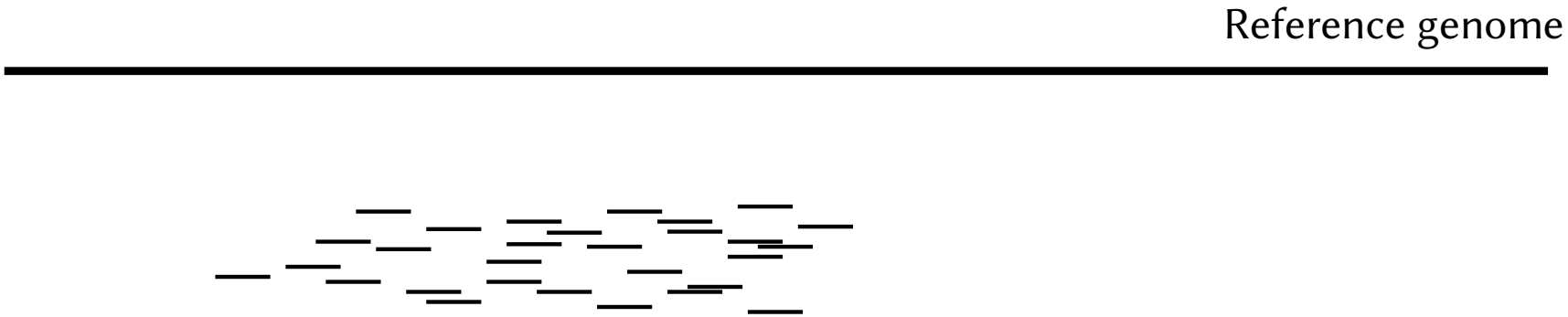
# To map or not to map?

Formation RNA-Seq – Bilille

Mikaël Salson
mikael.salson@univ-lille.fr

# RNA-Seq read mapping



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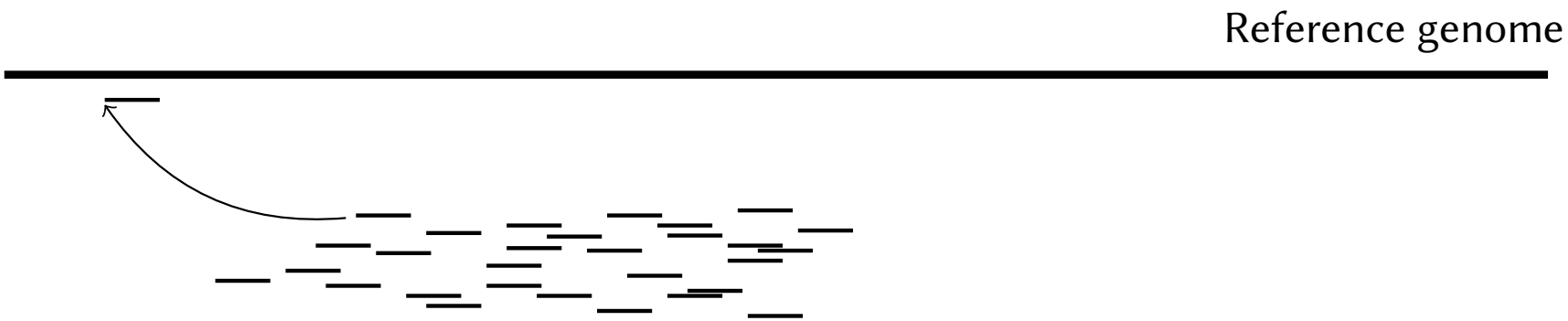

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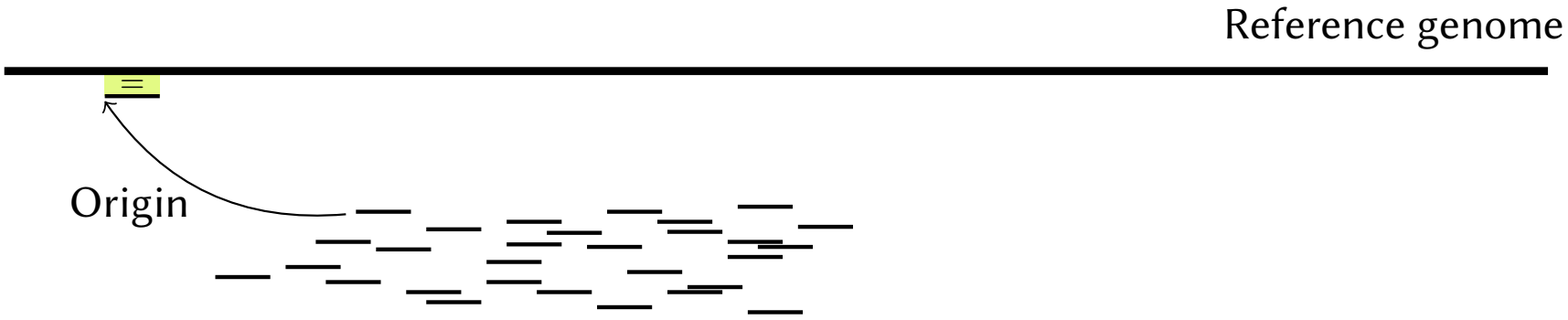

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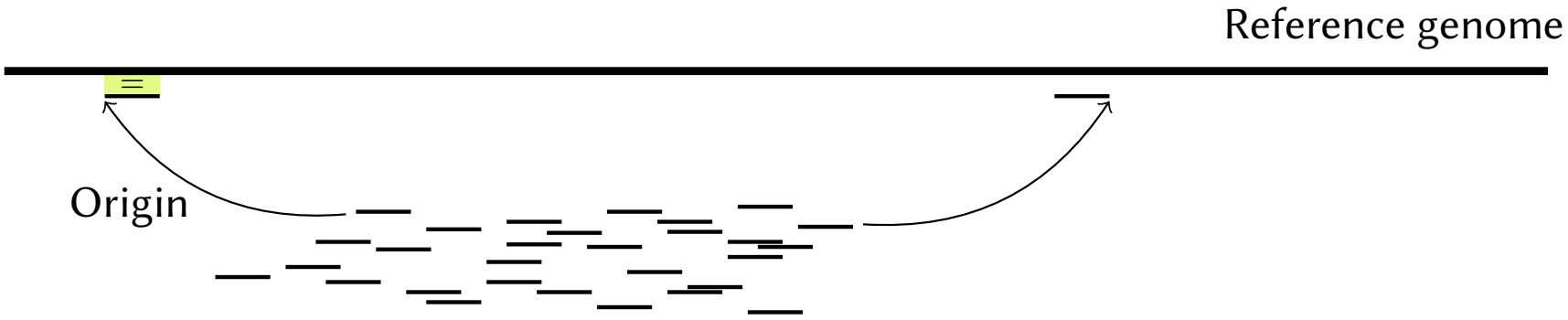

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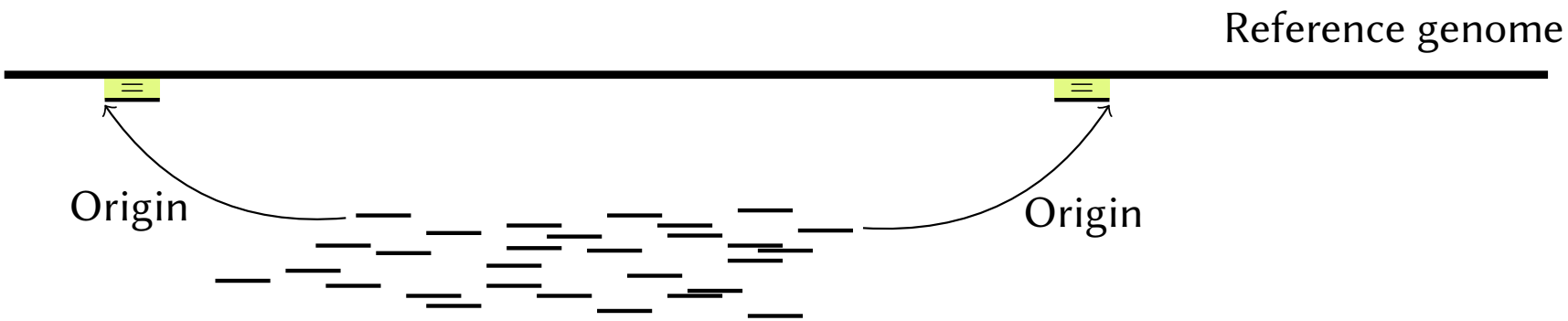

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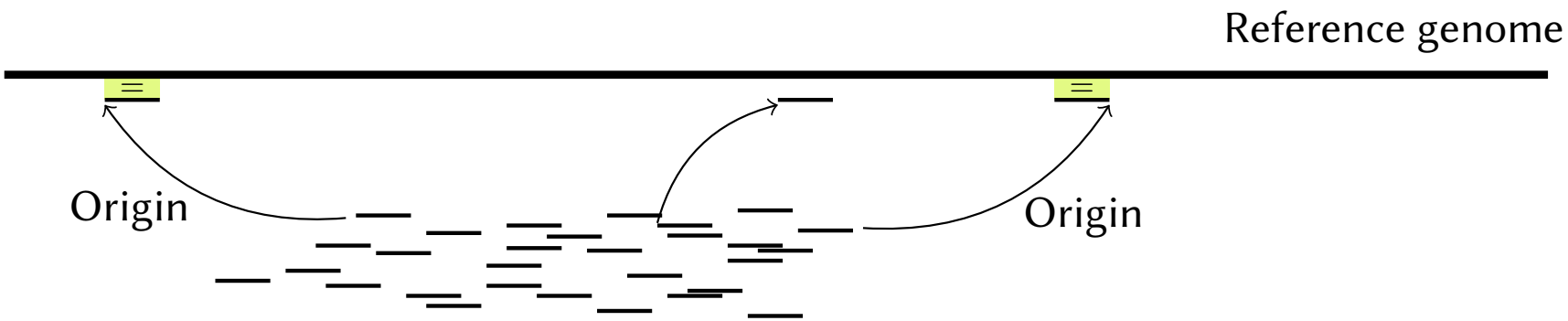

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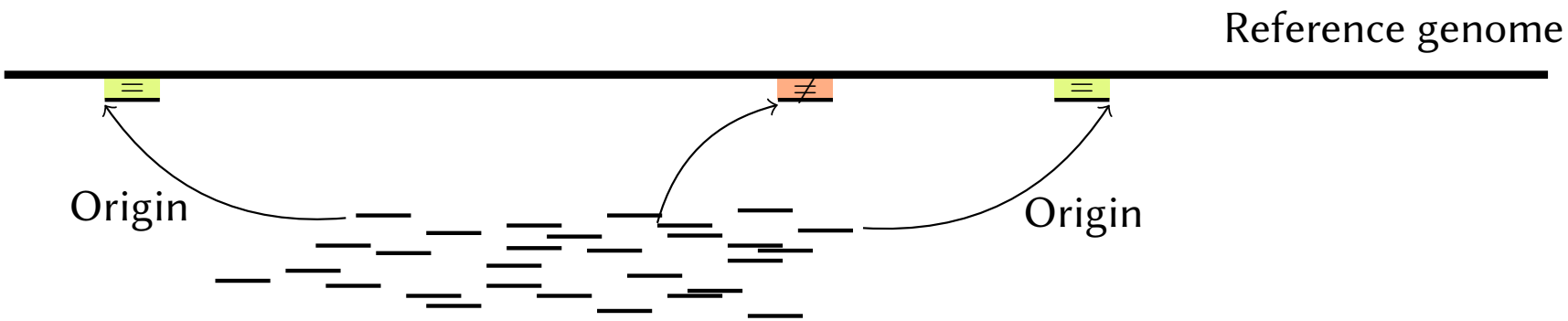

