Next Generation Genome Annotation

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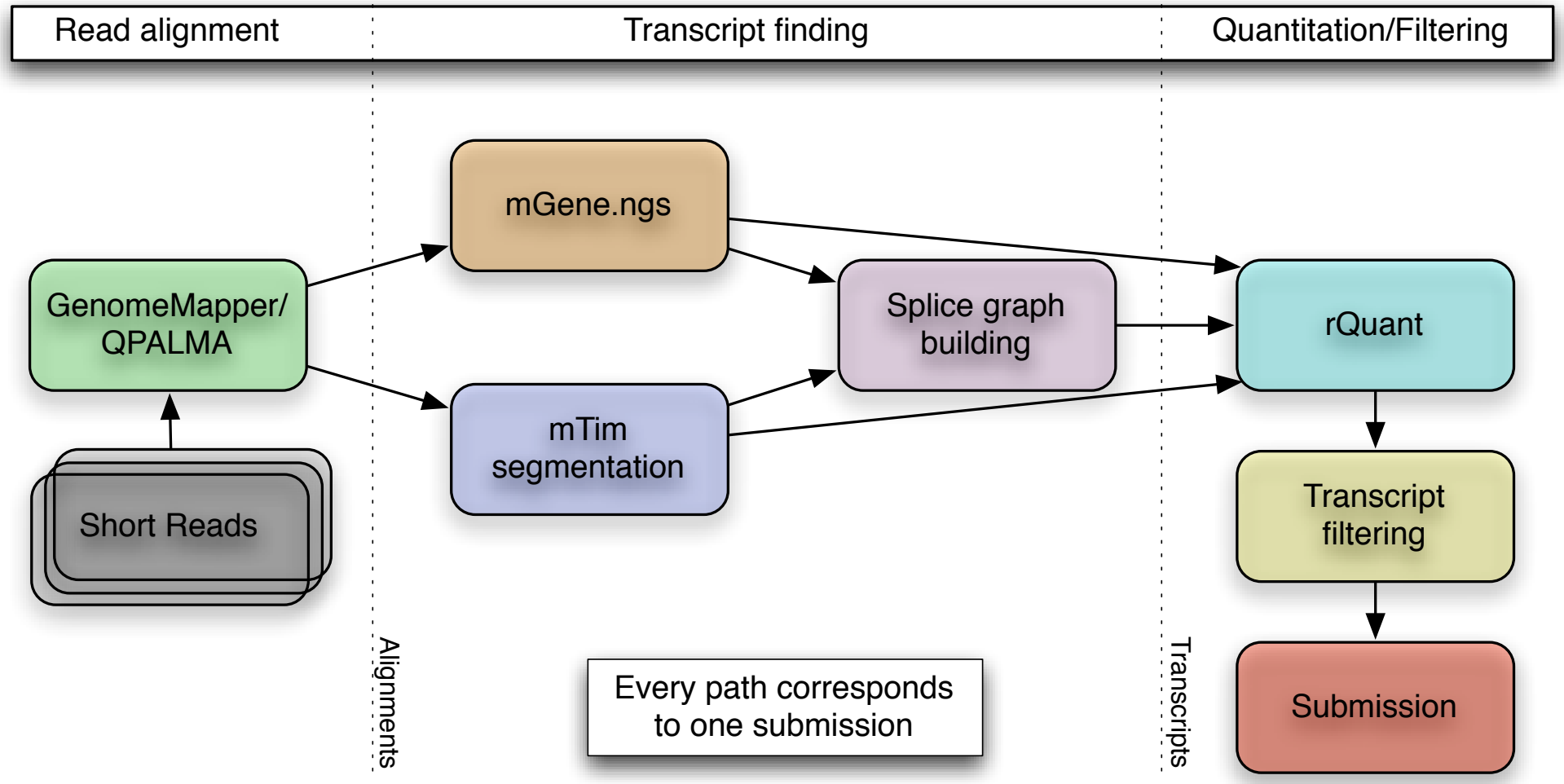
Tübingen, Germany

November 10, 2009

The Wellcome Trust Genome Campus, Hinxton

RGASP Overview (Tübingen)



Read Alignment – GenomeMapper/QPALMA

GenomeMapper for (unspliced) read mapping:

- Alignments based on GenomeMapper developed in Tübingen for the 1001 plant genome project (Schneeberger et al., 2009a)
- k-mer based index, well suited for smaller genomes with many mismatches/gaps

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QPALMA for spliced read alignments:

- GenomeMapper identifies seed regions for *spliced alignments*
- Alignments are performed using QPALMA (De Bona et al., 2008)
- QPALMA is individually adapted to every SR dataset

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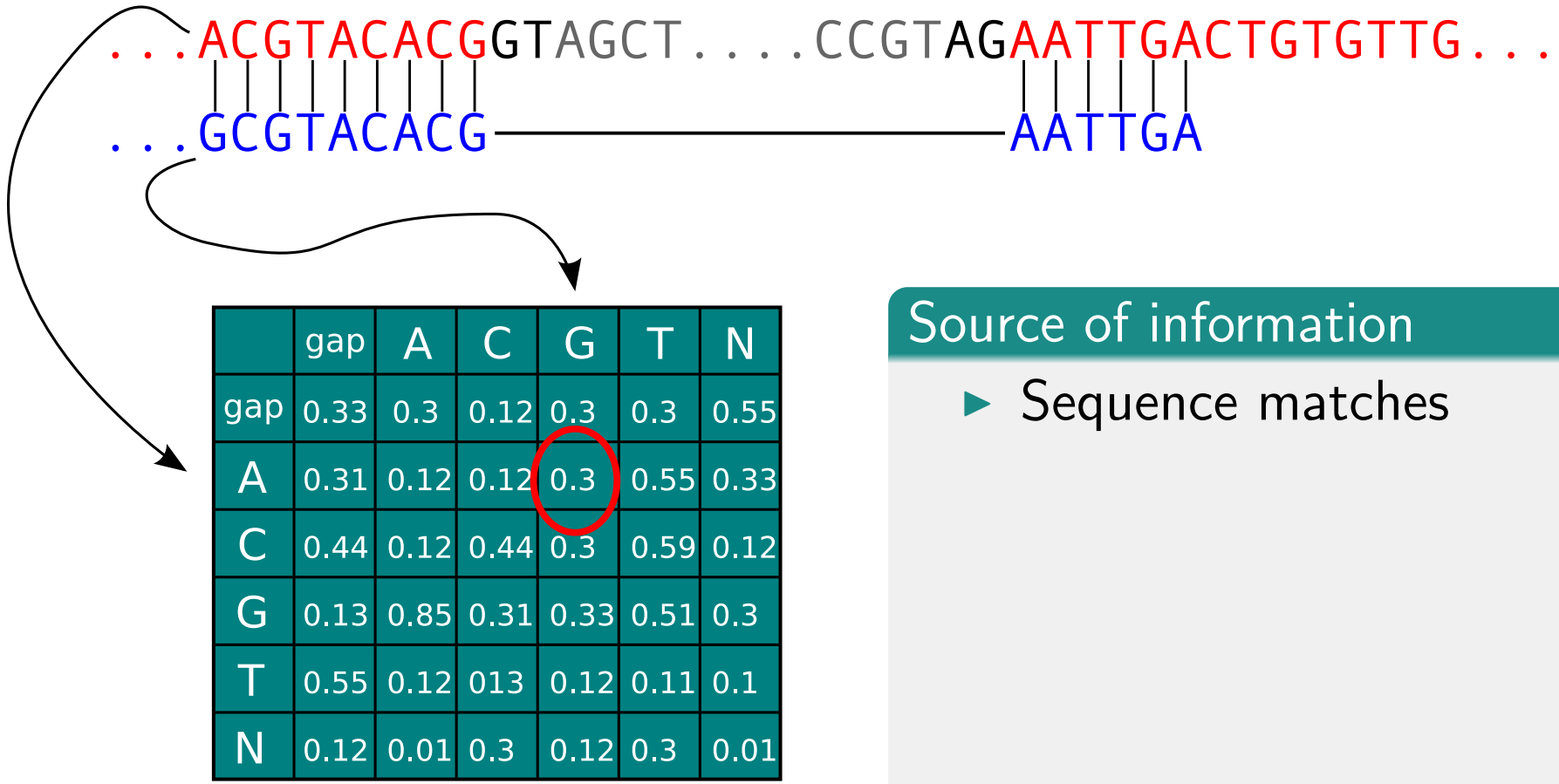
Web server available at `http://galaxy.tuebingen.mpg.de`.

