Machine Learning Methods for RNA-seq-based Transcriptome Reconstruction

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Friedrich Miescher Laboratory

Max Planck Society, Tübingen, Germany

NGS Bioinformatics Meeting, Paris (March 24, 2010)



Friedrich Miescher Laboratory
of the Max Planck Society



Discovery of the Nuclein
(Friedrich Miescher, 1869)









Discovery of Nuclein:

- from lymphocyte & salmon
- "multi-basic acid" (≥ 4)

"If one . . . wants to assume that a single substance . . . is the specific cause of fertilization, then one should undoubtedly first and foremost consider nuclein" (Miescher, 1874)

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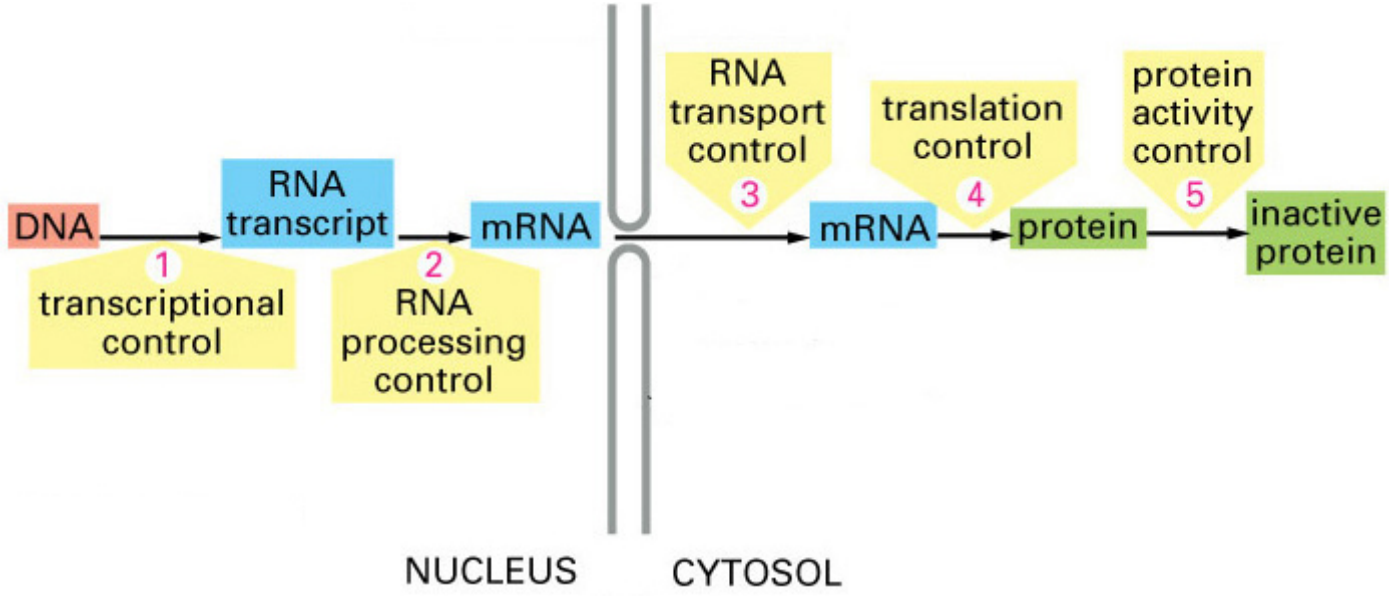
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Learning about the Transcriptome

⇝ What is encoded on the genome and how is it processed?



Computational Point of View

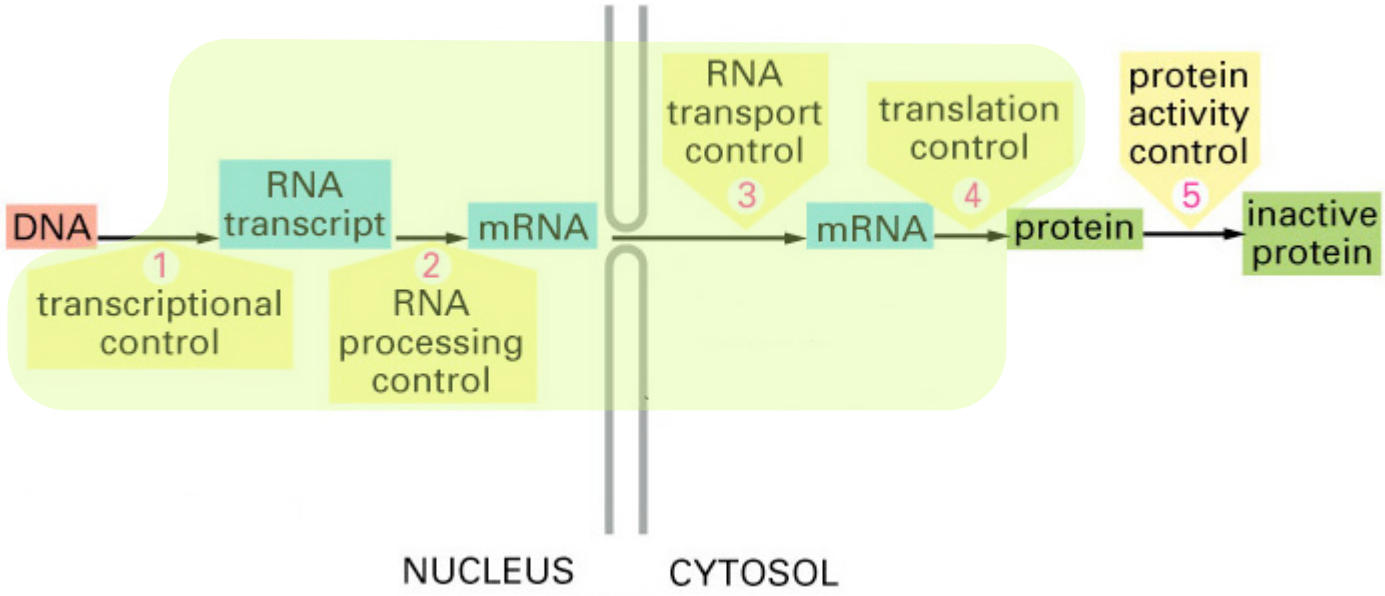
- How to learn to predict what these processes accomplish?
- How well can we predict it from the available information?

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- What can we not predict yet? What is missing?
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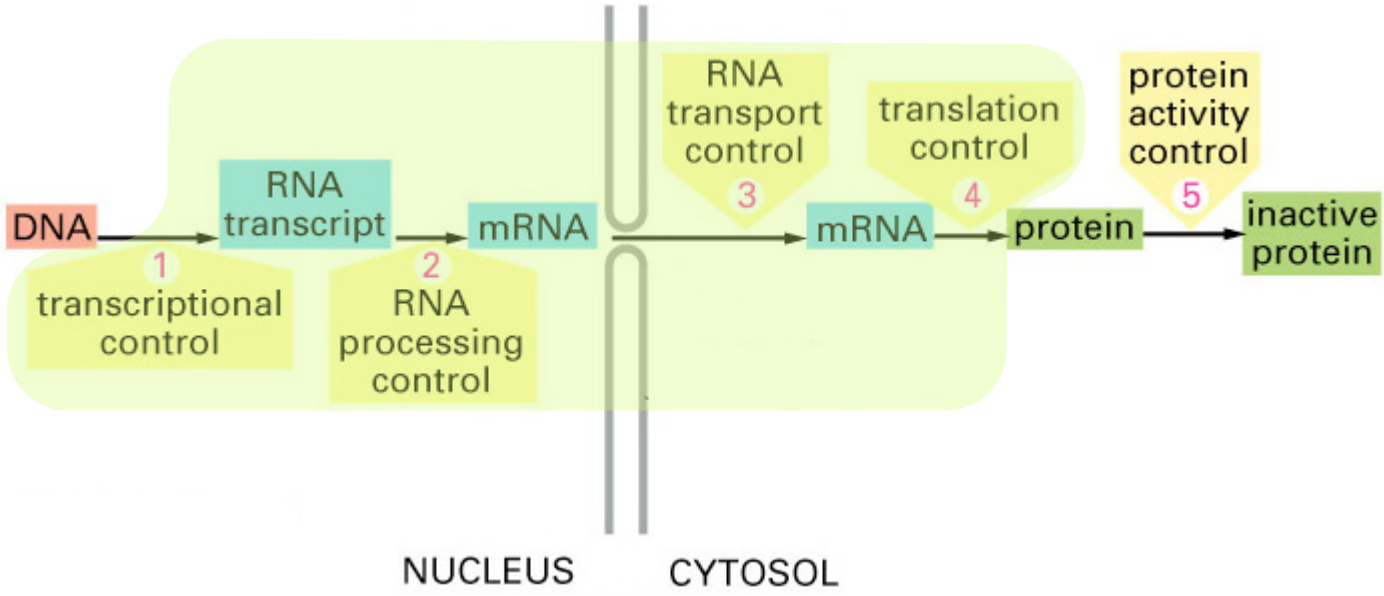
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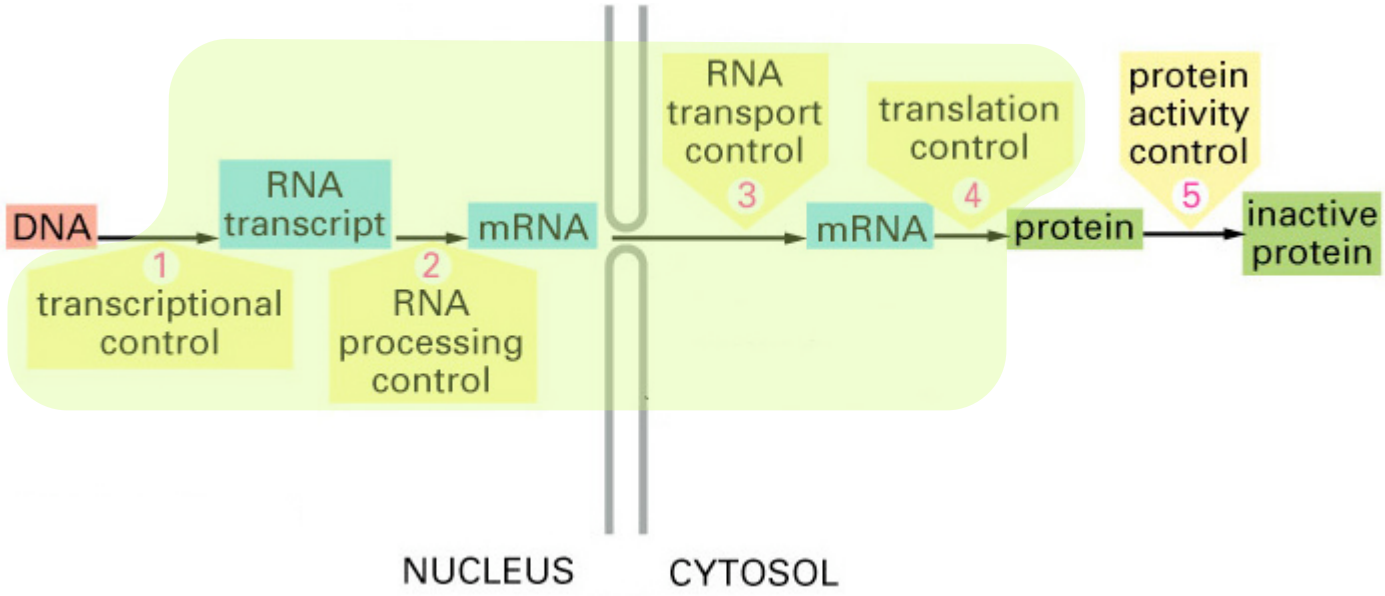
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Machine Learning
Learning from empirical observations



Given: Observations of some complex phenomenon

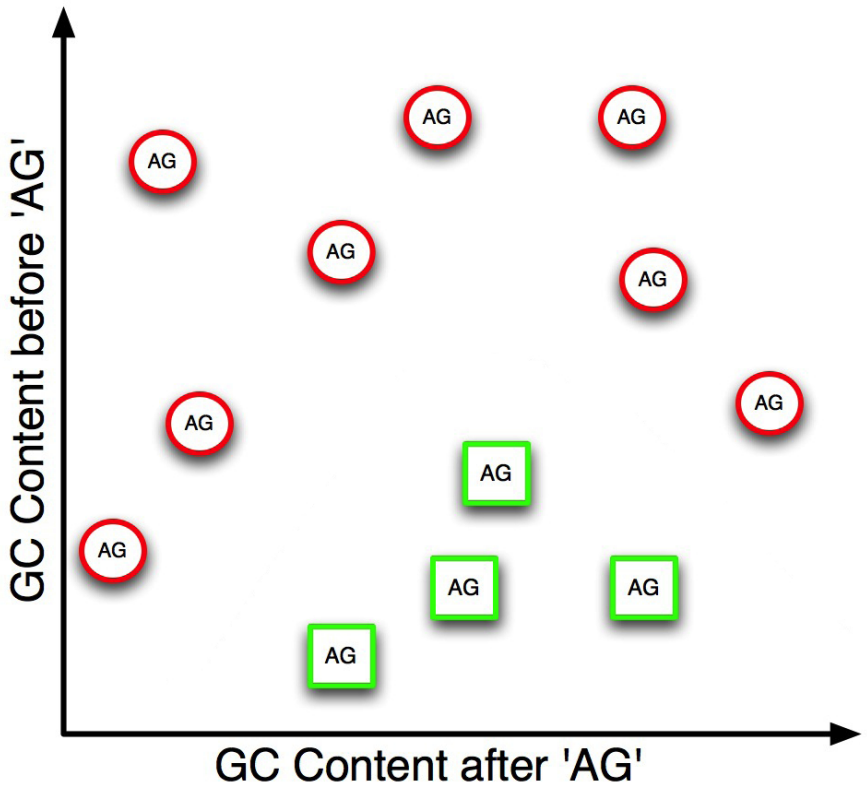
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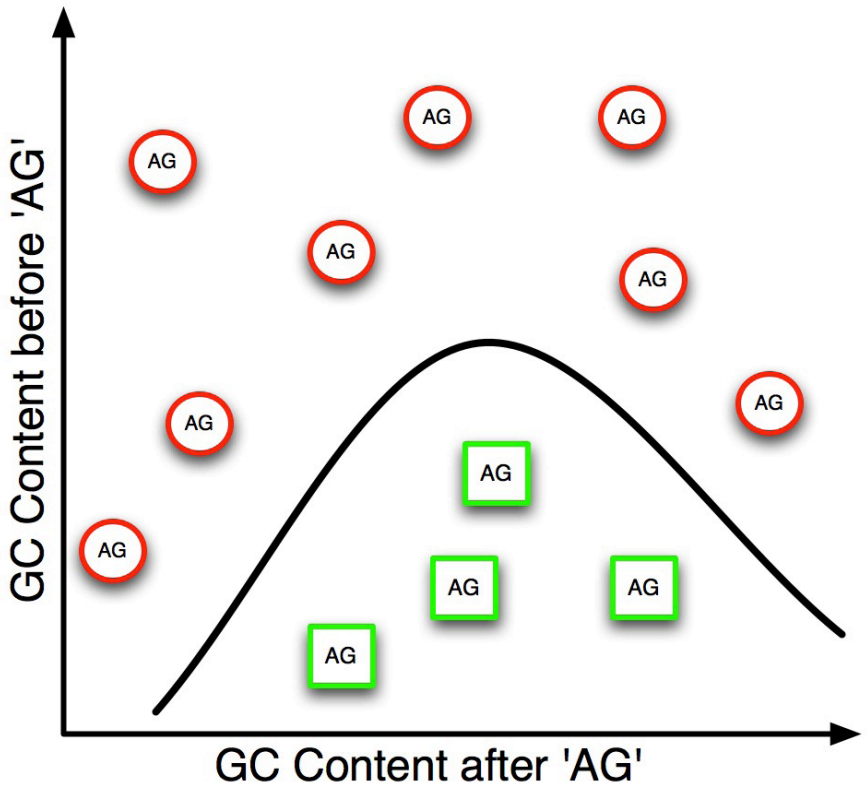
Two different classes of observations

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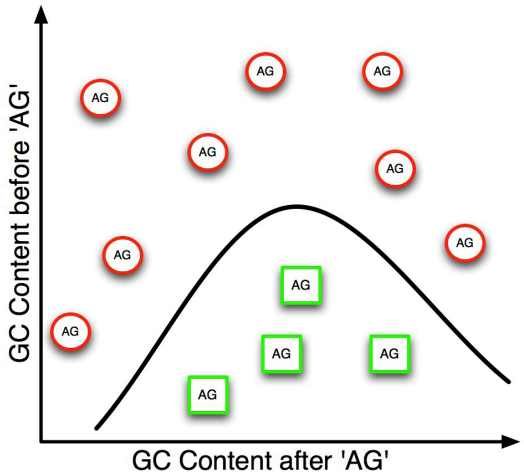


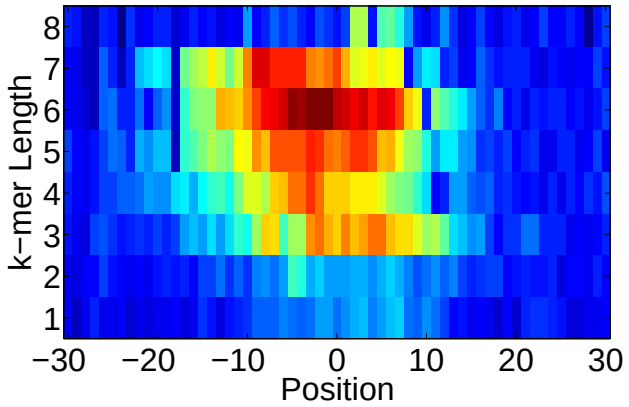
Inferred classification rule

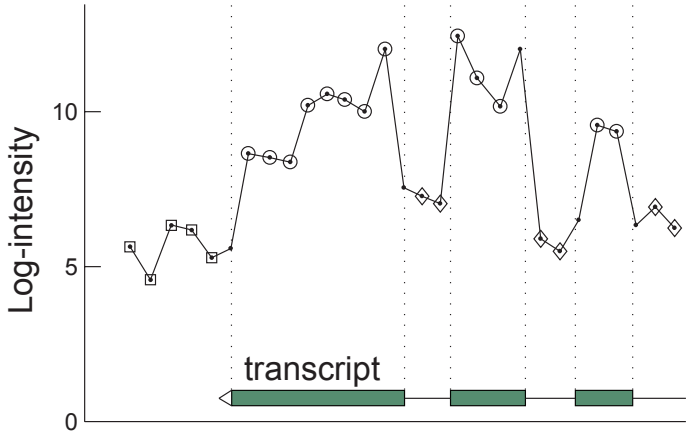
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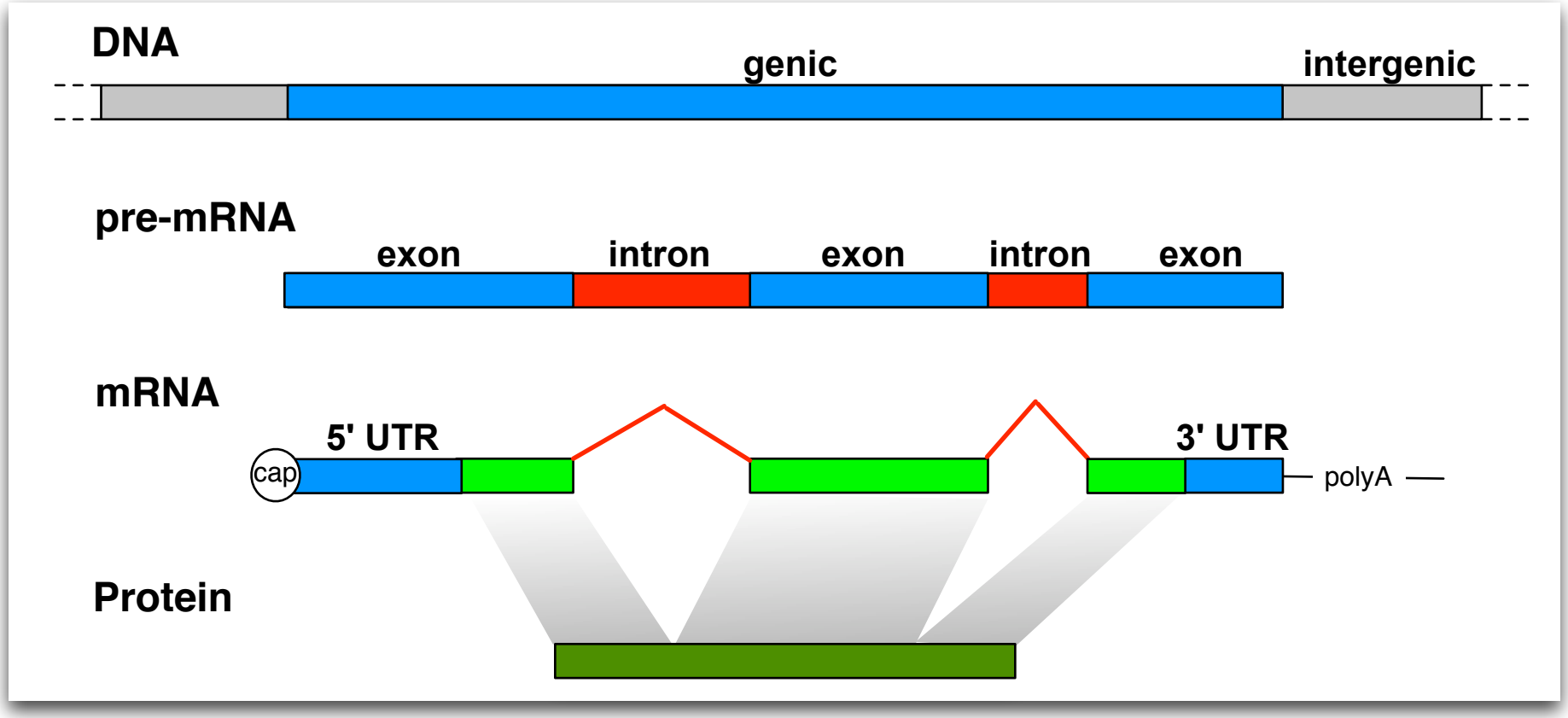
1. Large scale sequence classification
2. Analysis and explanation of learning results
3. Sequence segmentation & structure prediction





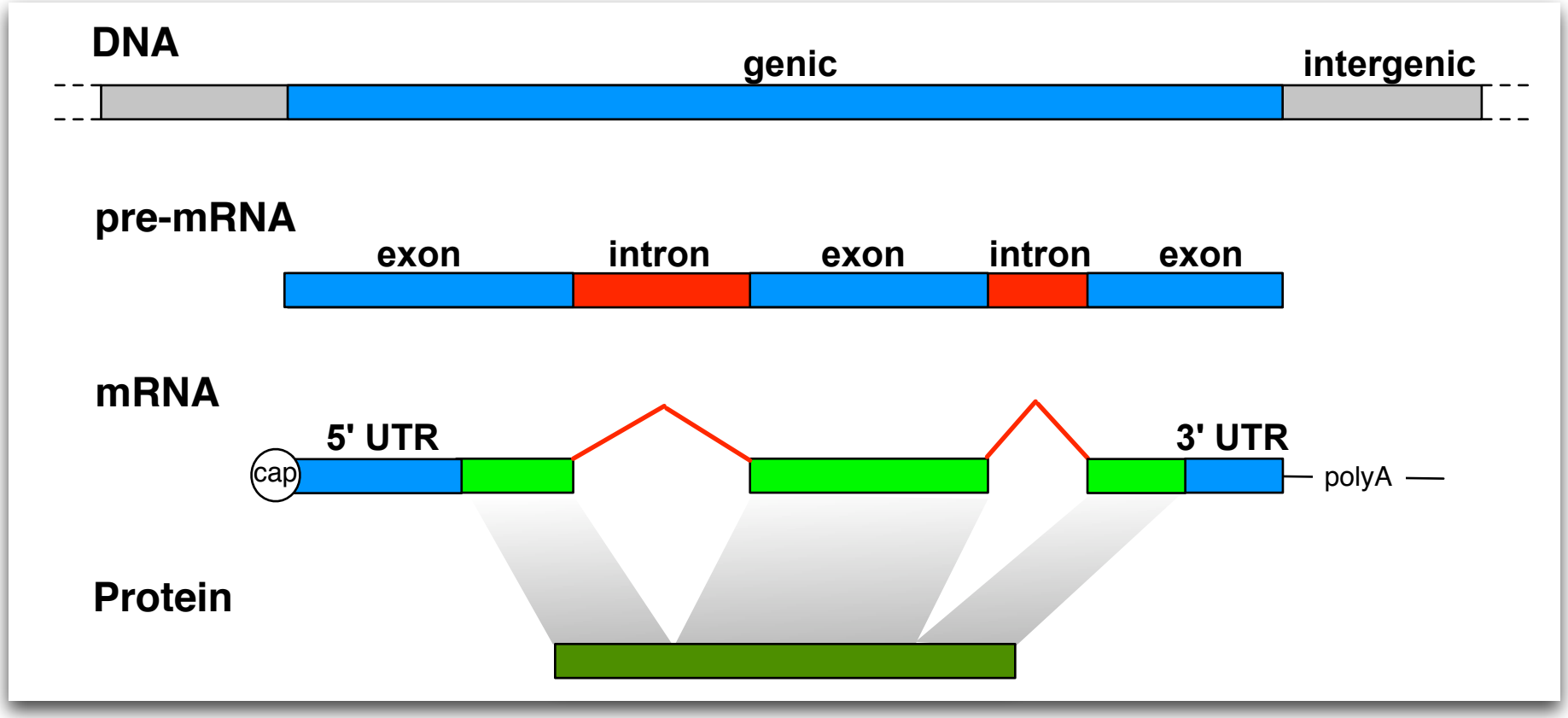


Computational Transcriptome Prediction
(a.k.a. Computational Gene Finding)



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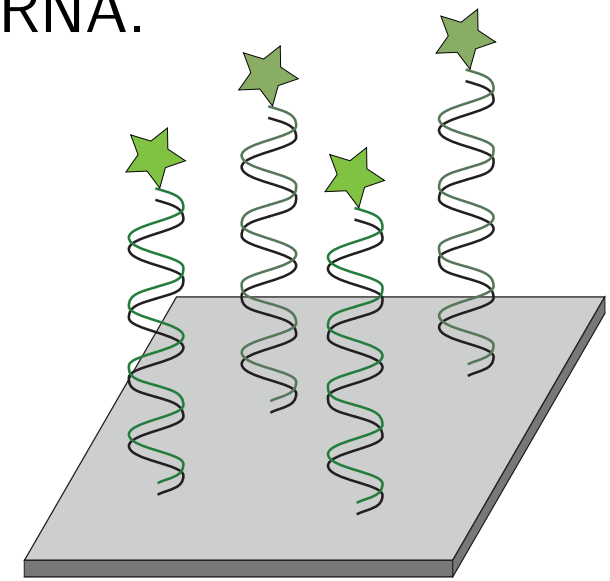
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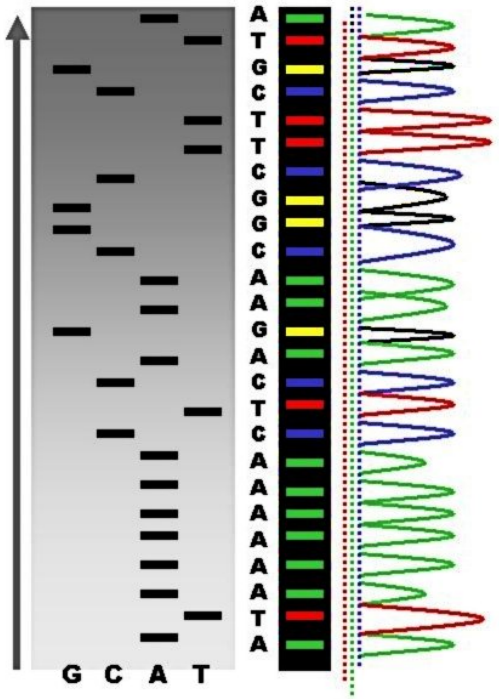
Getting closer to reality...

DNA Microarrays

Oligonucleotide probes immobilized on a glass slide hybridize to complementary labeled target RNA.



cDNA Sequencing



[Wikipedia]

Experimental methods have there inherent limitations and biases

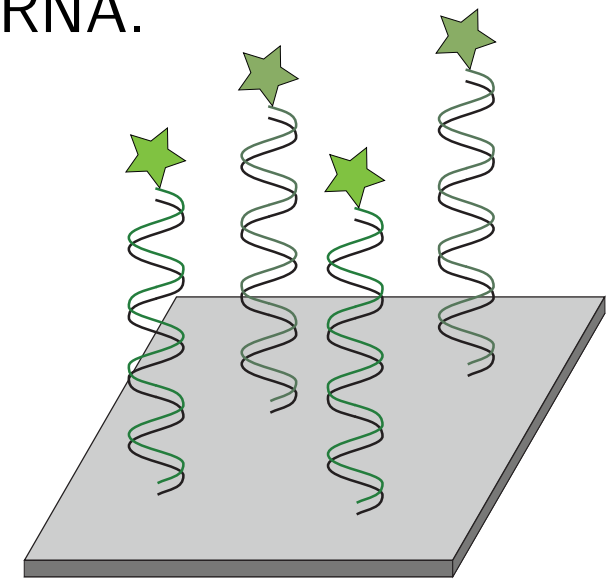
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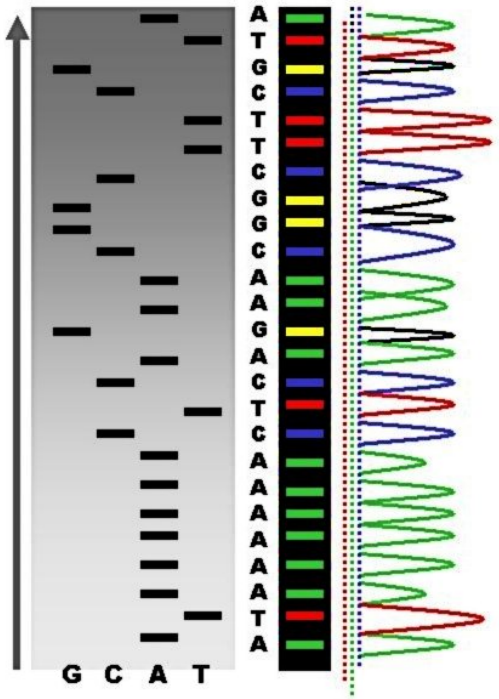
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Key Research Questions

- Characterize an organism's full complement of genes
  - $\Rightarrow$ Find new (possibly noncoding) genes
  - $\Rightarrow$ Compare genes among organisms
- Characterize transcript isoforms
  - $\Rightarrow$ Find new alternative splice forms / transcript ends
- Monitor transcriptome changes between tissues or in response to environmental changes (e.g. stress)
  - $\Rightarrow$ Identify significant expression changes
- Understand transcriptome regulation
  - $\Rightarrow$ Knock-out / knock-down analysis of regulators
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RNA-Seq allows ...

