Computational Analysis of Next-Generation Sequencing Data

For Advancing Cancer Research

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Center For Health Informatics And Bioinformatic, New York University

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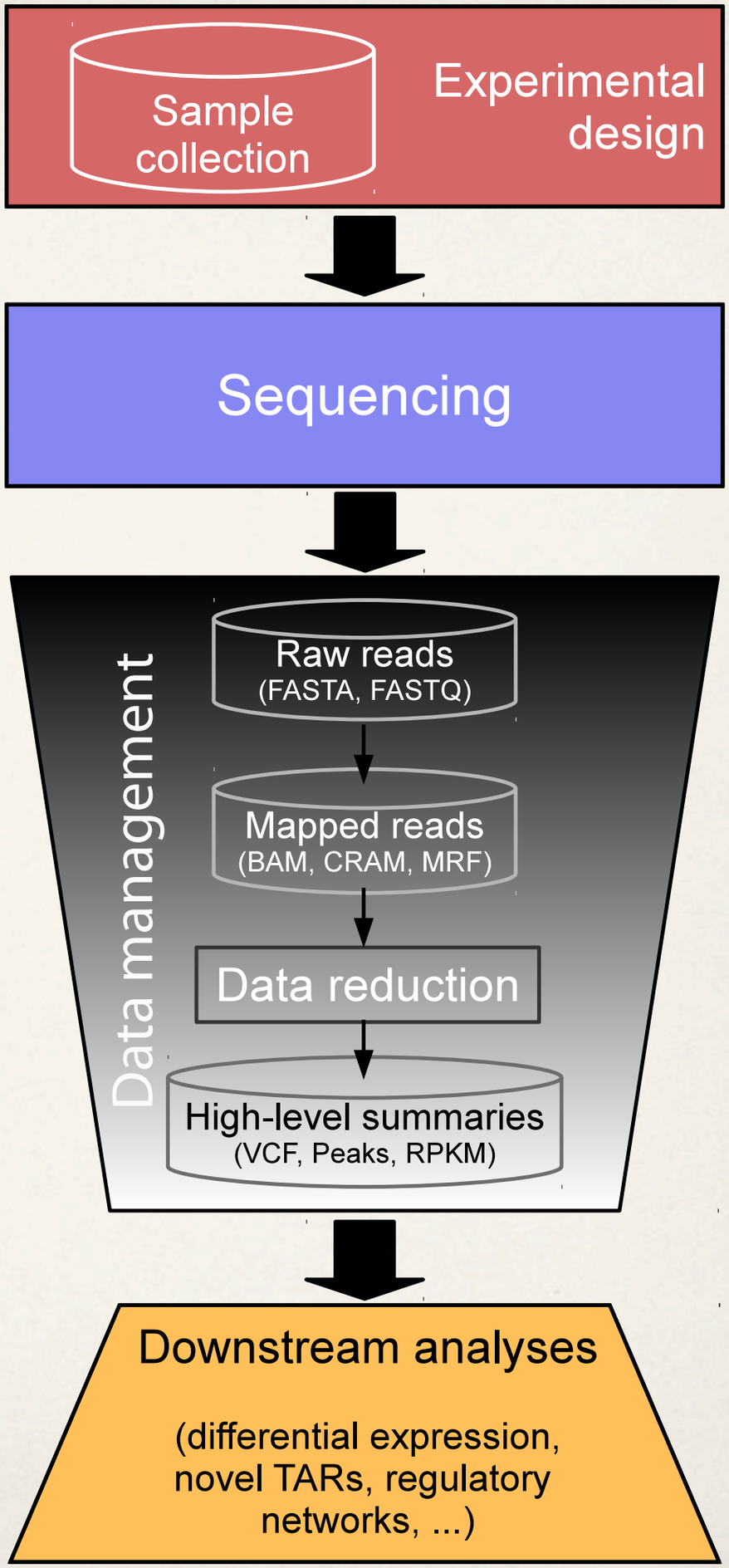
  - Functional genomics:
    - RNA-seq; CLIP-seq; ChIP-seq; chromosome conformation capture; bisulfite sequencing, etc.

Schematic of a NGS experiment



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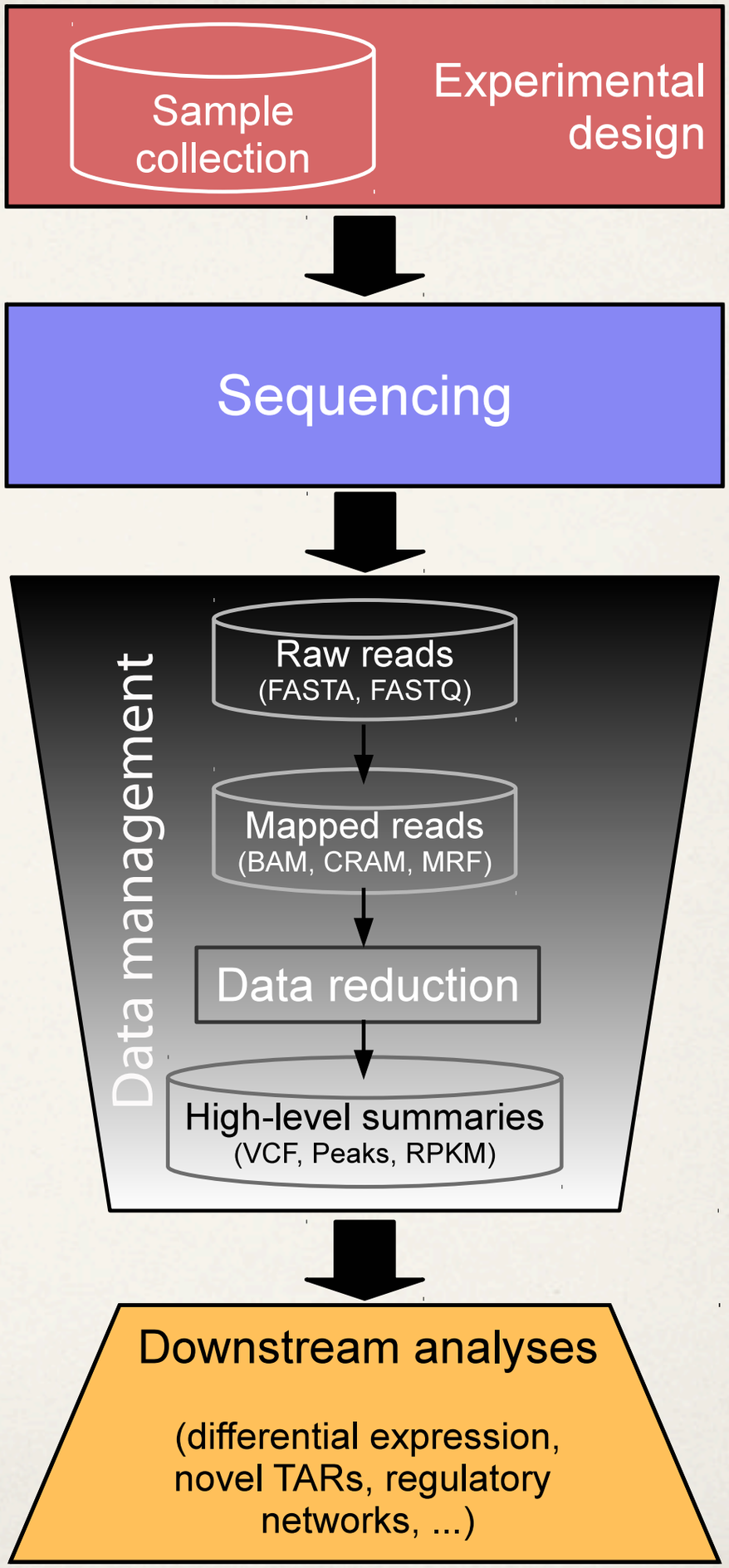

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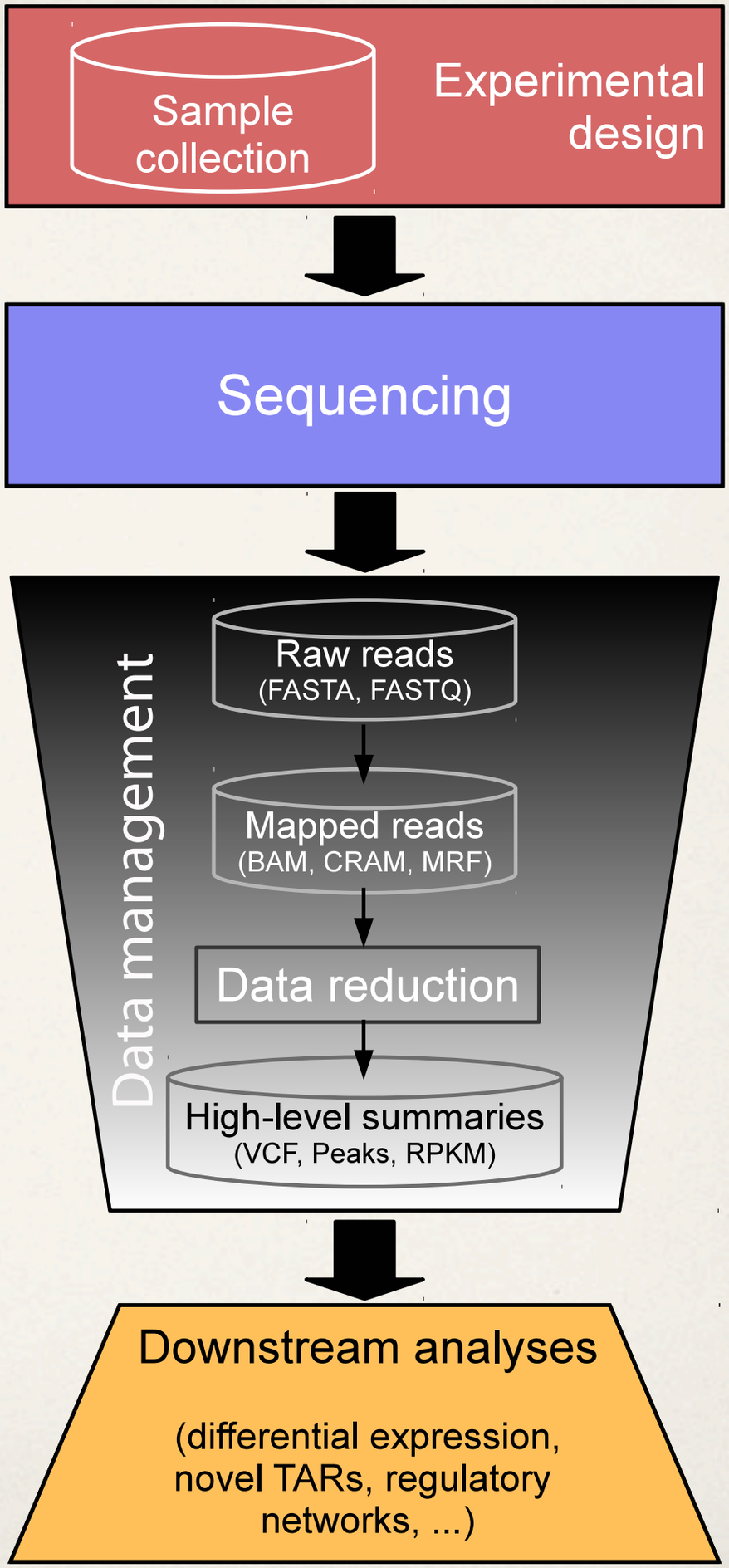

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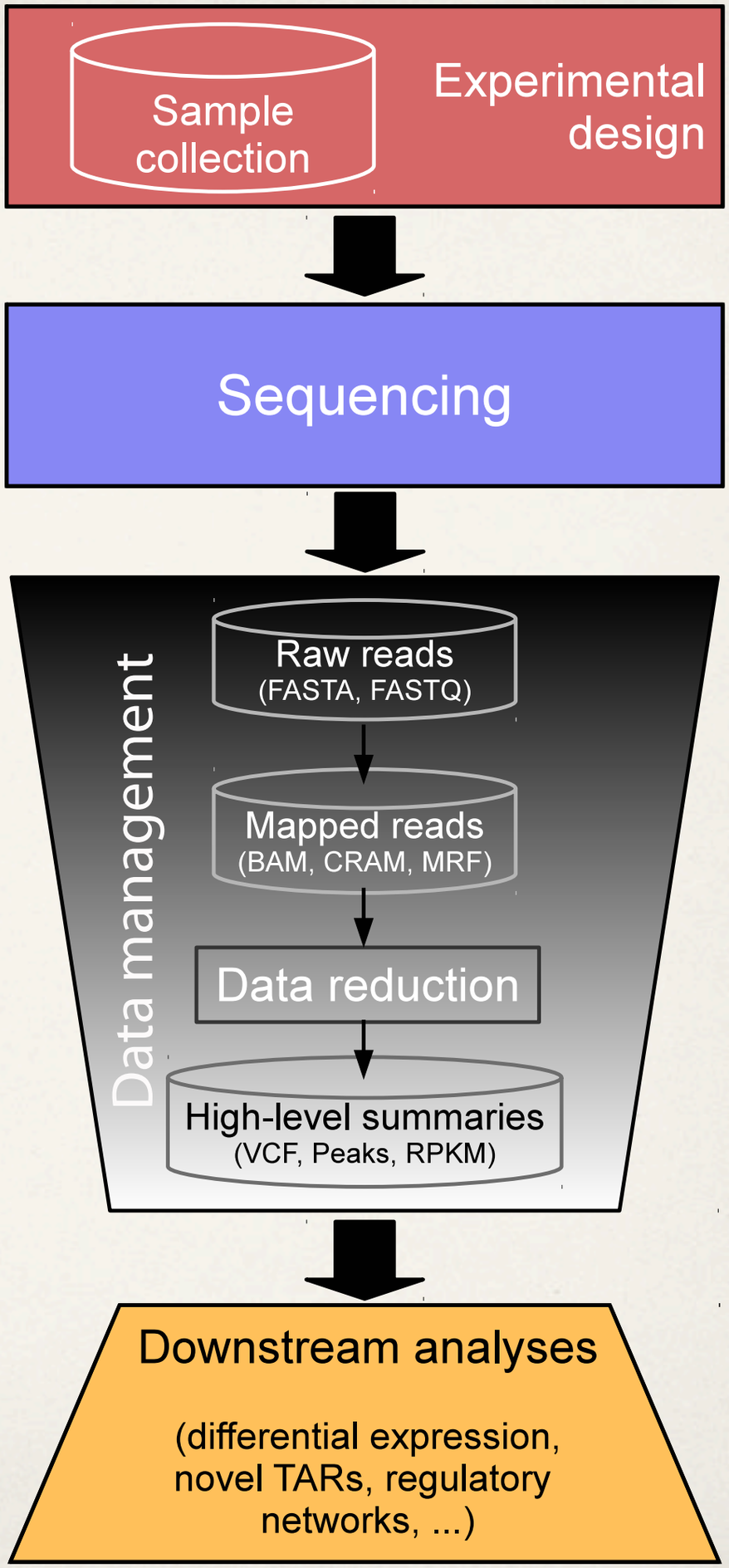

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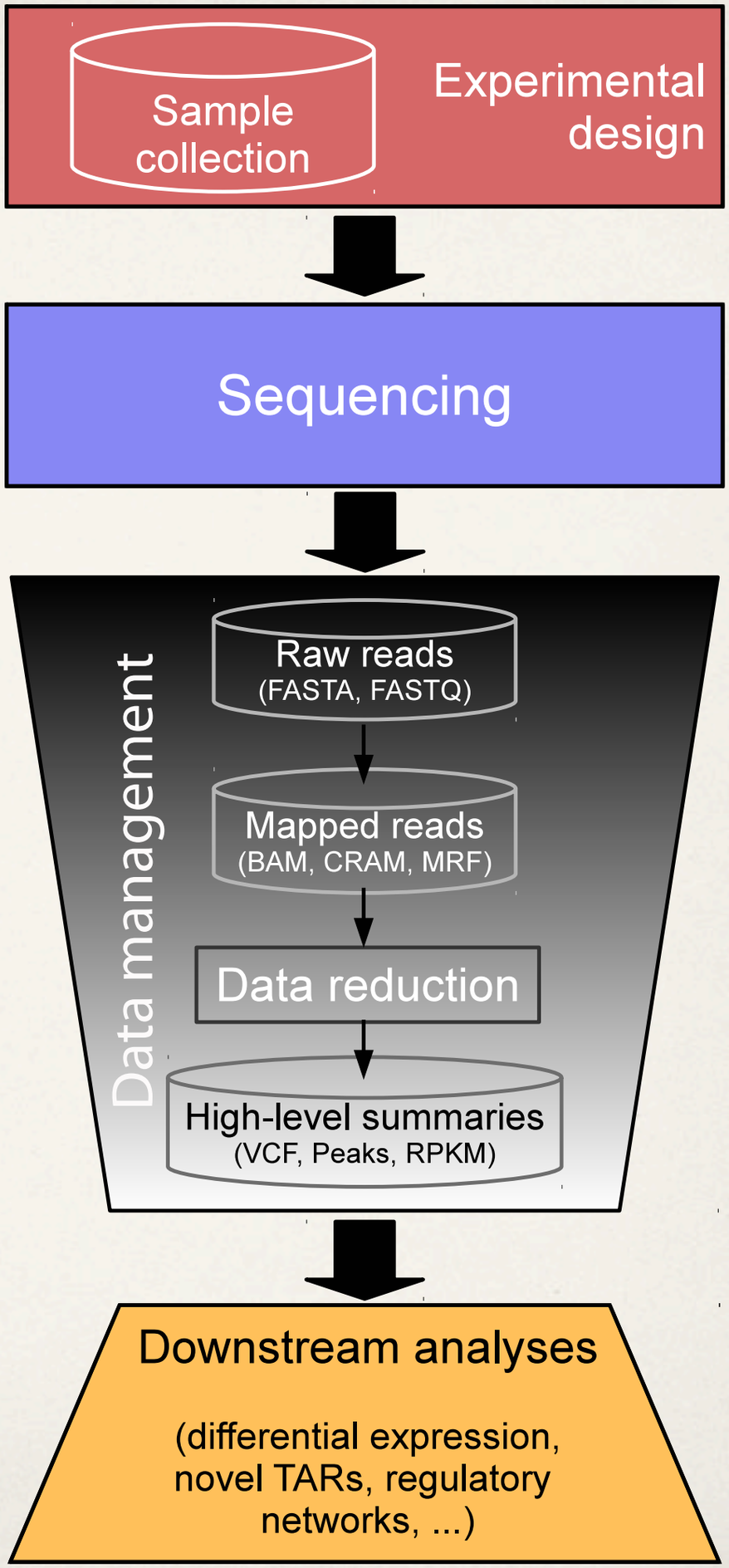

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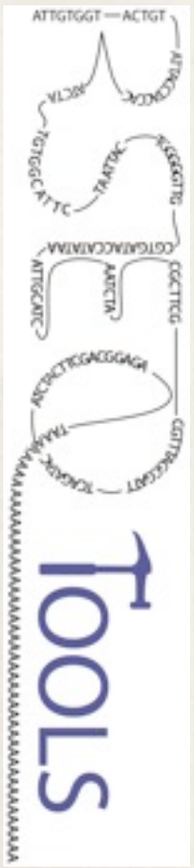
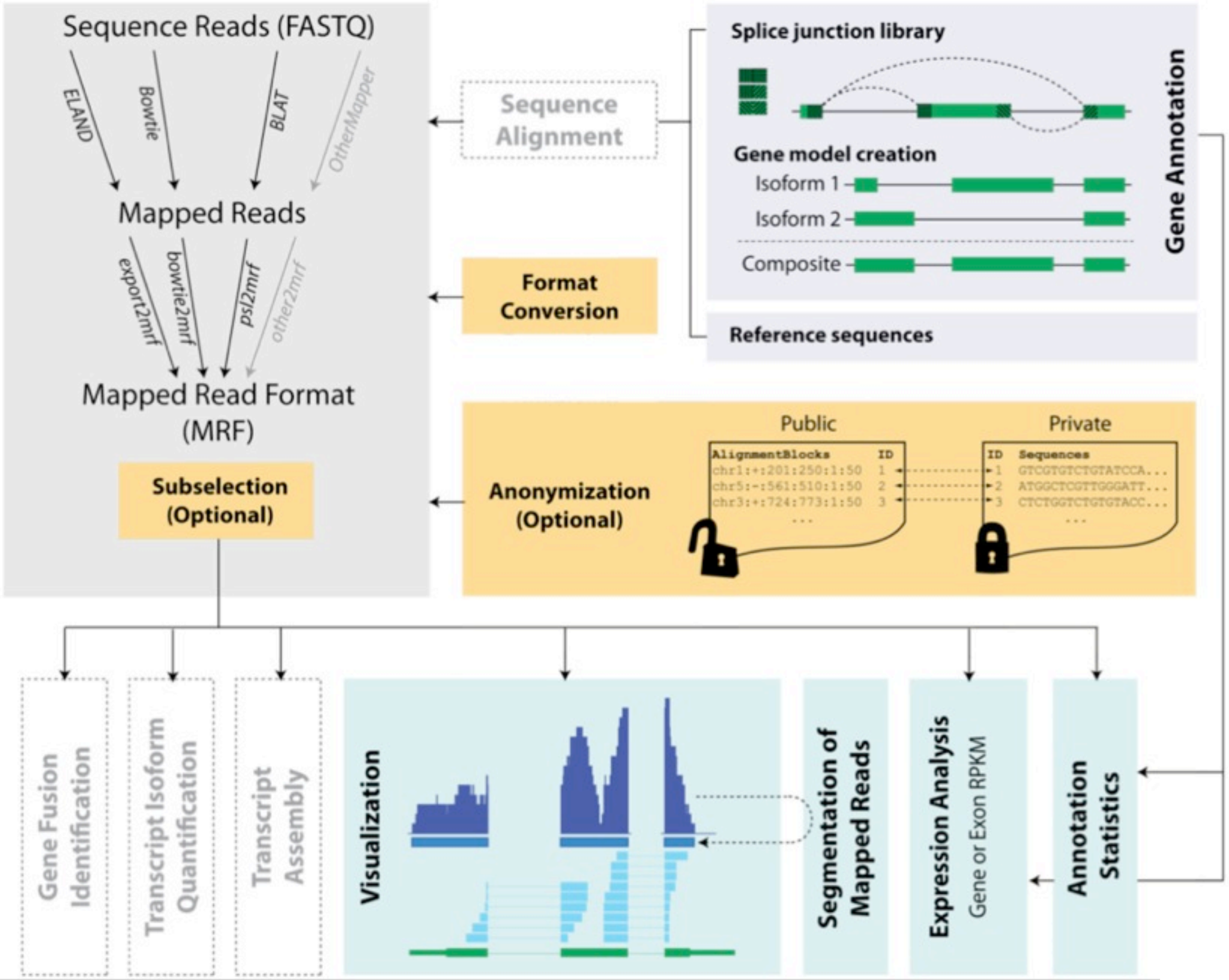
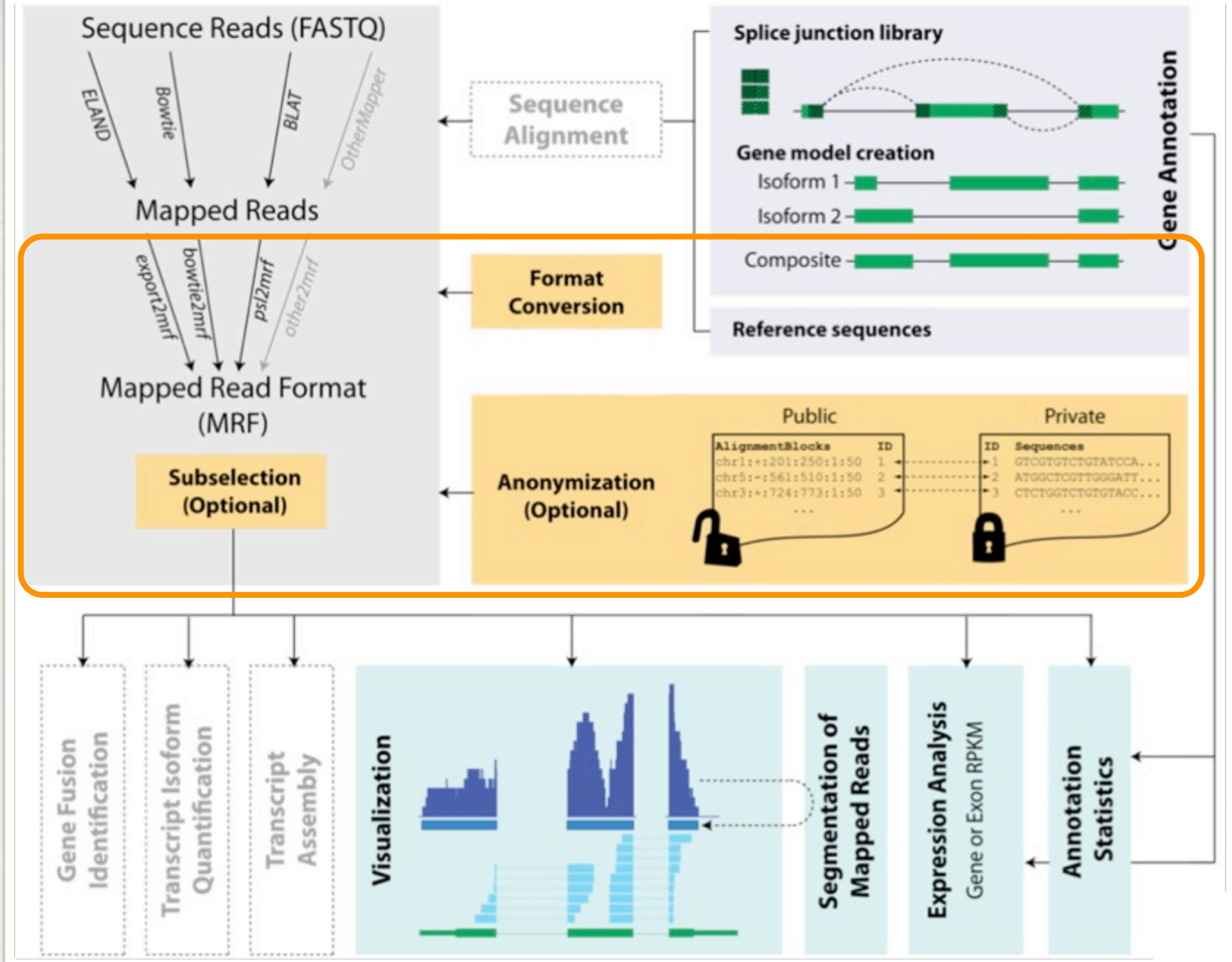
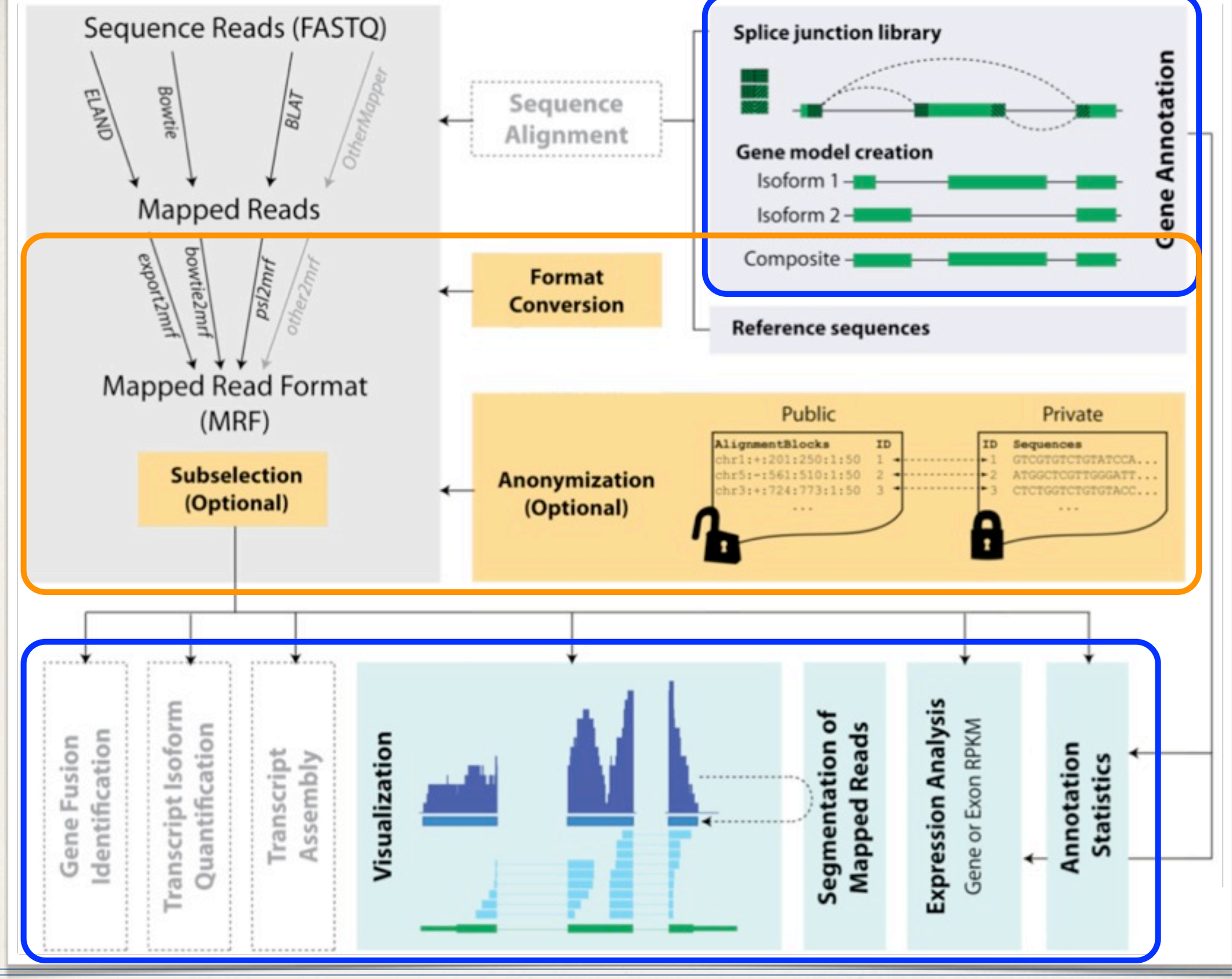


Habegger,* Lukas, Andrea Sboner*, et al. "RSEQtools: a Modular Framework to Analyze RNA-Seq Data Using Compact, Anonymized Data Summaries." Bioinformatics 2011 27(2);281–3







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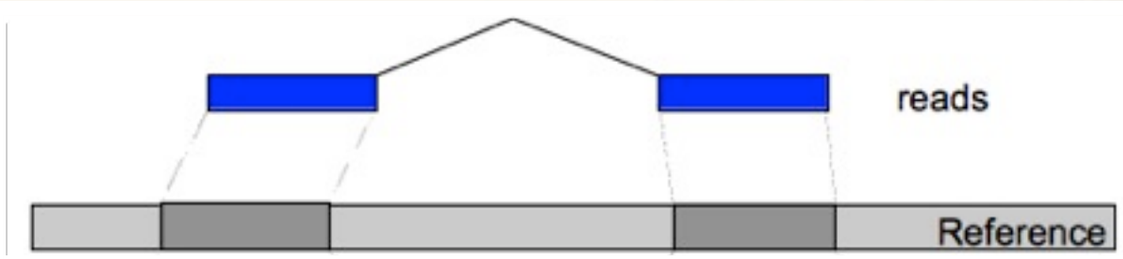

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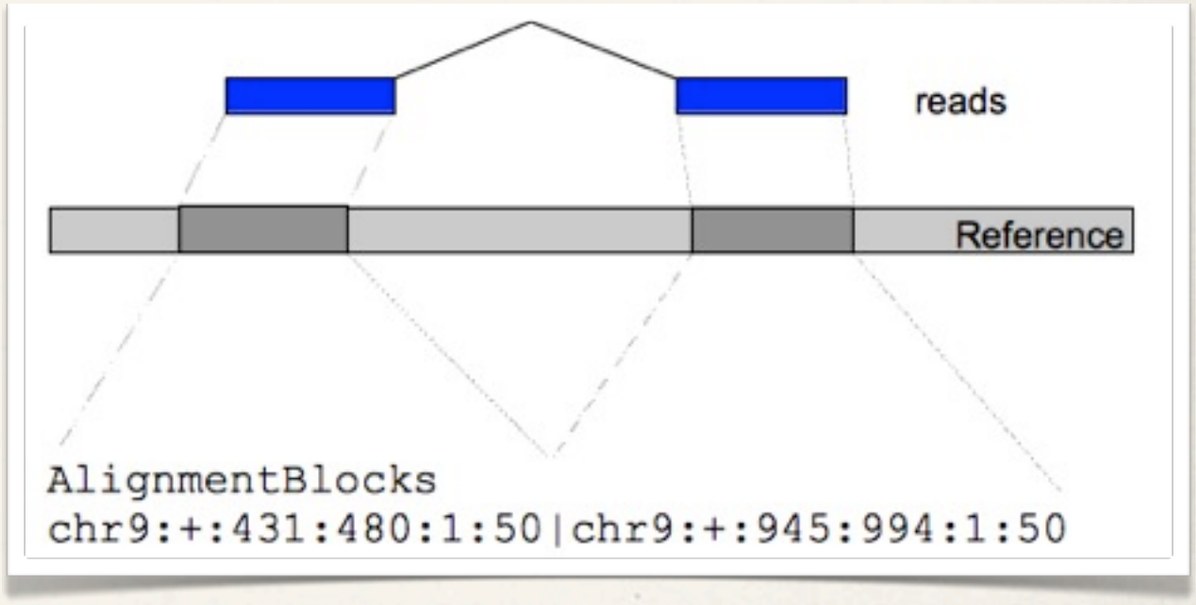

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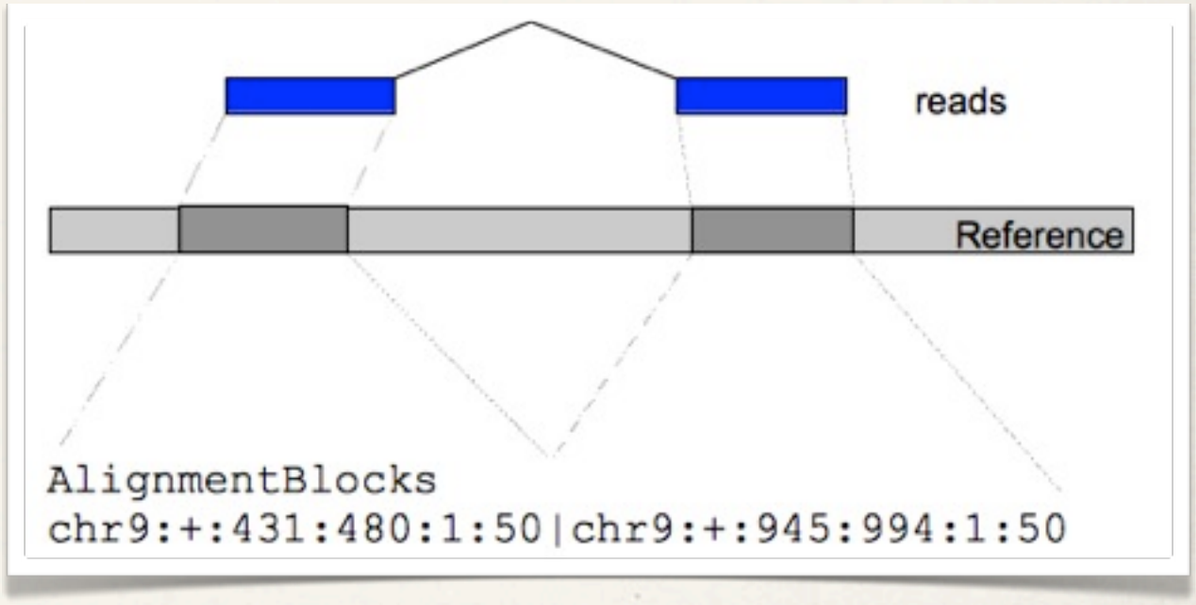

