A Roadmap to De-novo Assembly of Animal Genomes

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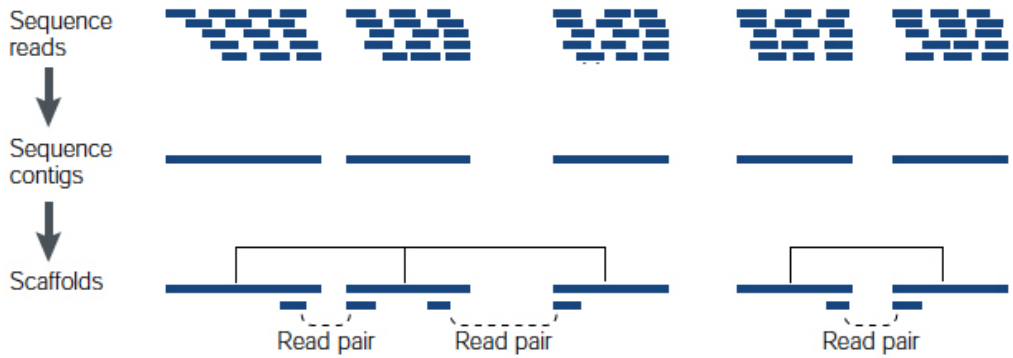
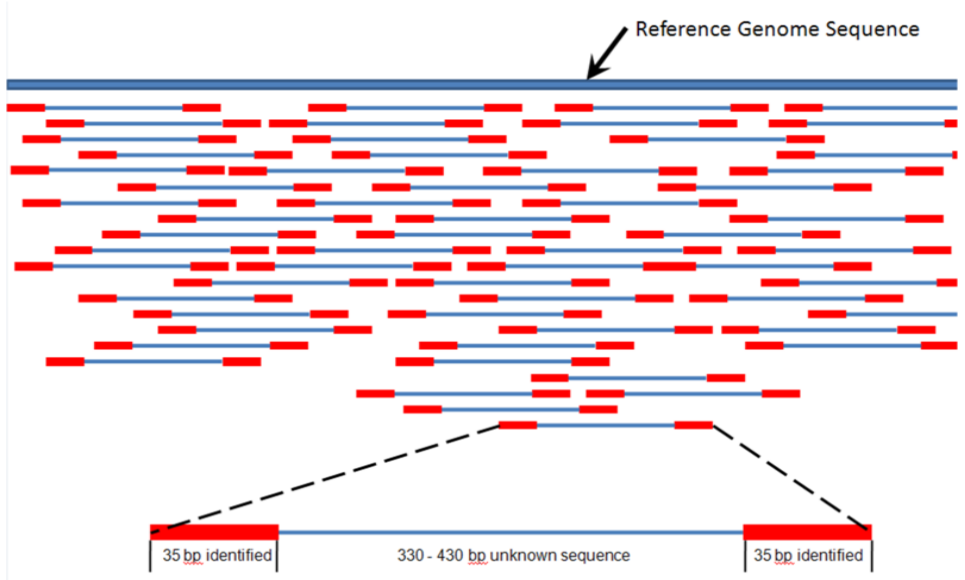
March 18th, 2015

