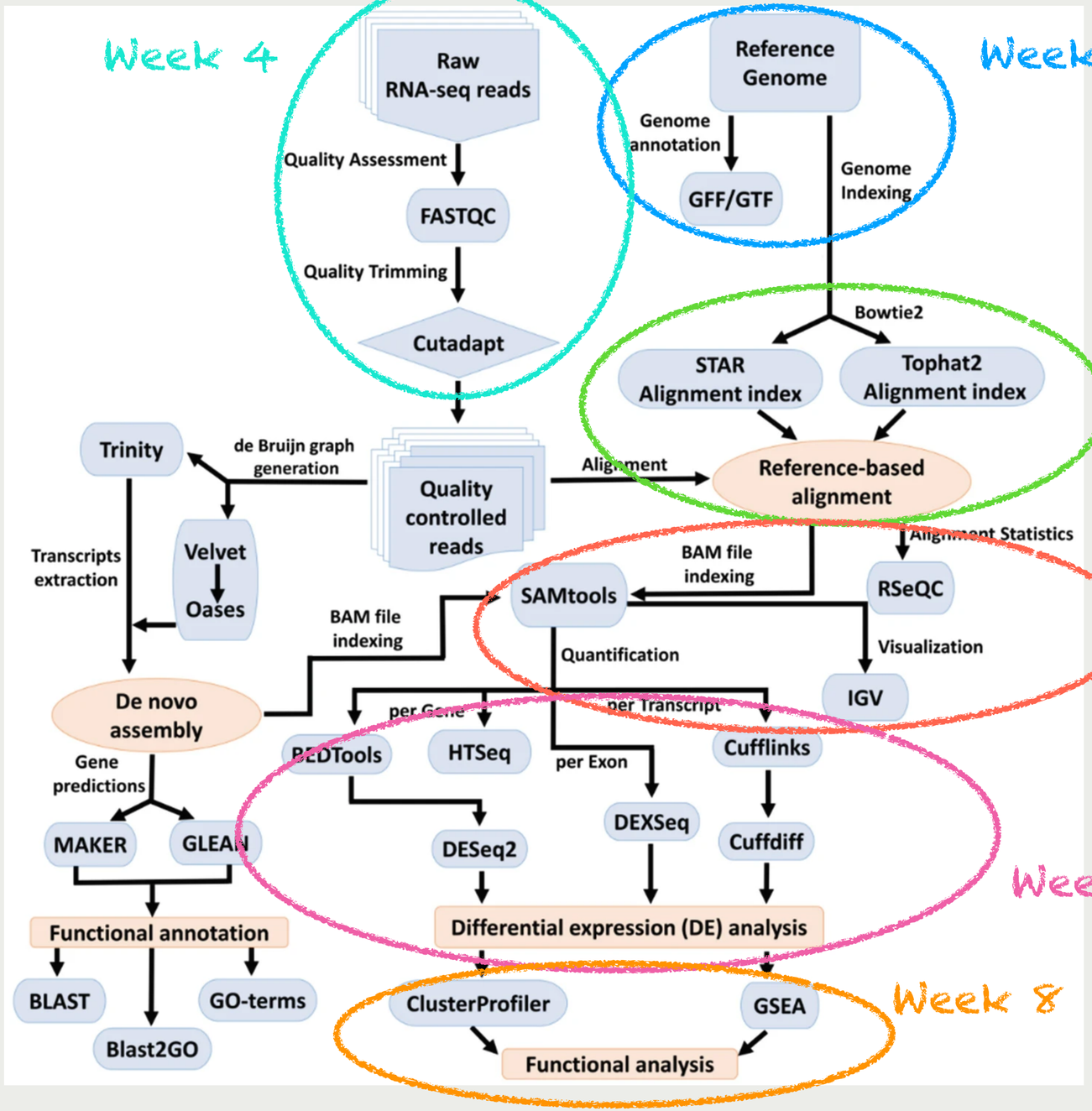
BIOL 343
Applied Bioinformatics I
Alignment/Mapping

Dr. Nic Wheeler

Learning Objectives

You will be able to:

1.



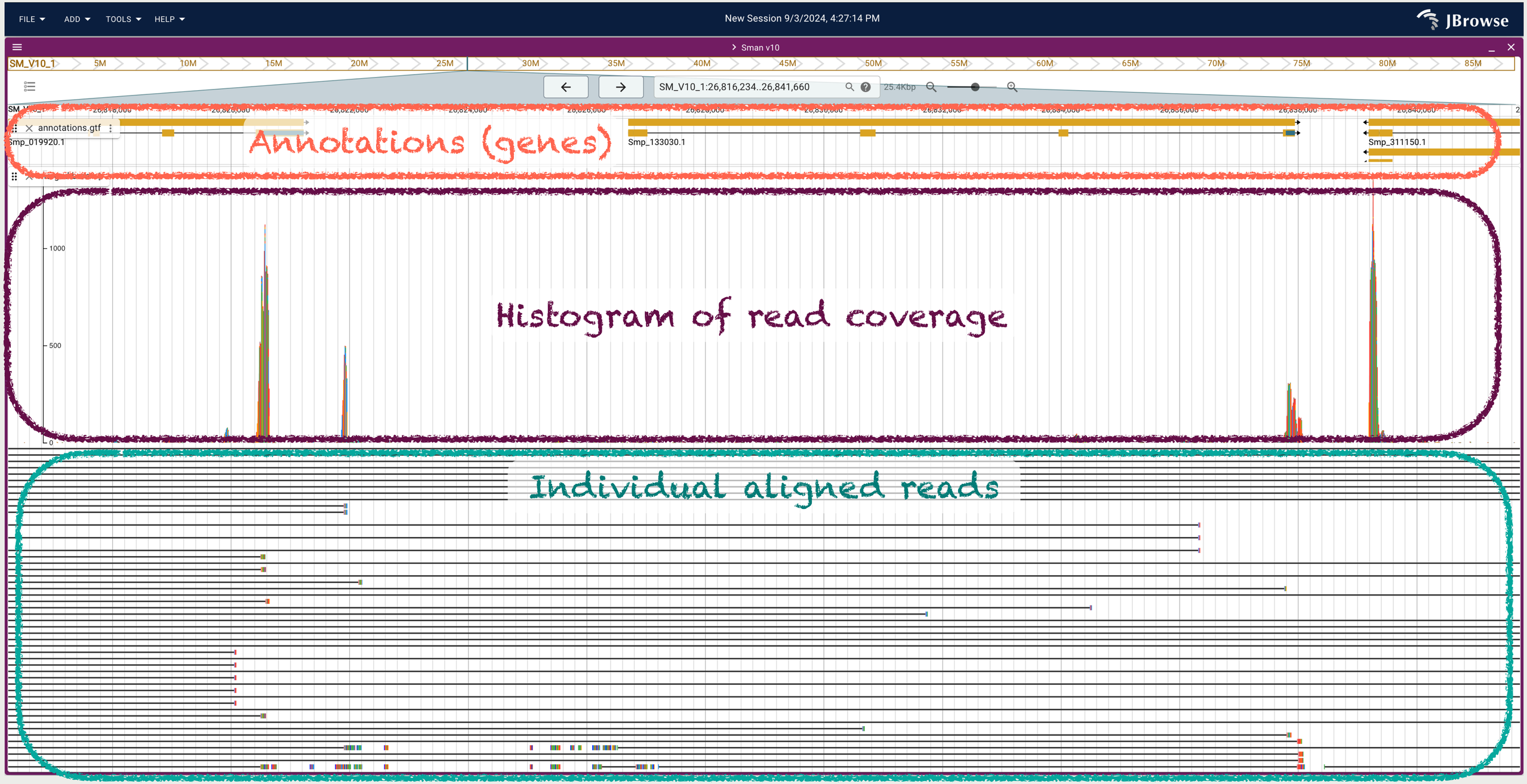
Bharti 2021, Next Generation Sequencing and Analysis

