Genome assembly with ALLPATHS-LG: How to make it work

BROAD INSTITUTE

How to use ALLPATHS-LG

What you will need:

- High-quality data
- Libraries of different sizes
- Long mate-pair links (40kb): difficult to make libraries!!
- A **BIG** compute server: recommended at least 1TB of RAM

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 3)

`in_libs.csv` is a comma separated value (CSV) file.
For clarity, blanks and tabs are allowed and ignored.

The first line describes the field names, listed below.
Each subsequent line describes a library.

`library_name` - a unique name for the library.

Each physically different library should have a different name!

Libraries – in_libs.csv (2 of 3)

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 (3 of 3)

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 – required format

Each BAM or FASTQ file contains paired reads from *one* library.

Data from a single library can be split between files.
Example, one file for each Illumina lane sequenced.

For FASTQ format, the paired reads can be divided in two files (`readsA.fastq`, `readsB.fastq`), or, if in a single file (`reads.fastq`), must be interleaved:

```
    pair1_readA
    pair1_readB
    pair2_readA
    pair2_readB
    …
```

Input files – in_groups.csv

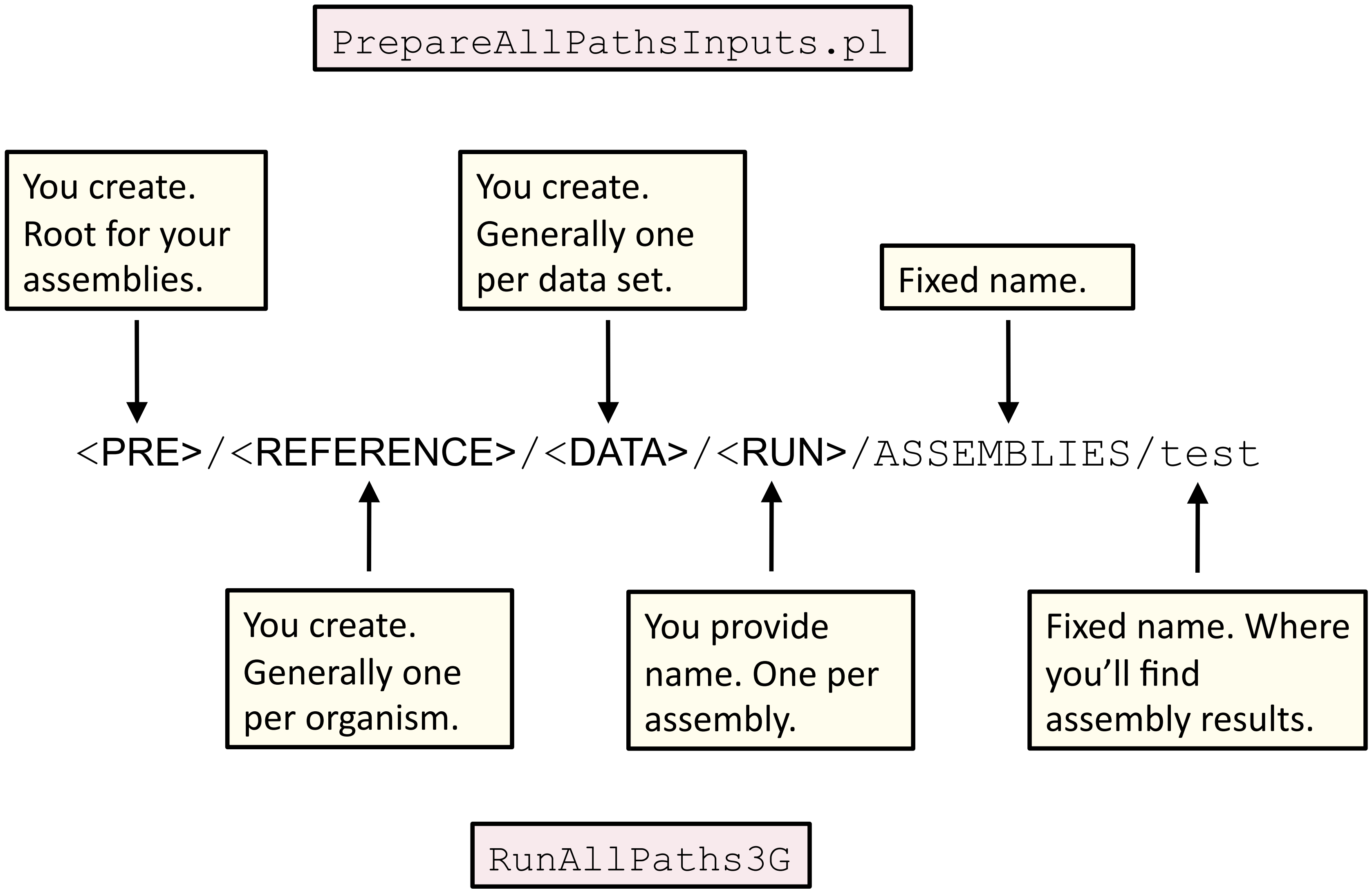
Each line in `in_groups.csv` comma separated value file, corresponds to a BAM or FASTQ file you wish to import for assembly.
The library name must match the names in `in_libs.csv`.