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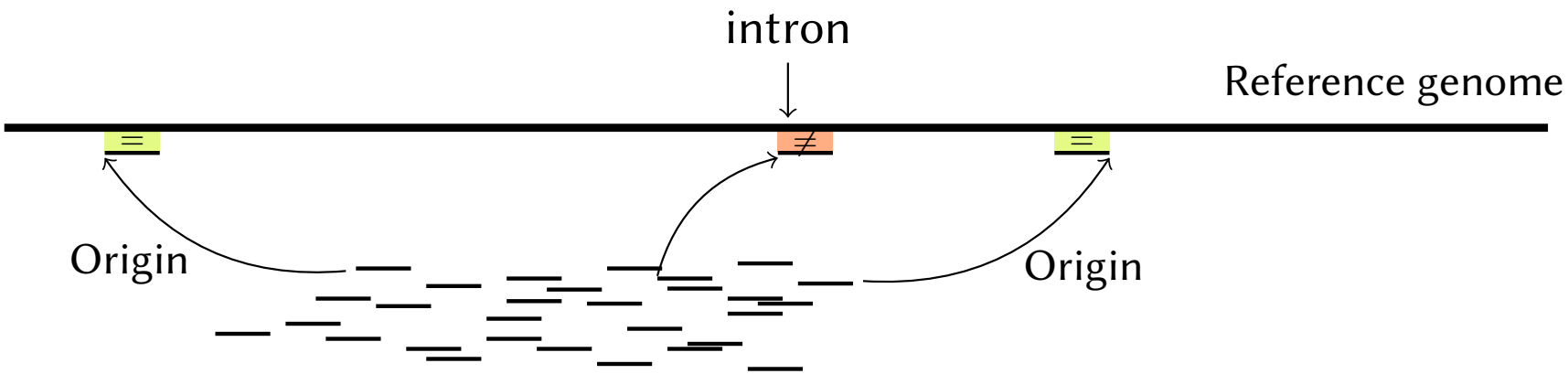

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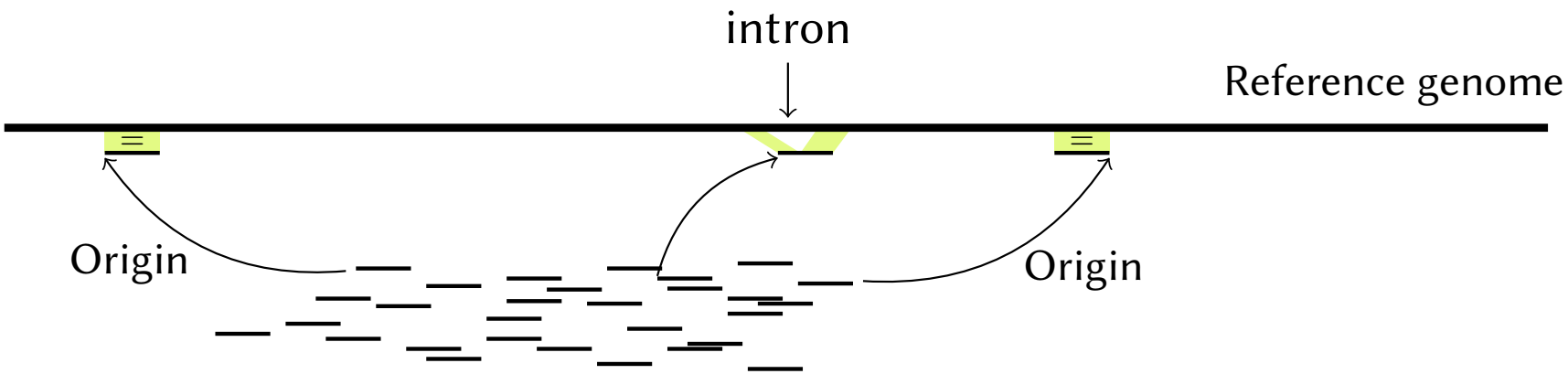

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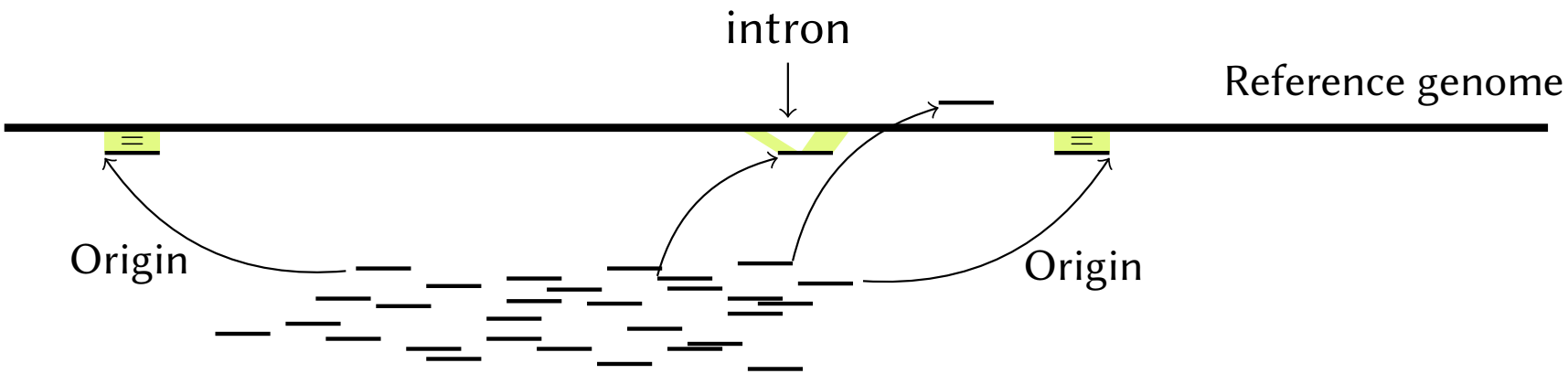

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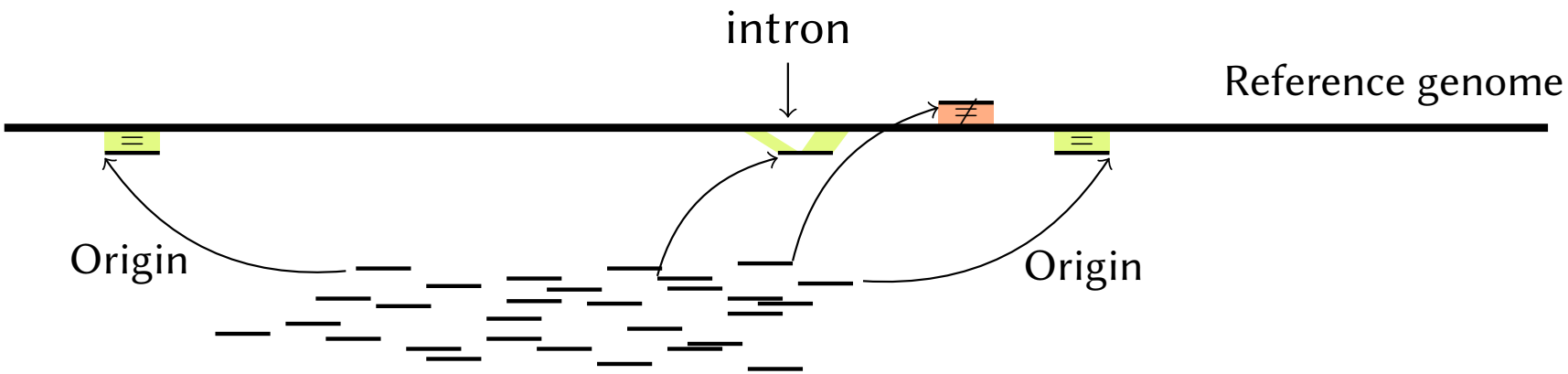

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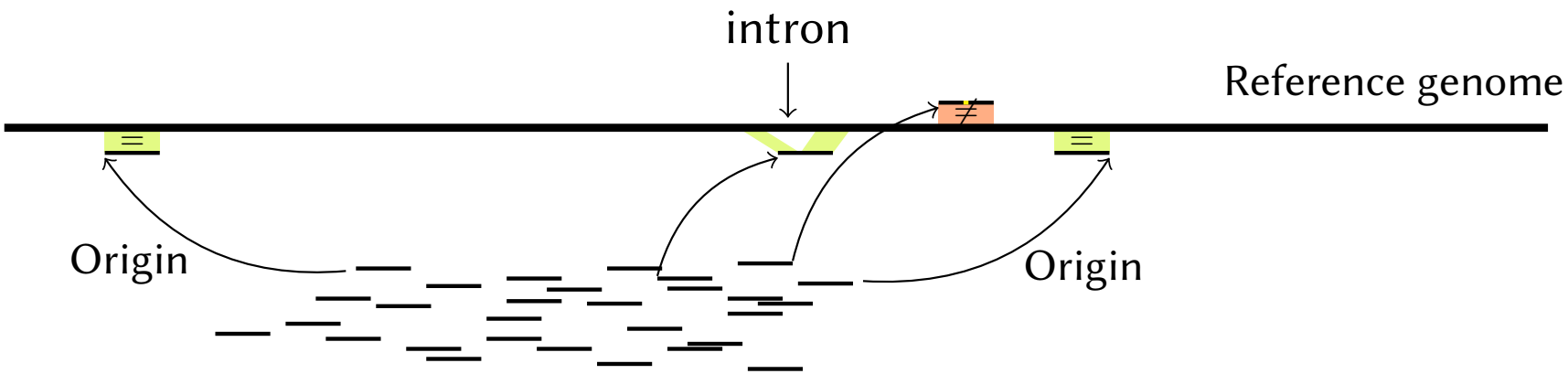

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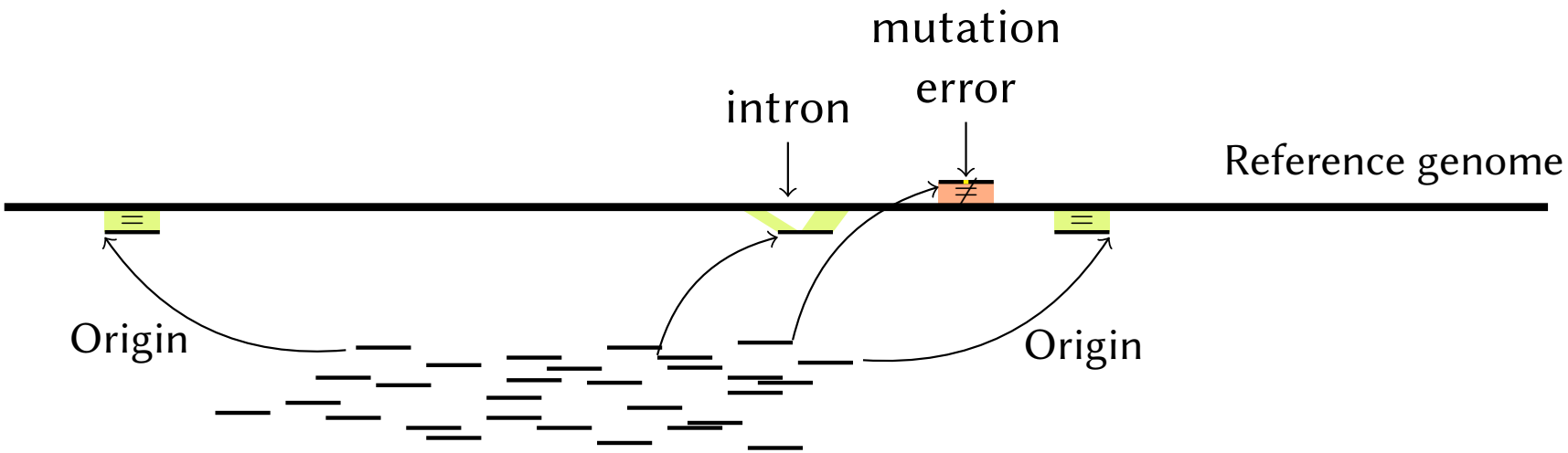

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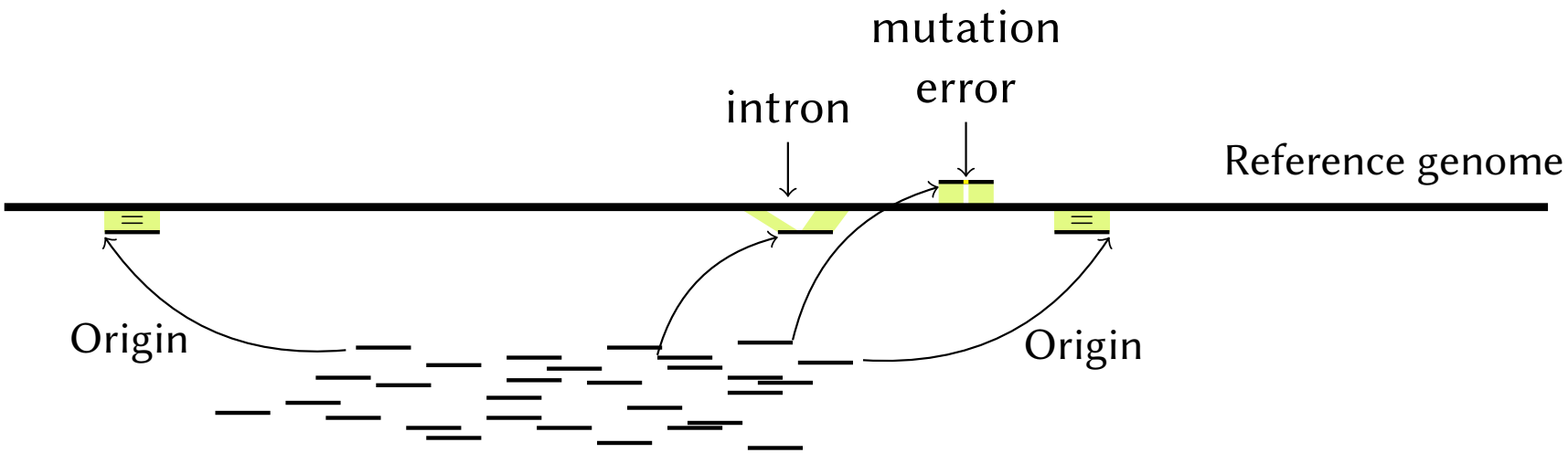