Alignment is the most important step in RNA-seq analysis

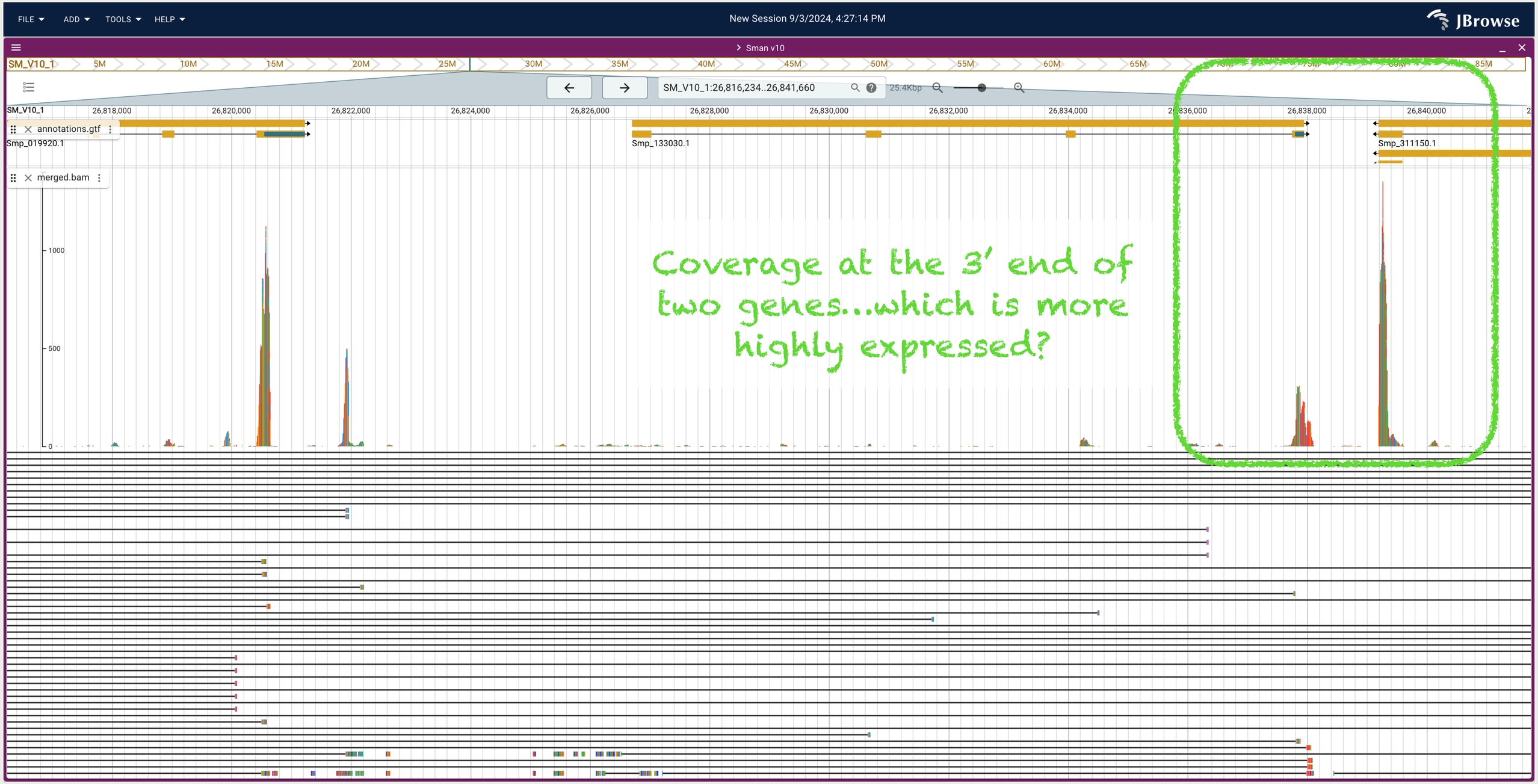
Counting (also important) and DEG ID relies on high-confidence mapping

- Recall the goal of our RNA-seq experiments…
  - Treatment vs Control
  - Mutant vs Wild type
  - ***Identify differentially expressed genes (DEGs)***
- DEGs will be identified using statistical tests comparing ***expression values*** of transcripts/genes
- Expression values will be calculated based on the number of reads that ***align/map*** to a given genomic locus