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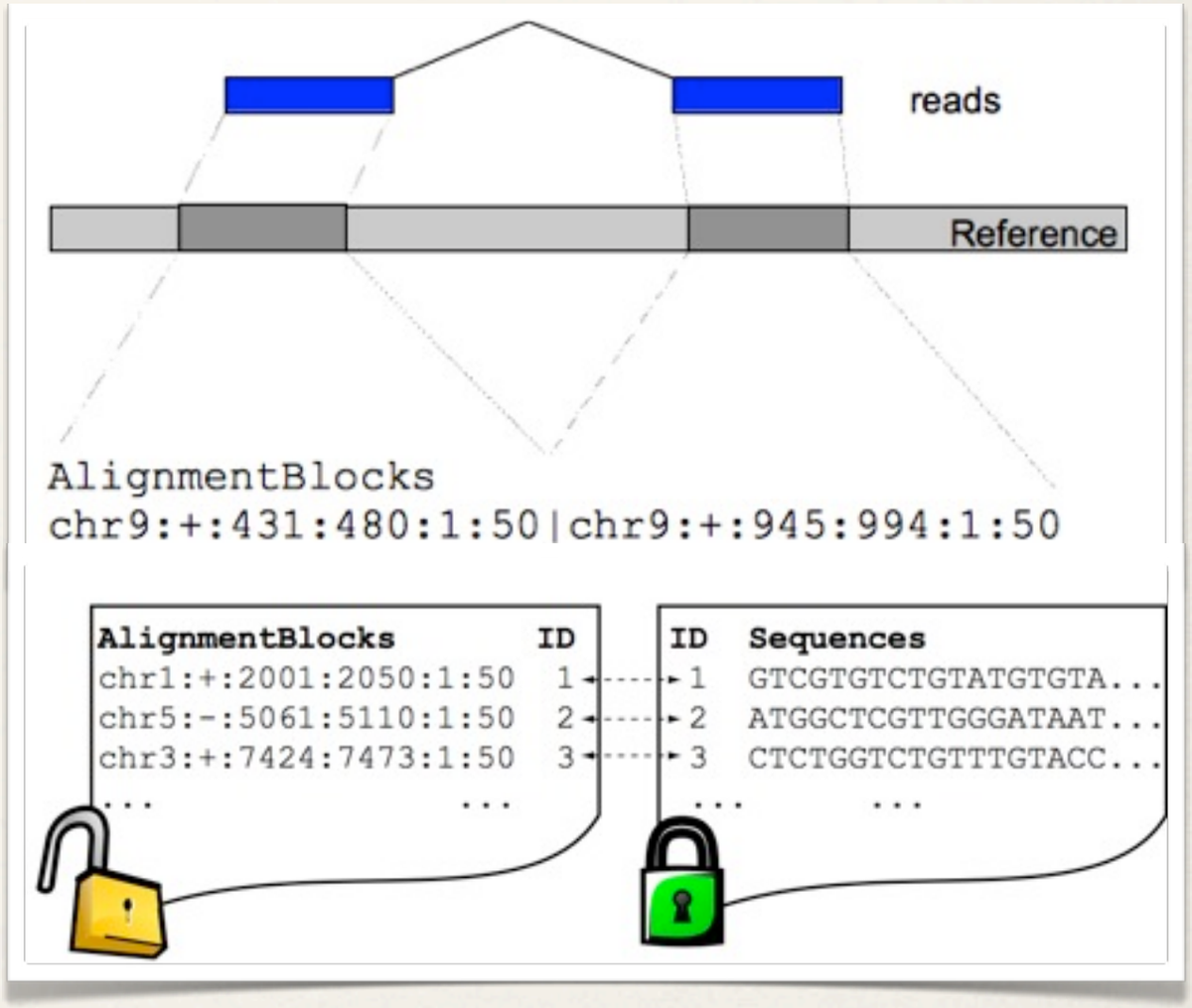

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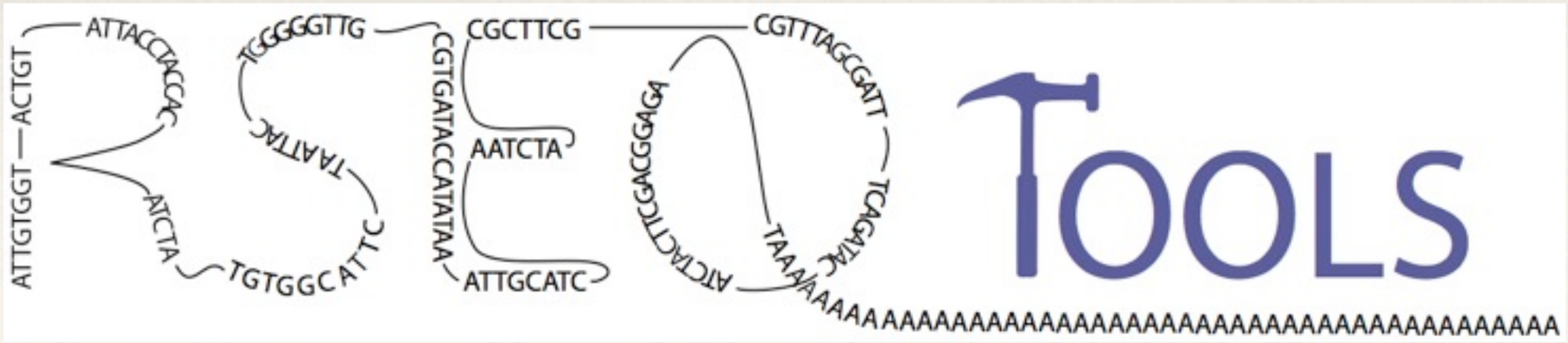

Purpose	Program	Time to process	File Sizes (uncompressed)	Notes
Alignment + conversion	ELAND2	~1 day	Export: 4.2Gb	Processing of one flow cell (8 lanes)
	export2mrf	~2 minutes	MRF: 400Mb	Number of mapped reads: ~12M
Quantification	mrfQuantifier	45 seconds	Gene expression values: 3.5Mb	GENCODE composite gene models (~22,000)
Visualization	mrf2wig	~2 minutes	One WIG file per chromosome: 1Mb - 150Mb	Signal track of mapped reads normalized per million mapped reads
	mrf2gff	45 seconds	One GFF file per chromosome: 100Kb - 16Mb	To visualize splice junction reads

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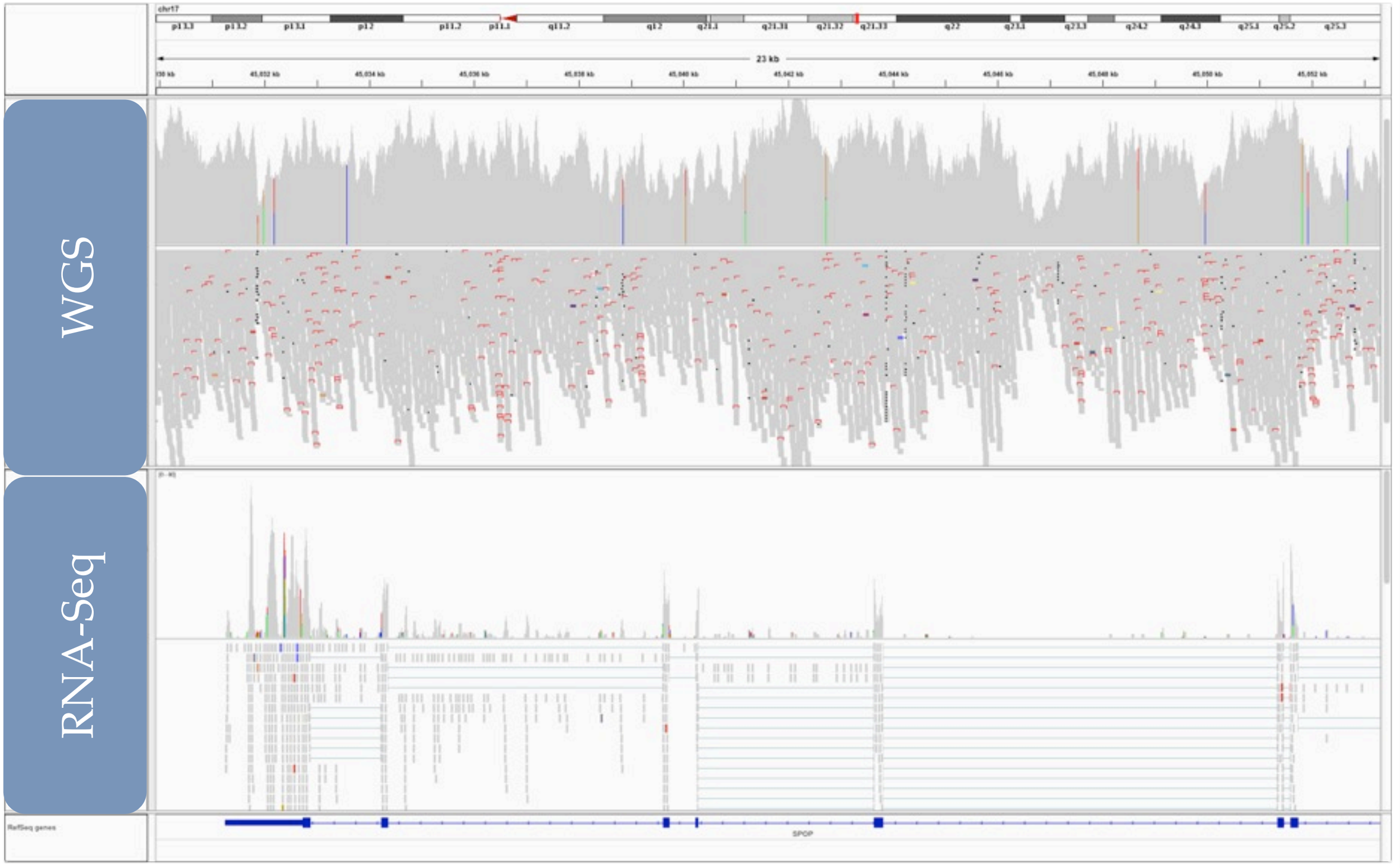

Visualizing mapped reads

e.g. via the Integrative Genome Viewer

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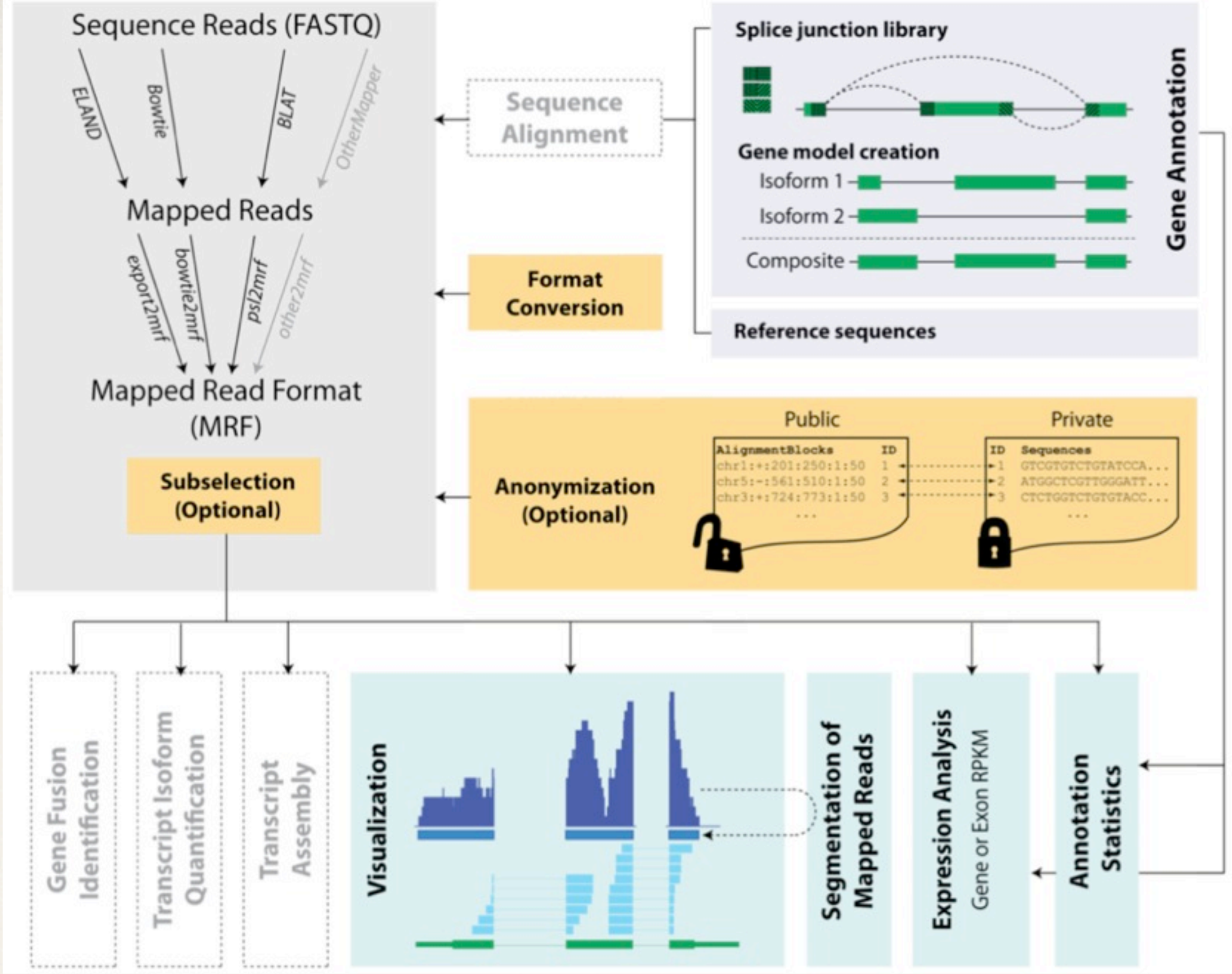

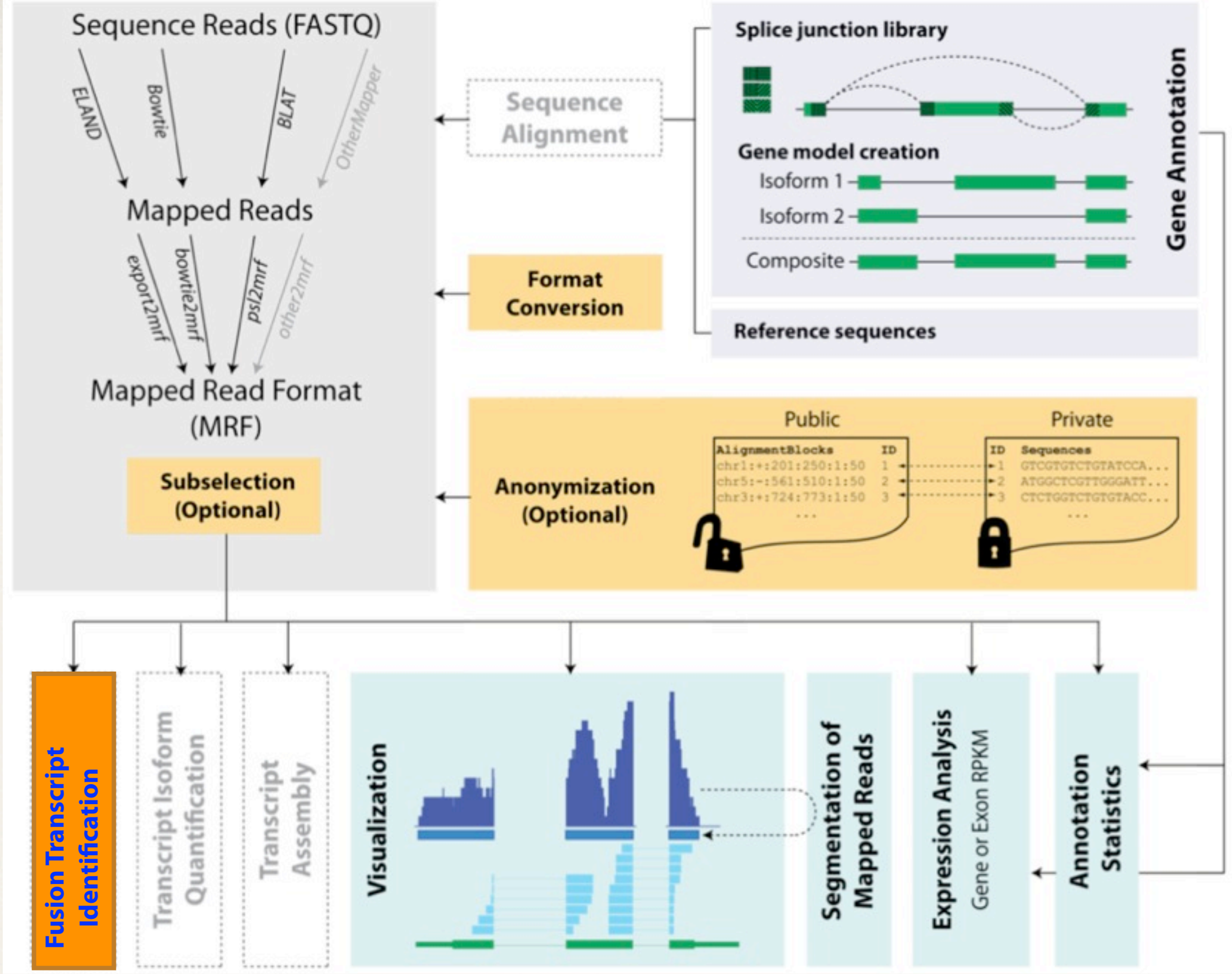

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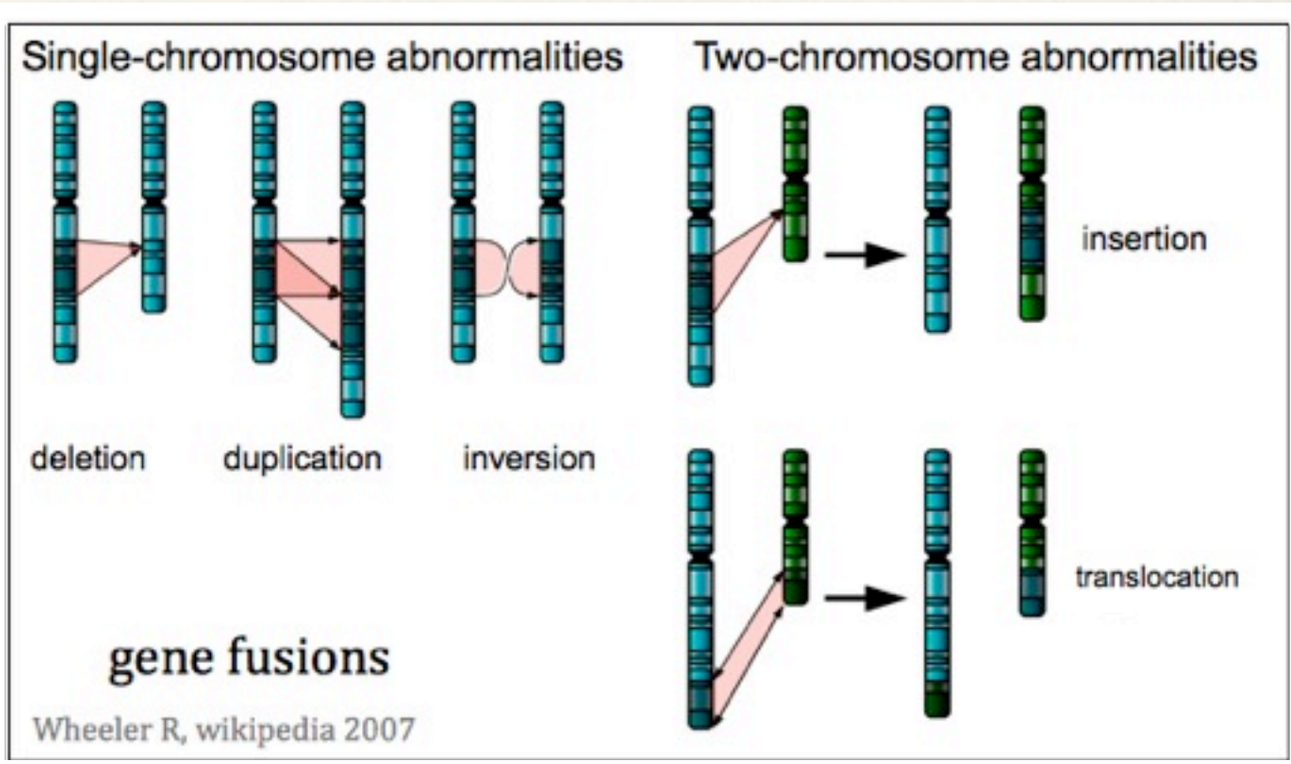

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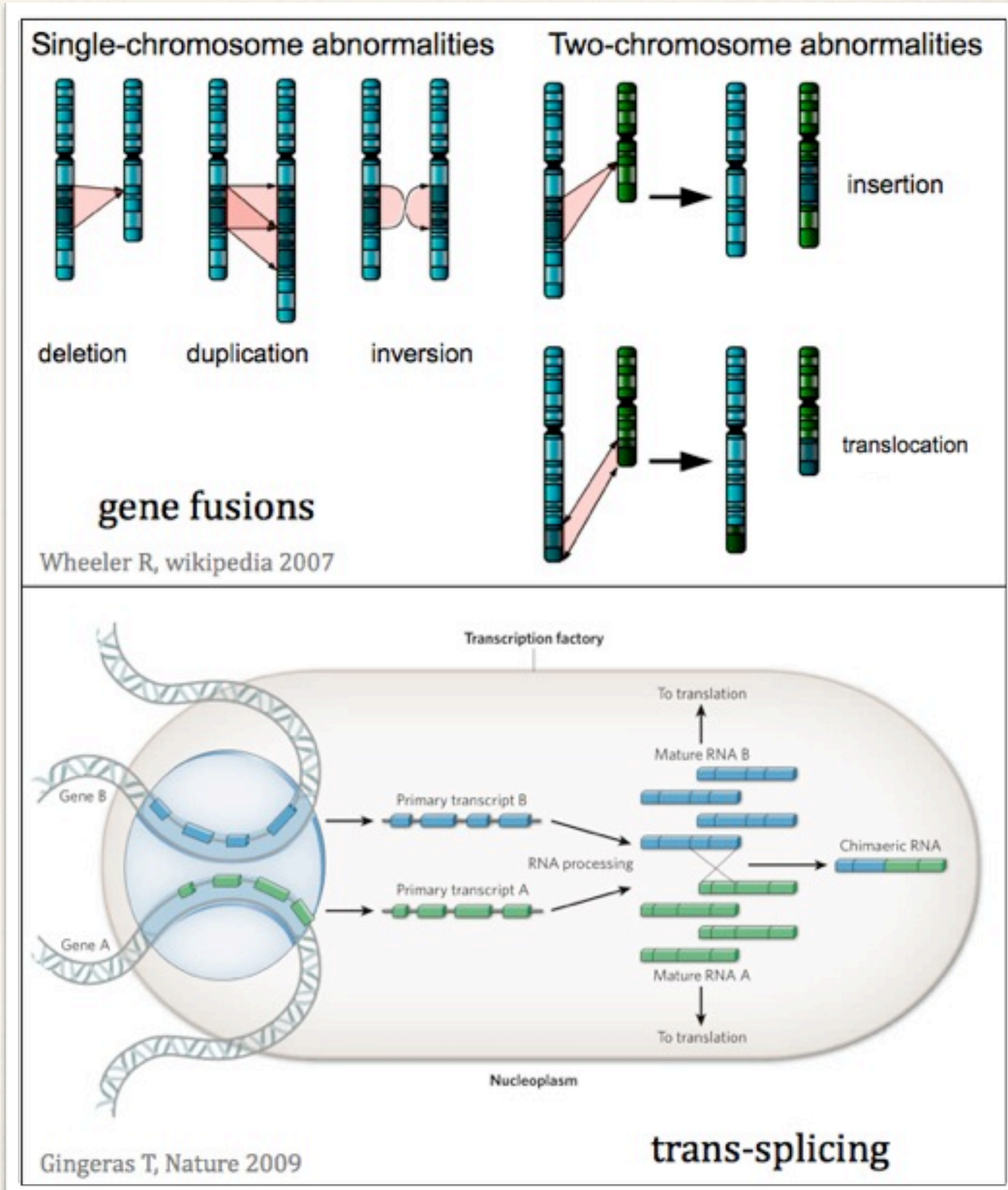
  - post-transcriptional events, i.e. *trans-splicing*



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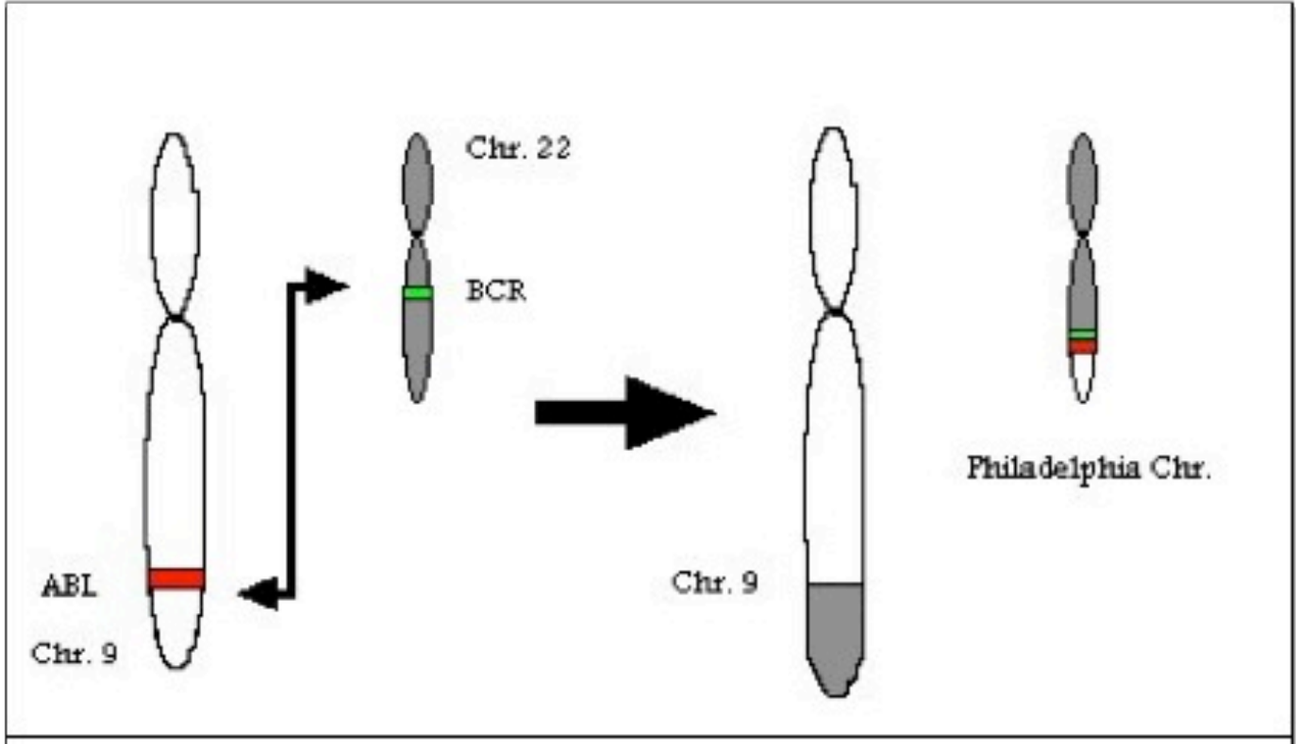

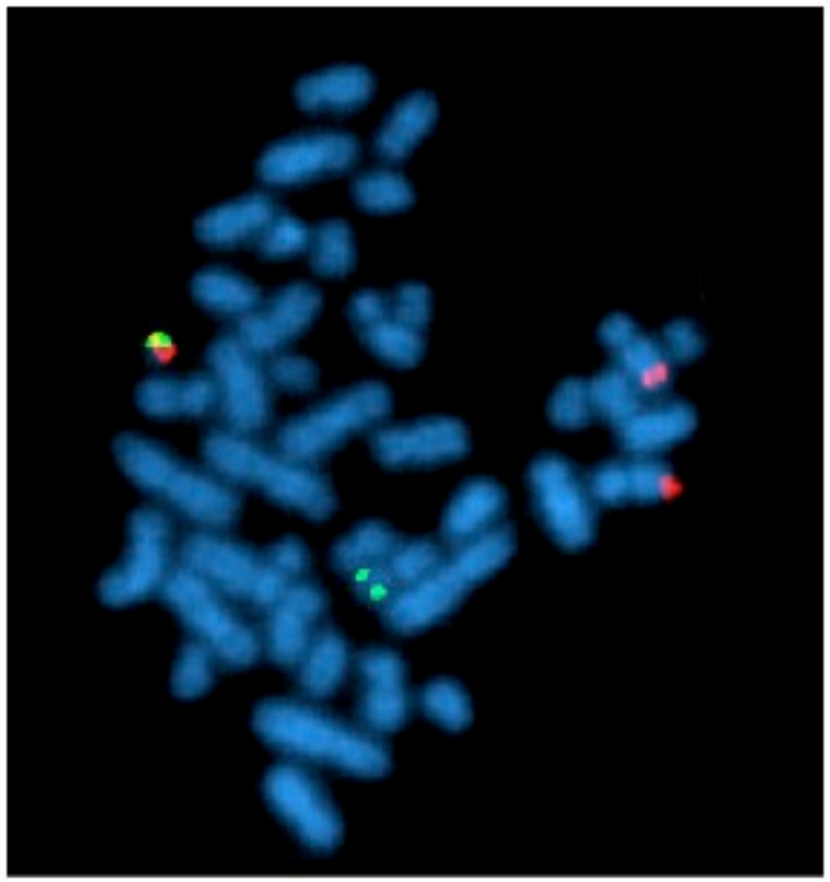

BCR-ABL gene fusion:
schematic and FISH

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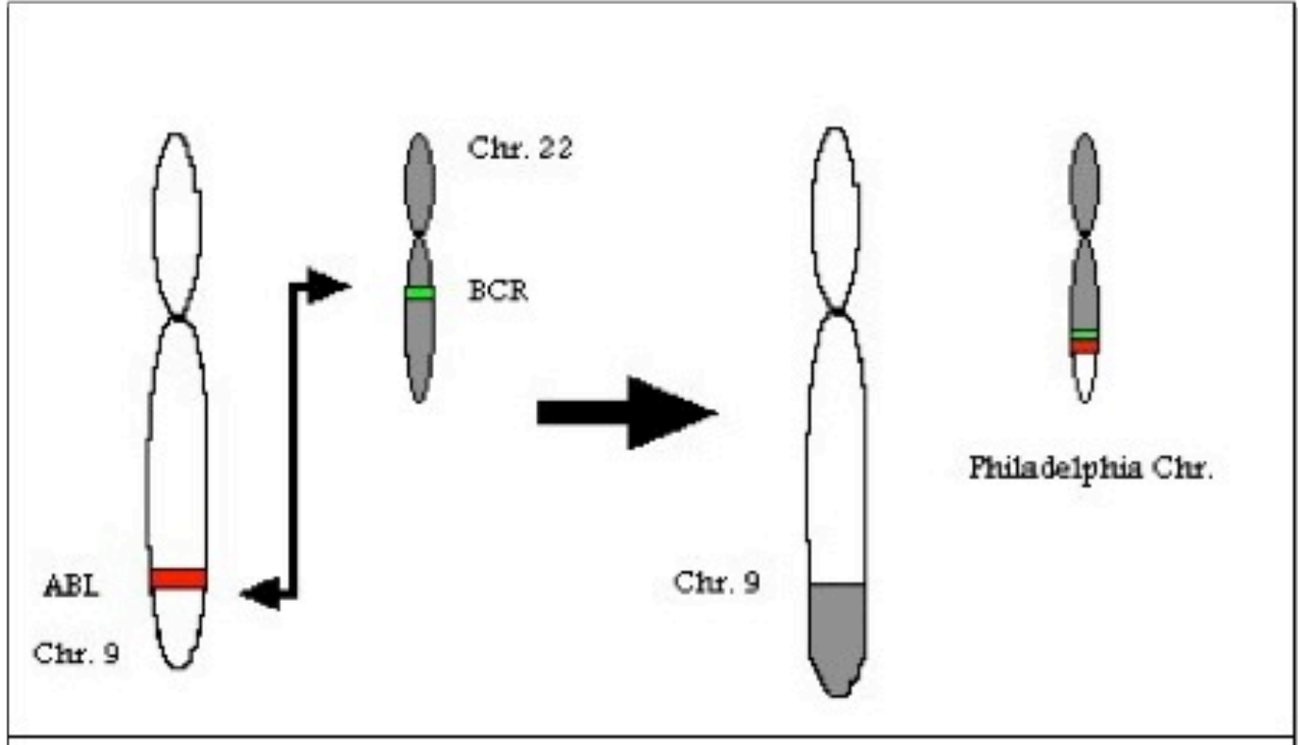