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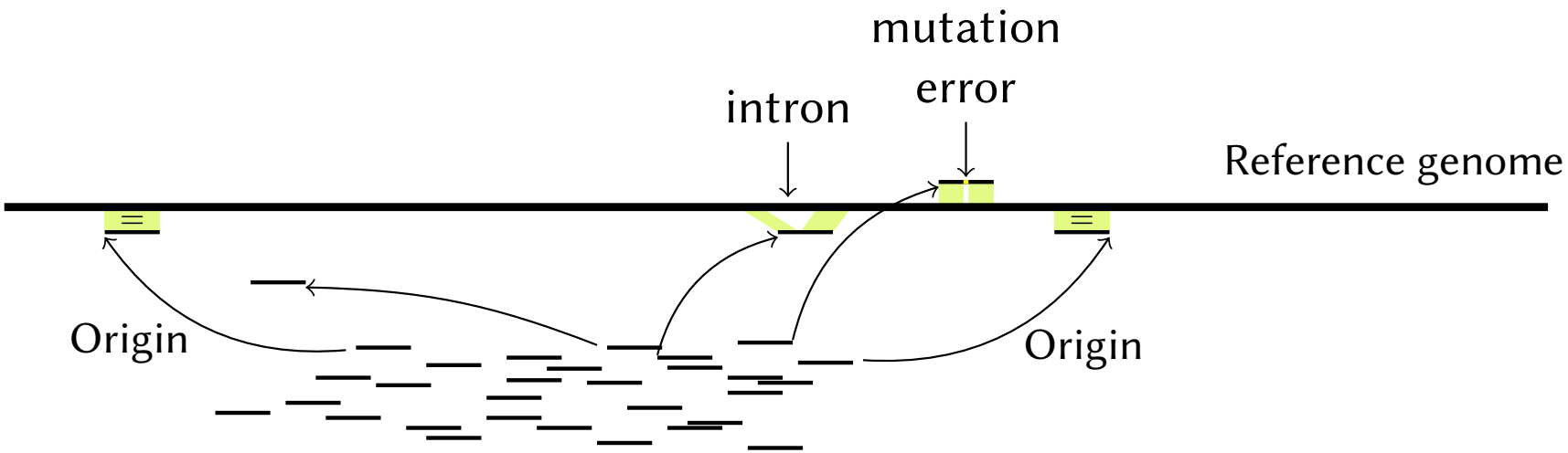

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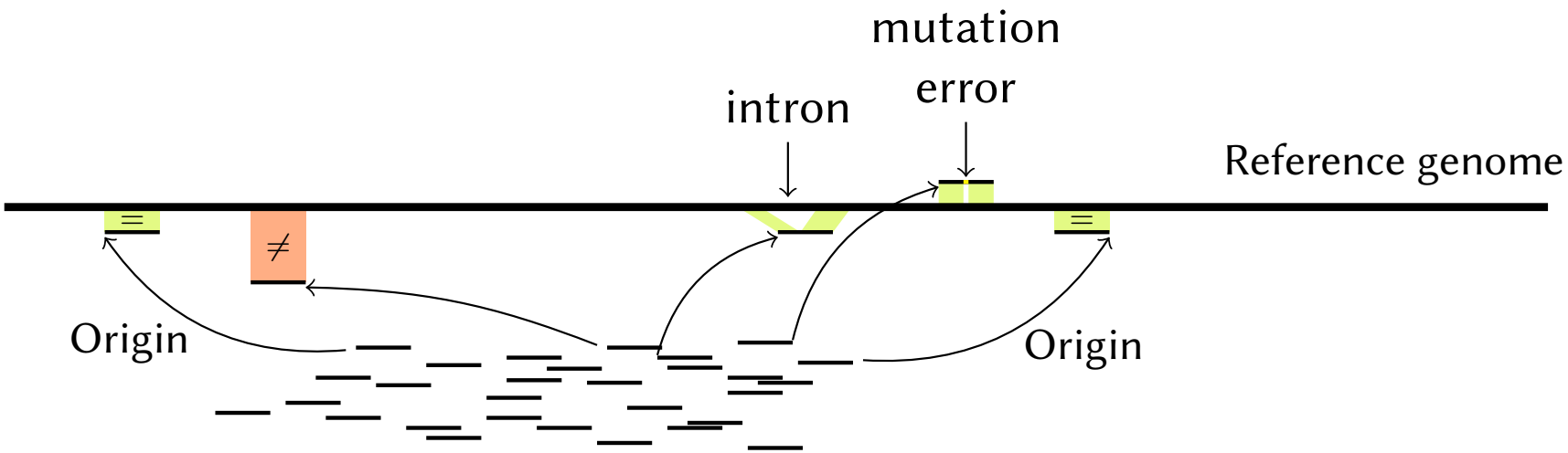

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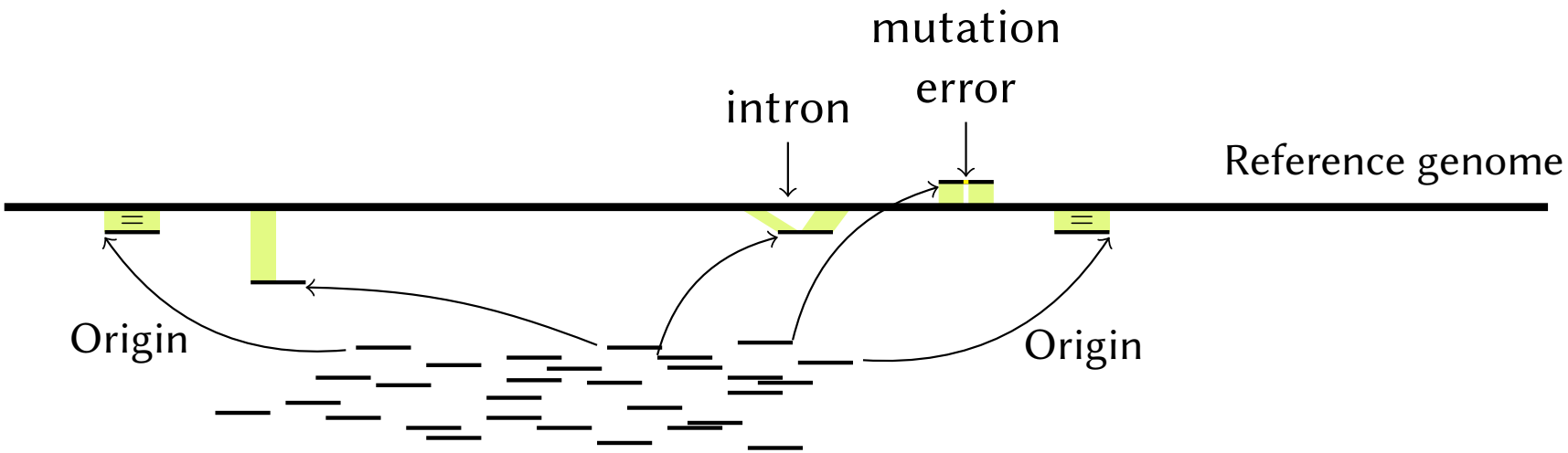

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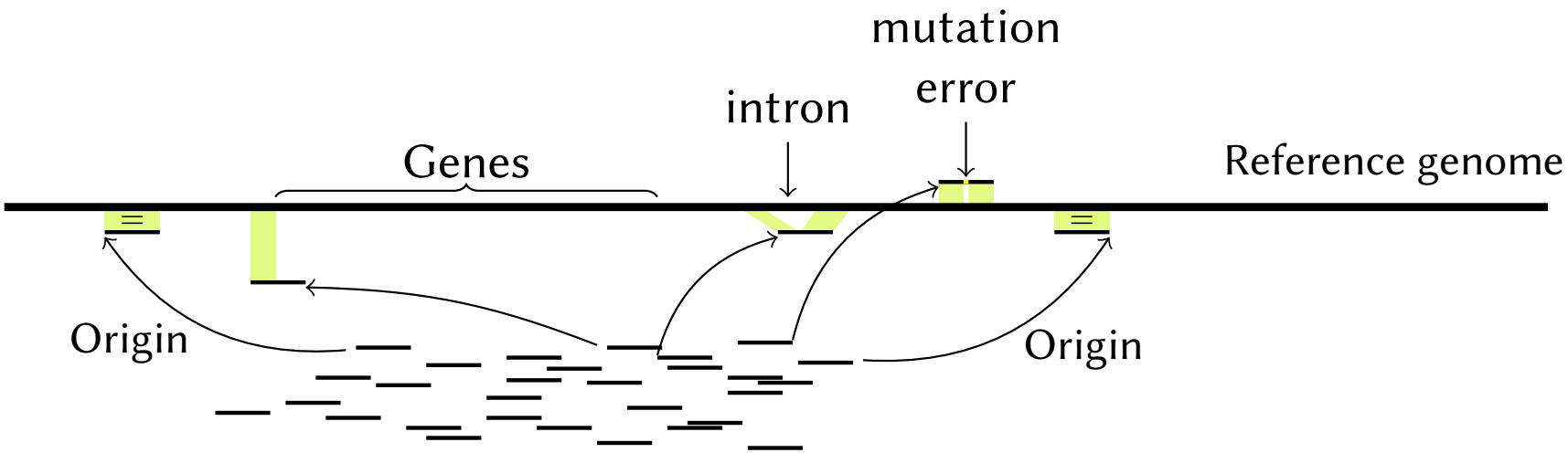

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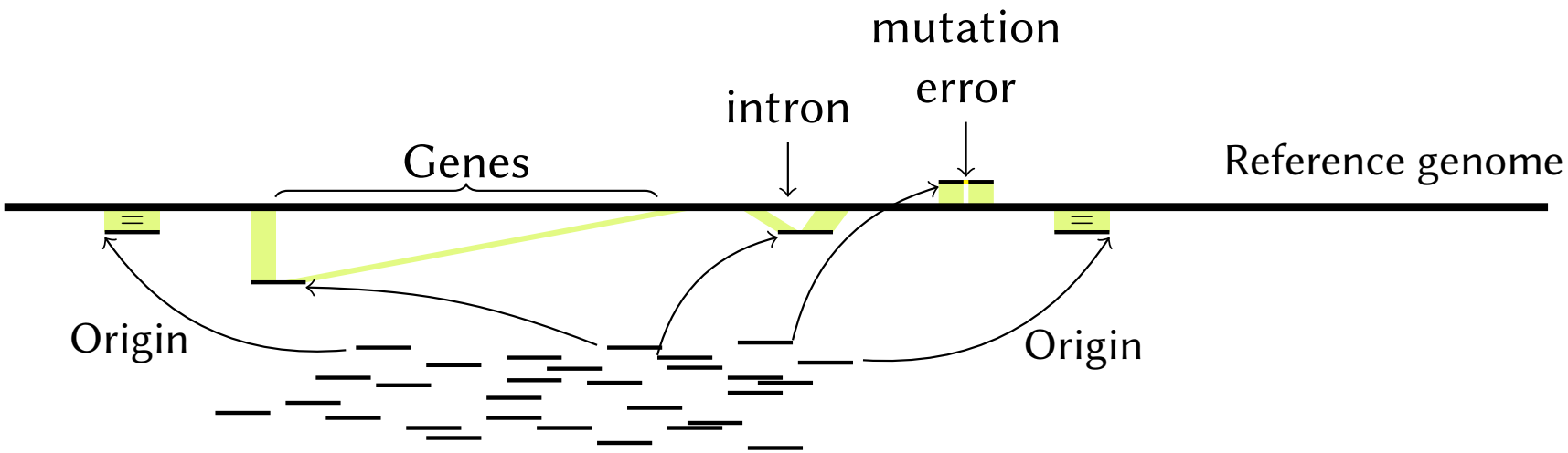