Where we're going…



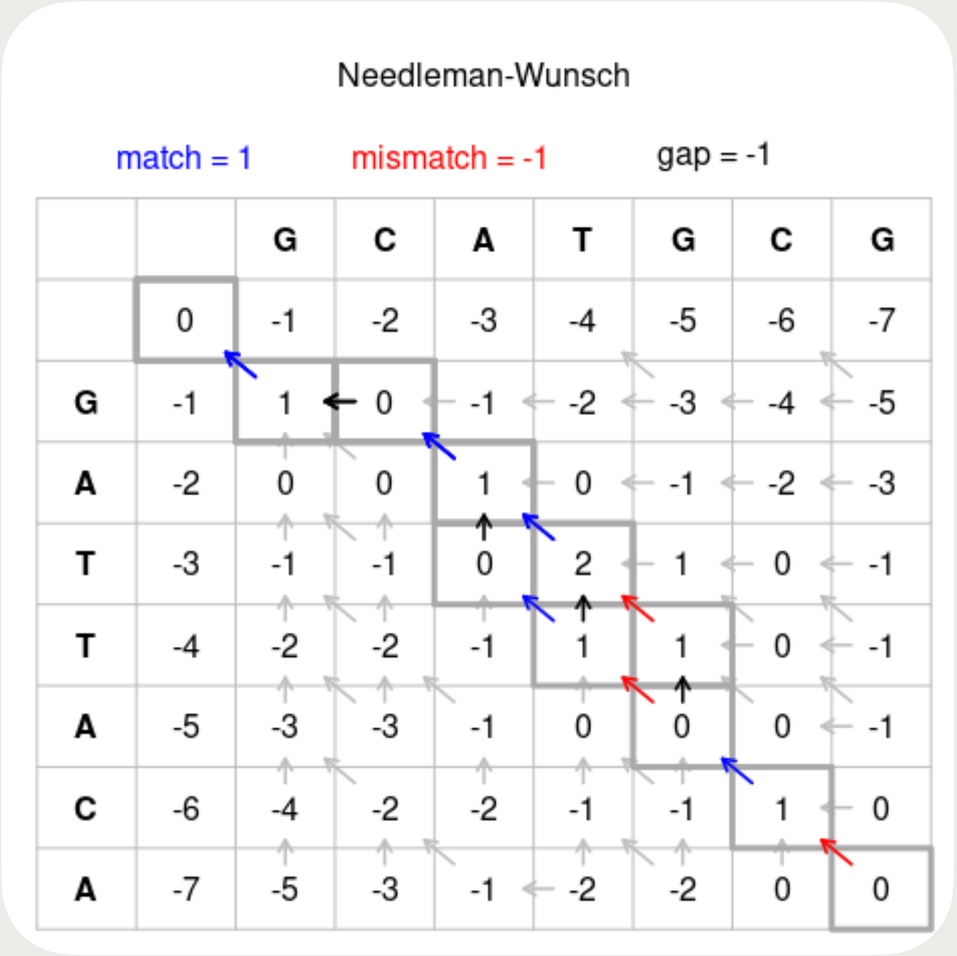
Where we're going…



Types of alignment algorithms

Needleman-Wunsch and…

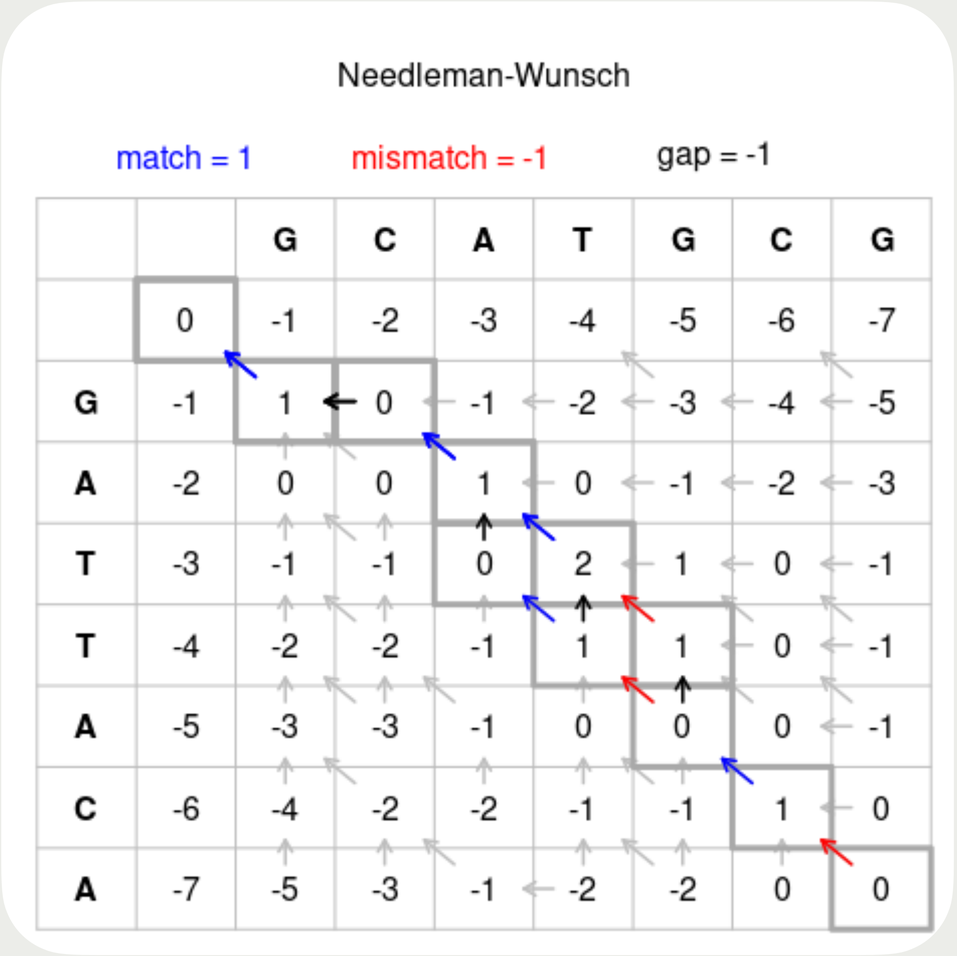
- Needleman-Wunsch (global alignment) and Smith-Waterman (local alignment)
  - Dynamic programming
  - Mismatch penalty (transitions or transversions)
  - Gap penalty



Types of alignment algorithms

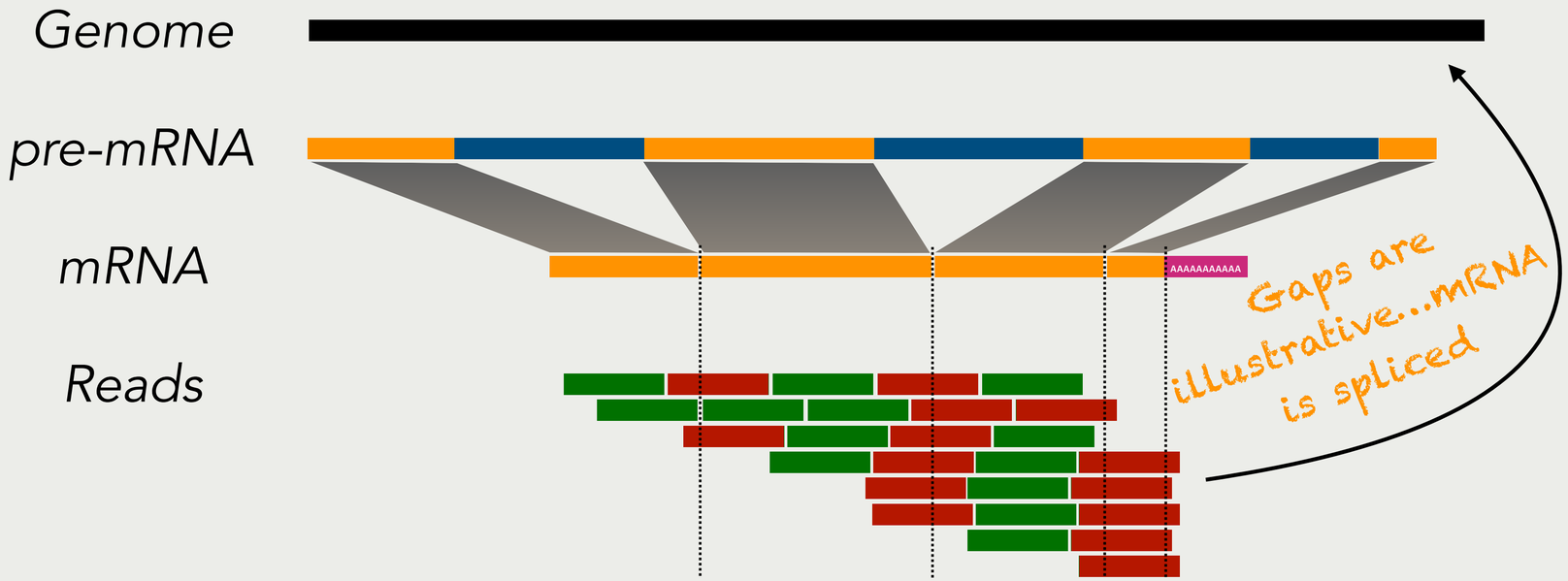
Needleman-Wunsch and…

- Problems
  - Genomes are *huge* strings with lots of repetition
  - Short reads are likely to align many locations
  - Reads should align with massive gaps if spanning an intron
- Solution - Suffix array (STAR) *or* Burrow-Wheelers transform and FM-index (HISAT)



