

Aligners

J Fass | 21 June 2017

Definitions

Assembly:

I've found the shredded remains of an important document; put it back together!

Definitions

Alignment:

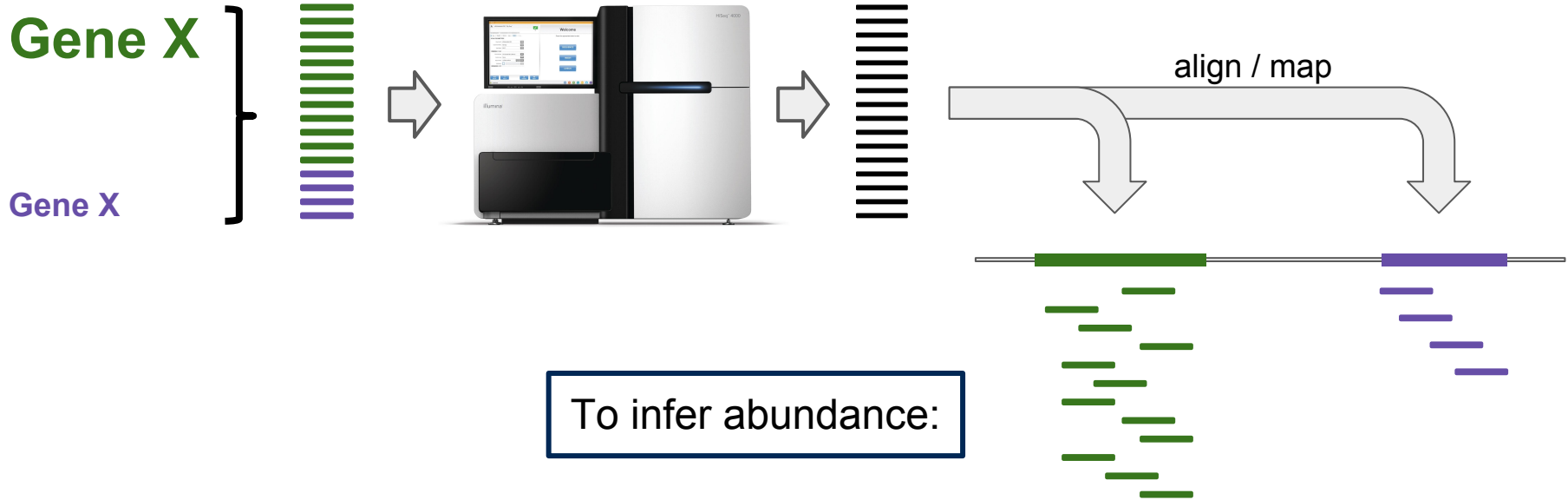
Somebody plagiarized parts of my document; where did they copy paragraphs from and where did each of the words come from?

Definitions

Mapping:

Somebody plagiarized parts of my document; where did they copy paragraphs from ~~and where did each of the words come from?~~

Why align (or map)?



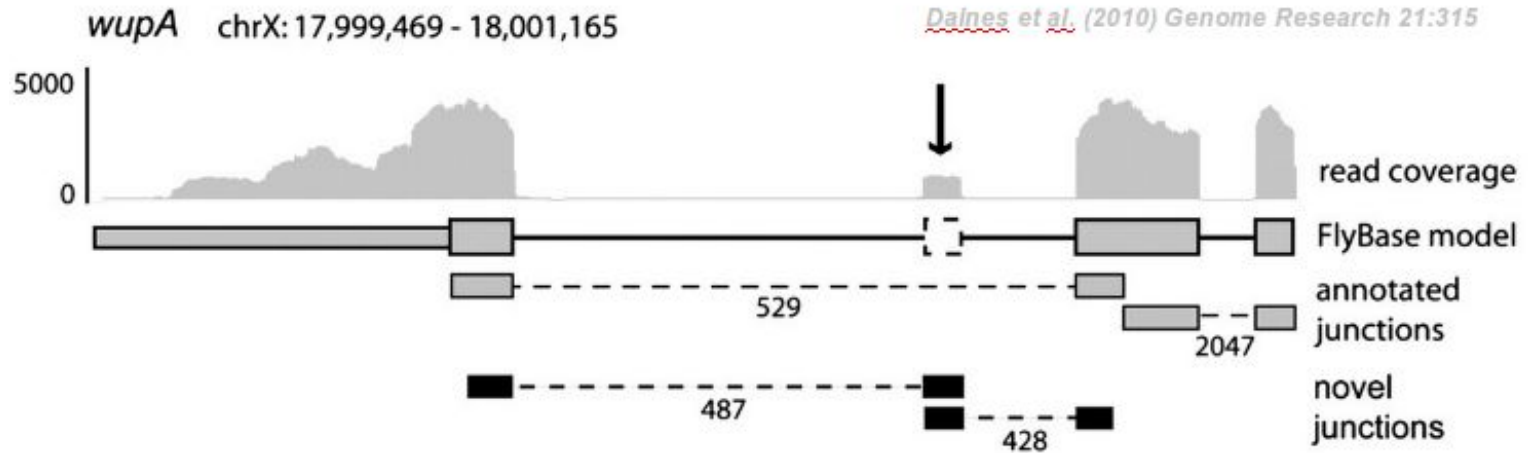
Why align (or map)?



