# RNA-Seq Module 8
# Alignment

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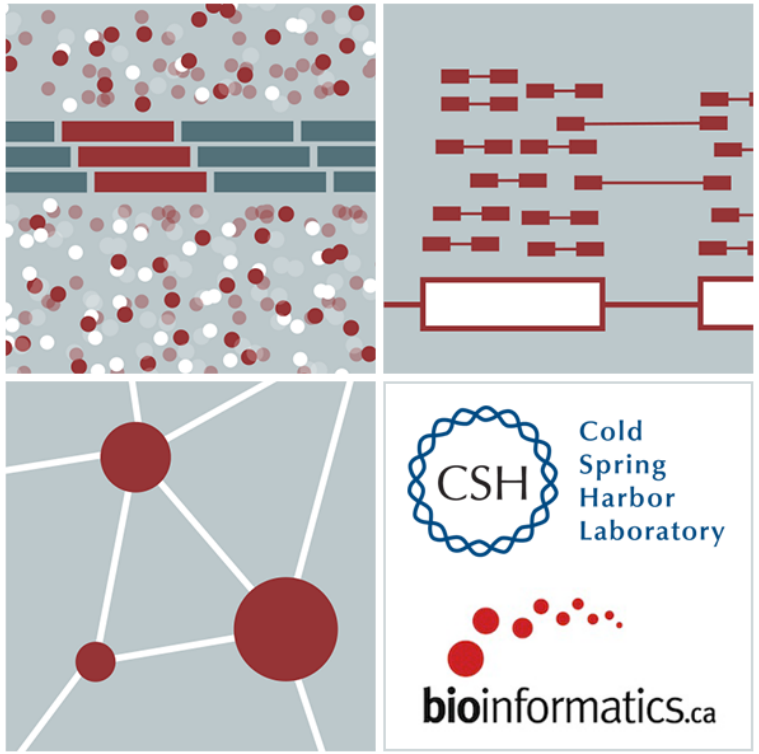



THE GENOME INSTITUTE
at Washington University

# Alignment - How does it work?

- Alignment is about fitting individual pieces (reads) into the correct part of the puzzle
- The human genome project gave us the picture on the box cover (the reference genome)
- Imperfections in how the pieces fit can indicate changes to a copy of the picture

Reference: AGCCTGAGACCGTAAAAAAGTCAAG

| | | | | | | | | | | | | | | | | | | | | |

A read sequence: GAGACCGTAAAAACGTC

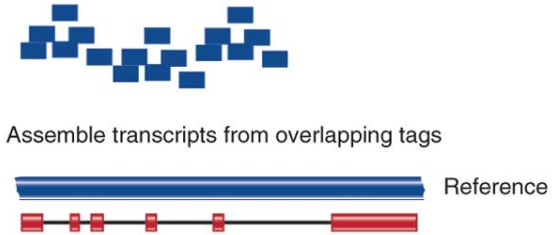
A variant!

# RNA-seq alignment challenges

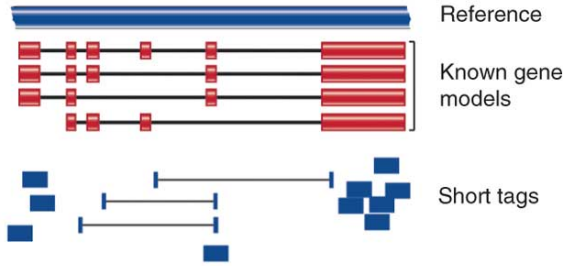
- Computational cost
  - 100's of millions of reads
- Introns!
  - Spliced vs. unspliced alignments
- Can I just align my data once using one approach and be done with it?
  - Unfortunately probably not