Read Alignment – QPALMA



	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
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Source of information

- Sequence matches

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

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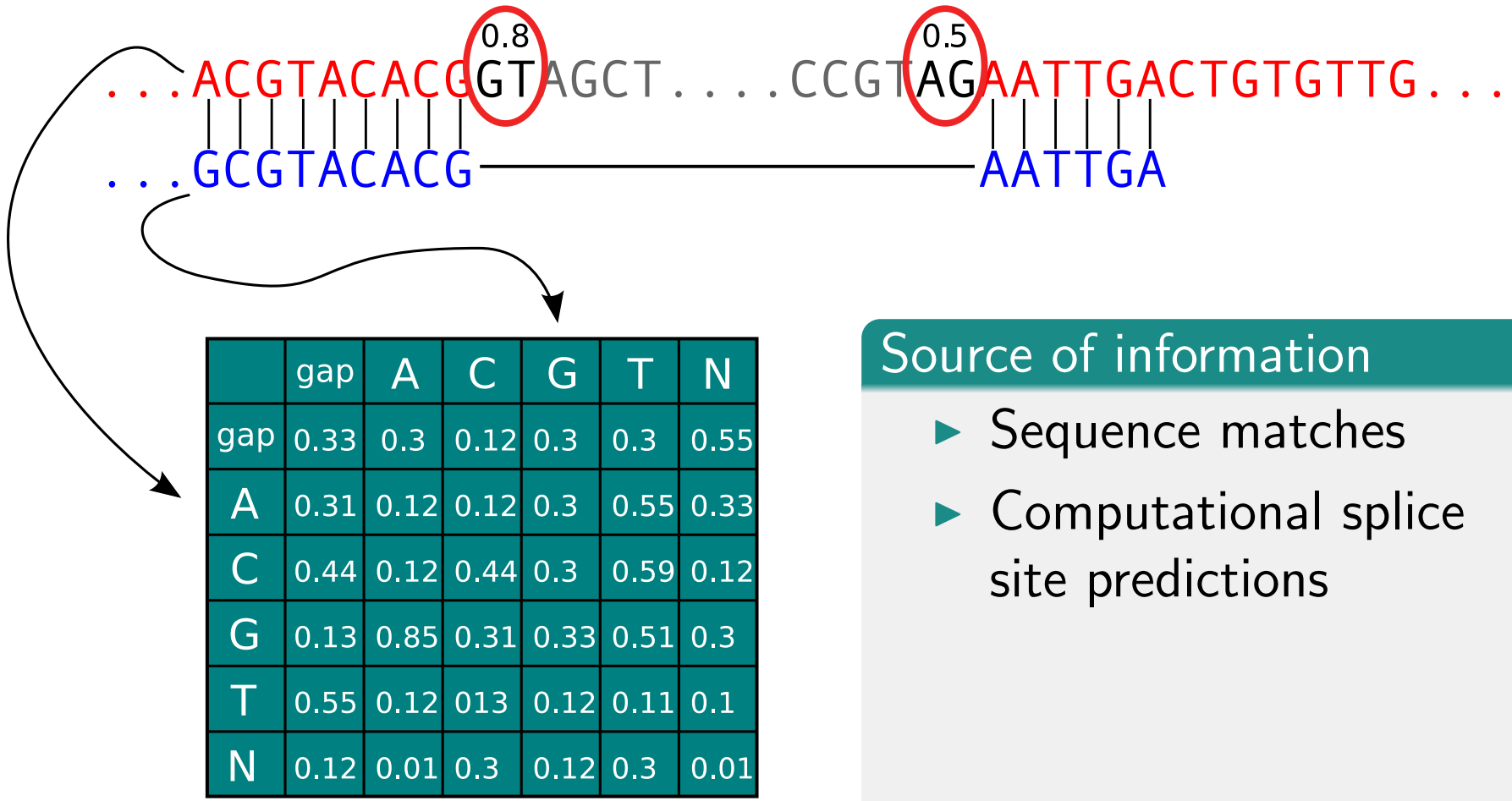