Splice-aware alignment

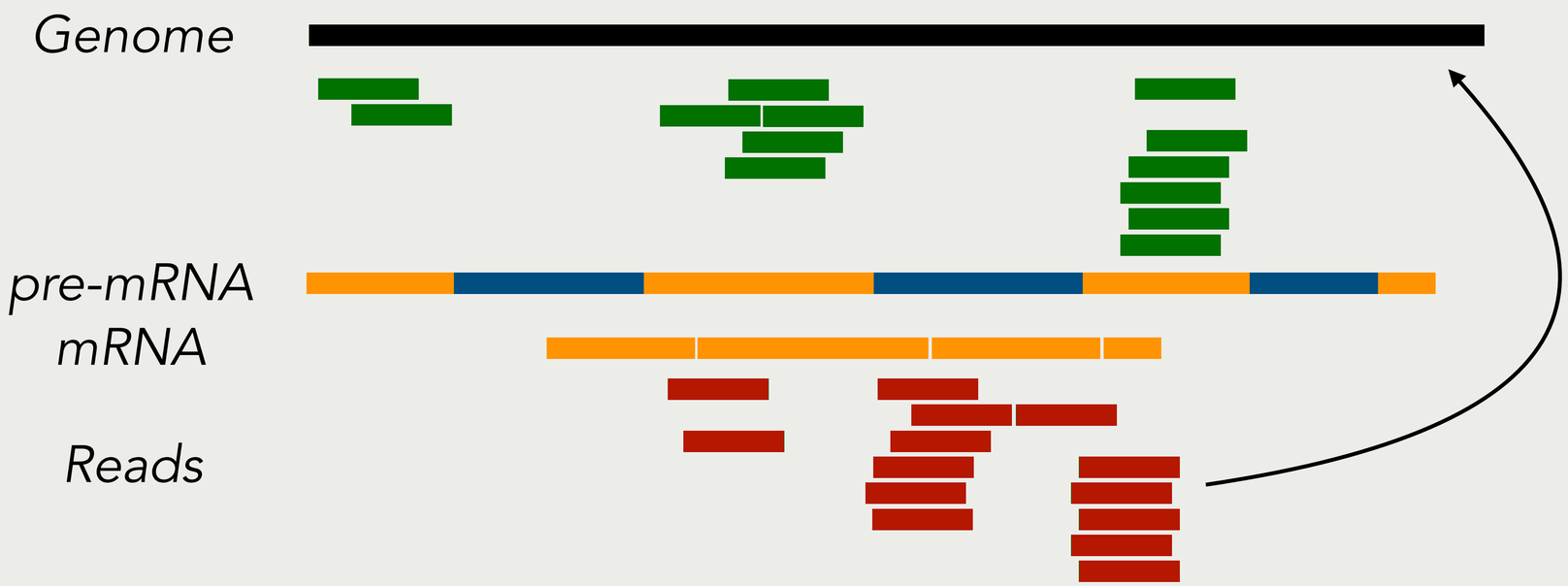
Gaps are large and encouraged



But, reads aren't aligned to a transcriptome (mRNAs), but a genome

Splice-aware alignment

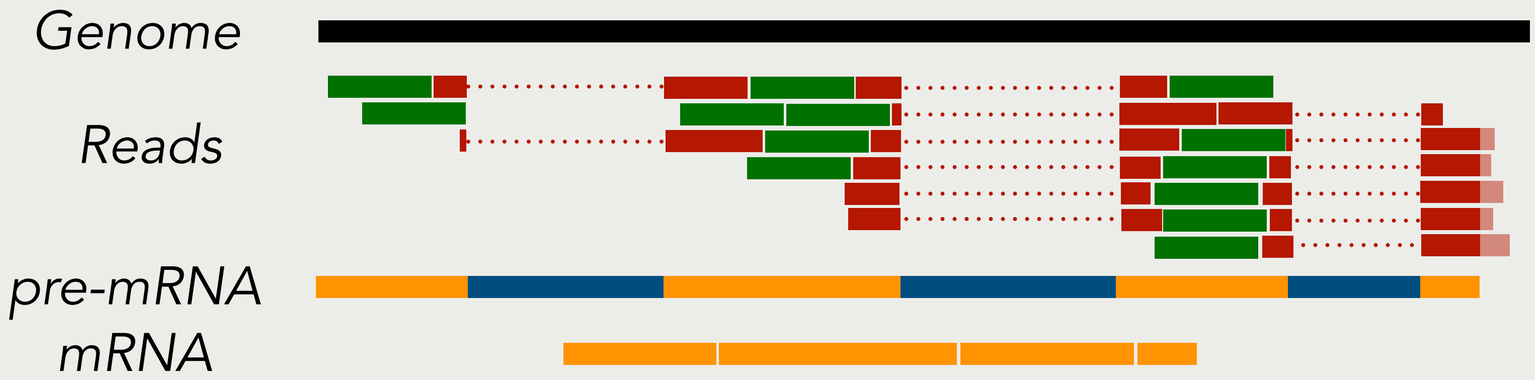
Gaps are large and encouraged



But, reads aren't aligned to a transcriptome (mRNAs), but a genome

Splice-aware alignment

Gaps are large and encouraged



Large gaps - representing introns - and reads from poly(A) tails don't align

Two (main) approaches to splice-aware alignment

STAR and HISAT

STAR

Spliced Transcripts Alignment to a Reference

Published in 2013

40574 citations

Requires a lot of RAM; ultra fast

HISAT

Hierarchical Indexing for Spliced Alignment of Transcripts

Published in 2015

17667 citations

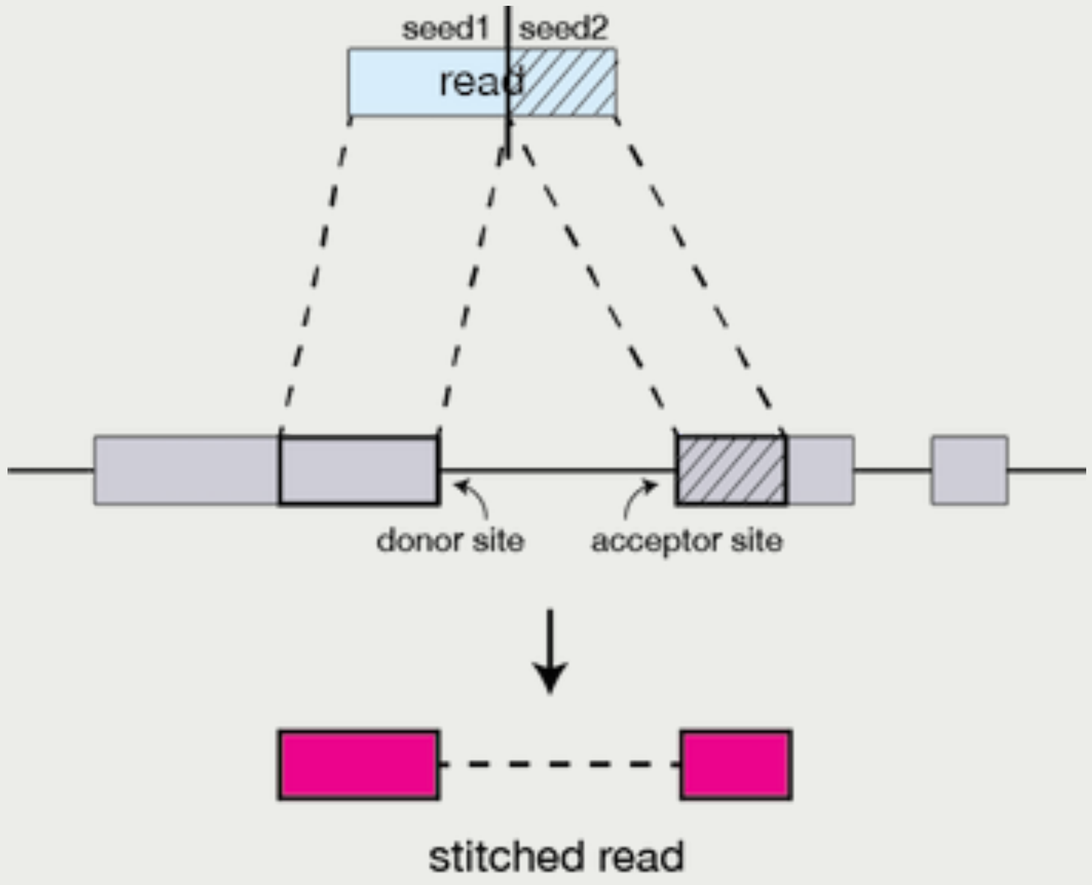
Less RAM needed; still fast

Index/compress/transform (or some combination of all) the reference genome to make searches faster