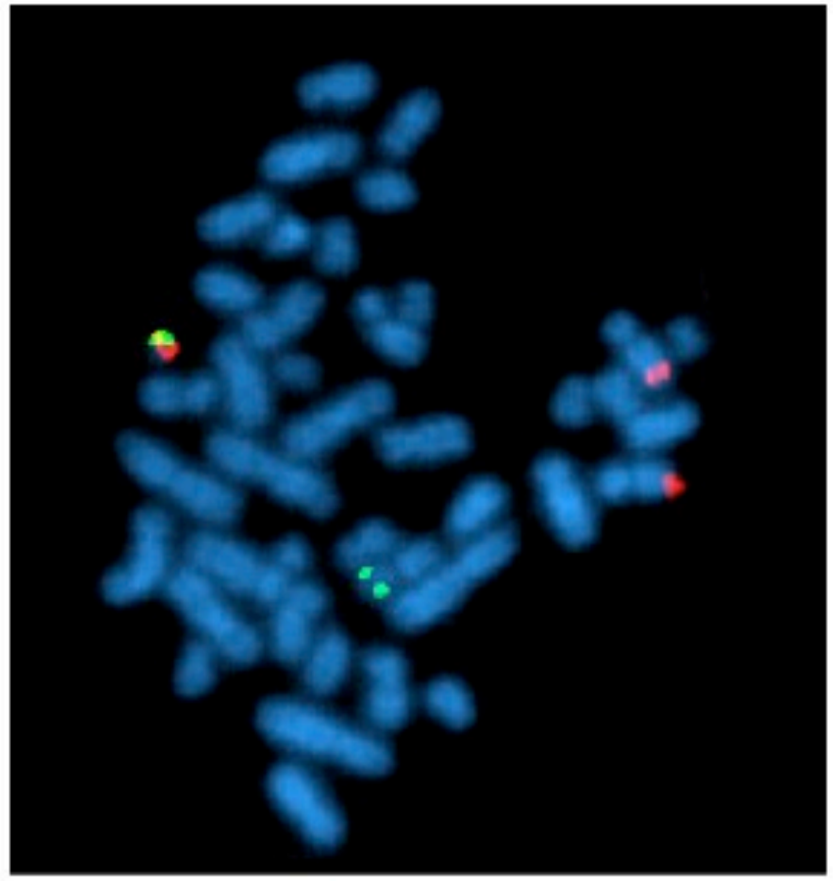

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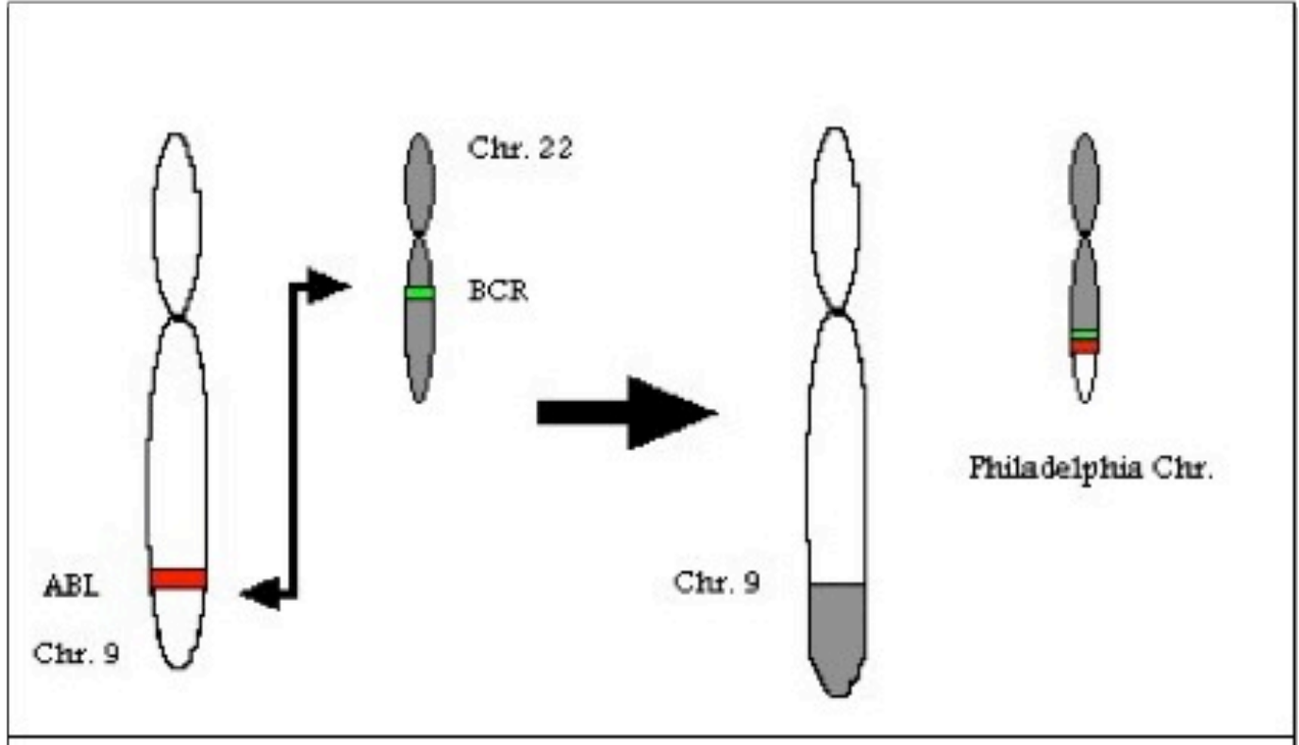
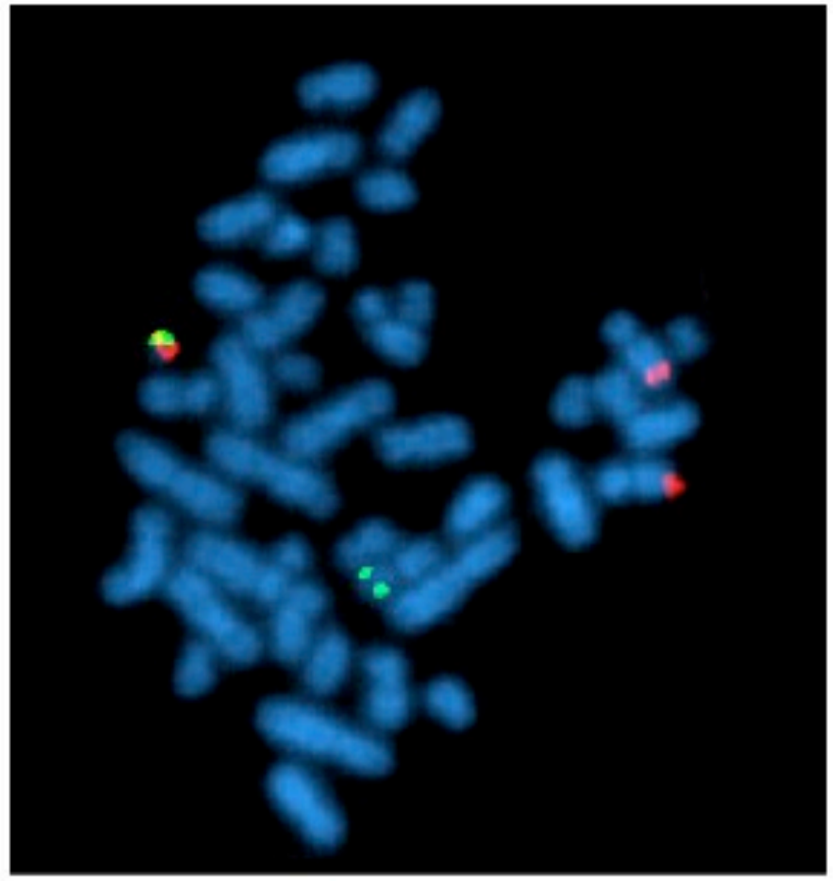
  - Ex: (IgH locus)-MYC in Burkitt's lymphoma (cMYC over-expressed)





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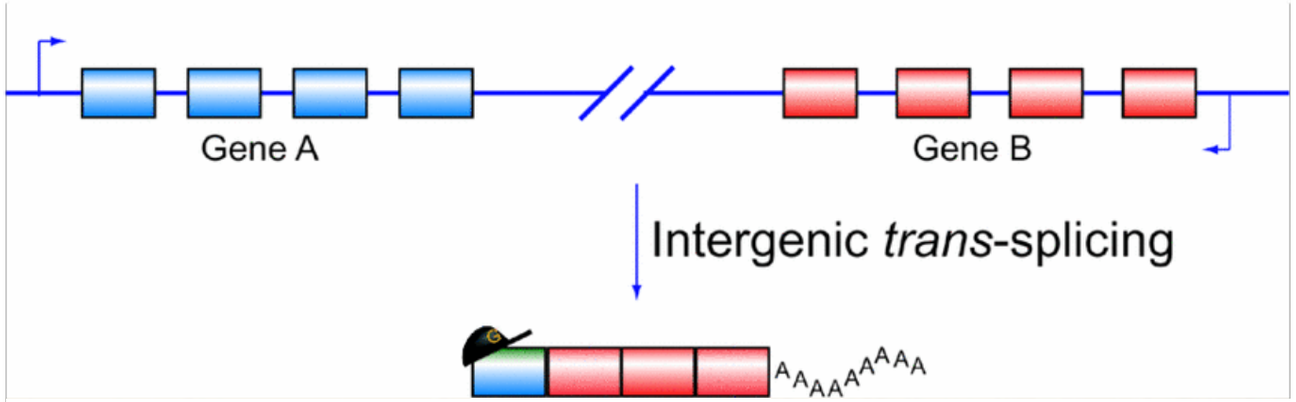

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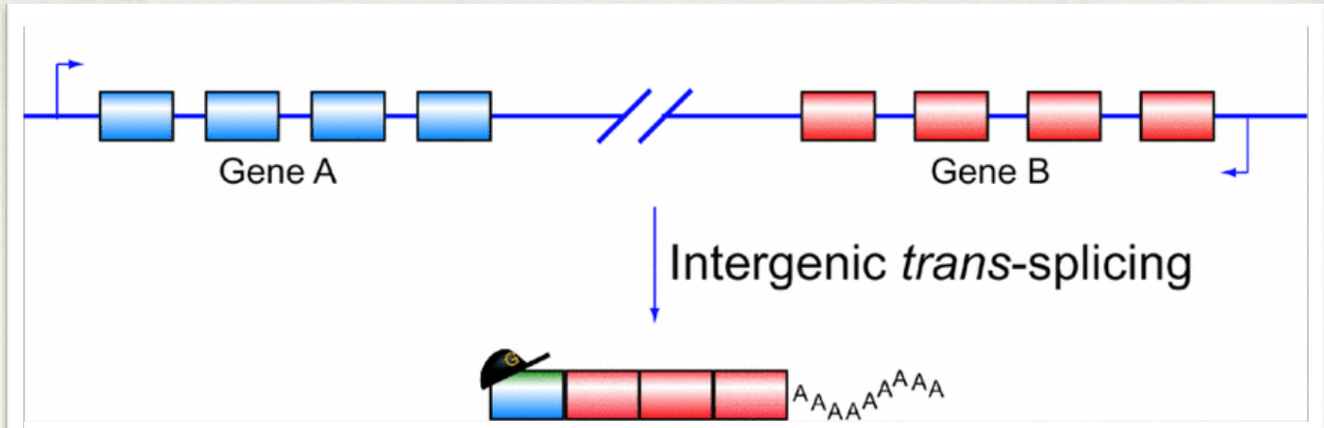

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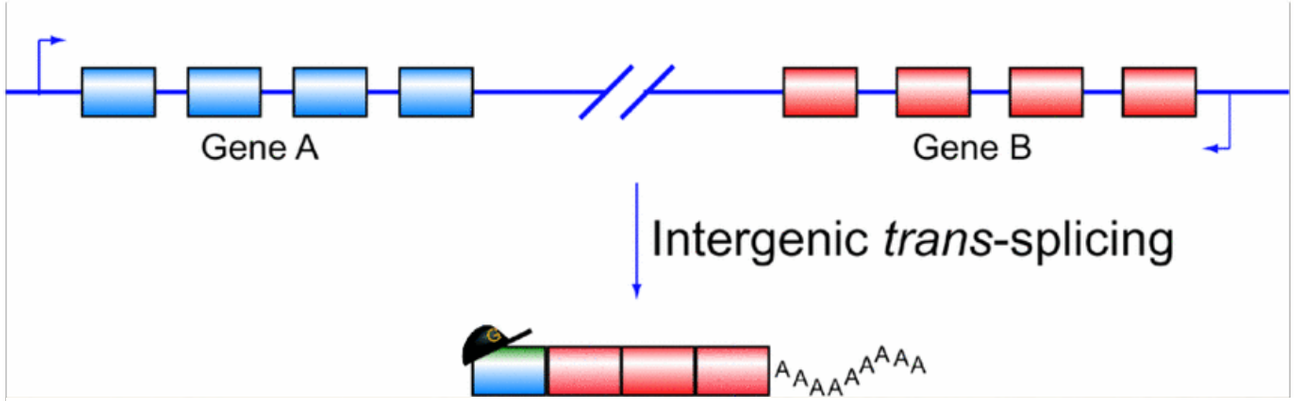

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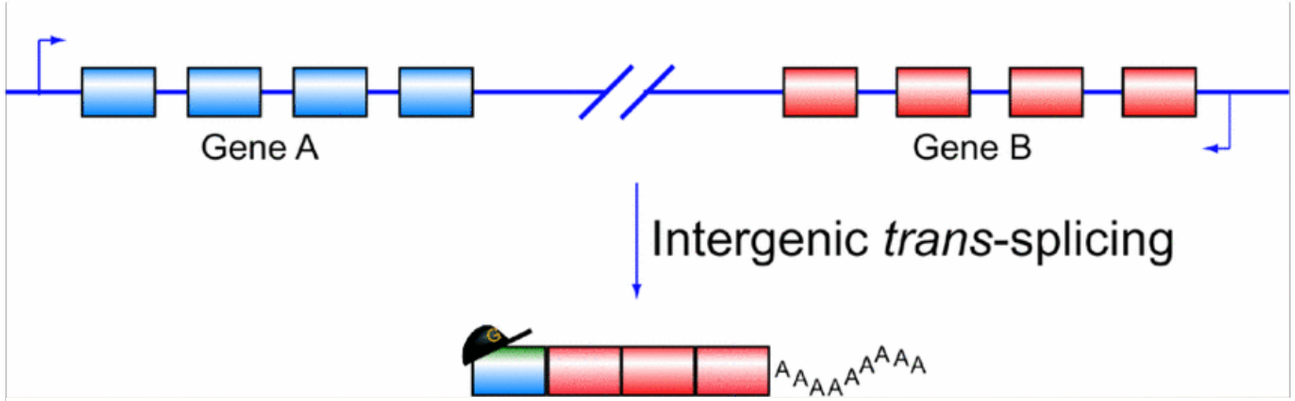

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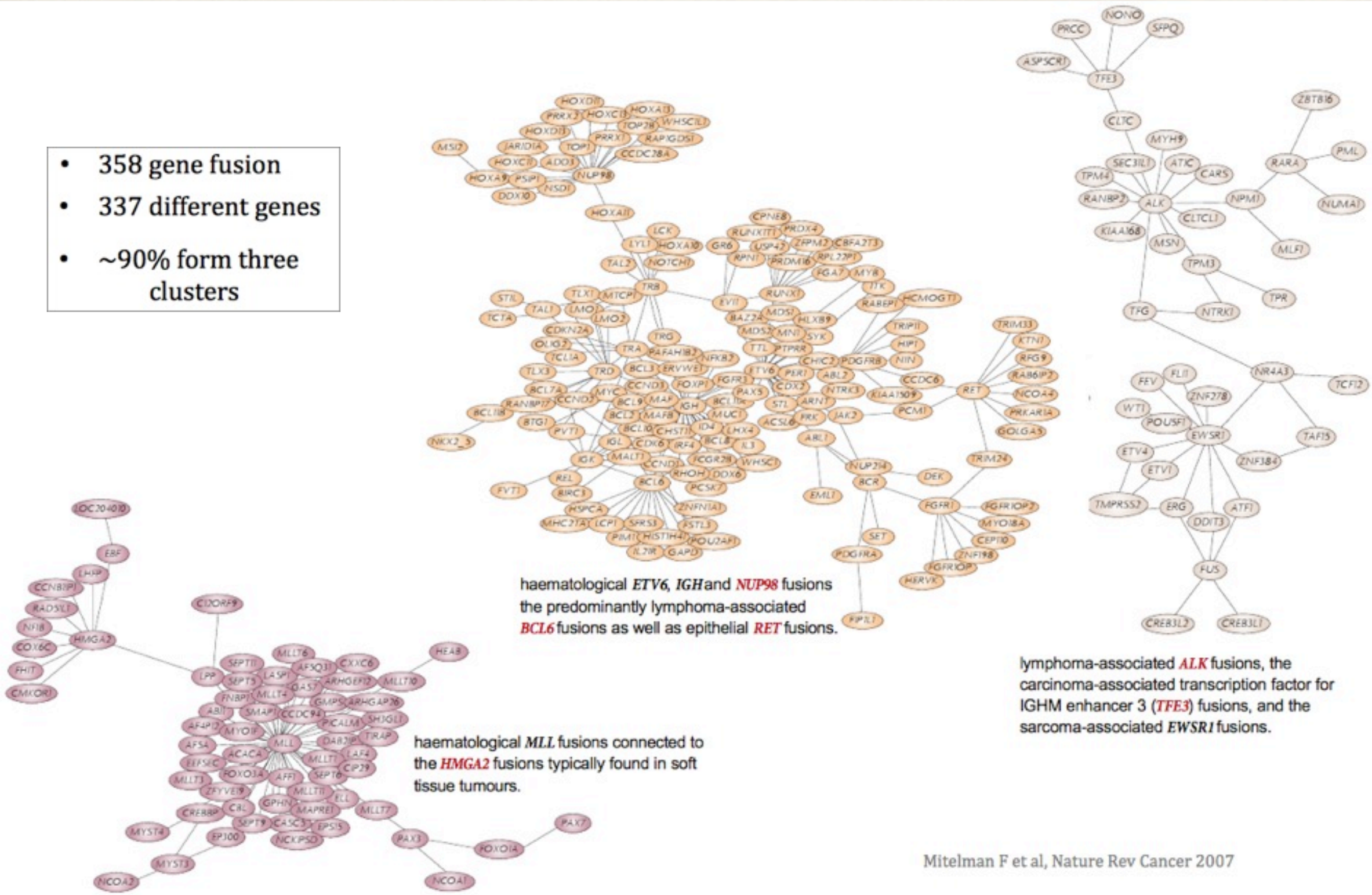
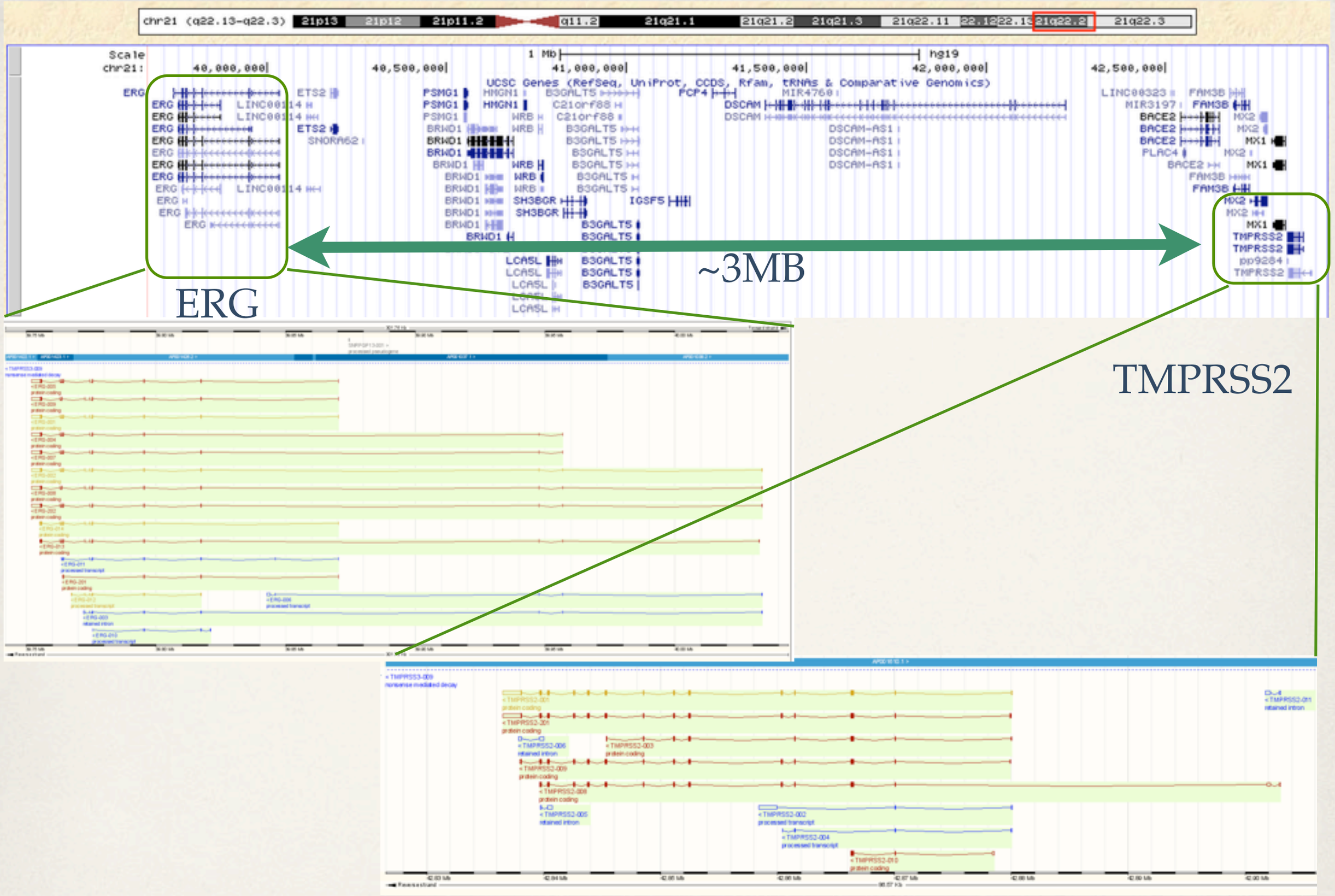
- 65% of protein-coding genes have distal 5' transcription start sites (ENCODE pilot)

- 358 gene fusion
- 337 different genes
- ~90% form three clusters



How many different gene fusions do we know?

What about prostate cancer?



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EDITORIAL

The Prostate Cancer Muddle

Published: March 19, 2009 The New York Times

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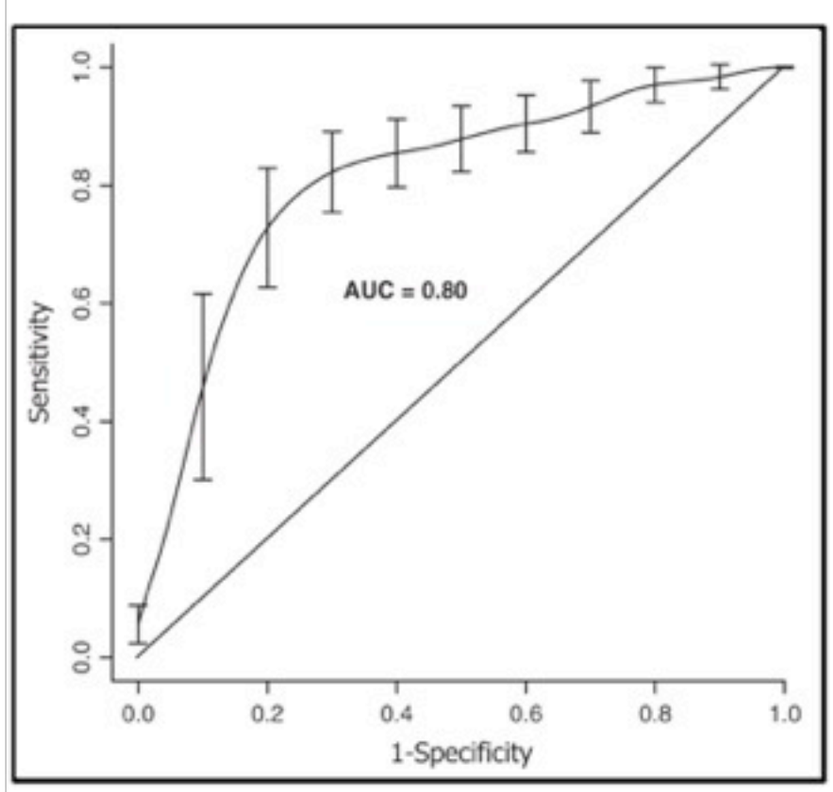
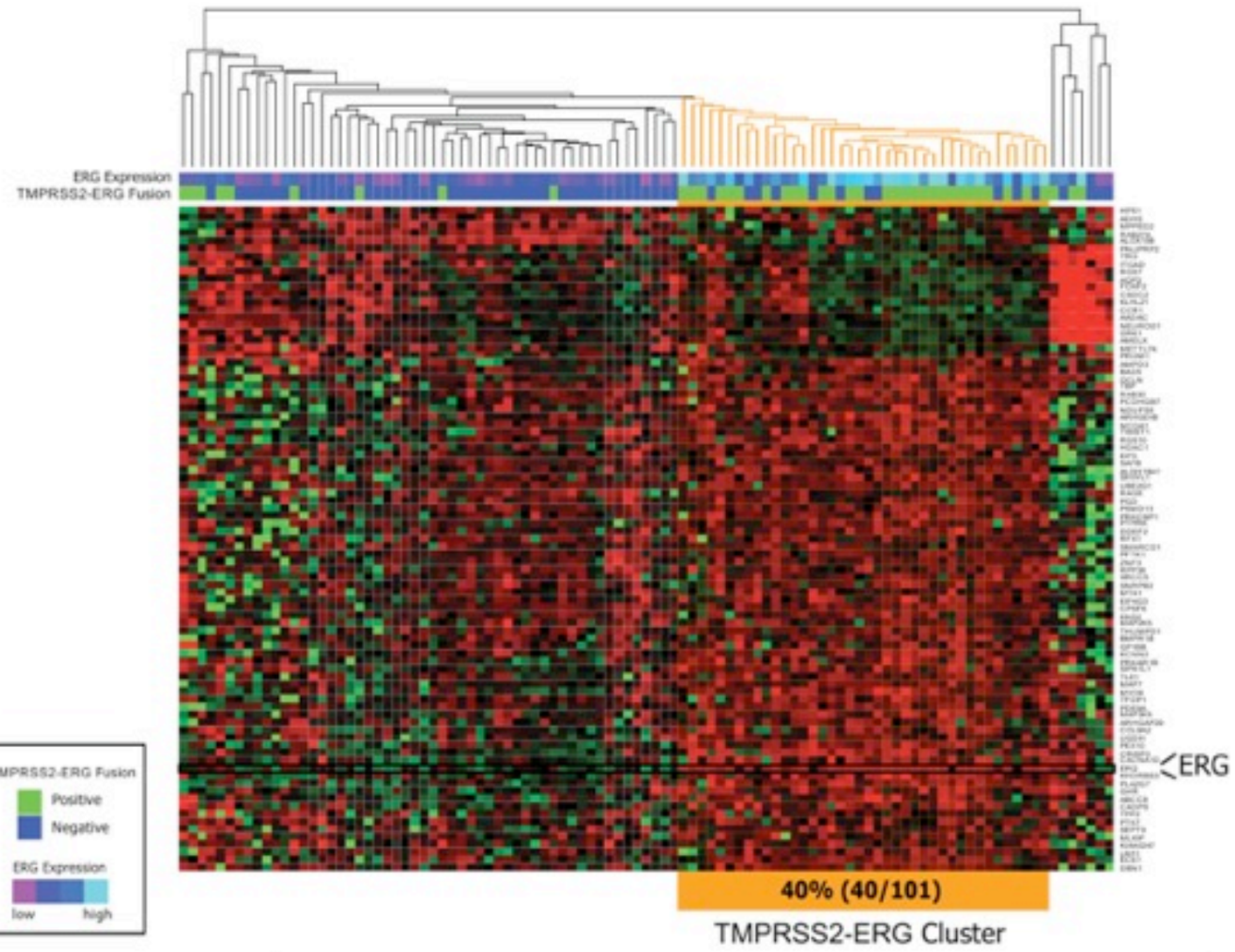
Are fusions important for outcome prediction?

ERG rearranged cases as a subclass of prostate cancer?

ERG rearrangement status:
87-gene signature

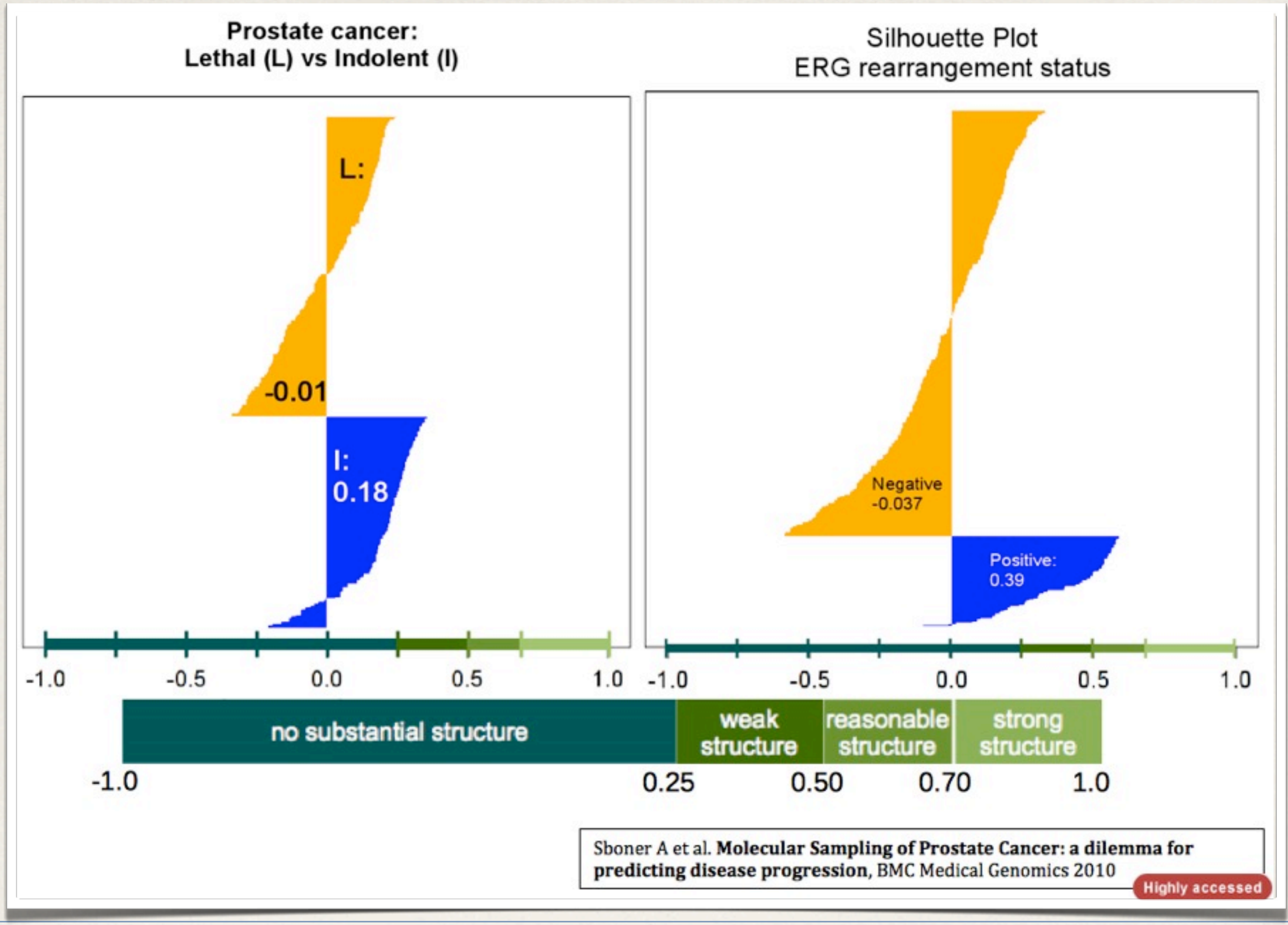




Estrogen-Dependent Signaling in a Molecularly Distinct Subclass of Aggressive Prostate Cancer

J Natl Cancer Inst 2008;100:815–825

ERG rearranged subclass is more homogeneous than lethal/indolent PCa



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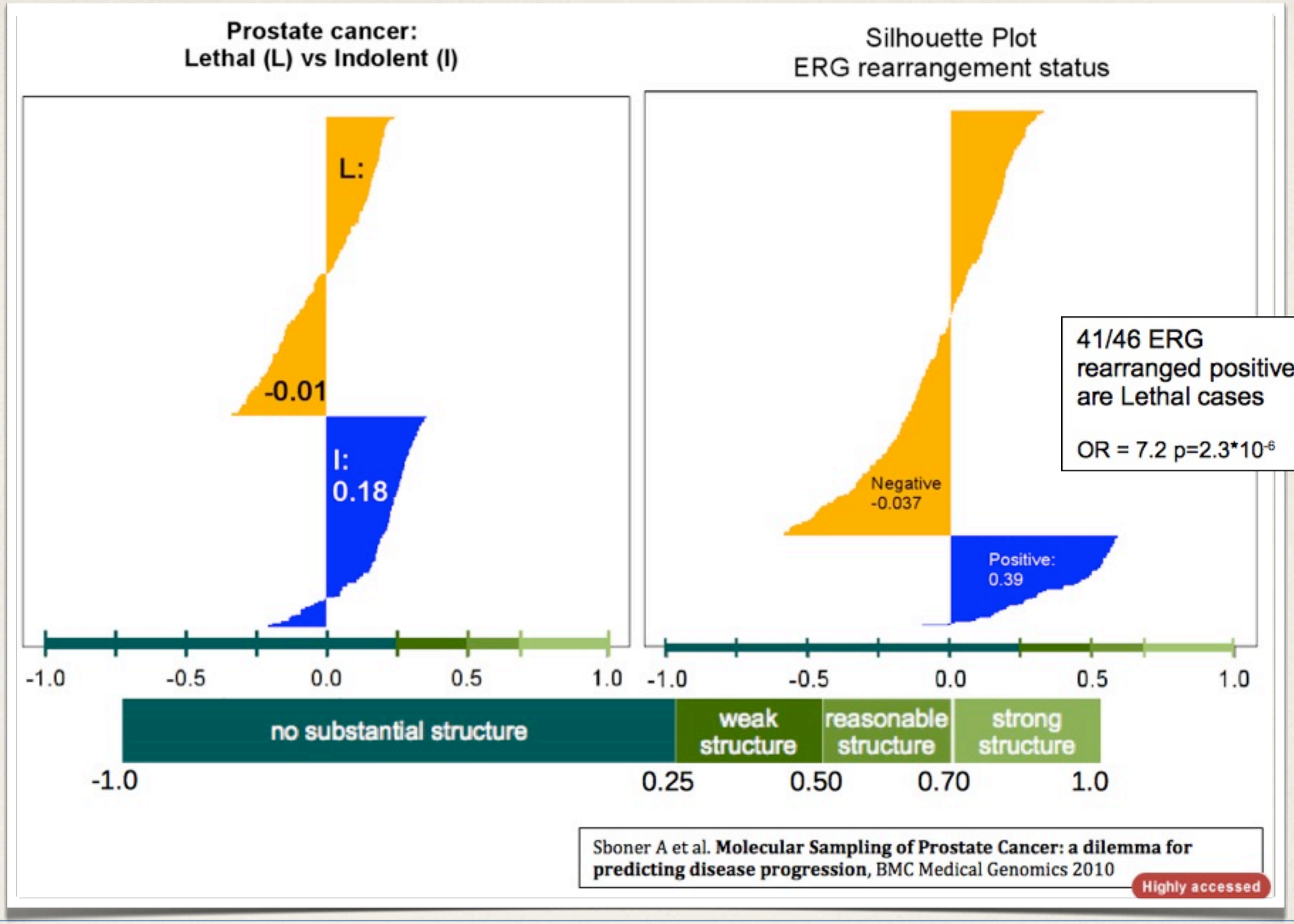

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Are gene fusions key elements in prostate cancer?

- *Hypothesis I*: additional gene fusions may be present in prostate cancer

- *Hypothesis II*: can those fusions help better define the molecular landscape of disease development and progression?