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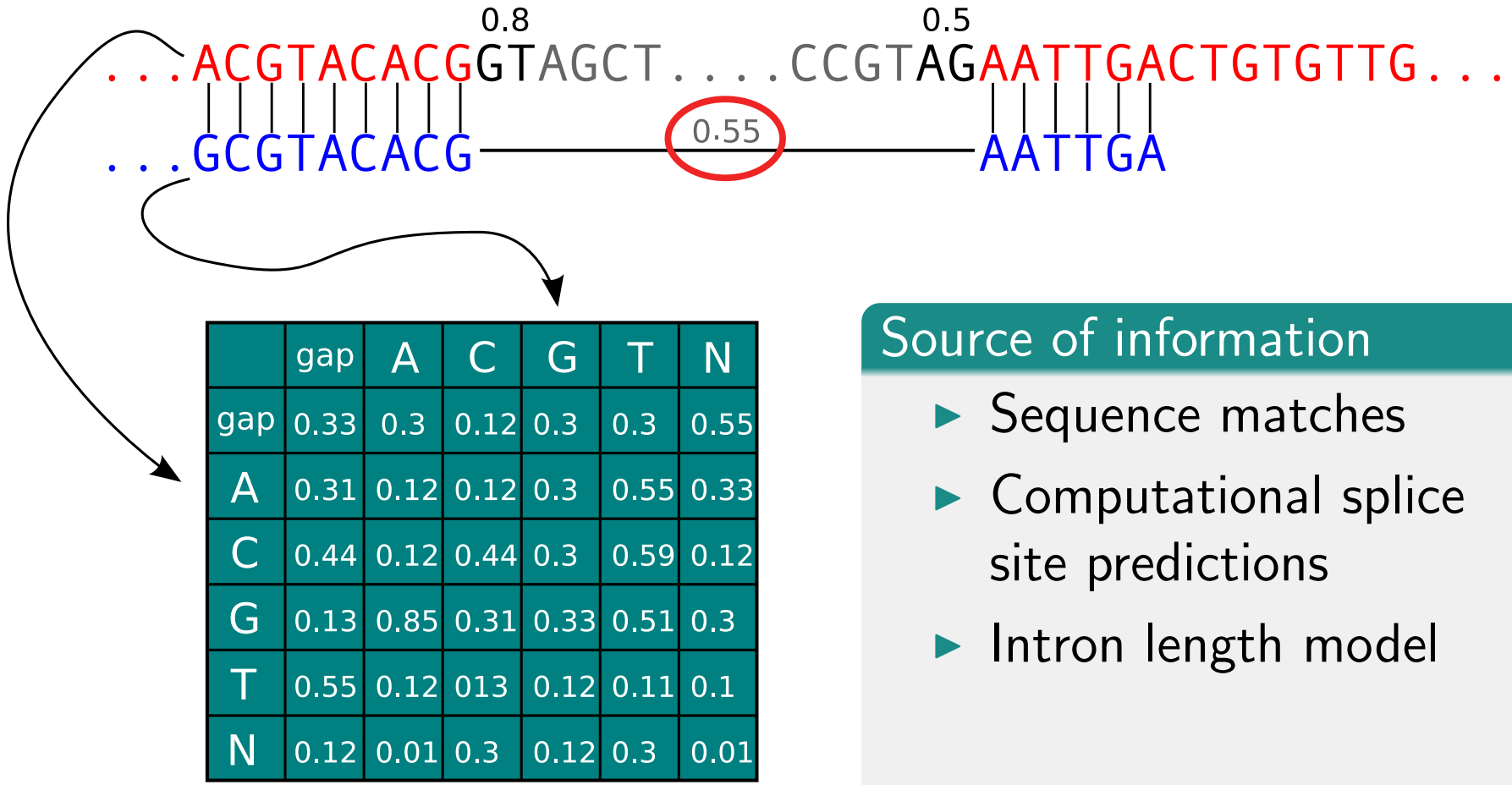

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- Computational splice site predictions
- Intron length model

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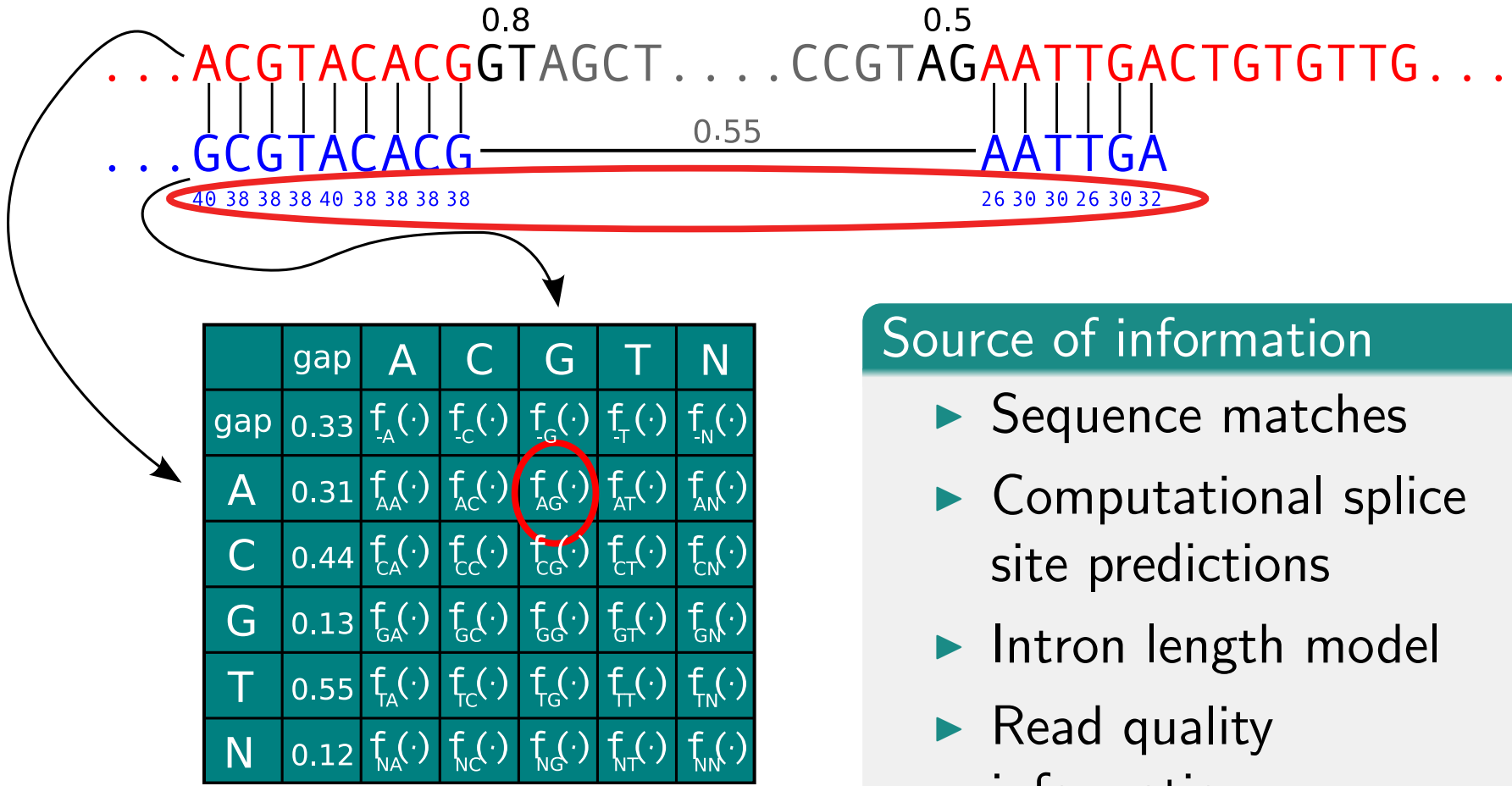