- High-throughput transcriptome measurements
- Qualitative studies
  - New transcripts
  - Altered expression across tissues, conditions etc.
- Quantitative studies at high resolution

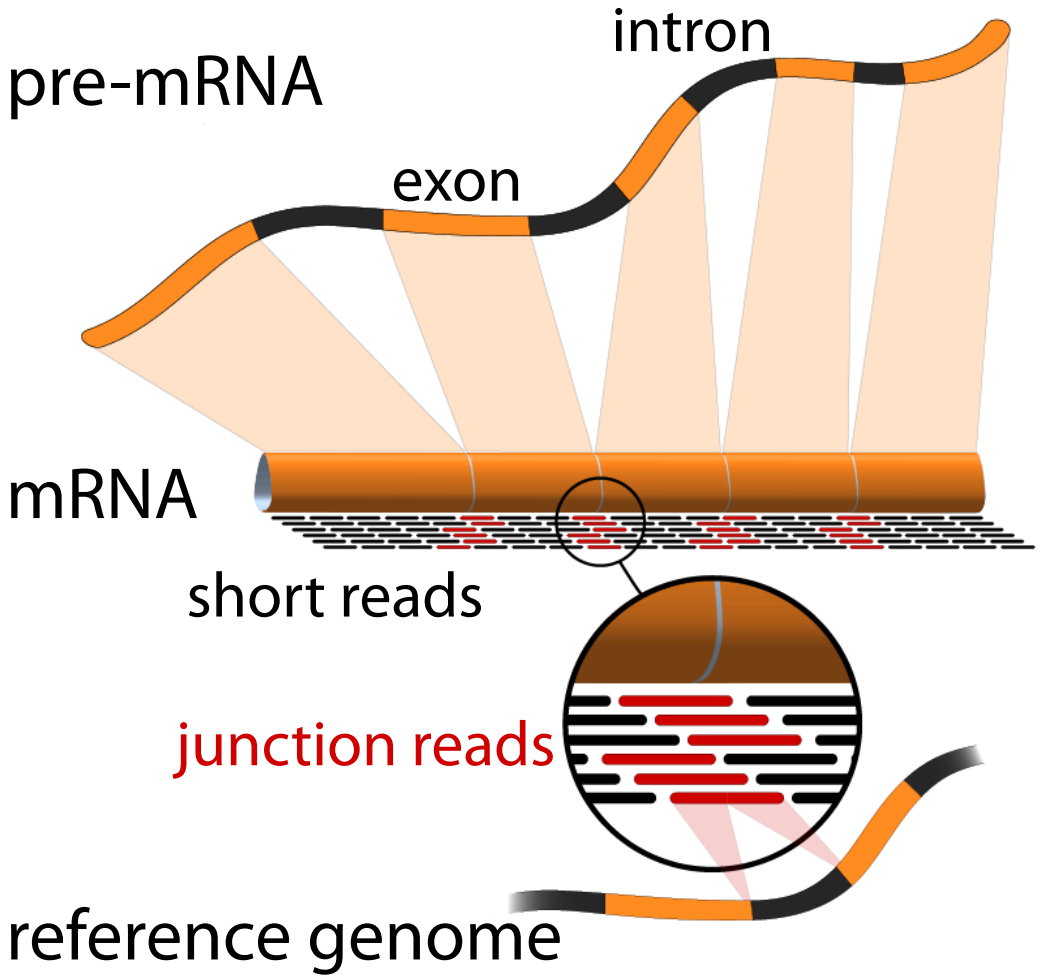


Figure adapted from Wikipedia

Motivation: Obtain complete transcriptome for further analyses

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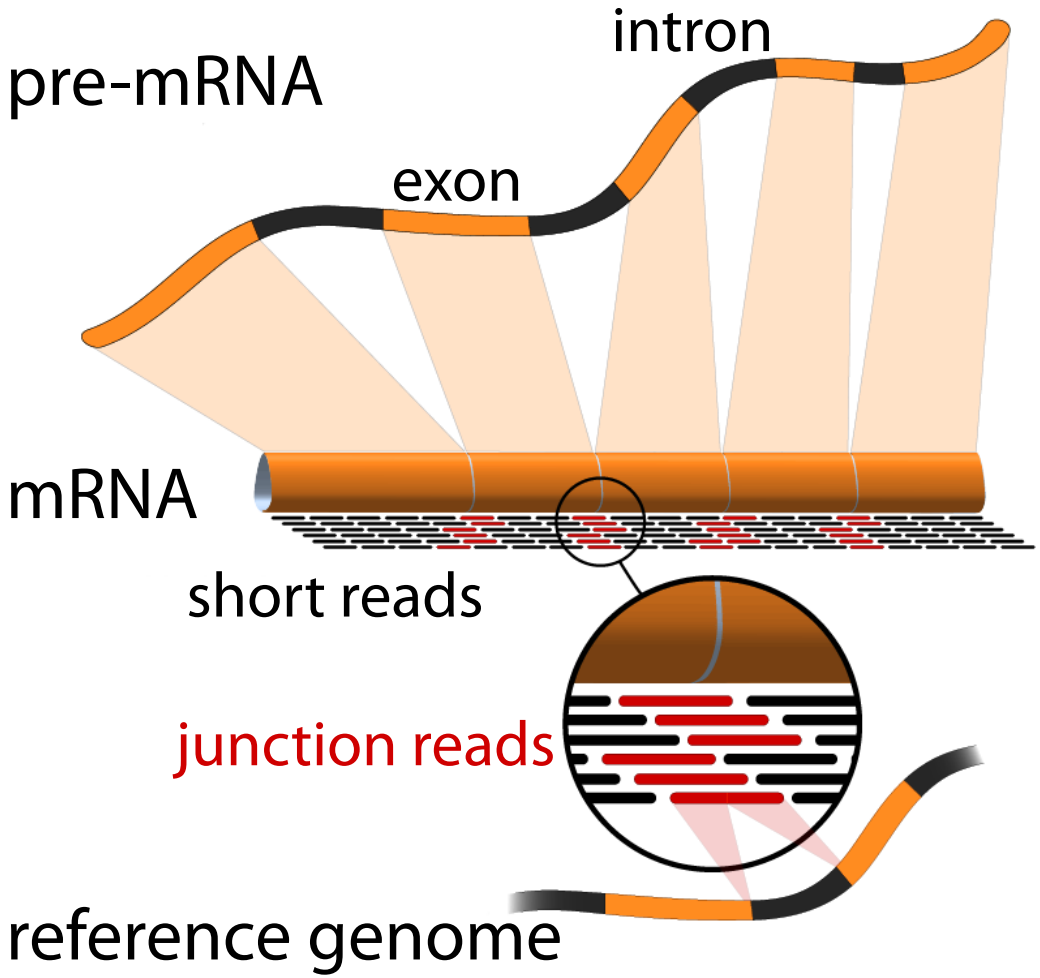


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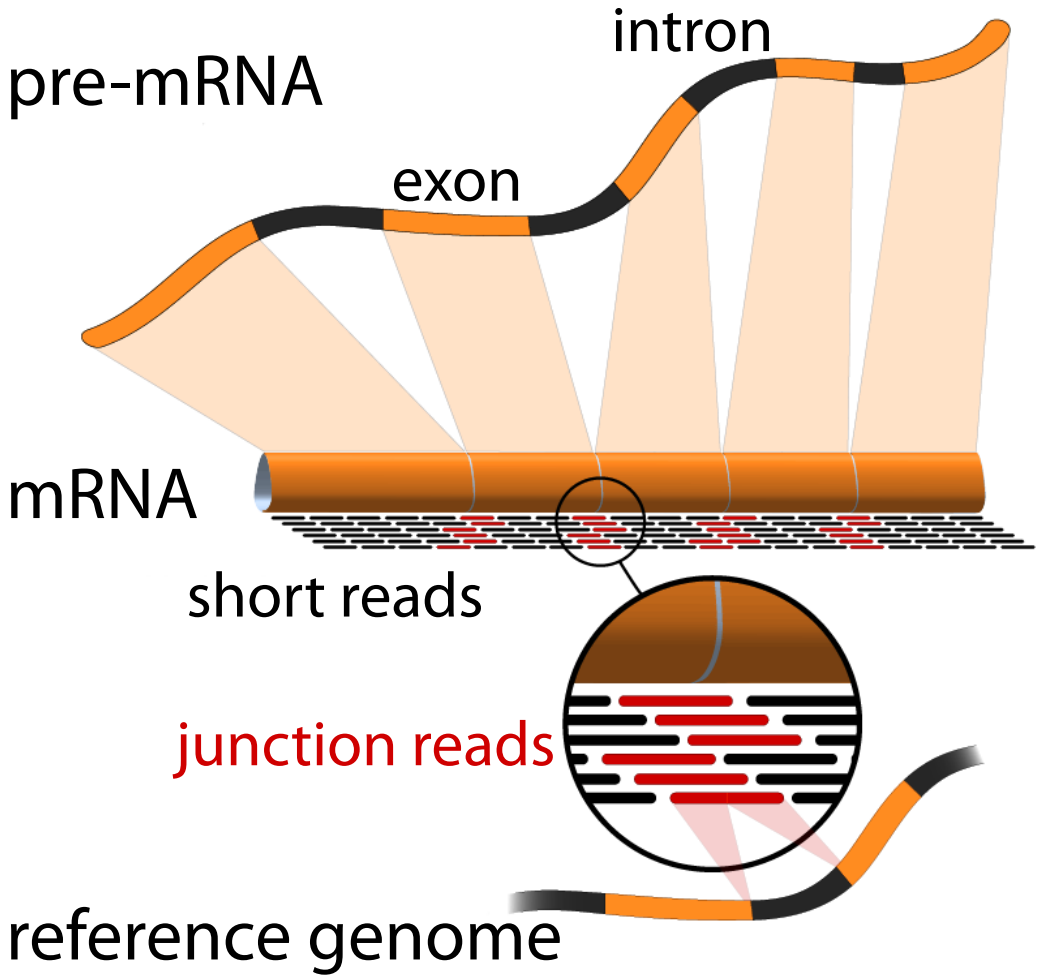


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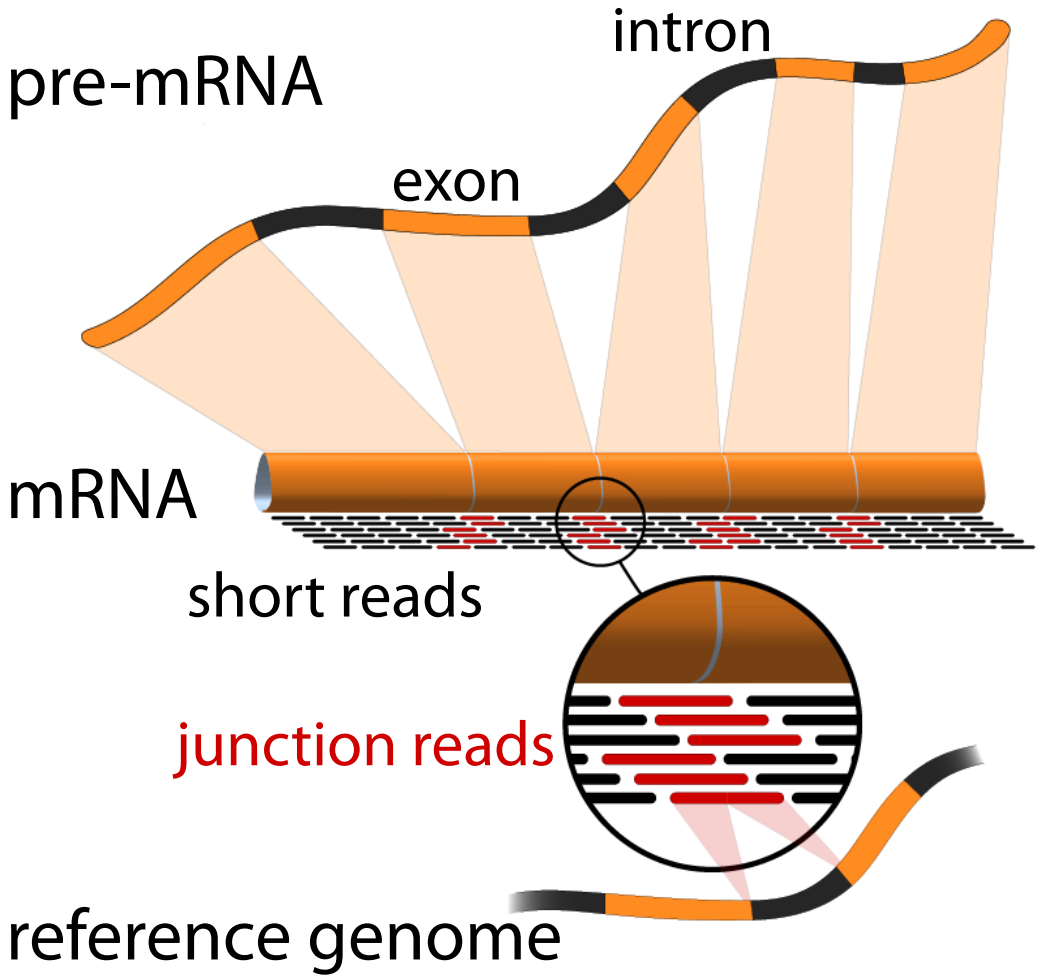
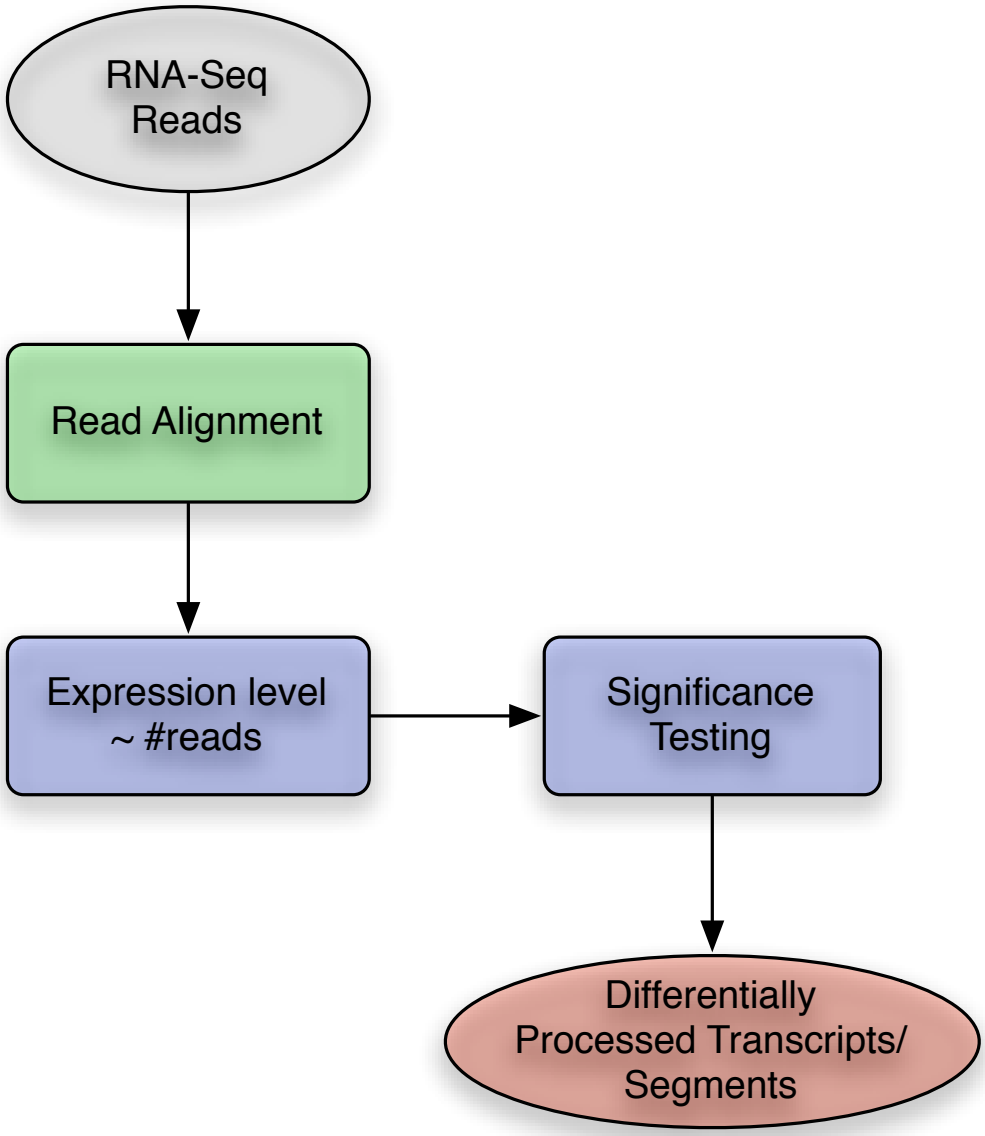


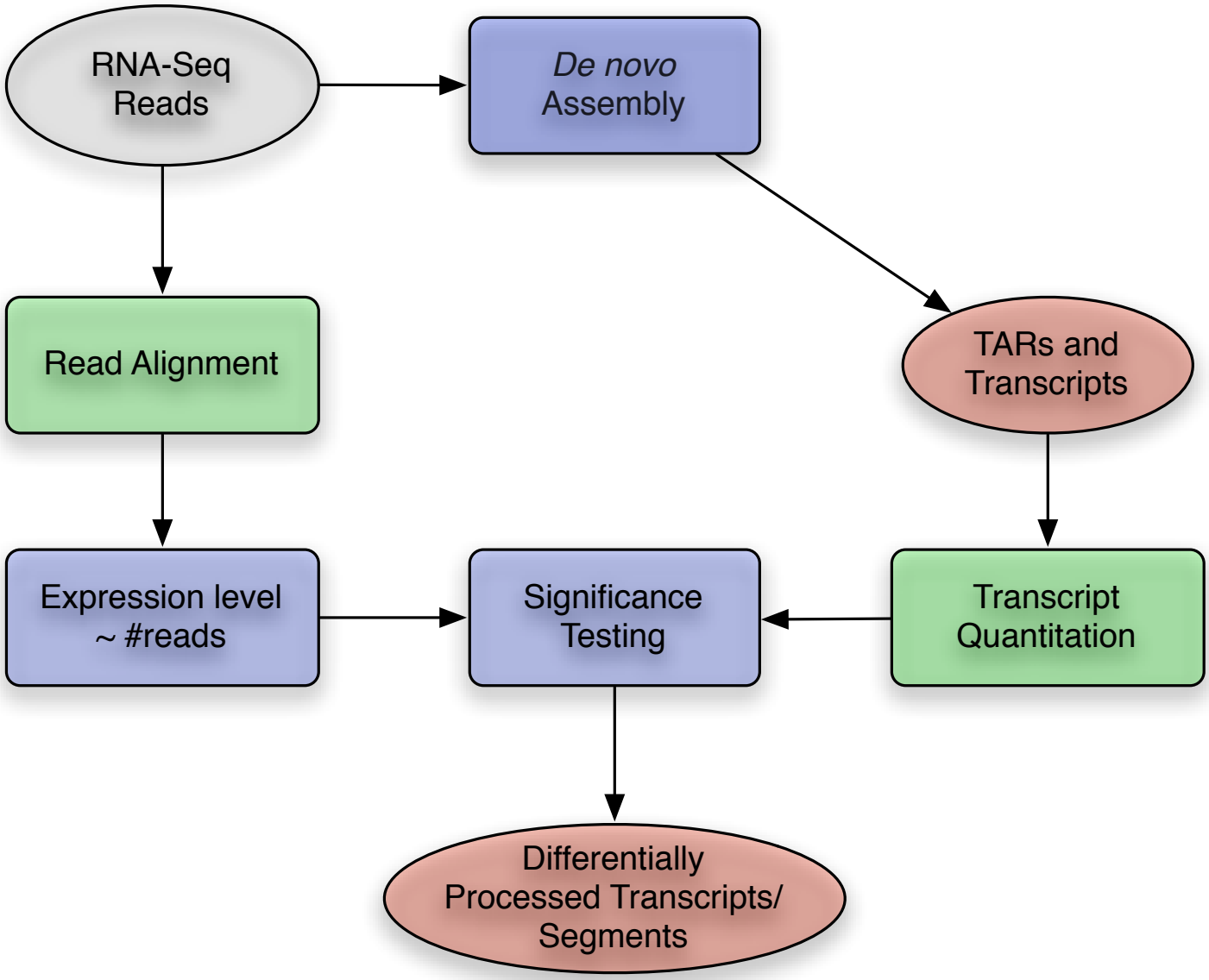
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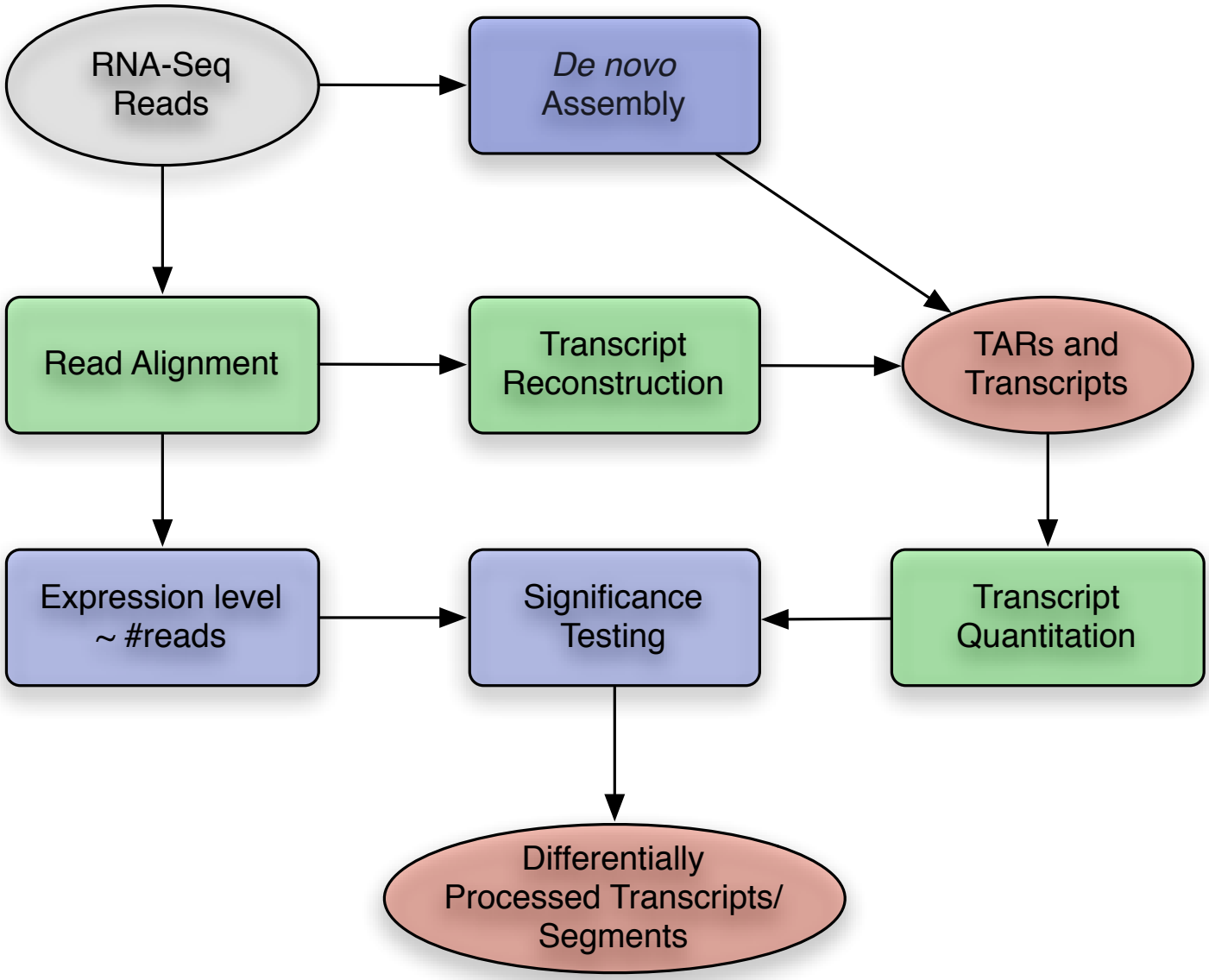
Common RNA-Seq Analysis Steps



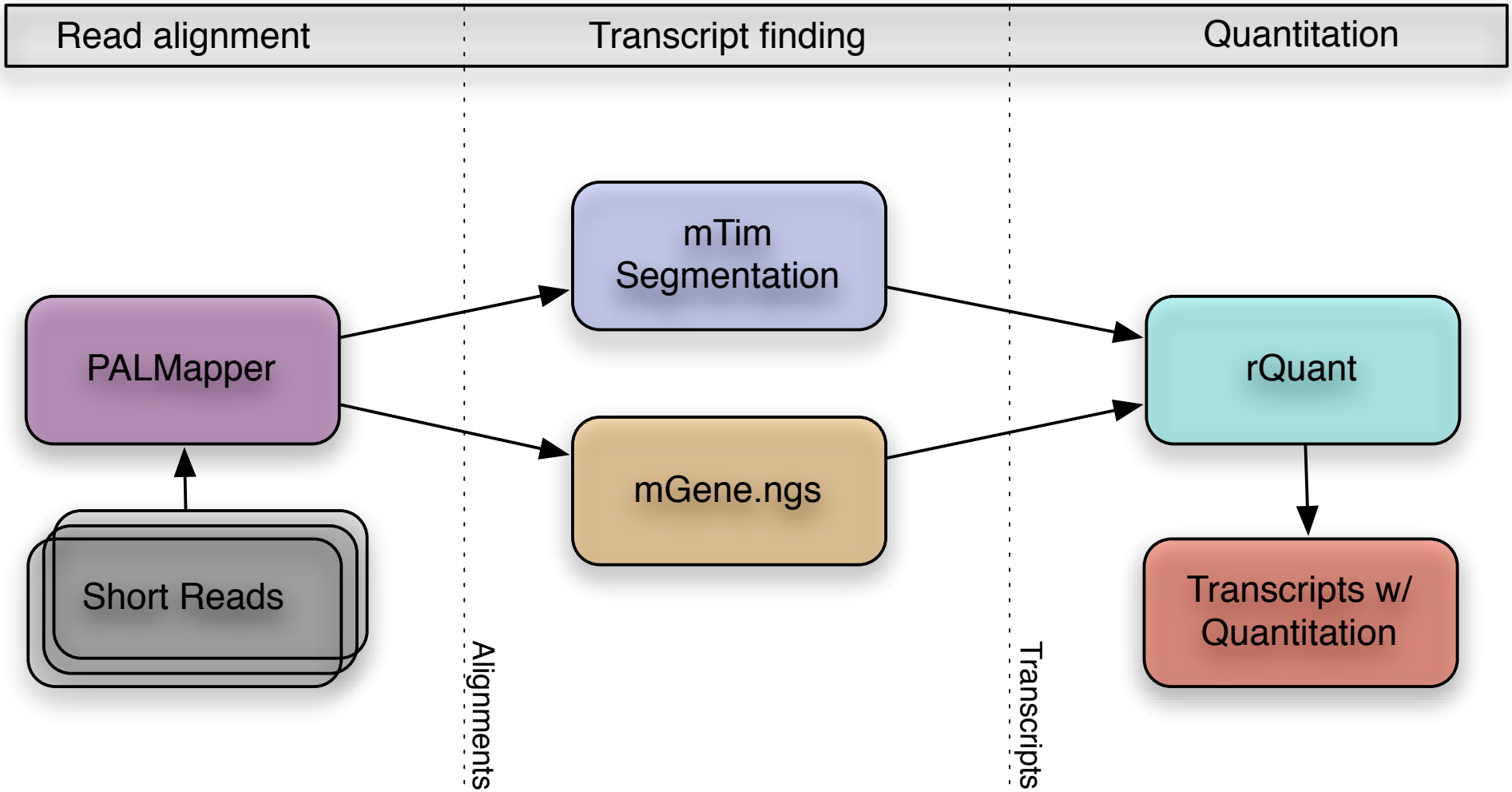
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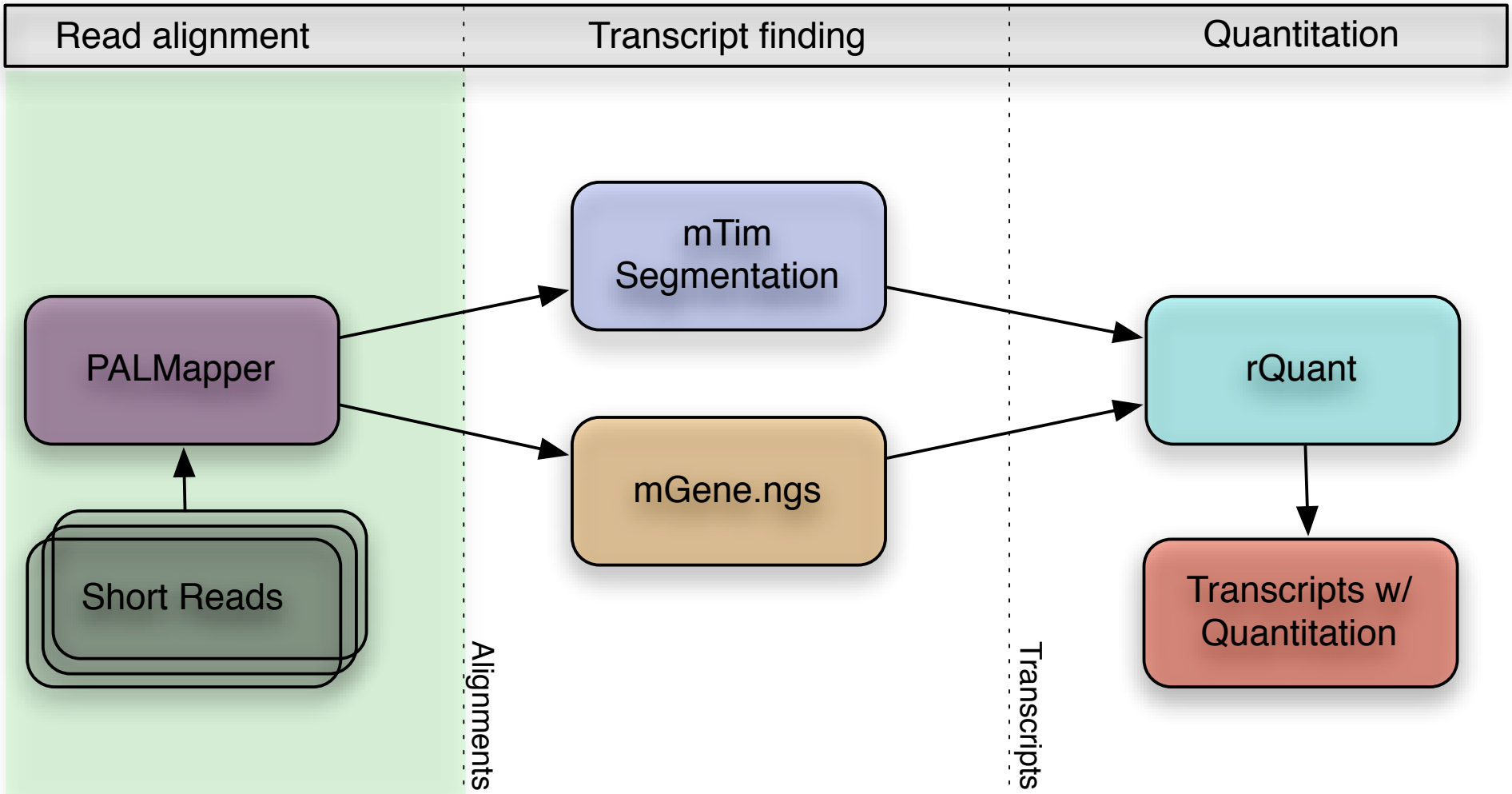
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RNA-Seq Pipeline Overview