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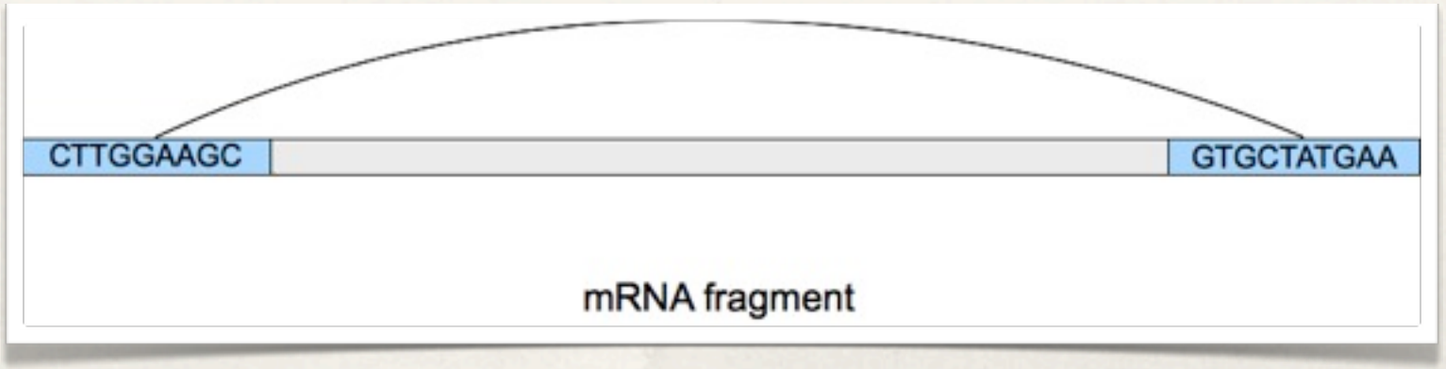
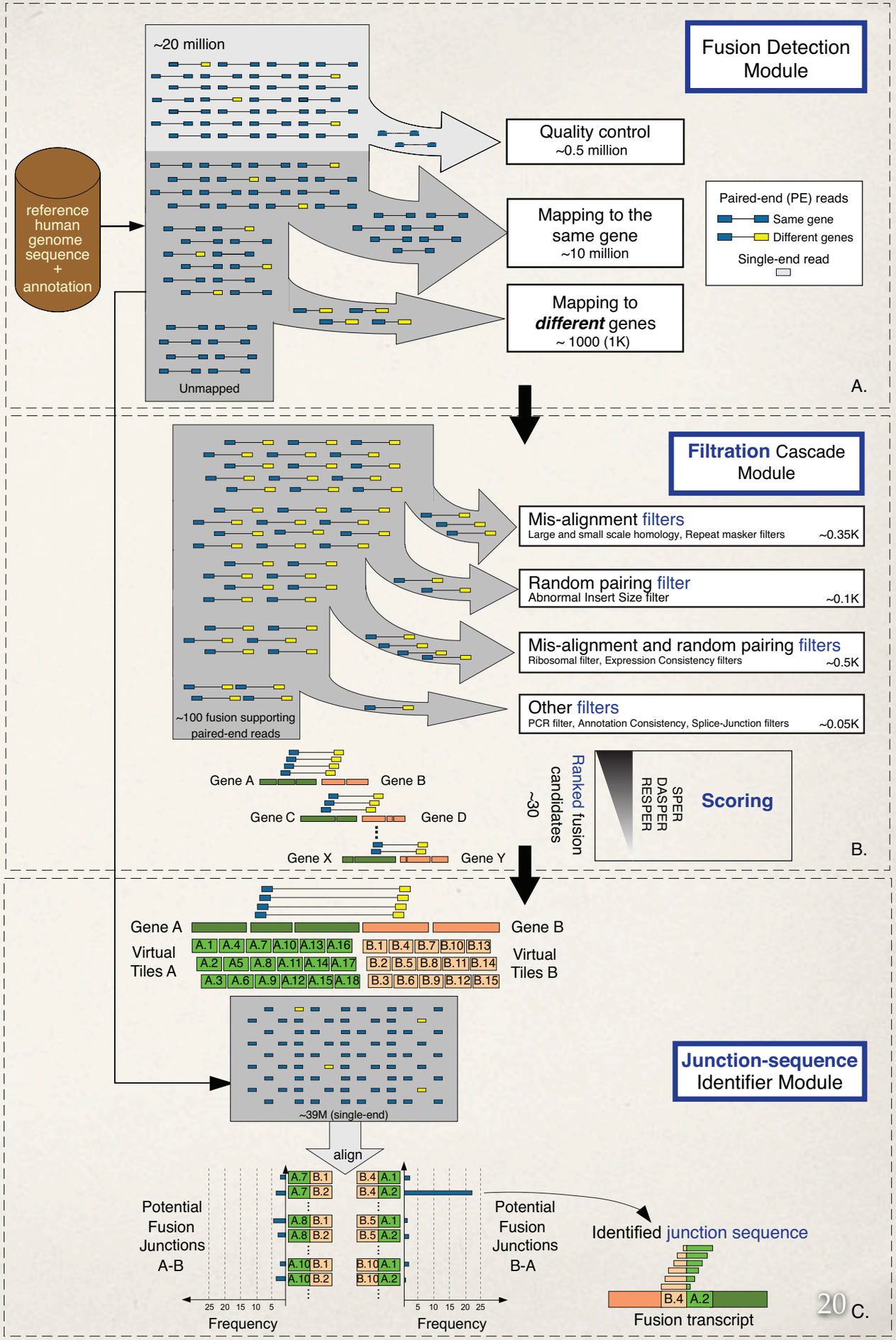
- Next-generation sequencing can address this challenge directly, especially with:

  - *Paired-end* RNA-Seq: keeping connectivity information



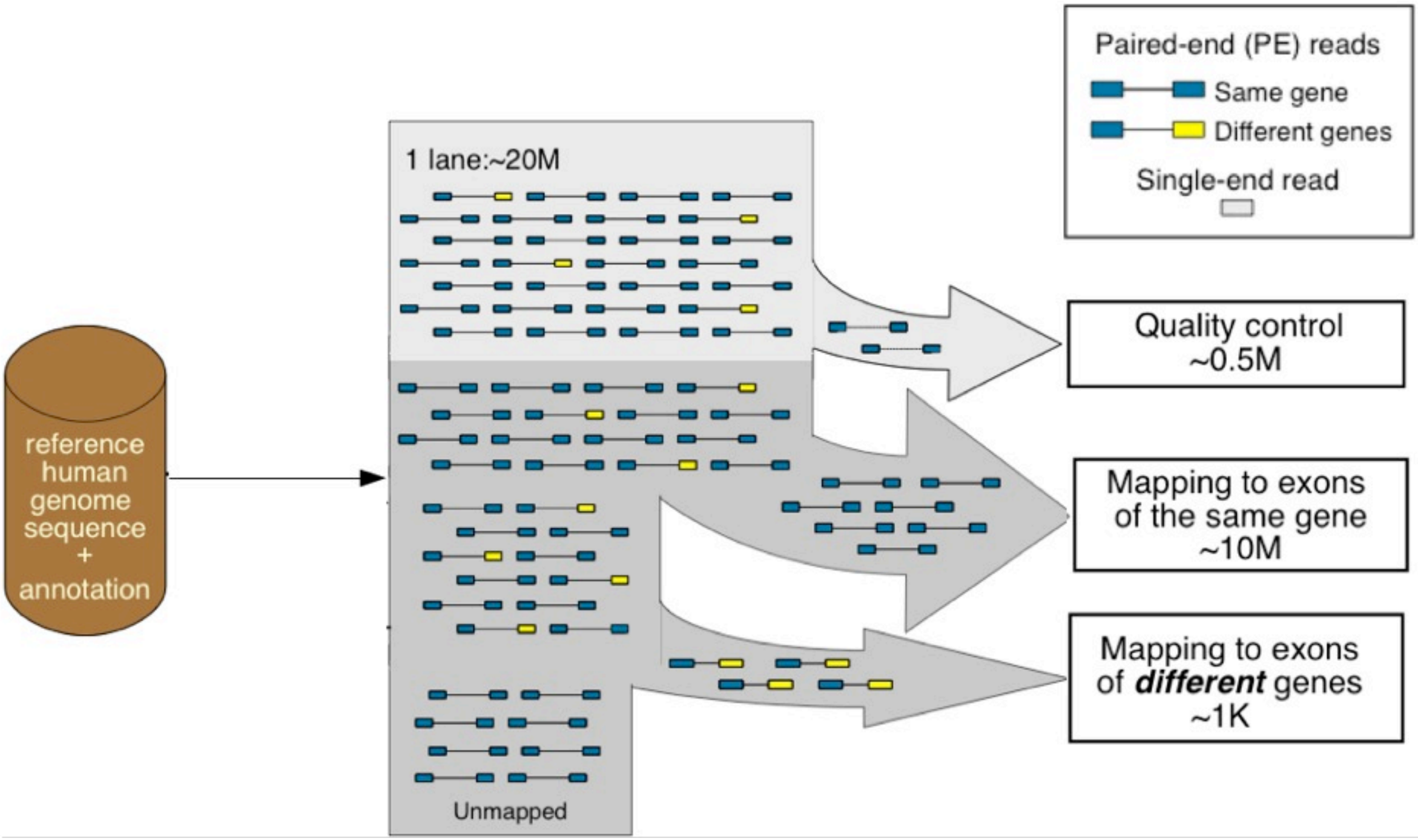
FusionSeq: a computational modular framework

- Fusion Detection Module
- Filtration Cascade Module
- Junction-sequence identifier module



Sboner, Andrea, et al. "FusionSeq: a Modular Framework for Finding Gene Fusions by Analyzing Paired-End RNA-Sequencing Data." Genome Biology 11, no. 10 (2010): R104.

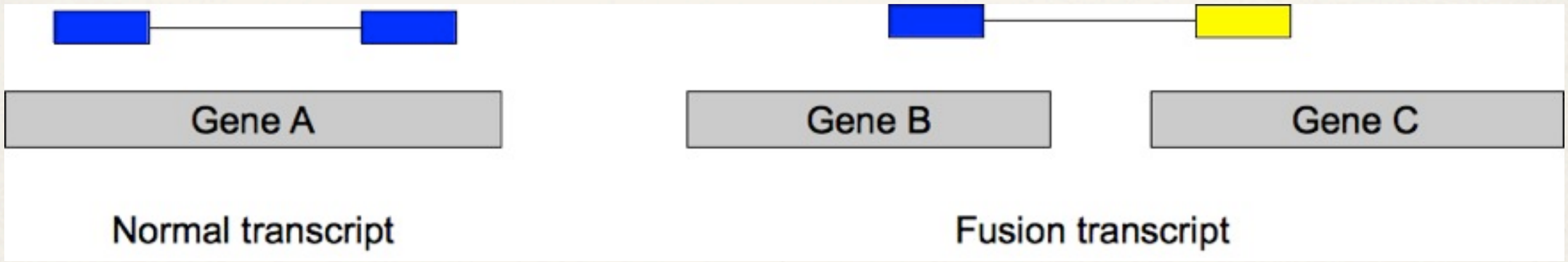
Highly accessed

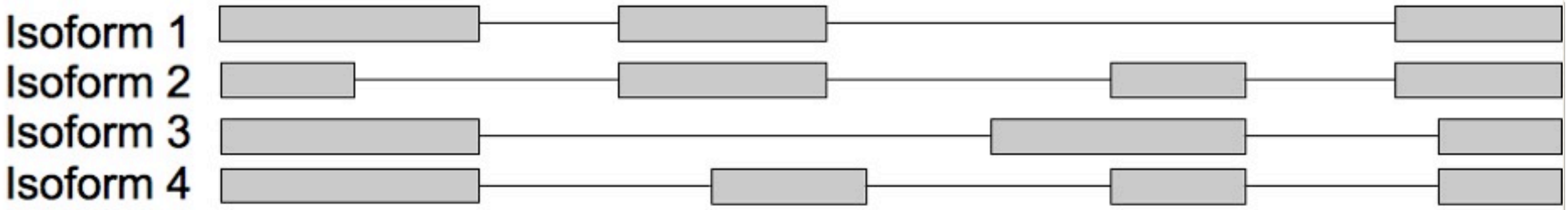


Fusion Detection Module

How to identify fusion transcripts?

- *Straightforward*:
  - If the two ends map to different genes, then we have a potential fusion transcript





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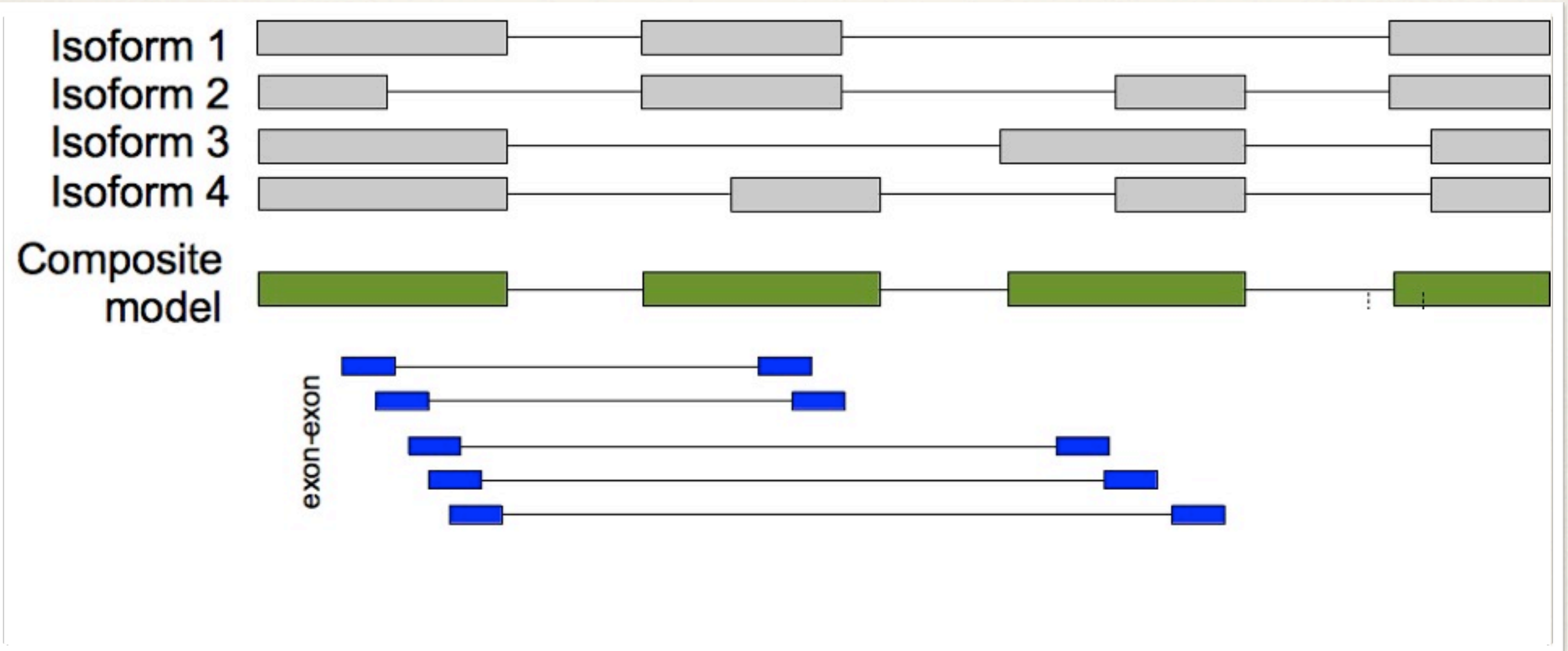

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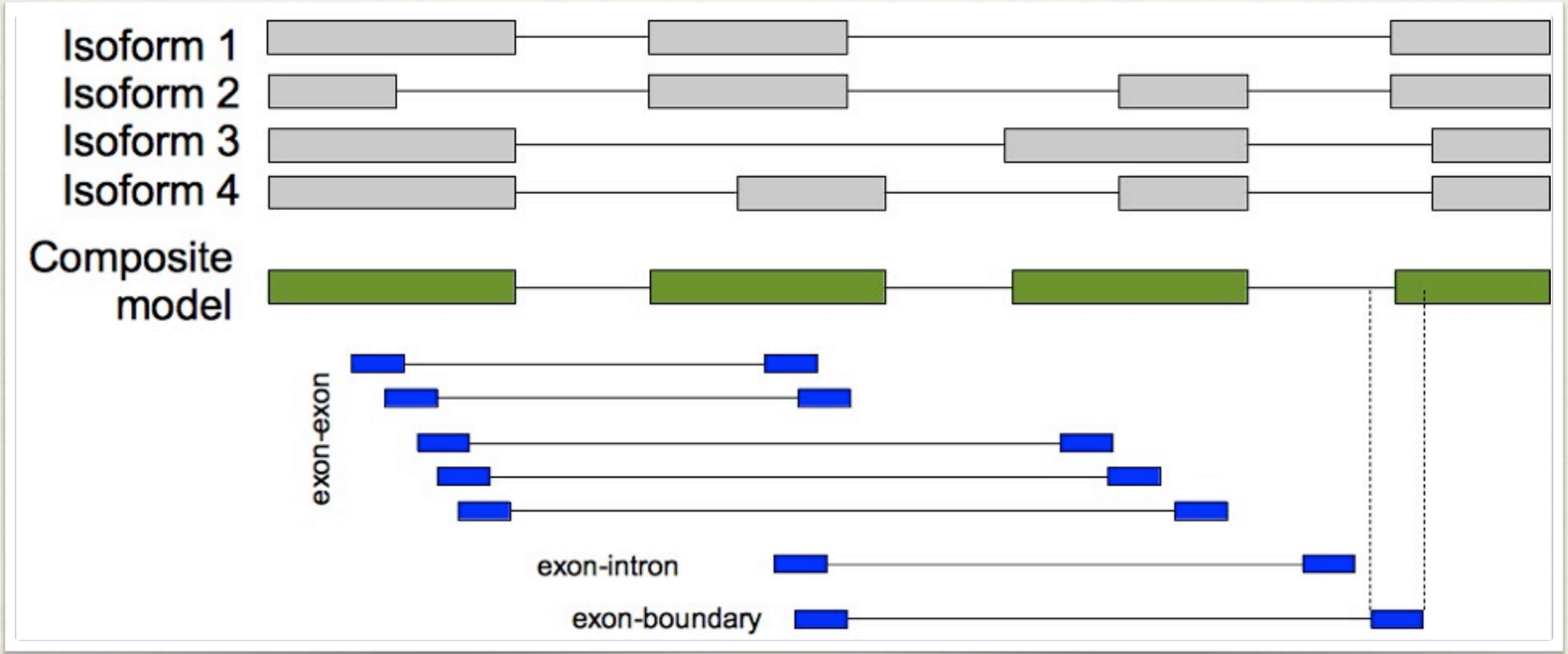

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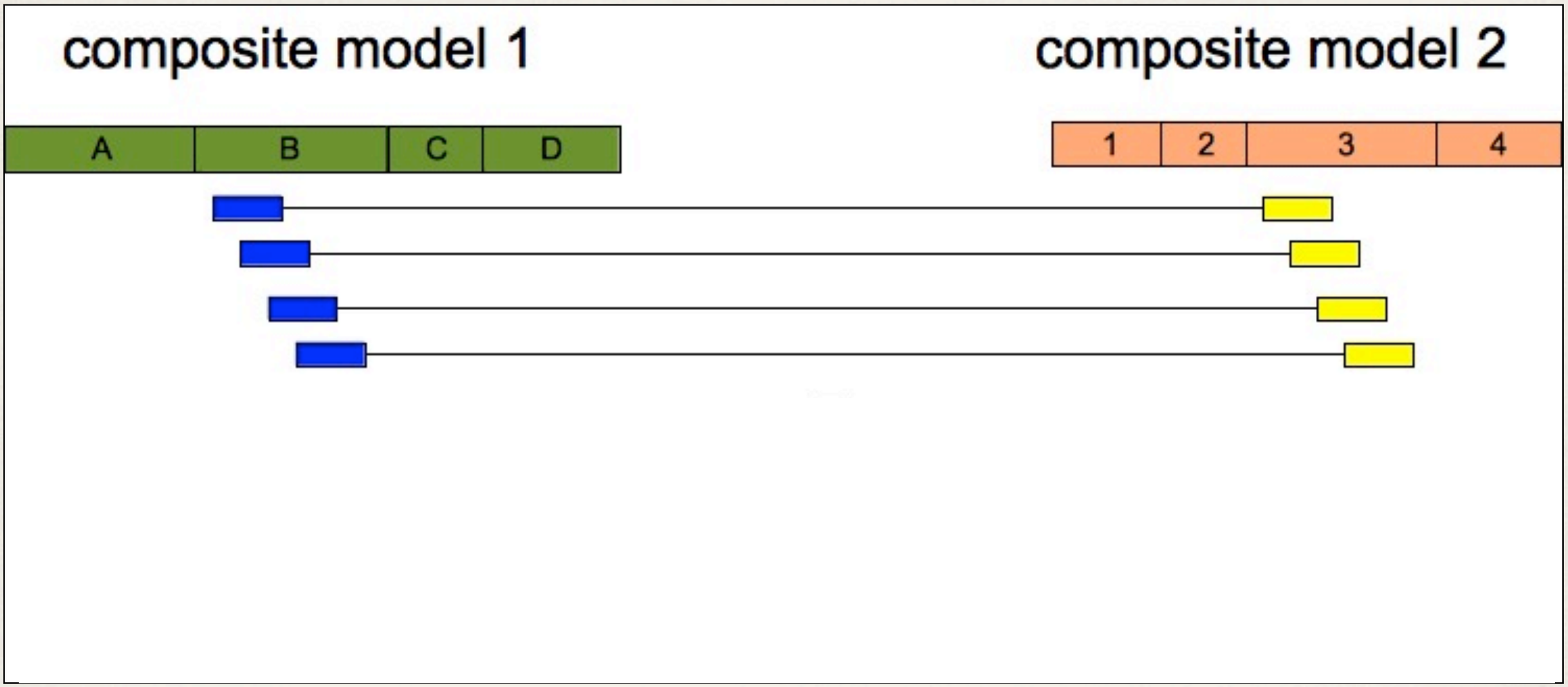
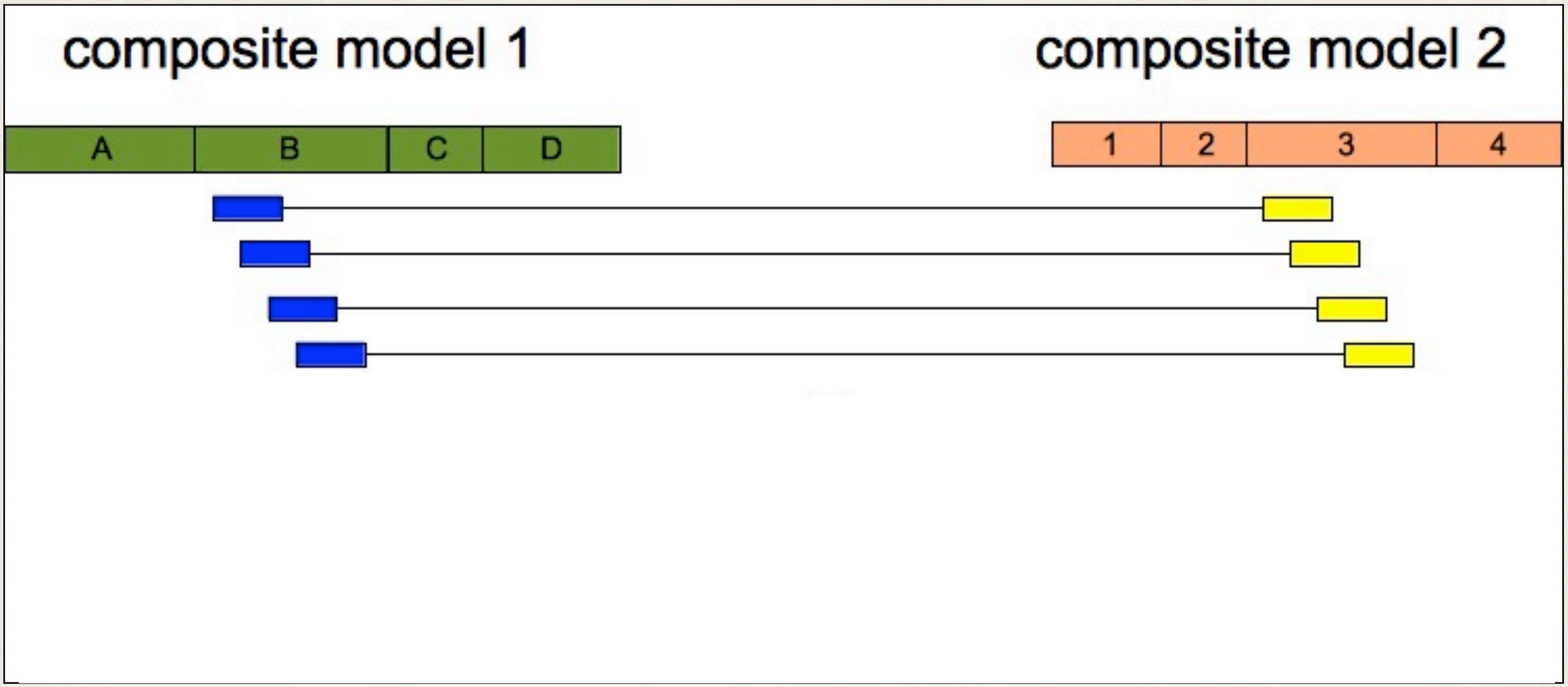


Finding fusion transcripts



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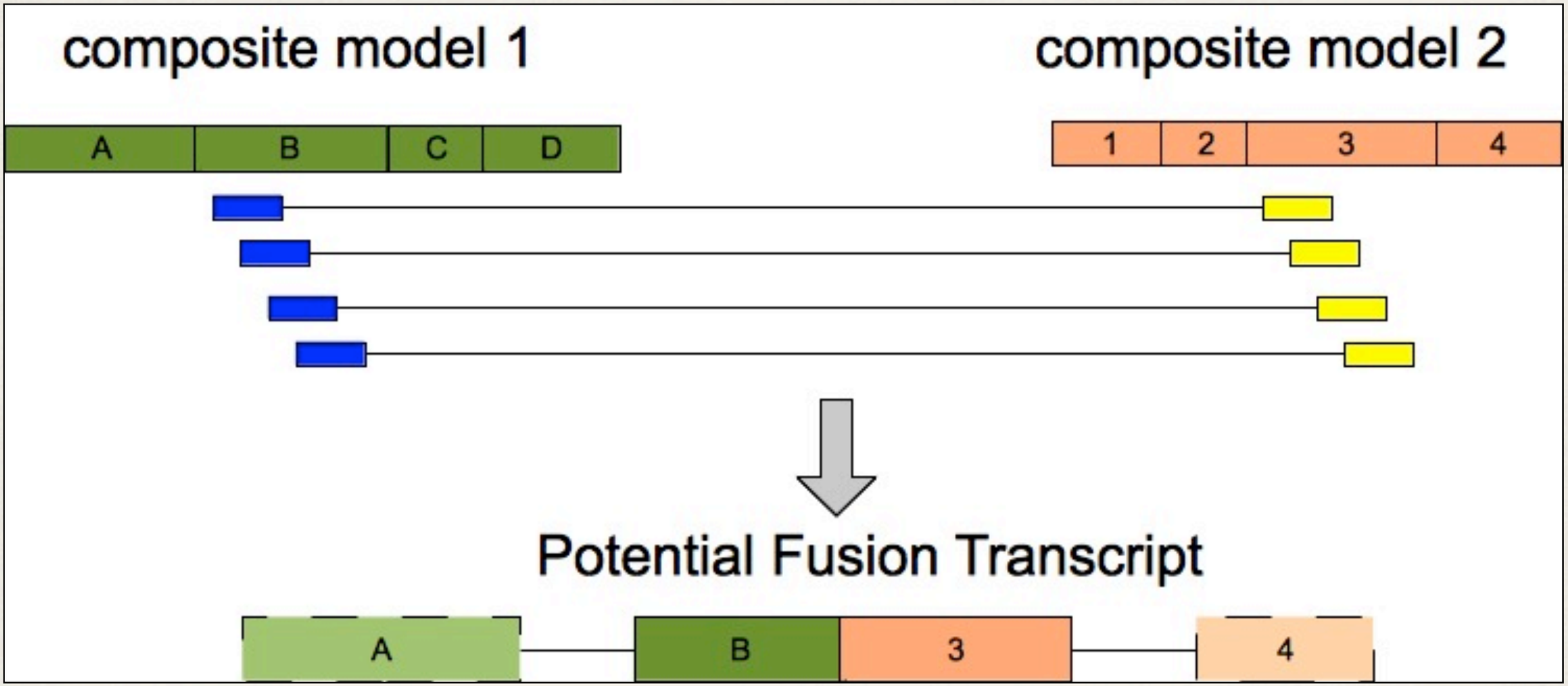
Finding fusion transcripts



- Each PE read can be assigned to one “gene”
- *Potential Fusion Transcripts*: if pair belong to different genes

Finding fusion transcripts

Not an ideal world: sources of errors

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- *Mis-alignments*
  - Base caller error
  - SNPs
  - RNA editing
  - Sequence similarity (paralogs, pseudogenes)

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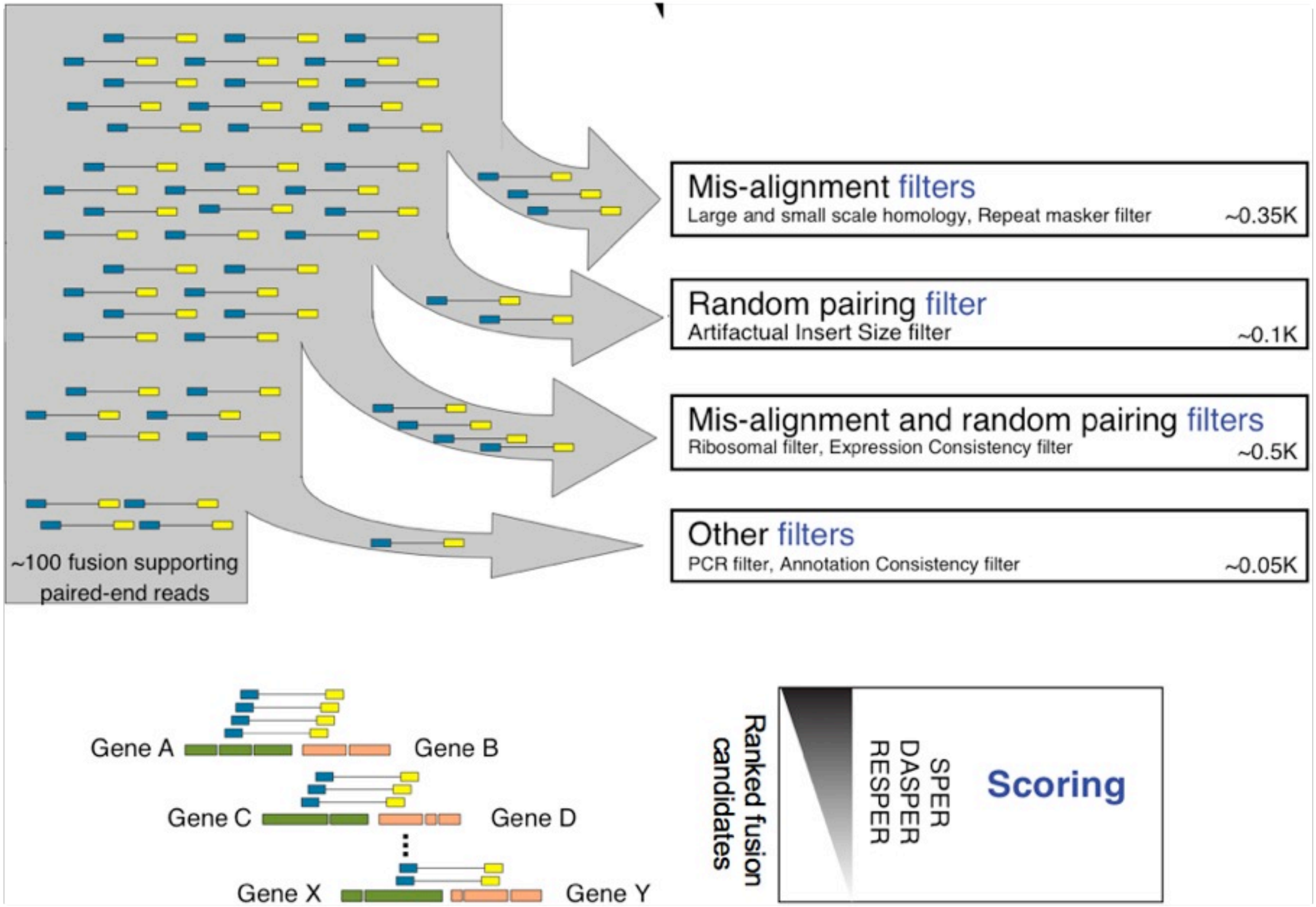
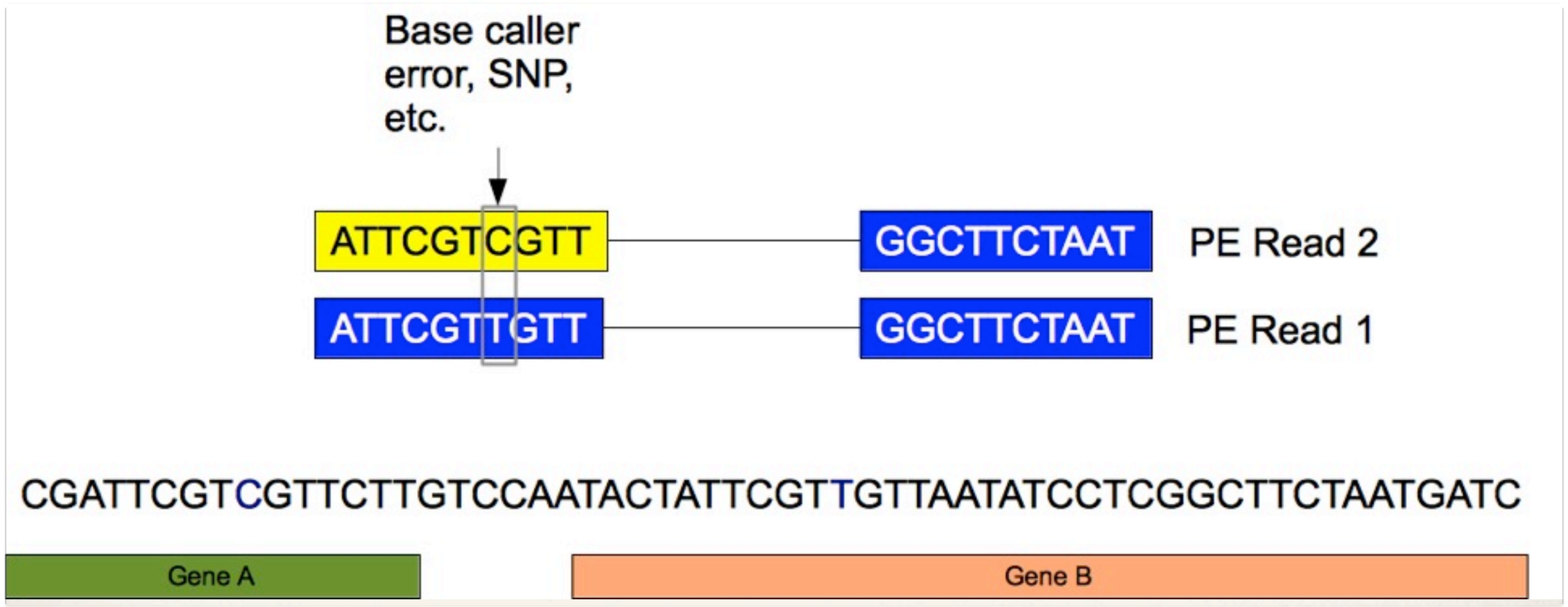
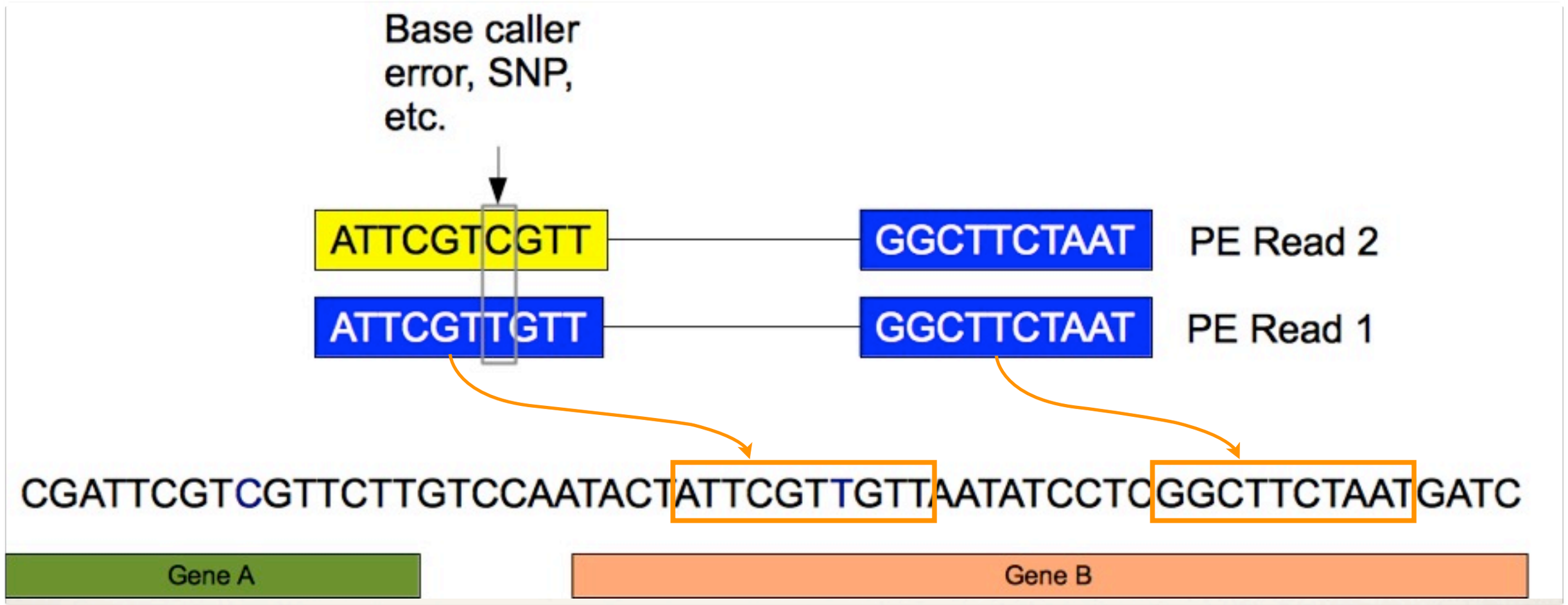
- *Combination of mis-alignment and random pairing*
- *PCR amplification, gene annotation inconsistencies/incompleteness*