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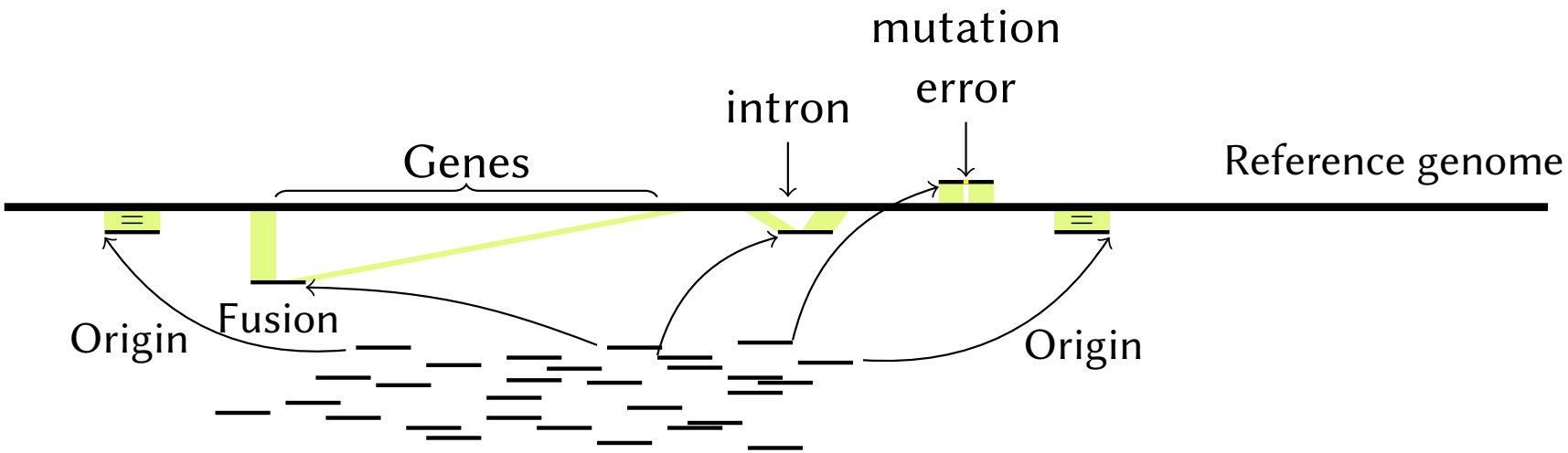

# Split reads don't align contiguously to the genome

Reads

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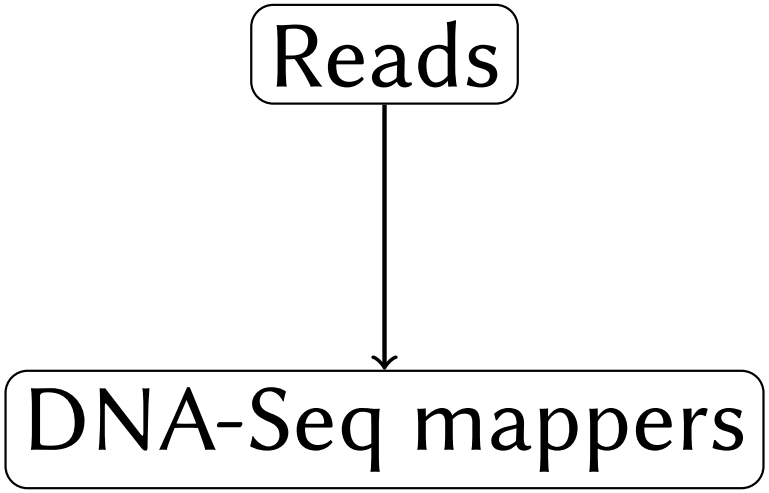

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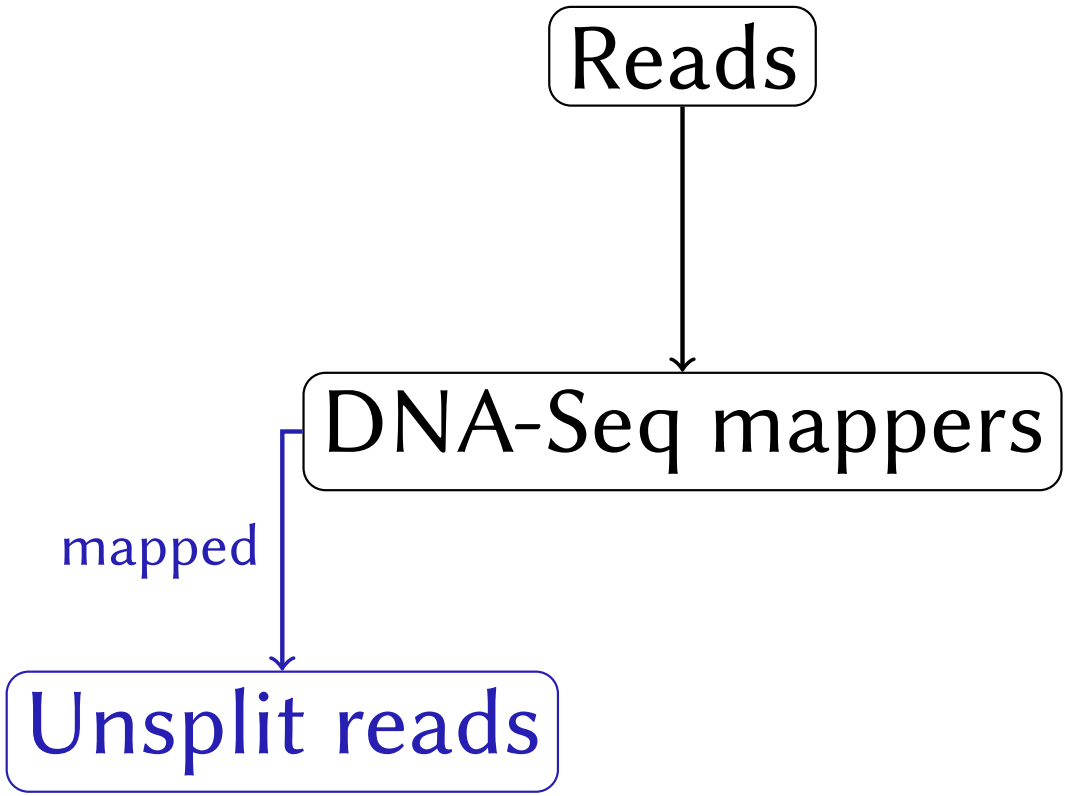

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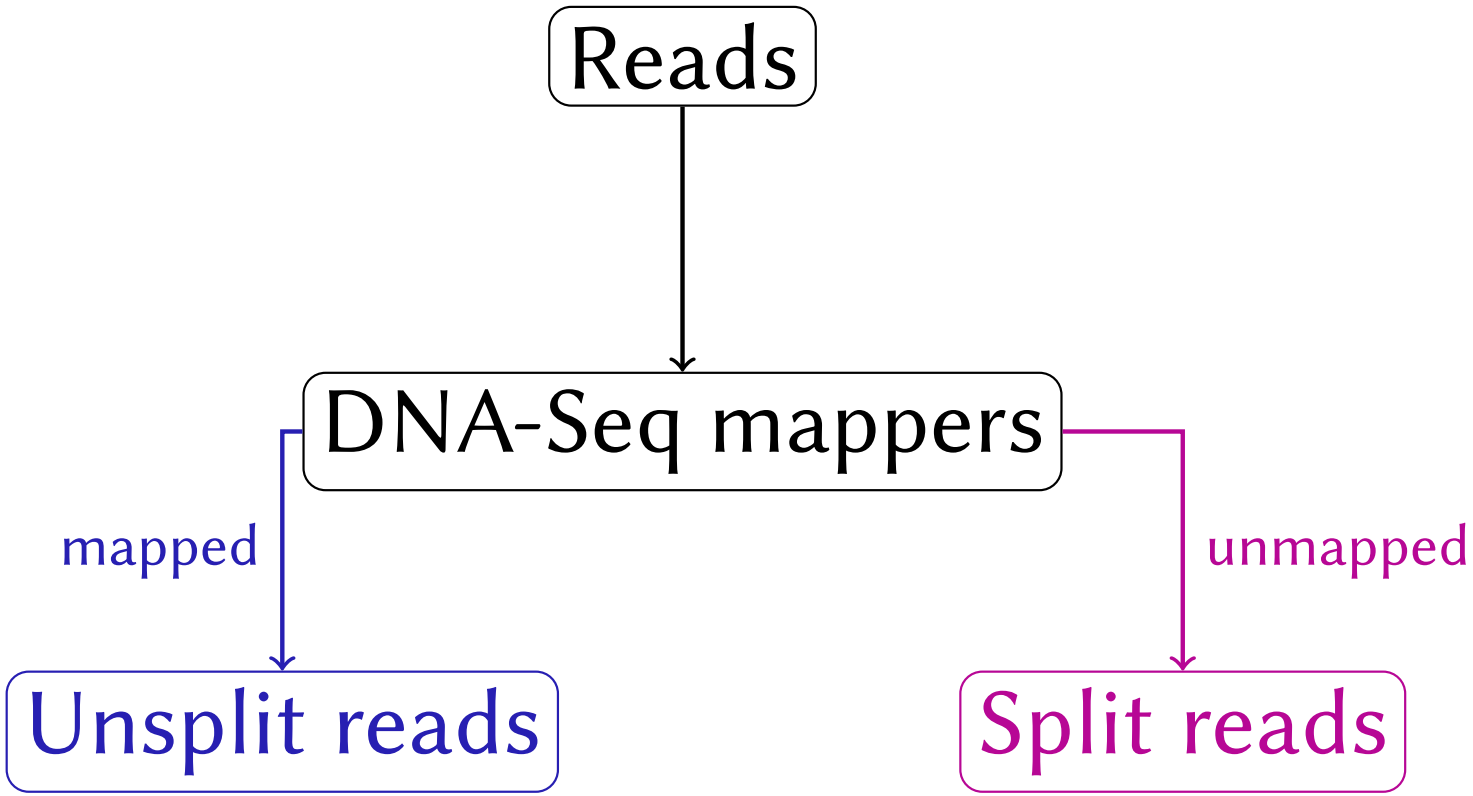