De-novo Assembly

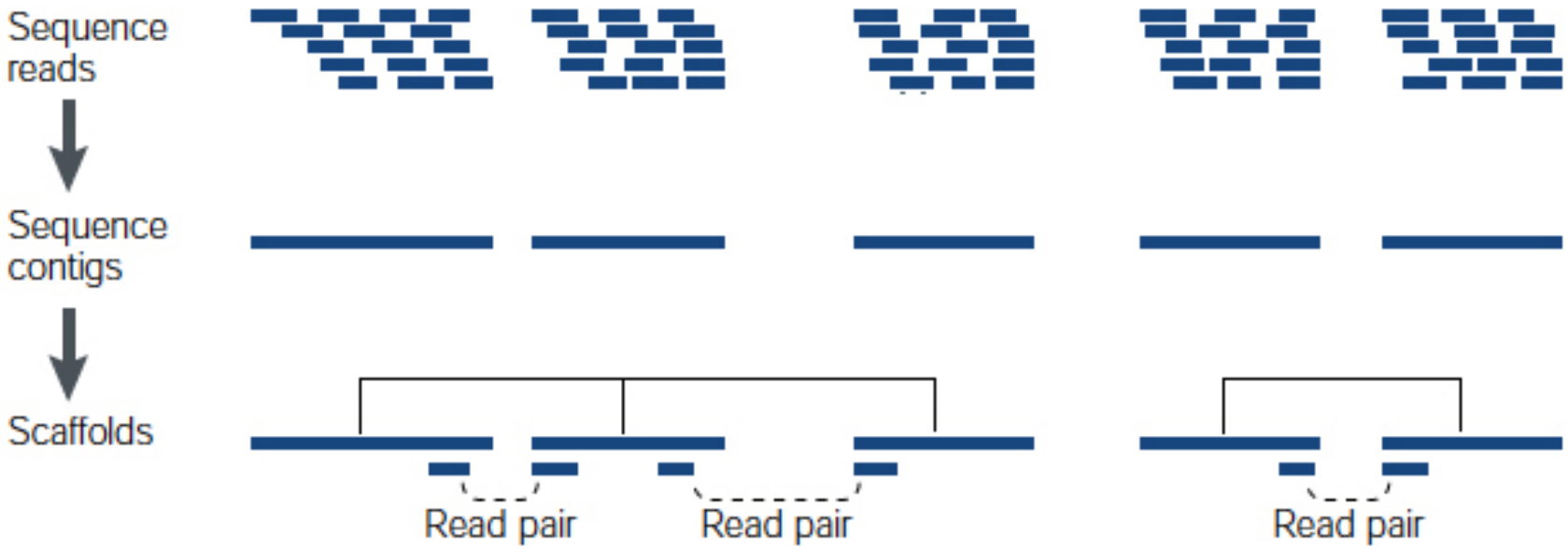


Changed after Green 2001

Reference-based Mapping

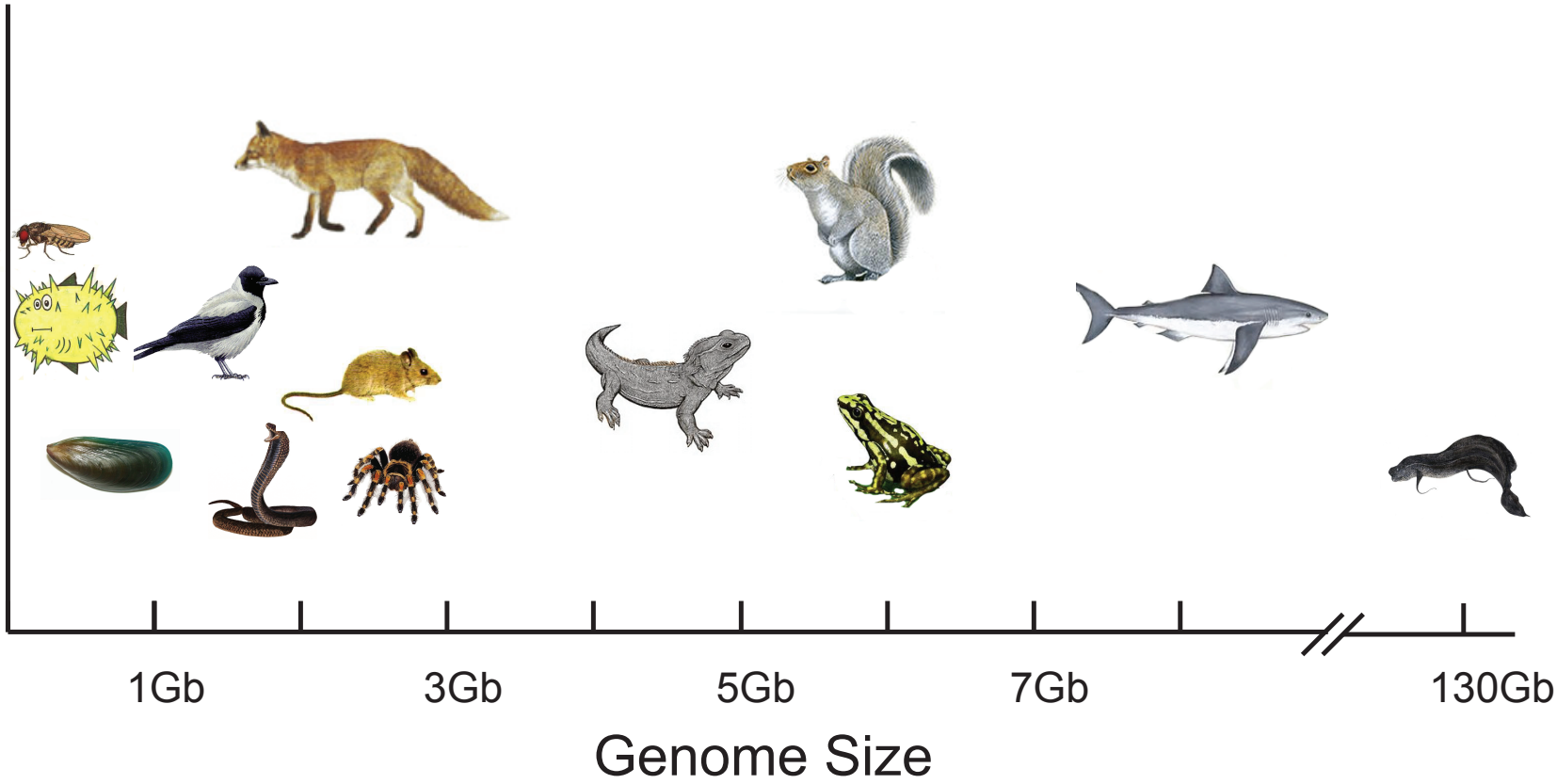


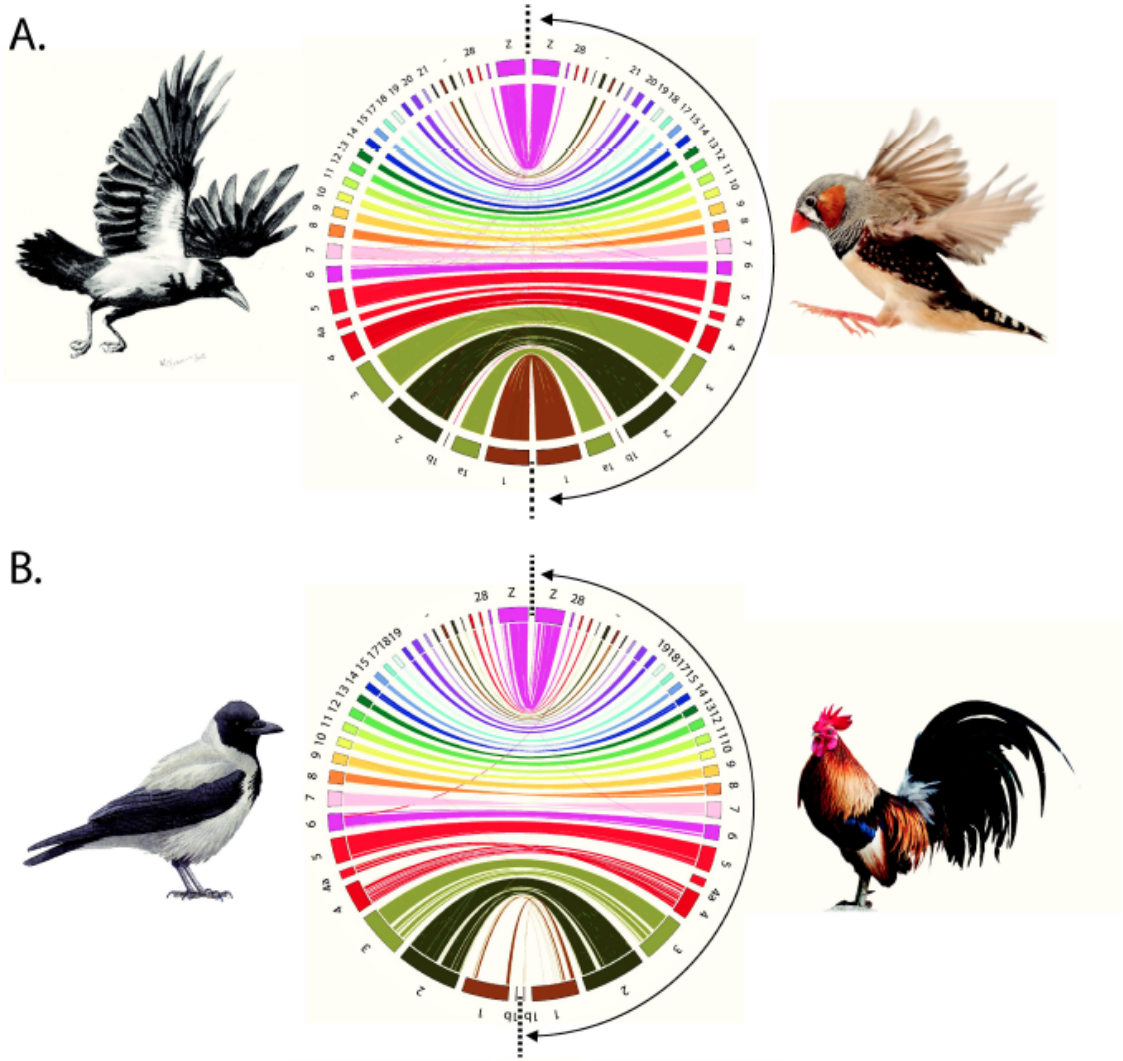
Wikipedia



Changed after Green 2001

- Expected Genome Size
- Expected Repeat Content
- Expected Heterozygosity
- Diploid or Polyploid?


1Gb
3Gb
5Gb
7Gb
130Gb
Genome Size

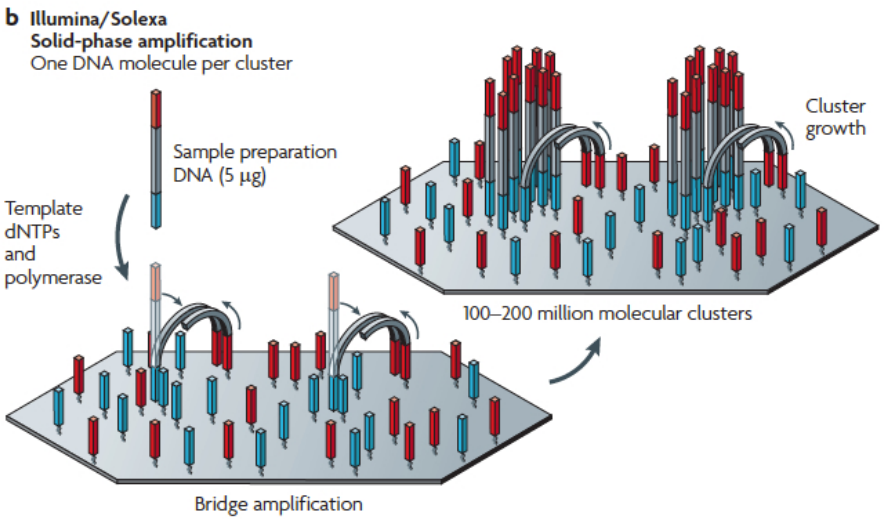


Poelstra et al. 2014

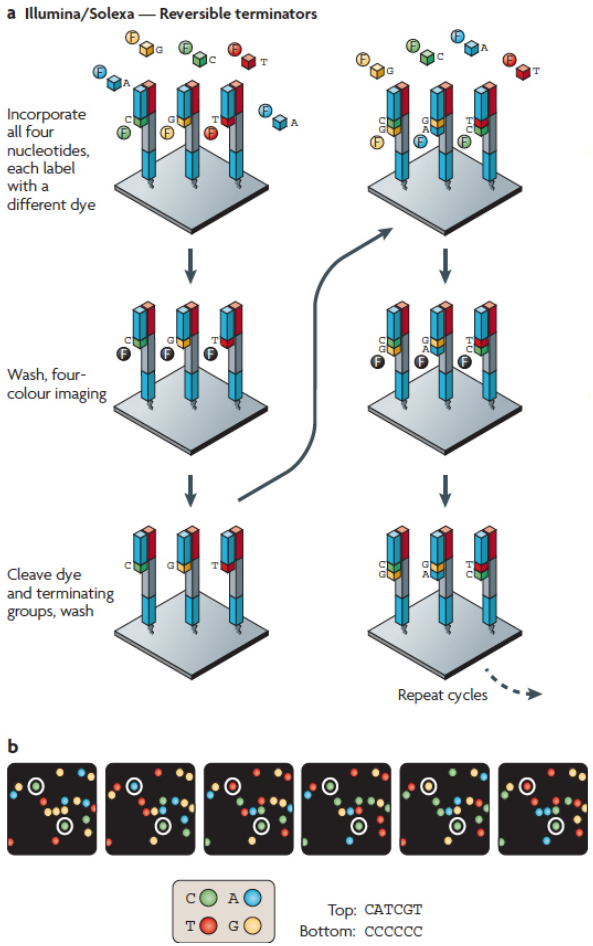
- Expected Genome Size
- Expected Repeat Content
- Expected Heterozygosity
- Diploid or Polyploid?

- First Generation Sequencing
  - Sanger Sequencing
- Second Generation Sequencing (*PCR Needed*)
  - Illumina: MiSeq & HiSeq
  - Roche: 454
  - Life Science: IONtorrent & IONproton
  - ABI: SOLiD
- Third Generation Sequencing (*Single Molecule Sequencing*)
  - Helicos Biosciences: Heliscope
  - Pacific Biosciences: PacBio RS II
  - Oxford Nanopore: MinION & GridION