ATGATAGCATCGTCGGGTGTCTGCTCAATAATAGTGCCGTATCATGCTGGTGTTATAATCGCCGCATGACATGATCAATGG
CAATAAAAGTGCCGTATCATGCTGGTGTTACAATCGCCGCA
CGTATCATGCTGGTGTTACAATCGCCGCATGACATGATCAATGG
TGTCTGCTCAATAAAAGTGCCGTATCATGCTGGTGTTACAATC
ATCGTCGGGTGTCTGCTCAATAAAAGTGCCGTATCATG--GGTGTTATAA
CTCAATAAGAGTGCCGTATCATG--GGTGTTATAATCGCCGCA
GTTATAATCGCCGCATGACATGATCAATGG

To measure variation

Why align (or map)?



To discover transcribed sequence.

More Definitions: “Global” and “local”

Global aligners try to align all provided sequence, end to end, both “query” and “subject / target” ...

E.g.

- Aligning two *Salmonella* genomes
- Aligning human and gorilla orthologous coding regions

“Global” and “local”

Local aligners try to find “hits” or chains of hits *within* each provided sequence ...

E.g.

- Finding mitochondrial “splinters” in nuclear chromosomes
- Finding genes that share a domain with a gene of interest

“Glocal ... ?”

Short read aligners generally assume that the *whole read* came from somewhere within the target (reference) sequence ...

... so, ***global*** with respect to the read, and ***local*** with respect to the reference.

Short Read (Non-splicing) Aligners

Li, H and Homer, N (2010) *Briefings in Bioinformatics* 11:473

“A survey of sequence alignment algorithms for next-generation sequencing”

Table 1:

Popular short-read alignment software

| Program | Algorithm | SOLiD | Long ^a | Gapped | PE ^b | Q ^c |
|------------------------|---------------|------------------|-------------------|------------------|-----------------|----------------|
| Bfast | hashing ref. | Yes | No | Yes | Yes | No |
| Bowtie | FM-index | Yes | No | No | Yes | Yes |
| BWA | FM-index | Yes ^d | Yes ^e | Yes | Yes | No |
| MAQ | hashing reads | Yes | No | Yes ^f | Yes | Yes |
| Mosaik | hashing ref. | Yes | Yes | Yes | Yes | No |
| Novoalign ^g | hashing ref. | No | No | Yes | Yes | Yes |

These two were fastest, at ~7 Gbp (vs human) per CPU day
... HiSeq 2500 generated 50-100 Gbp per day (at the time)

(Fall '12-'13) ... 150-180 Gbp per day

(Summer '16) ... 600 Gbp per day

(Summer '17) ... 1-3 Tbp per day

<https://www.illumina.com/systems/sequencing-platforms.html>

Burrows-Wheeler Aligners

Burrows-Wheeler Transform used in bzip2 file compression tool; FM-index (Ferragina & Manzini) allow efficient finding of substring matches within compressed text – algorithm is *sub-linear* with respect to time and storage space required for a certain set of input data (reference genome, essentially).

Reduced memory footprint, faster execution.