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(PALMapper = QPALMA + GenomeMapper)



GenomeMapper for (unspliced) read mapping:

- Alignments based on GenomeMapper developed in Tübingen for the 1001 plant genome project [Schneeberger et al., 2009]
- *k*-mer based index, well suited for smaller genomes

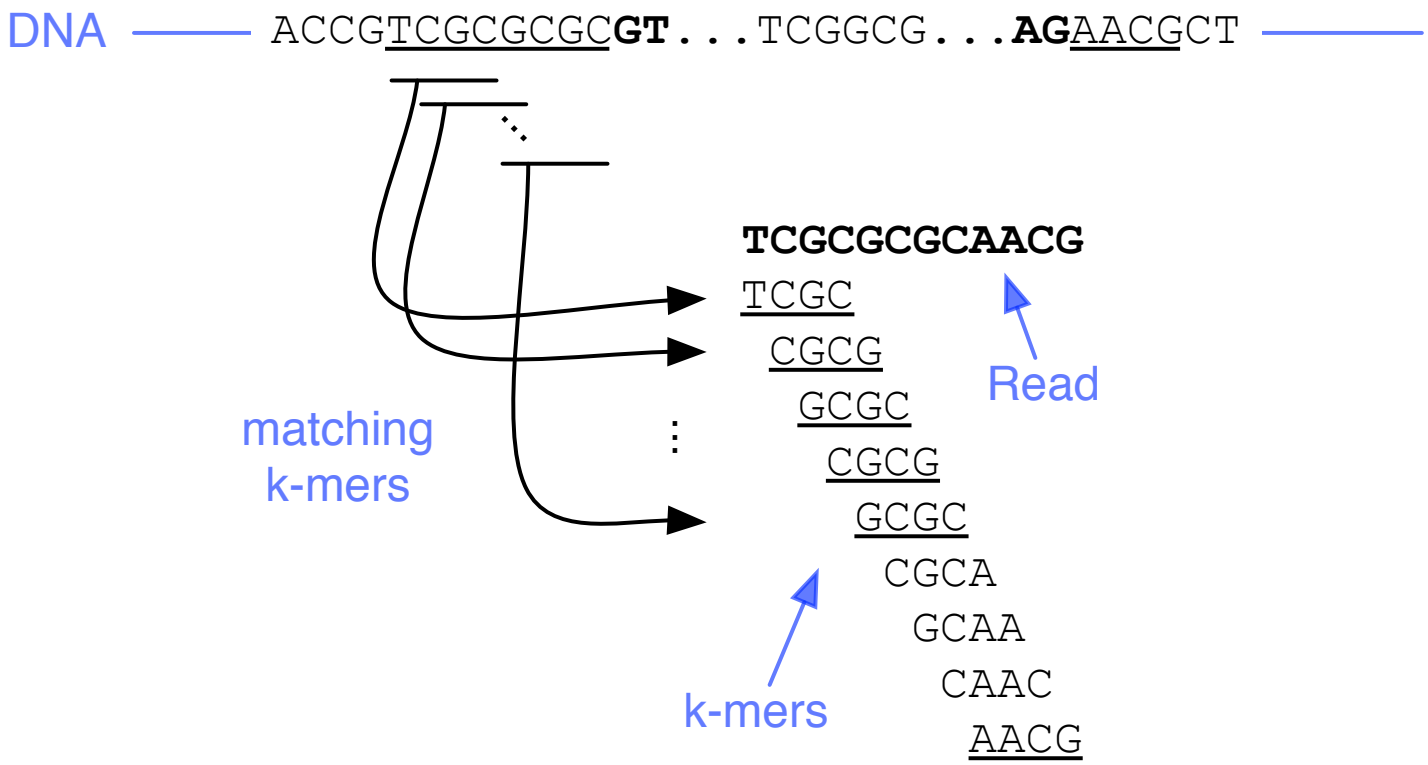
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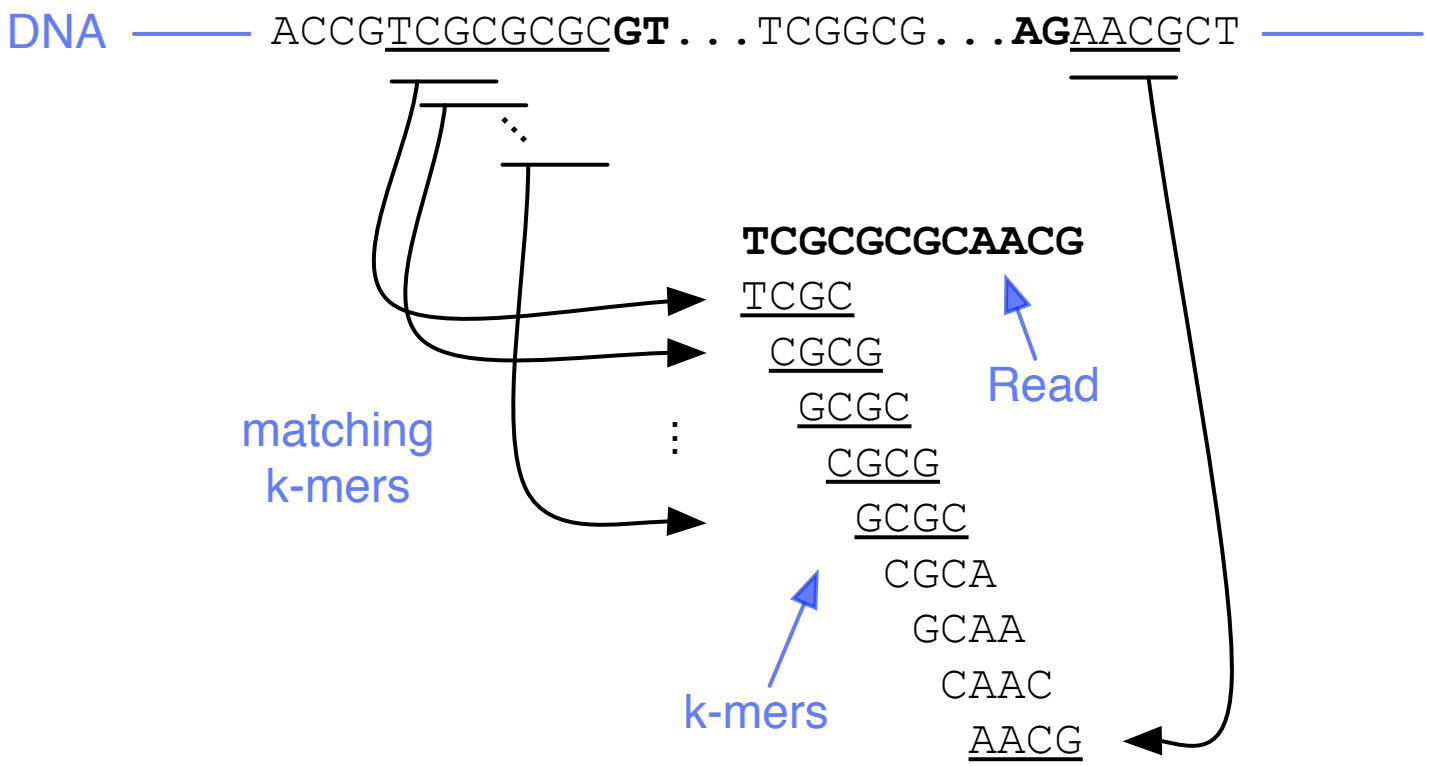


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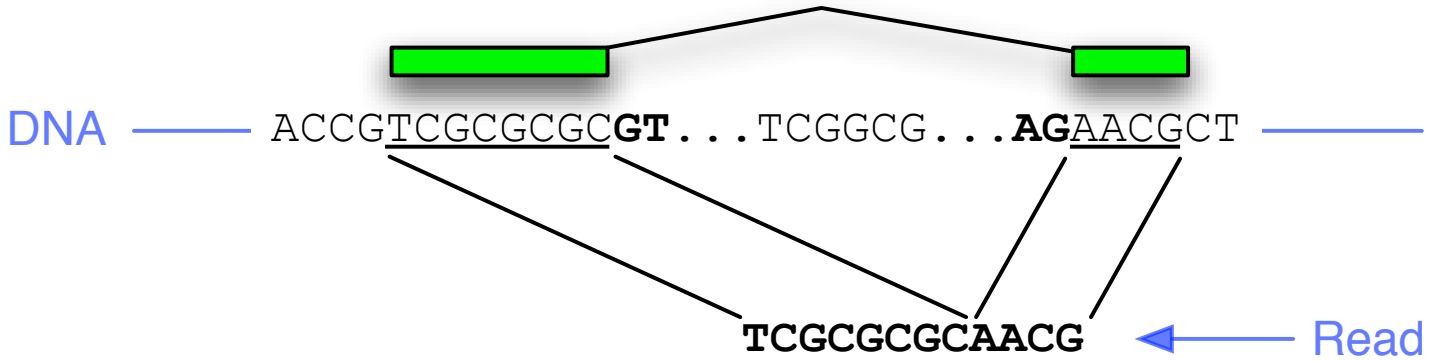
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QPALMA for spliced read alignments:

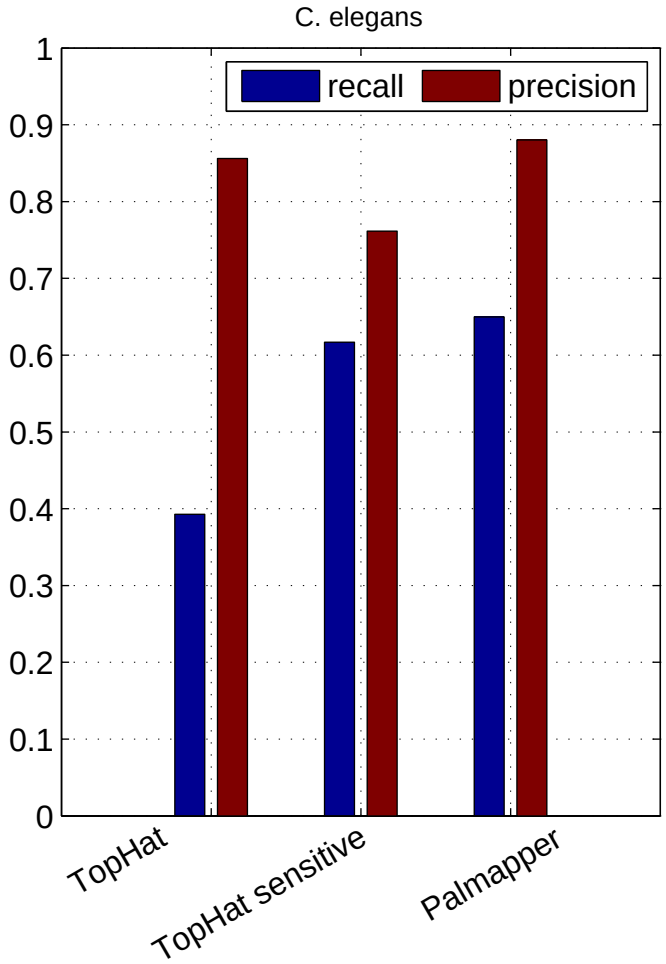
- GenomeMapper identifies *seed regions*
- *Spliced alignments* by QPALMA [De Bona et al., 2008]



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PALMapper Accuracy Evaluation

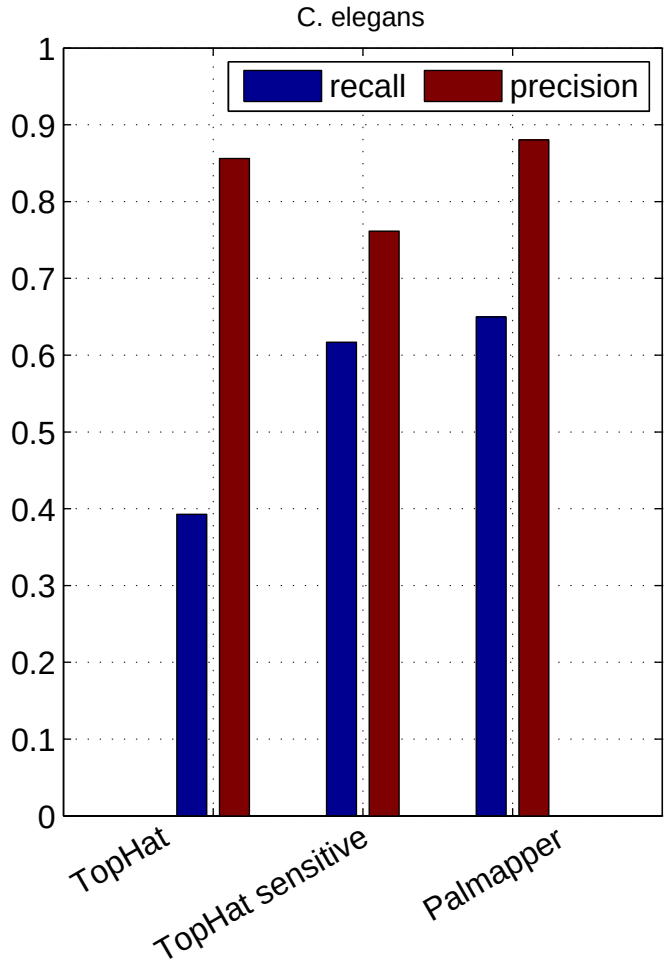
How accurately can PALMapper identify introns?



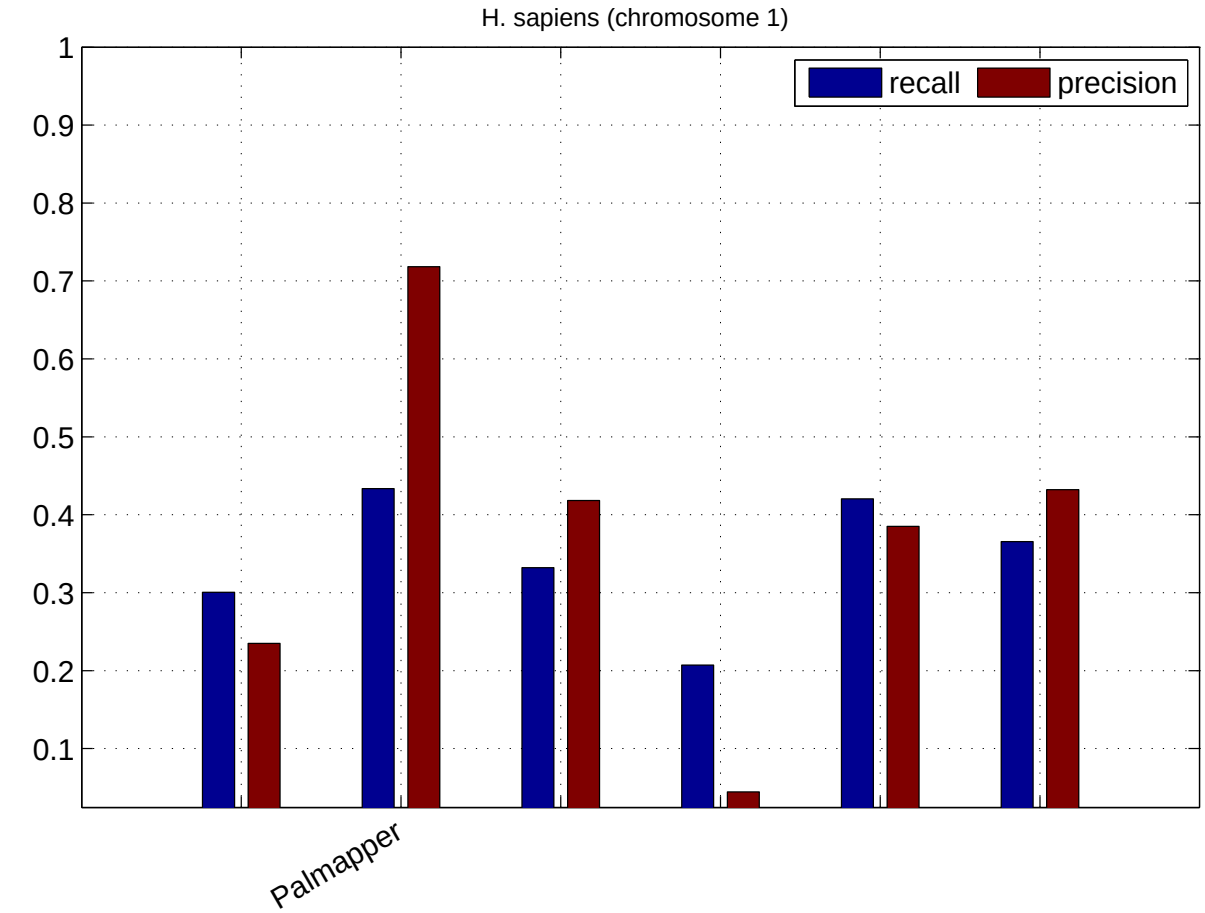
PALMapper (3.5h) and TopHat (3.5h/10h) aligning 24M reads

PALMapper Accuracy Evaluation

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Comparison of PALMapper with other alignment programs within the RGASP project (preliminary)

QPALMA: Extended Smith-Waterman Scoring



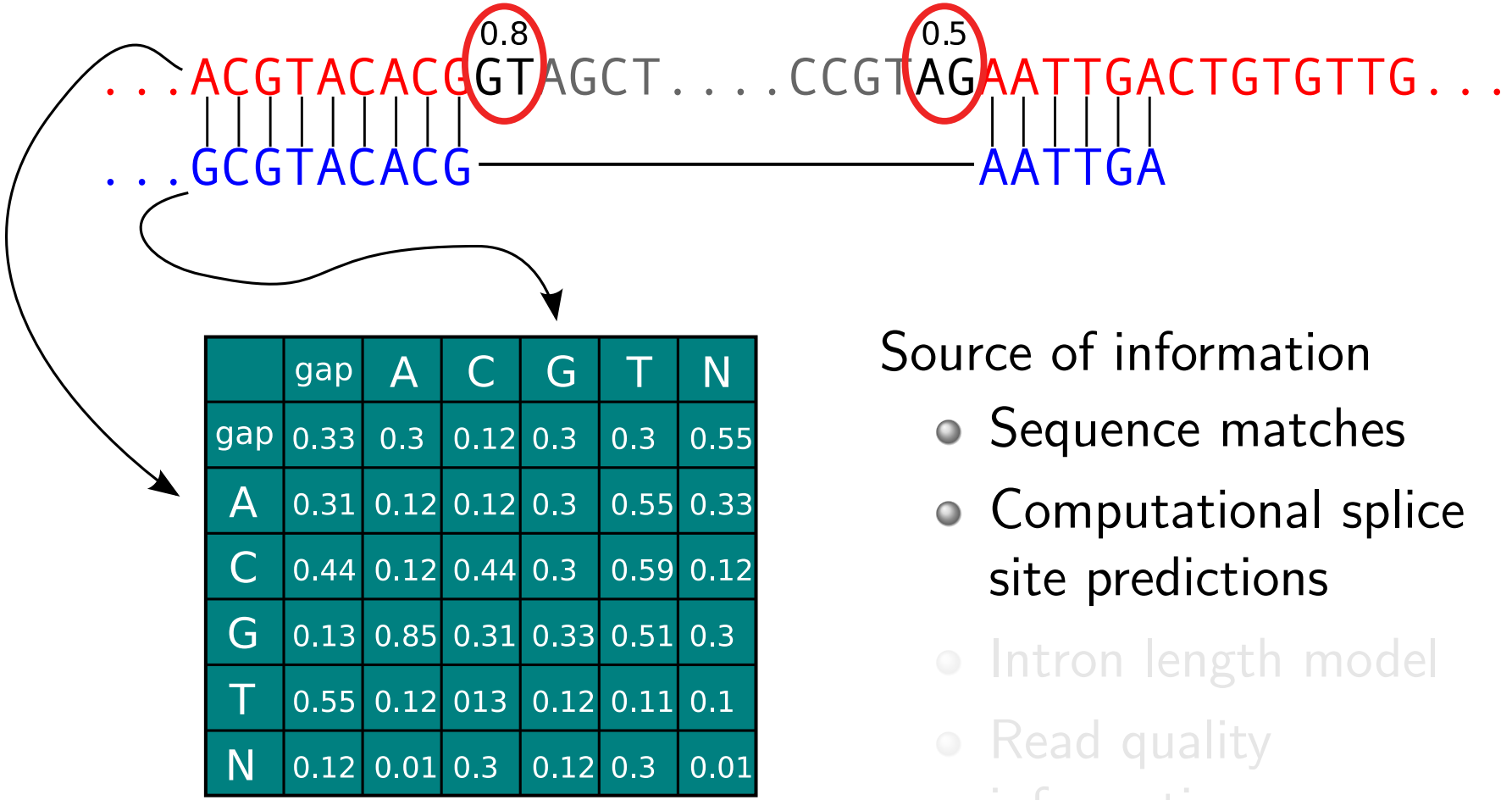
	gap	A	C	G	T	N
gap	0.33	0.3	0.12	0.3	0.3	0.55
A	0.31	0.12	0.12	0.3	0.55	0.33
C	0.44	0.12	0.44	0.3	0.59	0.12
G	0.13	0.85	0.31	0.33	0.51	0.3
T	0.55	0.12	013	0.12	0.11	0.1
N	0.12	0.01	0.3	0.12	0.3	0.01

Source of information

- Sequence matches
- Computational splice site predictions
- Intron length model
- Read quality information

Classical scoring $f : \Sigma \times \Sigma \rightarrow \mathbb{R}$

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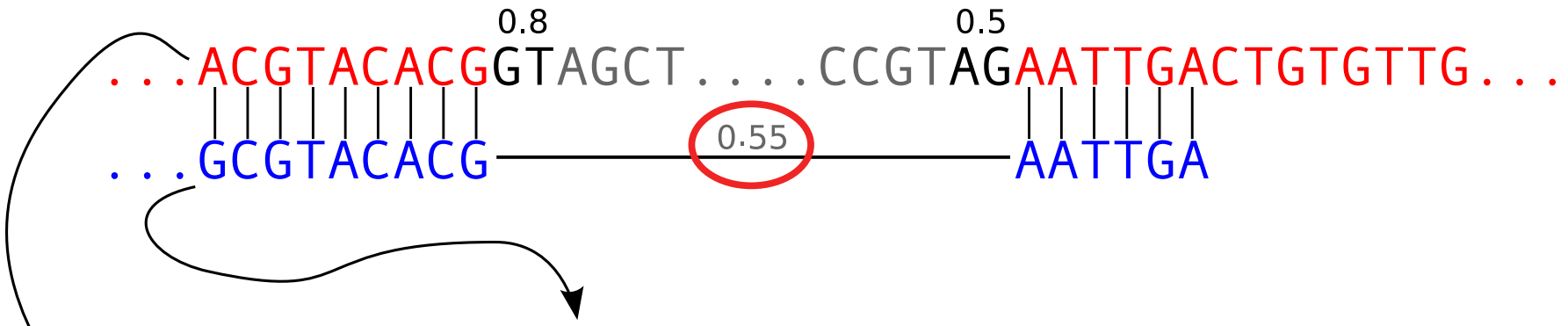
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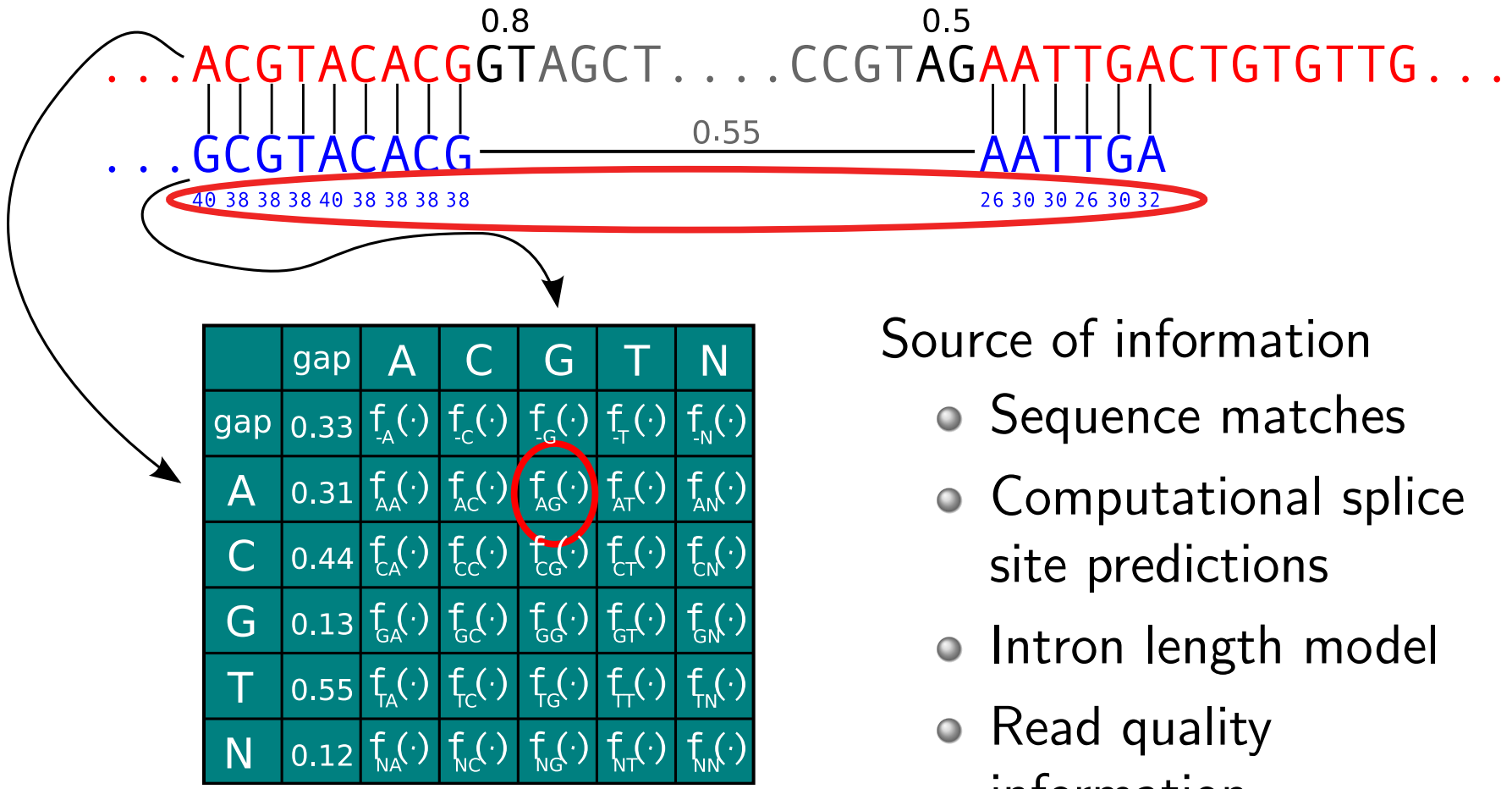
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Quality scoring $f : (\Sigma \times \mathbb{R}) \times \Sigma \rightarrow \mathbb{R}$

[De Bona et al., 2008]

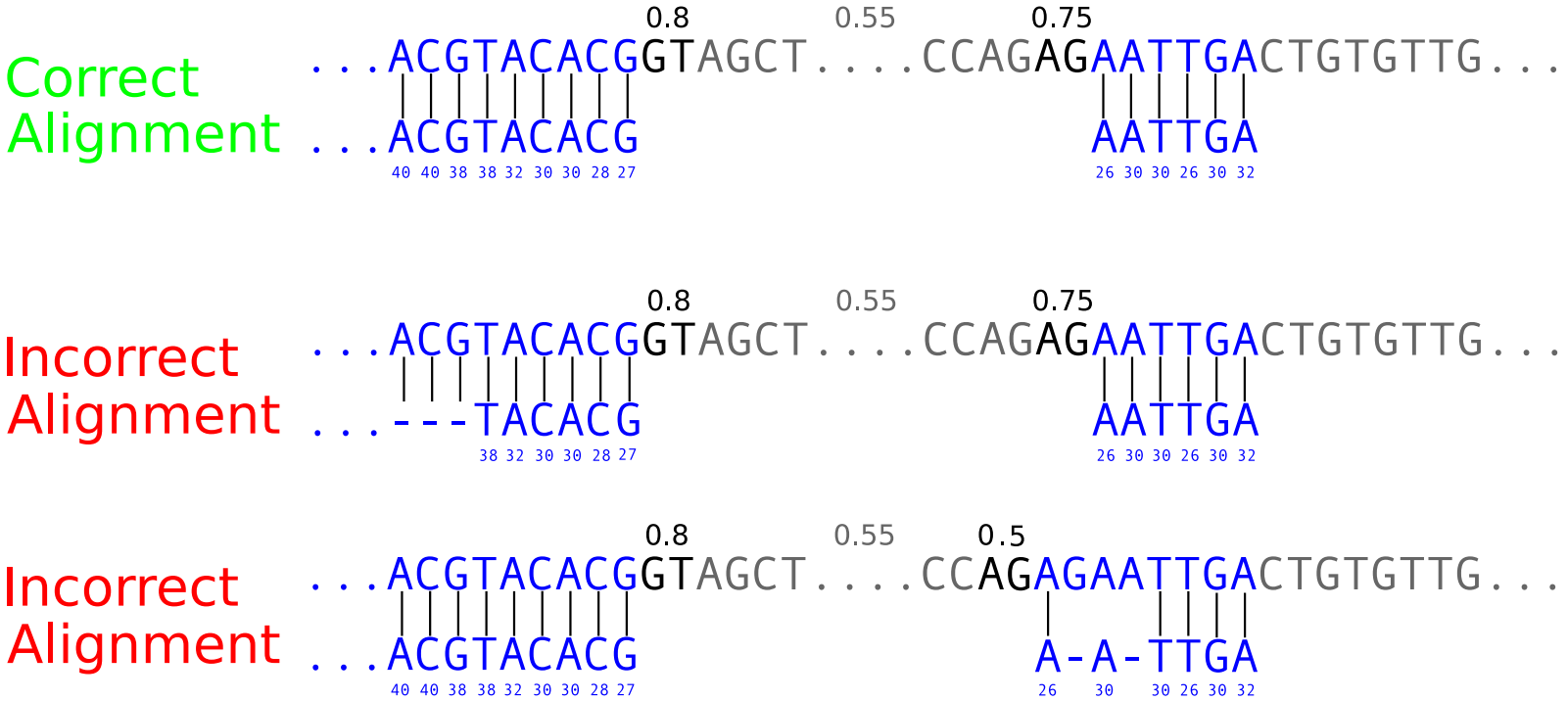
Scoring Parameter Inference



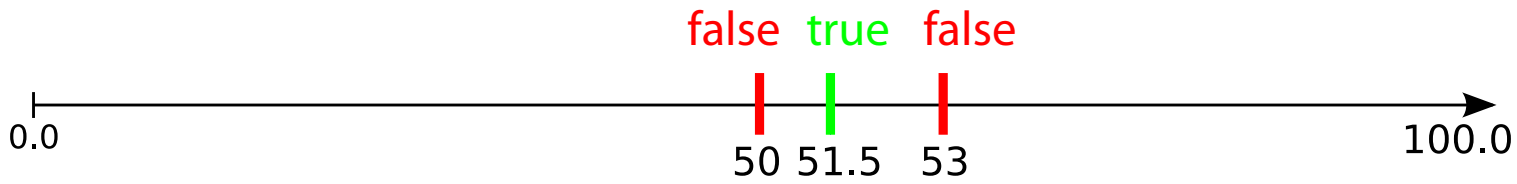
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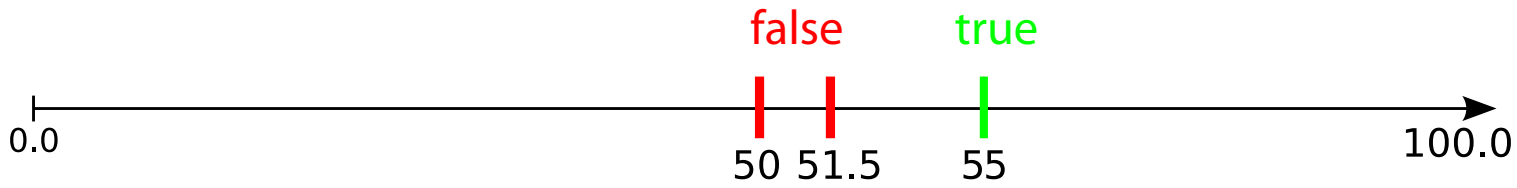


Cartoon: Maximize the Margin



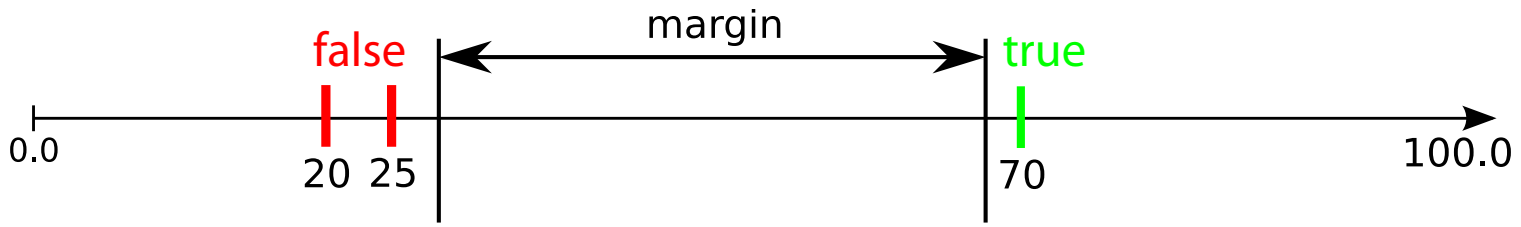
- Correct alignment is **not** highest scoring one
- Correct alignment is highest scoring one
- Can we do better?