Illumina Sequencing

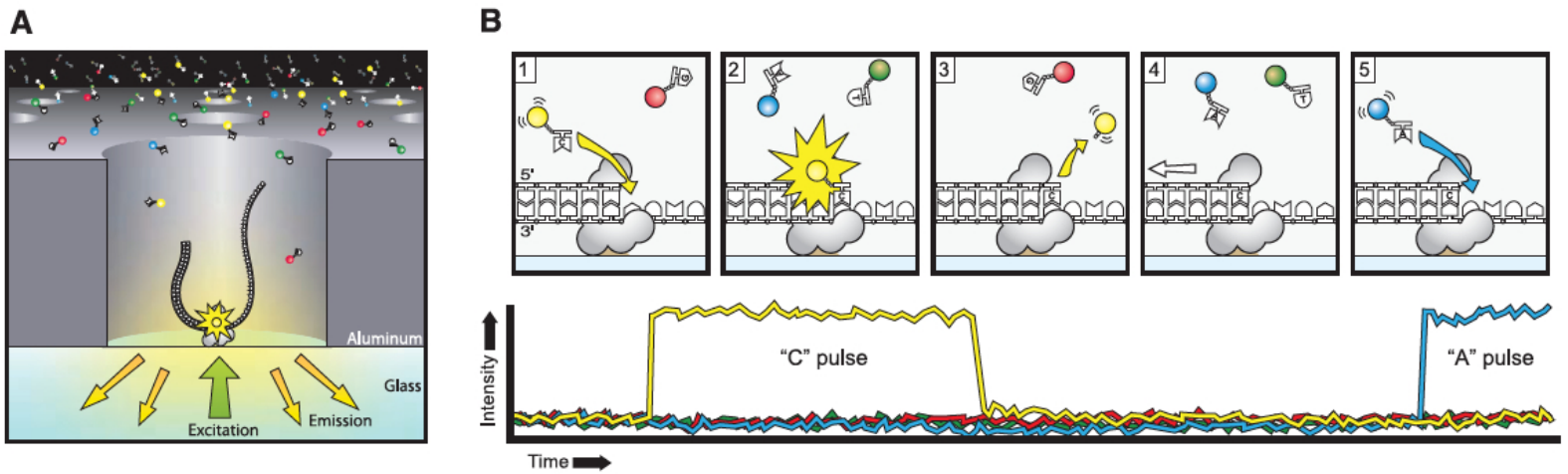


Metzker 2010



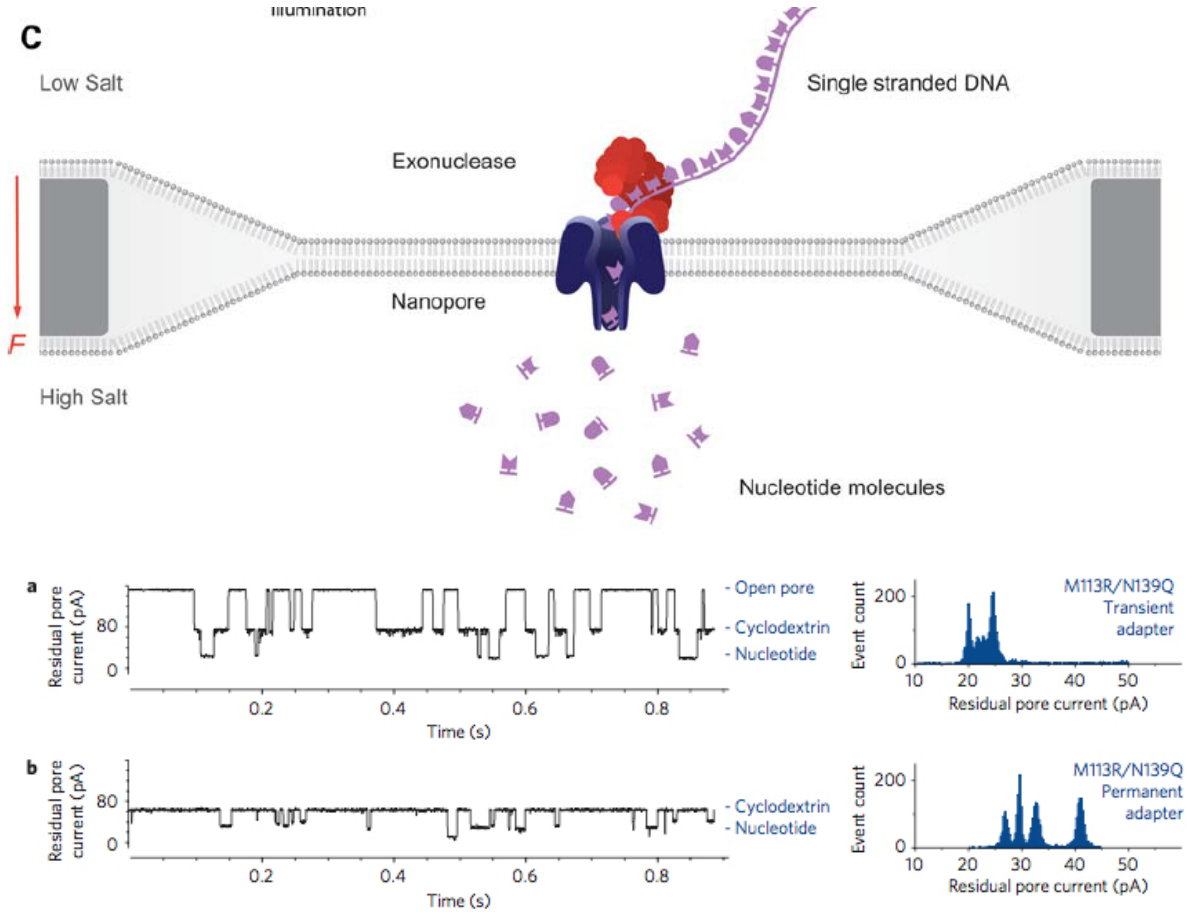
Metzker 2010

Pacific Biosciences



Eid et al. 2009

Oxford Nanopore



Schadt et al. 2010; Clarke et al. 2009

- First Generation Sequencing
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  - Illumina: MiSeq & HiSeq
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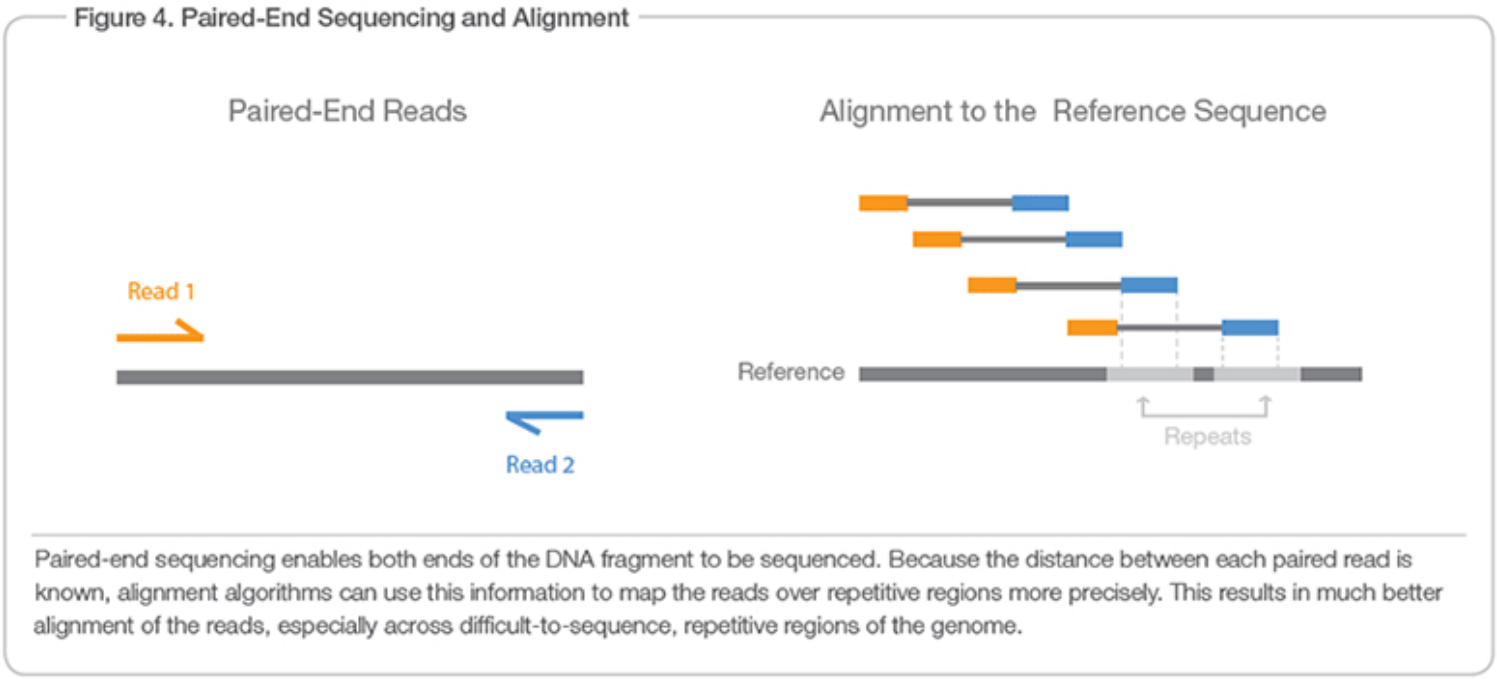
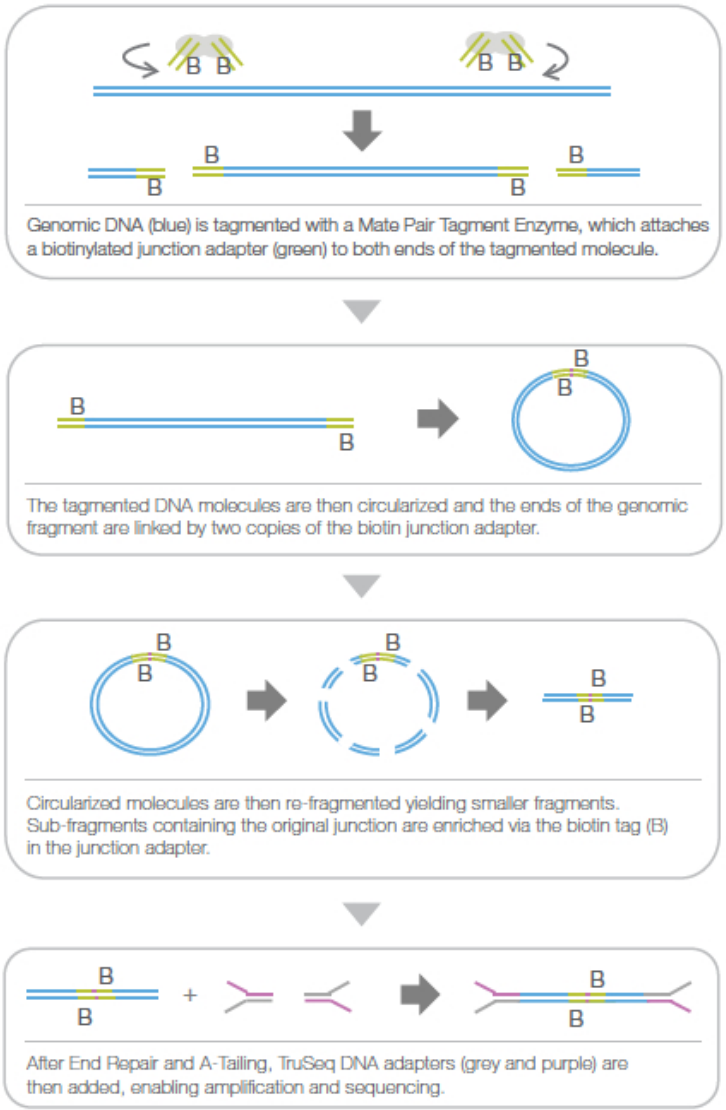
Illumina Paired-end Sequencing Libraries



Illumina Homepage

Illumina Mate Pair Sequencing Libraries



Allpaths-LG Recipe

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**

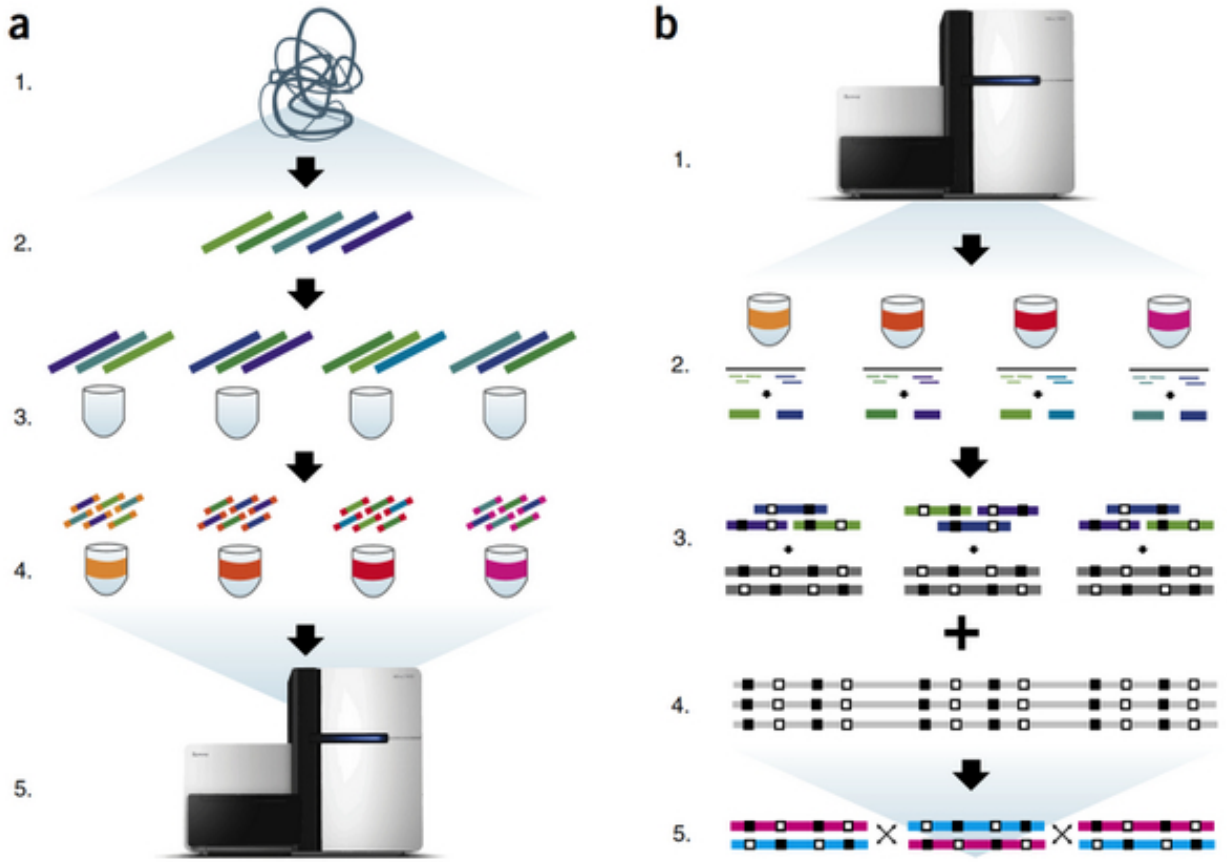
Custom Recipe for 1.3Gb Bird Genome (<15% Repeats)

- 180bp PE - 1 lane HiSeq (30x coverage)
- 650bp PE - 1 lane HiSeq (30x coverage)
- 5kb/8kb MP - 1 lane HiSeq (20x coverage)