Two (main) approaches to splice-aware alignment

STAR

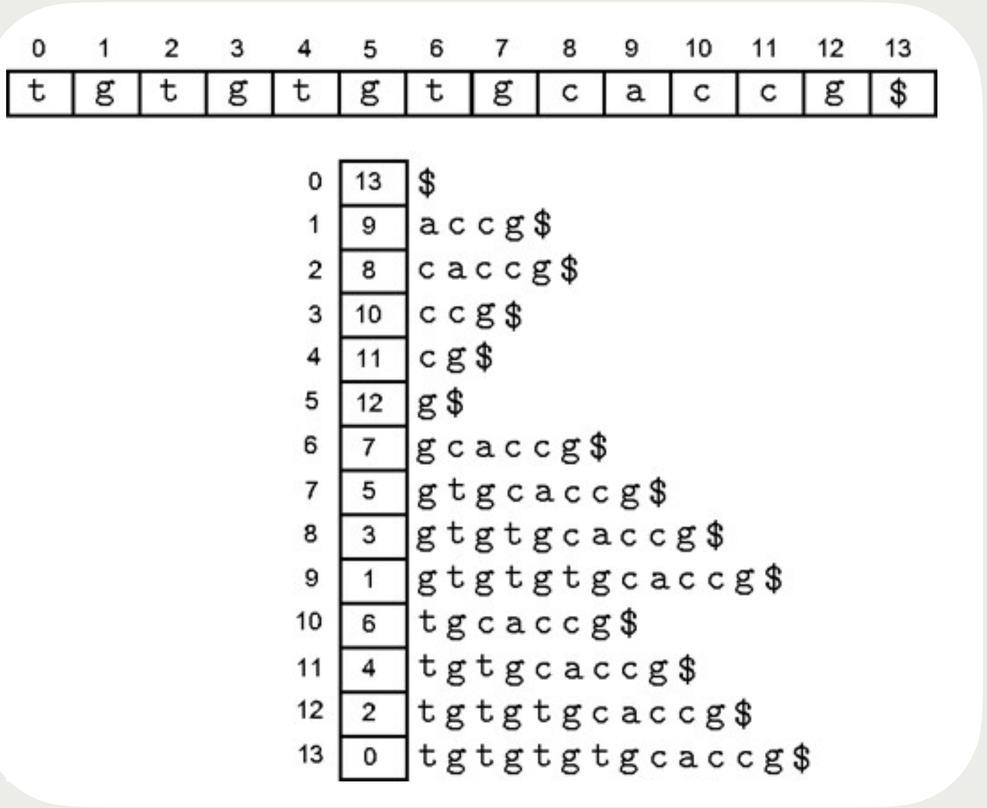
1. Starting from the first base, find the Maximal Mappable Prefix (MMP, seed 1) of the read
    - The end of seed 1 will map to a splice donor
2. Find the MMP of the remainder of the read
    - The end of seed 2 will map to a splice acceptor
3. Cluster and stitch the seeds based on proximity (if multiple seeds 1/2, stitch those that are closest together)



Two (main) approaches to splice-aware alignment

STAR

- Uses a suffix array of the reference genome
  - Every substring of the genome sorted lexicographically
  - Given a search string *P*, two binary searches to find the boundaries
    - *gtg* - binary search to find boundary 1 at index 5, binary search to find boundary 2 at index 9
    - Counts the number of occurrences (9-7+1)
- Many developments (ongoing) in 1) generating the SA and 2) searching the SA



0	1	2	3	4	5	6	7	8	9	10	11	12	13
t	g	t	g	t	g	t	g	c	a	c	c	g	$

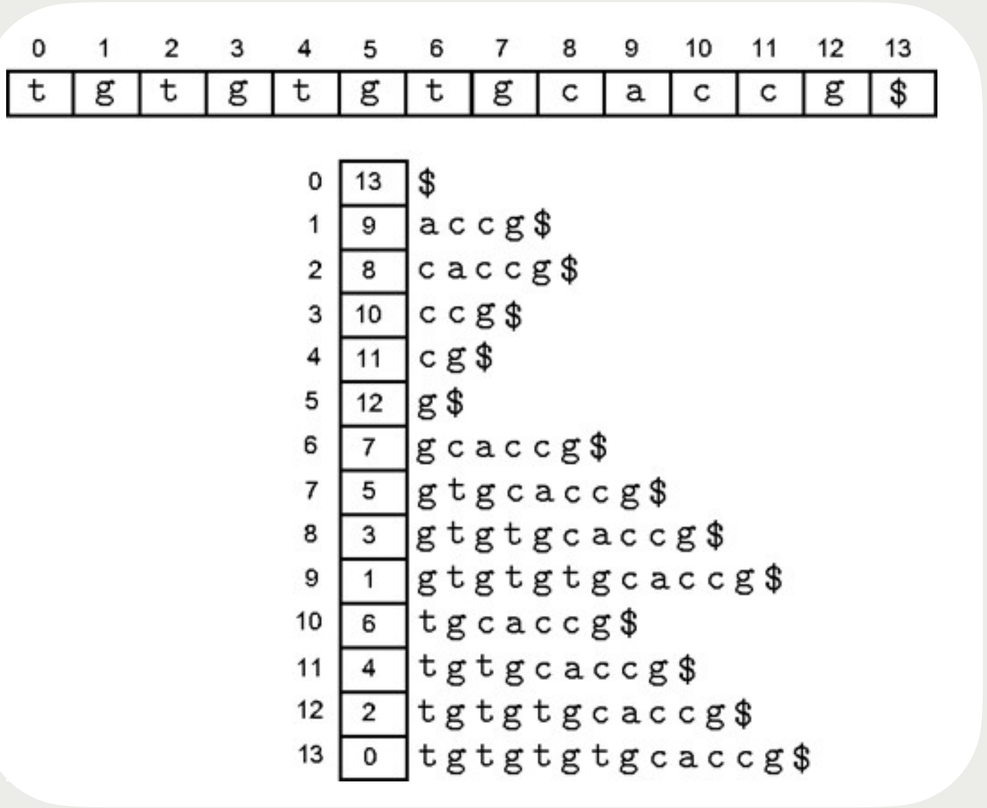
0	13	$
1	9	a c c g $
2	8	c a c c g $
3	10	c c g $
4	11	c g $
5	12	g $
6	7	g c a c c g $
7	5	g t g c a c c g $
8	3	g t g t g c a c c g $
9	1	g t g t g t g c a c c g $
10	6	t g c a c c g $
11	4	t g t g c a c c g $
12	2	t g t g t g c a c c g $
13	0	t g t g t g t g c a c c g $

Shrestha 2014, Briefings in Bioinformatics

Two (main) approaches to splice-aware alignment

STAR

- Only two binary searches - ultrafast!
- Suffix array of a large genome (16x) - very big RAM!
  - 32 GB RAM for human genome
- Generating suffix array - kinda slow!
- For more on suffix arrays, see https://www.cs.cmu.edu/~dga/csa.pdf