`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
```

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.
RunAllPaths3G

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>
    HOSTS=<list of hosts to be used in parallel>
```

- `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.

(continued)

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

Linearized graph assemblies

Example of an assembly in efasta format

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

Metrics, output and diagnostics

```
final.assembly.efasta
final.contigs.efasta
final.contigs.fastb
final.summary
final.assembly.fasta
final.contigs.fasta
final.rings
final.superb
```

```
assembly_stats.report
library_coverage.report
```

Metric: N50

"length-weighted median"

⇒ 50% of sequences are this long or longer

Things that can go wrong

- Not enough RAM
- Not enough CPU time (allpaths can resume from where it died!)

- Artifacts in the data

Computational requirements

- 64-bit Linux

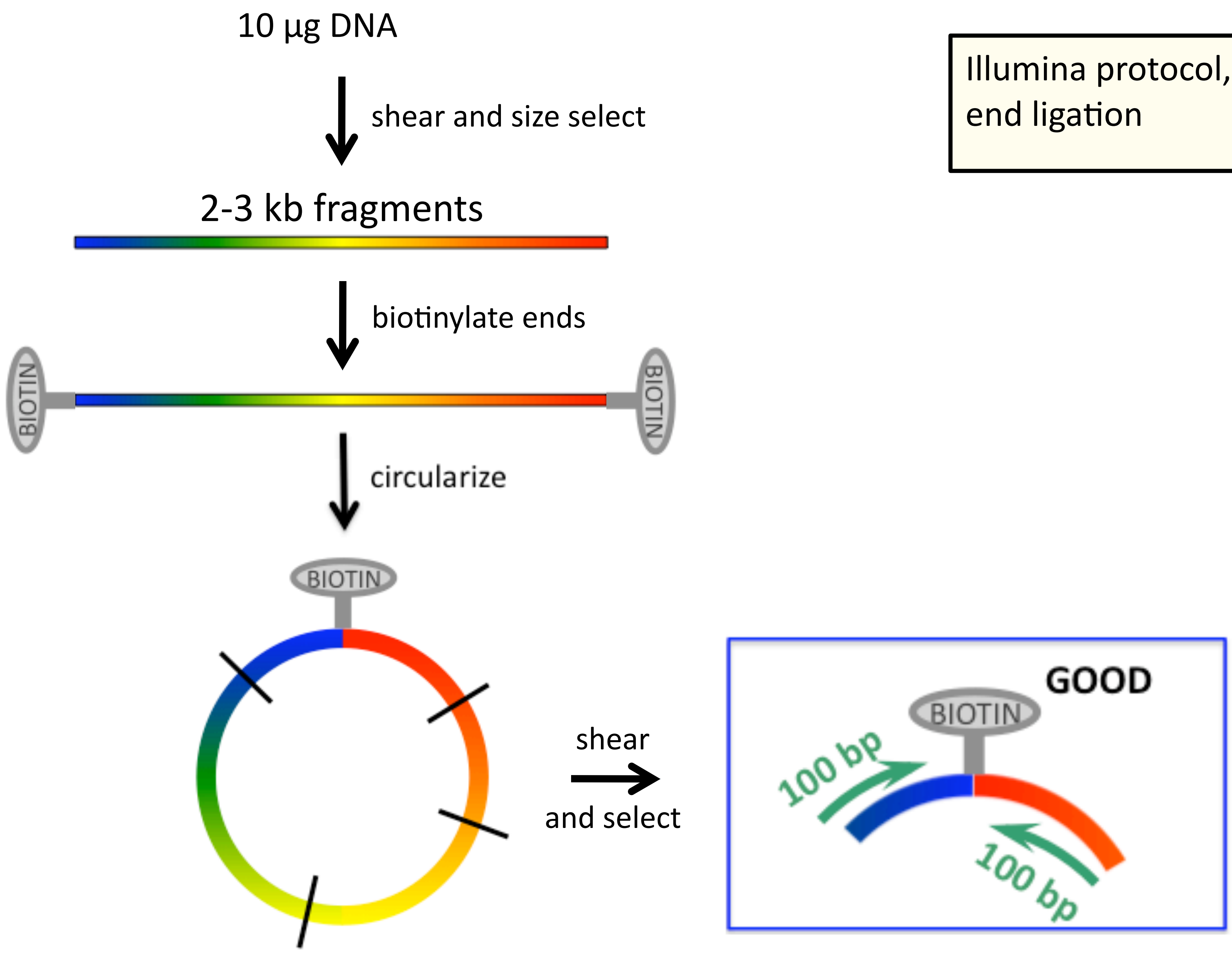
- runs multi-threaded on a single machine