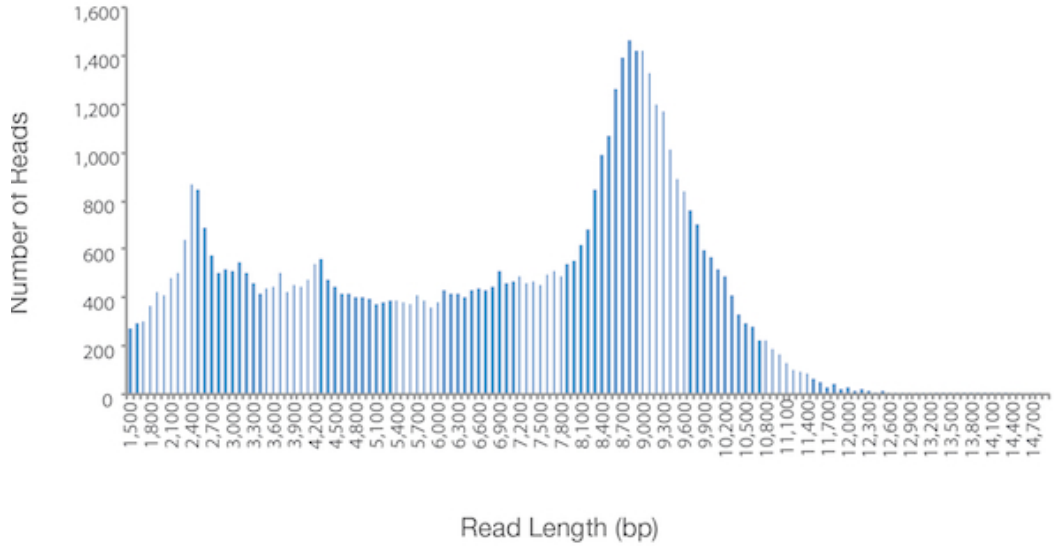
Custom Recipe for 2.5Gb Mammal Genome (<50% Repeats)

- 180bp PE - 4 lanes HiSeq (50x coverage)
- 3kb MP - 4 lanes HiSeq (40x coverage)
- 8kb/15kb MP - 1 lane (10x coverage)

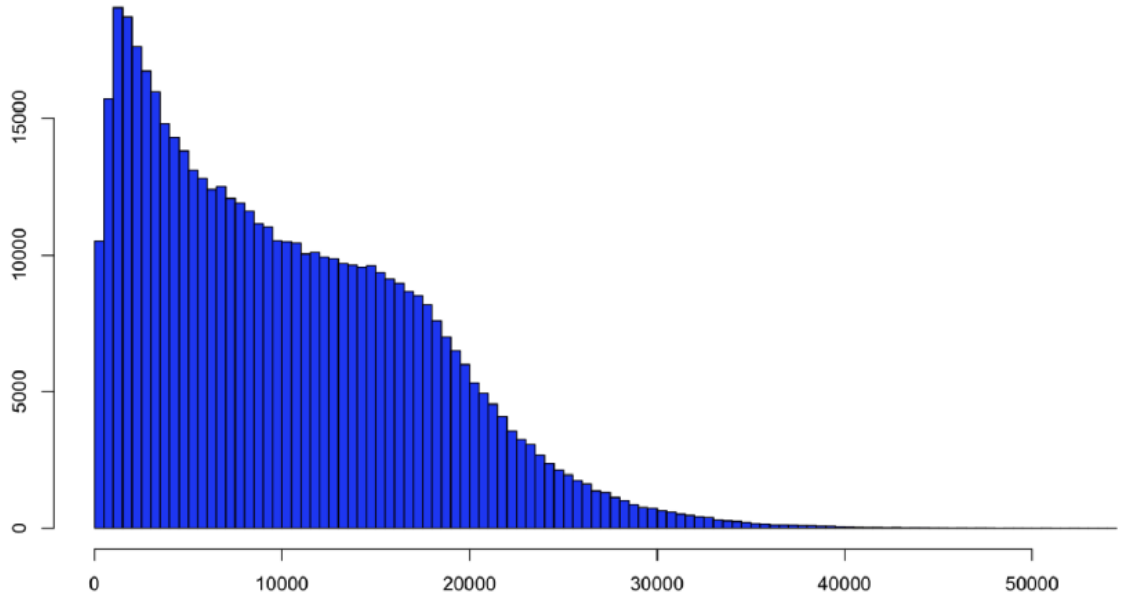
TruSeq Synthetic Long-Read Libraries (Moleculo)