Cartoon: Maximize the Margin



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- Correct alignment is highest scoring one
- Can we do better?

Cartoon: Maximize the Margin



- Use technique motivated by SVMs ("large-margin")
- Idea: Enforce a margin between correct and incorrect examples
- One has to solve a big quadratic problem

How Can We Generate Data for Training?



- How do we obtain true alignments for training QPalma?
- Simulate *realistic* transcriptome reads with known origin

Strategy:

1. Estimate relationship between quality score and error probability from given reads
2. Use annotation of a few genes to simulate spliced reads
3. Introduce errors according to error model using quality strings from given read set
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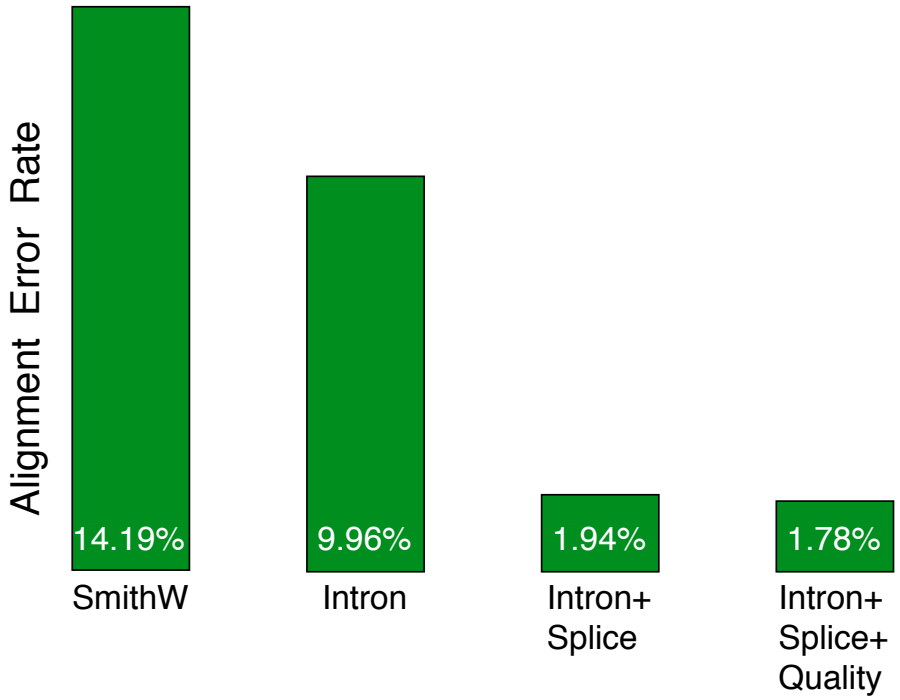
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QPALMA RNA-Seq Read Alignment

Generate set of artificially spliced reads

- Genomic reads with quality information
- Genome annotation for artificially splicing the reads
- Use 10,000 reads for training and 30,000 for testing

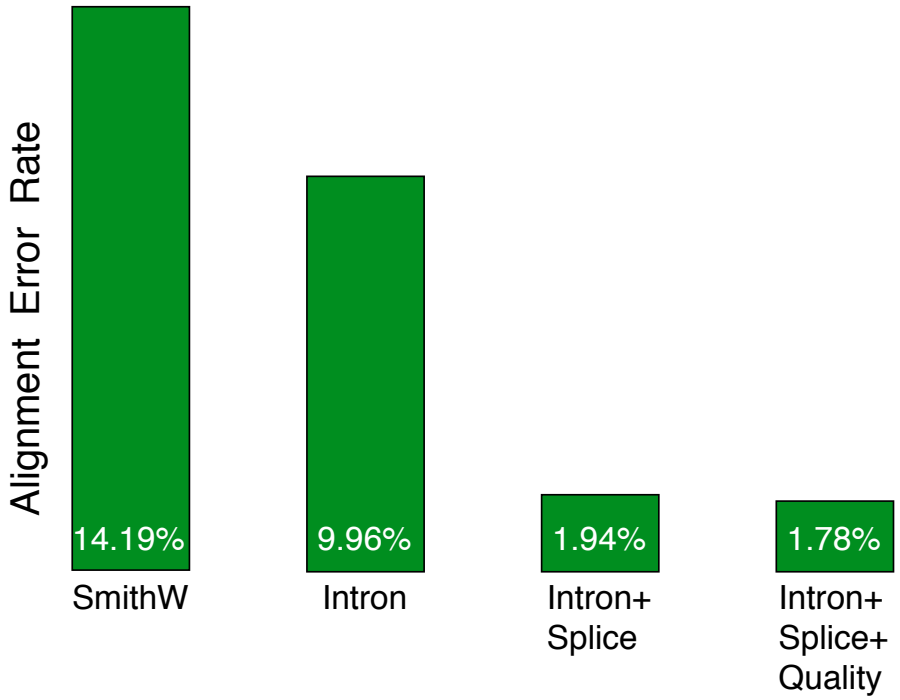


[De Bona et al., 2008]

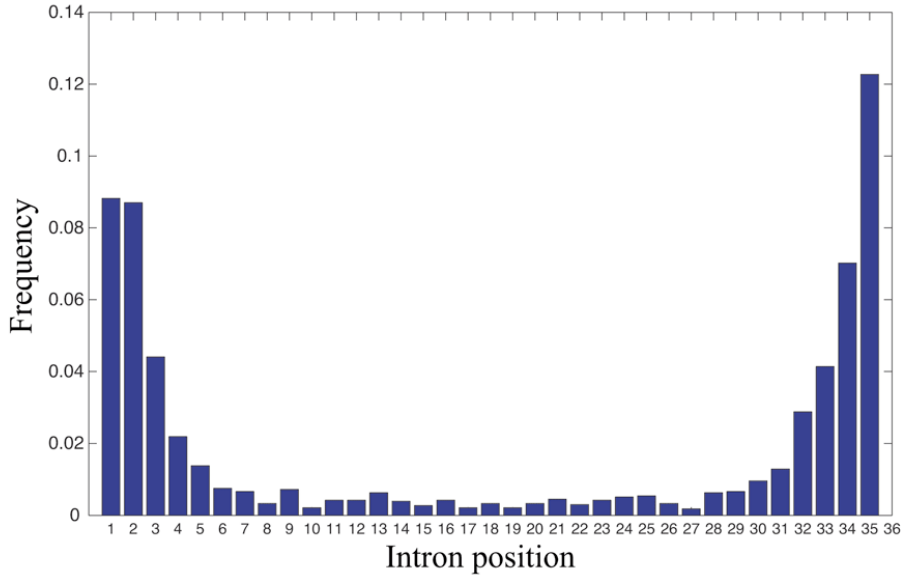
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Error vs. intron position

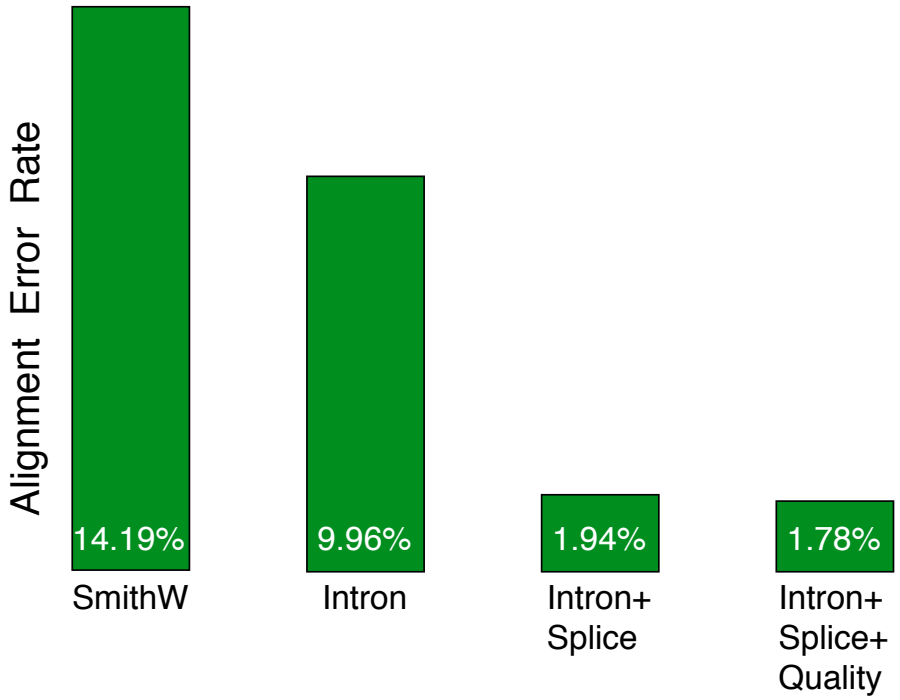


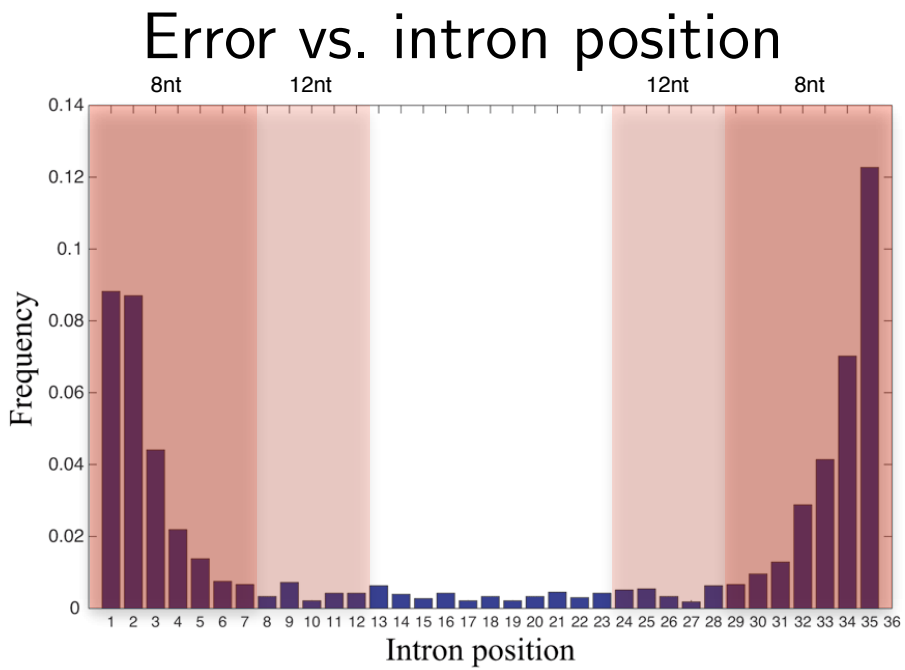
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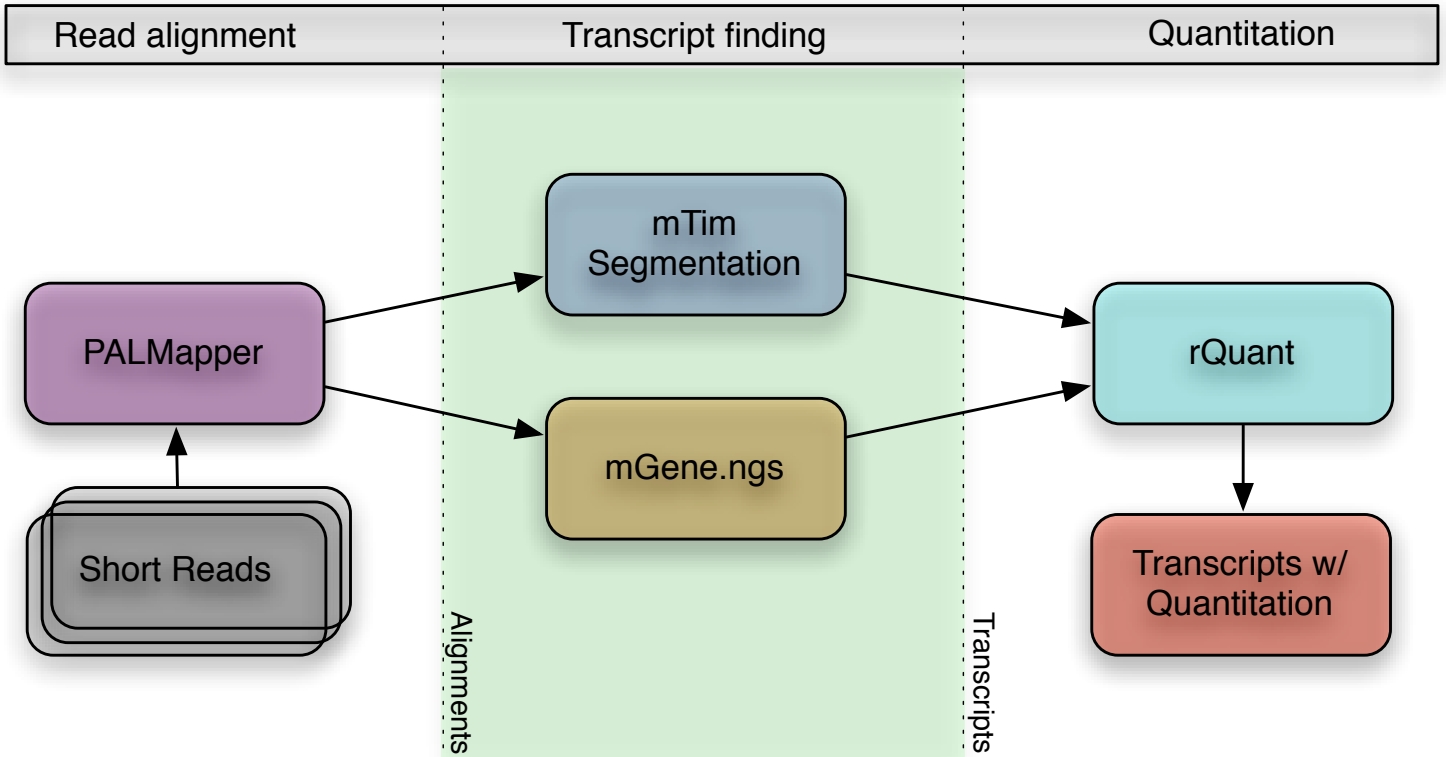
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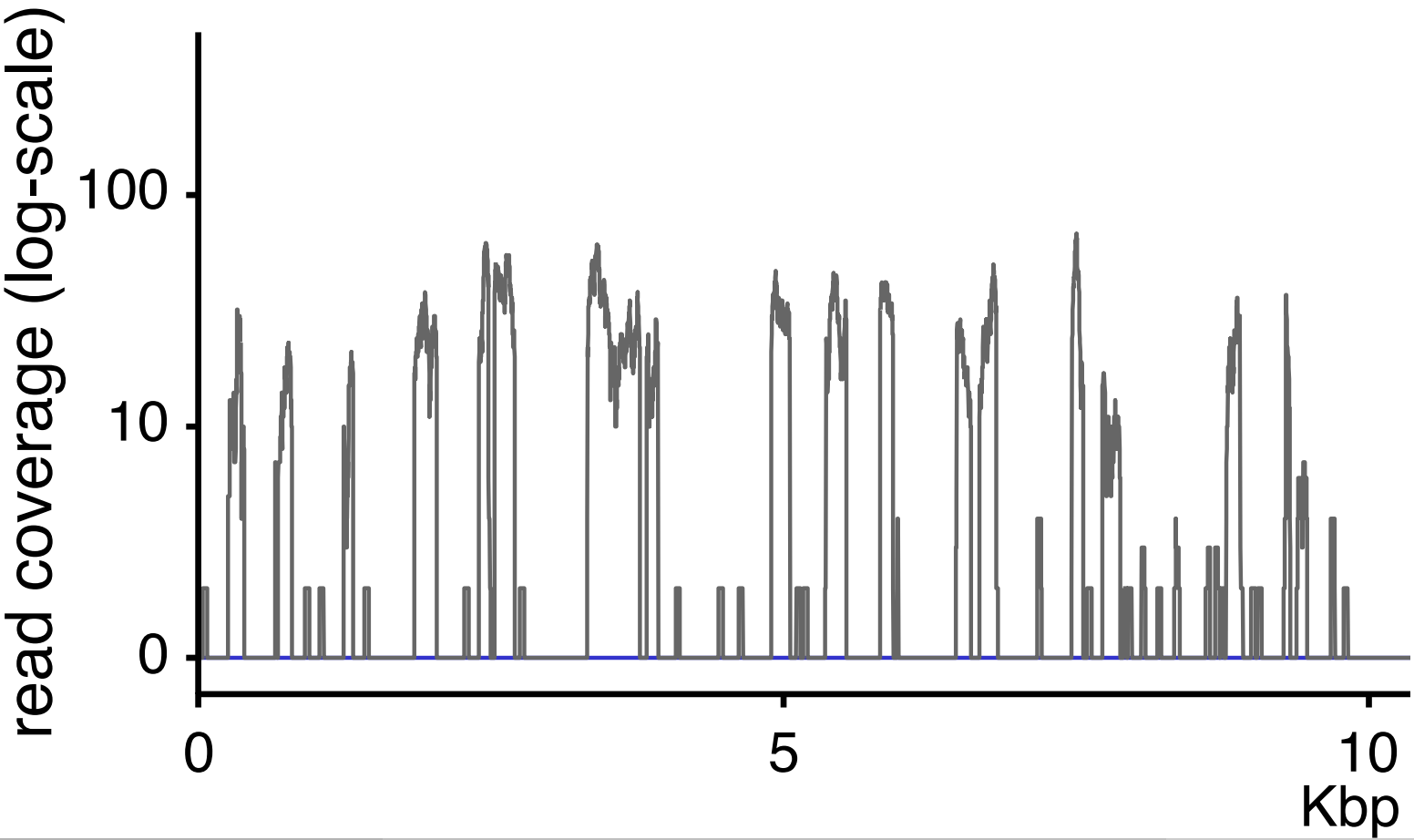
Step 2: Transcript Prediction



A. Coverage segmentation algorithm **mTiM** for general transcripts (no coding bias/assumption)
B. Extension of the mGene gene finding system to use NGS data for protein coding transcript prediction (**mGene.ngs**)

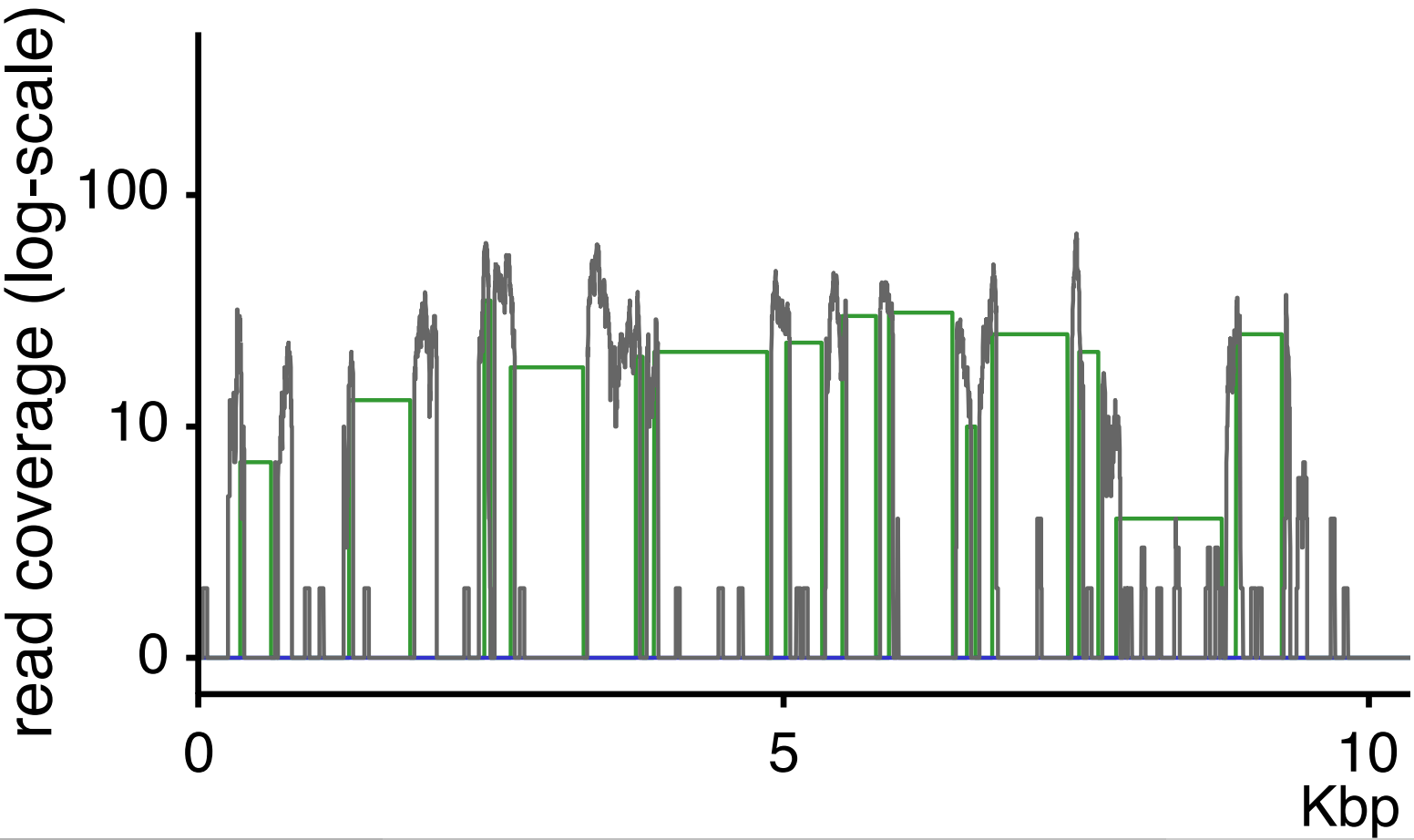
mTiM: Read Coverage Segmentation

Goal: Characterize each base as *intergenic*, *exonic*, or *intronic*



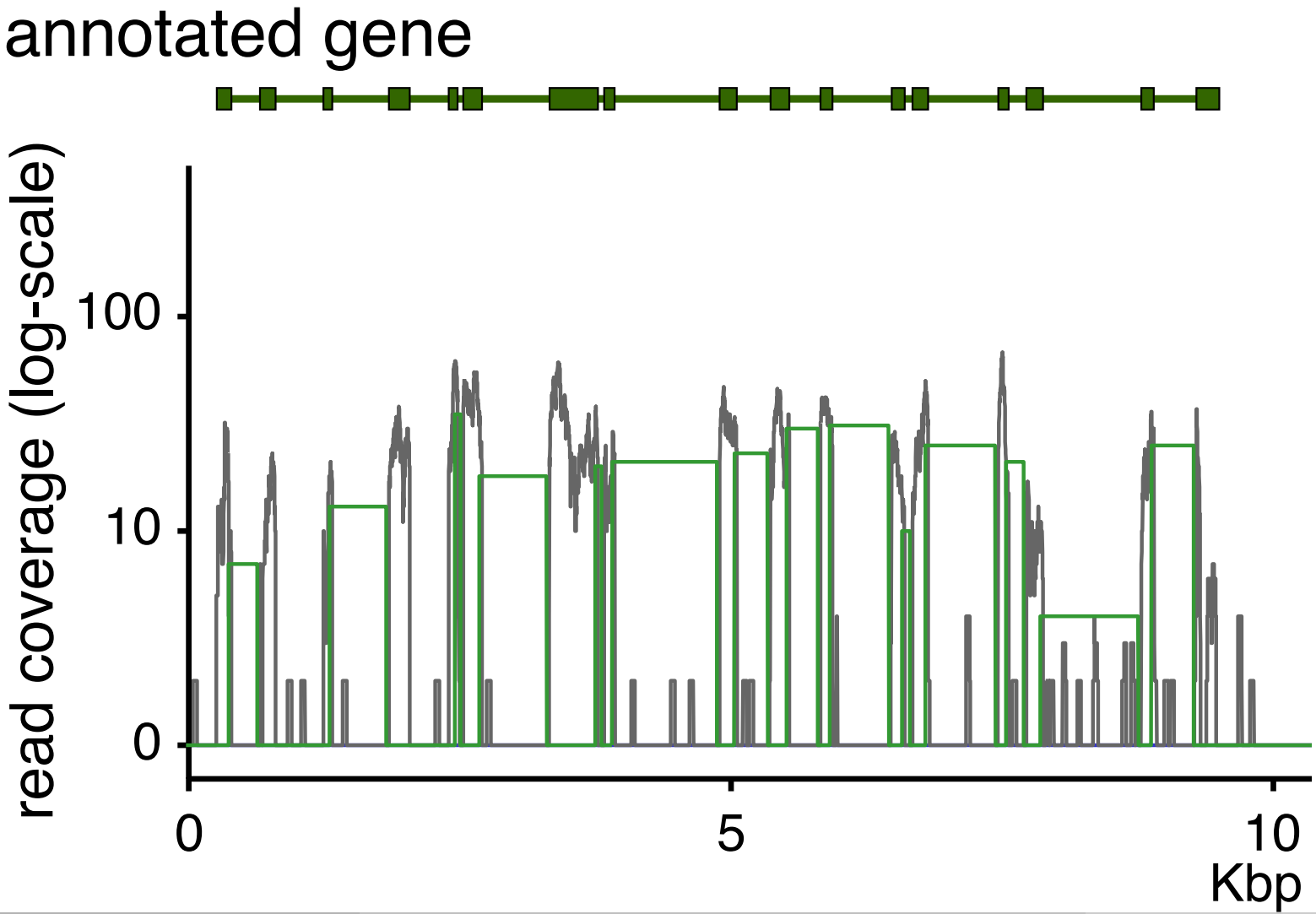
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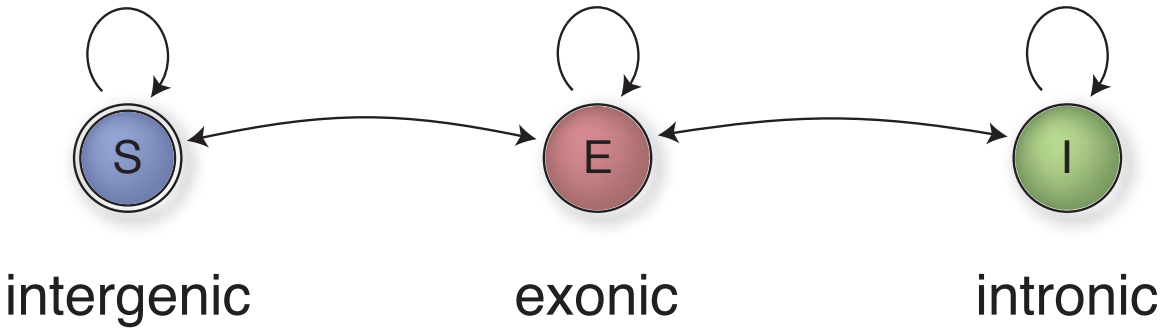