Source of information

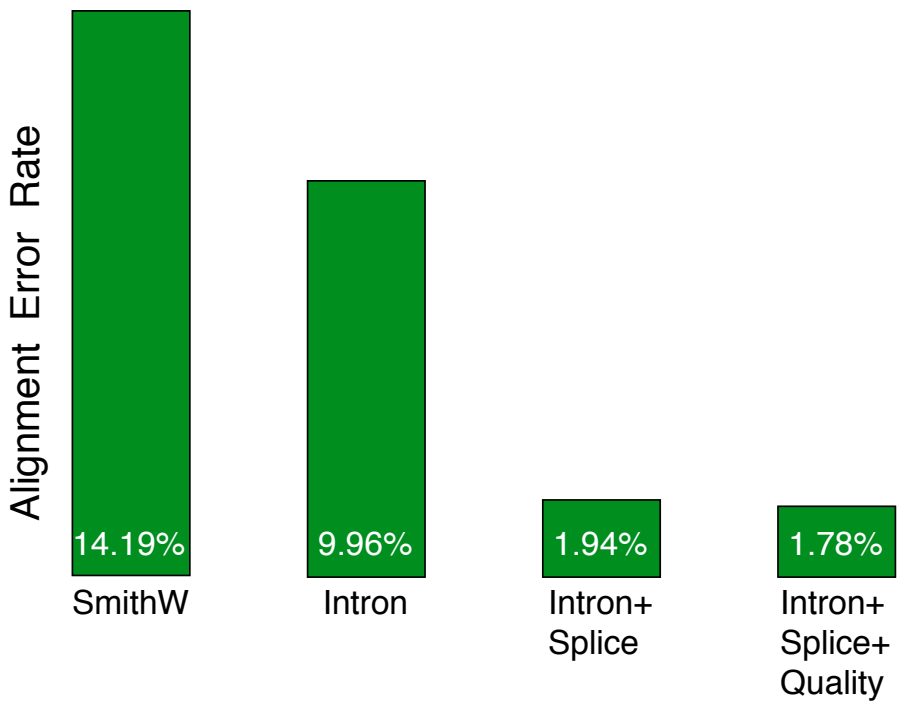
- Sequence matches
- Computational splice site predictions
- Intron length model
- Read quality information

Quality scoring $f : (\Sigma \times \mathbb{R}) \times \Sigma \longrightarrow \mathbb{R}$ (De Bona et al., 2008)

RNA-Seq Read Alignment – QPALMA

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



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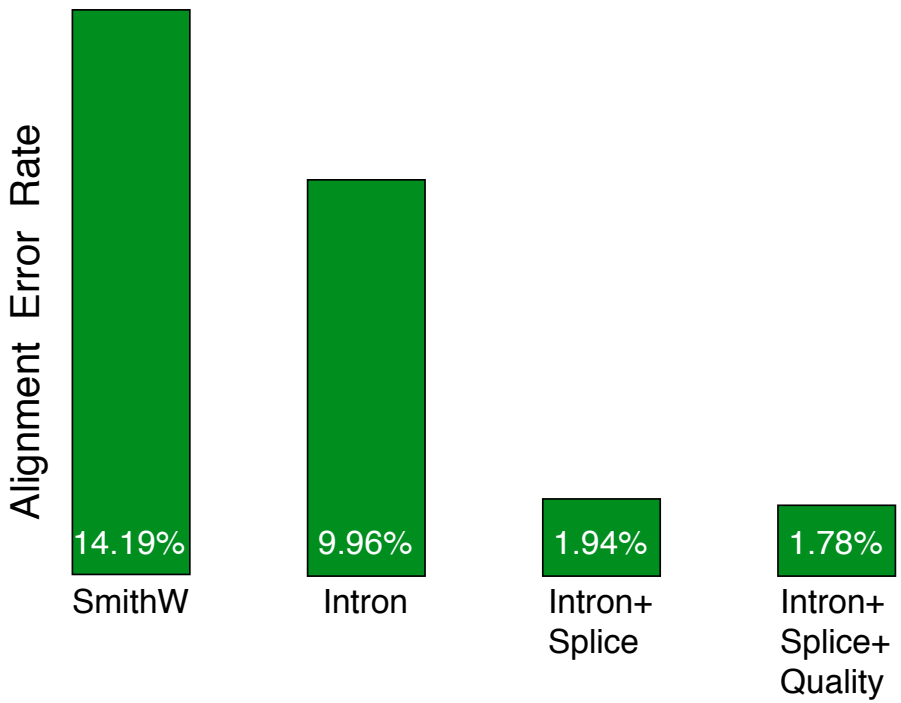
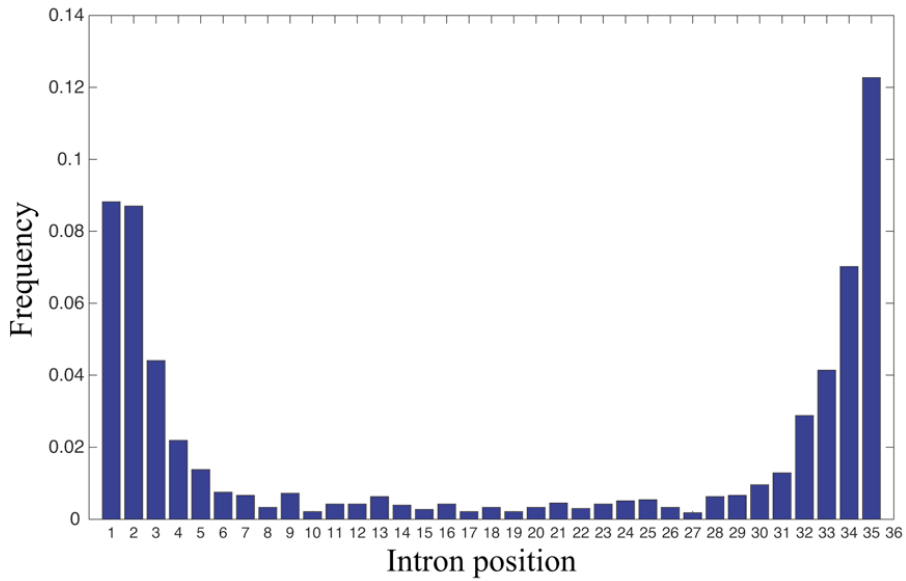