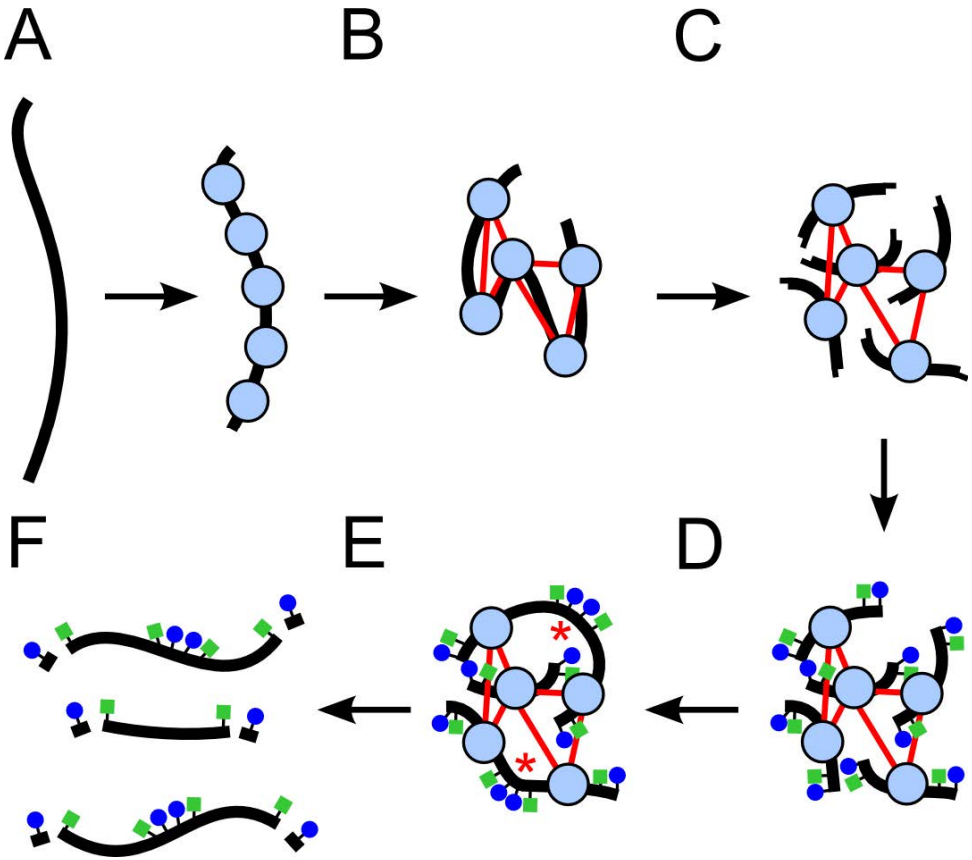


Moleculo



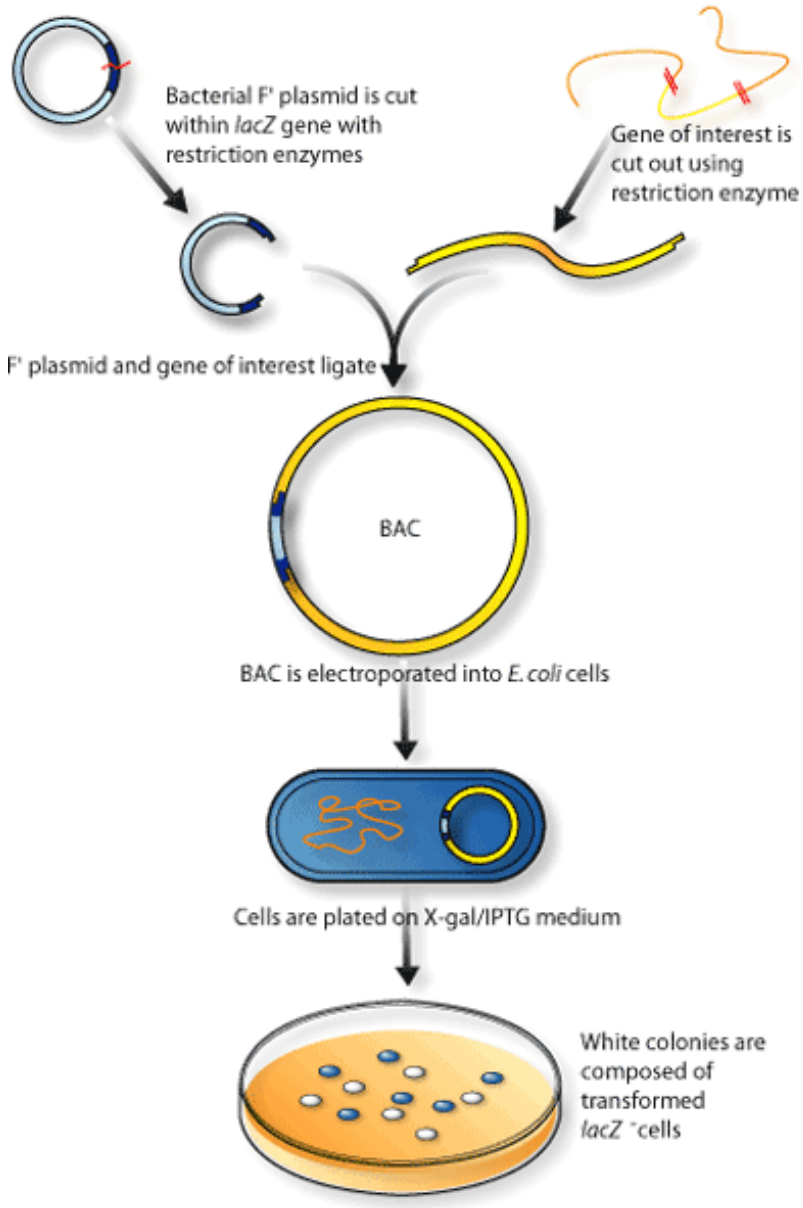
PacBio

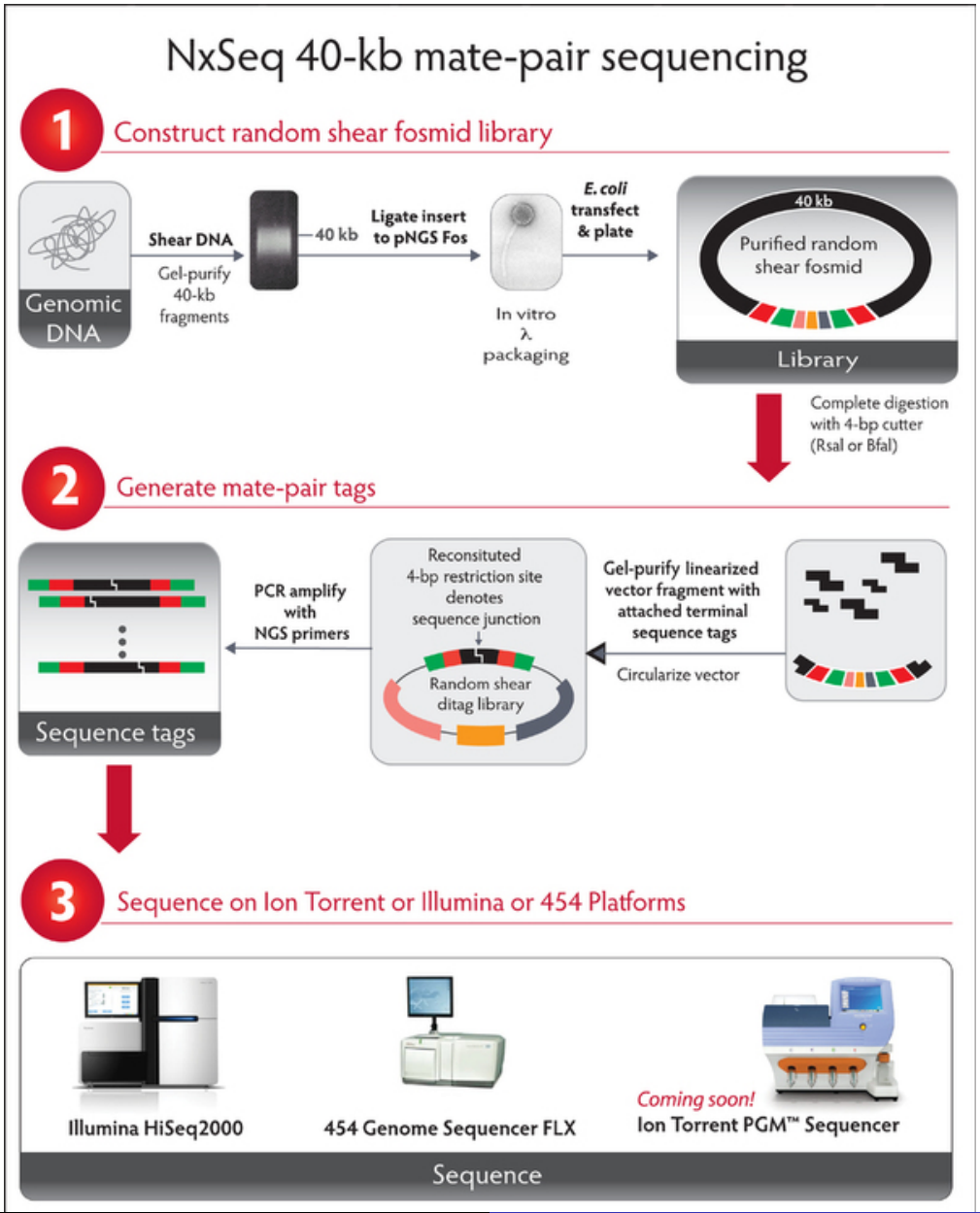




Putnam et al. 2015 (ArXiv)

- Fosmid Vector
  - Bacterial F-plasmid
  - <40kb Insert Size
- Bacterial Artificial Chromosome (BAC)
  - <300kb Insert size




NxSeq 40-kb mate-pair sequencing
1
Construct random shear fosmid library
Genomic DNA
Shear DNA
Gel-purify 40-kb fragments
40 kb
Ligate insert to pNGS Fos
In vitro λ packaging
E. coli transfect & plate
40 kb
Purified random shear fosmid
Library
Complete digestion with 4-bp cutter (RsaI or BfaI)
2
Generate mate-pair tags
Sequence tags
PCR amplify with NGS primers
Reconstituted 4-bp restriction site denotes sequence junction
Random shear ditag library
Gel-purify linearized vector fragment with attached terminal sequence tags
Circularize vector
3
Sequence on Ion Torrent or Illumina or 454 Platforms
Illumina HiSeq2000
454 Genome Sequencer FLX
Coming soon!
Ion Torrent PGM™ Sequencer
Sequence

Read Quality Assessment

- Base Quality

  Phred Score: Q_{Phred} = -10 $\log_{10}$ P(error)

  e.g. a Phred score of 20 translates to a 1% error rate

- GC Content
- Sequence Duplication Levels
- Kmer Content
- ...

Software Tools

- FastQC
- Preqc

Fastq Format

```
@HISEQ:119:C42B3ACXX:7:1101:2009:2249 2:N:0:CGACCTG
TCTTGGGGACAGGGAATTTCATTTCCAAATGAAAATCCTCAAAGAACGCCTTTTATTTTACAGGAGGCTGTATATCTTAGCCAAAGTGGTAGATCGGAAGA
+
BB<BFFFBFB<BFF7BBF<BF<FBB<FFF<FFBFF<BFFFFBF'BF<7BB<BFBFB<BFFFFF<FFFFFF<BBFB<BB<BBBBBBB7<B<BB<77<BBB77
```

Quality Score Encoding

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 .........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
 .............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII...........................
 ..............................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.........................
 ..LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                          |    |        |                              |                     |
33                          59   64       73                            104                   126
 0........................26...31.......40
                           -5....0........9.............................40
                                0........9.............................40
                                   3.....9.............................40
 0.2......................26...31........41

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

Read Quality Assessment

- Base Quality

  Phred Score: Q_{Phred} = -10 $\log_{10}$ P(error)

  e.g. a Phred score of 20 translates to a 1% error rate

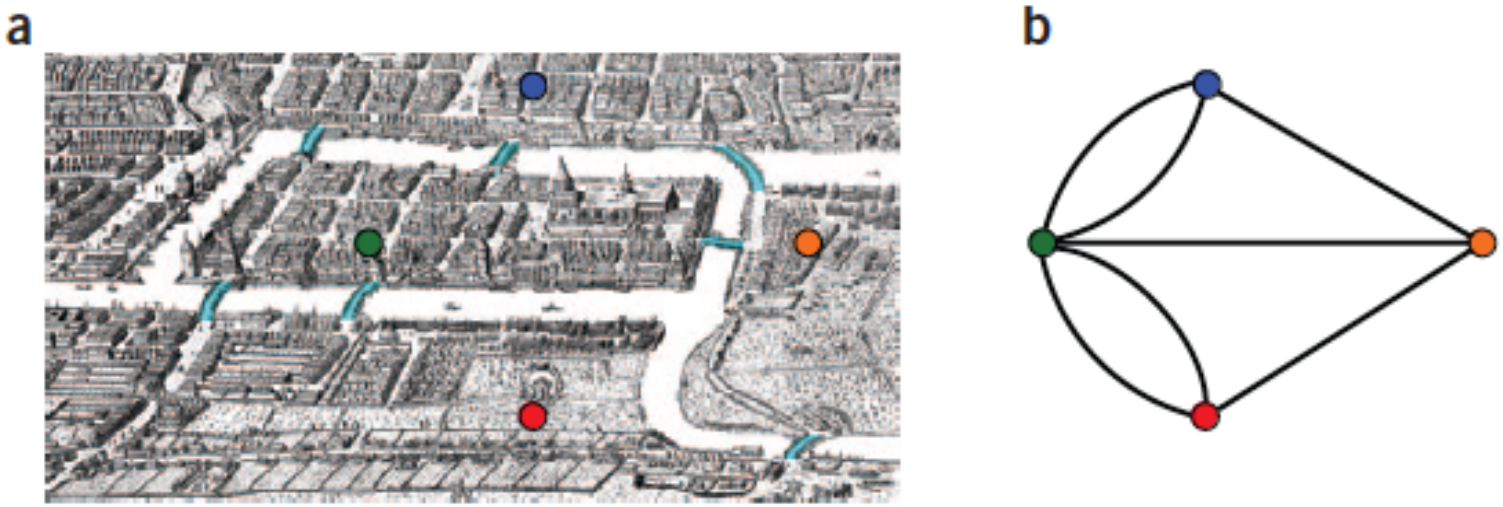
- GC Content
- Sequence Duplication Levels
- Kmer Content
- ...

Software Tools

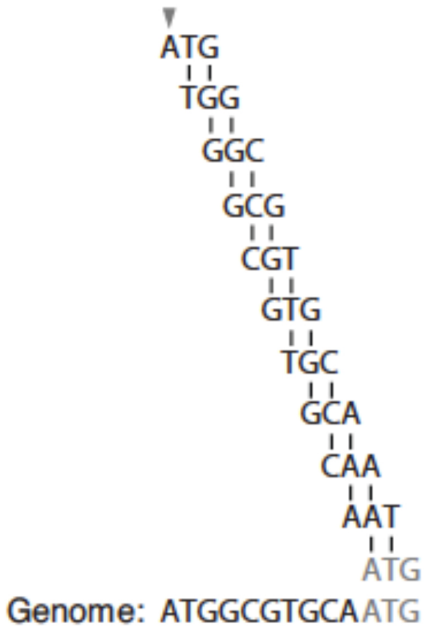
- FastQC
- Preqc

- Adapter and Low Quality Base Trimming
  - Skewer (Jiang et al. 2014)
  - AdapterRemoval (Lindgreen 2012)
  - Trimmomatic (Bolger et al. 2014)
- Contamination Filtering
  - Blast
  - Allpaths-LG
    - Removing Low Frequency k-mers
    - Discarding Scaffolds Shorter than 1kb

Bridges of Koenigsberg problem (Graph Theory by Euler)