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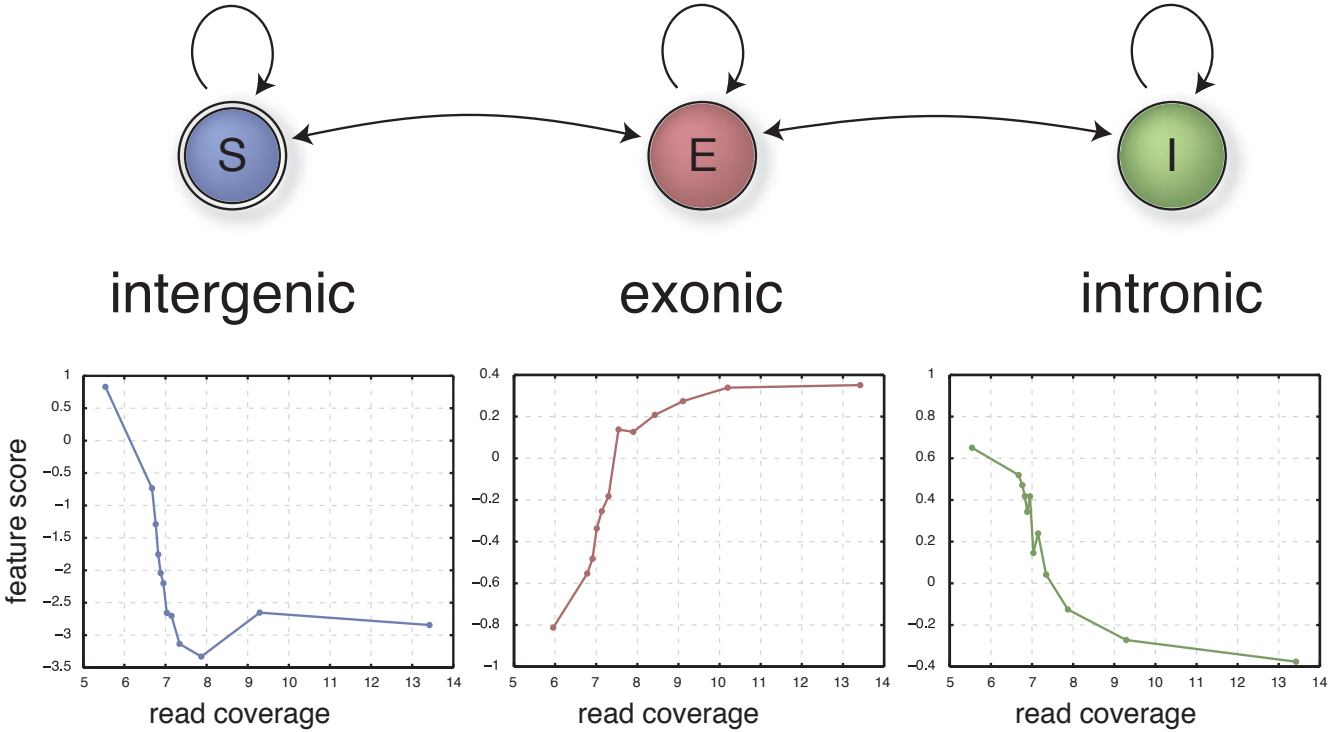
The mTiM Segmentation Approach



- Learn to associate a state with each position given its read coverage and local context
- HM-SVM training: Optimize transformations: signal → score
- Extension: Score spliced reads and splice sites

(G. Zeller et al., 2008; G. Zeller et al., in prep., 2009)

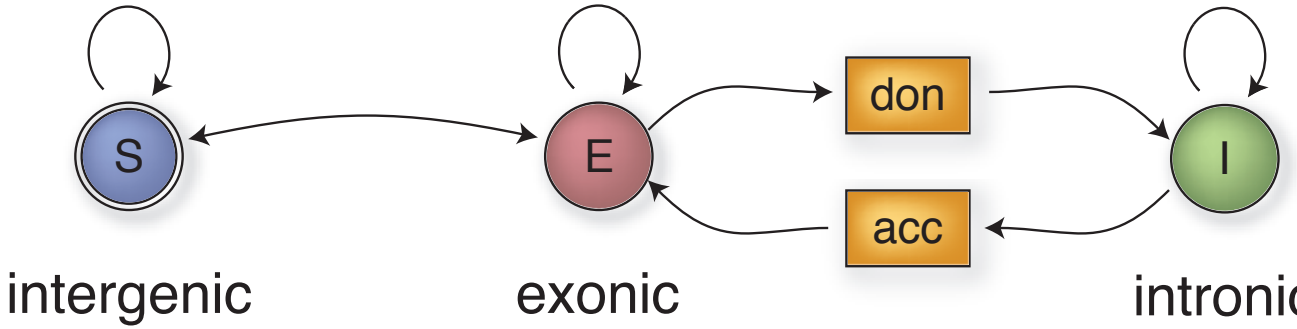
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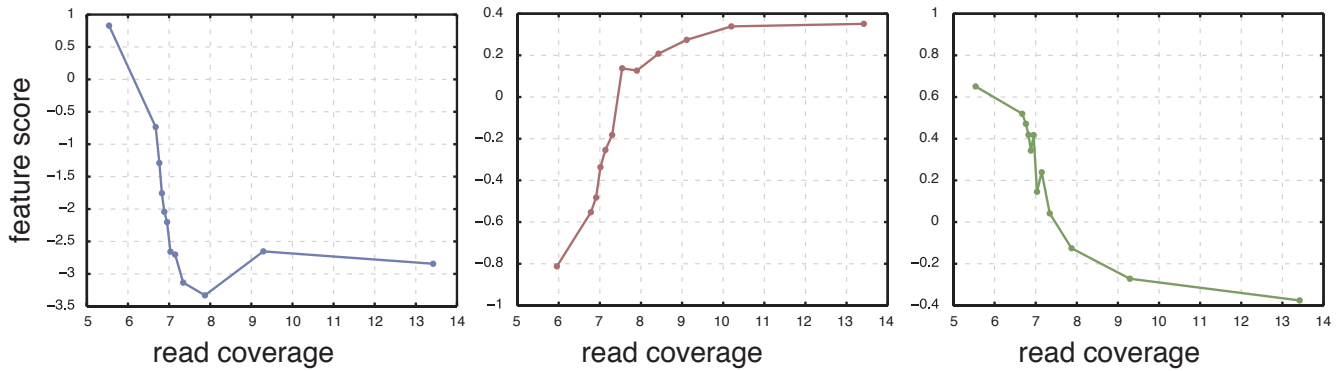


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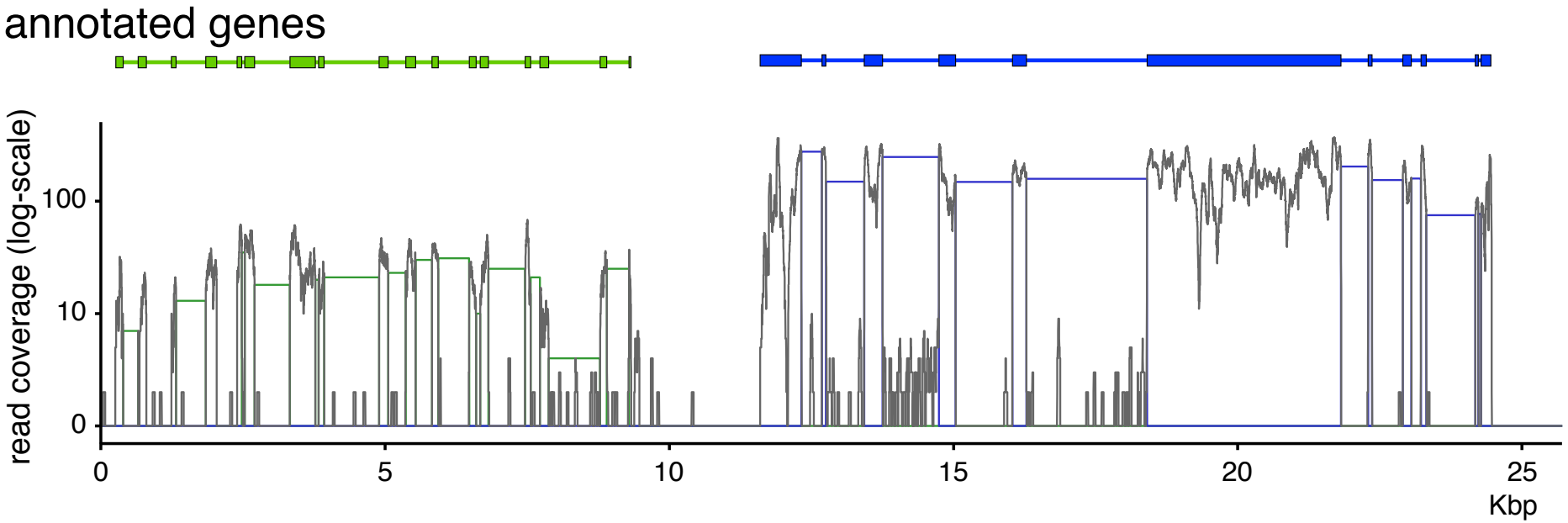


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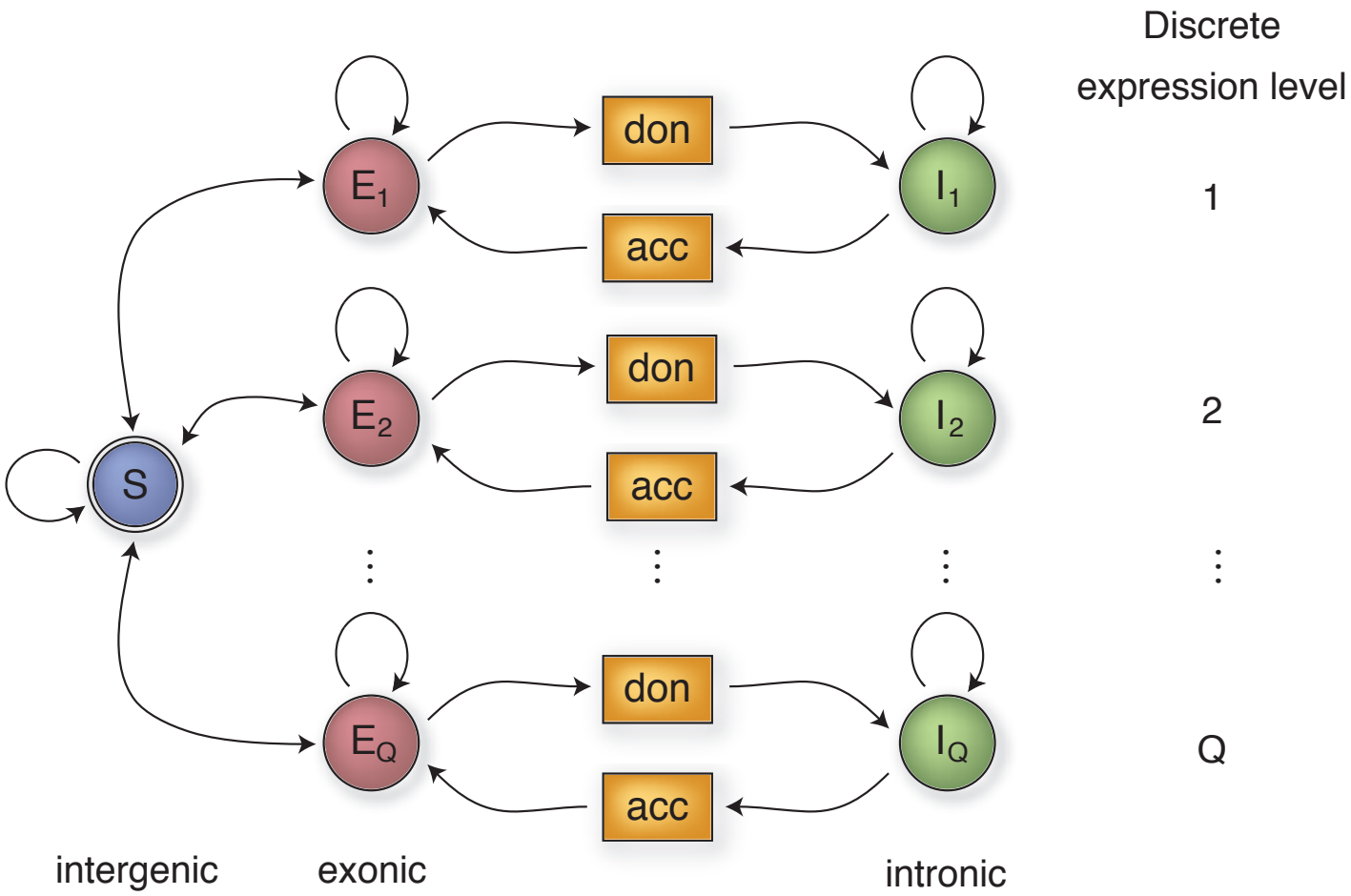
(G. Zeller et al., 2008; G. Zeller et al., in prep., 2009)

The mTiM Segmentation Approach

Idea: Assume uniform read coverage within exons of same transcript



The mTiM Segmentation Approach



Carry "expression level" information between exons of same transcript

(G. Zeller et al., 2008; G. Zeller et al., in prep., 2010)

Discriminative training of HM-SVMs



$f : \mathbb{R}^\star \rightarrow \Sigma^\star$

given a sequence of hybridization measurements $\boldsymbol{\chi} \in \mathbb{R}^\star$
predicts a state sequence (path) $\boldsymbol{\sigma} \in \Sigma^\star$

Discriminant function $F_{\boldsymbol{\theta}} : \mathbb{R}^\star \times \Sigma^\star \rightarrow \mathbb{R}$ such that for decoding: $f(\boldsymbol{\chi}) = \underset{\boldsymbol{\sigma} \in \mathcal{S}^\star}{\operatorname{argmax}} F_{\boldsymbol{\theta}}(\boldsymbol{\chi}, \boldsymbol{\sigma})$.

Training:
For each training example $(\boldsymbol{\chi}^{(i)}, \boldsymbol{\sigma}^{(i)})$, enforce a large margin of separation

$$F_\theta(\boldsymbol{\chi}^{(i)}, \boldsymbol{\sigma}^{(i)}) - F_\theta(\boldsymbol{\chi}^{(i)}, \overline{\boldsymbol{\sigma}}) \geq \rho$$

between the correct path $\boldsymbol{\sigma}^{(i)}$ and *any* other wrong path $\overline{\boldsymbol{\sigma}} \neq \boldsymbol{\sigma}^{(i)}$.

A quadratic programming problem (QP) is solved to optimize θ.

[???]

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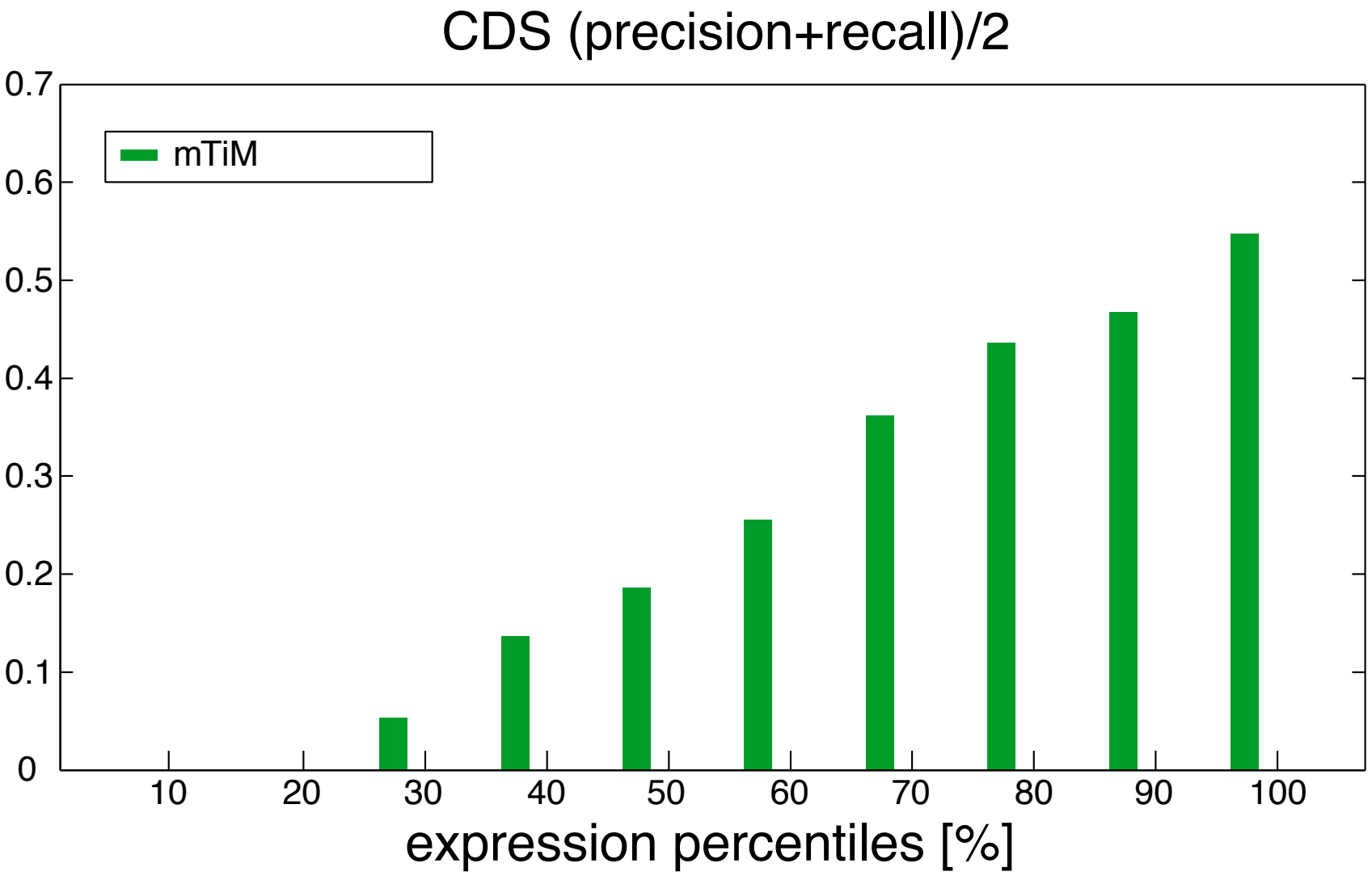
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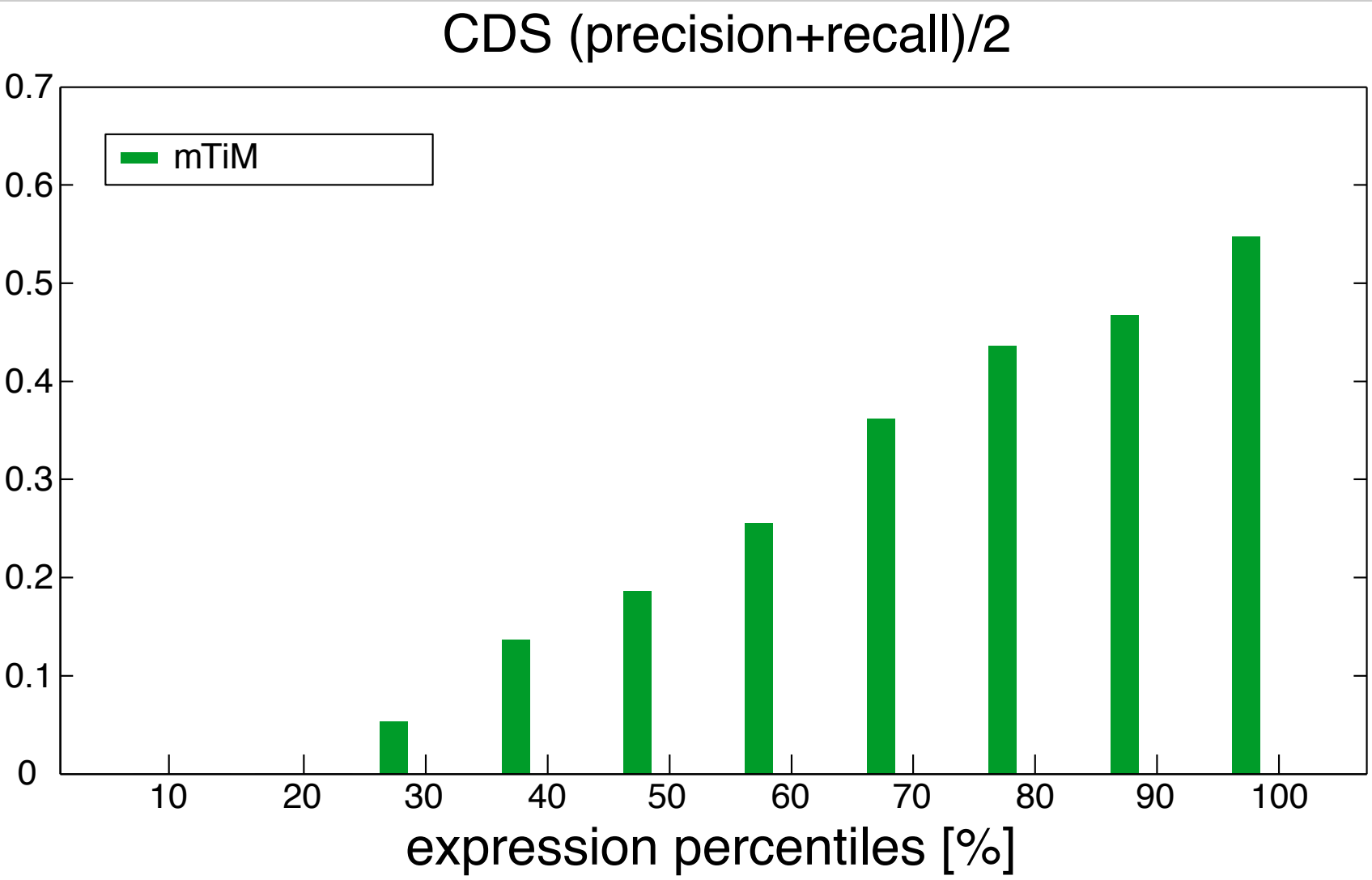
[???]

Preliminary Evaluation (*C. elegans*)



Sensitivity heavily depends on read density

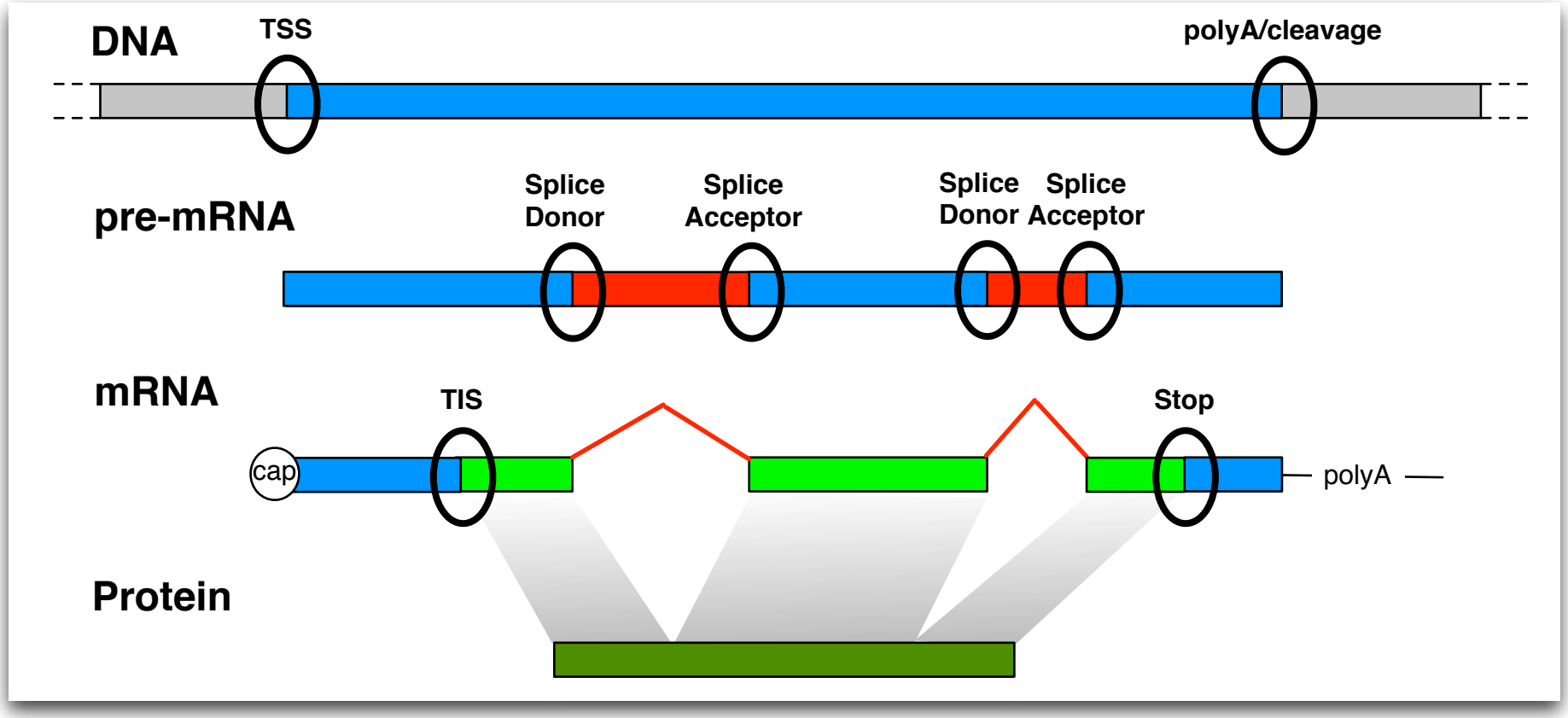
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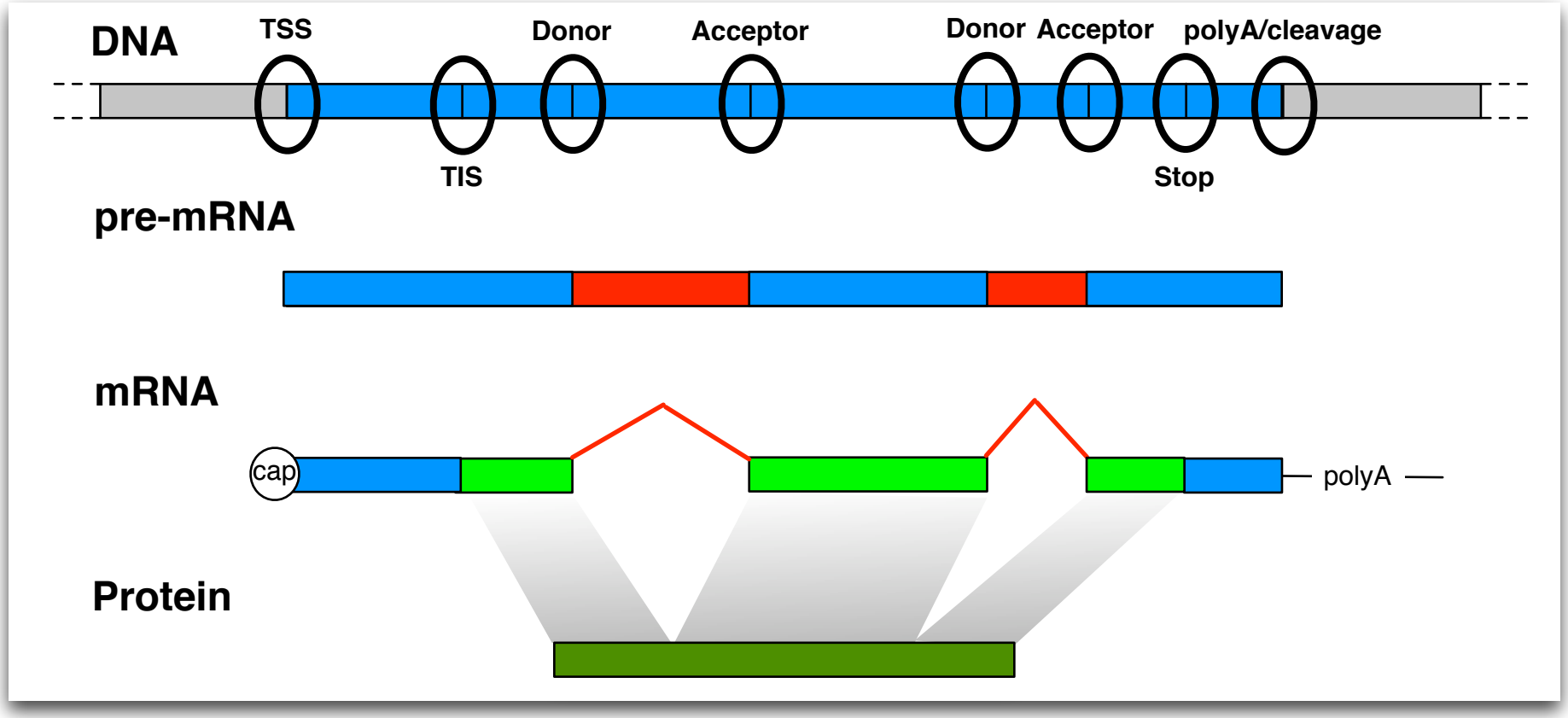
Computational Gene Finding