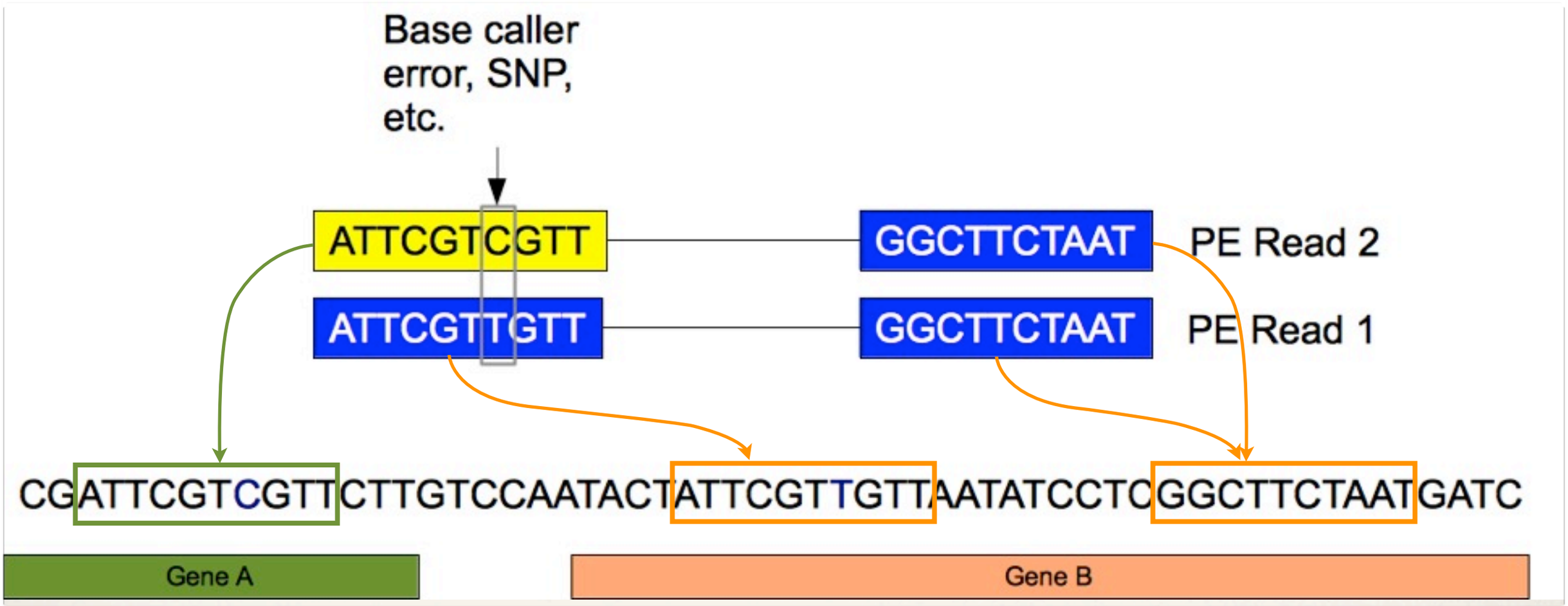
Filtration Cascade Module



Mis-alignment



Mis-alignment



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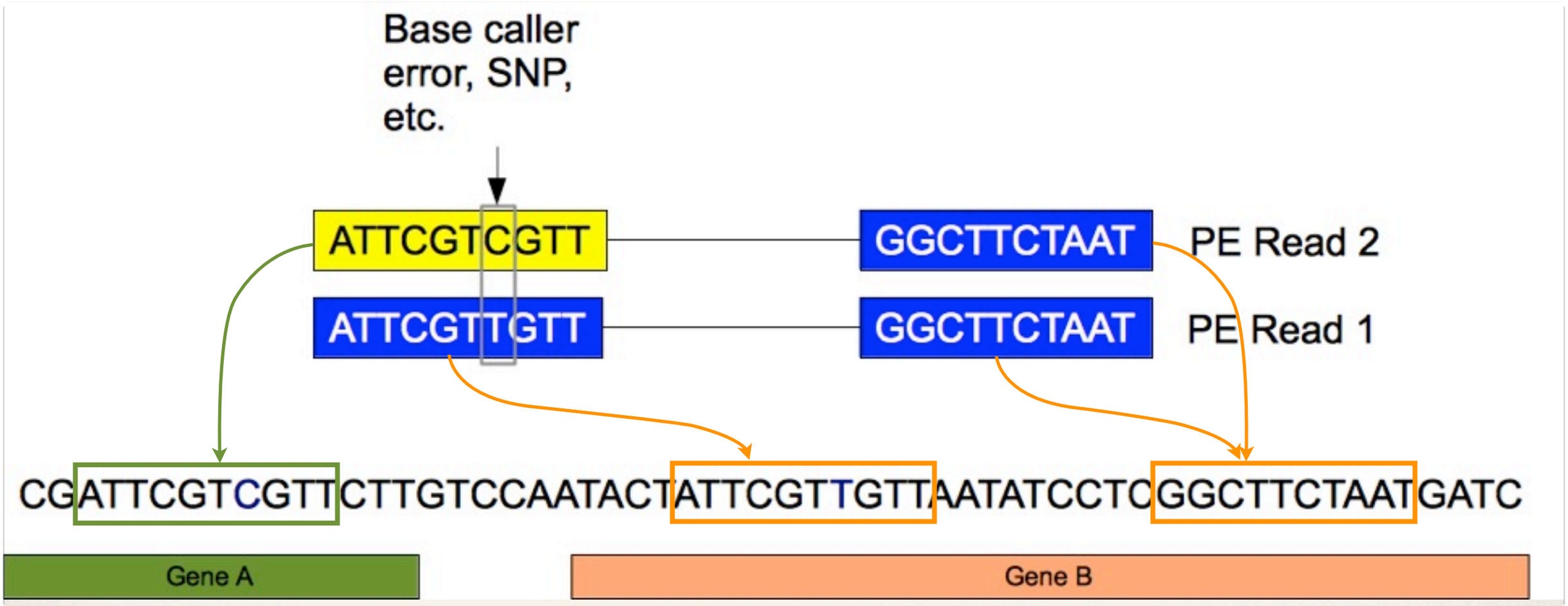

- *Large-scale*: similarity at the gene level → TreeFam to exclude paralogs

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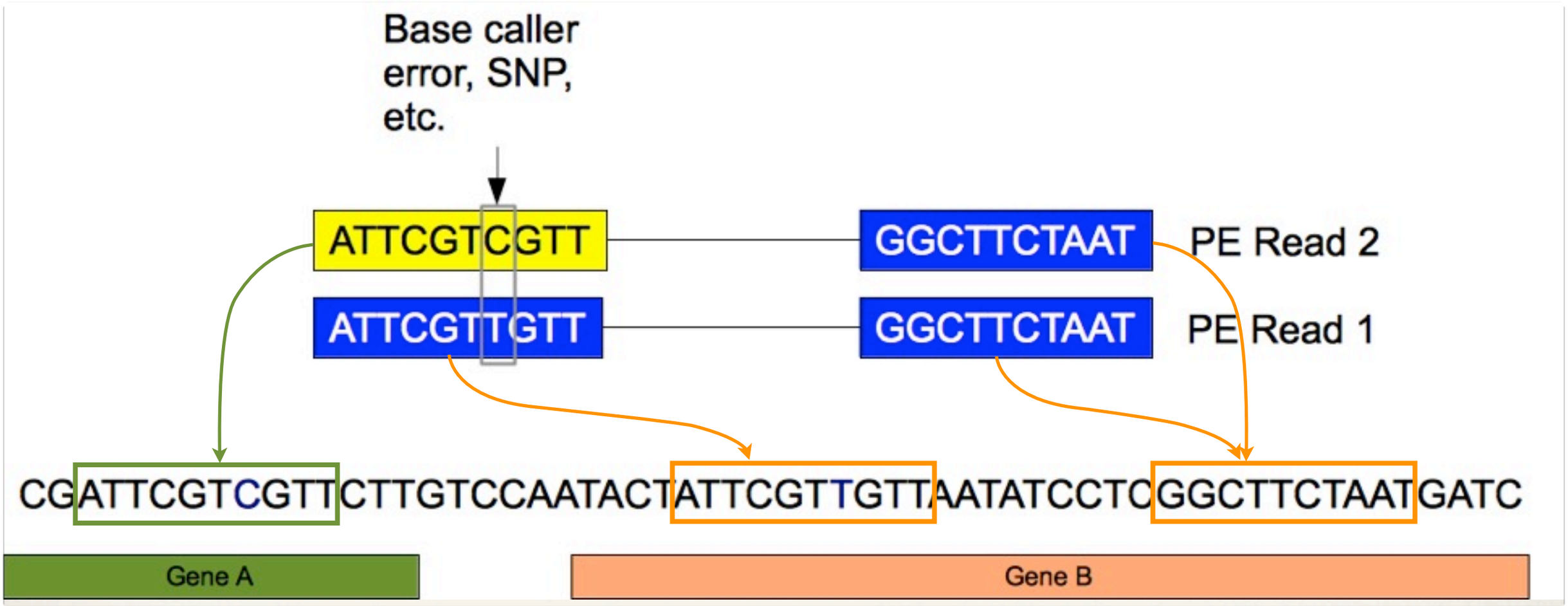
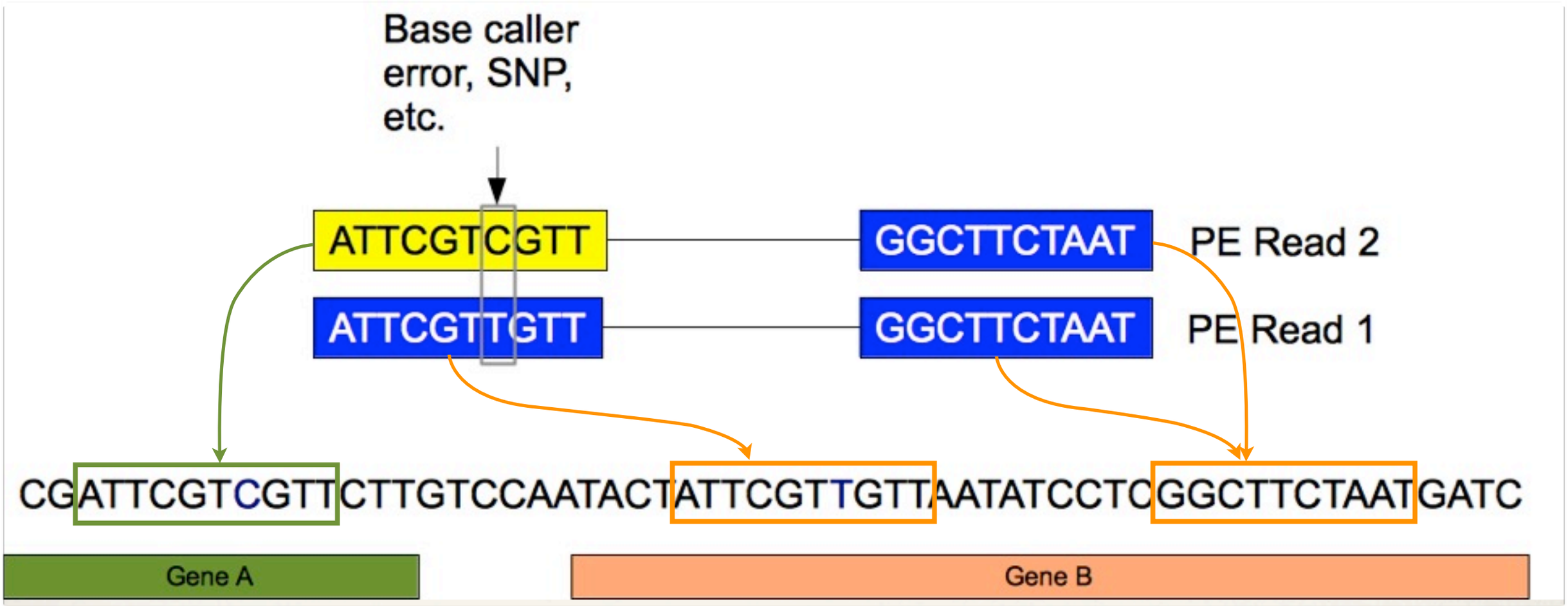


- *Large-scale*: similarity at the gene level → TreeFam to exclude paralogs
- *Small-scale*: similarity of smaller regions within the genes → 2-step approach using bowtie and BLAT

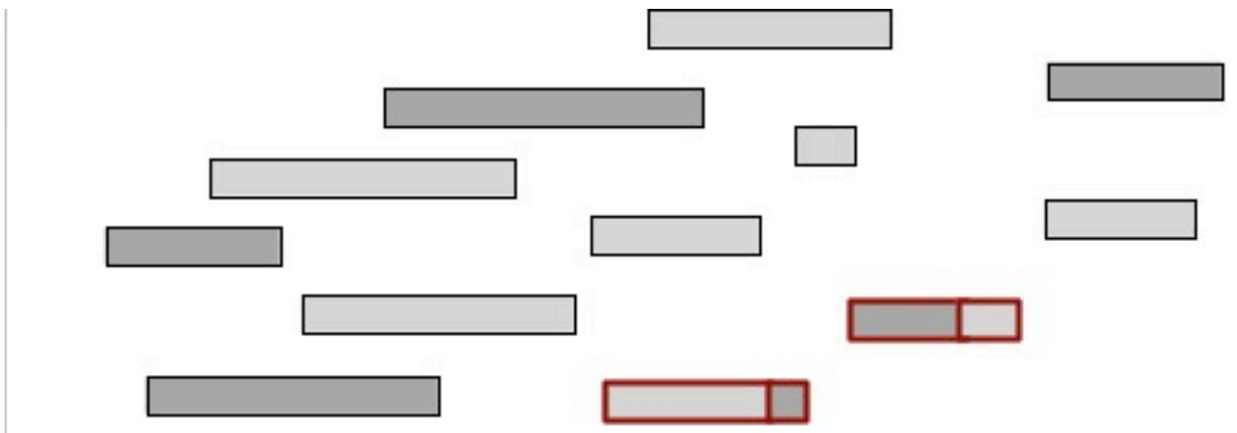
Mis-alignment



- *Large-scale*: similarity at the gene level → TreeFam to exclude paralogs
- *Small-scale*: similarity of smaller regions within the genes → 2-step approach using bowtie and BLAT
- *Repetitive region*: reads mapping to low-sequence complexity

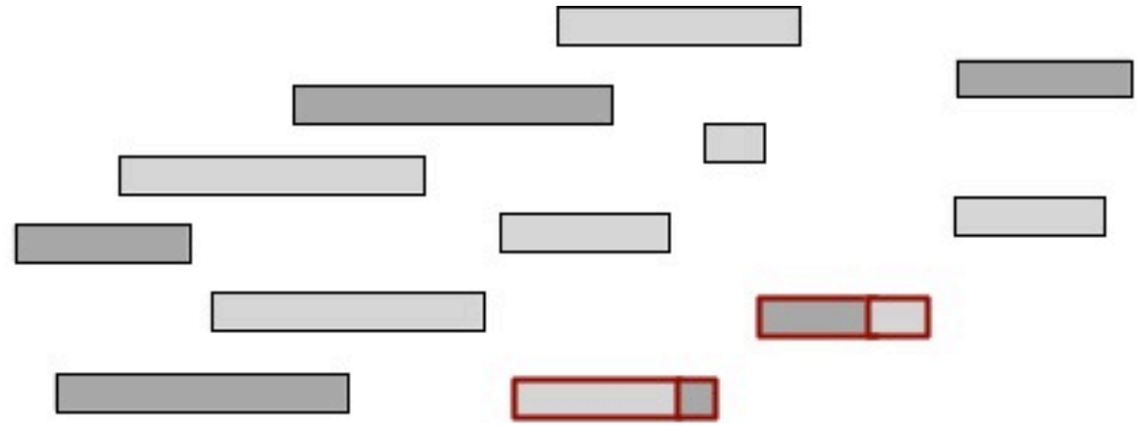
Mis-alignment

Random pairing of transcript fragments



Random pairing of transcript fragments

- Inefficient A-tailing → random joining of transcript fragments → artificial chimeric transcripts



Random pairing of transcript fragments

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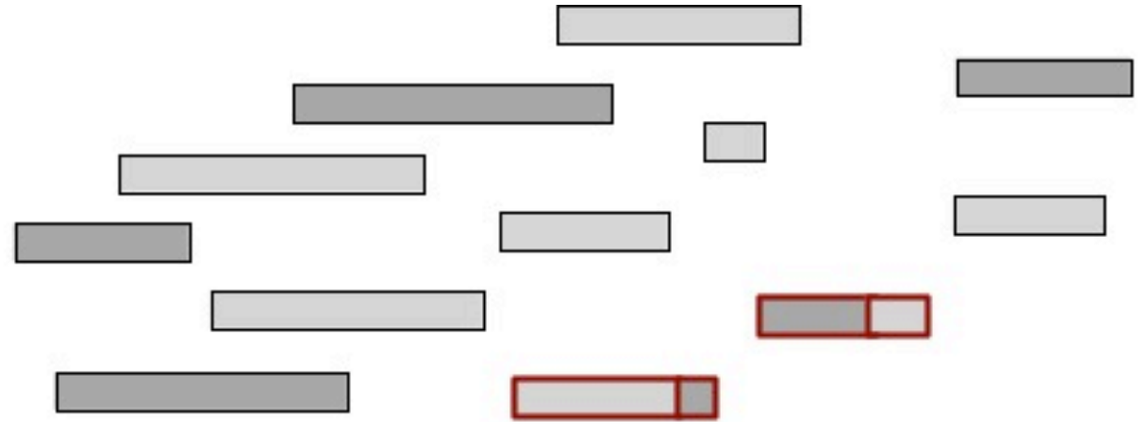
- How to remove them?



Random pairing of transcript fragments

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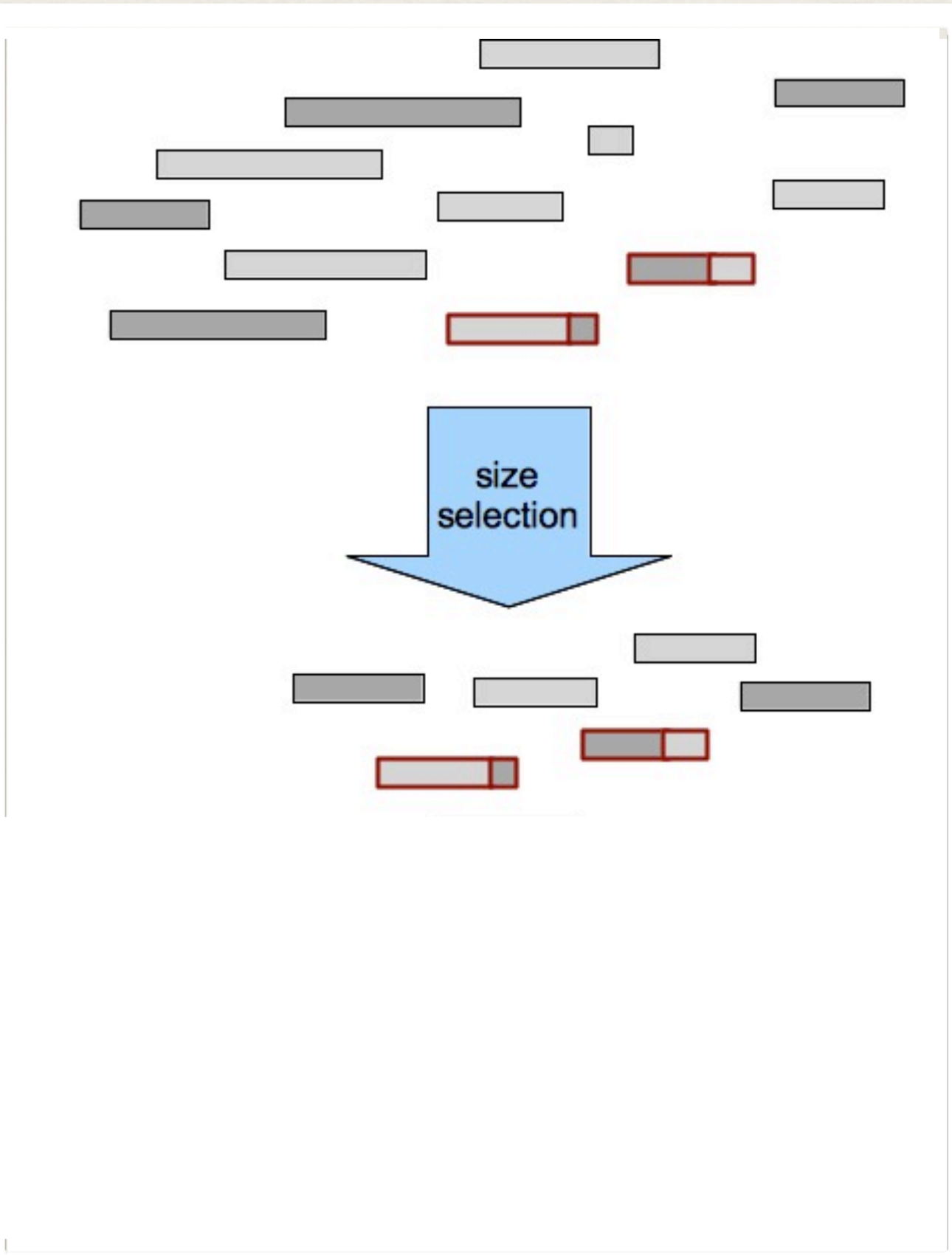
  - *Observation 1*: preparation protocol requires a size-selection step, i.e. all transcript fragments have similar size



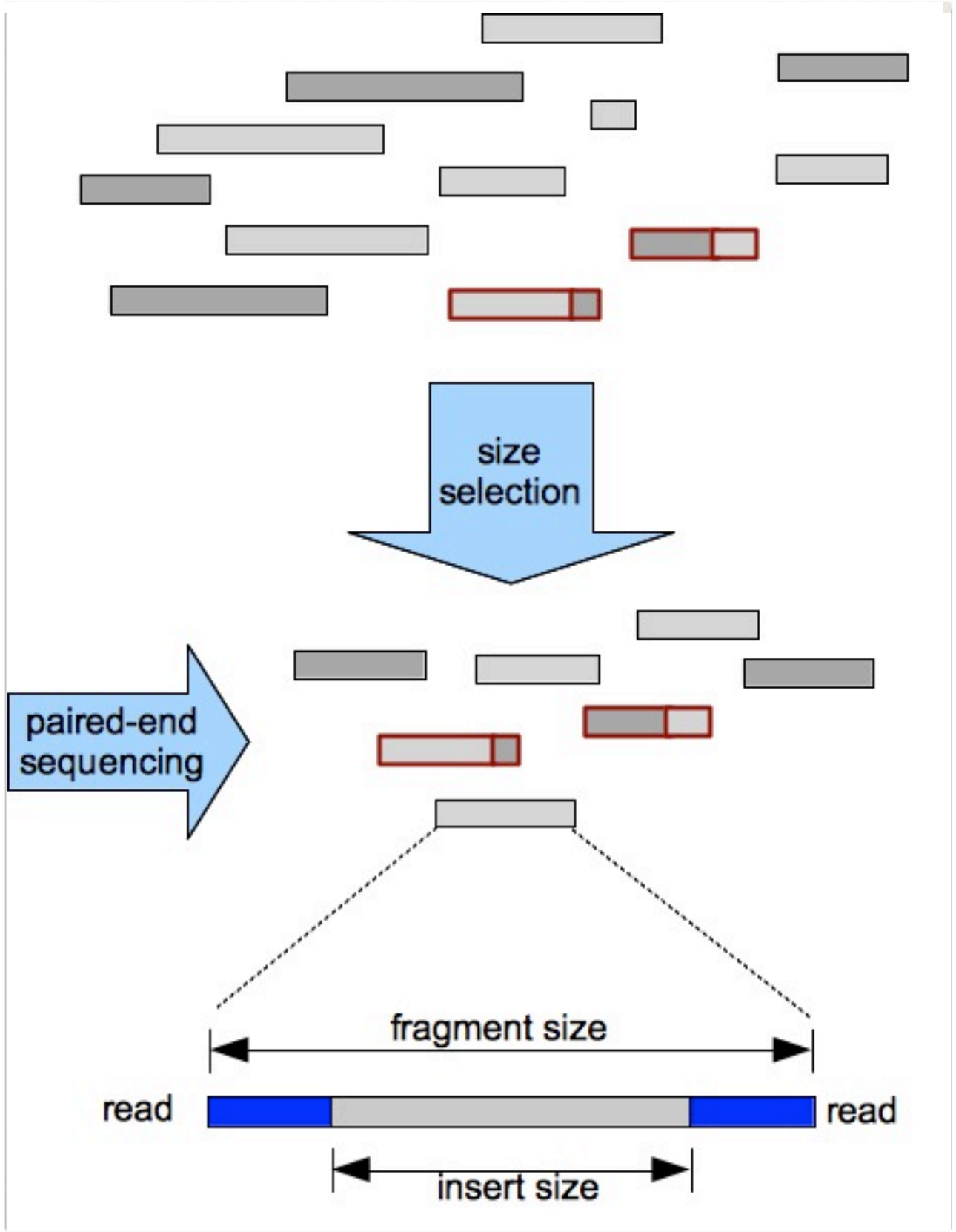
Random pairing of transcript fragments

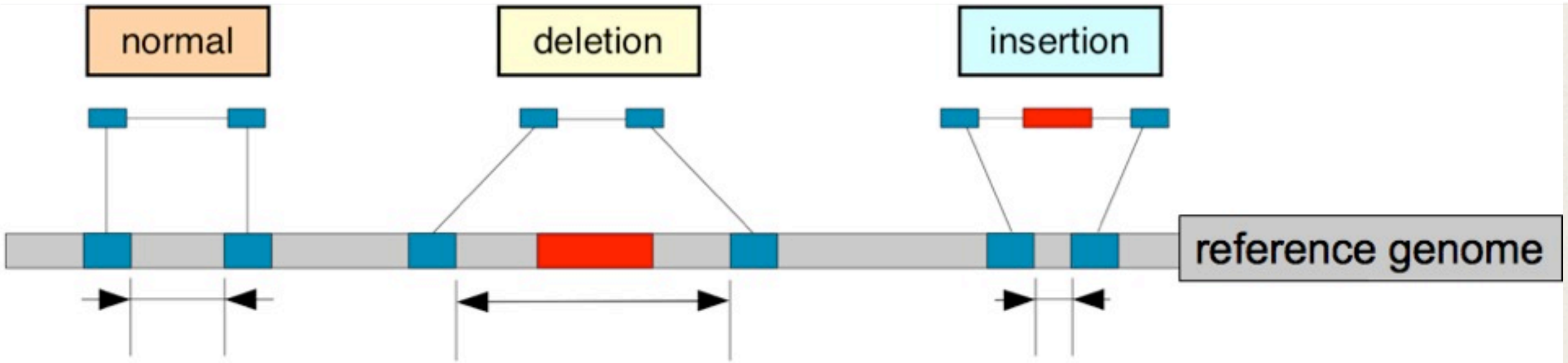
- Inefficient A-tailing → random joining of transcript fragments → artificial chimeric transcripts

- How to remove them?

  - *Observation 1*: preparation protocol requires a size-selection step, i.e. all transcript fragments have similar size

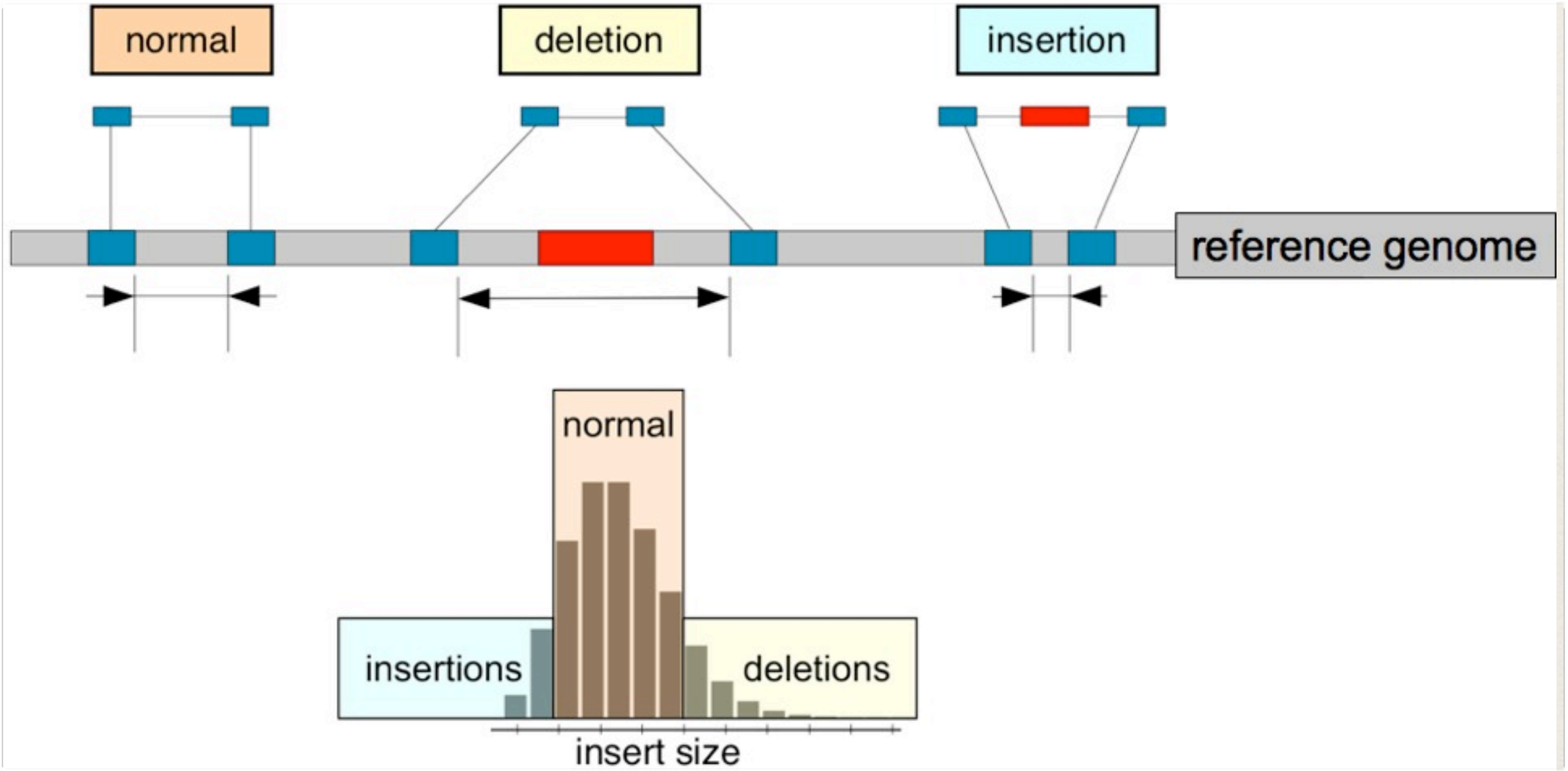
  - *Observation 2:* the insert-size distribution of the PE reads should be roughly the same for all PE reads





Insert-size distribution concept

Detecting structural variations (SVs)