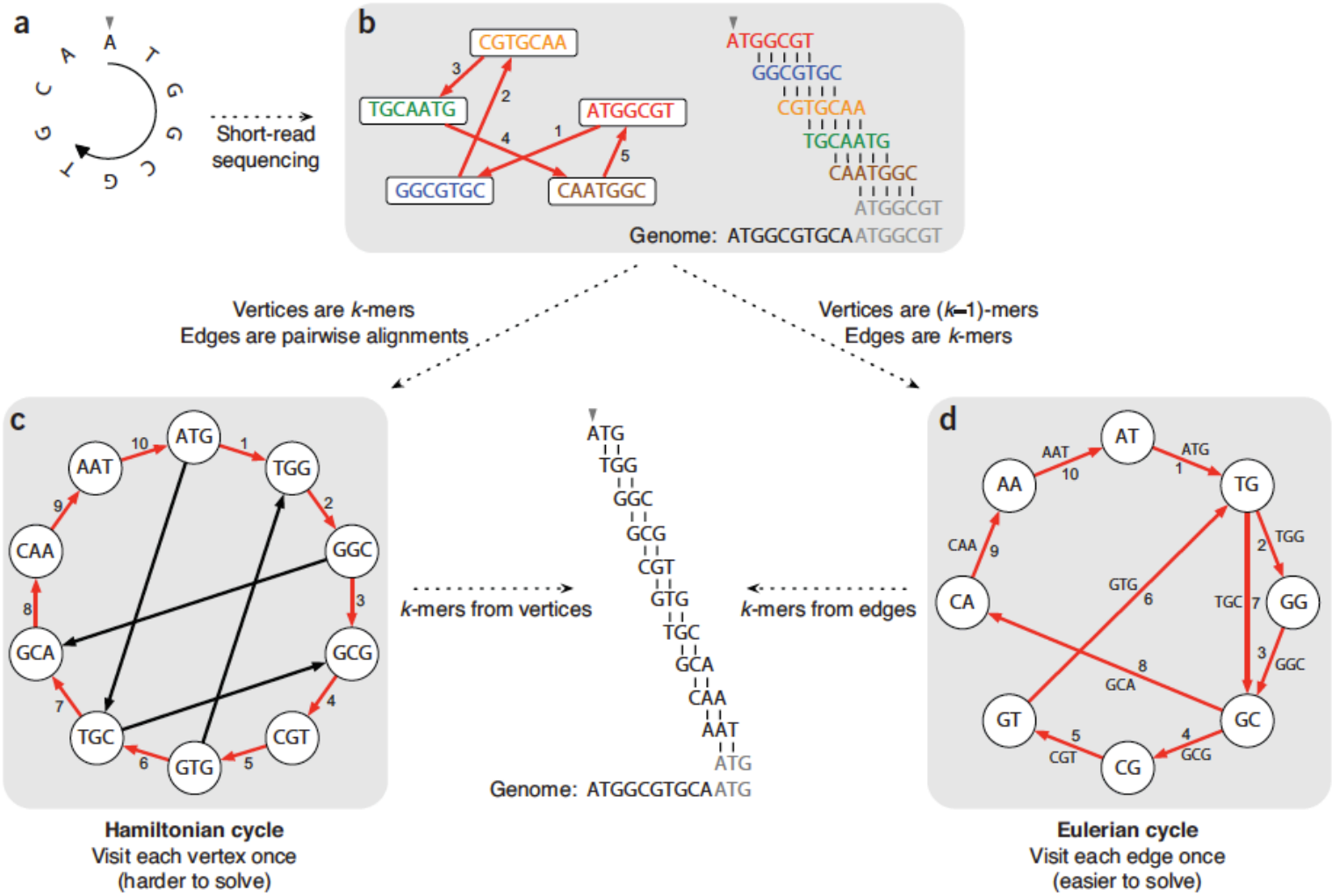


Compeau et al. 2011



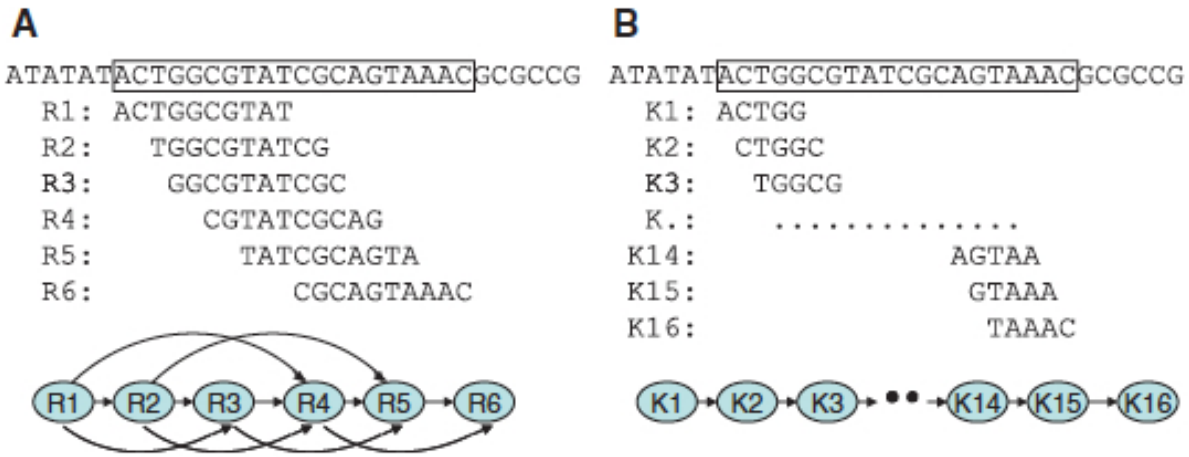
Definition: Kmer

Short, unique element of DNA sequence of length n



Compeau et al. 2011

Overlap-layout-consensus vs. *de Bruijn* graph



Li et al. 2011, Briefings in Functional Genomcis

Overlap-layout-consensus based Tools:

SGA (Simpson and Durbin 2012), Celera assembler (Myers et al. 2002)

de Bruijn graph based Tools:

Allpaths-LG (Gnerre et al. 2011), Abyss (Simpson et al. 2009), SOAPdenovo (Luo et al. 2012)

Short Read Assembler

- Allpaths-LG (Gnerre et al. 2011)
- Abyss (Simpson et al. 2009)
- SOAPdenovo (Luo et al. 2012)
- Platanus (Kajitani et al. 2014)
- SSAKE (Warren et al. 2007)
- SGA (Simpson and Durbin 2012)
- Celera assembler (Myers et al. 2002)
- MaSuRCA (Zimin et al. 2013)

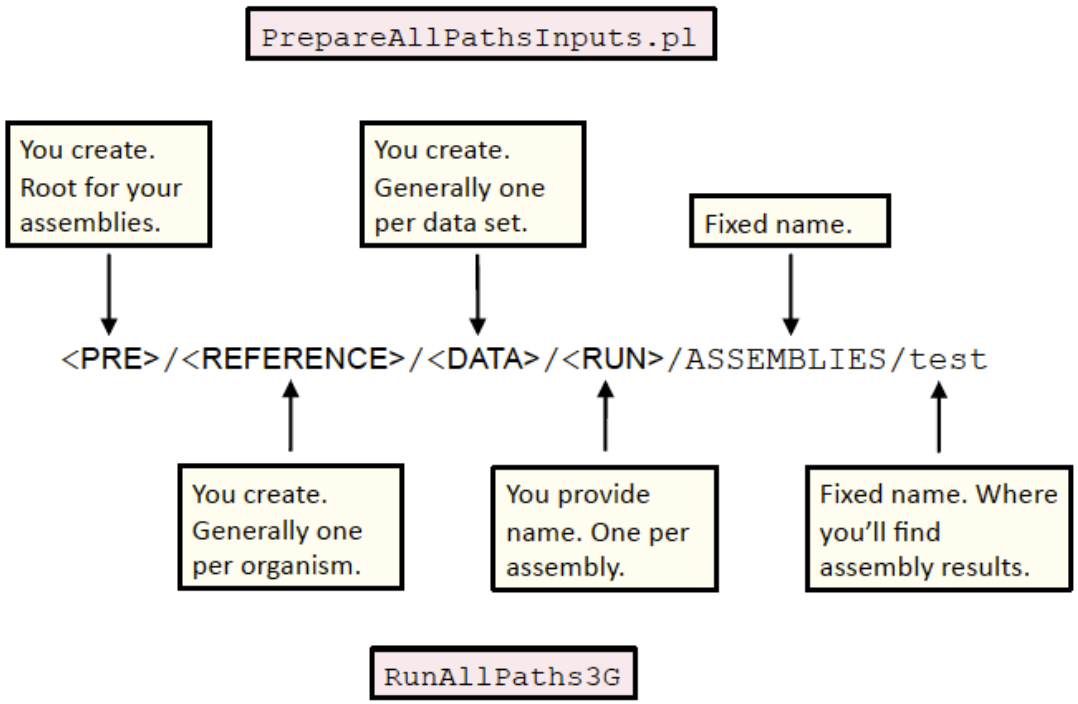
Long Read Assembler

- Allpaths-LG (Gnerre et al. 2011)
- DBG2OLC (Ye et al. 2014)
- PBcR - Celera Assembler (Berlin et al. 2014)

Short or Long Read Scaffolder

- SSPACE (Boetzer et al. 2011)
- PBjelly (English et al. 2012)

- Input Data
  - BAM, FASTQ, FASTA/QUALA
- Input Files
  - in_libs.csv
  - in_groups.csv
  - ploidy.txt



Schatz Presentation

```
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>
```

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

Schatz Presentation

- Output Files
  - final.assembly.fasta
  - final.assembly.efasta
  - final.contigs.fasta
  - final.contigs.efasta
  - final.summary
  - final.rings
  - final.superb
  - assembly_stats.report
  - library_coverage.report
  - assembly.report

http://soap.genomics.org.cn/soapdenovo.html

Config file:

```
max_rd_len=100
[LIB]
avg_ins=450
reverse_seq=0
asm_flags=3
rd_len_cutoff=100
rank=1
pair_num_cutoff=3
map_len=32
q1=/scratch/stefan/bop/lyco_soap/1_140430_BC4682ANXX_P974_101_1.fastq.gz
q2=/scratch/stefan/bop/lyco_soap/1_140430_BC4682ANXX_P974_101_2.fastq.gz
[LIB]
avg_ins=5000
reverse_seq=1
asm_flags=3
rd_len_cutoff=100
rank=2
pair_num_cutoff=5
map_len=35
q1=/scratch/stefan/bop/lyco_soap/3_140723_AC49LVACXX_P1326_101_1.fastq.gz
q2=/scratch/stefan/bop/lyco_soap/3_140723_AC49LVACXX_P1326_101_2.fastq.gz
```

Output Files

- lycocorax_soap.scafSeq
- lycocorax_soap.contig
- lycocorax_soap.err
- lycocorax_soap.scafStatistics

ABySS command

```
abyss-pe k=60 np=20 name=little_eagle_k60 lib='pe1' mp='mp1 mp2'
pe1='D23CCACXX_NZGL00439_Little_Eagle_200bp_L006_R1_001.fastq.gz
D23CCACXX_NZGL00439_Little_Eagle_200bp_L006_R2_001.fastq.gz'
mp1='C1WMWACXX_NZGL00439_Little_Eagle_MP5kb_L007_R1_001.fastq.gz
C1WMWACXX_NZGL00439_Little_Eagle_MP5kb_L007_R2_001.fastq.gz'
mp2='HOMTRADXX_NZGL00439_Little_Eagle_MP5kb_L001_R1_001.fastq.gz
HOMTRADXX_NZGL00439_Little_Eagle_MP5kb_L001_R2_001.fastq.gz'
```

Output Files

- little_eagle-unitigs.fa
- little_eagle-contigs.fa
- little_eagle-scaffolds.fa

Get Assembly Stats:

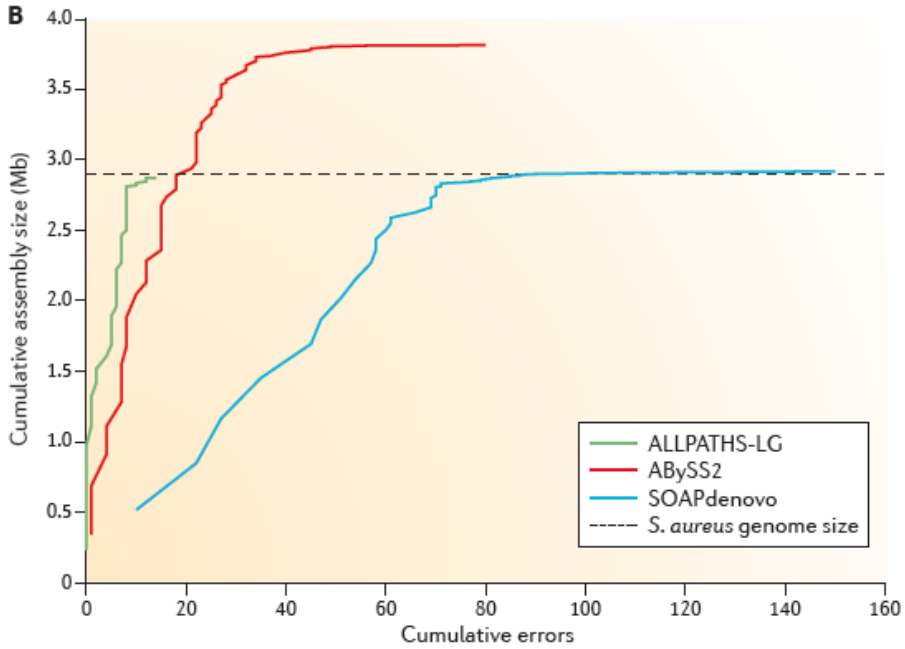
abyss-fac little_eagle-scaffolds.fa

n	n:500	n:N50	min	N80	N50	N20	max	sum	
577130	20211	526	500	167980	573013	1234088	7692772	1.13e9	little_eagle-scaffolds.fa

- By annotation: CEGMA (Parra et al. 2007)
- Using RNA transcripts: Baa.pl (Ryan 2013/4, Arxiv)
- *De novo* likelihood-based measures (LAP; Ghodsi et al. 2013)
- Feature Response Curve (FRC; Vezzi et al. 2012)
- Assembly Statistics