BWA

BWA is a fast gapped aligner. Long read aligners (bwasw and mem) also fast, and can perform well for 454, Ion Torrent, Sanger, and PacBio reads. BWA is actively maintained and has a strong user community.

bio-bwa.sourceforge.net

‘bwa aln’ (BWA “backtrack”) for reads < 70 bp

‘bwa bwasw’

‘bwa mem’ (seeds with *maximal exact matches*, extends via *Smith-Waterman*)

Bowtie

(now Bowtie 2) ... comparable to BWA.

Bowtie is part of a suite of tools (Bowtie, Tophat, Cufflinks, CummeRbund) that address RNAseq experiments.

<http://bowtie-bio.sourceforge.net>

Written by same folks as Tophat ... so, full compatibility.

Tophat2

Aligns full reads to genome, to determine “coverage islands.” Creates simulated spliced exons based on these islands, then aligns remaining short reads to the simulated cDNA (to find reads crossing splice junctions).

STAR

Spliced Transcripts Aligned to a Reference

Aligns short reads and full length cDNA

Similar to BWA MEM algorithm, but searches uncompressed version (suffix array) of genome (faster, but more RAM required!). Claims better sensitivity and specificity than previous short read aligners, and 50x speed vs TopHat2. Requires ~27 GB RAM for human genome!

HISAT2

Improved replacement for Tophat2, multiple high level and low level indexes of compressed sequence (similar to graph-based assemblers using De Bruijn graphs to represent overlapping k-mers). Graph structure can represent multiple sequence paths, i.e. a population of references, allowing rapid genotyping of samples.

HISAT2 is currently probably the best combination, *in a full aligner*, of speed (faster than STAR) and memory (~4 GB for human genome).

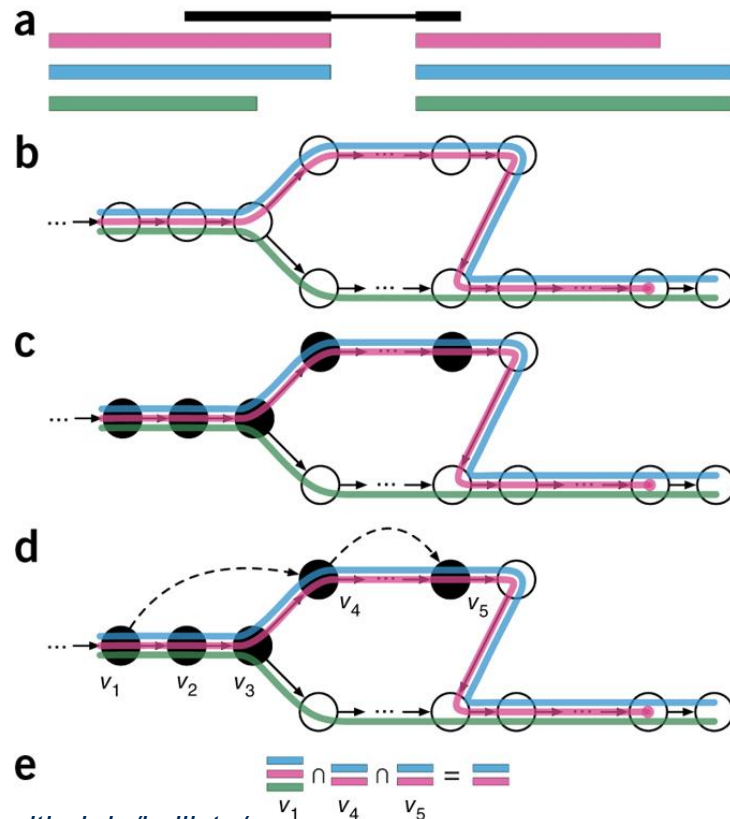
Kim, Langmead, Salzberg (2015) *Nature Methods* 12:357

Kallisto

A pseudoaligner (mapper? see Pachter [blog post](#)) that compares read k-mers (overlapping subsequences) to a *transcriptome de Bruijn graph* (T-DBG) to find transcripts compatible with the read. Also uses expectation maximization (EM) and bootstrapping to determine most likely transcript and abundance uncertainty.

Bray ... Pachter (2016) *Nat Biotech* 34:525

See [sleuth](#) for DGE analysis.