# Three RNA-seq mapping strategies

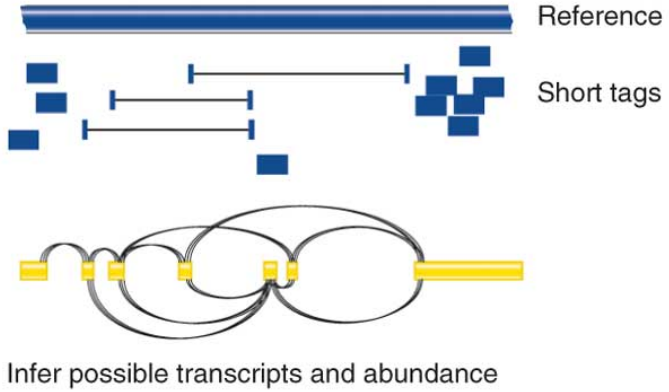
## De novo assembly




## Align to transcriptome




## Align to reference genome

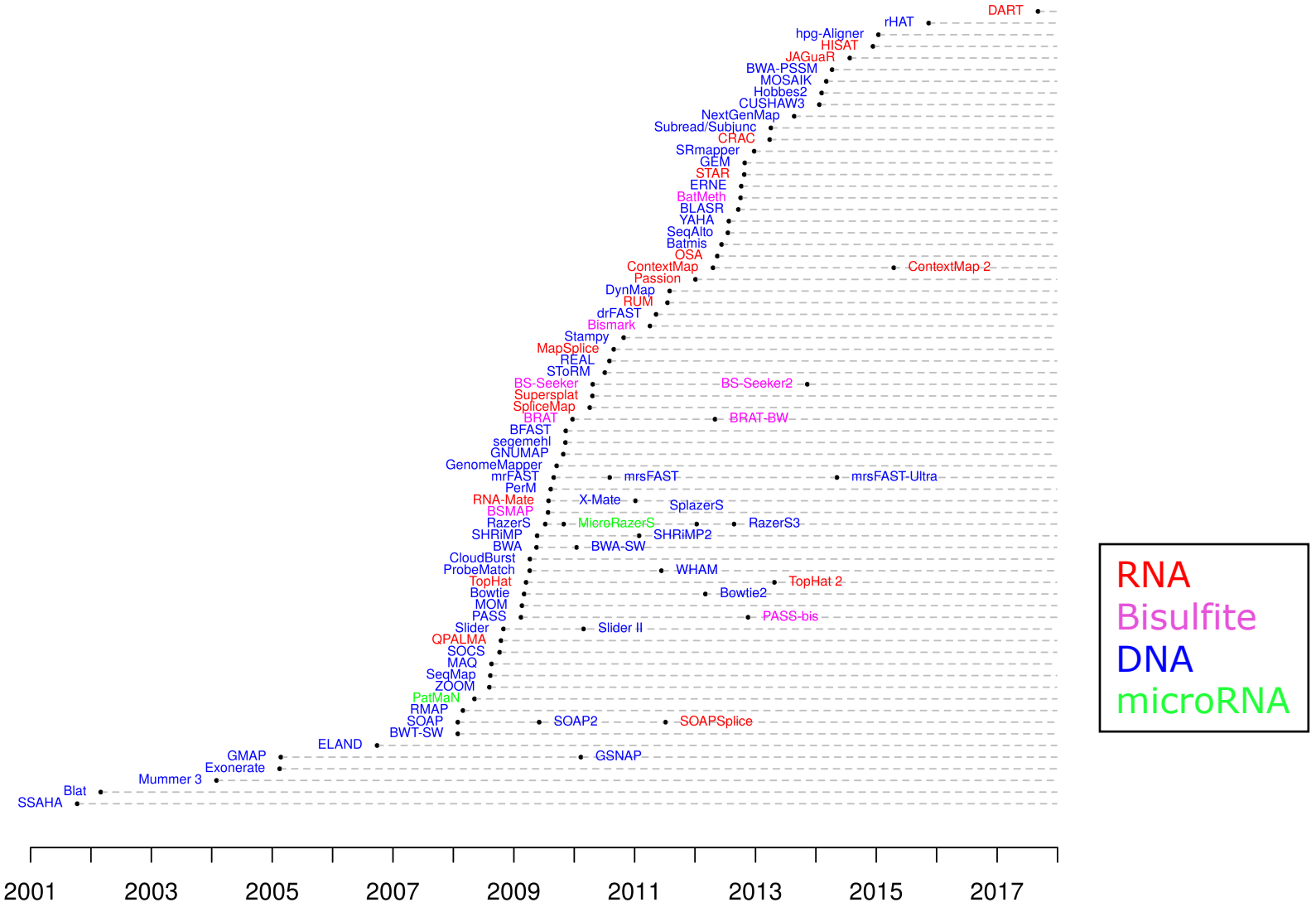



Diagrams from Cloonan & Grimmond, Nature Methods 2010

# Which alignment strategy is best?

- De novo assembly
  - If a reference genome does not exist for the species being studied
  - If complex polymorphisms/mutations/haplotypes might be missed by comparing to the reference genome
- Align to transcriptome
  - If you have short reads (< 50bp)
- Align to reference genome
  - All other cases
- Each strategy involves different alignment/assembly tools