Definition: **N50** contig length also called **L50**

A contig N50 is calculated by first ordering every contig by length from longest to shortest. Next, starting from the longest contig, the lengths of each contig are summed, until this running sum equals one-half of the total length of all contigs in the assembly. The contig N50 of the assembly is the length of the shortest contig in this list. (Yandel and Ence 2012)



Features:

- Mate-pair Orientations and Separations
- Repeat Content by k-mer Analysis
- Depth-of-coverage
- Correlated Polymorphism in the Read Alignments
- Read Alignment Breakpoints

- By annotation: CEGMA (Parra et al. 2007)
- Using RNA transcripts: Baa.pl (Ryan 2013/4, Arxiv)
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Gap Filling

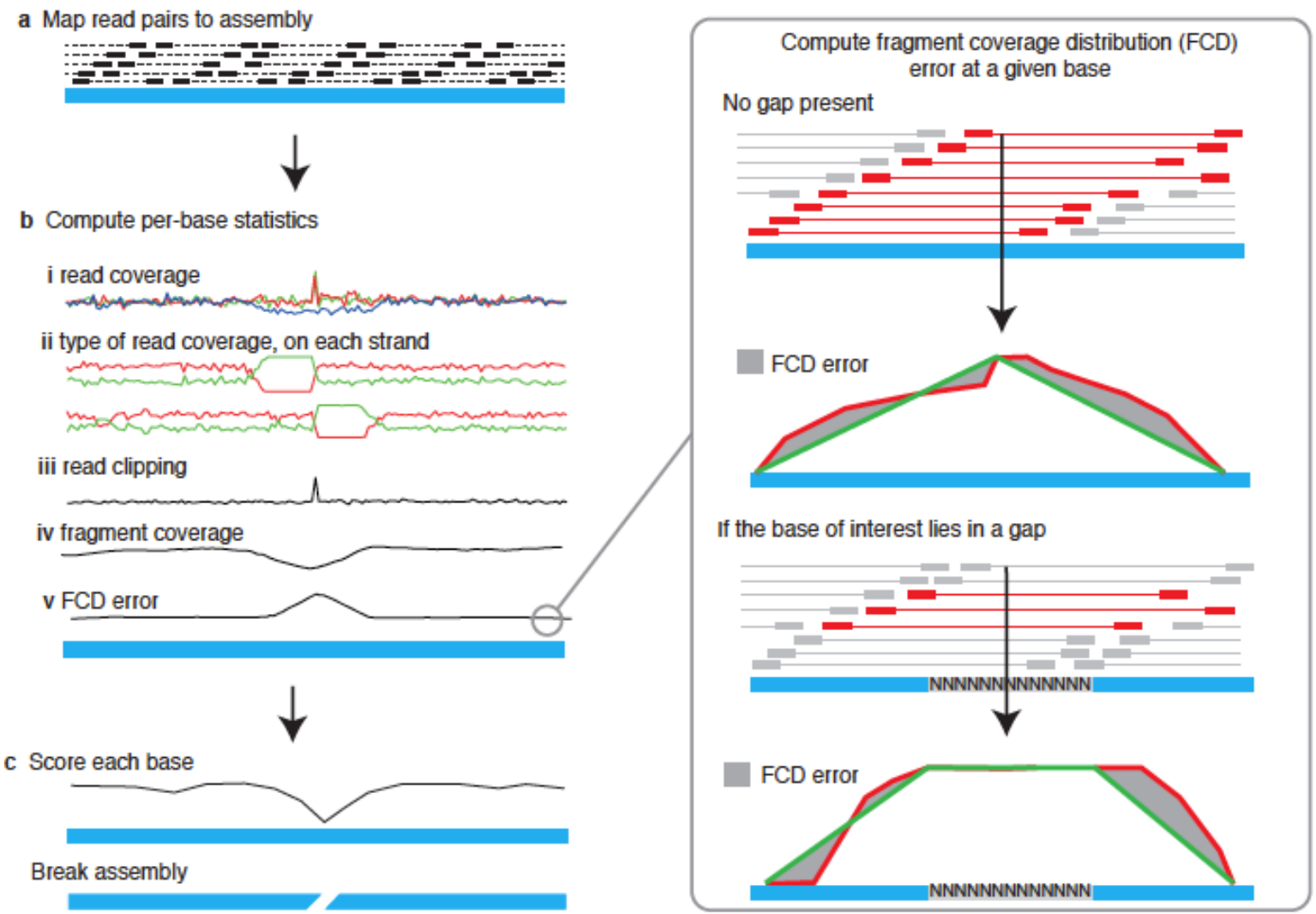
- GapCloser (Luo et al. 2012)
- GapFiller (Nadalin et al. 2011)

Resolving Mis-Assemblies

- REAPR (Hunt et al. 2013)
- NxRepair (Murphy et al. 2014)

Genome Merging

- Metassembler (Wences and Schatz 2015, Arxiv)
- GAM-NGS (Vicedomini et al. 2013)


a Map read pairs to assembly
b Compute per-base statistics
i read coverage
ii type of read coverage, on each strand
iii read clipping
iv fragment coverage
v FCD error
c Score each base
Break assembly
Compute fragment coverage distribution (FCD) error at a given base
No gap present
FCD error
If the base of interest lies in a gap
NNNNNNNNNNNNN
FCD error
NNNNNNNNNNNNN

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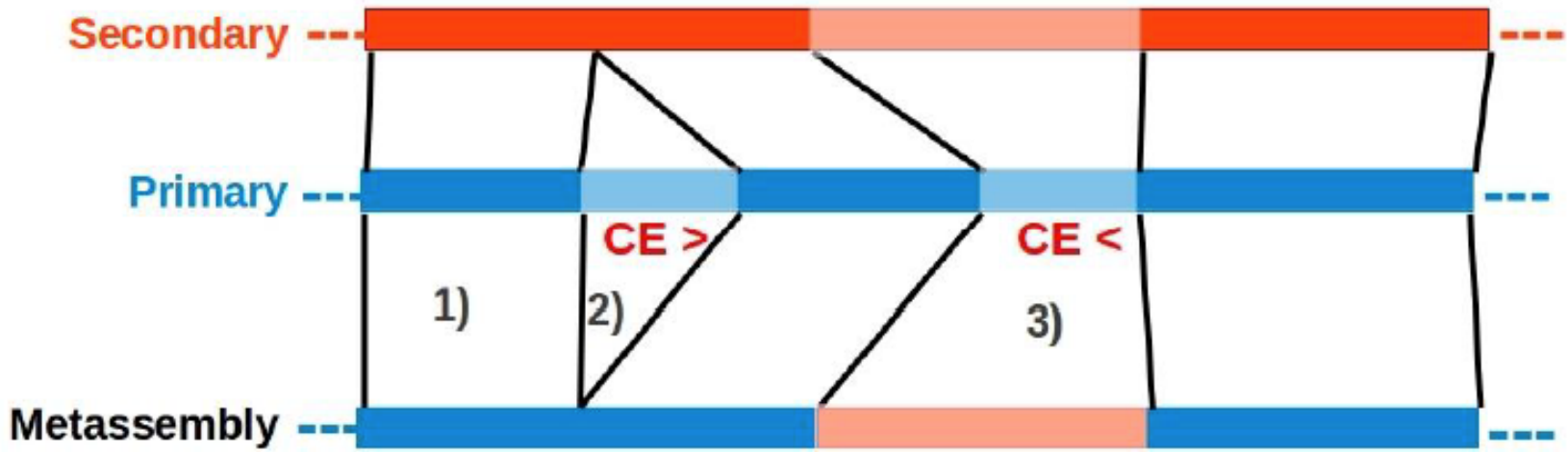

(Wences and Schatz 2015, Arxiv)

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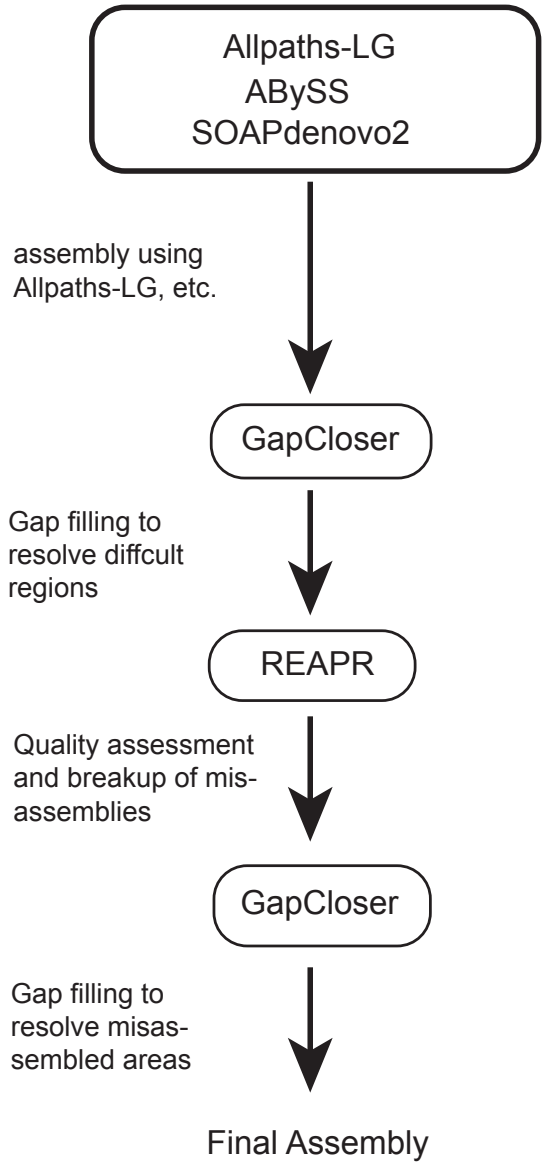
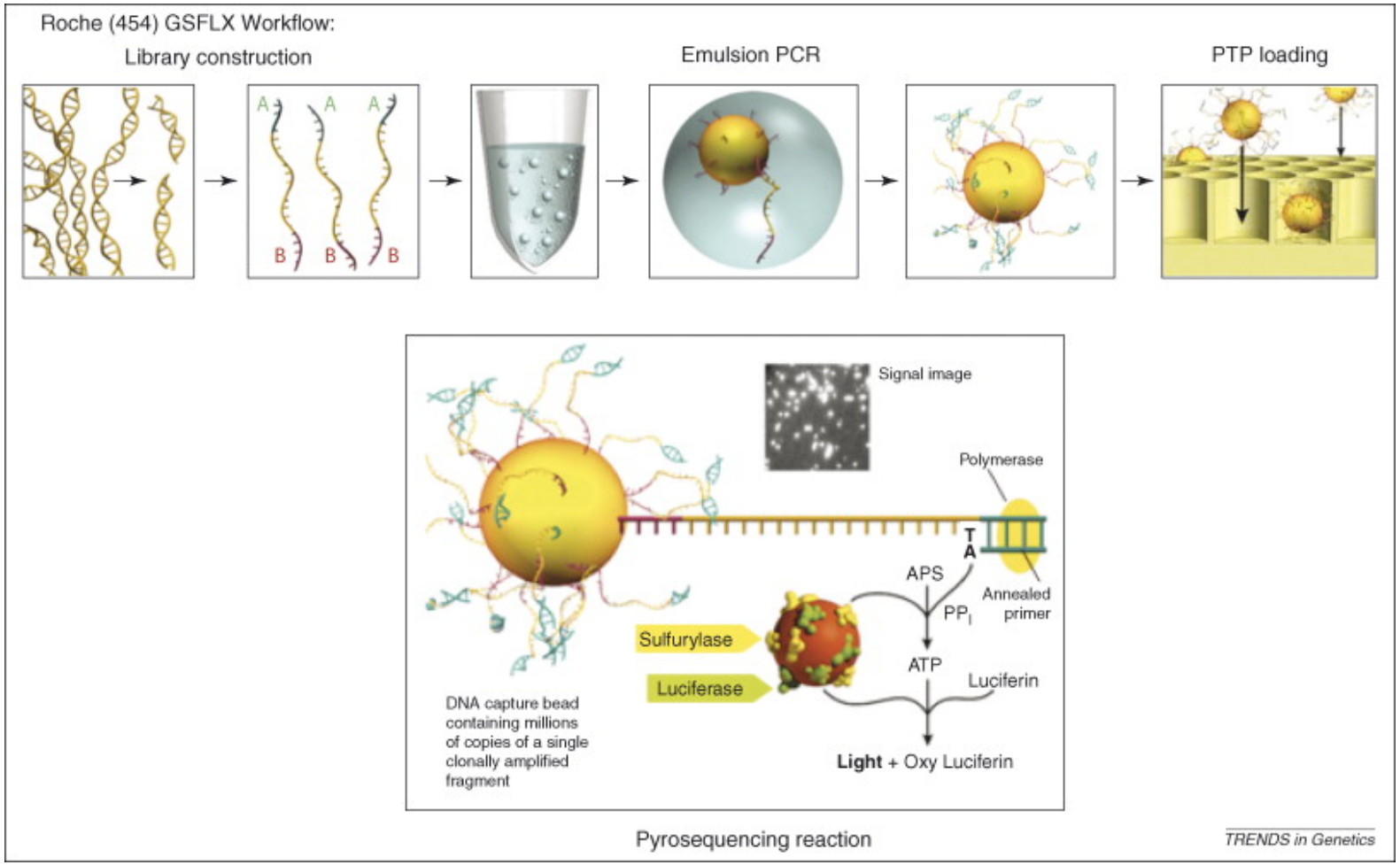