0	1	2	3	4	5	6	7	8	9	10	11	12	13
t	g	t	g	t	g	t	g	c	a	c	c	g	$

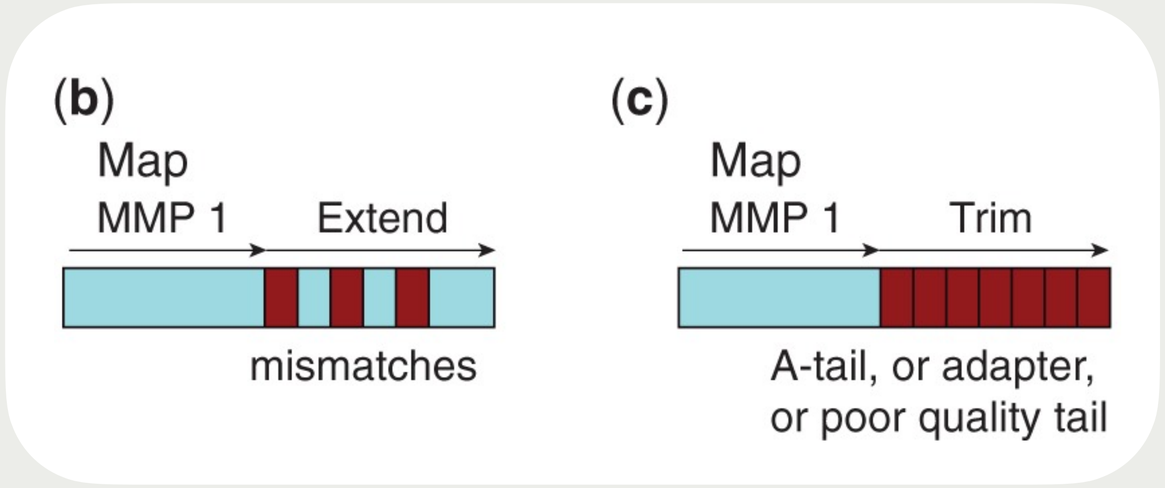
0	13	$
1	9	a c c g $
2	8	c a c c g $
3	10	c c g $
4	11	c g $
5	12	g $
6	7	g c a c c g $
7	5	g t g c a c c g $
8	3	g t g t g c a c c g $
9	1	g t g t g t g c a c c g $
10	6	t g c a c c g $
11	4	t g t g c a c c g $
12	2	t g t g t g c a c c g $
13	0	t g t g t g t g c a c c g $

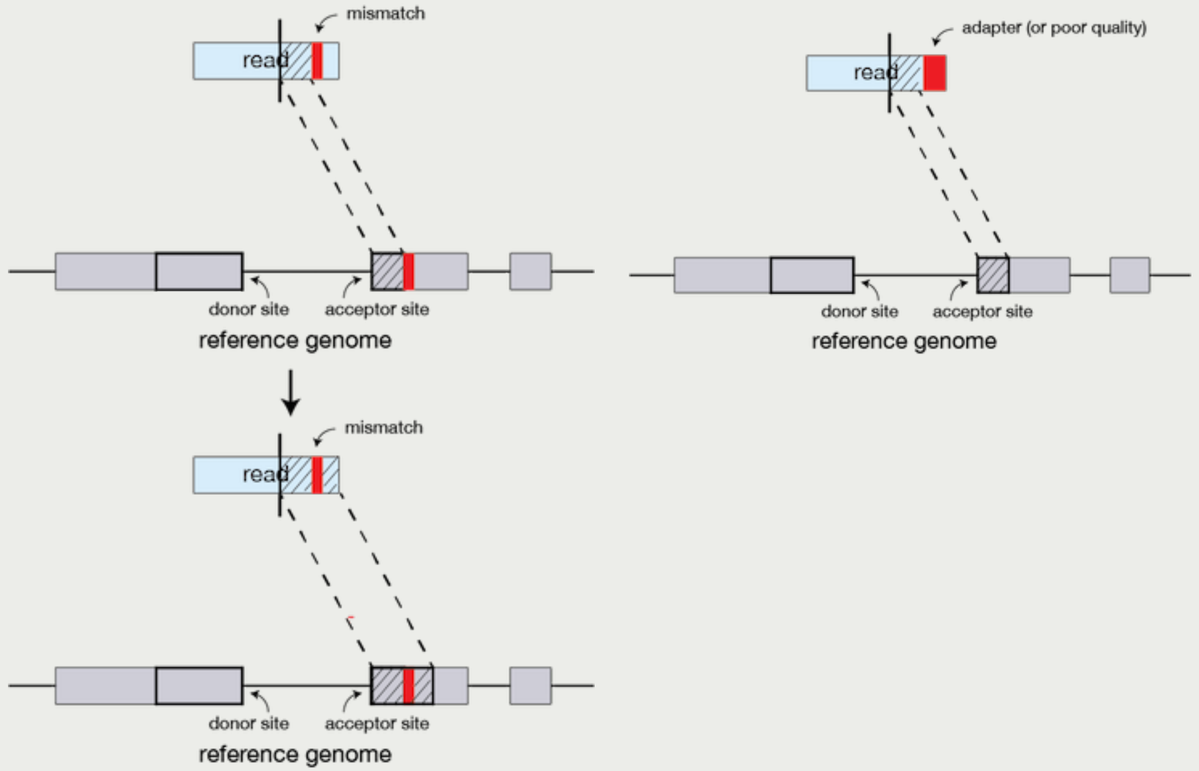
Shrestha 2014, Briefings in Bioinformatics

Two (main) approaches to splice-aware alignment

STAR

- Other advantages to this approach:
  - Robust to mismatches - MMPs can be extended
  - Can trim (***soft clip***) if extension of MMP results in many mismatches