⇝ Labeling the Genome



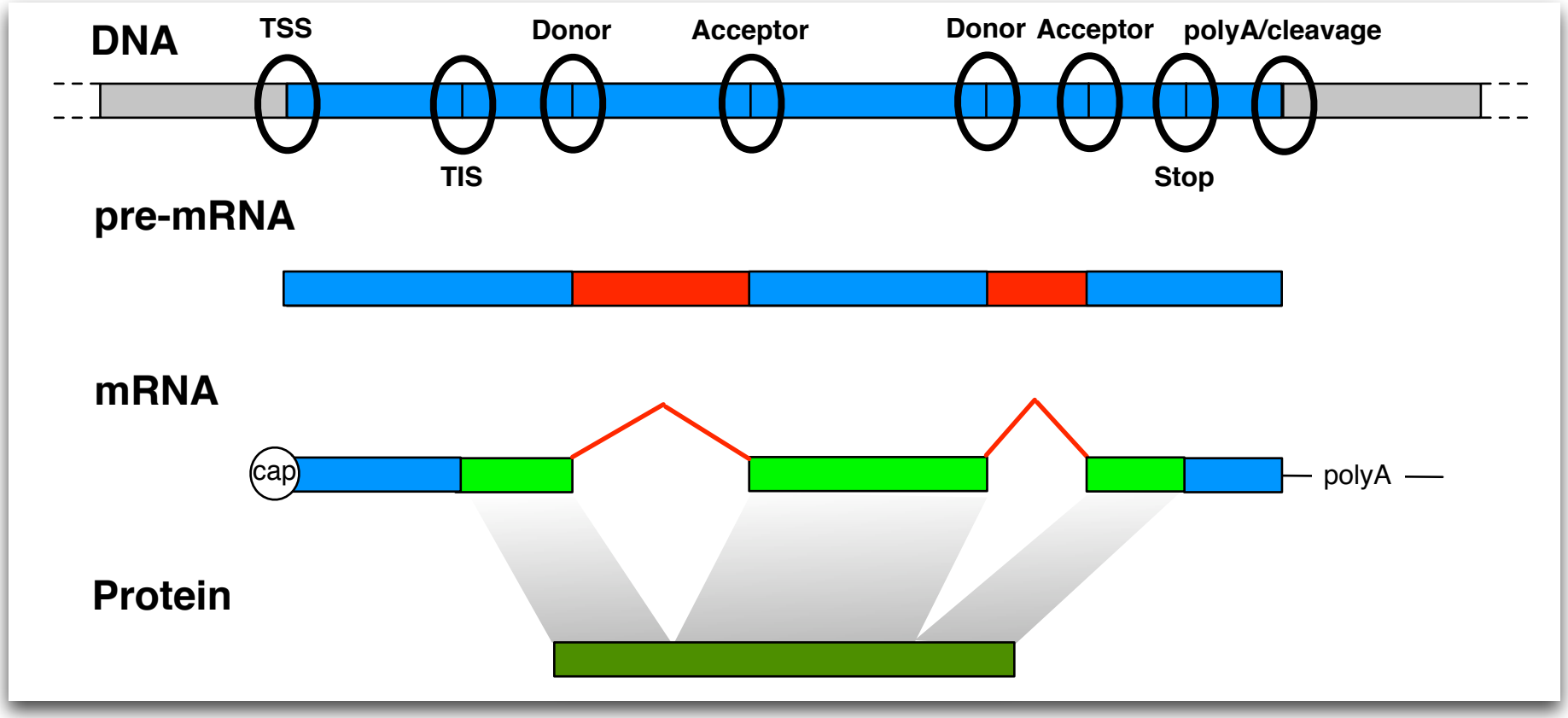
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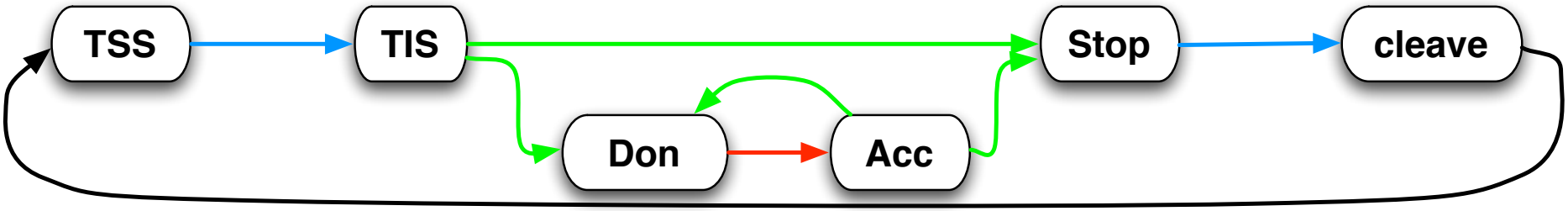
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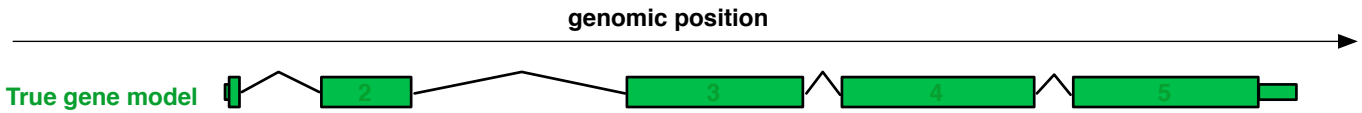
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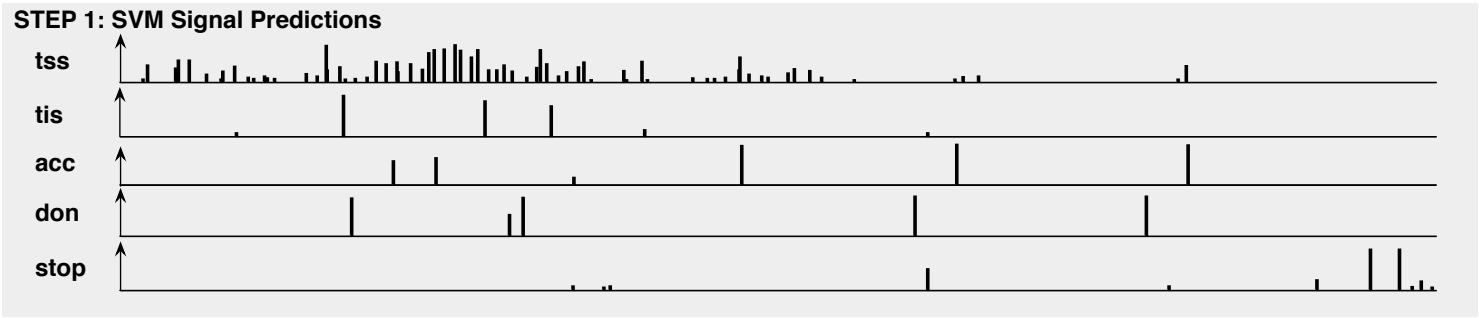
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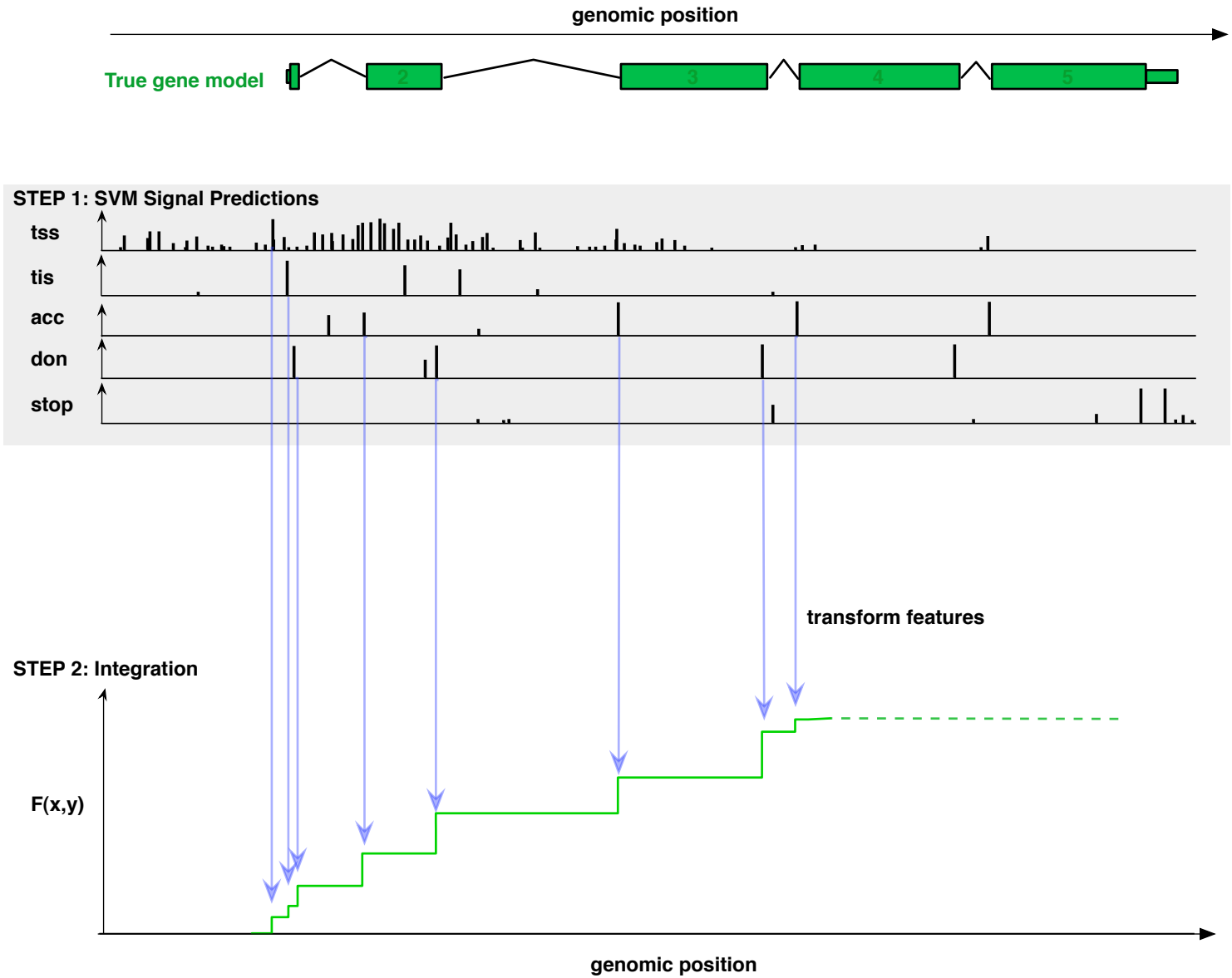
mGene-based Transcript Prediction



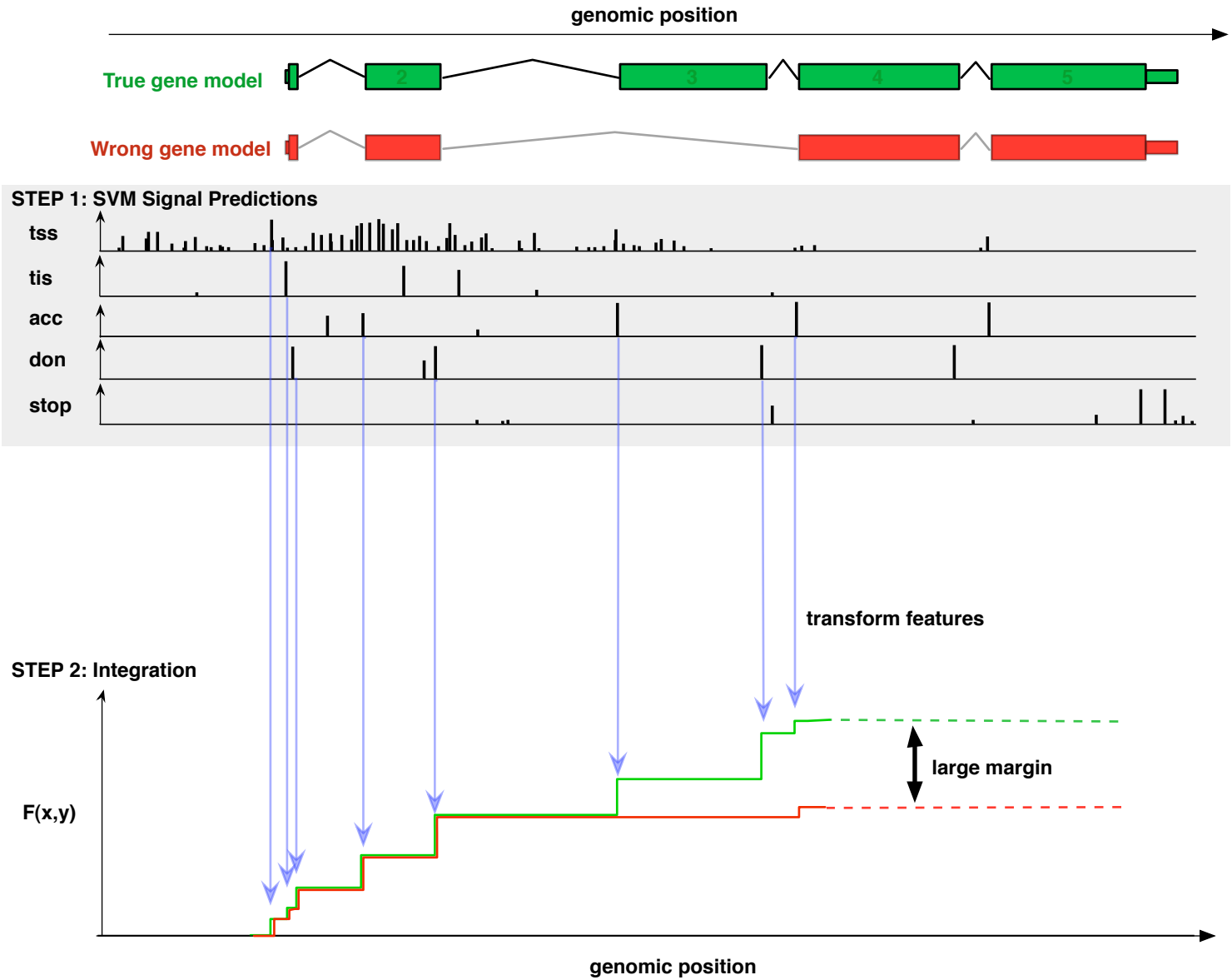




mGene-based Transcript Prediction



mGene-based Transcript Prediction



Learning to use Expression Measurements

Two approaches:

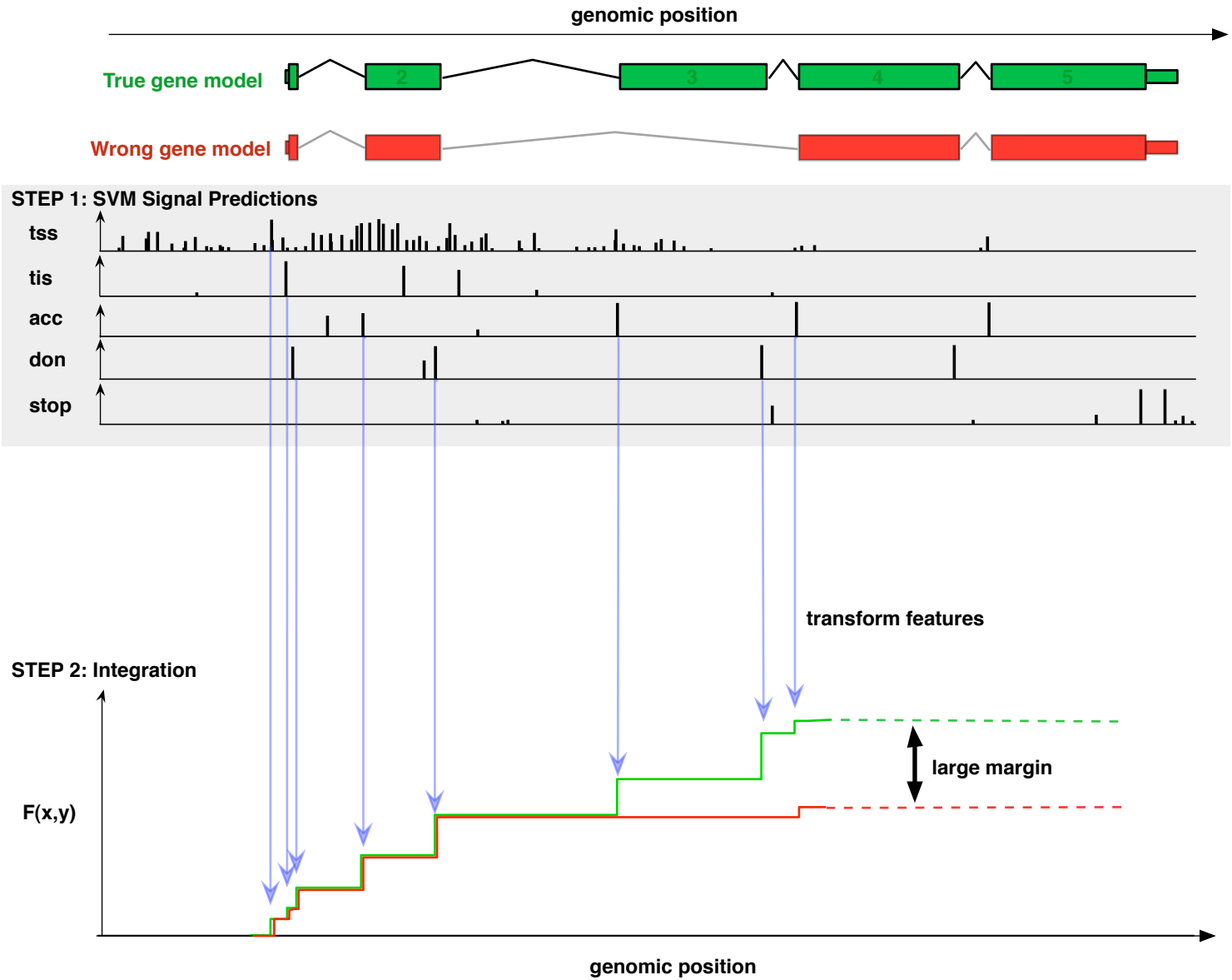
- Heuristic to incorporate ESTs/reads/tiling array measurements to *refine predictions*
- Directly *use evidence during learning* to learn to appropriately weight its importance

	Exon Level			Transcript Level		
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ab initio	82.3	82.6	82.5	43.1	49.5	46.1
ESTs heuristic	85.3	84.7	85.0	49.5	56.4	52.7
ESTs trained	84.8	85.8	**85.3**	50.5	57.8	**53.9**

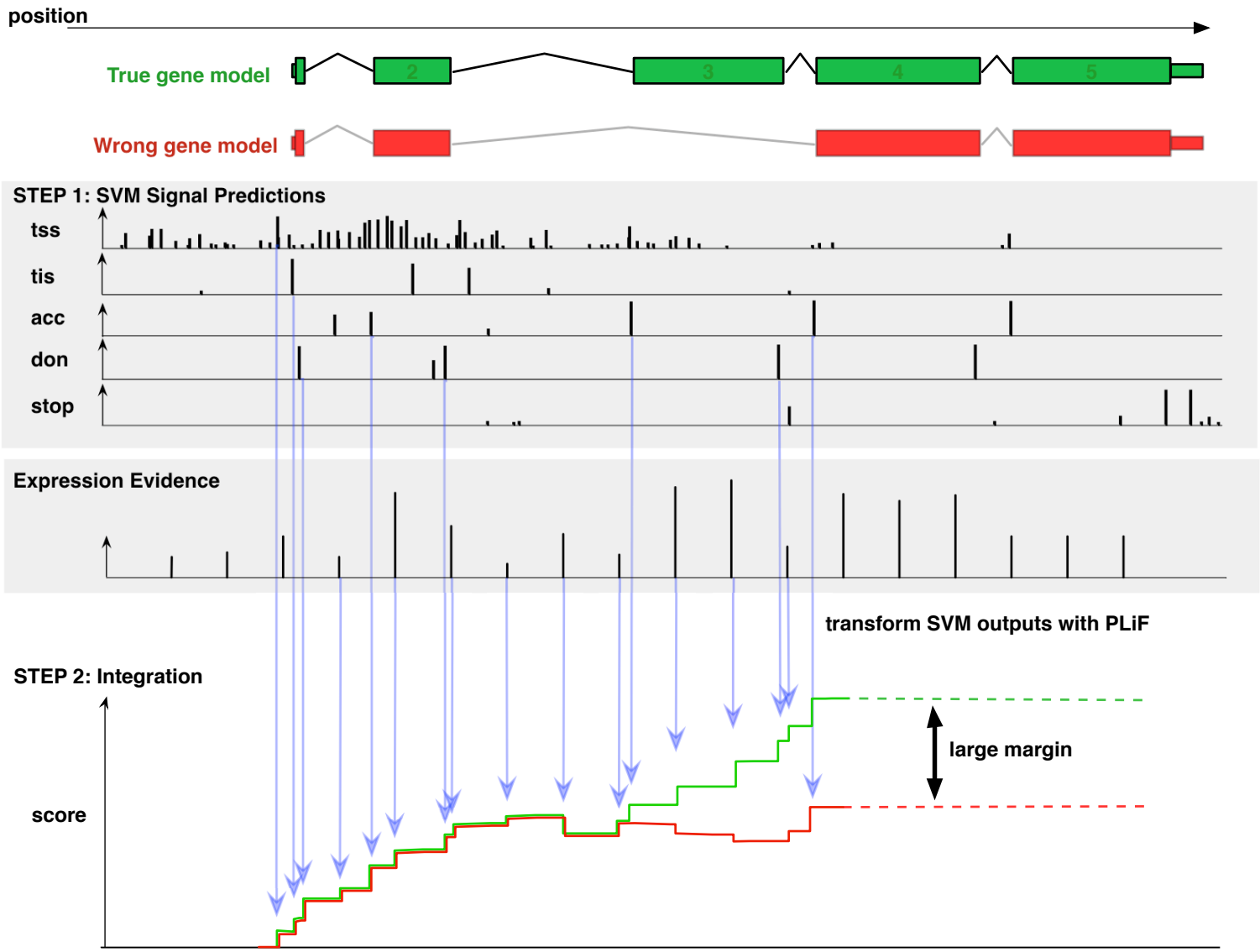
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Behr et al., in pre., 2010

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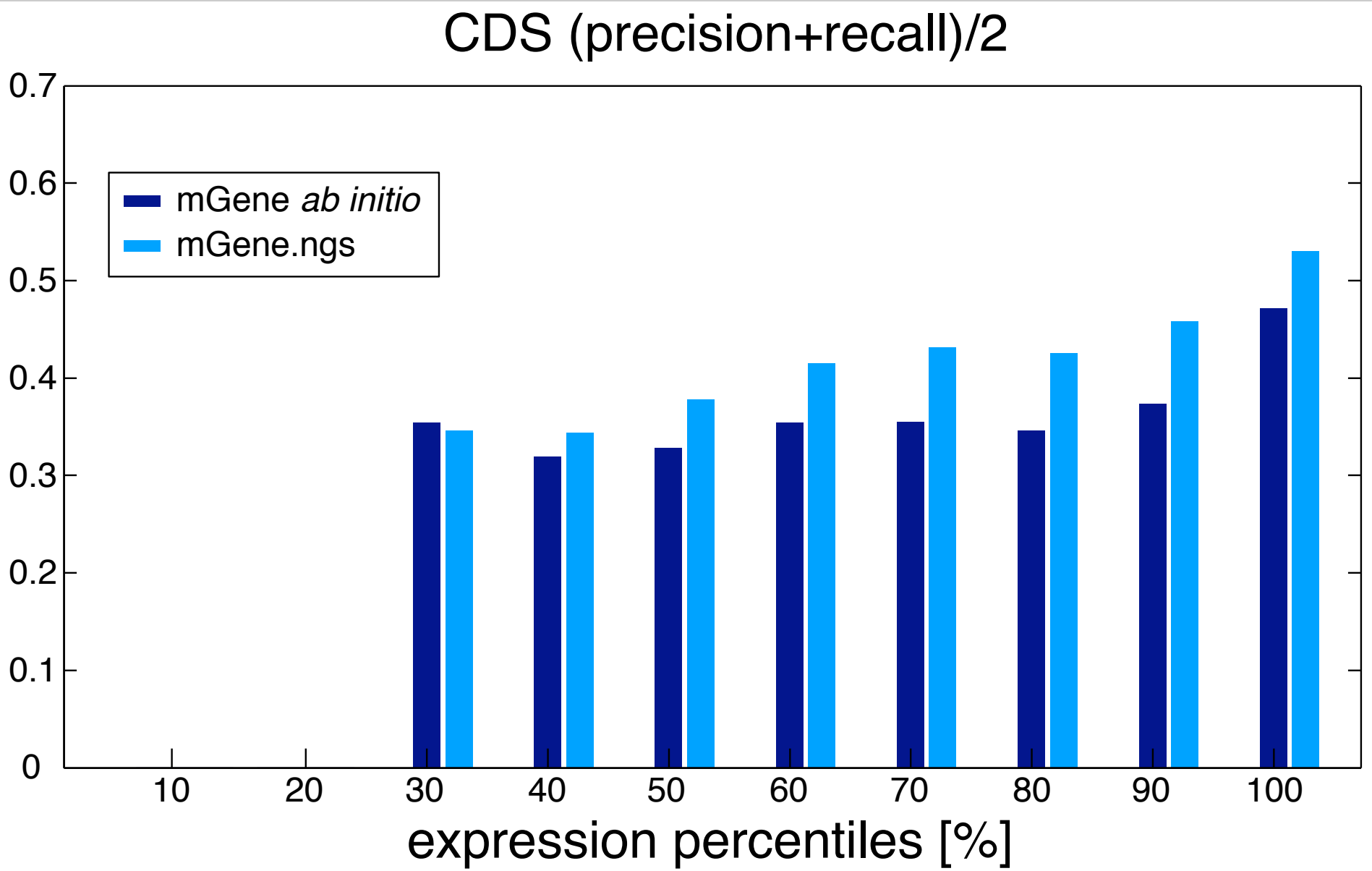
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RNA-Seq/ESTs trained	84.7	**86.9**	85.8	50.3	60.5	**54.9**

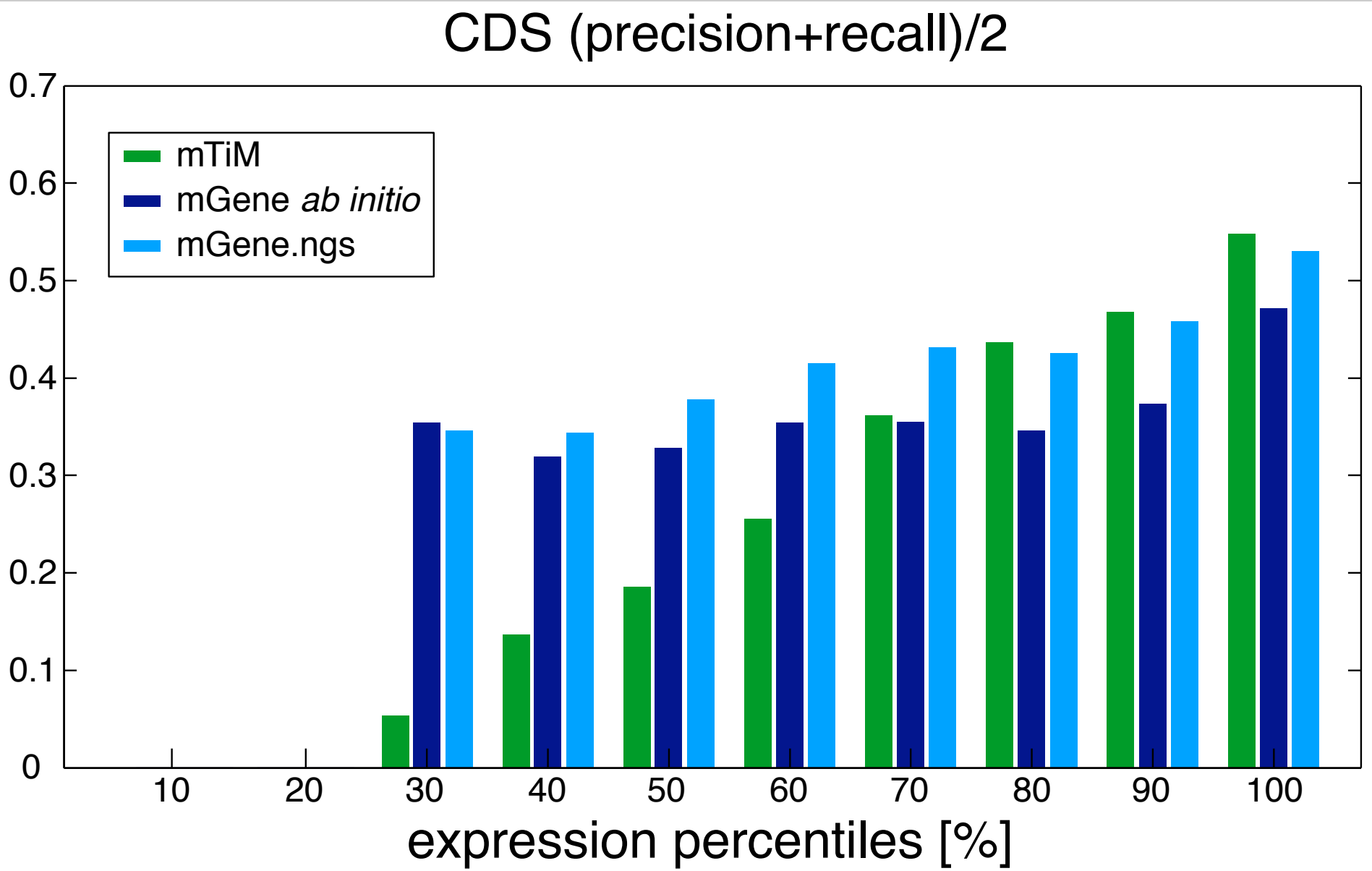
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Digestion