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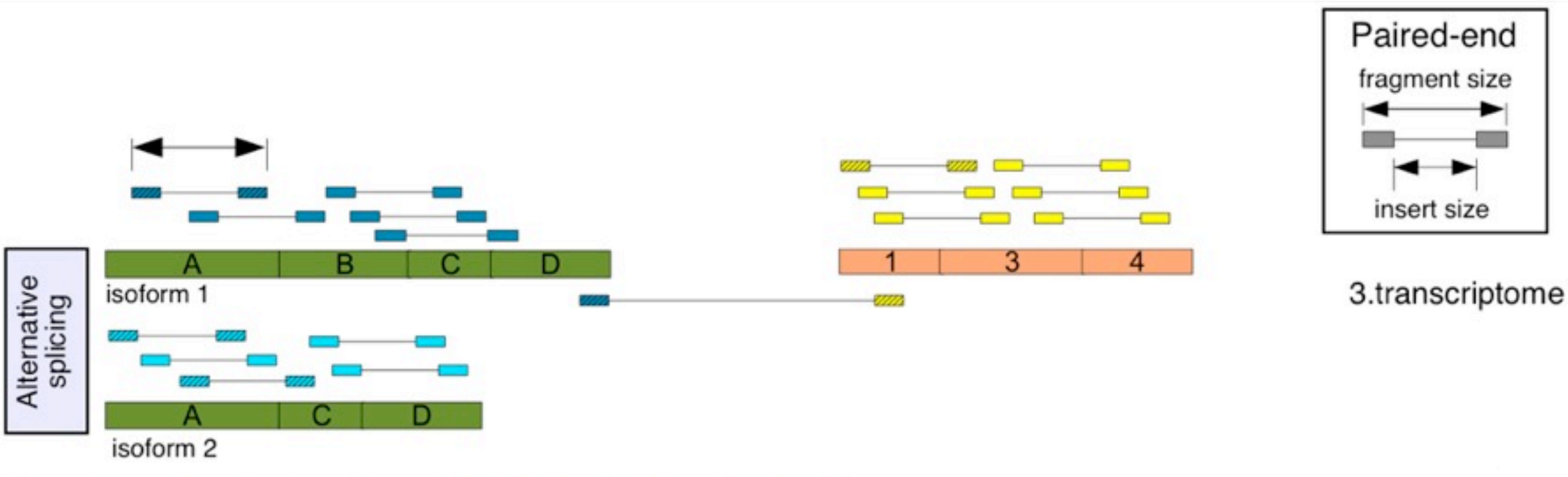
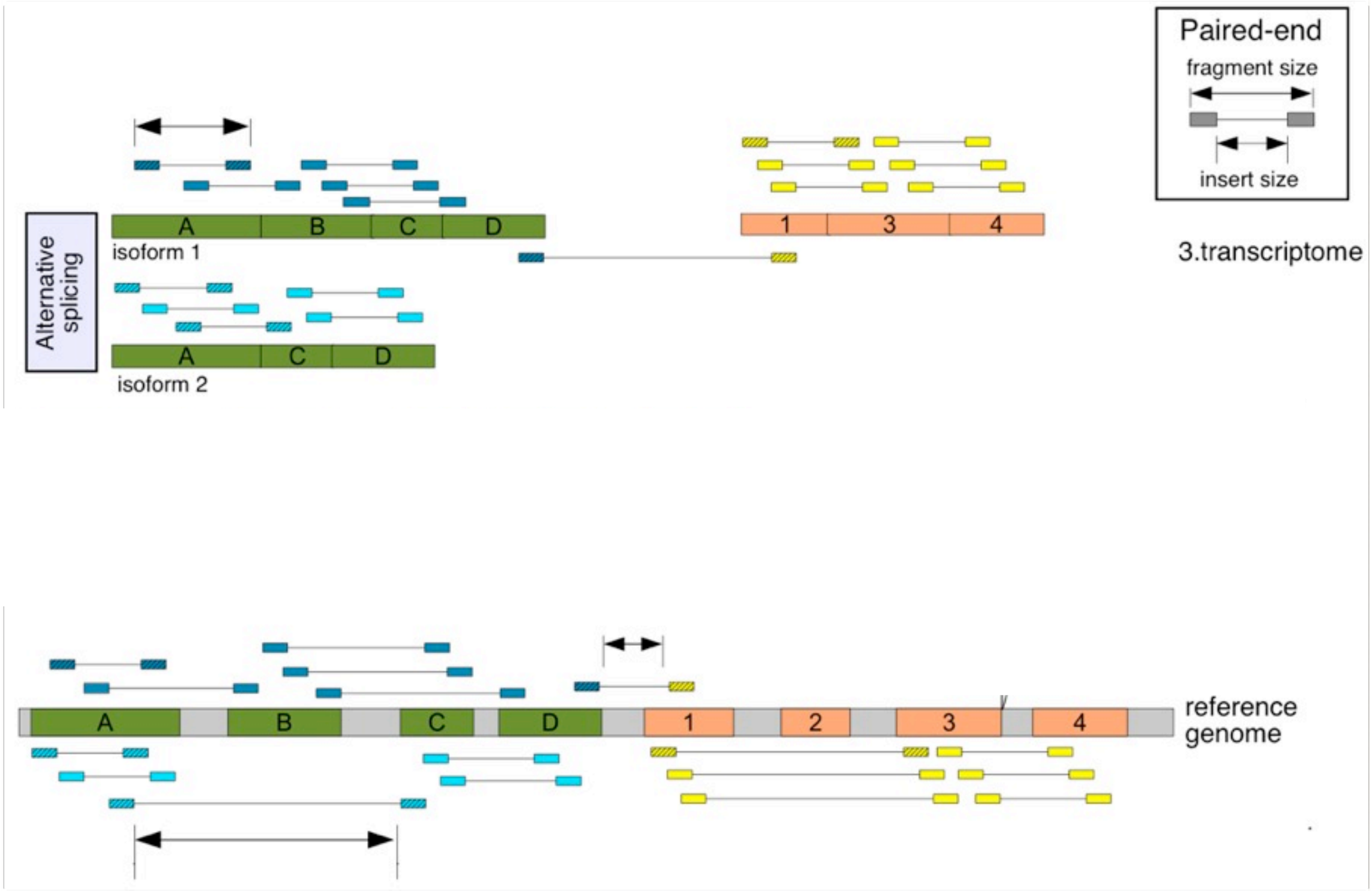


Complexity of applying the insert-size analysis to the transcriptome



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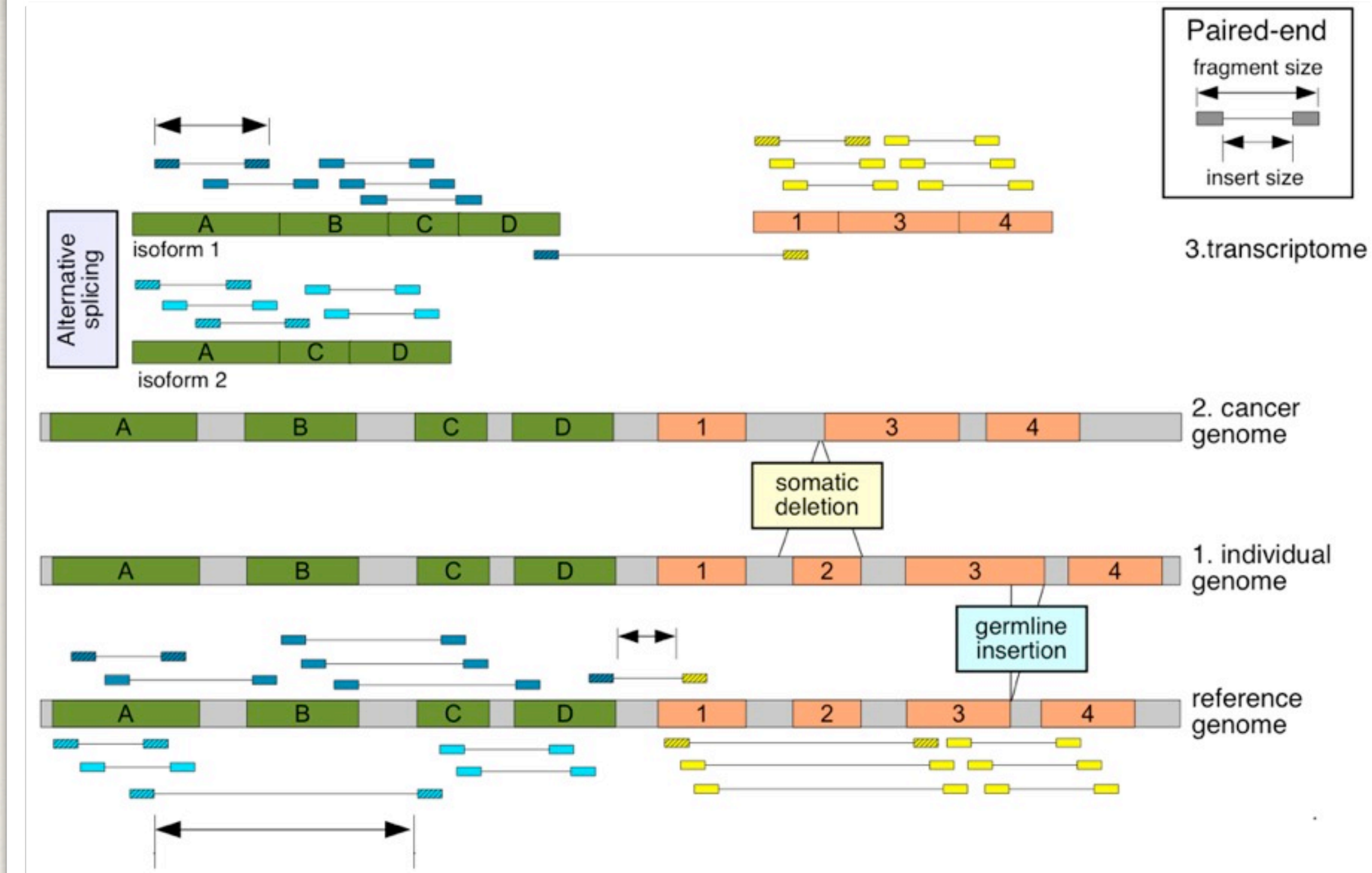

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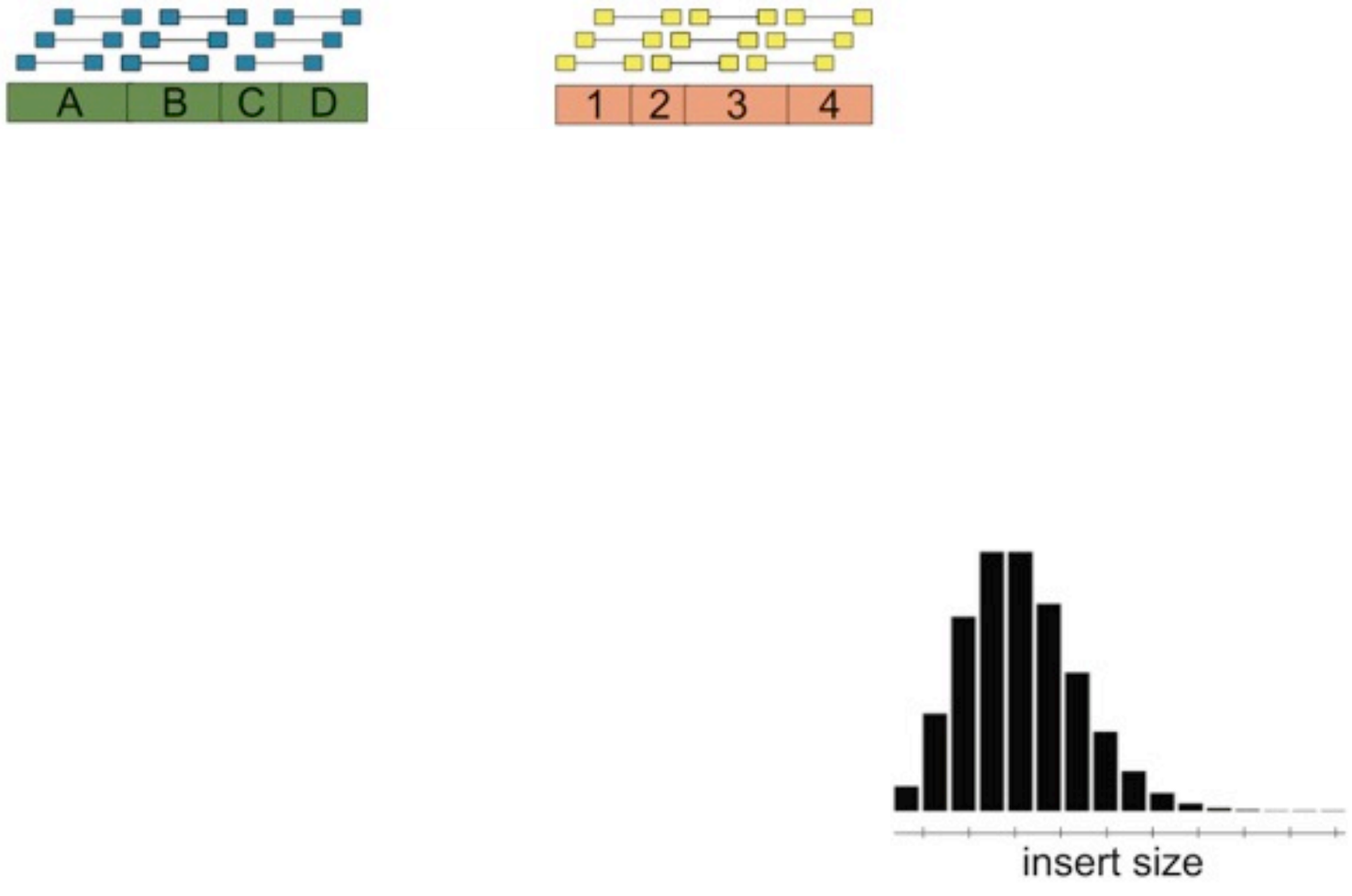
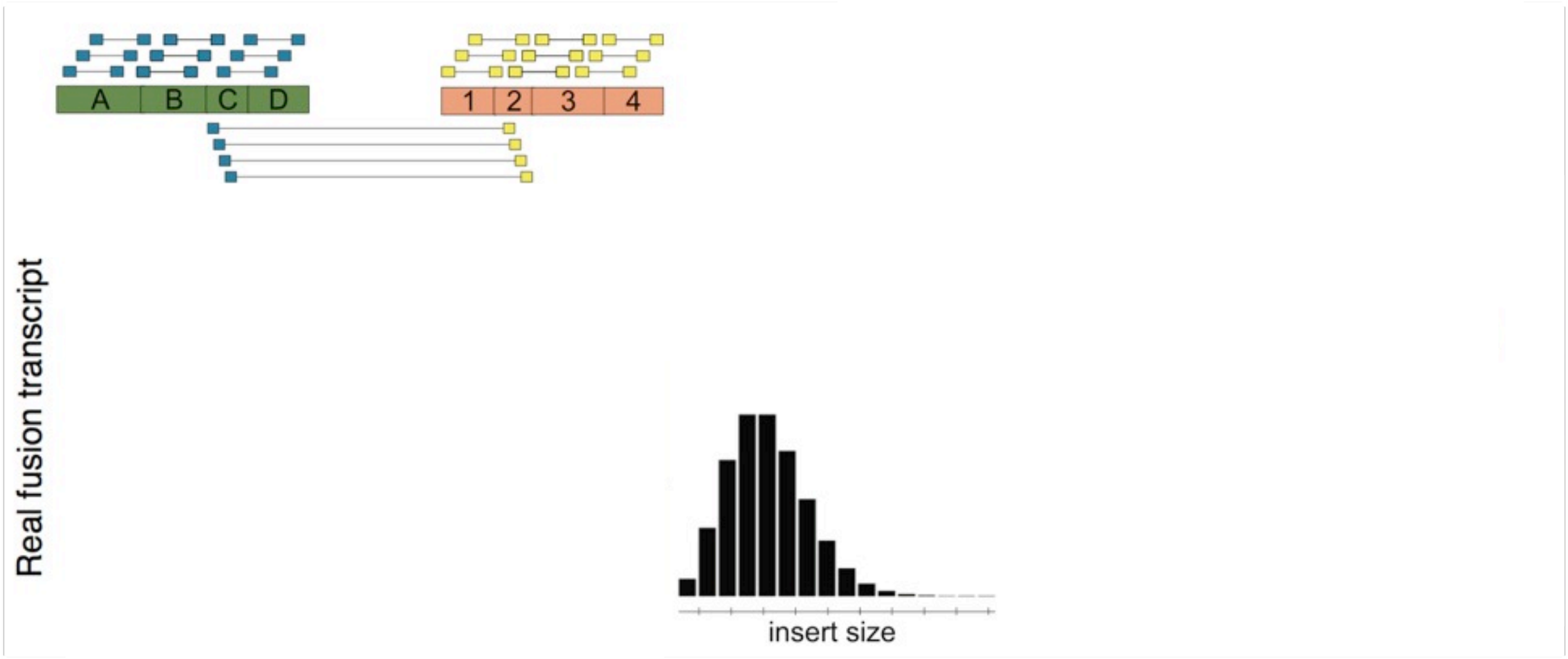


“Rescuing” the insert-size concept for transcriptome analysis

Minimal fusion transcript fragment



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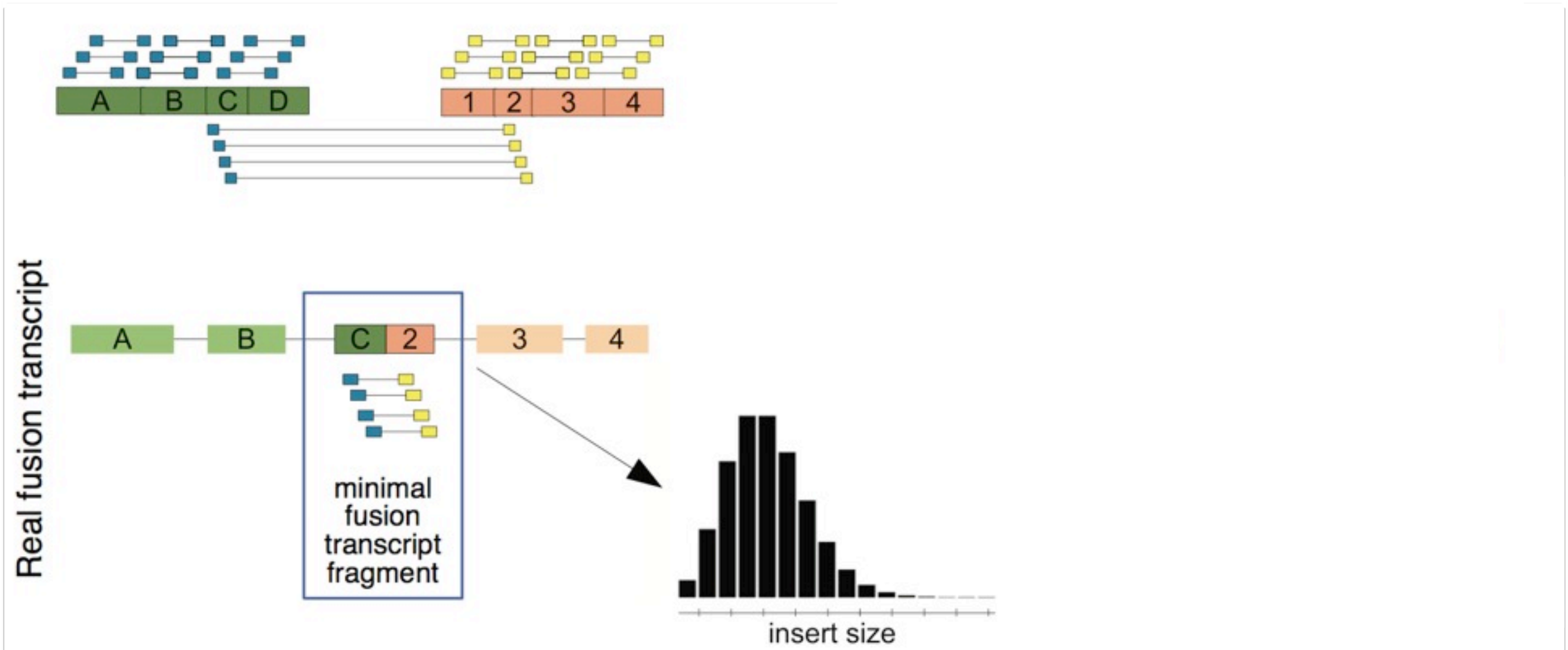

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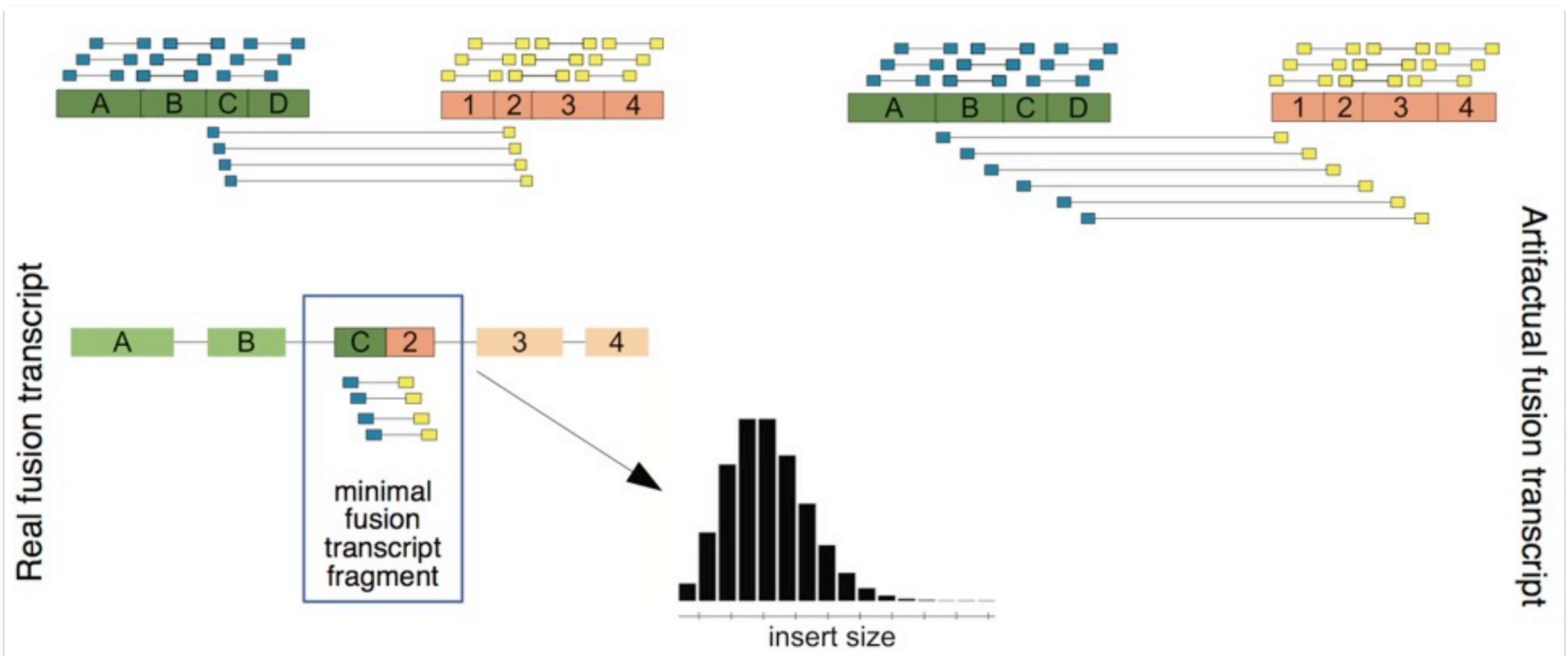

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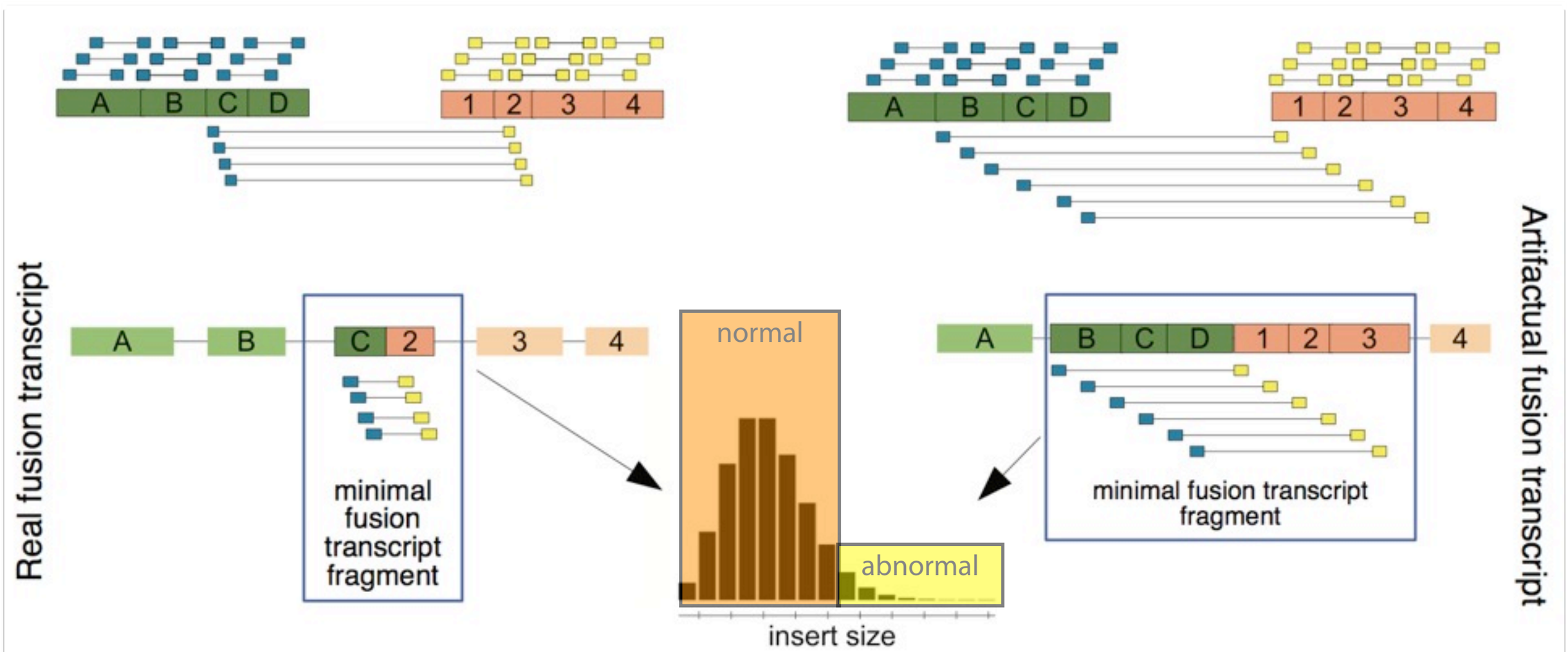
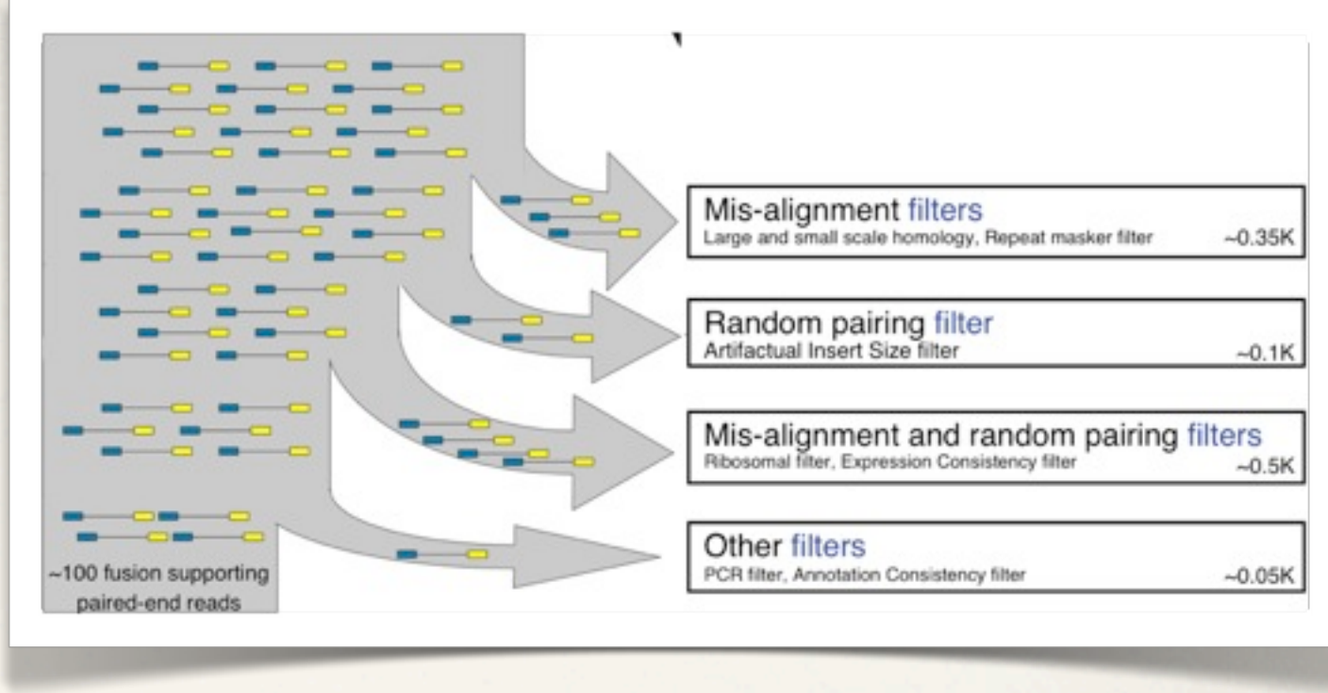


Permutation test: the probability that the inter-transcript insert-size distribution is compatible with the intra-transcript one.

"Rescuing" the insert-size concept for transcriptome analysis

Minimal fusion transcript fragment

Mis-alignment and random pairing filters



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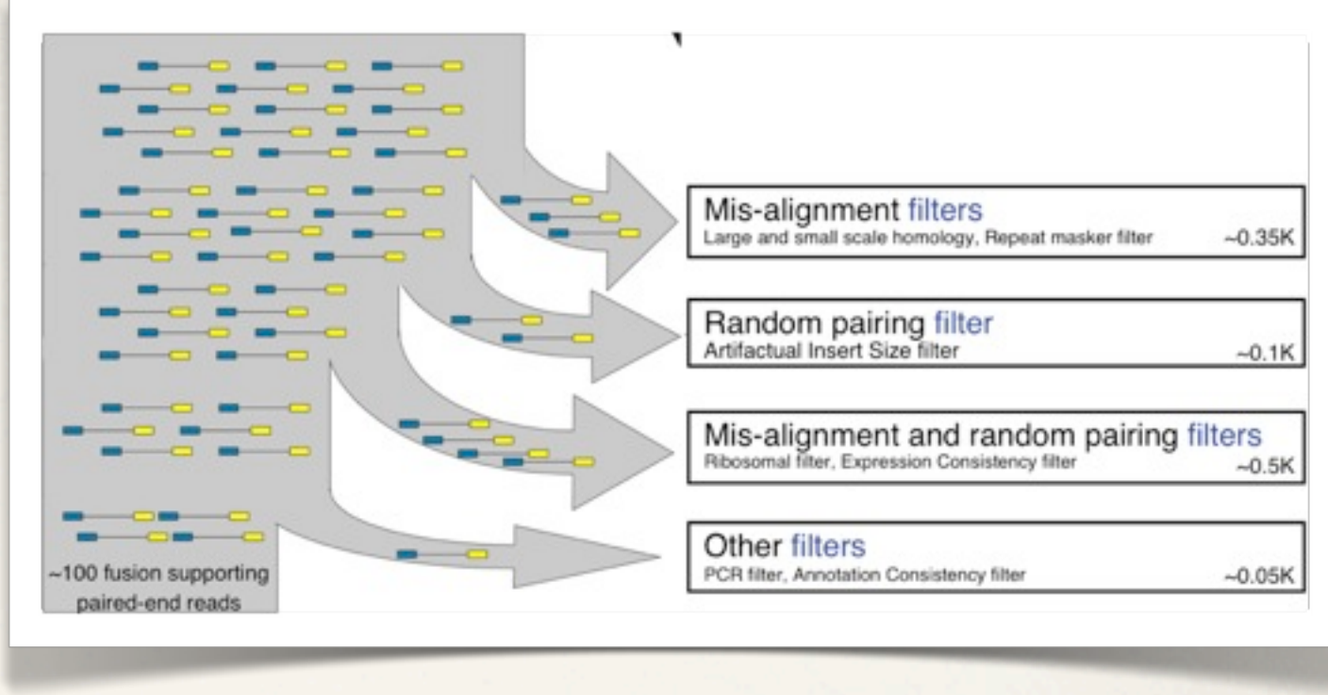

- *Observation 1*: highly expressed genes are more likely to generate random chimeric transcripts

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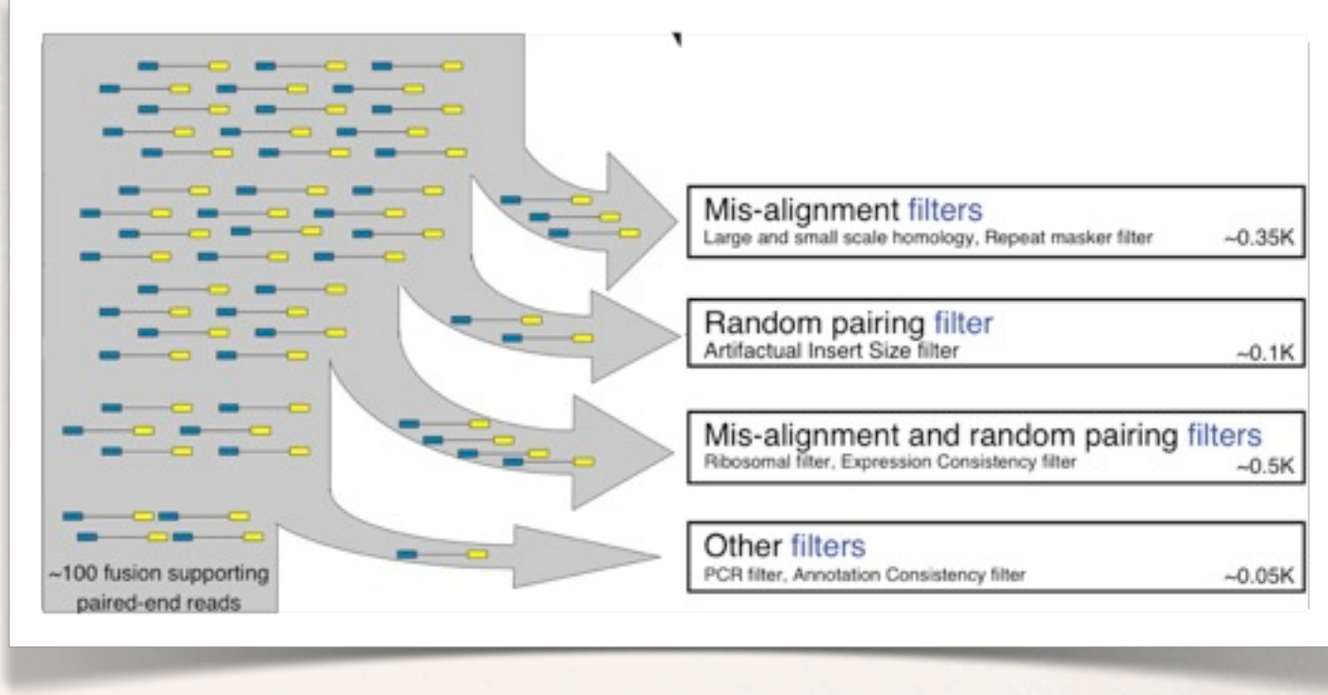

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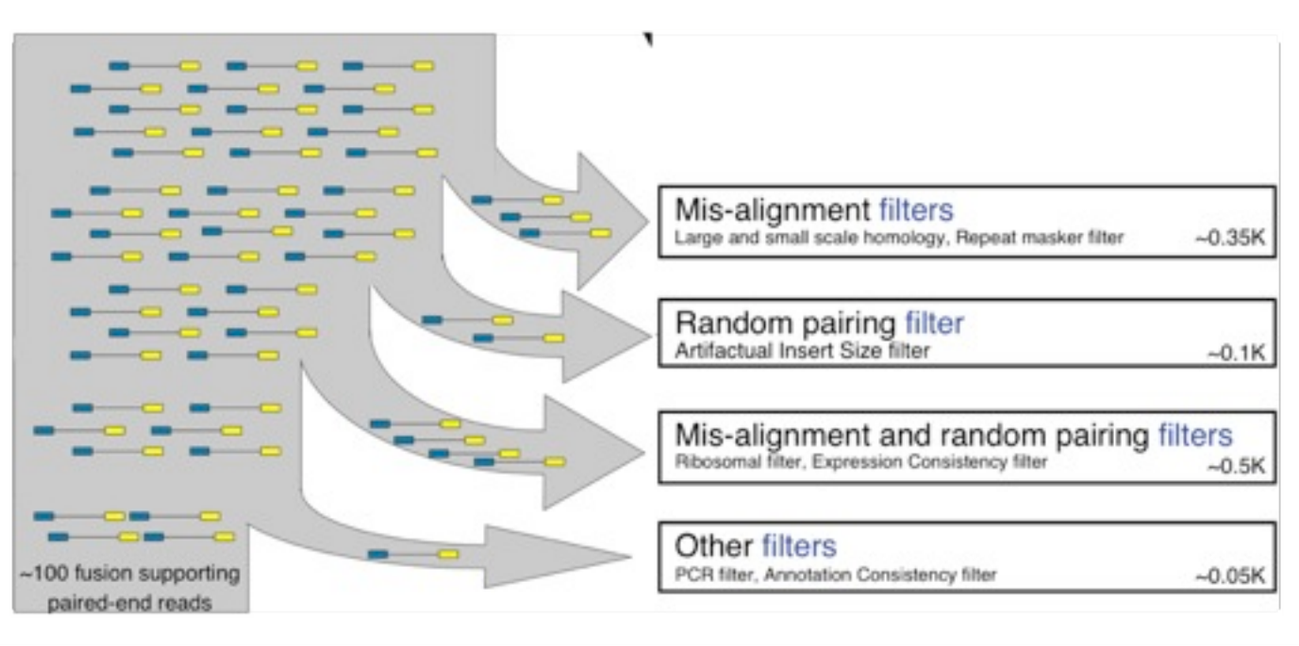
Mis-alignment and random pairing filters