Error vs. intron position



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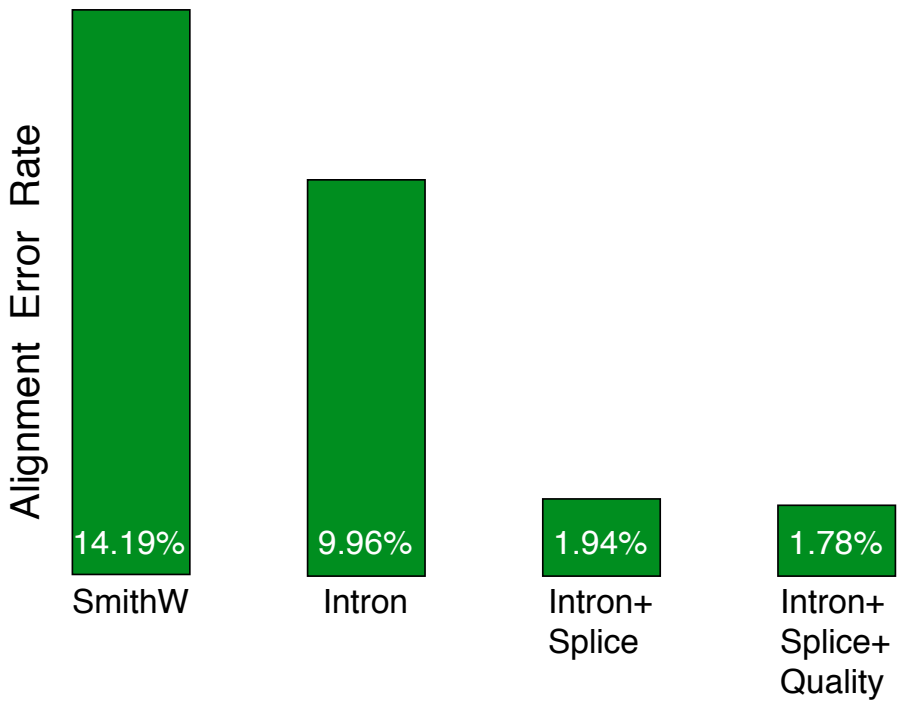
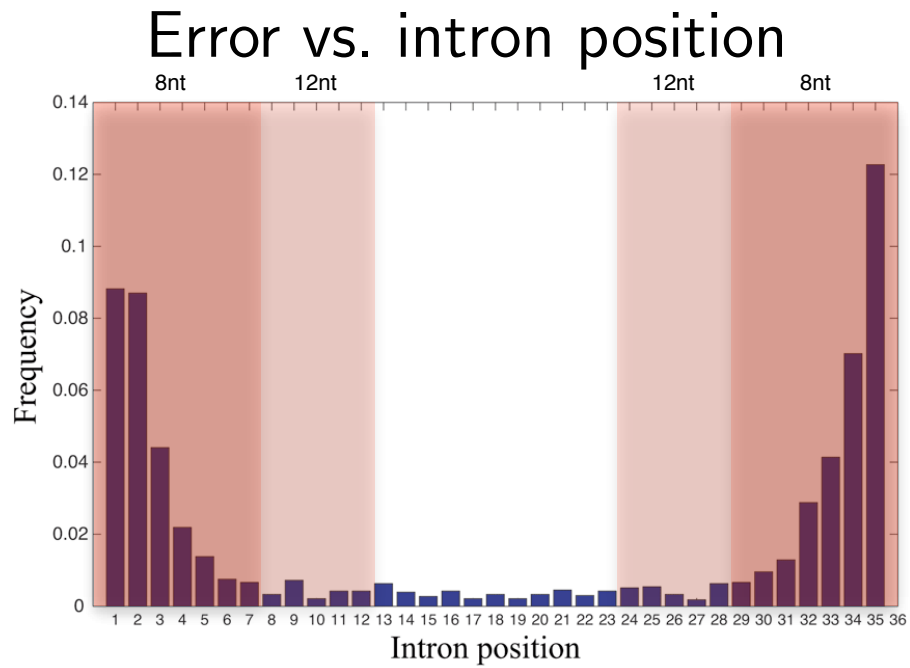




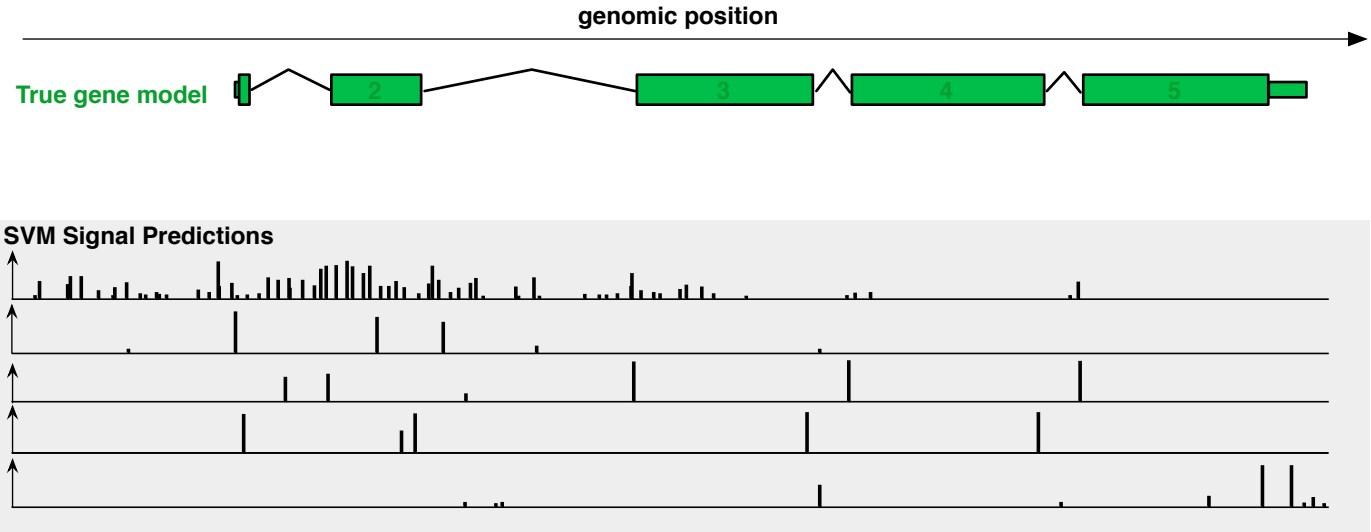
(De Bona et al., 2008)