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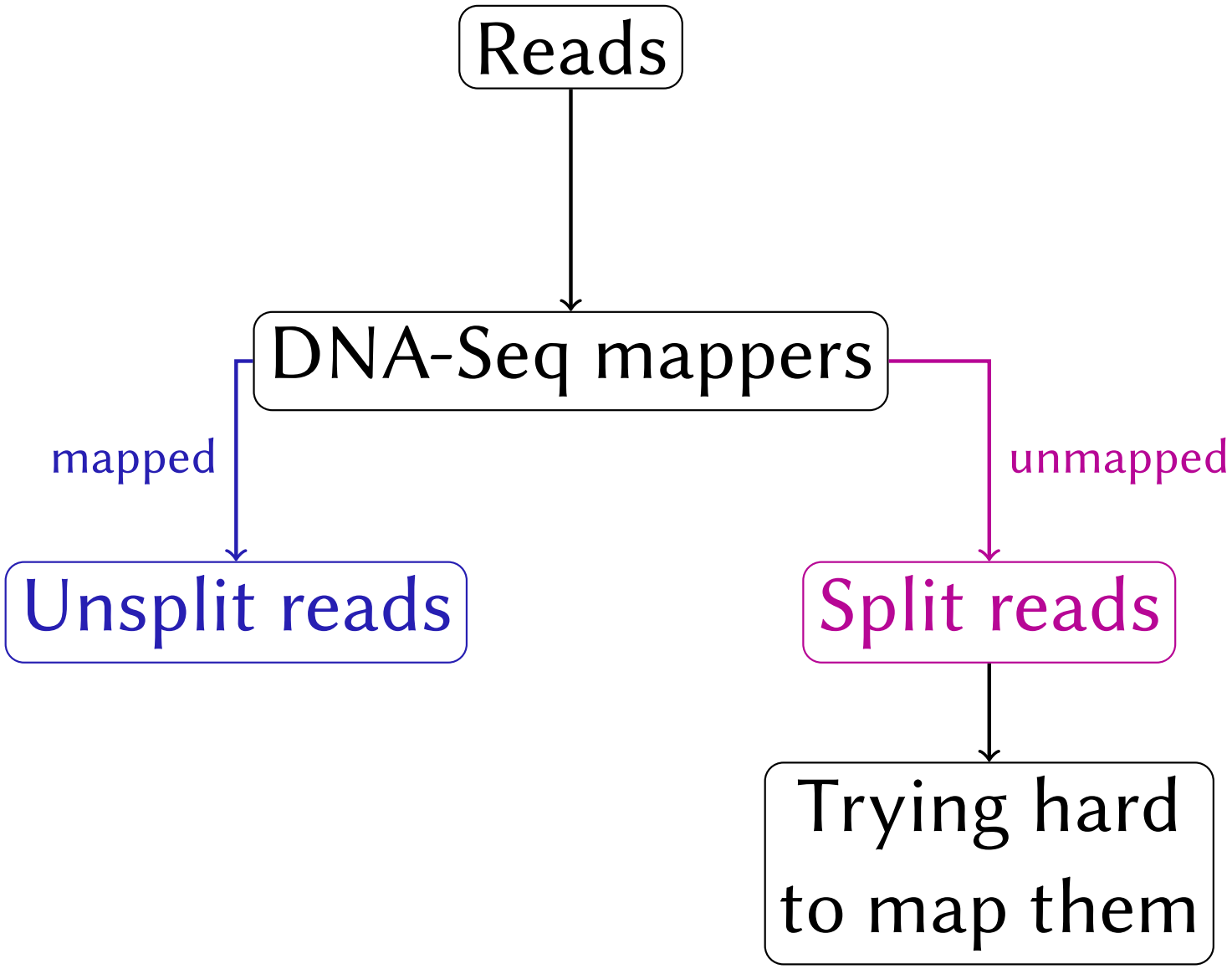
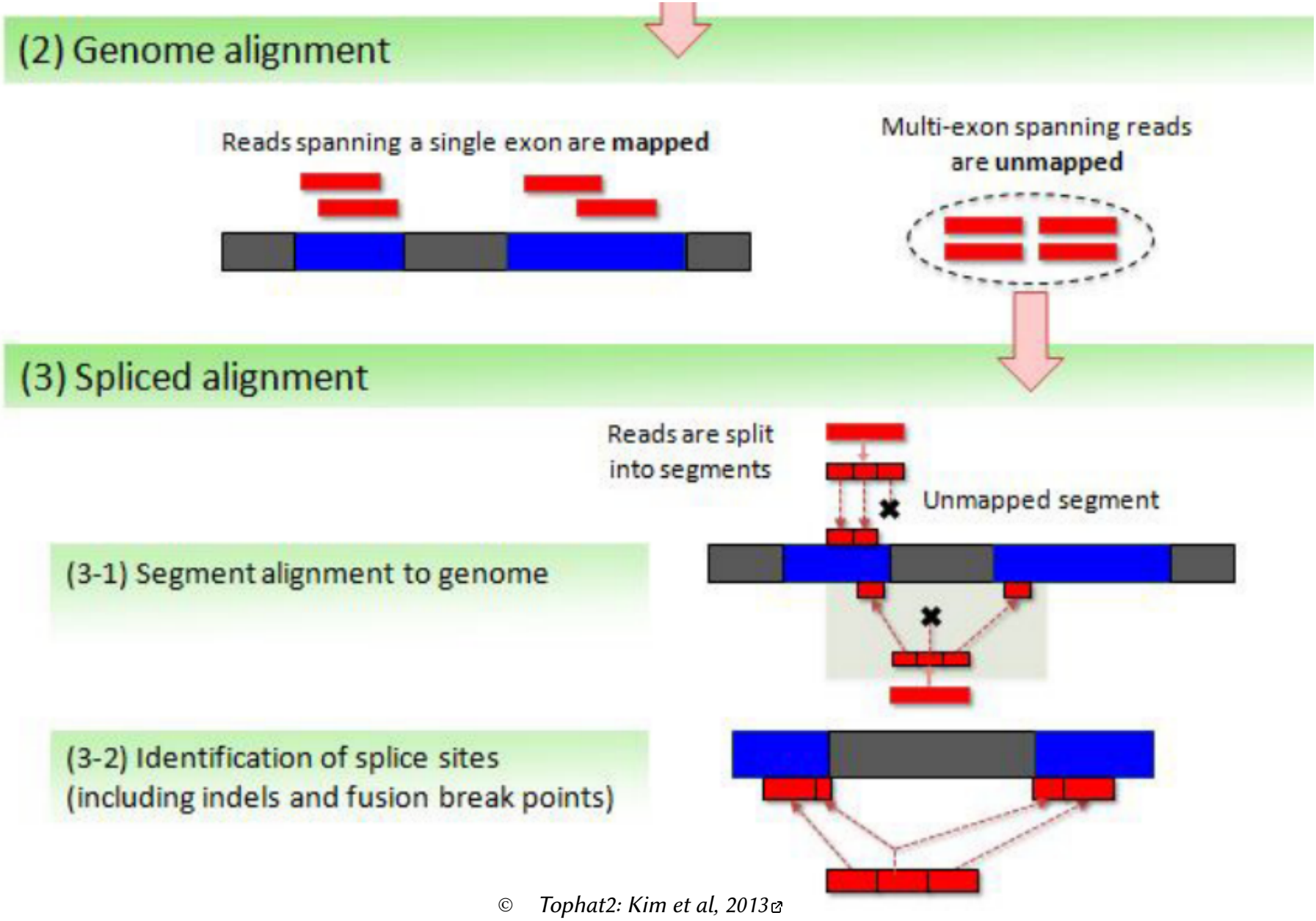
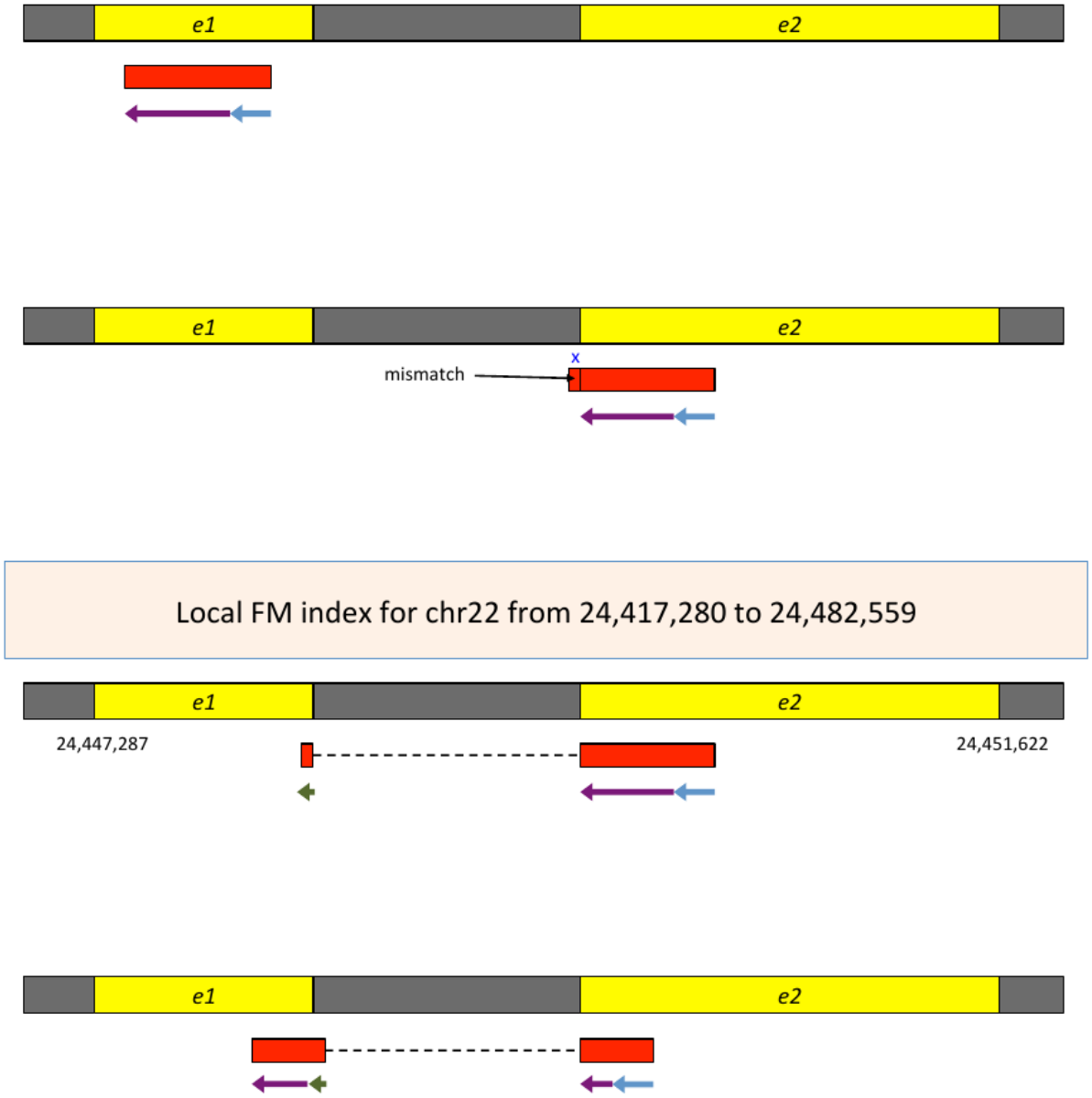


# Mapping split reads by… splitting them – TopHat2



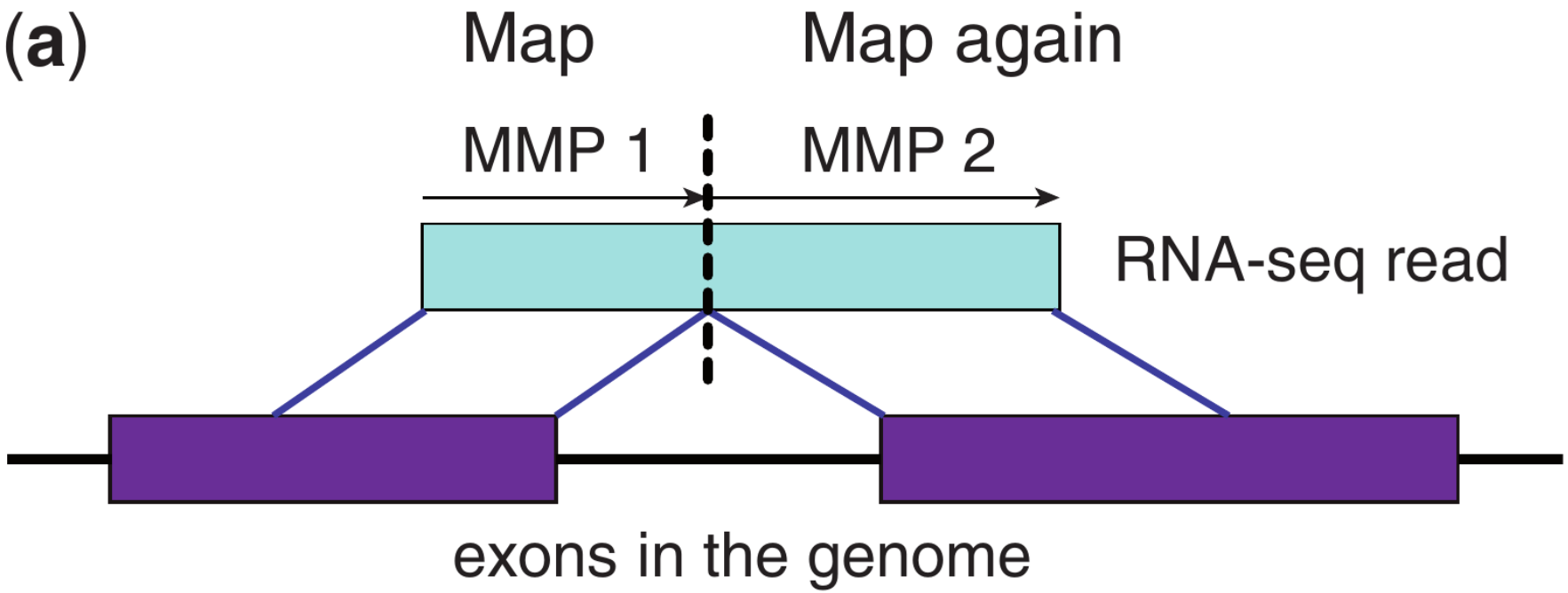

© *Tophat2: Kim et al, 2013*

# Mapping all reads by splitting them – HISAT2, STAR




© *HISAT: Kim, Langmead, Salzberg, Nat. Methods, 2015*

# Mapping all reads by splitting them – HISAT2, STAR




© *STAR: Dobin et al, Bioinformatics, 2013*

# Mapping methods

| | |
|---|---|
| **TopHat2** | Exact contiguous fixed-lengh |
| **HISAT** | Maximal mappable suffix |
| **STAR** | Maximal mappable prefix |