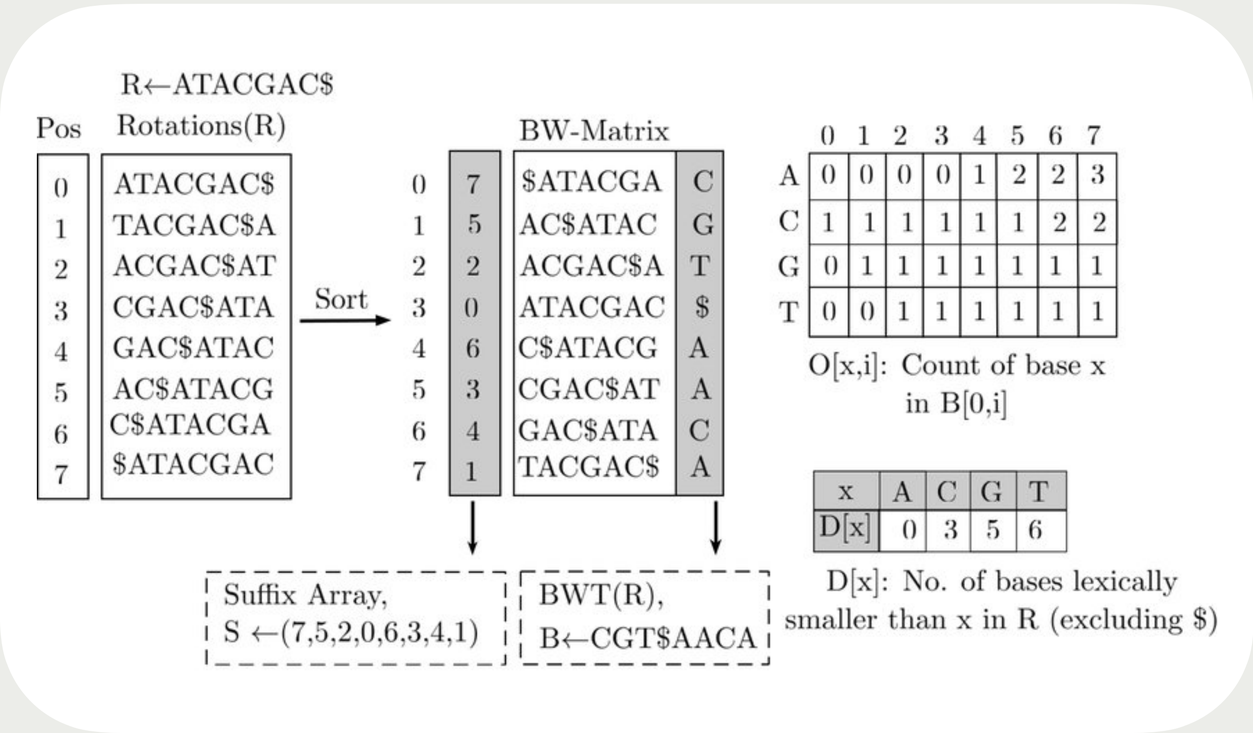
Dobin 2013, Bioinformatics

hbctraining.github.io

Two (main) approaches to splice-aware alignment

HISAT

- Burrows-Wheeler Tranform (BWT) to losslessly and reversibly compress the genome
- Stored in an FM-index
  - Related to a SA (compressed SA)
  - Search time is independent of genome length (not true of SA)
  - Includes the SA, the BWT, a “milestone table,” and a count array (C)
- Substrings can quickly be located and counted

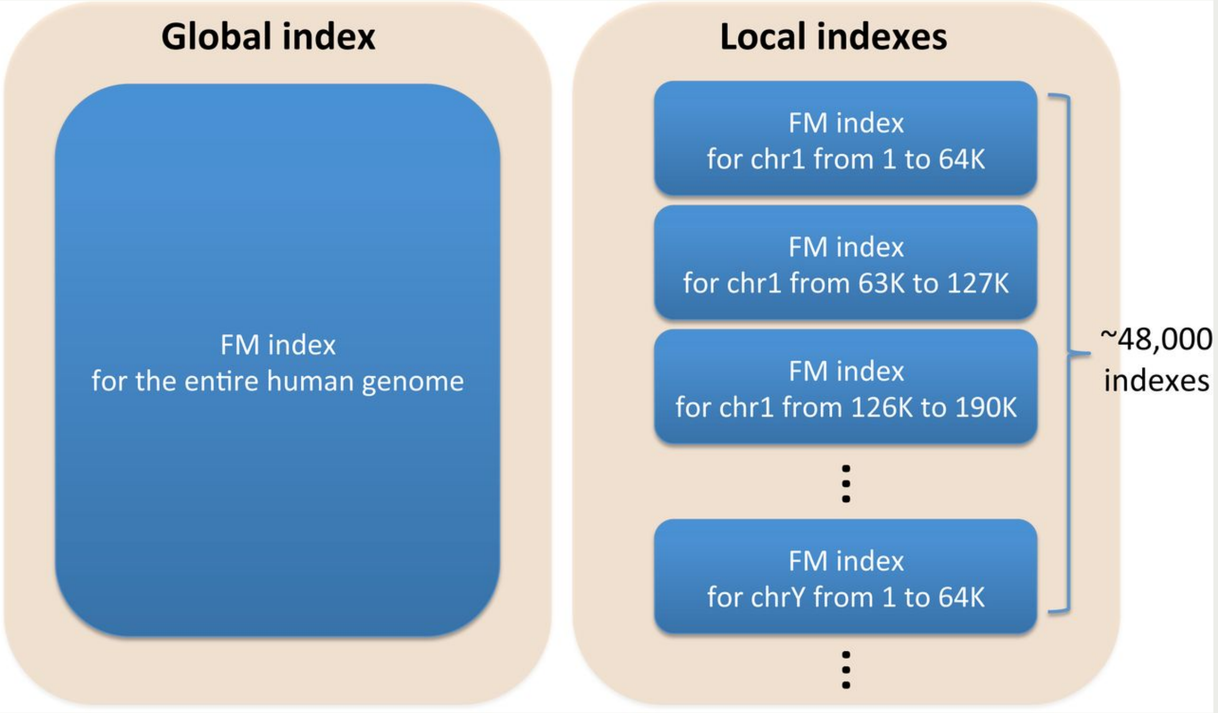


Vasimuddin 2019, arXiv

Two (main) approaches to splice-aware alignment

HISAT

- How to make splice-aware?
- HISAT approach - two types of FM-indexes:
  - Global - entire genome
  - Local - 64,000 bp with 1,024 bp overlaps (~48,000 for the human genome)
    - 90% of human introns fit within a single local index
    - 8 bp will map ~1 time in a local index
- 4 GB total space

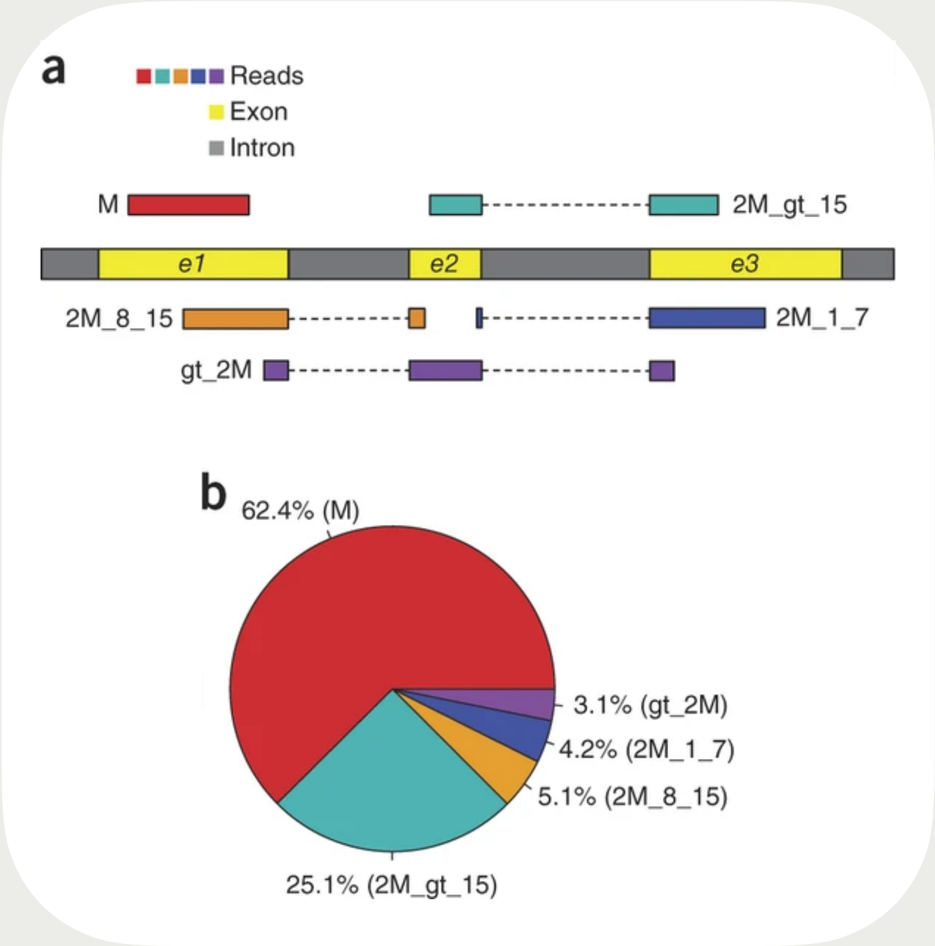


Kim 2014, bioRxiv

Two (main) approaches to splice-aware alignment

HISAT

1. Align part of each to the global FM-index
2. Align remainder of the read to a smaller 64,000 bp index (presumably contains the next exon)
   - Depending on length of match of Alignment #1, adaptive strategy with different algorithms
   - All include global search, local search, and read extension