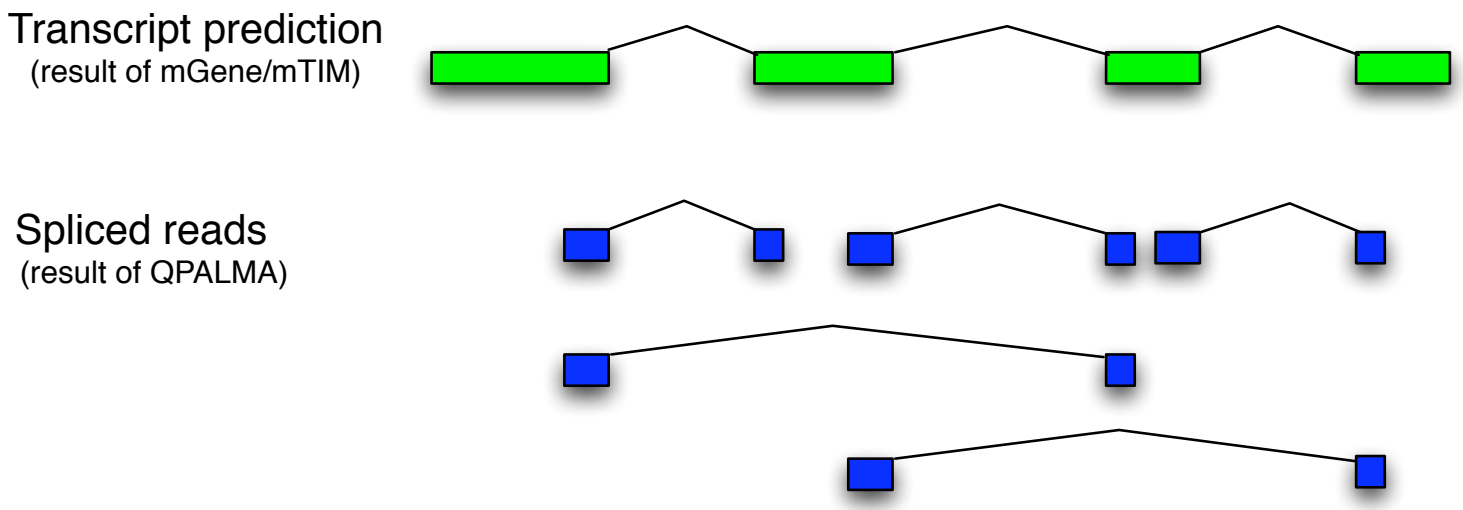
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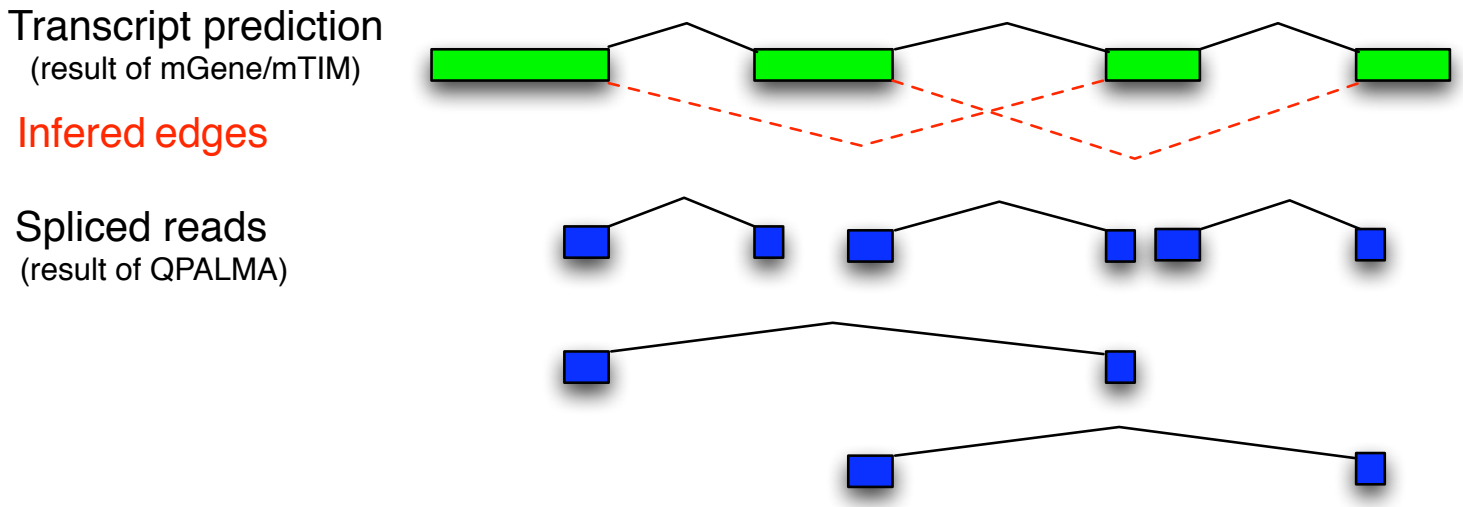
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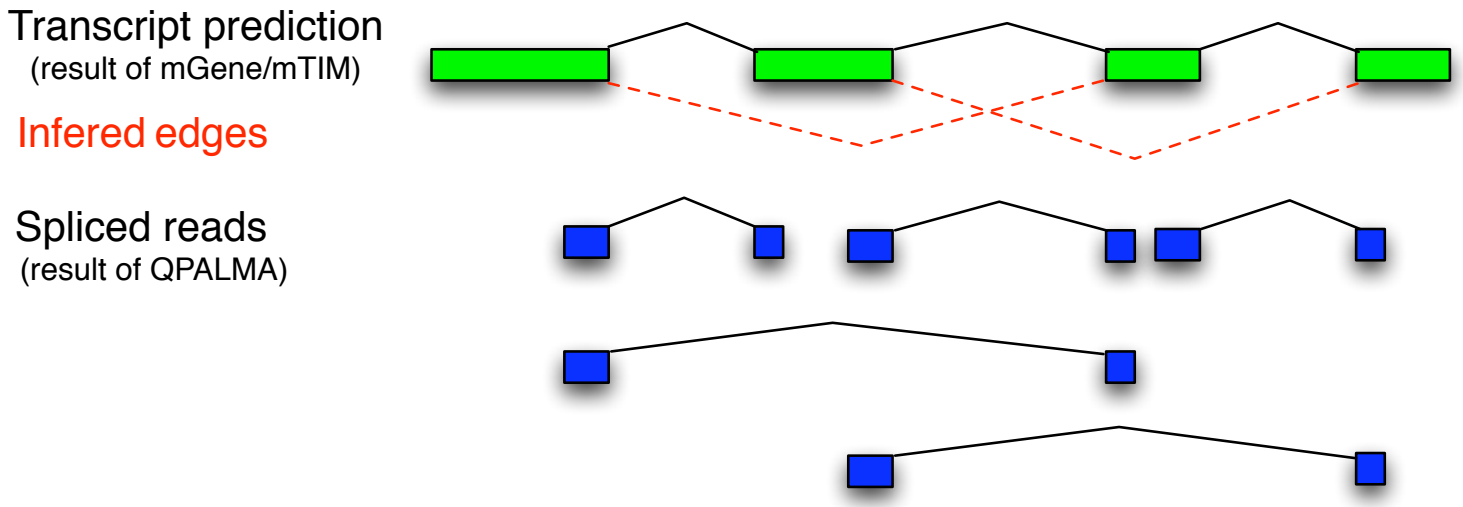
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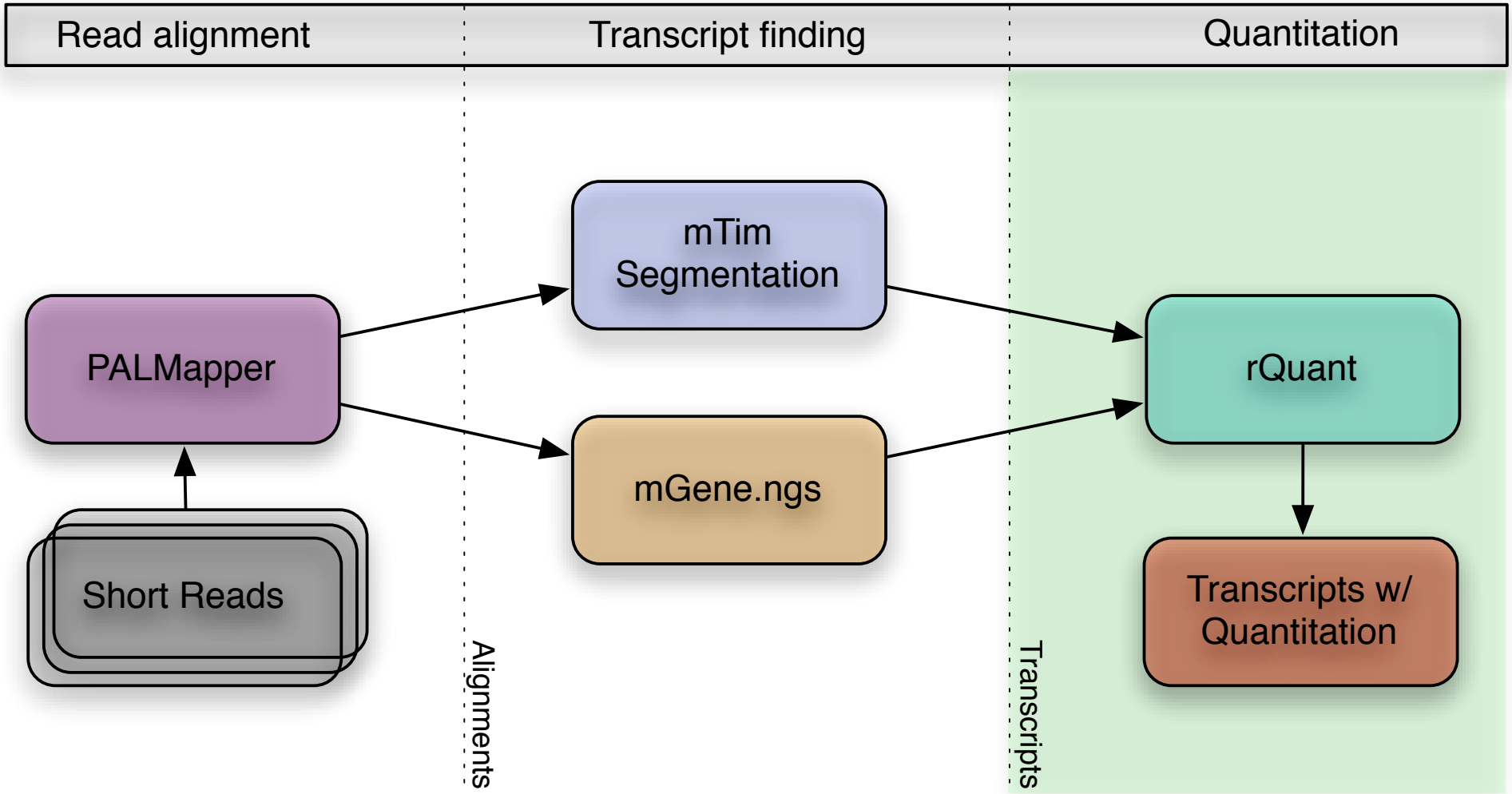
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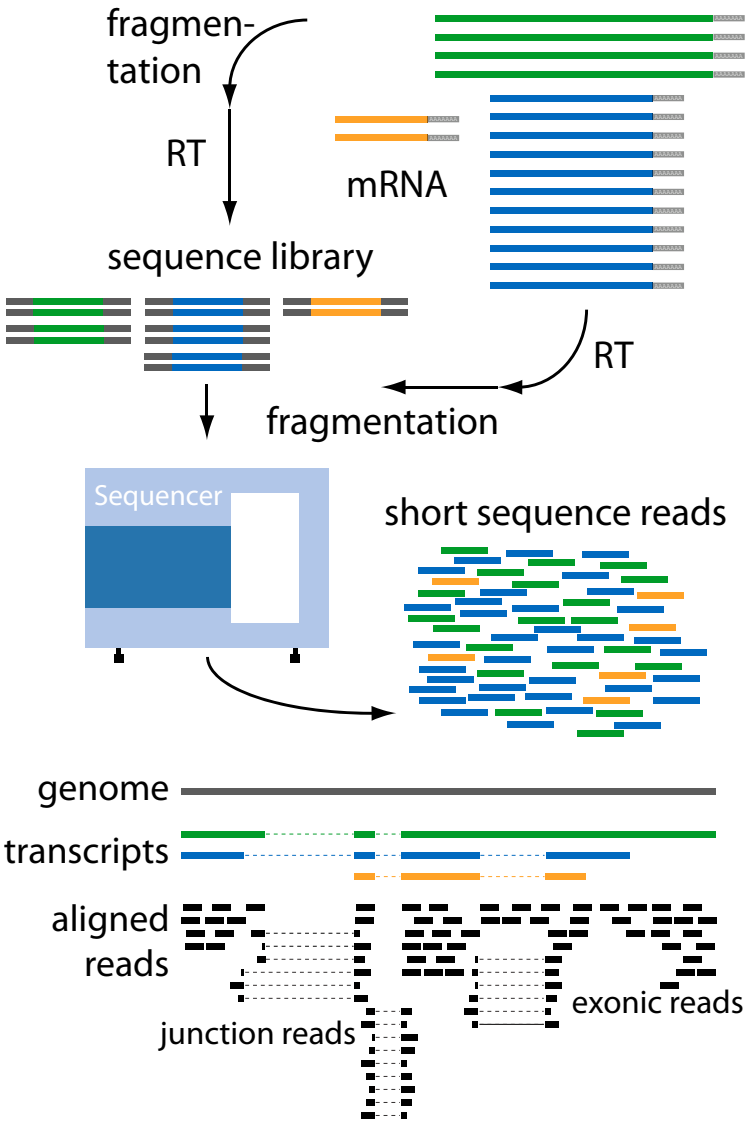


- Paths through splicing graph define *alternative transcripts*

RNA-Seq Pipeline Overview

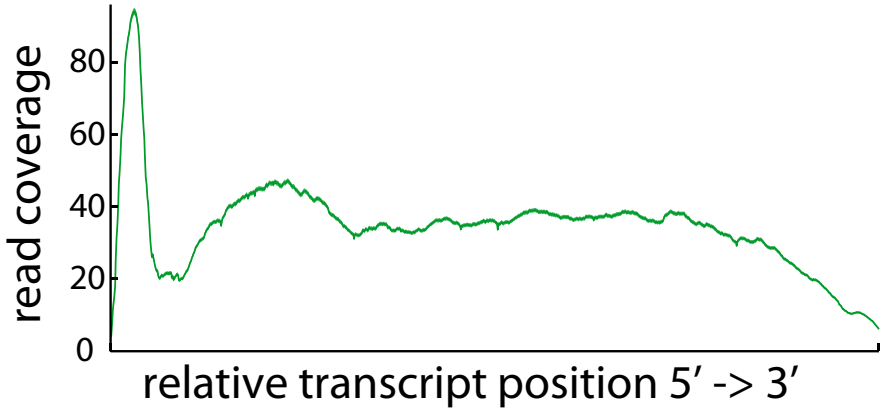


RNA-Seq Biases and Quantitation



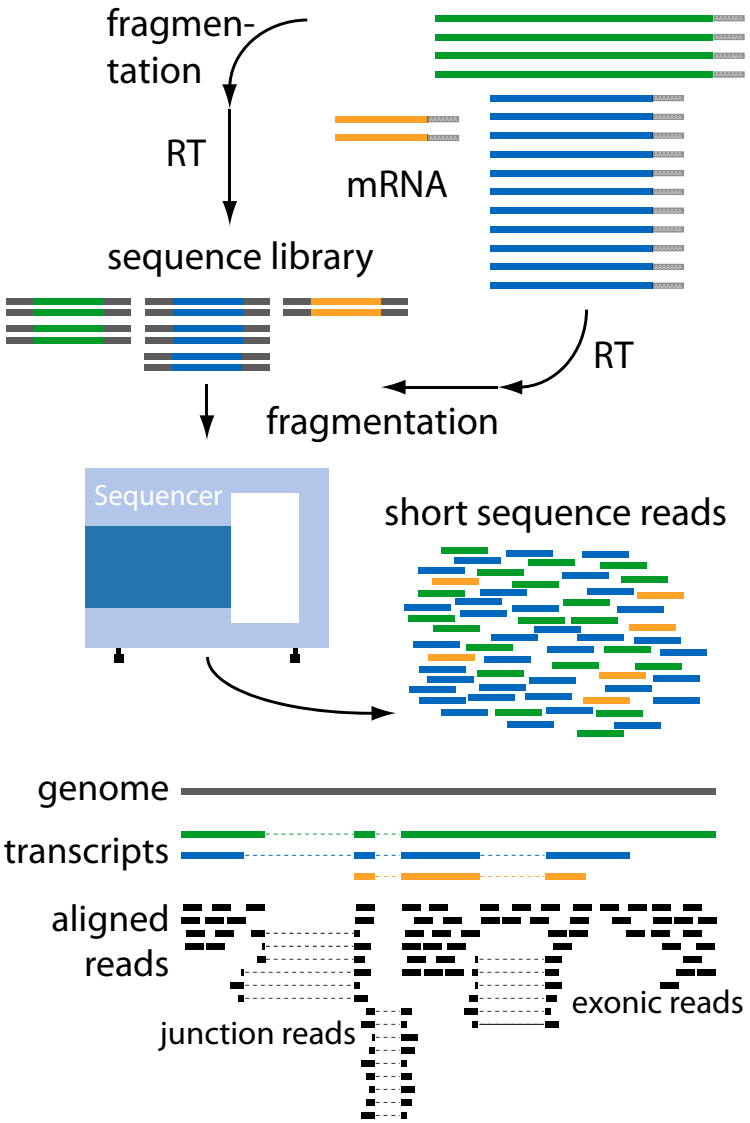
Biases due to ...

- cDNA library construction
- Sequencing
- Read mapping



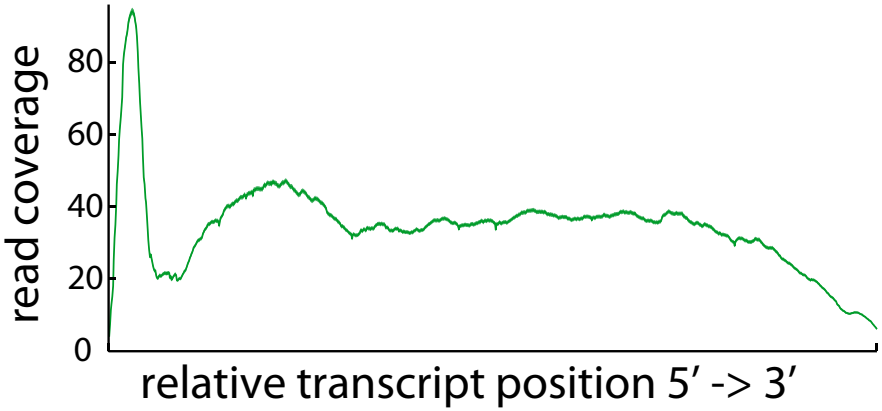
(average over annotated transcripts of length ≈1kb for the *C. elegans* SRX001872 dataset)

RNA-Seq Biases and Quantitation



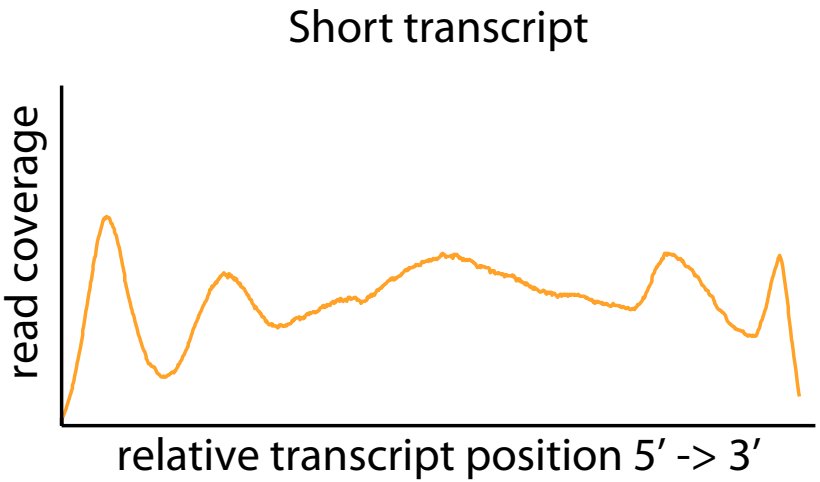
Biases due to ...

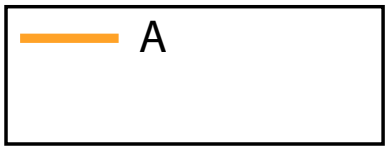
- cDNA library construction
- Sequencing
- Read mapping



(average over annotated transcripts of length ≈1kb for the *C. elegans* SRX001872 dataset)

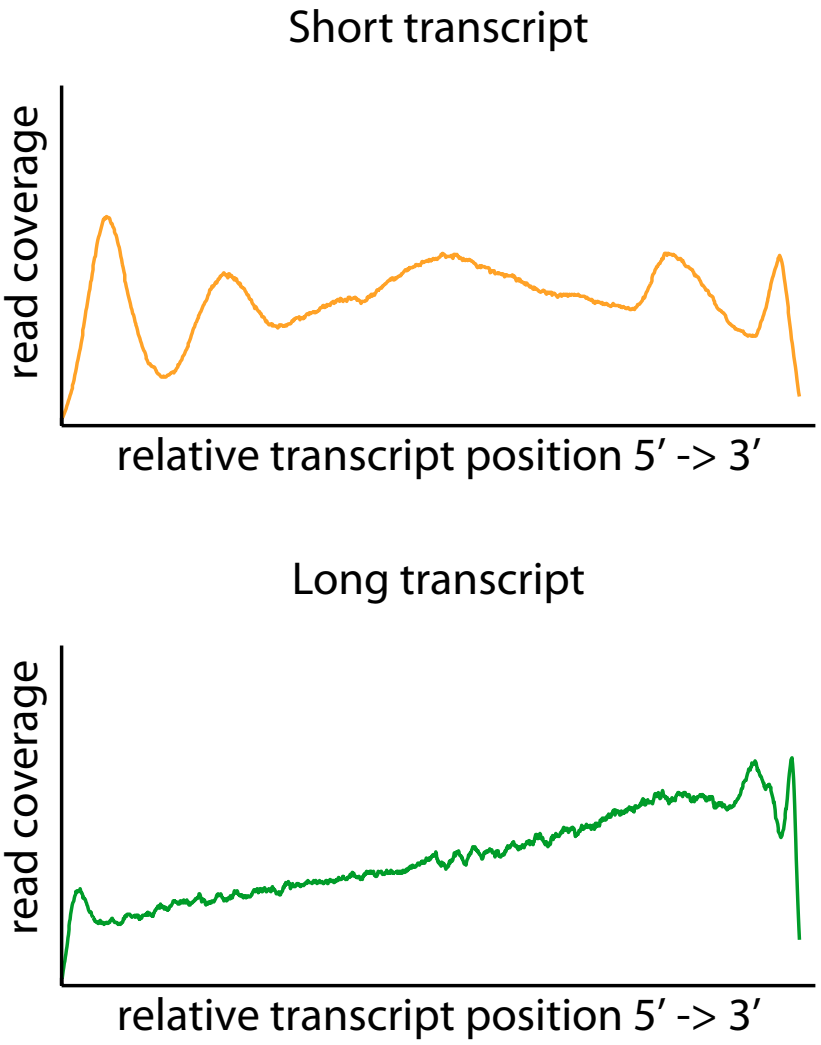
rQuant – Basic Idea





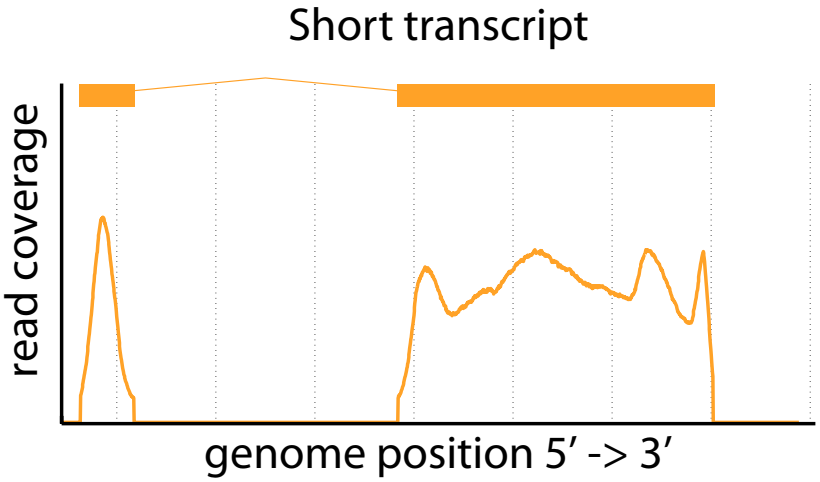
$$M_i = w_A A_i + w_B B_i \quad \Rightarrow \quad \min_{w_A, w_B} \sum_i \ell(M_i, R_i)$$

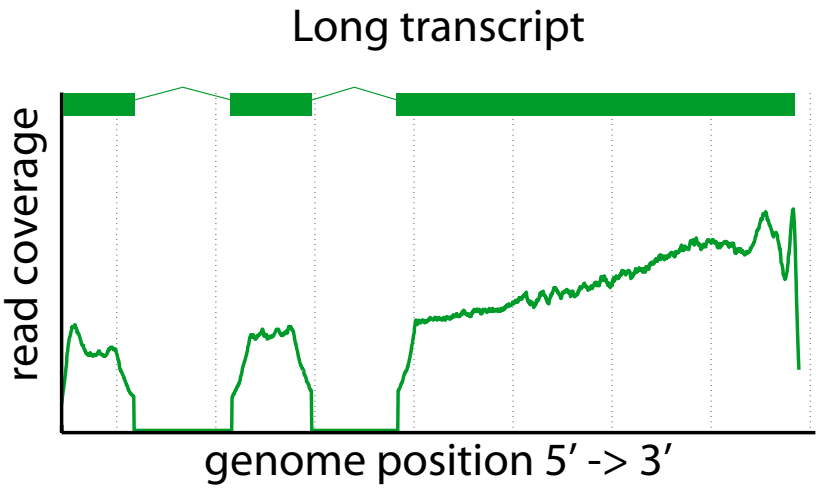
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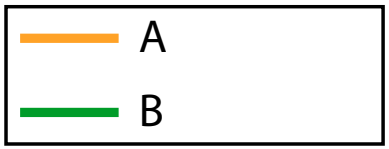


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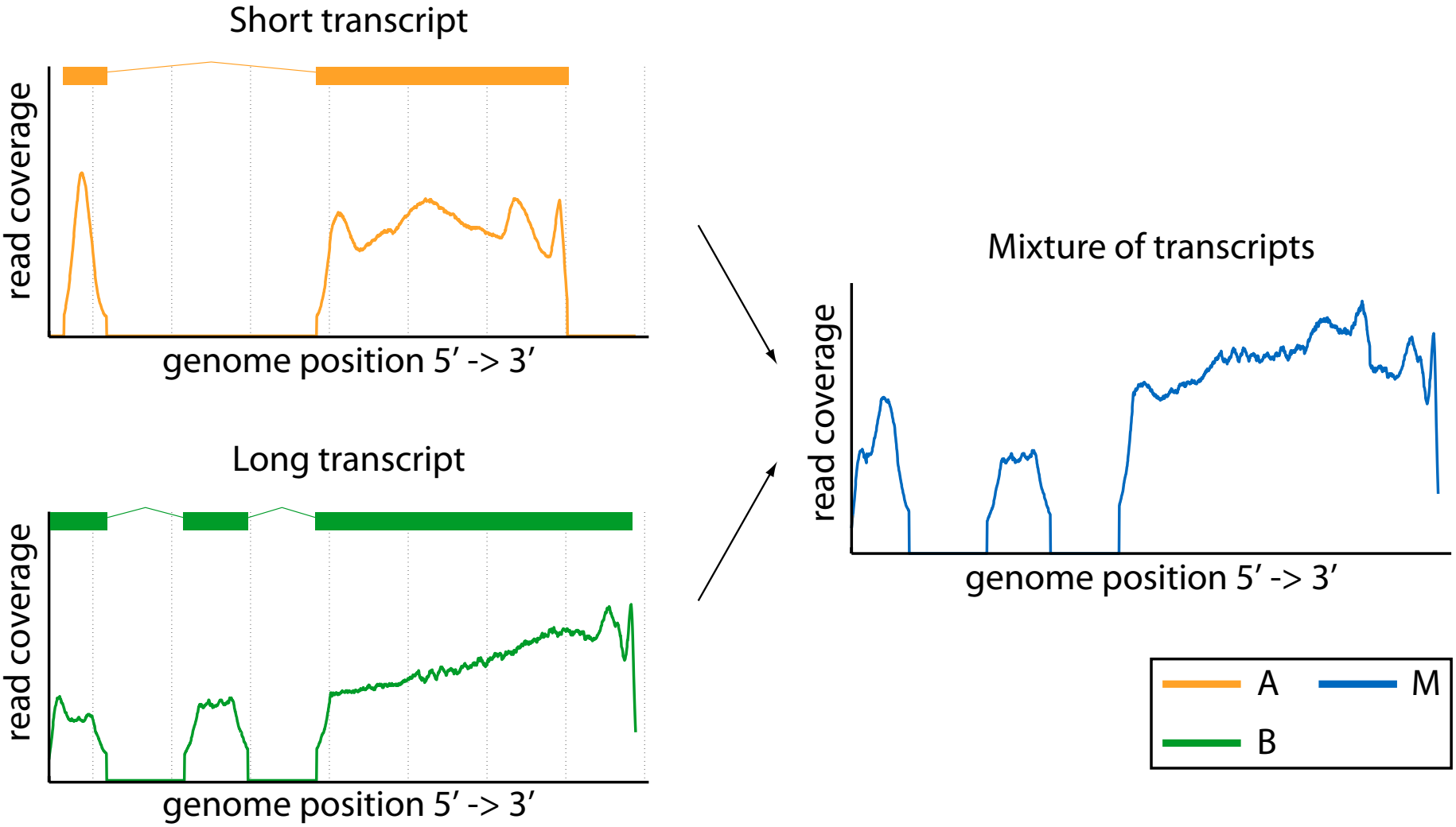