Step 2: Transcript Prediction

1. Extension of *mGene* gene finding system to use NGS data for protein coding transcript prediction
2. Coverage segmentation algorithm *mTIM* for general transcripts (no coding bias/assumption)
3. Splice graph construction by extending splice graph with spliced reads (connecting exons)

Approaches 1 & 2 use read coverages and spliced reads.
Approach 3 uses existing transcripts and spliced reads.

mGene-based Transcript Prediction I



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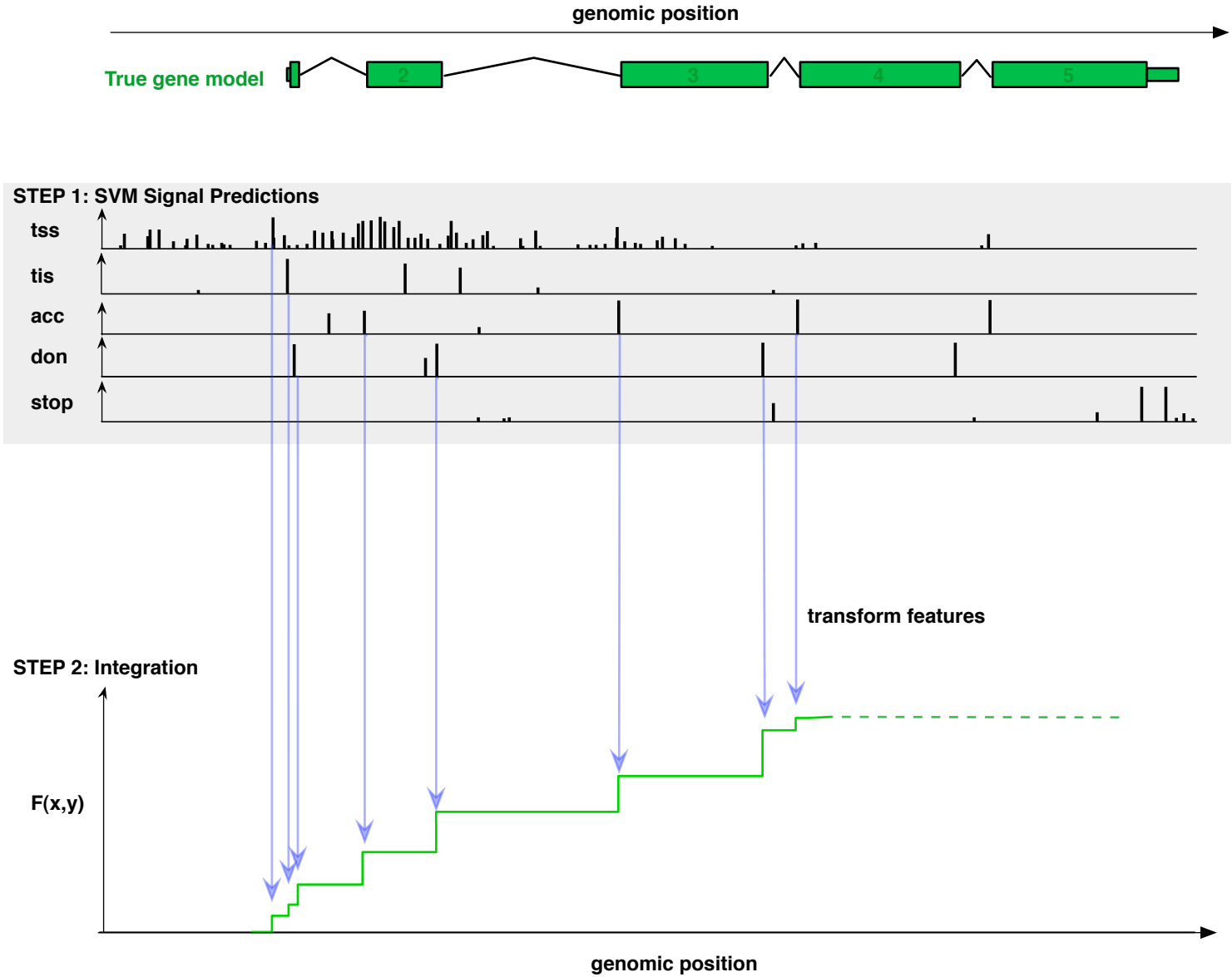

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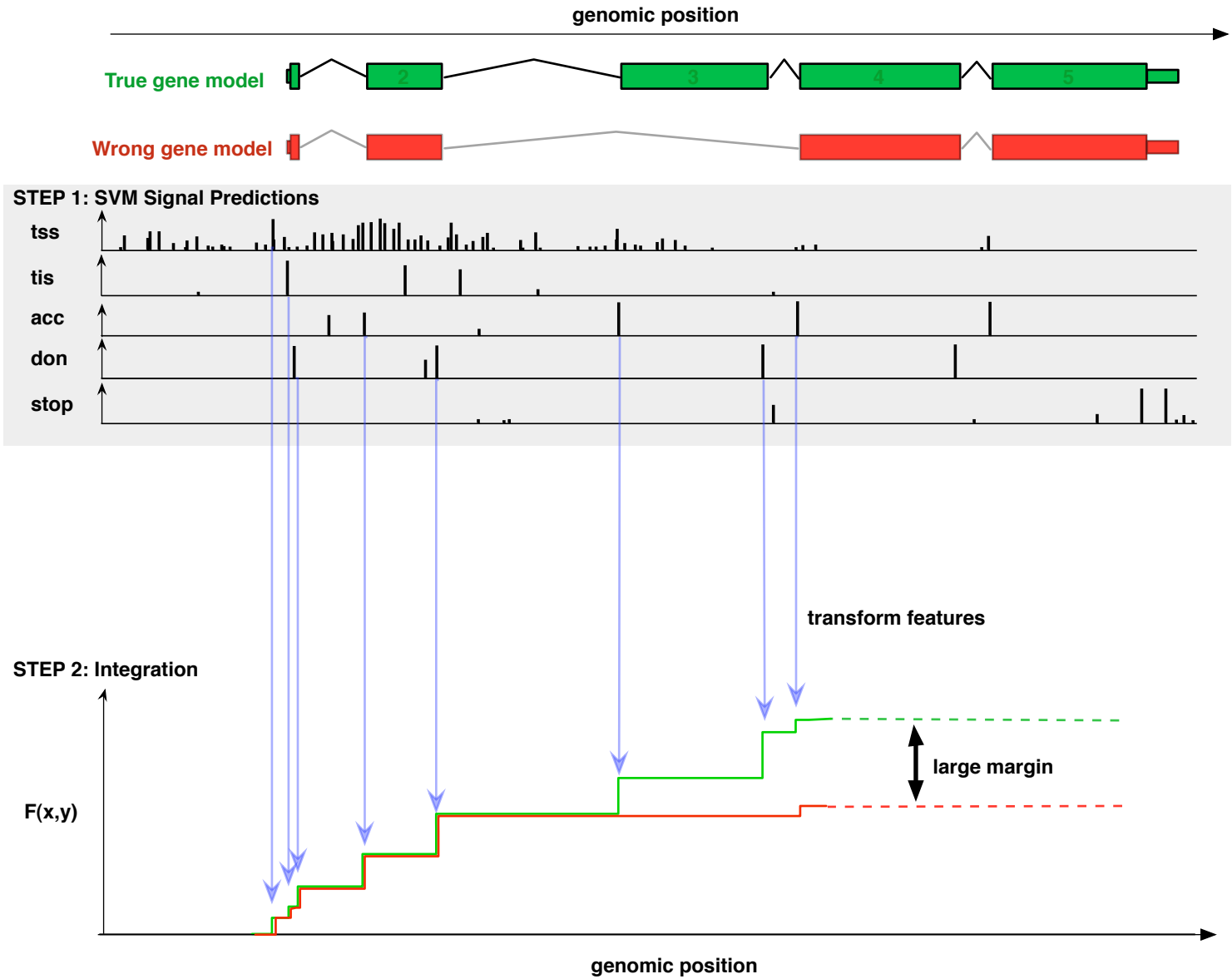