# Specificities of the approaches

## Mapping methods

| | |
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| **TopHat2** | Exact contiguous fixed-lengh |
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## Indexing methods

| | |
|---|---|
| **TopHat2** | FM-index |
| **HISAT** | Multiple FM-indices |
| **STAR** | Suffix Array |

# Indexing methods

$$T = \overset{0}{\mathrm{C}}\ \overset{1}{\mathrm{T}}\ \overset{2}{\mathrm{A}}\ \overset{3}{\mathrm{G}}\ \overset{4}{\mathrm{T}}\ \overset{5}{\mathrm{T}}\ \overset{6}{\mathrm{A}}\ \overset{7}{\mathrm{G}}\ \overset{8}{\$}$$

# Indexing methods

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| $T =$ | C | T | A | G | T | T | A | G | $ |

| TS | 8 | 6 | 2 | 0 | 7 | 3 | 5 | 1 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| | $ | A | A | C | G | G | T | T | T |
| | | G | G | T | $ | T | A | A | T |
| | | $ | T | A | | T | G | G | A |
| | | | T | G | | A | $ | T | G |
| | | | A | T | | G | | T | $ |
| | | | G | T | | $ | | A | |
| | | | $ | A | | | | G | |
| | | | | G | | | | $ | |

# Indexing methods

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| $T =$ | C | T | A | G | T | T | A | G | $ |

| TS | 8 | 6 | 2 | 0 | 7 | 3 | 5 | 1 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| | $ | A | A | C | G | G | T | T | T |
| | C | G | G | T | $ | T | A | A | T |
| | T | $ | T | A | C | T | G | G | A |
| | A | C | T | G | T | A | $ | T | G |
| | G | T | A | T | A | G | C | T | $ |
| | T | A | G | T | G | $ | T | A | C |
| | T | G | $ | A | T | C | A | G | T |
| | A | T | C | G | T | T | G | $ | A |
| | G | T | T | $ | A | A | T | C | G |

# Indexing methods

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| $T =$ | C | T | A | G | T | T | A | G | $ |

| TS | 8 | 6 | 2 | 0 | 7 | 3 | 5 | 1 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| | $ | A | A | C | G | G | T | T | T |
| | C | G | G | T | $ | T | A | A | T |
| | T | $ | T | A | C | T | G | G | A |
| | A | C | T | G | T | A | $ | T | G |
| | G | T | A | T | A | G | C | T | $ |
| | T | A | G | T | G | $ | T | A | C |
| | T | G | $ | A | T | C | A | G | T |
| | A | T | C | G | T | T | G | $ | A |
| | **G** | **T** | **T** | **$** | **A** | **A** | **T** | **C** | **G** |

Burrows-Wheeler Transform

# What approach is the best? (slide courtesy of J. Audoux)

NATURE METHODS | ANALYSIS

## Simulation-based comprehensive benchmarking of RNA-seq aligners

Giacomo Baruzzo, Katharina E Hayer, Eun Ji Kim, Barbara Di Camillo, Garret A FitzGerald & Gregory R Grant

METHOD | OPEN ACCESS

## A benchmark for RNA-seq quantification pipelines

Mingxiang Teng, Michael I. Love, Carrie A. Davis, Sarah Djebali, Alexander Dobin, Brenton R. Graveley, Sheng Li, Christopher E. Mason, Sara Olson, Dmitri Pervouchine, Cricket A. Sloan, Xintao Wei, Lijun Zhan and Rafael A. Irizarry

0940-1 | © Teng et al. 2016

NATURE METHODS | ANALYSIS OPEN

## Systematic evaluation of spliced alignment programs for RNA-seq data

Pär G Engström, Tamara Steijger, Botond Sipos, Gregory R Grar
RGASP Consortium, Gunnar Rätsch, Nick Goldman, Tim J Hubb
Roderic Guigó & Paul Bertone

Article | OPEN

## Comparative assessment of methods for the fusion transcripts detection from RNA-Seq data

Am J Hum Genet. 2013 Oct 3; 93(4): 641–651. PMCID: PMC3
doi: 10.1016/j.ajhg.2013.08.008

## Reliable Identification of Genomic Variants from RNA-Seq Data

Robert Piskol,[1] Gokul Ramaswami,[1] and Jin Billy Li[1,*]

Author information ▶ Article notes ▶ Copyright and License information ▶

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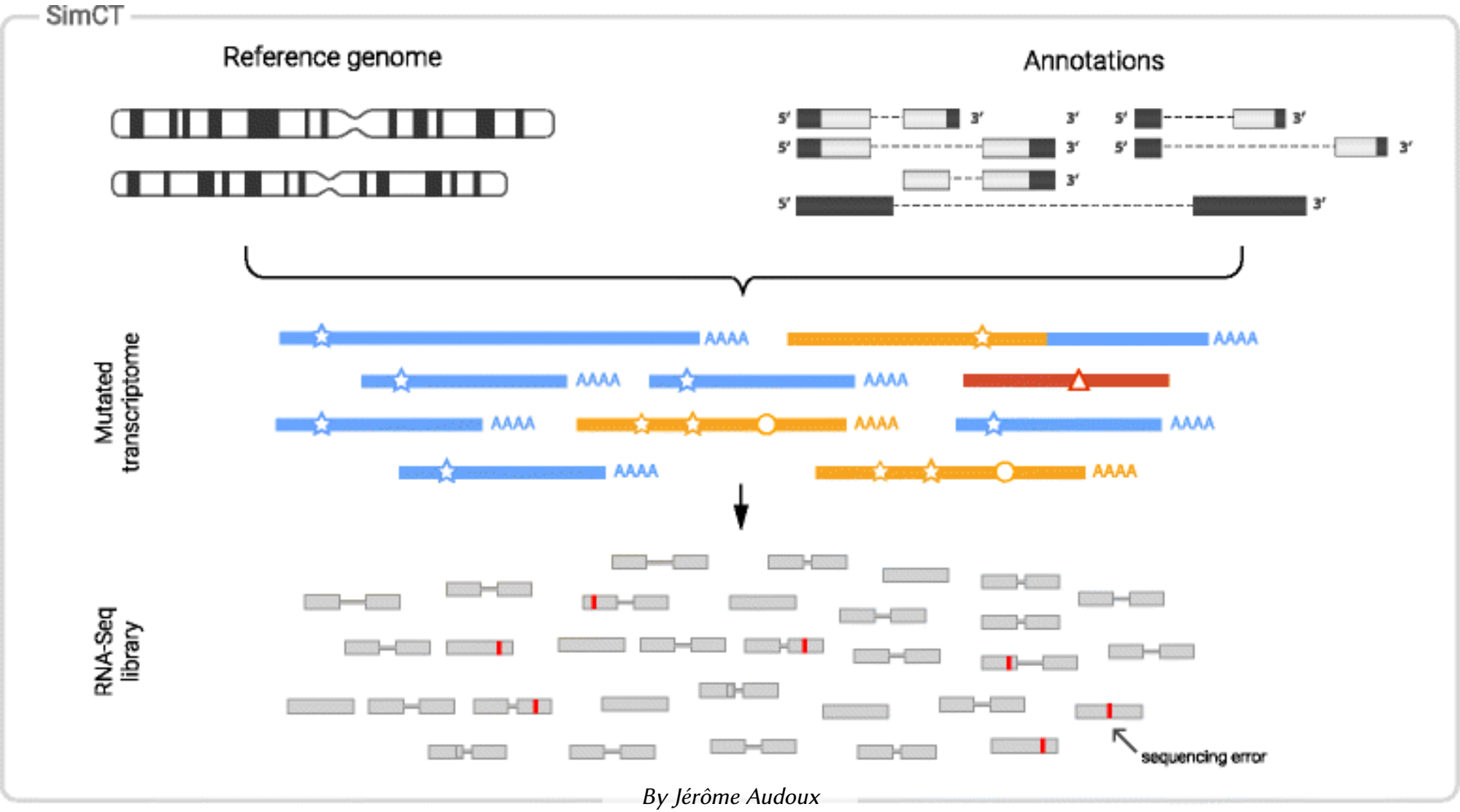
# Benchmarking RNA-Seq aligners

Audoux *et al*, BMC Bioinformatics, 2017




*By Jérôme Audoux*

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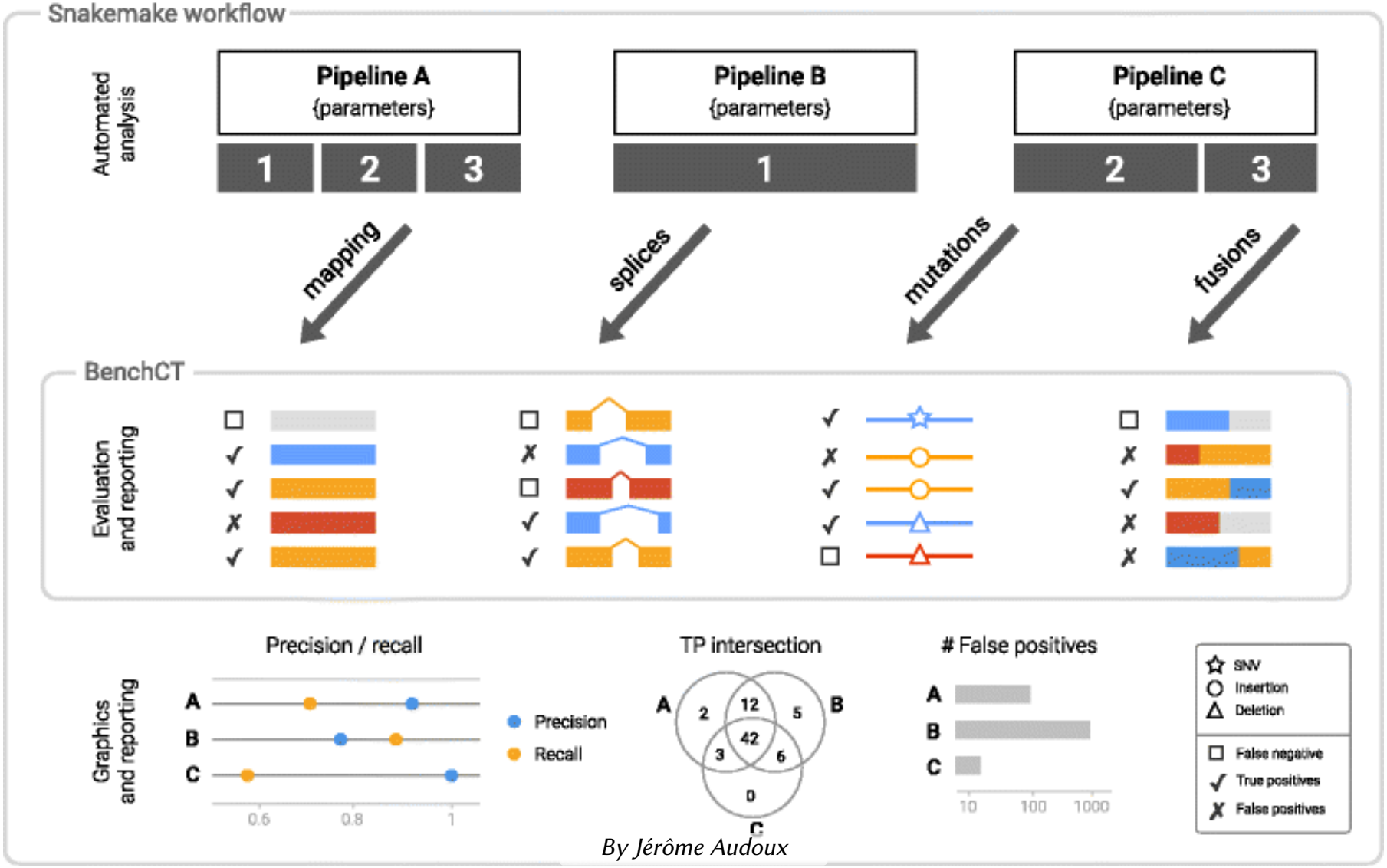