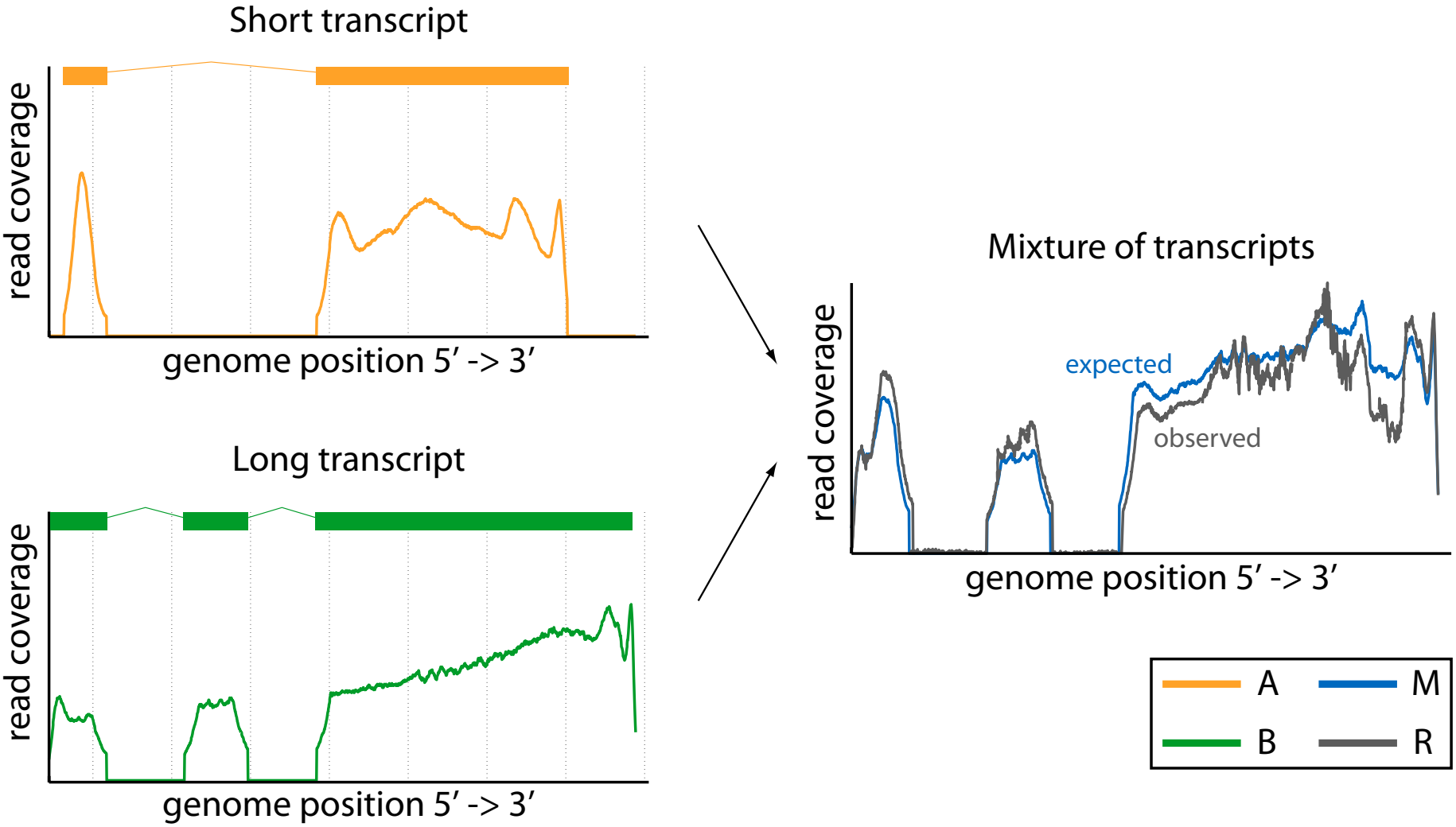
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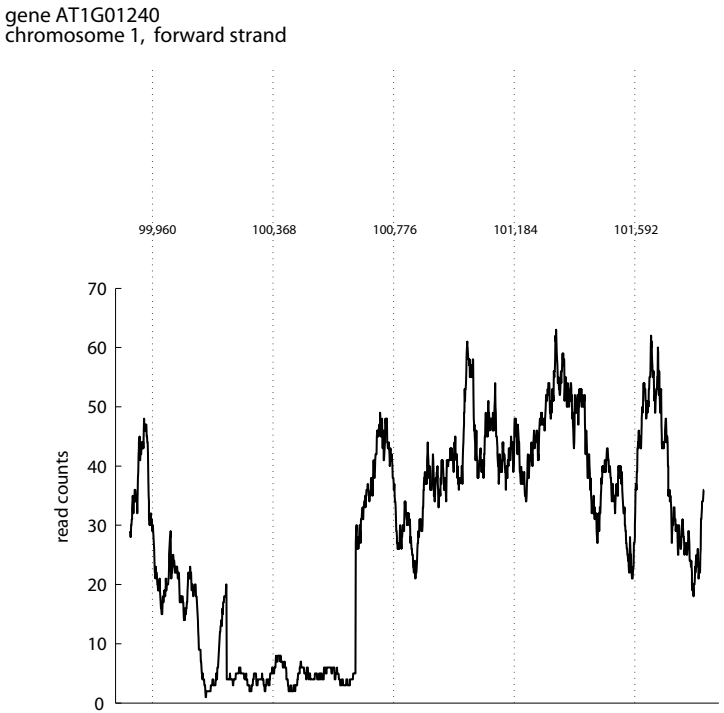
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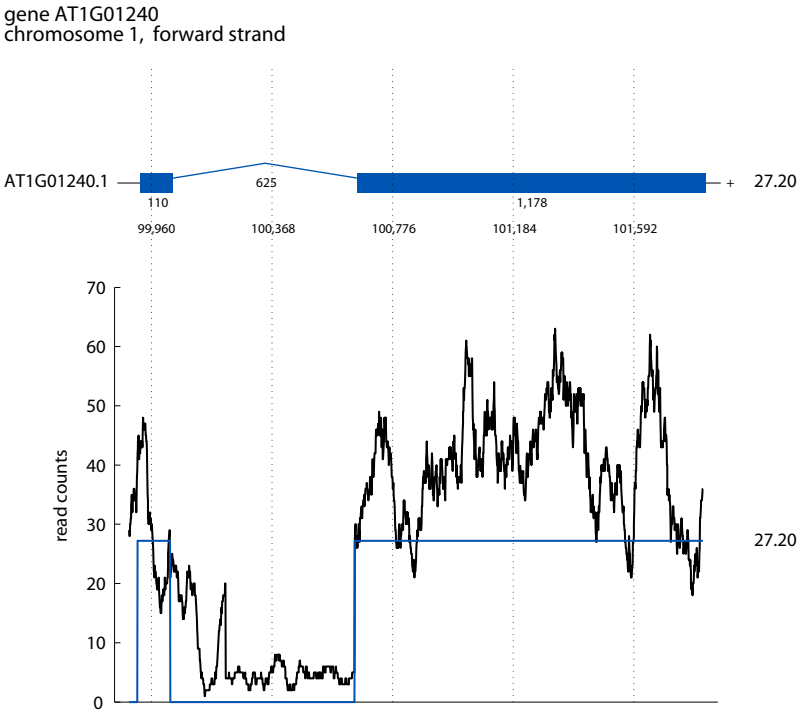
rQuant – Iterative Algorithm

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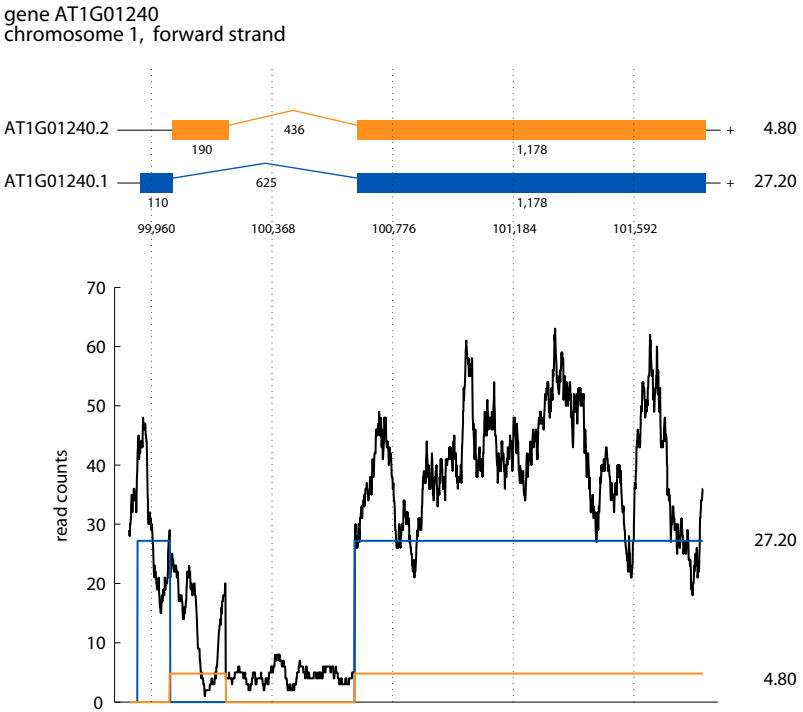
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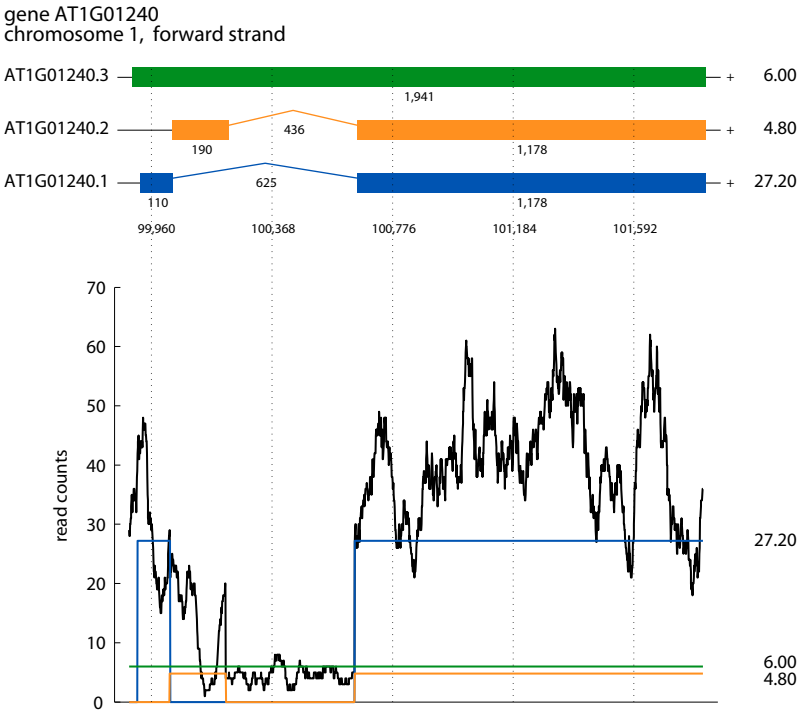
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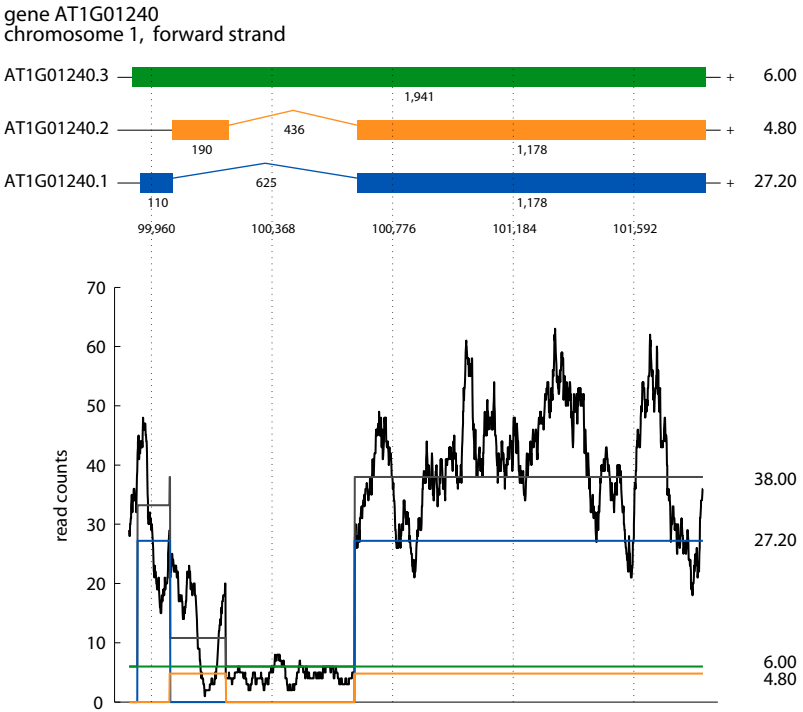
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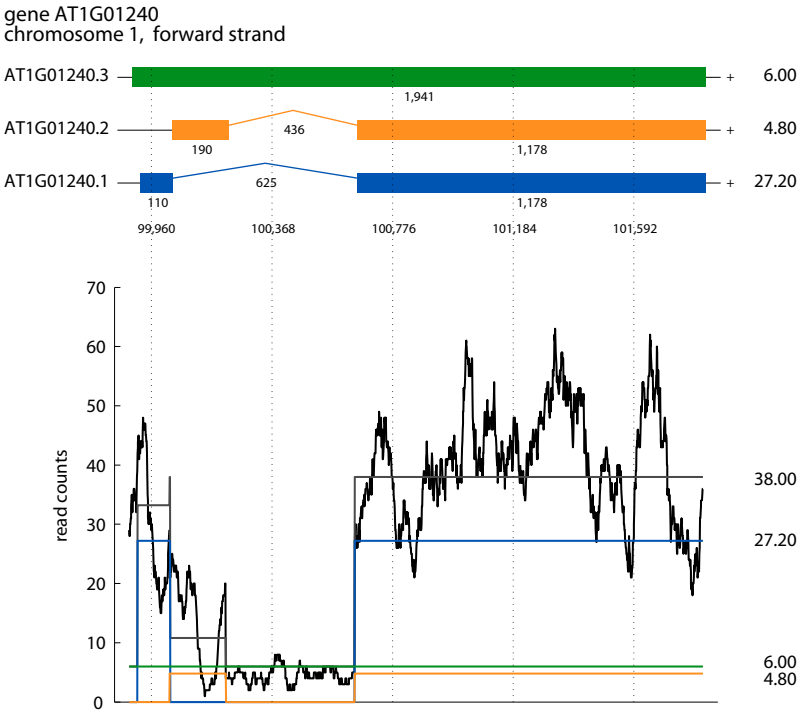
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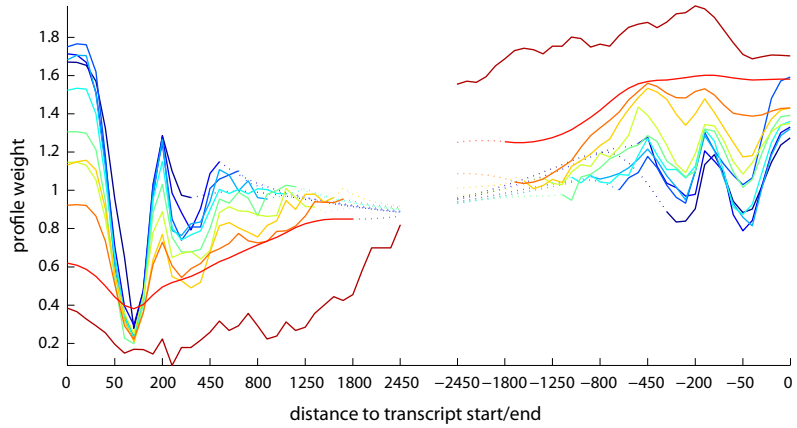
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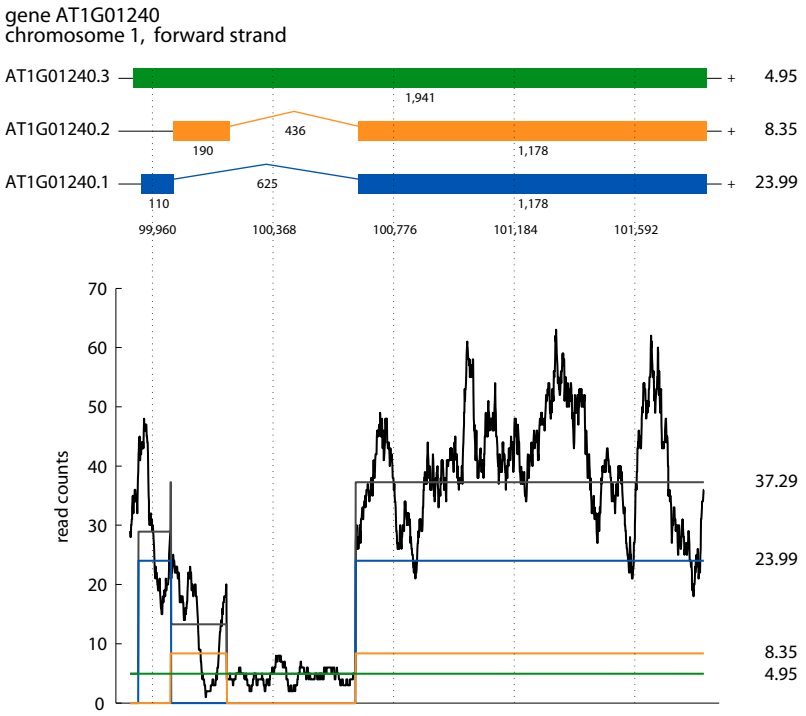
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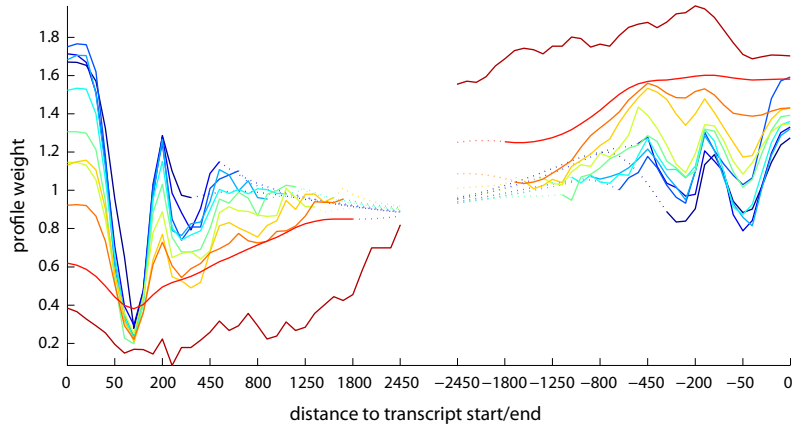




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rQuant Evaluation I



rQuant: Position-wise with profiles (estimating library and mapping bias)

compared to

- Position-wise, without profiles
- Segment-wise, without profiles (e.g., Jiang and Wong [2009])
- Segment-wise, with profiles (e.g. Flux Capacitor [Sammeth, 2009a])

Estimate transcript abundances

- Using simulated data for *A. thaliana* (Flux Simulator [Sammeth, 2009b])
- Subset of alternatively spliced genes

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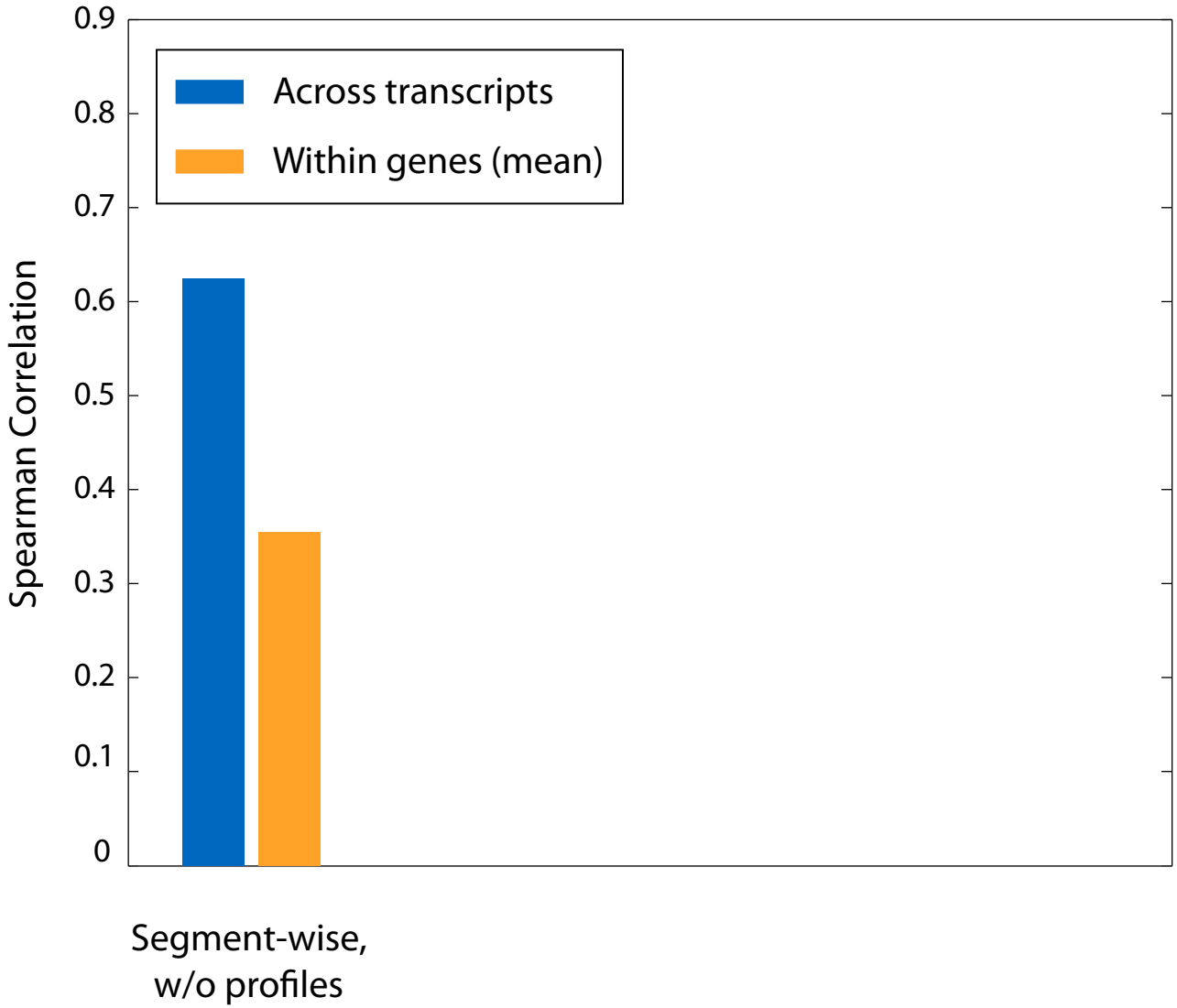
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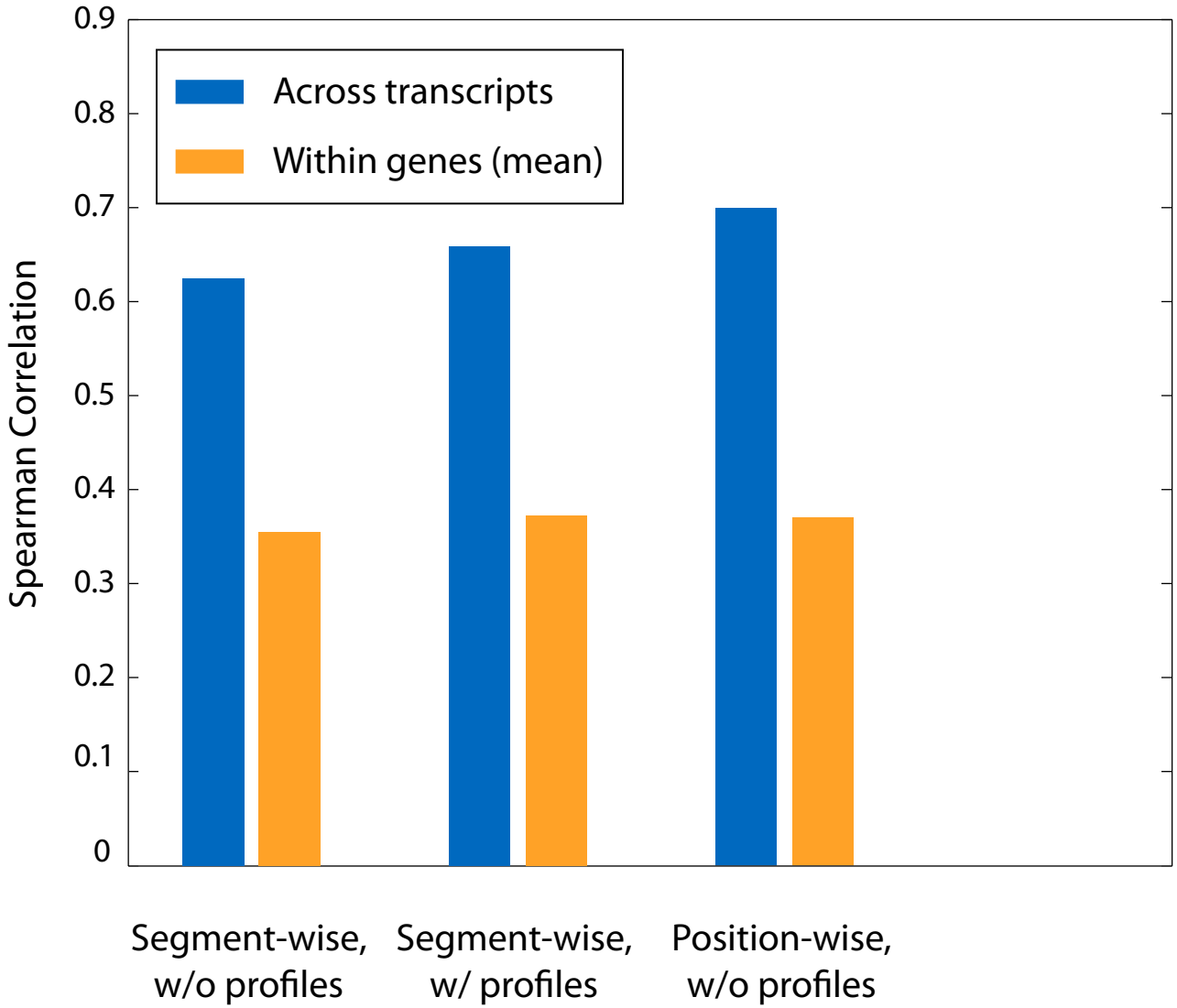
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rQuant Evaluation II



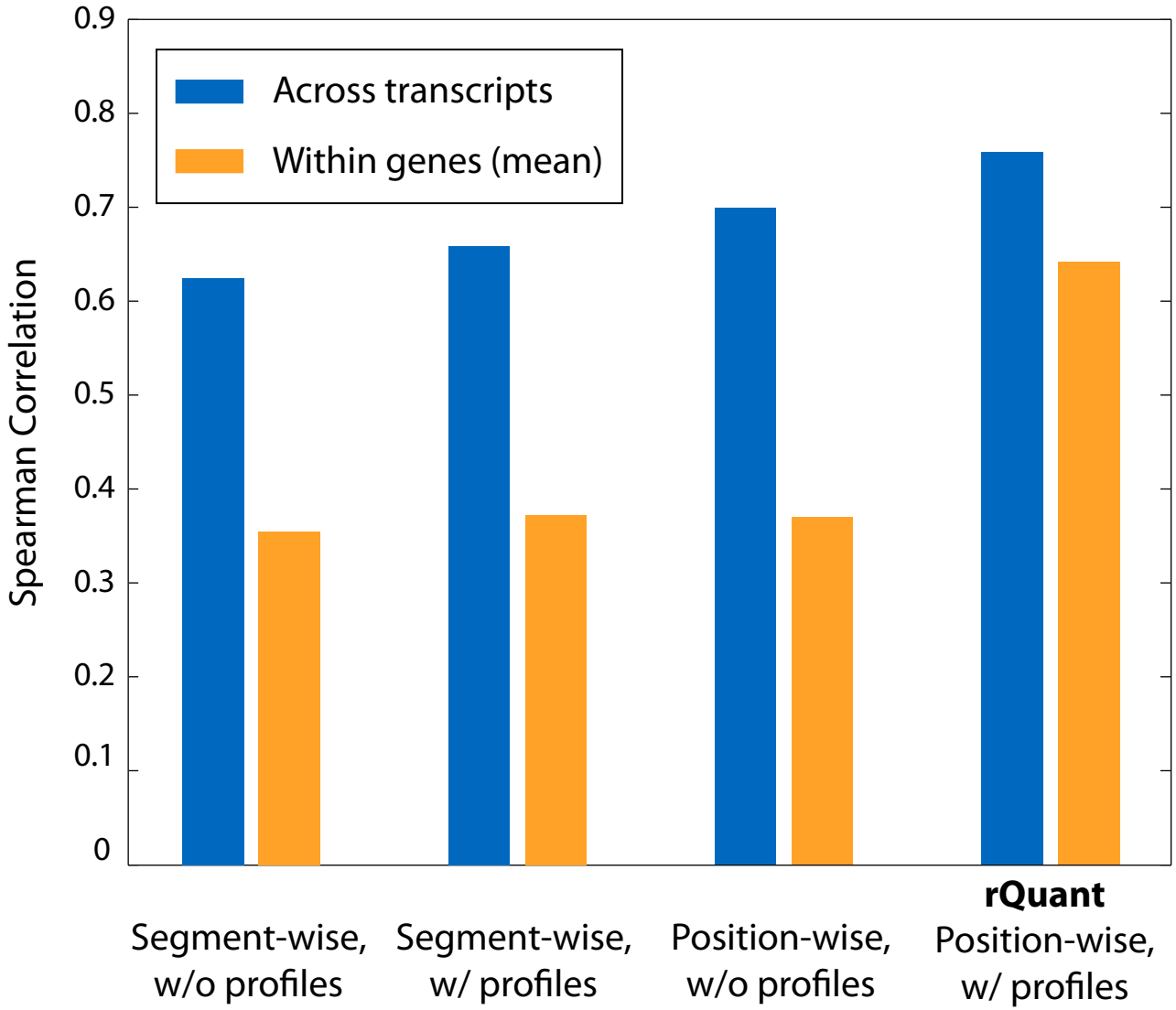
(Bohnert et al., submitted, 2010)

rQuant Evaluation II



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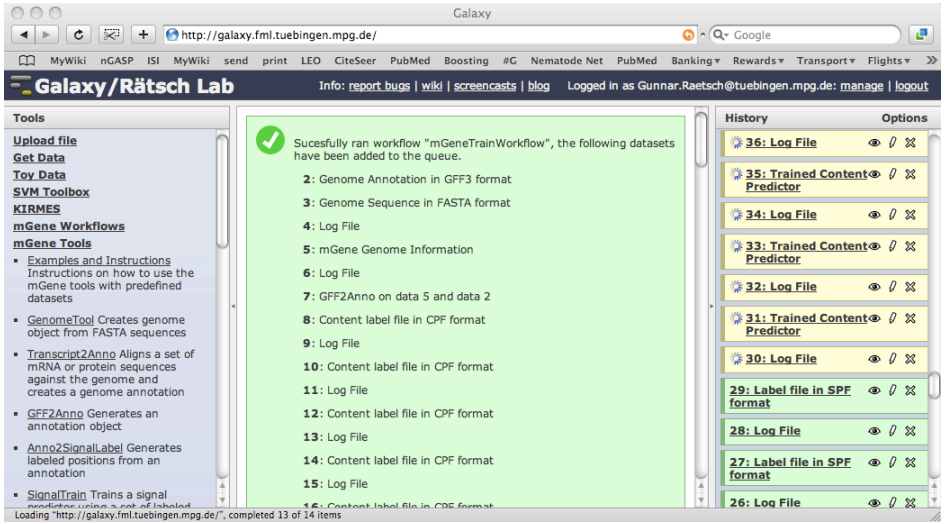


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Galaxy-based Web Services for NGS Analyses

Galaxy-based web service http://galaxy.fml.mpg.de

- PALMapper http://fml.mpg.de/raetsch/suppl/palmapper
- mGene http://mgene.org/web
- mTIM http://fml.mpg.de/raetsch/suppl/mtim (in prep.)
- rQuant http://fml.mpg.de/raetsch/suppl/rquant/web



(Rätsch et al., in preparation, 2010)

Summary

- PALMapper
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  - Outperforms many other read mappers in intron accuracy
- mTiM
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