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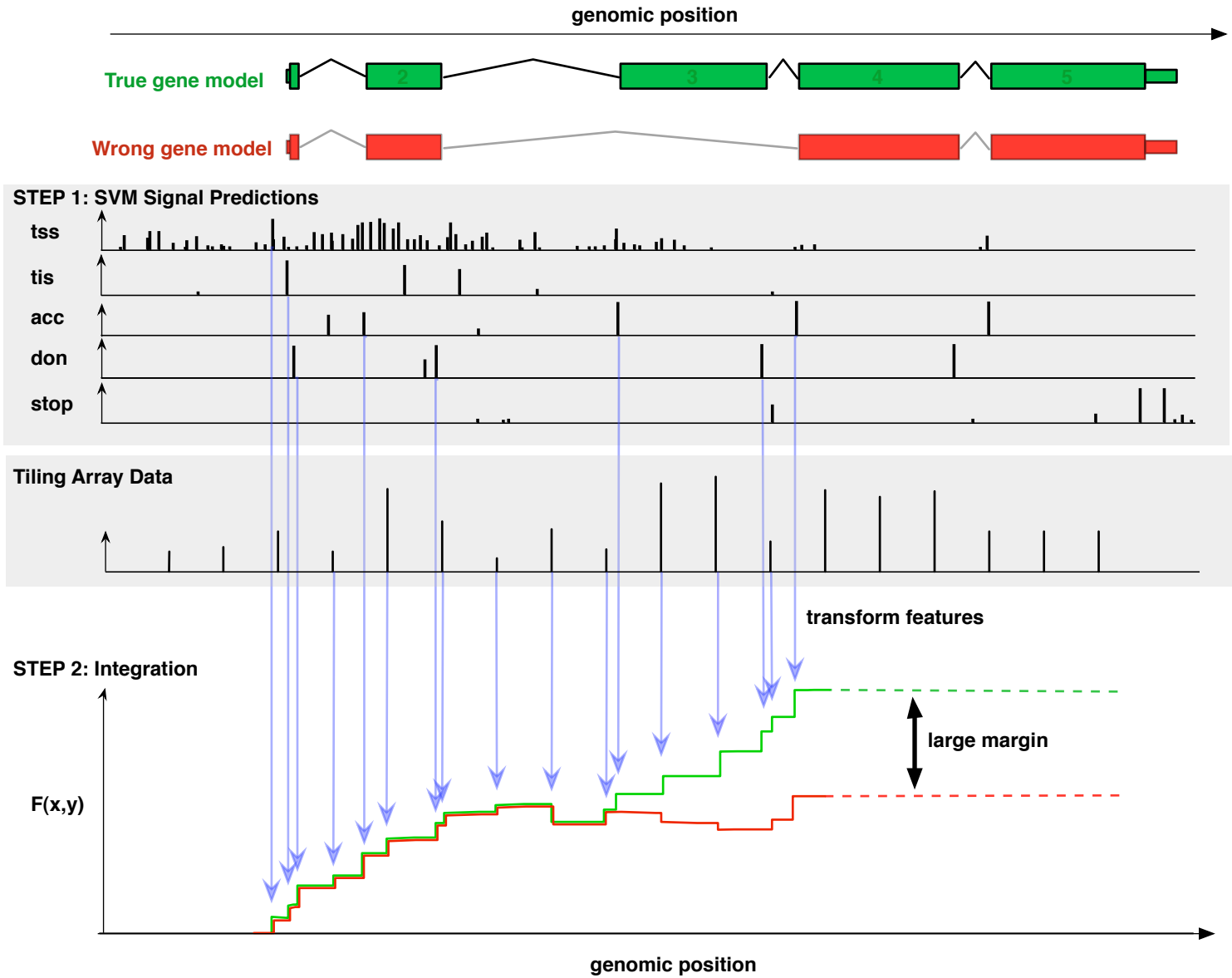

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Results for A. thaliana: (Comparison with known gene models)