*By Jérôme Audoux*

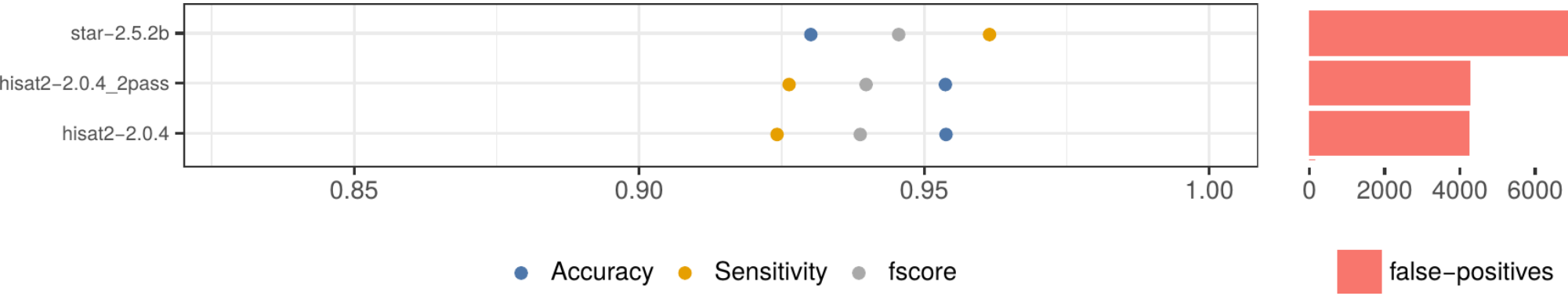
# Sensitivity/accuracy of read mappers

*By Jérôme Audoux*

# STAR offers the best trade-off for splice detection

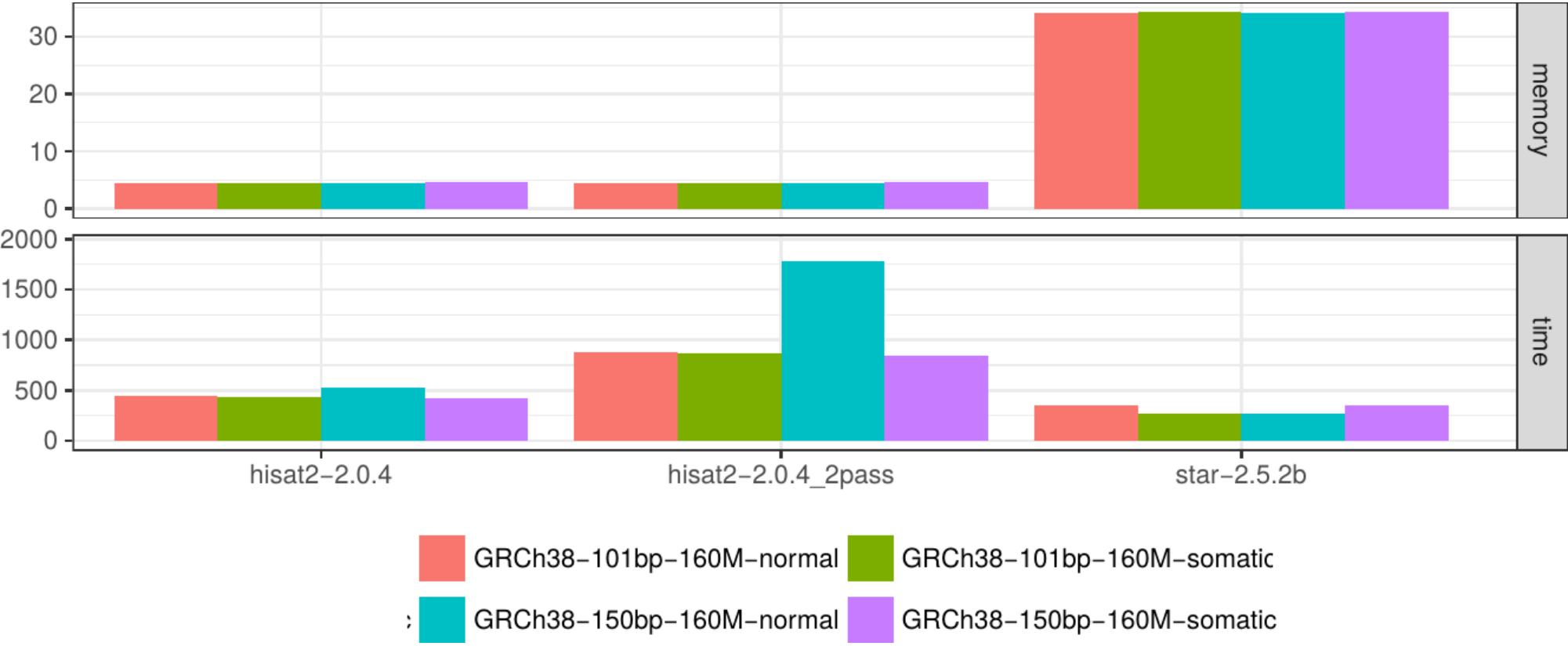
Splicing

160M 150bp reads from GRCh38




*By Jérôme Audoux*

# Space/time for read mappers




*By Jérôme Audoux*

# Many people uses TopHat2

($>$ 10,623 citations in Scholar, $> 1,000$ citations in 2021 only)

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**but don't**

# Many people uses TopHat2

($>$ 10,623 citations in Scholar, $>$ 1, 000 citations in 2021 only)

## but don't

### On TopHat2 website (since Feb 2016)

TopHat2 « *is now largely superseded by HISAT2 which provides the same core functionality (i.e. spliced alignment of RNA-Seq reads), in a more accurate and* ***much more efficient*** *way* » .

# Do you really need to map reads?

Does it matter to have a base pair precision location for hundreds of millions of reads?

# Alignment-free RNA-seq quantification

Quantifying transcripts may not require alignment

Kallisto

Bray et al, Nat. Biotechnology, 2016

Salmon

Patro et al, Nat. Methods, 2017

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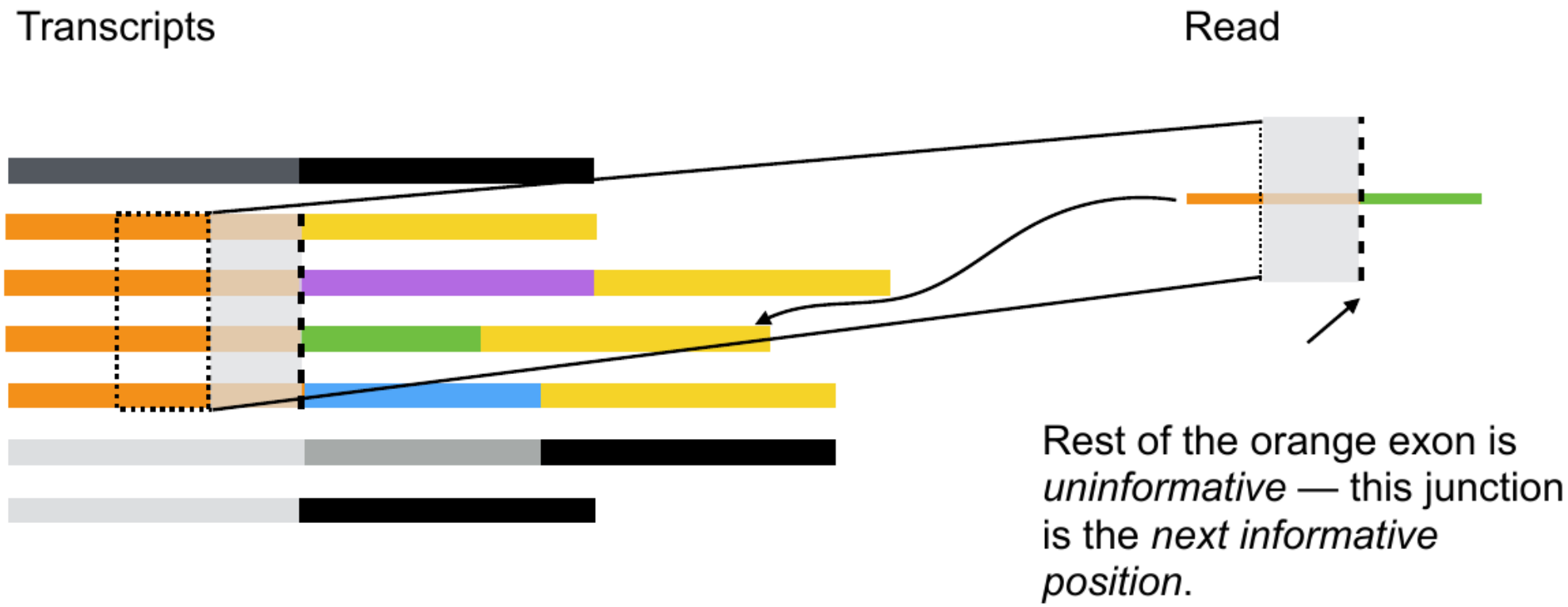
Two orders of magnitude faster than TopHat+Cufflinks

# How to quantify without aligning?




© *Rob Patro (Salmon)*

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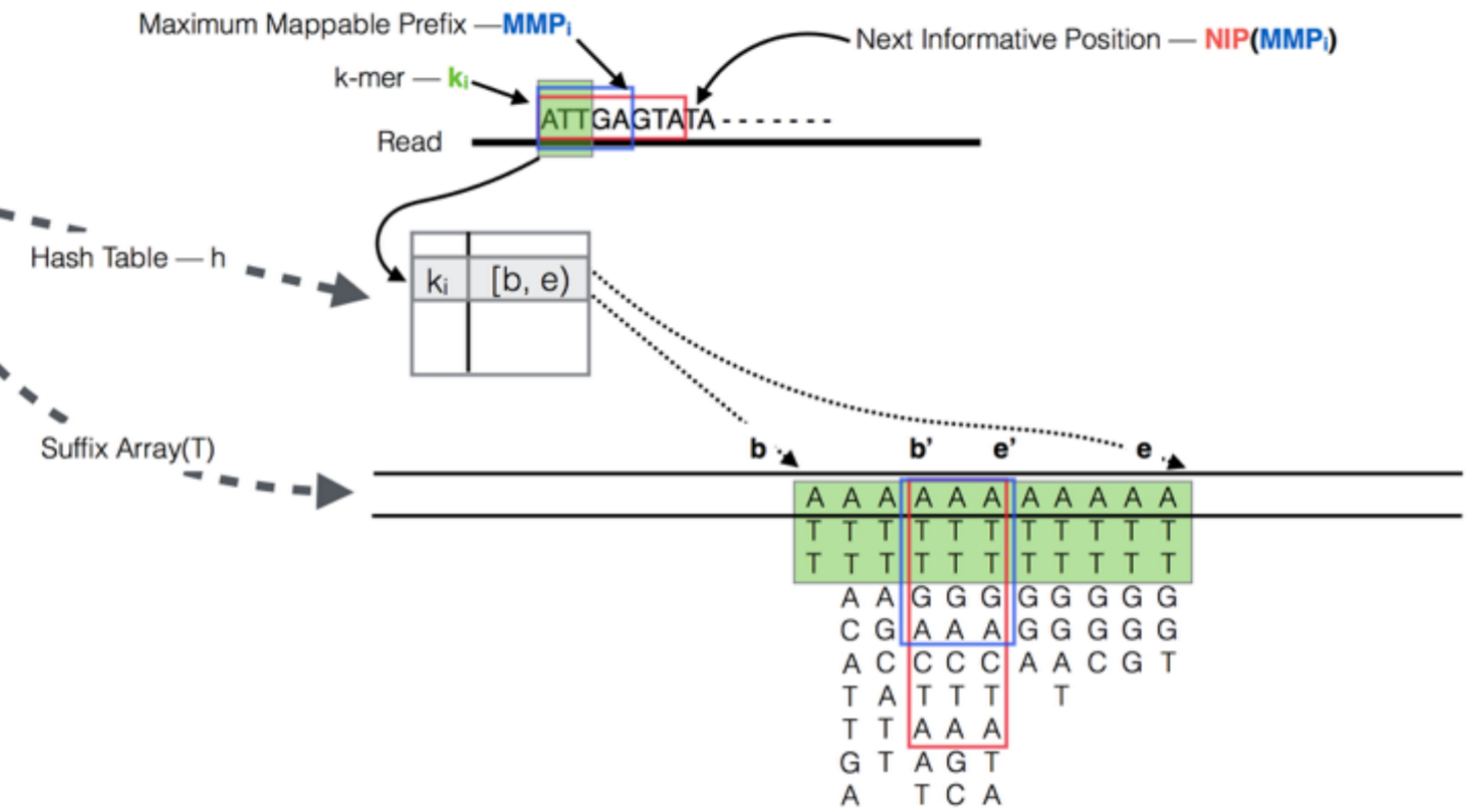