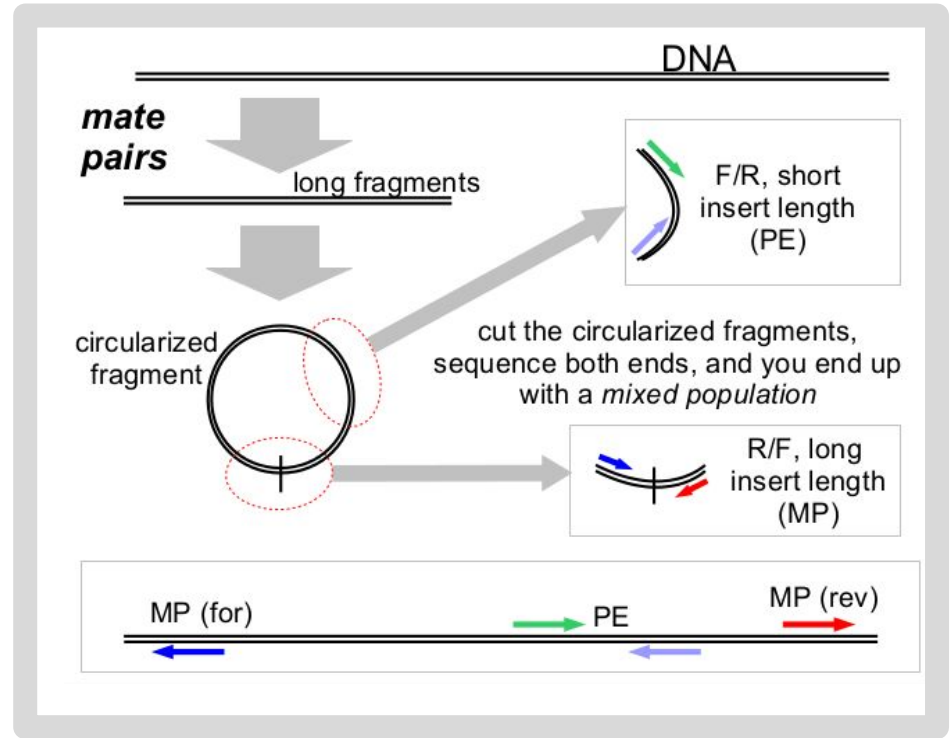
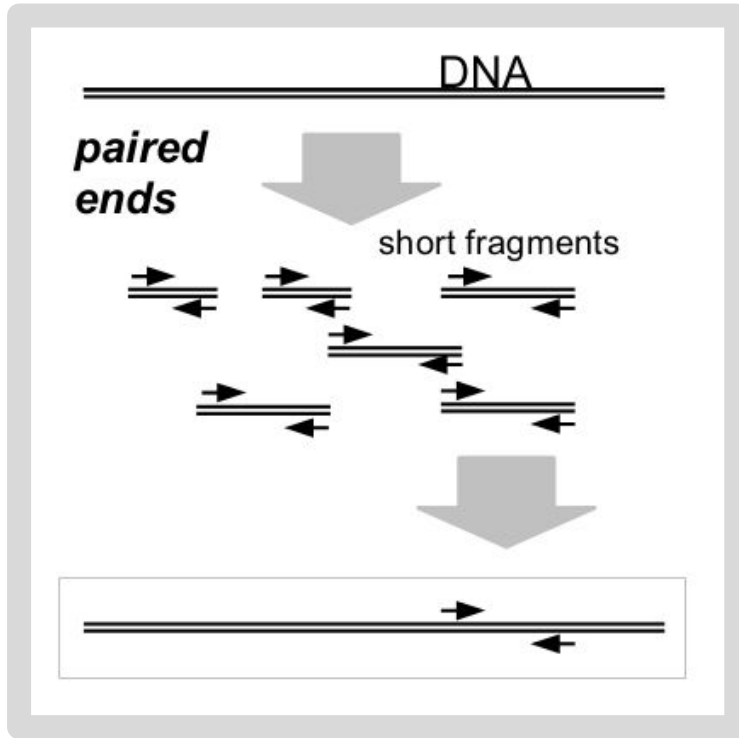
<http://pachterlab.github.io/kallisto/>
<http://pachterlab.github.io/sleuth/>

Salmon

Supersedes Sailfish, an older k-mer based transcript analysis tool. Performs *quasi-mapping* to a set of transcripts (not genome), similar in methods to kallisto. Also performs bias correction for multiple modes of bias (sequence, position) to more accurately determine abundance.