# Which read aligner should I use?

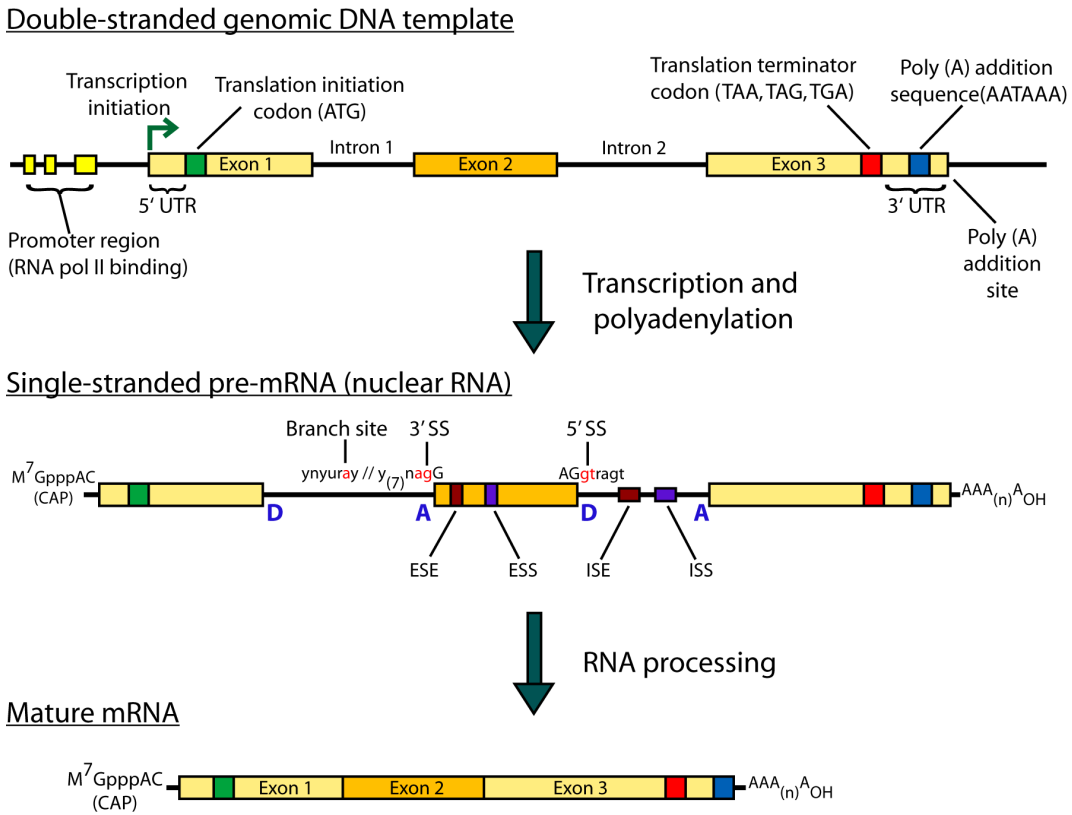



RNA
Bisulfite
DNA
microRNA

http://wwwdev.ebi.ac.uk/fg/hts_mappers/

# Should I use a splice-aware or unspliced mapper?

- RNA-seq reads may span large introns
- The fragments being sequenced in RNA-seq represent mRNA - introns are removed
- But we are usually aligning these reads back to the reference genome
- Unless your reads are short (<50bp) you should use a splice-aware aligner
  - HISAT2, STAR, MapSplice, etc.



# HISAT/HISAT2

- HISAT is a ‘splice-aware’ RNA-seq read aligner
- Requires a reference genome
- Very fast

- Uses an indexing scheme based on the Burrows-Wheeler transform and the Ferragina-Manzini (FM) index
- Multiple types of indexes for alignment
  - a whole-genome FM index to anchor each alignment
  - numerous local FM indexes for very rapid extensions of these alignments.
  - Whole-genome indices with SNPs and known transcript structures accounted for

