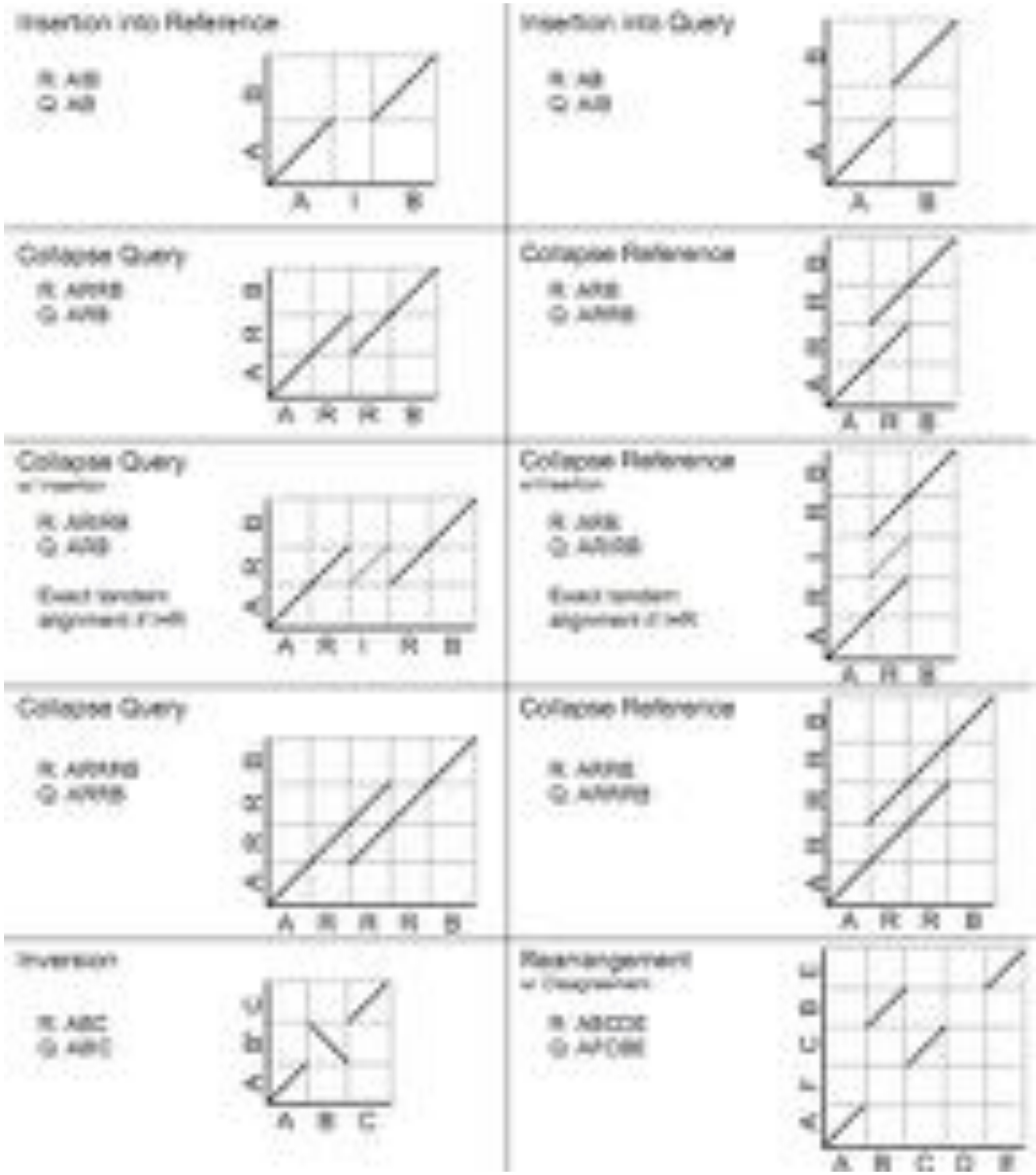
4. Get the results (four files).

```
% cd default/ASSEMBLIES/test/
% less final.{assembly,contigs}.{fasta,efasta}
```



Whole Genome Alignment with MUMmer

SV Types



- Different structural variation types / misassemblies will be apparent by their pattern of breakpoints
- Most breakpoints will be at or near repeats
- Things quickly get complicated in real genomes

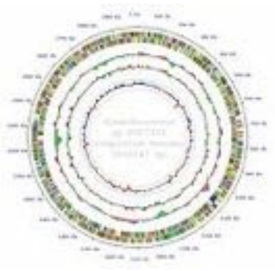
http://mummer.sf.net/manual/
AlignmentTypes.pdf

Find and decode

```
nucmer -maxmatch ref.fasta \
   default/ASSEMBLIES/test/final.contigs.fasta -p refctg
   -maxmatch     Find maximal exact matches (MEMs) without repeat filtering
   -p refctg     Set the output prefix for delta file

mummerplot --layout refctg.delta
   -r   Show the dotplot

show-coords -rclo refctg.delta
   -r   Sort alignments by reference position
   -c   Show percent coverage
   -l   Show sequence lengths
   -o   Annotate each alignment with BEGIN/END/CONTAINS

samtools faidx default/ASSEMBLIES/test/final.contigs.fasta

samtools faidx default/ASSEMBLIES/test/final.contigs.fasta \
    contig_XXX:YYY-ZZZ | ./dna-encode -d
```

See manual at http://mummer.sourceforge.net/manual

Resources



- Assembly Competitions
  - Assemblathon: http://assemblathon.org/
  - GAGE: http://gage.cbcb.umd.edu/

- Assembler Websites:
  - ALLPATHS-LG: http://www.broadinstitute.org/software/allpaths-lg/blog/
  - SOAPdenovo: http://soap.genomics.org.cn/soapdenovo.html
  - Celera Assembler: http://wgs-assembler.sf.net

- Tools:
  - MUMmer: http://mummer.sourceforge.net/
  - Quake: http://www.cbcb.umd.edu/software/quake/
  - AMOS: http://amos.sf.net

Questions?

http://schatzlab.cshl.edu/