Patro, Duggard, Kingsford (2015) *bioRxiv* <http://dx.doi.org/10.1101/021592>

General Alignment Parameters / Concepts



General Alignment Parameters / Concepts

Edit Distance:

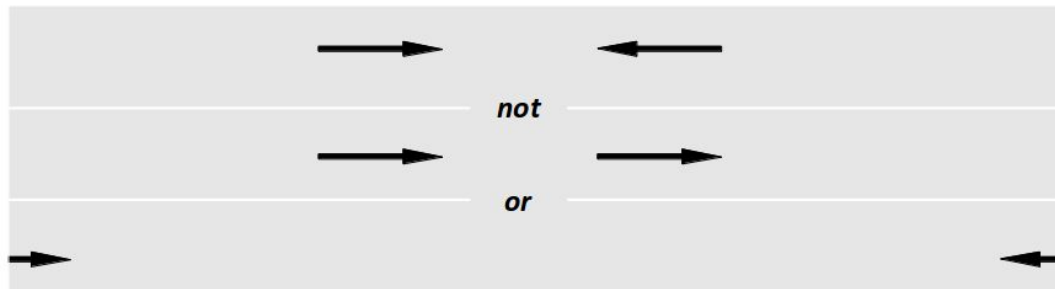
```
ATCGACCGCGCTAA-TATTAGTC . . .  
CGACGGCGCTAACTATTA
```

edit distance = 2

Mapping Quality:

prob. of incorrect position = $10^{-MQ/10}$... (BWA)

Proper Pairs:

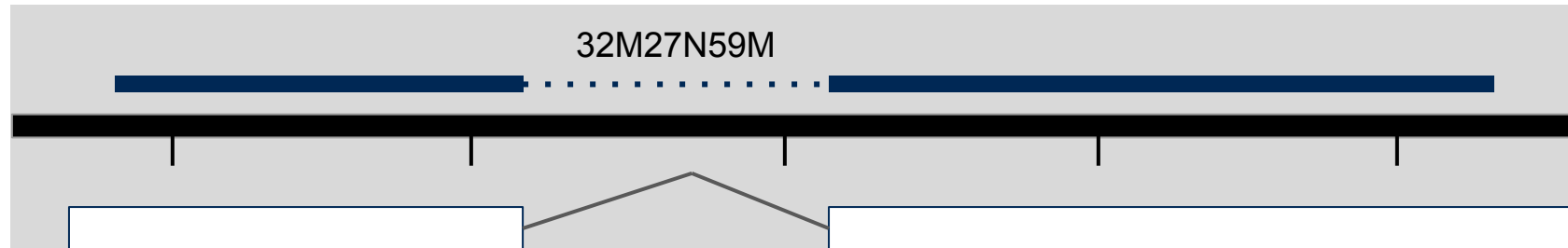


General Alignment Parameters / Concepts

Clipping:

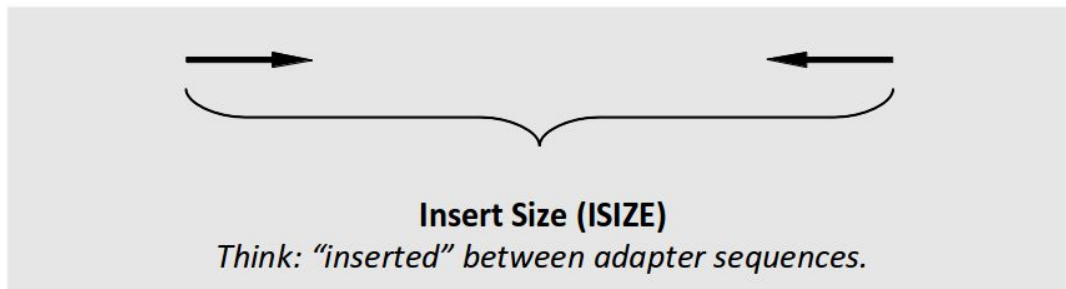


Splicing:

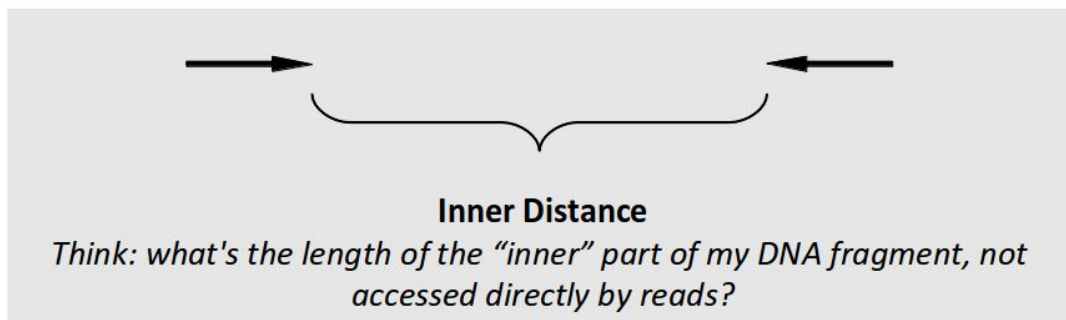


General Alignment Parameters / Concepts

Insert Size:



Inner Distance:



General Alignment Parameters / Concepts

Multimappers:

Reads that align *equally well* to more than one reference location.

Generally, multimappers are discounted in variant detection, and often discounted (ignored) in counting applications (RNA-Seq).

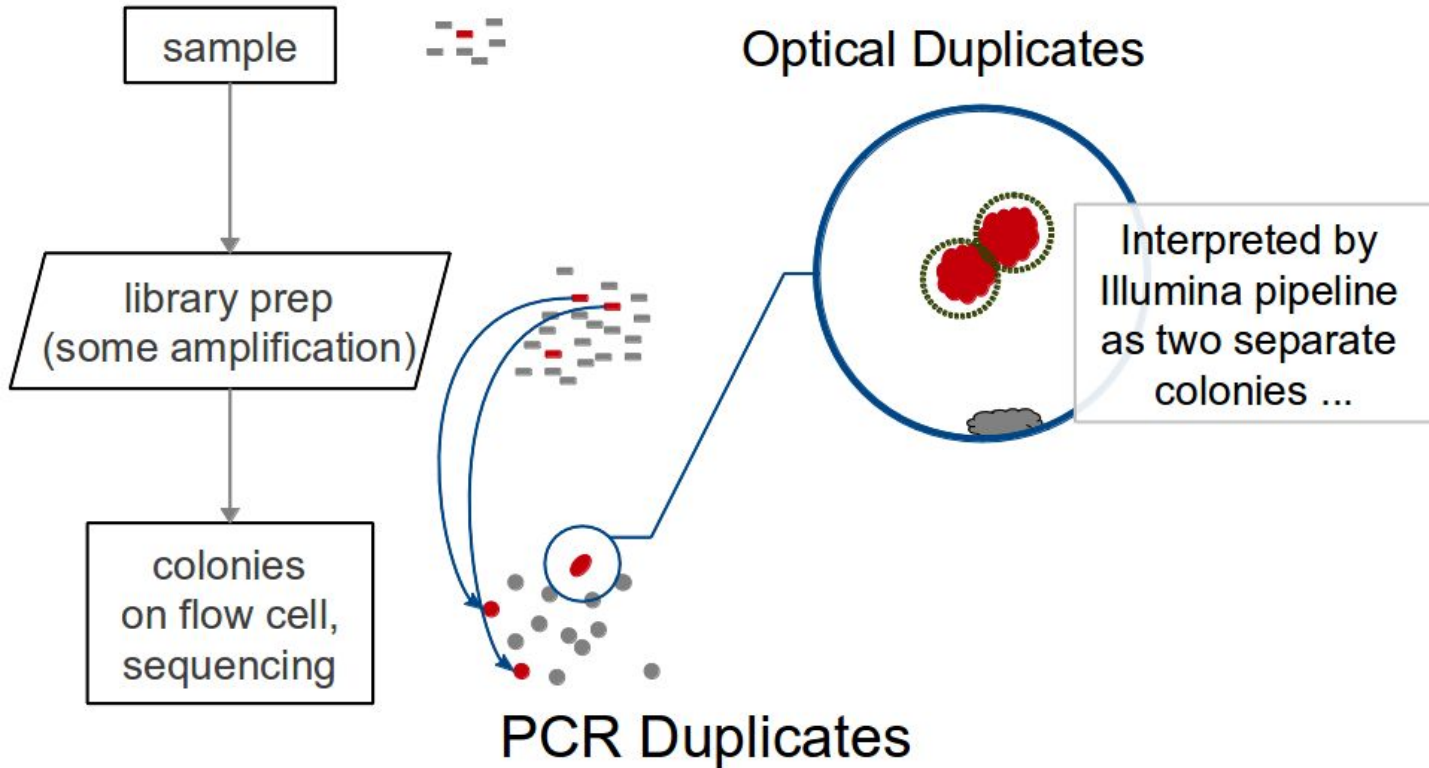
Note: *multimapper “rescue”* in some algorithms.