Allpaths-LG
ABySS
SOAPdenovo2
assembly using Allpaths-LG, etc.
GapCloser
Gap filling to resolve diffcult regions
REAPR
Quality assessment and breakup of mis-assemblies
GapCloser
Gap filling to resolve misas-sembled areas
Final Assembly

What is a good assembly and when is it finished?

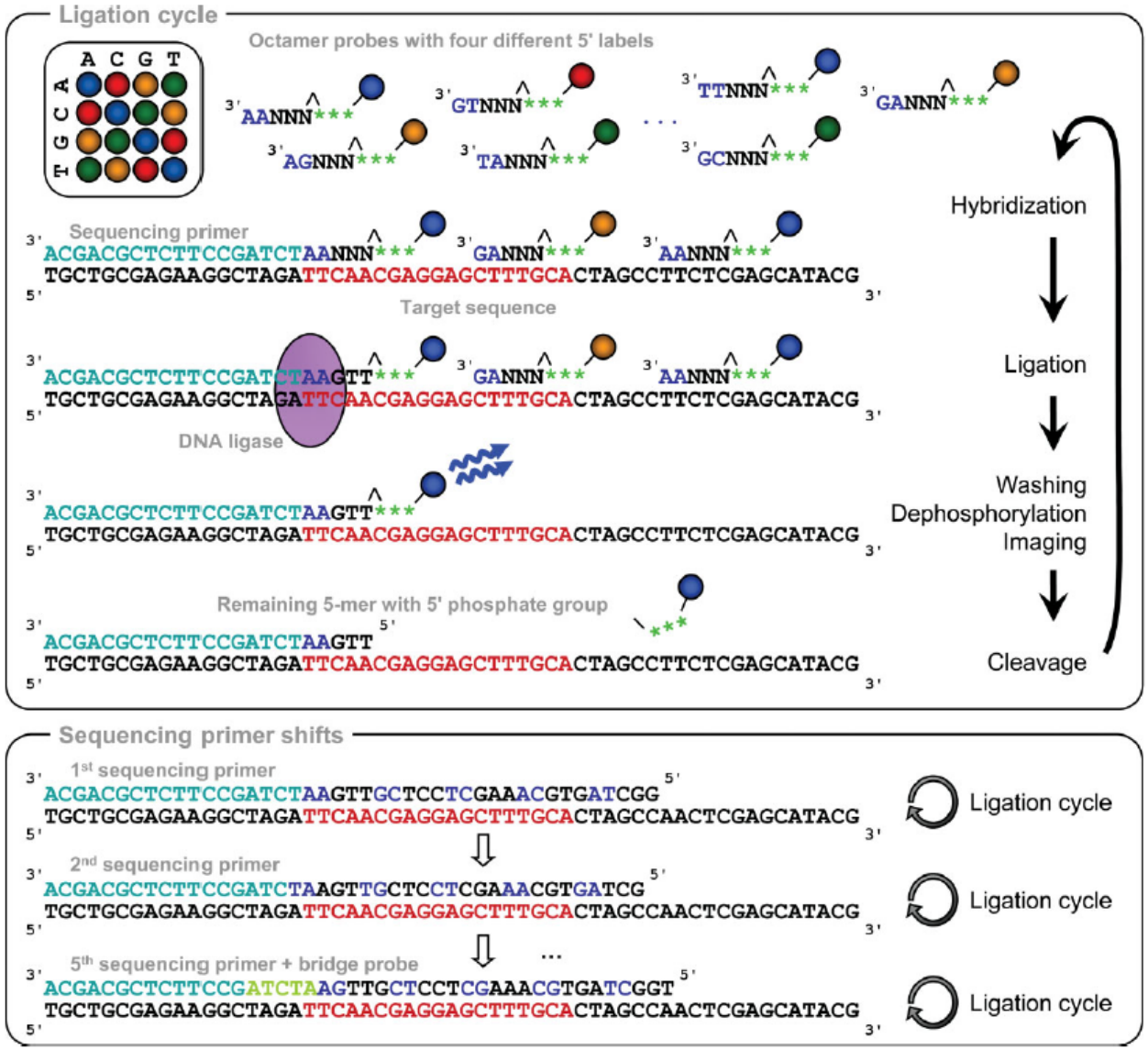
- No Finished Assemblies
  - >100 Euchromatic Gaps in Human Genome
  - *Drosophila melongaster* release 6.1 (More Centric Heterochromatic Sequences Added)
- Contig L50 Close to or Bigger than Average Gene Size
- Number of Scaffolds Should be Close to Number of Chromosomes

- Downstream Processing
  - Repeat Annotation
  - Gene Annotation
  - Mapping to get Diploid Genome

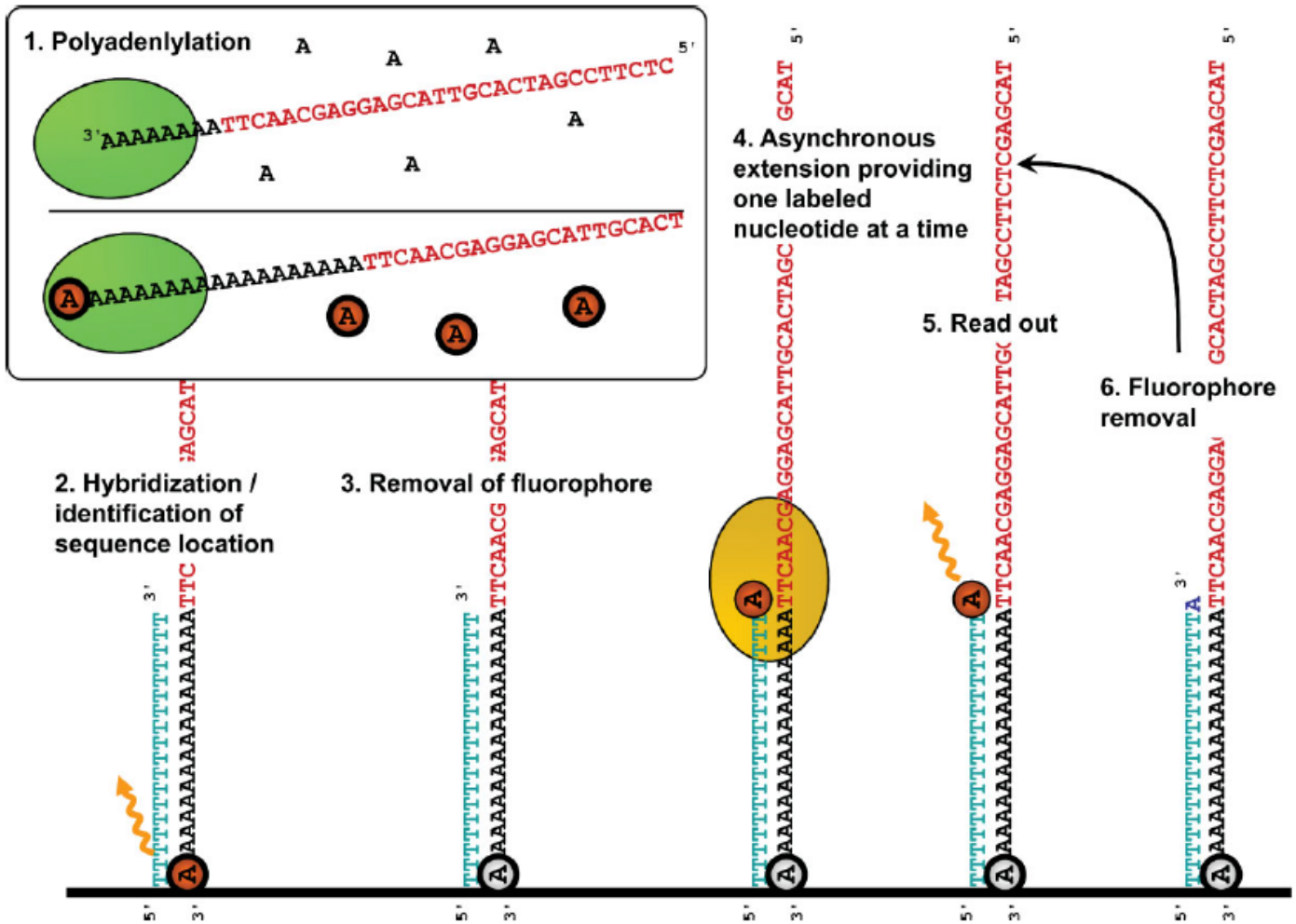
Roche 454


Roche (454) GSFLX Workflow:
Library construction
Emulsion PCR
PTP loading
A
B
Signal image
Polymerase
T
A
APS
PPi
Annealed primer
Sulfurylase
Luciferase
ATP
Luciferin
DNA capture bead containing millions of copies of a single clonally amplified fragment
Light + Oxy Luciferin
Pyrosequencing reaction
TRENDS in Genetics

ABI SOLiD


Ligation cycle
Octamer probes with four different 5' labels
A C G T
Hybridization
Sequencing primer
Target sequence
Ligation
DNA ligase
Washing
Dephosphorylation
Imaging
Remaining 5-mer with 5' phosphate group
Cleavage
Sequencing primer shifts
1st sequencing primer
Ligation cycle
2nd sequencing primer
Ligation cycle
5th sequencing primer + bridge probe
Ligation cycle

Helicos



Kircher and Kelso 2010