Kim et al. 2015. Nat Methods 12:357–360

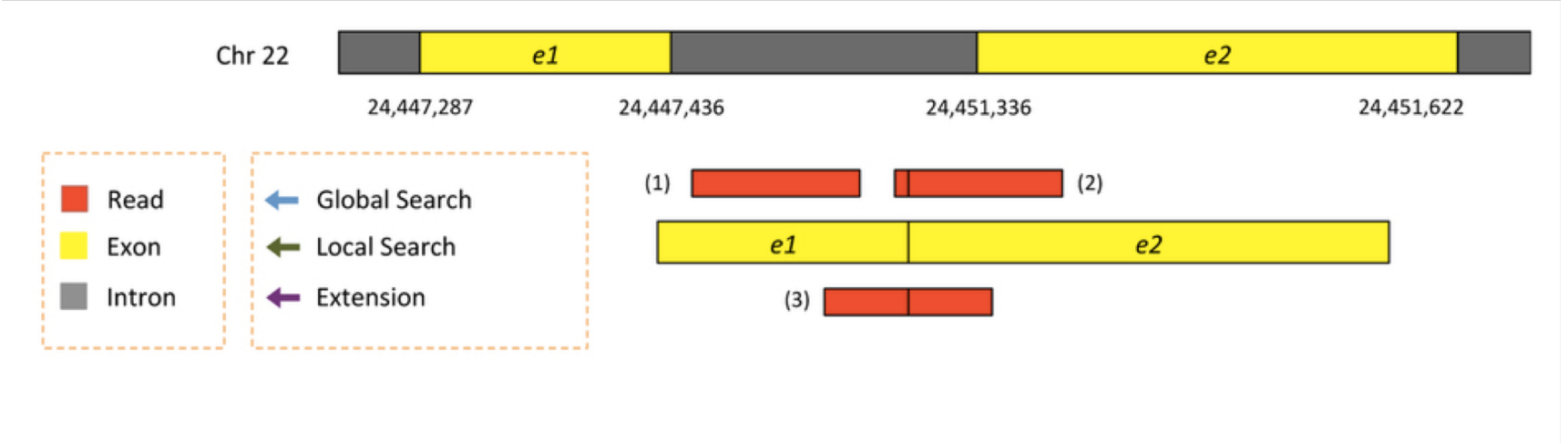
# HISAT/HISAT2 algorithm

- Uses a hierarchical indexing algorithm + several adaptive strategies
  - based on the position of a read with respect to splice sites

1) Find candidate locations across the whole genome first
   - mapping part of each read using the global FM index
   - Generally identifies one or a small number of candidates.
2) Do local alignment
   - selects one of ~48,000 local indexes for each candidate
   - uses it to align the remainder of the read.

- For paired reads, each mate is separately aligned
  - If a read fails to align, then the alignments of its mate are used as anchors to map the unaligned mate