Duplicates:

Reads or read pairs arising from the same library fragment, either during library preparation (PCR duplicates) or colony formation (optical duplicates).

Generally, duplicates are discounted in variant detection , and ignored in counting applications (RNA-Seq).

General Alignment Parameters / Concepts

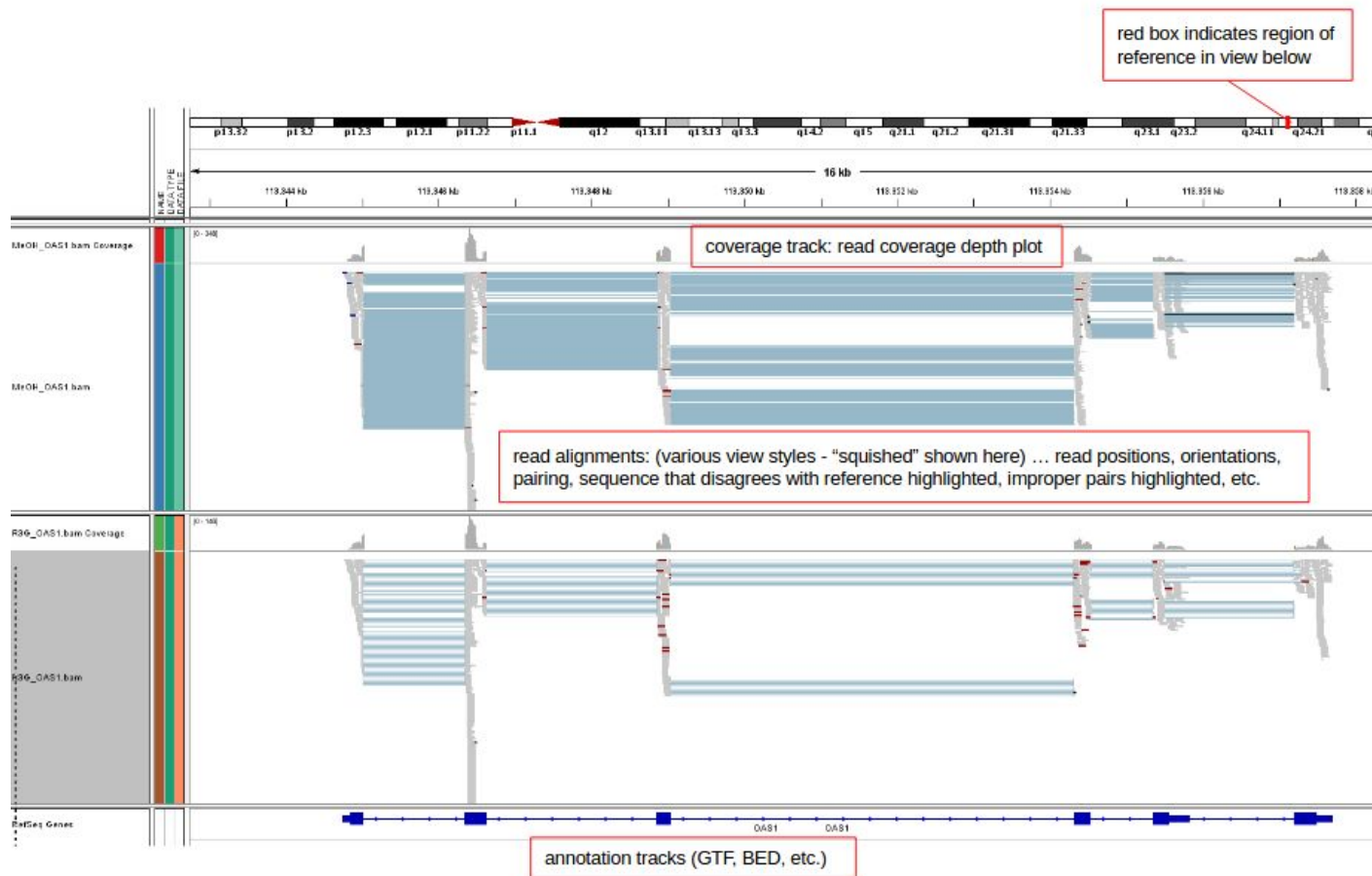


Alignment Viewers

- IGV (Integrated Genomics Viewer)
 - www.broadinstitute.org/igv/
- BAMview, tview (in SAMtools), IGB, GenomeView, SAMscope
- ...
- UCSC Genome Browser, GBrowse