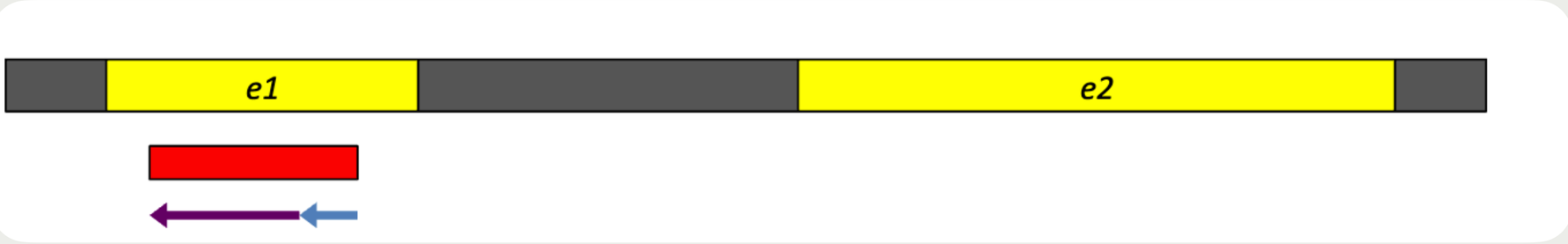
Kim 2015, Nature Methods

Two (main) approaches to splice-aware alignment

HISAT

Simple scenario - entire read fits within one exon

1. Align read to global FM-index until 28 nt at one location are exactly matching (slow)



2. Switch to extension mode for the remainder of the read (very fast)

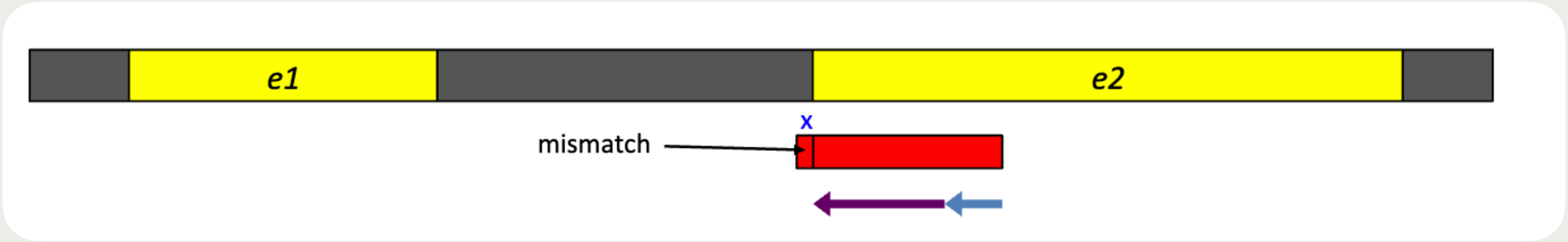
Kim 2015, Nature Methods

Two (main) approaches to splice-aware alignment

HISAT

Complex scenario - most of read fits in one exon

1. Align read to global FM-index until 28 nt at one location are exactly matching (slow)



2. Switch to extension mode until reaching a mismatch (very fast)

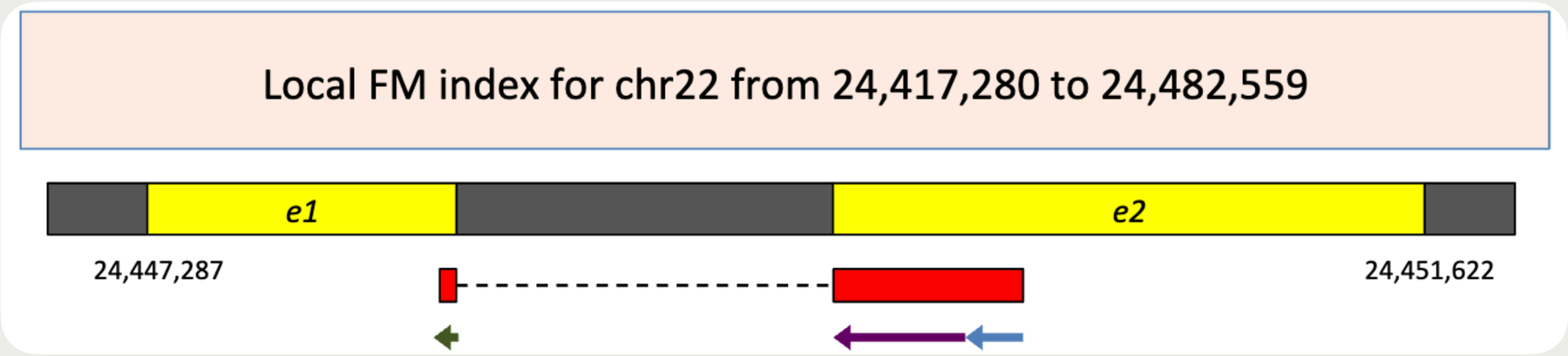
Kim 2015, Nature Methods

Two (main) approaches to splice-aware alignment

HISAT

Complex scenario - most of read fits in one exon

1. Align read to global FM-index until 28 nt at one location are exactly matching (slow)



2. Switch to extension mode until reaching a mismatch (very fast)
3. Align remainder of read to local FM-index

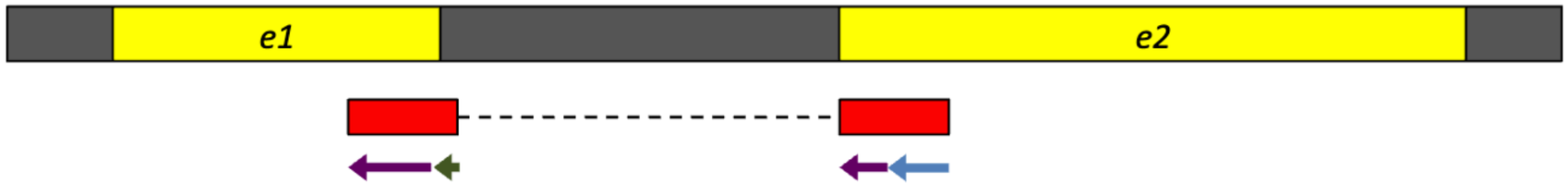
Kim 2015, Nature Methods

Two (main) approaches to splice-aware alignment

HISAT

Complex scenario - long anchors in two exons

1. Align read to global FM-index until 28 nt at one location are exactly matching (slow)



2. Switch to extension mode until reaching a mismatch (very fast)
3. Align 8 bp of unaligned portion of read to local FM-index (longer prefix if too many potential local alignments)
4. Switch to extension mode

Kim 2015, Nature Methods

Two (main) approaches to splice-aware alignment

STAR and HISAT

STAR

Requires a lot of RAM; ultra fast

Soft clips

Few parameters

HISAT

Less RAM needed; still fast

Soft clips

Some hard coded parameters (local index size ≈ max intron length; local search seed correlated with expected matches in local index)

If you have the RAM...go with STAR