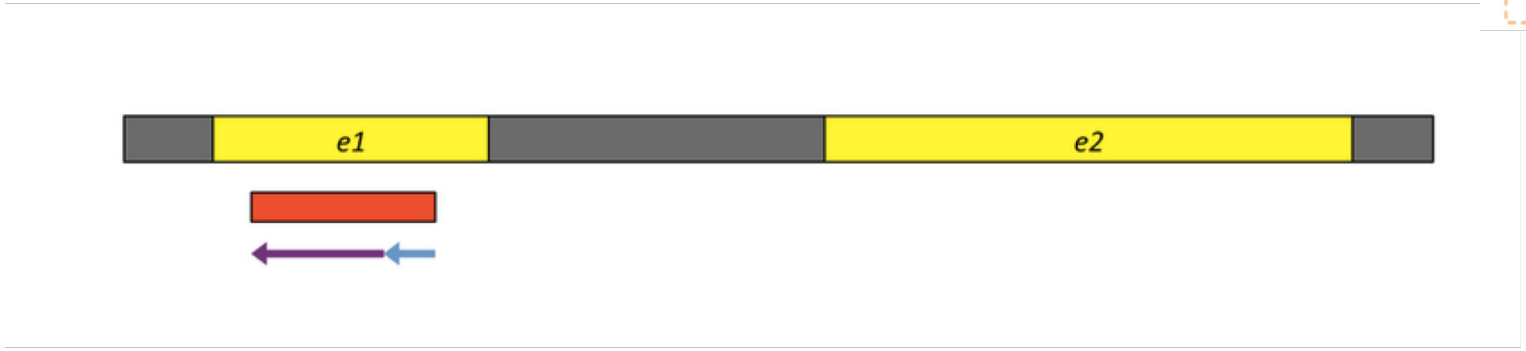
# HISAT2 Alignment




- Two exons from chr22
- Three reads

Kim et al. 2015. Nat Methods 12:357–360

# HISAT2 Alignment

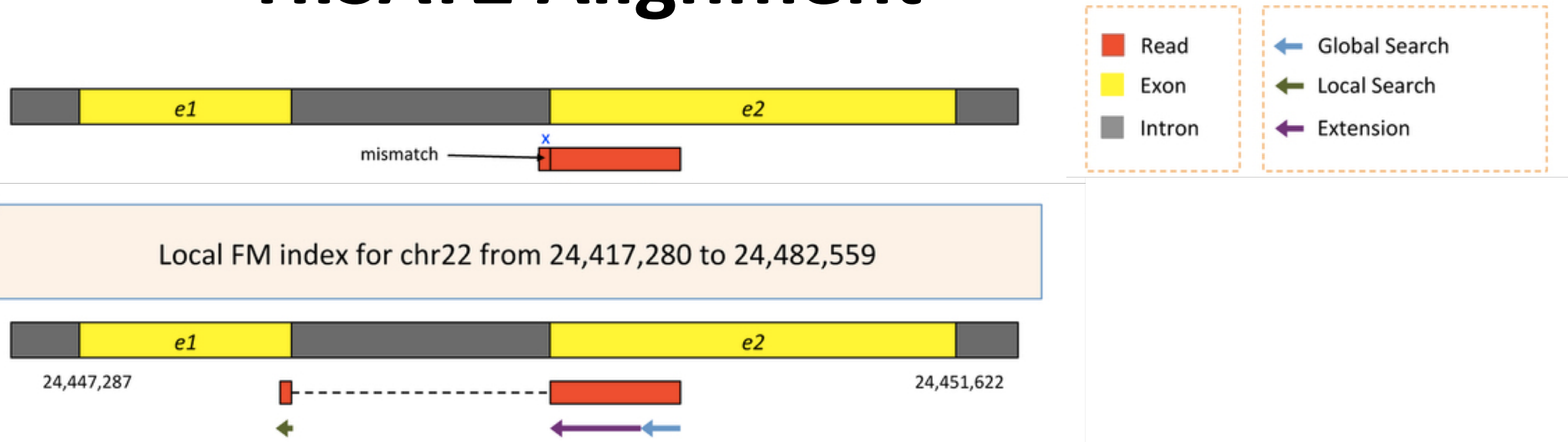


1) Search for read position with global FM index (slower)

2) Once at least 28bp and exactly one location switch to extension mode against reference genome (faster)

Kim et al. 2015. Nat Methods 12:357–360

# HISAT2 Alignment




1) Search for read position with global FM index (slower)

2) Extend until mismatch at 93bp (faster)

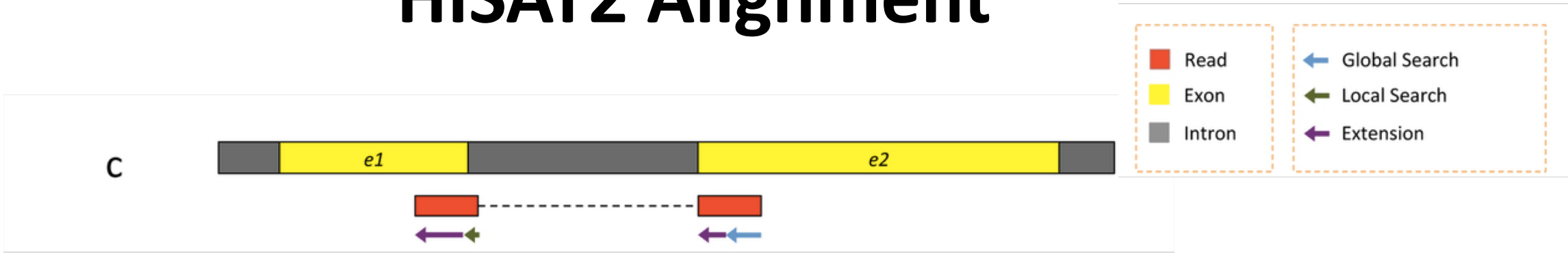
3) Switch to local FM index to align remaining 8bp

   - index covers only a small region, so we find just one match

4) Check for compatibility and combine into single spliced alignment

Kim et al. 2015. Nat Methods 12:357–360

# HISAT2 Alignment




1) global search until exactly one match of at least 28bp (slower)

2) Extend until mismatch at 51bp (faster)

3) switch to local FM index to align first 8bp of remaining read

    - If too many matches increase prefix size

4) Extend again

5) Check for compatibility and combine into single spliced alignment

Kim et al. 2015. Nat Methods 12:357–360

# Should I allow ‘multi-mapped’ reads?

- Depends on the application
- In *DNA* analysis it is common to use a mapper to randomly select alignments from a series of equally good alignments
- In *RNA* analysis this is less common
  - Perhaps disallow multi-mapped reads if you are variant calling
  - Definitely should allow multi-mapped reads for expression analysis with Cufflinks (and StringTie?)
  - Definitely should allow multi-mapped reads for gene fusion discovery

# What is the output of HISAT2?

- A SAM/BAM file
  - SAM stands for Sequence Alignment/Map format
  - BAM is the binary version of a SAM file

- Remember, compressed files require special handling compared to plain text files

- How can I convert BAM to SAM?
  - http://www.biostars.org/p/1701/

- Is HISAT2 the only mapper to consider for RNA-seq data?
  - http://www.biostars.org/p/60478/