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- *Solution*:

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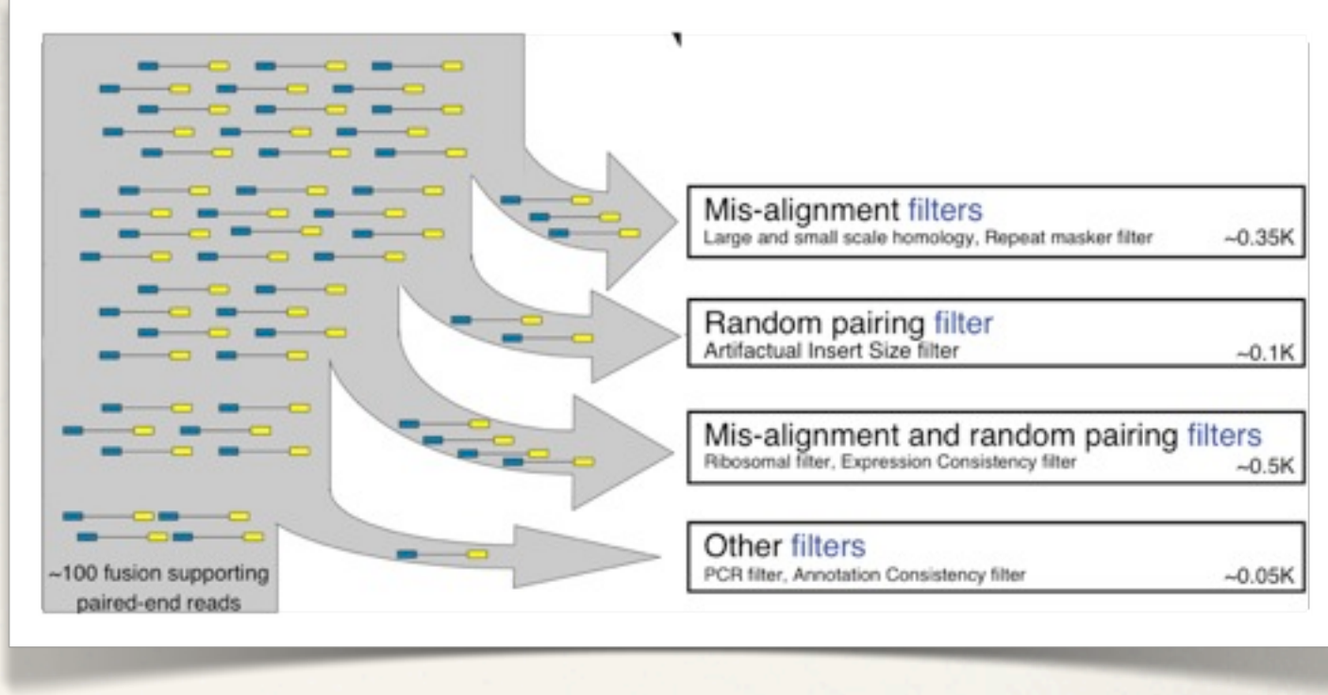

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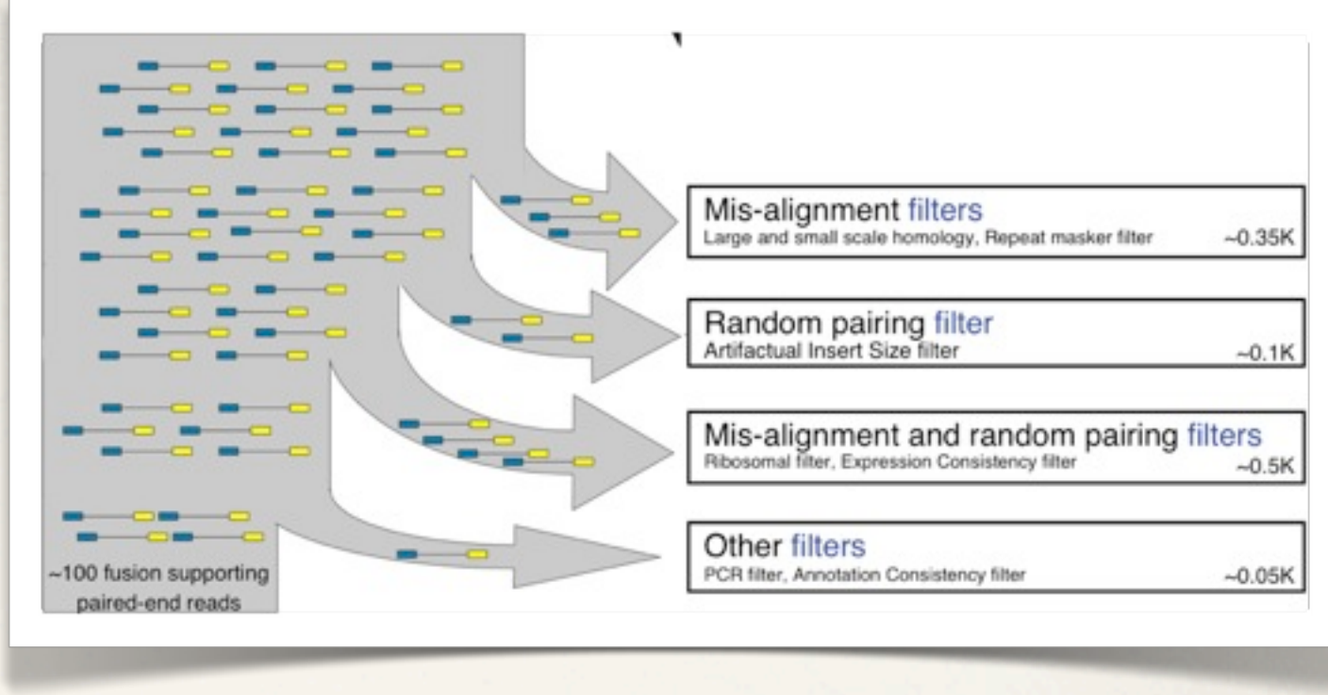
Mis-alignment and random pairing filters



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- *Solution*:
  - *Ribosomal* filter (highly expressed genes)
  - *Expression consistency* filter

Classifying the candidates

- Fusion candidates are classified based on the location of the two genes on the genome:

- *Inter-chromosomal*: genes on different chromosomes;

- *Intra-chromosomal*: genes on the same chromosome and strand, with other genes between them;

- *Read-through*: genes of the same chromosome and strand, and without other genes between them;

- *Cis*: genes on the same chromosome, but in opposite strands.

Scoring the candidates

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- Supportive PE Reads per million mapped reads (*SPER*)
  - Normalized number of *inter*-transcript PE reads (m_i)

$$SPER_i = \frac{m_i}{N_{mapped}} \cdot 10^6$$

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- How good is the observed SPER compared with the expected SPER?
  - Difference of observed SPER and analytically computed SPER (*DASPER*)
  - Ratio of observed SPER and empirically computed SPER (*RESPER*)

SPER expectations

Analytical

- Expected number of inter-transcript reads by chance (<m_{AB}>):

$$\langle SPER_i \rangle = \frac{\langle m_{AB} \rangle}{N_{mapped}} \cdot 10^6$$

- <m_{AB}> can be estimated from the joint probability:

$$\langle m_{AB} \rangle = P(A) \cdot P(B) \cdot N_{mapped} = \frac{m_A \cdot m_B}{N_{mapped}}$$

$$DASPER_i = SPER_i - \langle SPER_i \rangle$$

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$$DASPER_i = SPER_i - \langle SPER_i \rangle$$

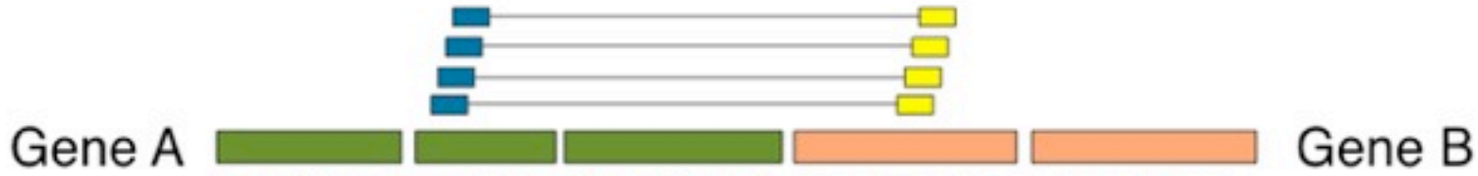
Empirical

- Average of the other candidates' SPER:

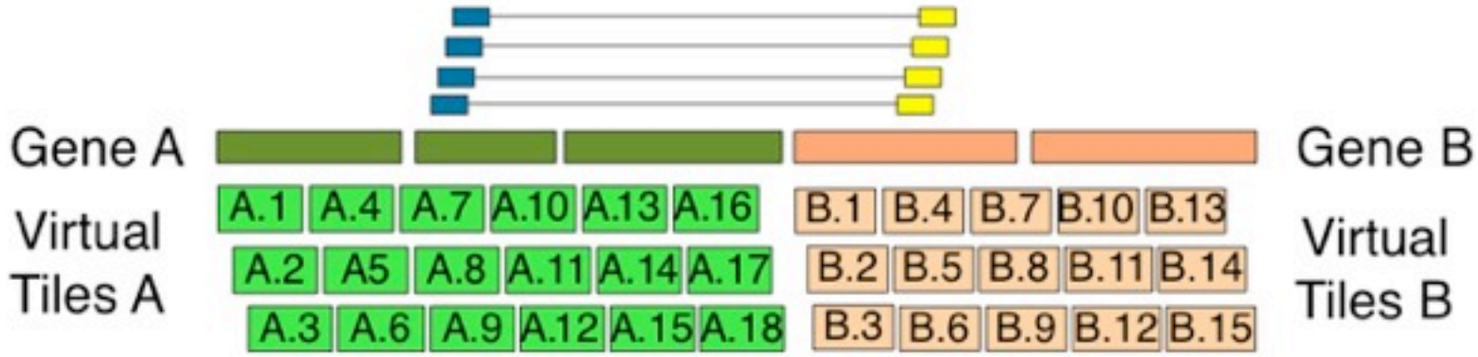
$$\overline{SPER} = \frac{1}{M} \cdot \sum_{j=1}^{M} SPER_j$$

- where M is the total number of fusion transcript candidates

$$RESPER_i = \frac{SPER_i}{\overline{SPER}}$$



Junction-Sequence Identifier Module



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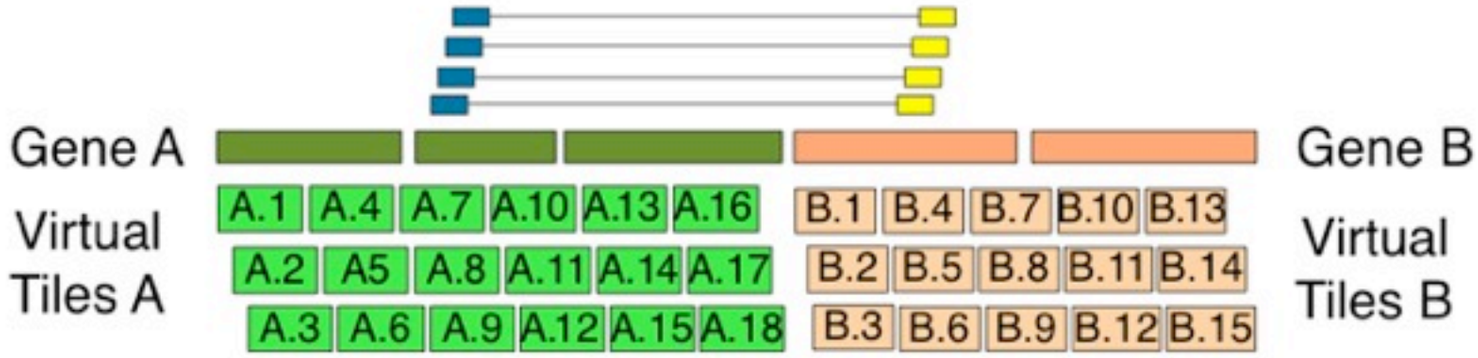

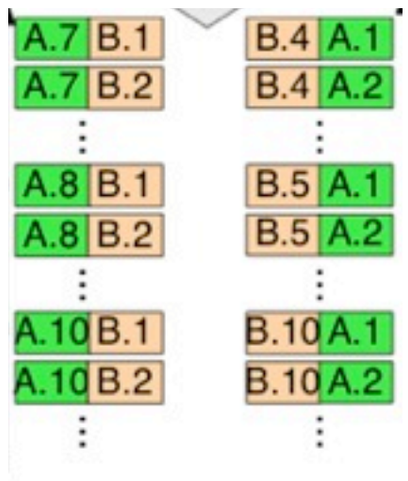

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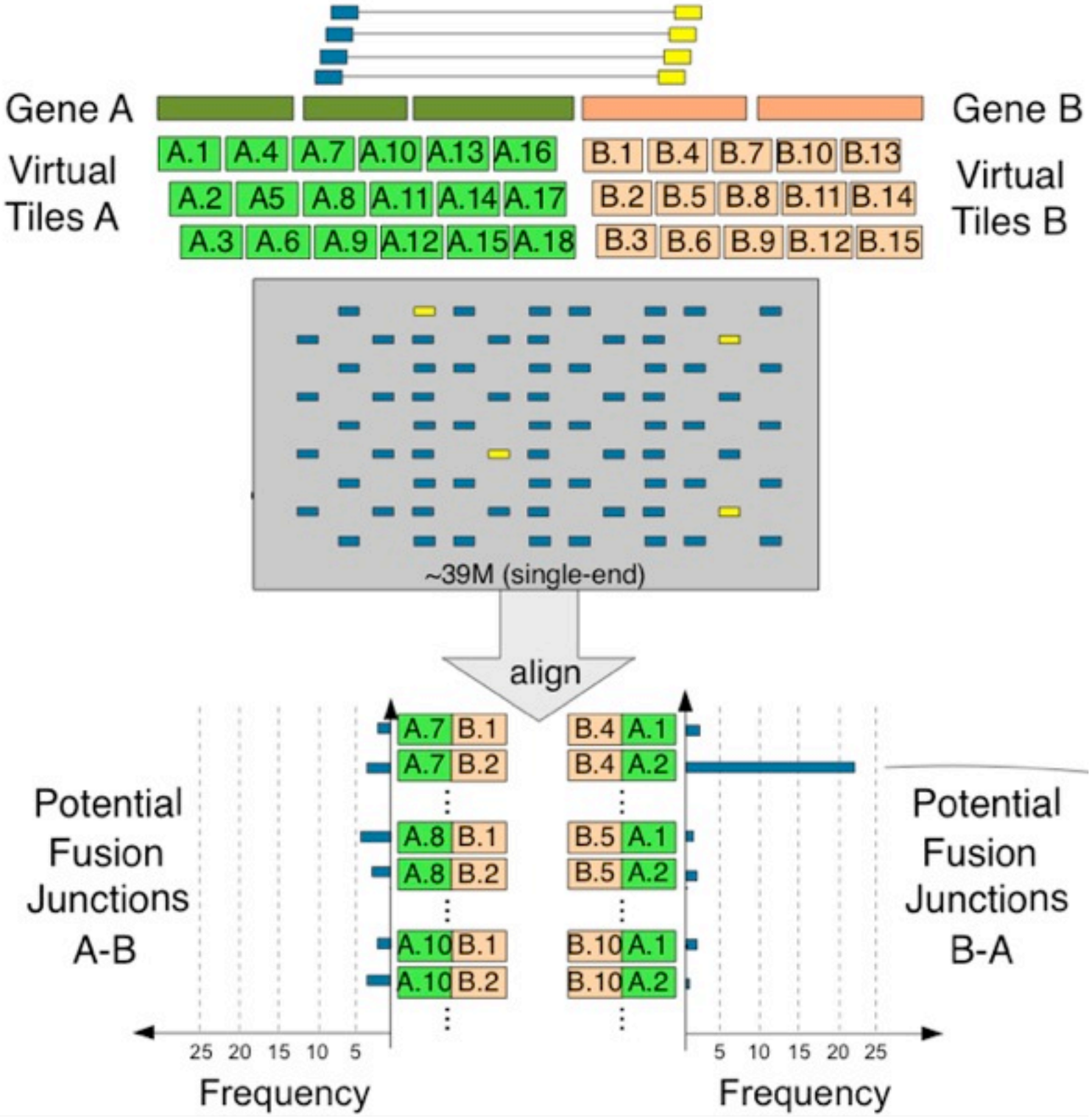

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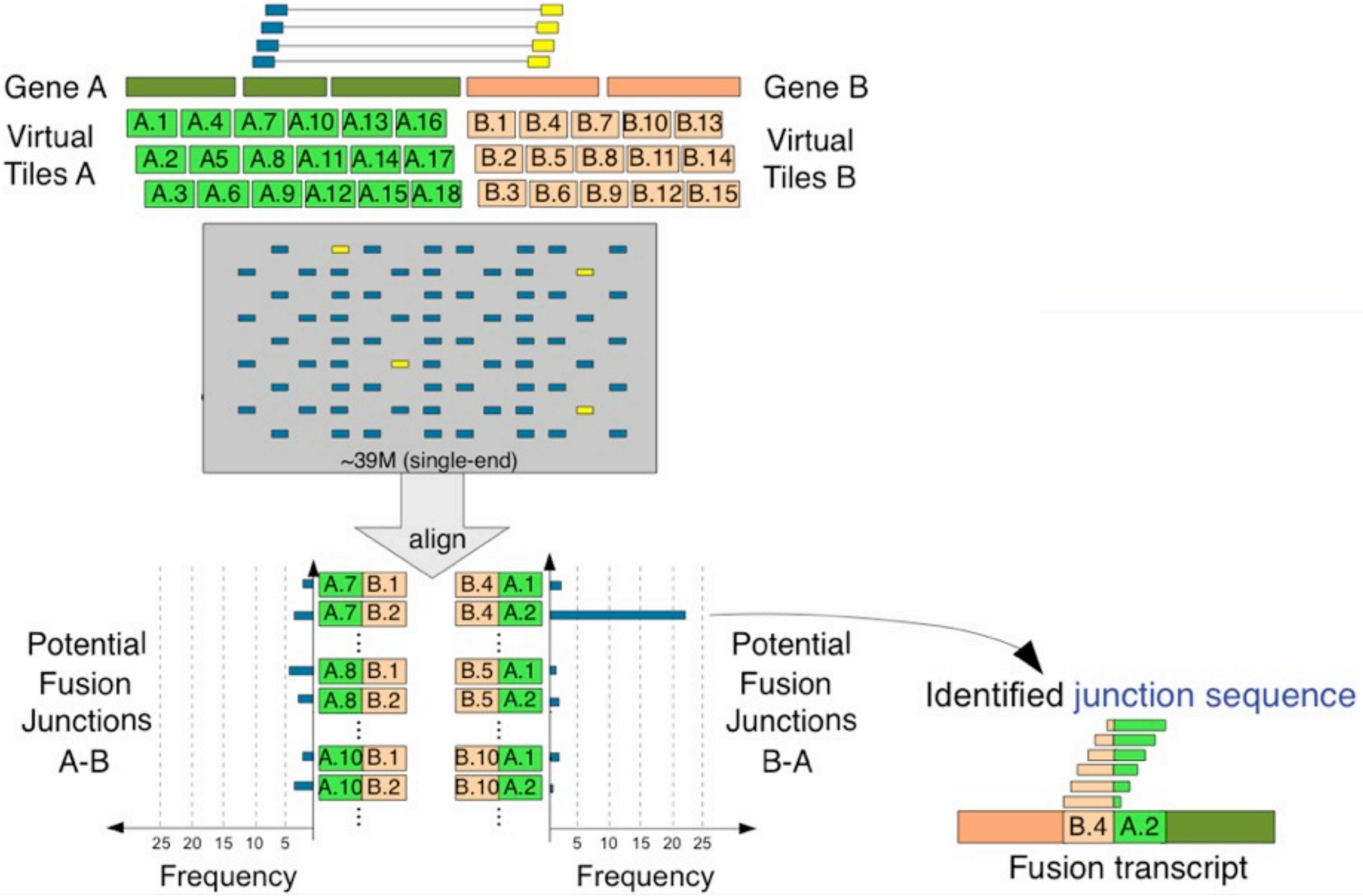

Junction-Sequence Identifier Module

Study design

- Well-characterized samples

Sample ID	Type (PCa=prostate cancer)	Known Fusion Type	Read size	Mapped PE reads
106_T	PCa	TMPRSS2-ERG	51	4,312,087
1700_D	PCa	TMPRSS2-ERG	51	6,805,776
580_B	PCa	TMPRSS2-ERG	36	5,211,234
99_T	PCa	NDRG1-ERG	36	1,515,444
2621_D	PCa	SLC45A3-ERG	54	11,899,985
1043_D	PCa	No known fusions	51	1,549,569
NCI-H660	PCa cell line	TMPRSS2-ERG	51	3,572,391
GM12878	Lymphoblastoid cell line	No known fusions	54	20,676,160

What is the effect of the filters?

- Total of 7,342 candidates

	Average number of identified candidate per sample	Average number of candidates per sample after filtering
Number	917.75	38
Range	451-1618	3-94

- 304 passed the filters, i.e. **96**% reduction

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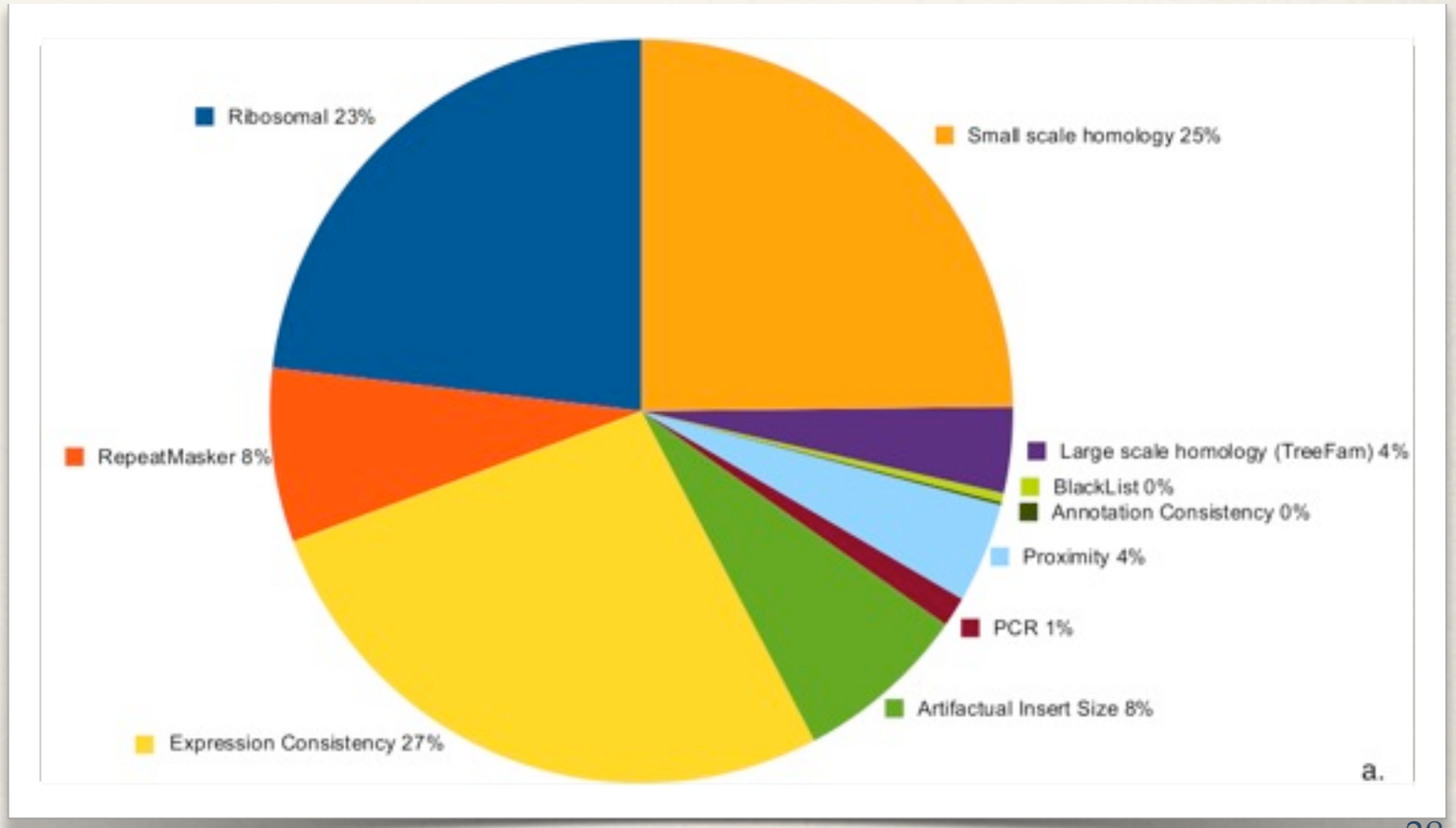
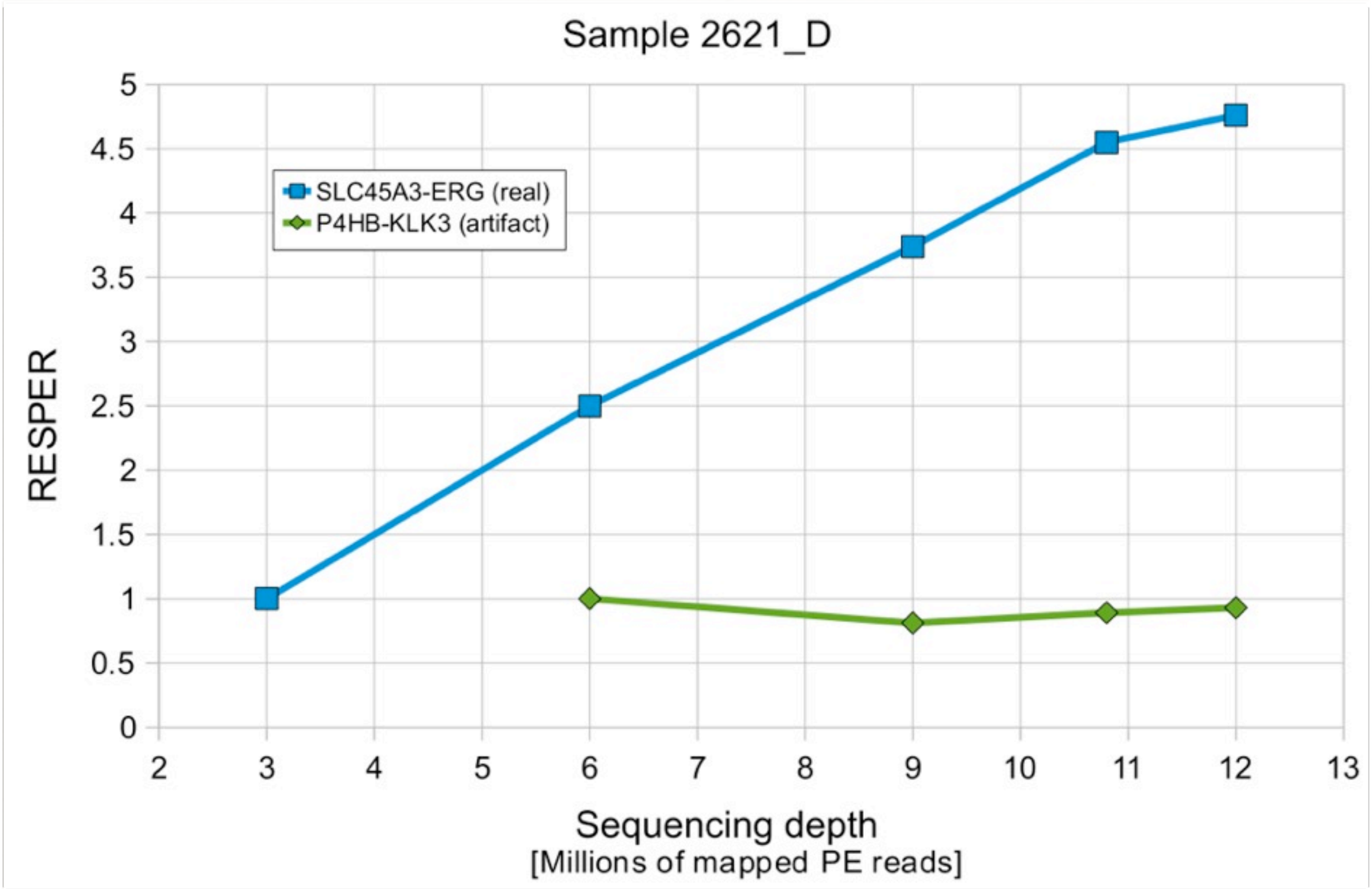
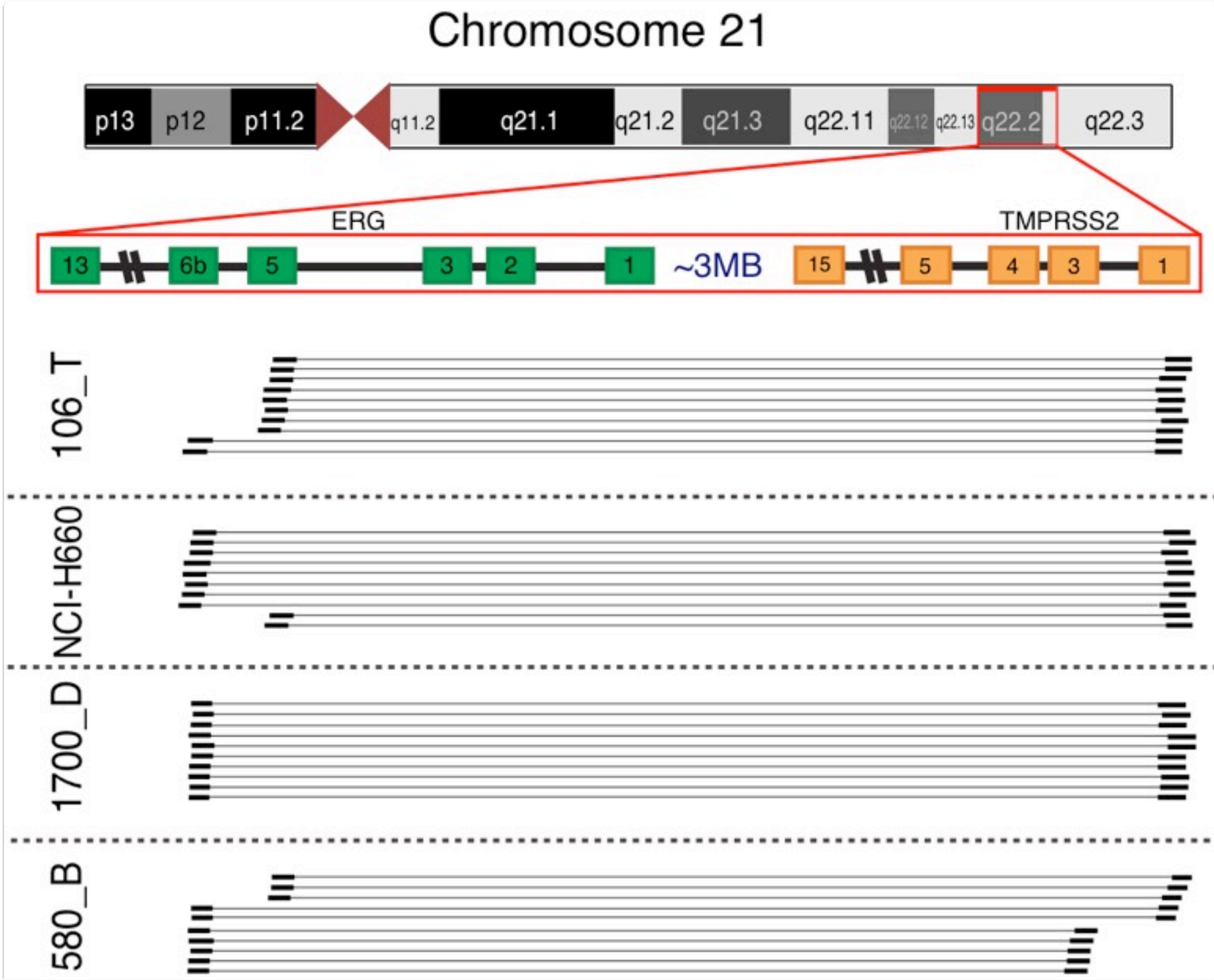
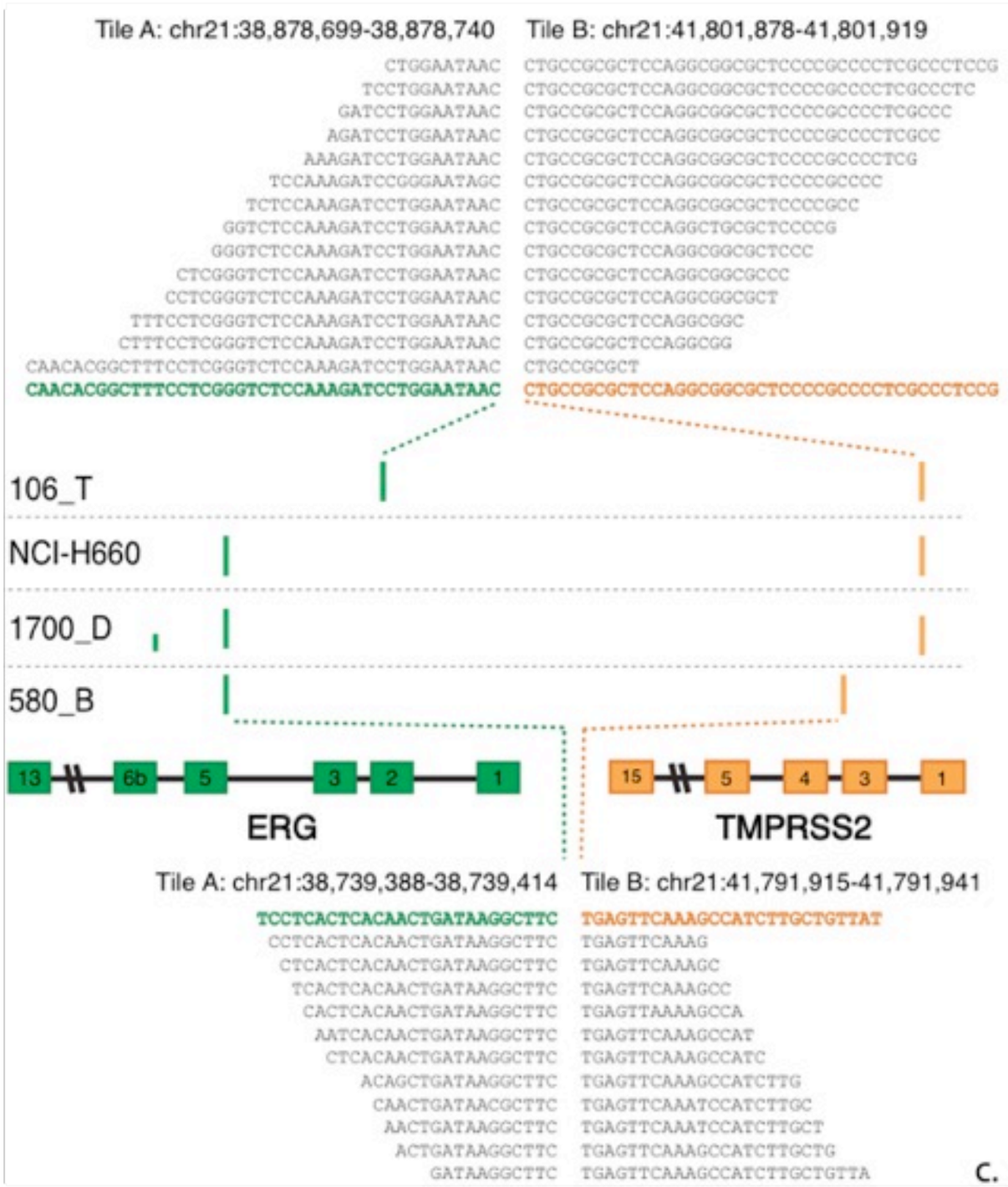




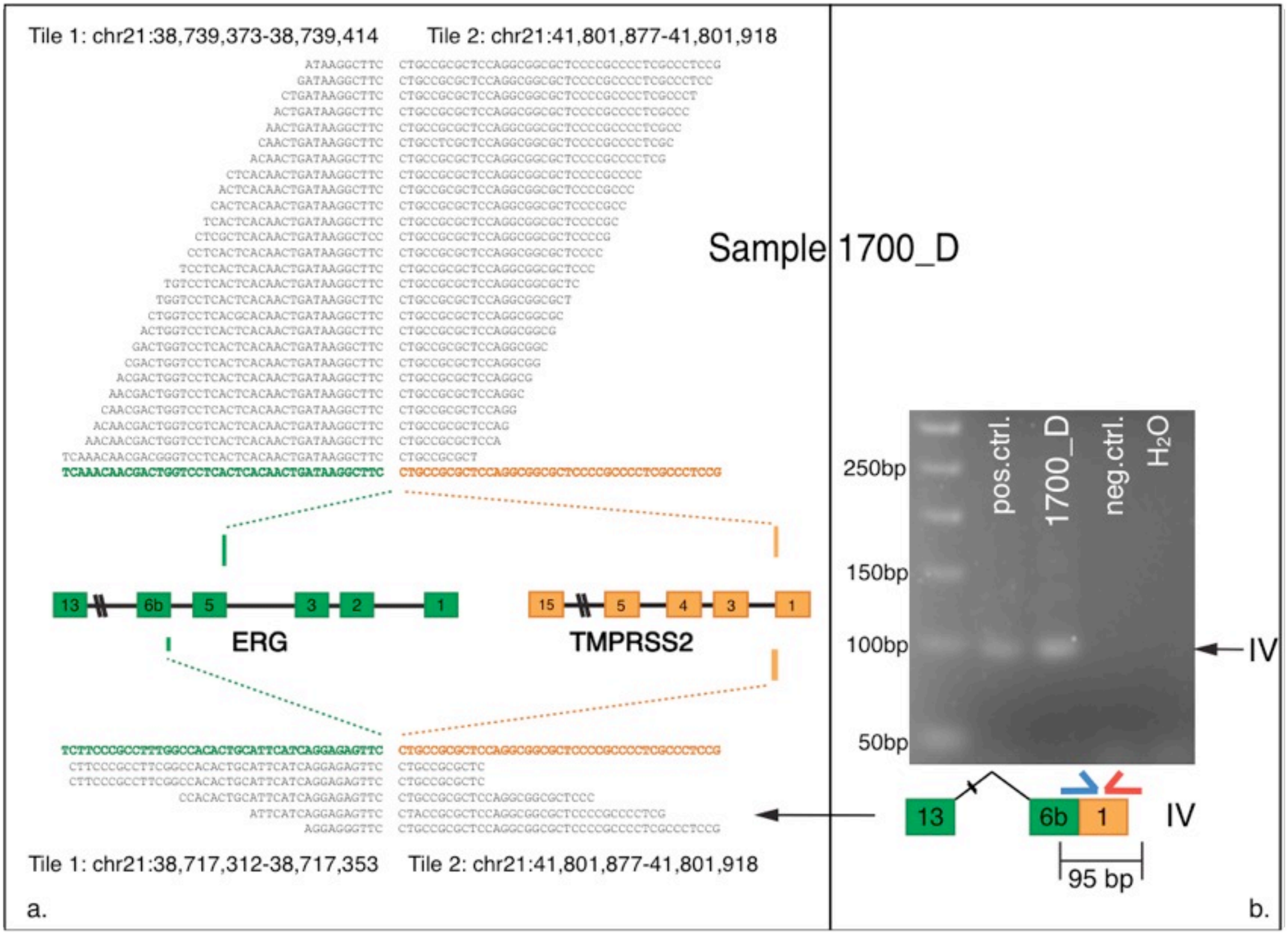
What is the effect of coverage?