IGV

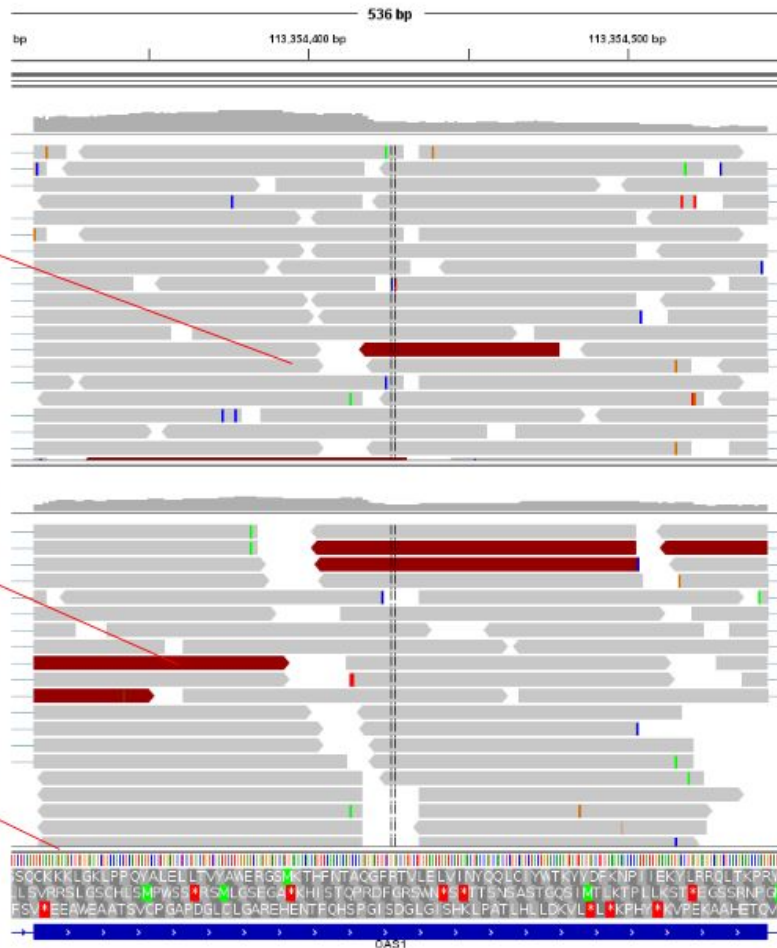


IGV

colored bases where they disagree with reference (substitution, indel, etc.)

improper pairs (mate aligns far away, in wrong orientation, or on another chromosome)

reference sequence, reading frames, etc.



IGV

More on IGV's interface, file formats, and display can be found here:

<http://www.broadinstitute.org/igv/AlignmentData>

More on interpreting and customizing IGV's display can be found here:

http://www.broadinstitute.org/software/igv/interpreting_insert_size