© *Rob Patro (Salmon)*

# Ultra fast methods with good results…

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« *With the exception of the underperforming Flux Capacitor and eXpress, we found that the other algorithms performed similarly.* »

Teng et al, Genome Biology, 2016

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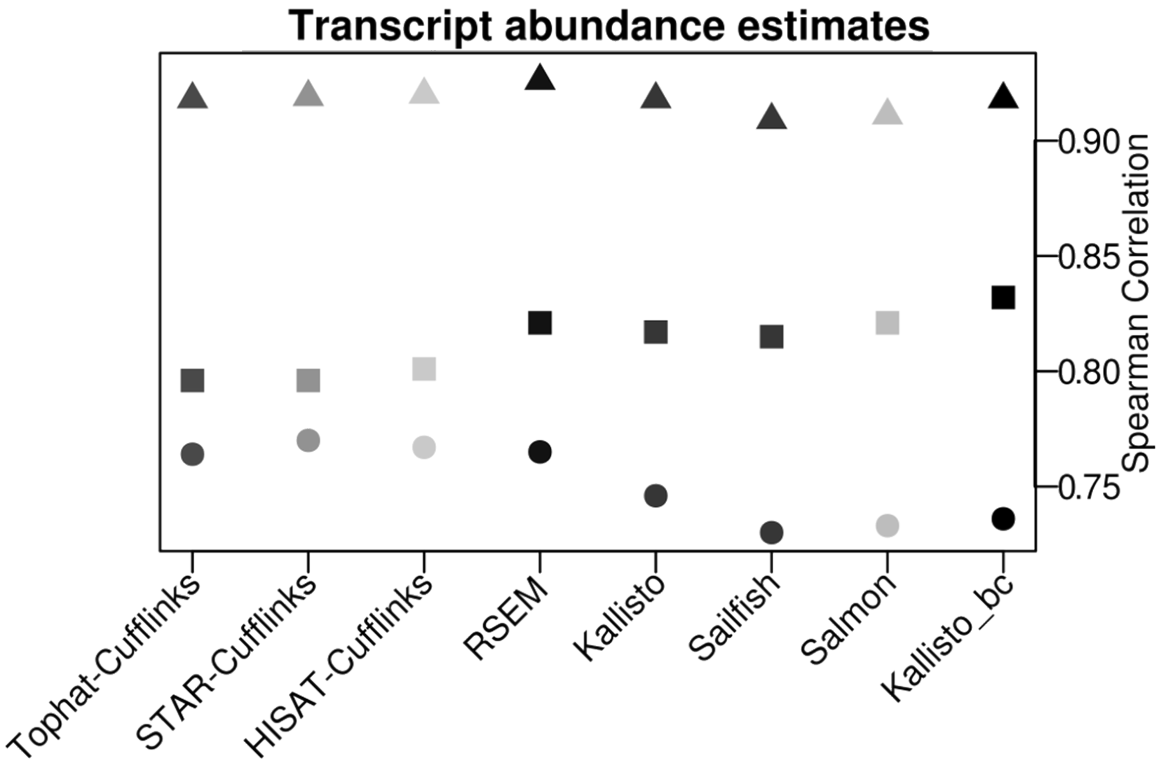



Germain et al, Nucleic Acid Research, 2016

# Ultra fast methods with good results…

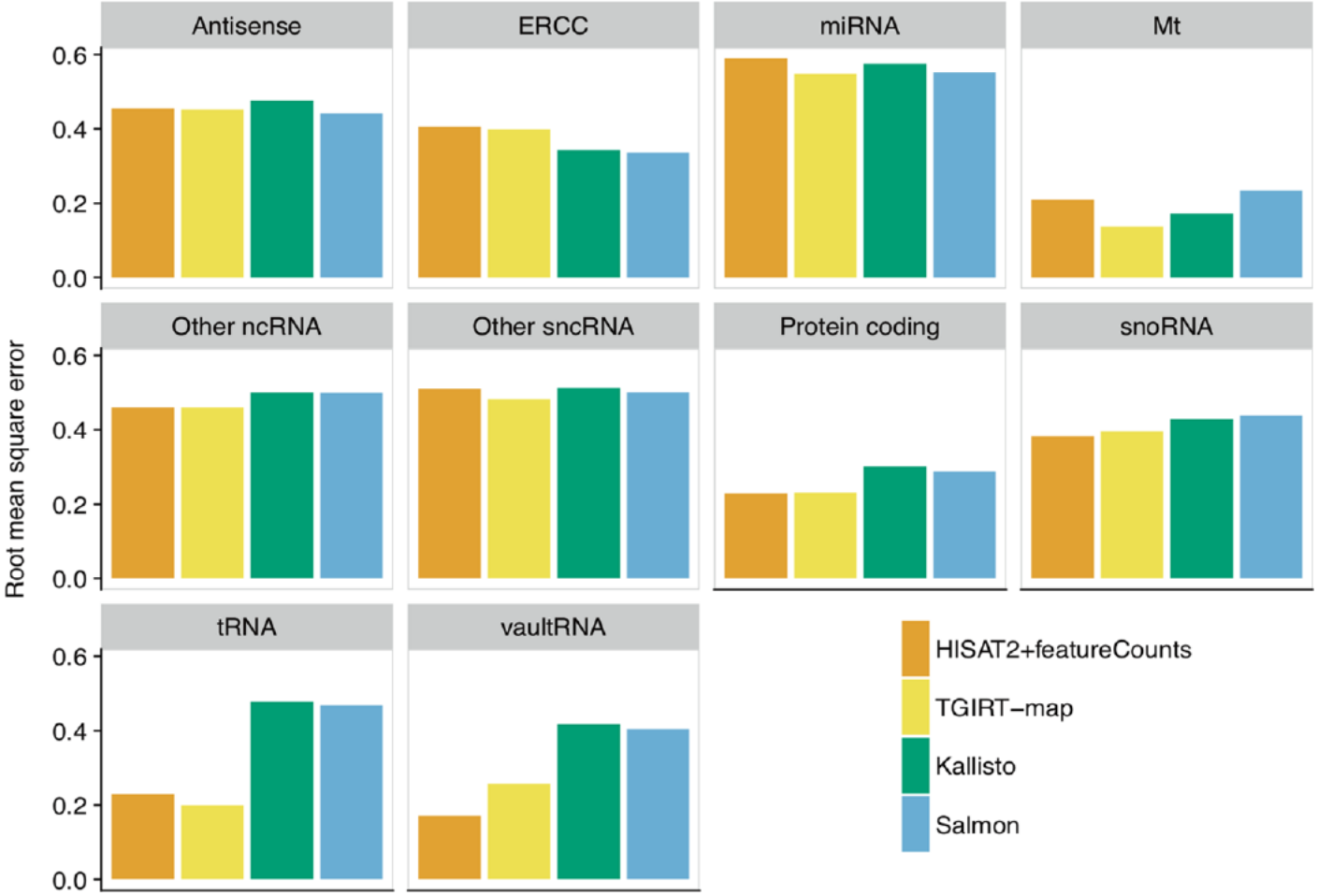
« *With the exception of the underperforming Flux Capacitor and eXpress, we found that the other algorithms performed similarly.* »

Teng et al, Genome Biology, 2016

« *It is particularly noteworthy that Salmon, which (like Sailfish and Kallisto) bypasses traditional alignment and thereby quantifies a single sample in a matter of minutes, had a comparable performance to Cufflinks and RSEM. Importantly, we confirmed these results using a variety of assays on both empirical and simulated data.* »

Germain et al, Nucleic Acid Research, 2016

# Good performances may not hold true for all the data




CC BY *Wu et al, 2018*

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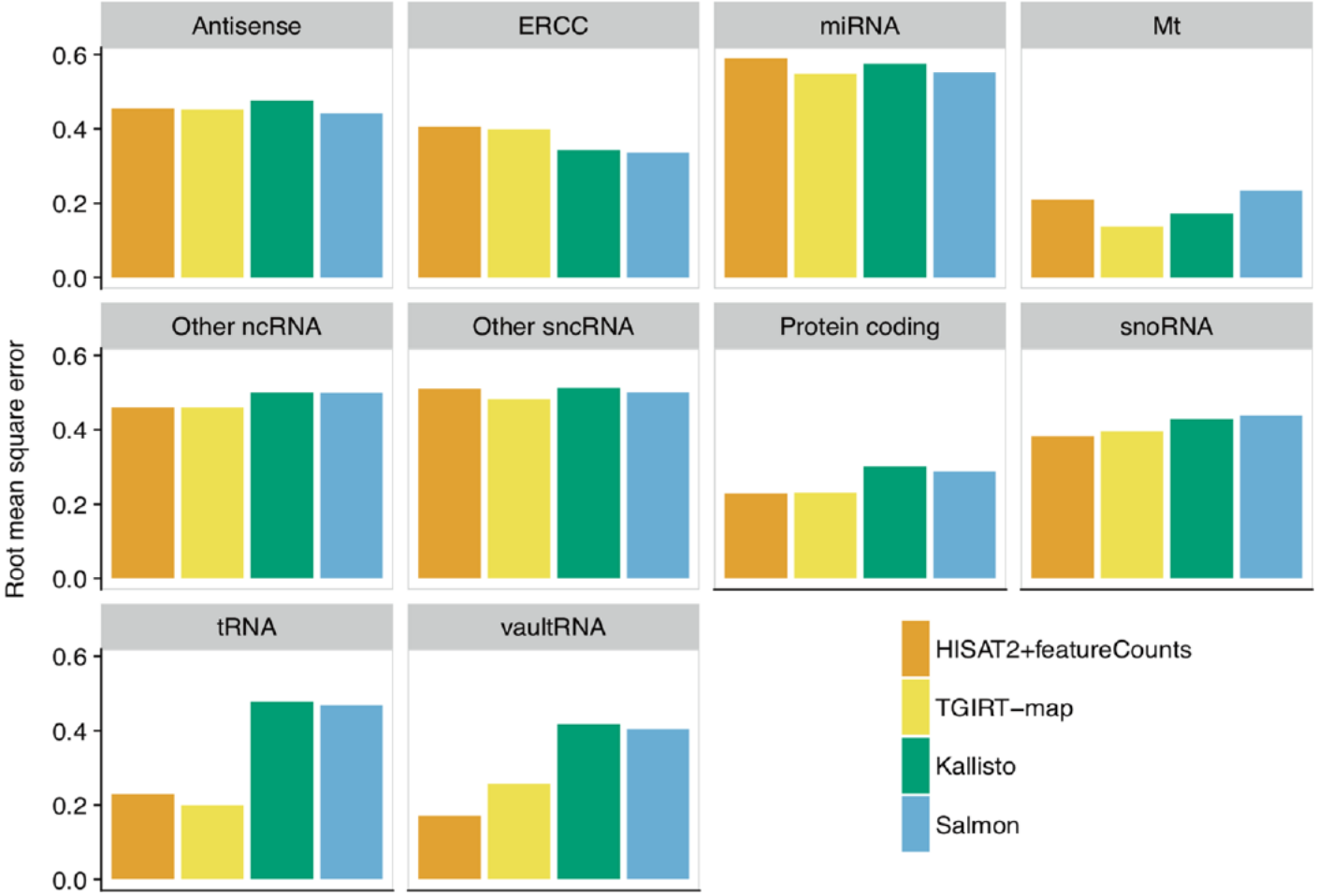


CC BY *Wu et al, 2018*

« *We have found that alignment-based tools were more accurate in quantifying lowly-expressed or small genes.* »

Wu *et al*, BMC Genomics, 2018

# Where the differences come from?

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1. Alignement vs pseudo-alignment

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   see Srivastava *et al*, 2020

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3. Quantification method

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When a read maps at multiple loci,
what transcript/gene should be counted?

See Deschamps-Francoeur *et al*, 2020 (thanks Pierre!)

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See Deschamps-Francoeur *et al*, 2020 (thanks Pierre!)

- None
  (*eg.* HTSeq-count, STAR genecount, featureCounts)

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- Expectation maximization
  (*eg.* RSEM, Salmon, Kallisto)

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