- memory requirements
  - about 160 bytes per genome base, implying
    - need 512 GB for mammal (Dell R315, 48 processors, €18,000)
    - 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)

Short jumping libraries (2-3 kb)

Illumina protocol, blunt-end ligation



Short jumping libraries (2-3 kb)

Problem 1. Many steps → many opportunities for failure.

Example: a reagent might degrade. (This has happened.)

Short jumping libraries (2-3 kb)

Problem 2. Many steps → many DNA losses.

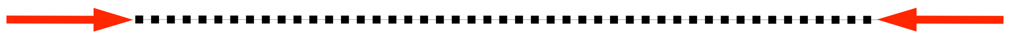
Here are *good* results for a mammalian genome:

Input: 10 μg DNA ⟷ ~3,000,000x physical coverage

Output: (if fully sequenced) ~3,000x physical coverage

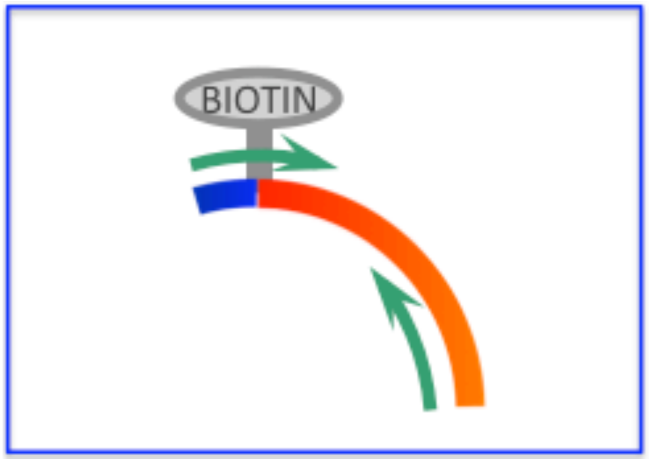
Loss: 99.9% (not including DNA between reads)

Small genomes are much easier!



Short jumping libraries (2-3 kb)

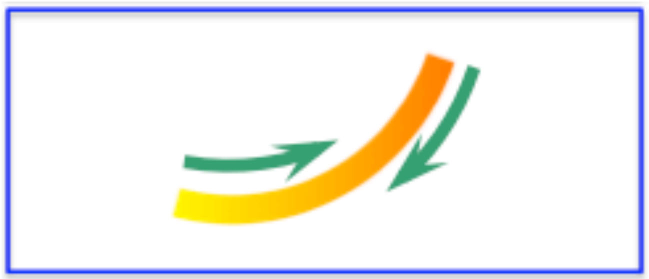
Problem 3. Read passes through circularization junction. This reduces the effective read length (and complicates algorithm).



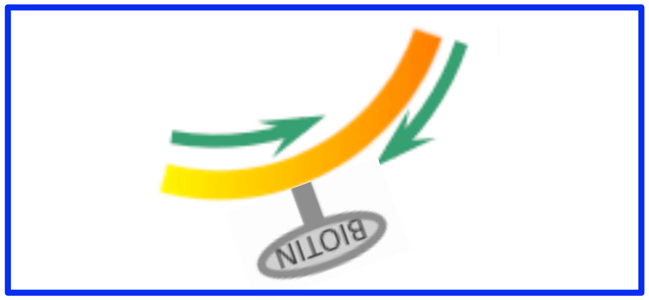
What might be done to reduce incidence of this:
shear circles to larger size and select larger fragments

Short jumping libraries (2-3 kb)

Problem 4. 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



Long jumping libraries (~6 kb)

Method 1. Instead of shearing circles, using EcoP15I restriction enzyme.

<u>Pros</u>
- demonstrated to work
- no artifacts

<u>Cons</u>
- read length = 26 bases

Method 2. Use Illumina blunt-end ligation protocol, but shear and size select larger fragments.

<u>Pros</u>
- long reads

<u>Cons</u>
- yield may be very low (probably not problem for small genomes)