TMPRSS2-ERG fusion positives



Junction-sequence identifier



Second isoform

Top candidates

Type	Sample ID	Fusion Candidate	RESPER
intra	**580_B**	**TMPRSS2-ERG**	**14.31**
intra	**1700_D**	**TMPRSS2-ERG**	**8.79**
intra	**106_T**	**TMPRSS2-ERG**	**3.97**
inter	**2621_D**	**SLC45A3-ERG**	**3.56**
inter	**1700_D**	**ERG-GMPR**	**2.05**
read-through	*1700_D*	*SLC16A8-BAIAP2L2*	*1.93*
read-through	*106_T*	*AK094188-AK311452*	*1.9*
read-through	*1700_D*	*ZNF473-FLJ26850*	*1.58*
read-through	*580_B*	*ZNF577-FLJ26850*	*1.58*
read-through	*1043_D*	*ZNF577-ZNF649*	*1.55*
read-through	*1700_D*	*CAMTA2-INCA1*	*1.35*
inter	1700_D	HDAC5	1.29
read-through	*1043_D*	*FLJ00248-LRCH4*	*1.27*
read-through	*1700_D*	*VMAC-CAPS*	*1.17*
read-through	*106_T*	*FLJ00248-LRCH4*	*1.16*
cis	1043_D	AX747861-FLI1	1.13
read-through	*106_T*	*TAGLN-AK126420*	*1.07*
inter	**580_B**	**PIGU-ALG5**	**1.07**
inter	**99_T**	**NDRG1-ERG**	**1.02**

Top candidates

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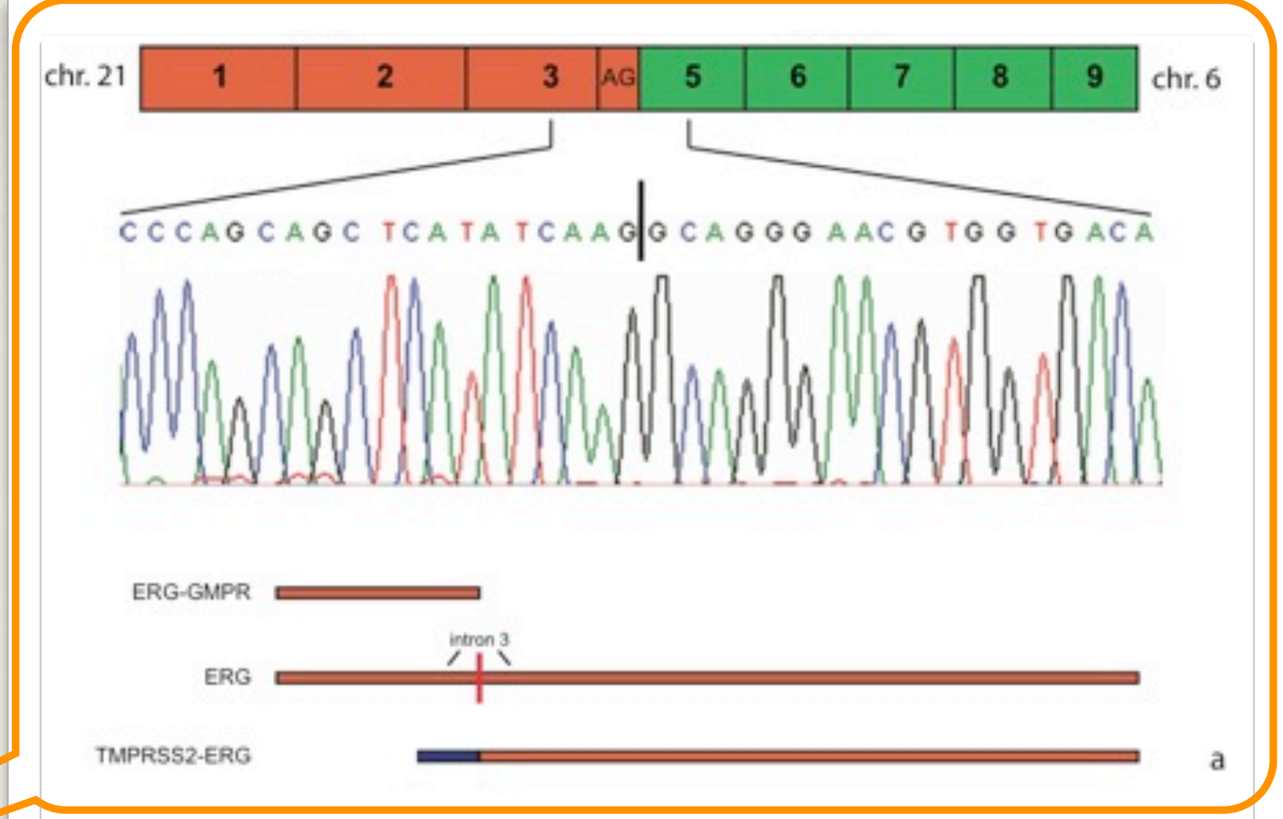
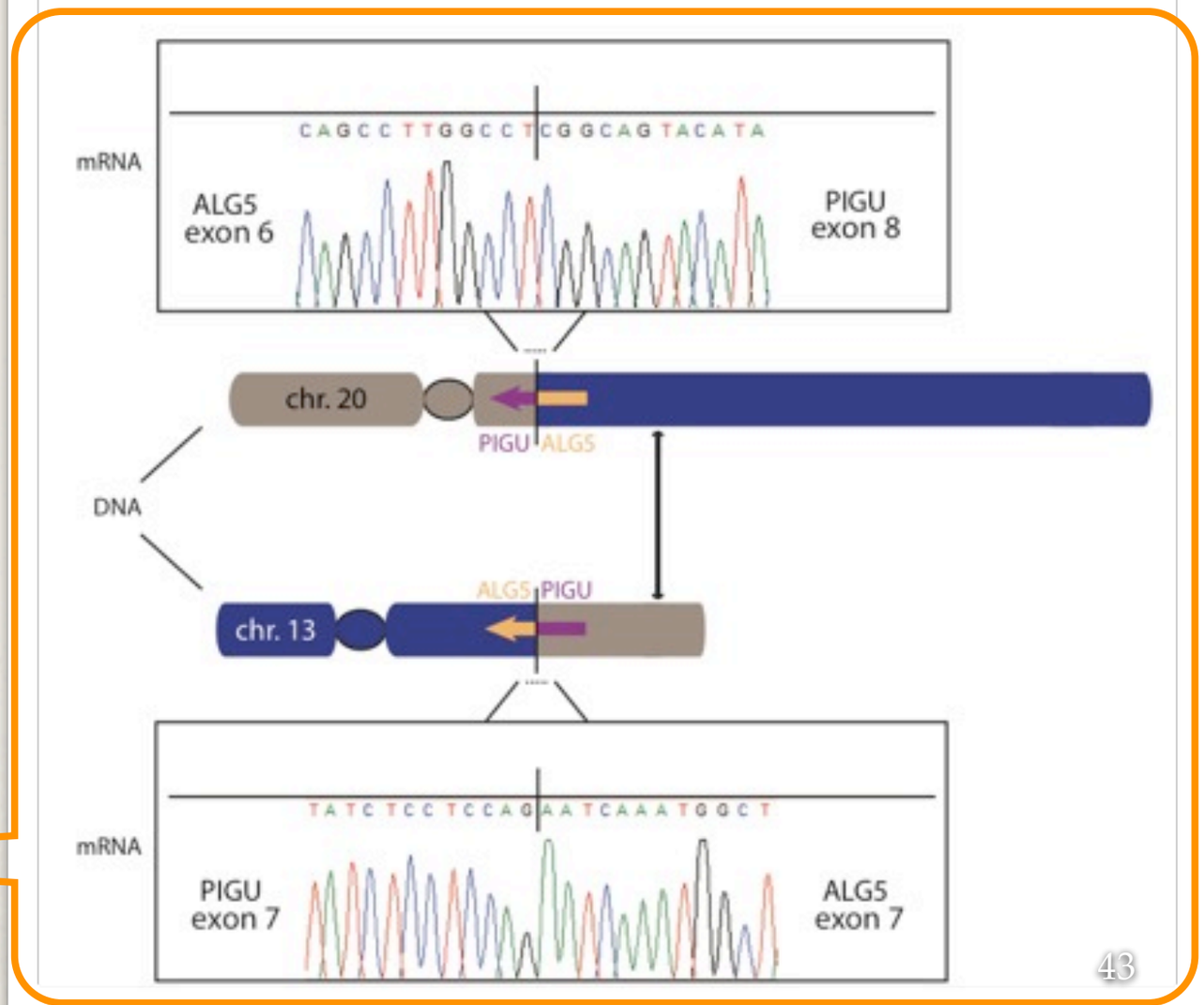
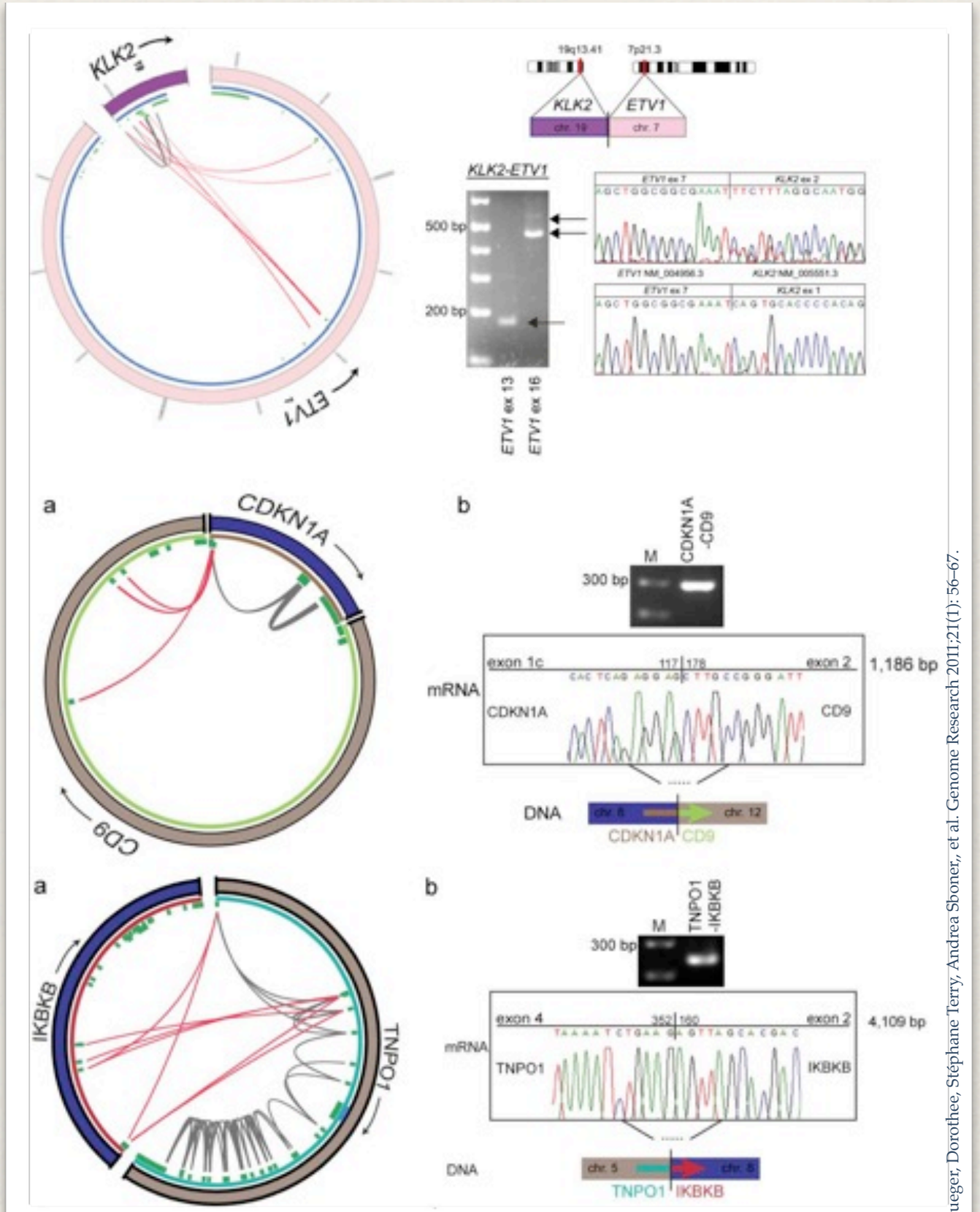


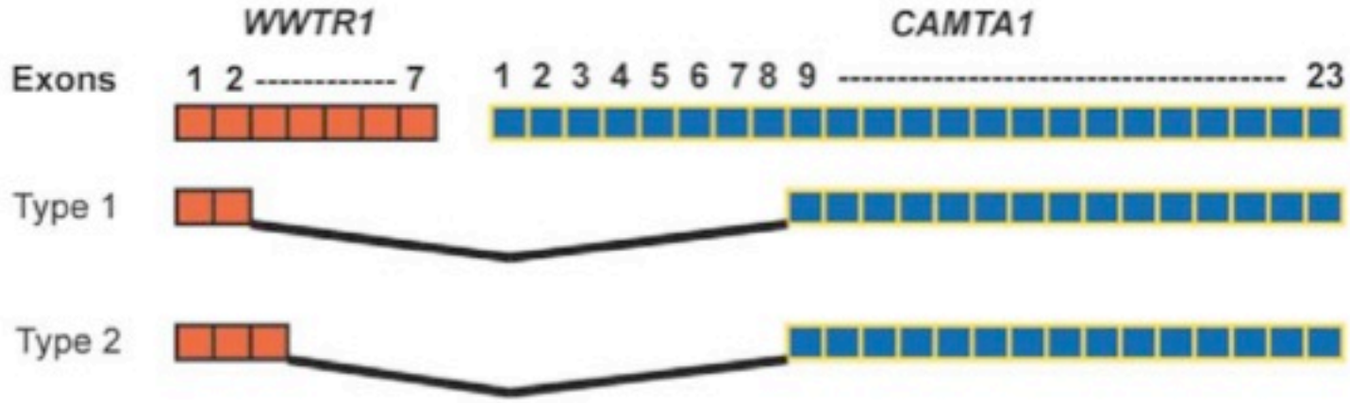


Other data sets?

- Larger prostate cancer data set
- Identified 7 novel fusion candidates
- Experimentally validated

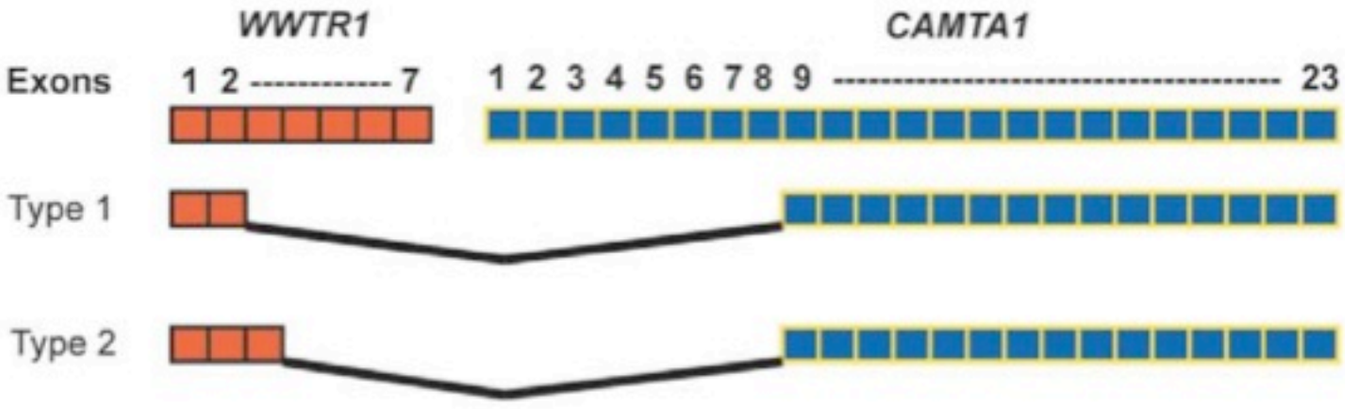


Pflueger, Dorothee, Stéphane Terry, Andrea Sboner,, et al. Genome Research 2011;21(1): 56–67.



Novel *defining* fusion gene in epitheliod hemangioendothelioma

Tanas, Munir R, Andrea Sboner,et al. "Identification of a Disease-Defining Gene Fusion in Epithelioid Hemangioendothelioma." Science Translational Medicine 3, no. 98 (August 31, 2011): 98ra82–98ra82.



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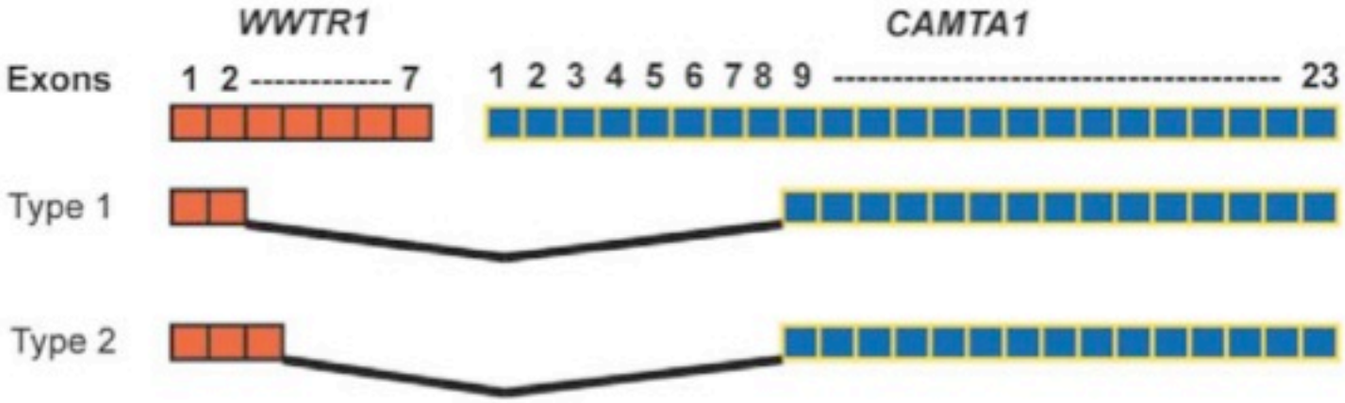

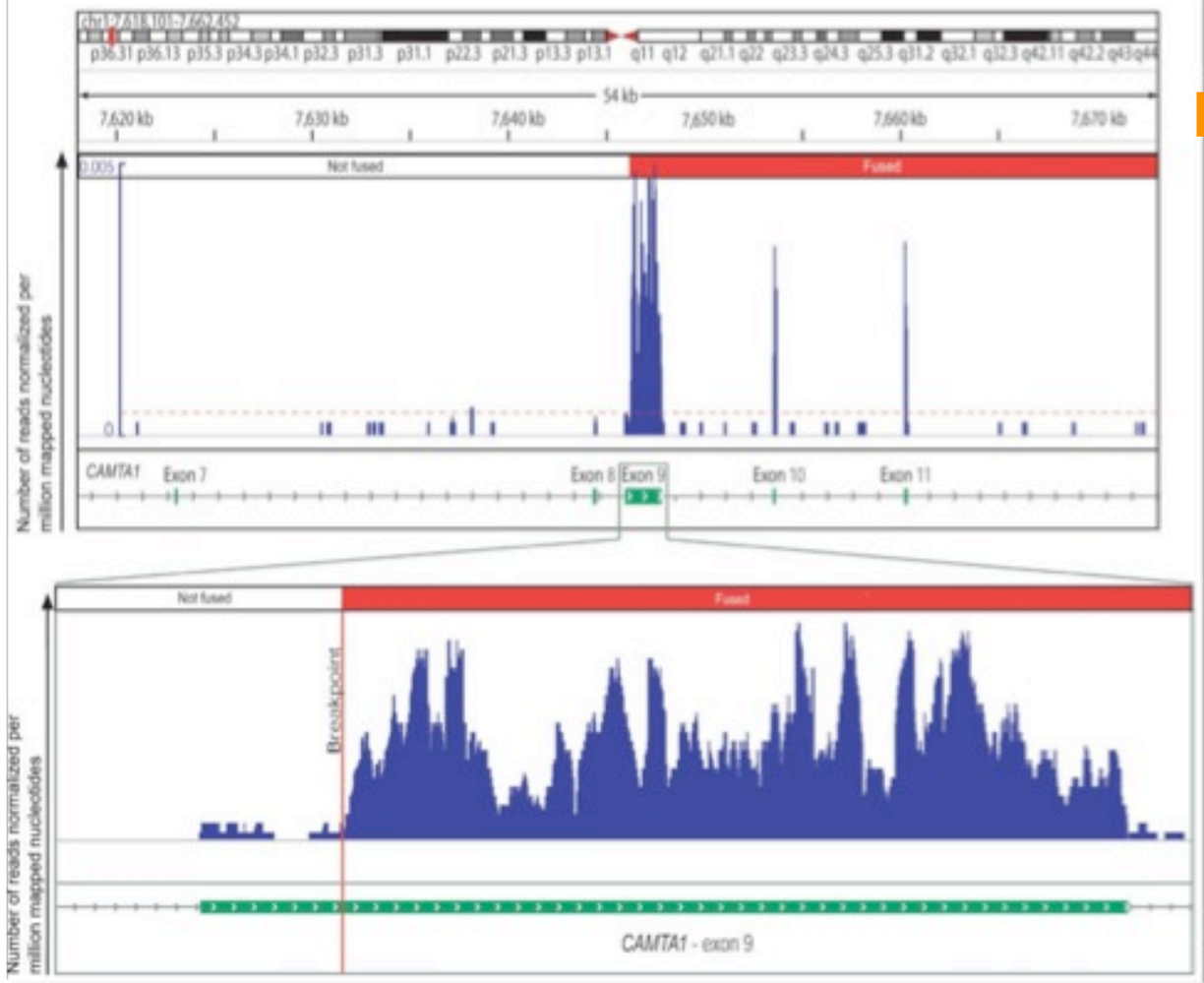

G	WWTR1		CAMTA1	
	Positive /total	%	Positive /total	%
Epithelioid hemangioendothelioma	42/47	89%	39/45	87%
Angiosarcoma, NOS	0/42	0%	0/39	0%
Epithelioid angiosarcoma	0/7	0%	0/7	0%
Intimal sarcoma	0/5	0%	0/3	0%
Kaposi's sarcoma	0/4	0%	0/4	0%
Malignant hemangioendothelioma, NOS	0/1	0%	0/1	0%
Retiform hemangioendothelioma	0/1	0%	0/1	0%
Kaposiform hemangioendothelioma	0/3	0%	0/2	0%
Epithelioid hemangioma	0/5	0%	0/4	0%
Arteriovenous malformation	0/2	0%	0/2	0%
Angiomatosis	0/1	0%	0/1	0%
Hemangioma, NOS	0/3	0%	0/3	0%
Capillary/pyogenic hemangioma	0/5	0%	0/5	0%
Cavernous hemangioma	0/5	0%	0/5	0%
Juvenile hemangioma	0/1	0%	0/1	0%
Spindle cell hemangioma	0/4	0%	0/4	0%
Synovial hemangioma	0/1	0%	0/1	0%
Intramuscular hemangioma	0/6	0%	0/5	0%
Littoral cell hemangioma	0/6	0%	0/2	0%
Malignant hemangiopericytoma	0/1	0%	0/1	0%
Hemangiopericytoma, NOS	0/1	0%	0/1	0%
Sinonasal hemangiopericytoma	0/1	0%	0/1	0%
Glomus tumor	0/1	0%	0/1	0%
Atypical glomus tumor	0/2	0%	0/2	0%
Lymphangioma	0/7	0%	0/7	0%
Lymphangioleiomyomatosis	0/1	0%	0/1	0%
Papillary endothelial hyperplasia	0/2	0%	0/2	0%
Total cases	165		151	

Tanas, Munir R, Andrea Sboner,et al. "Identification of a Disease-Defining Gene Fusion in Epithelioid Hemangioendothelioma." Science Translational Medicine 3, no. 98 (August 31, 2011): 98ra82–98ra82.

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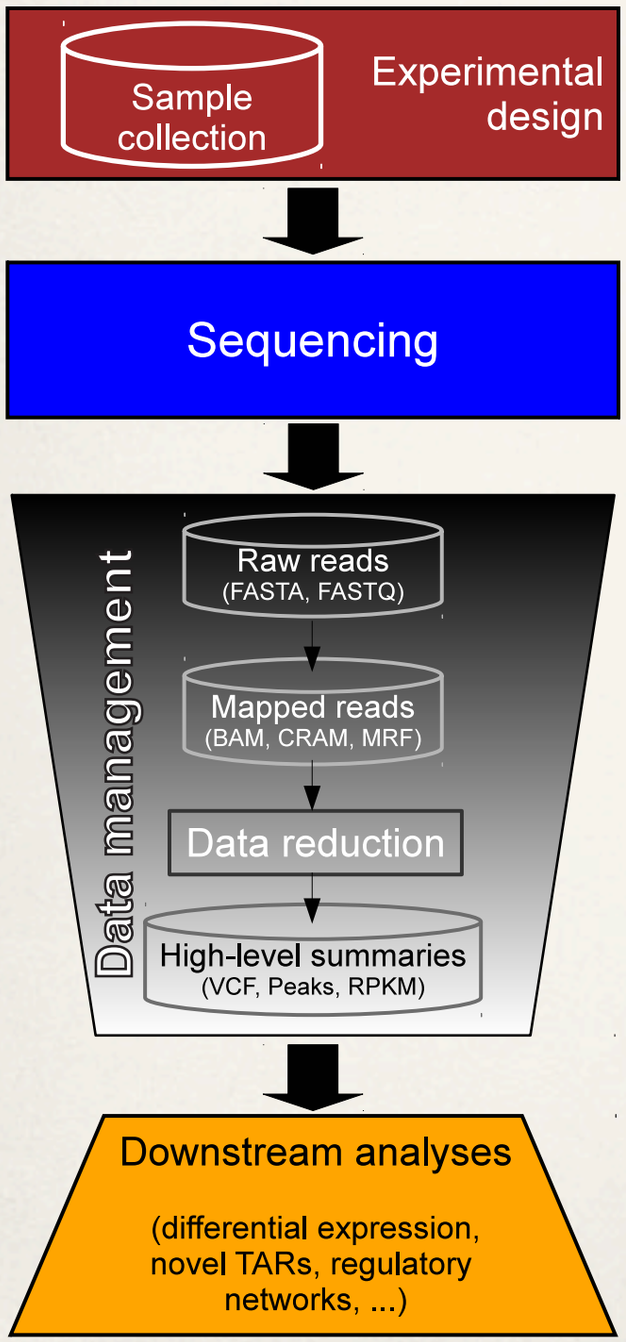
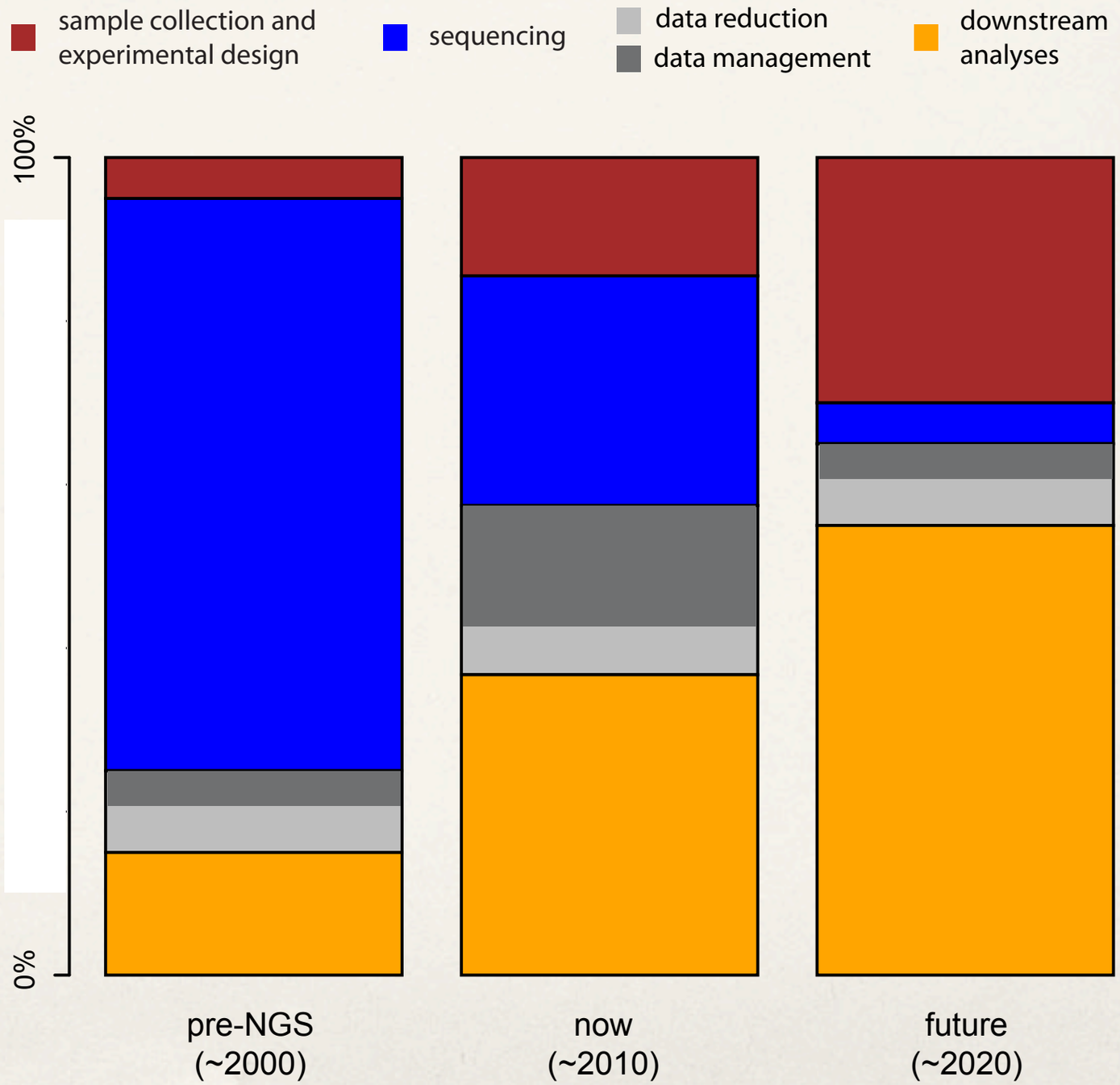
NGS future trends





Sboner, Andrea, et al. 2011. Genome Biology 12(8):25:125

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