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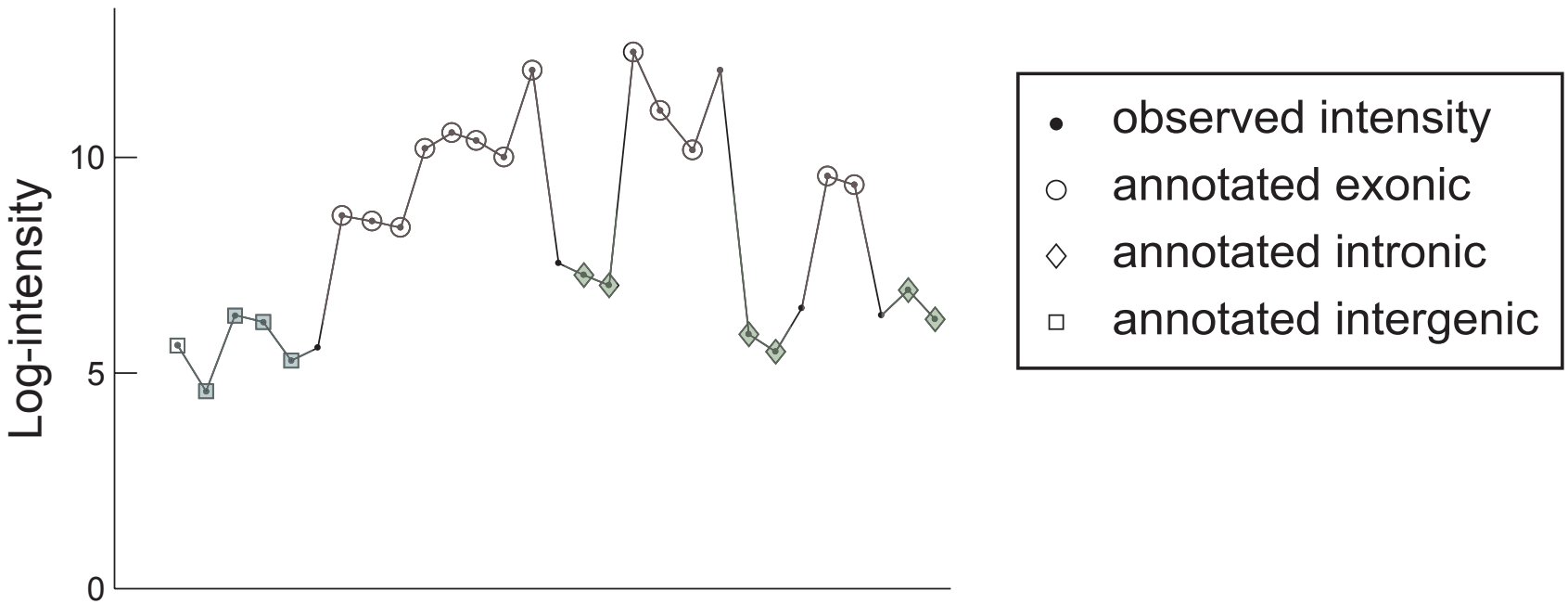
Similar observations for RGASP predictions.

Tiling Array/Read Coverage Segmentation

Goal: Characterize each "probe" as either intergenic, exonic or intronic



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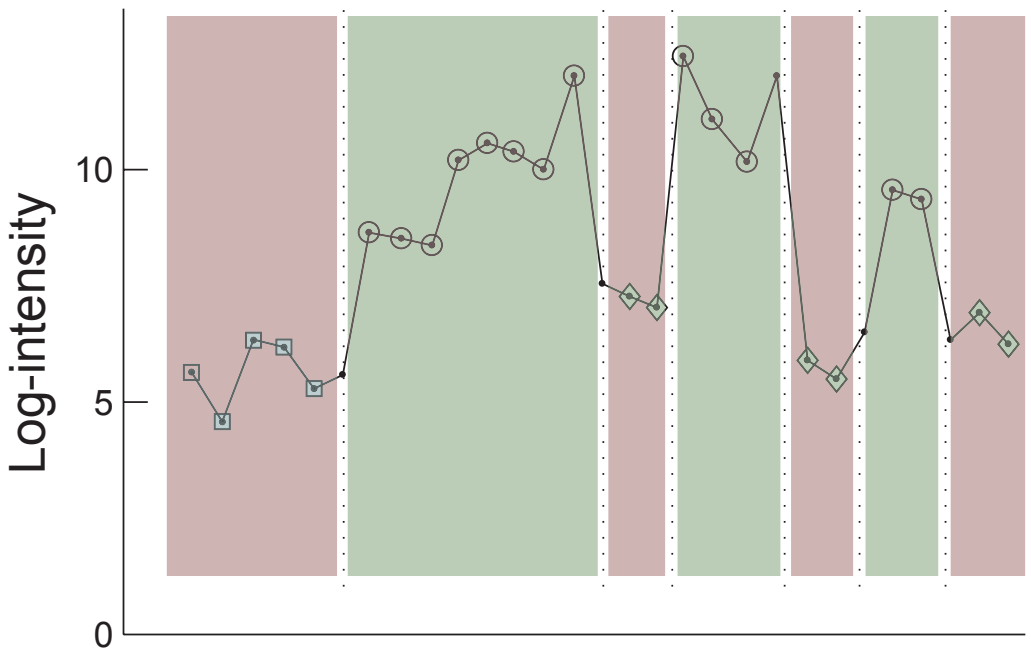

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- annotated exonic
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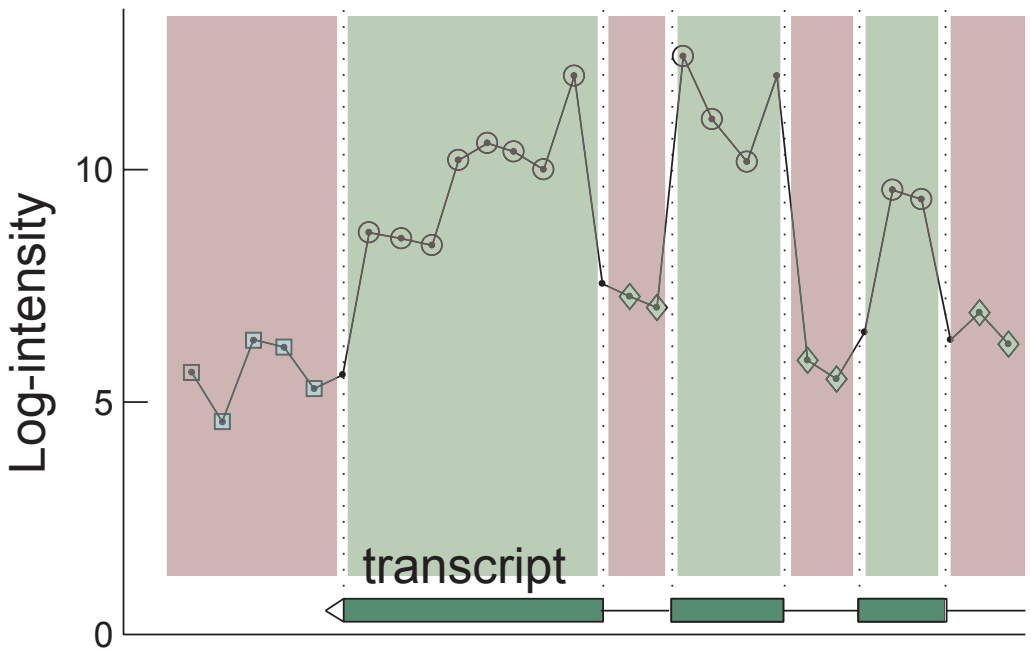

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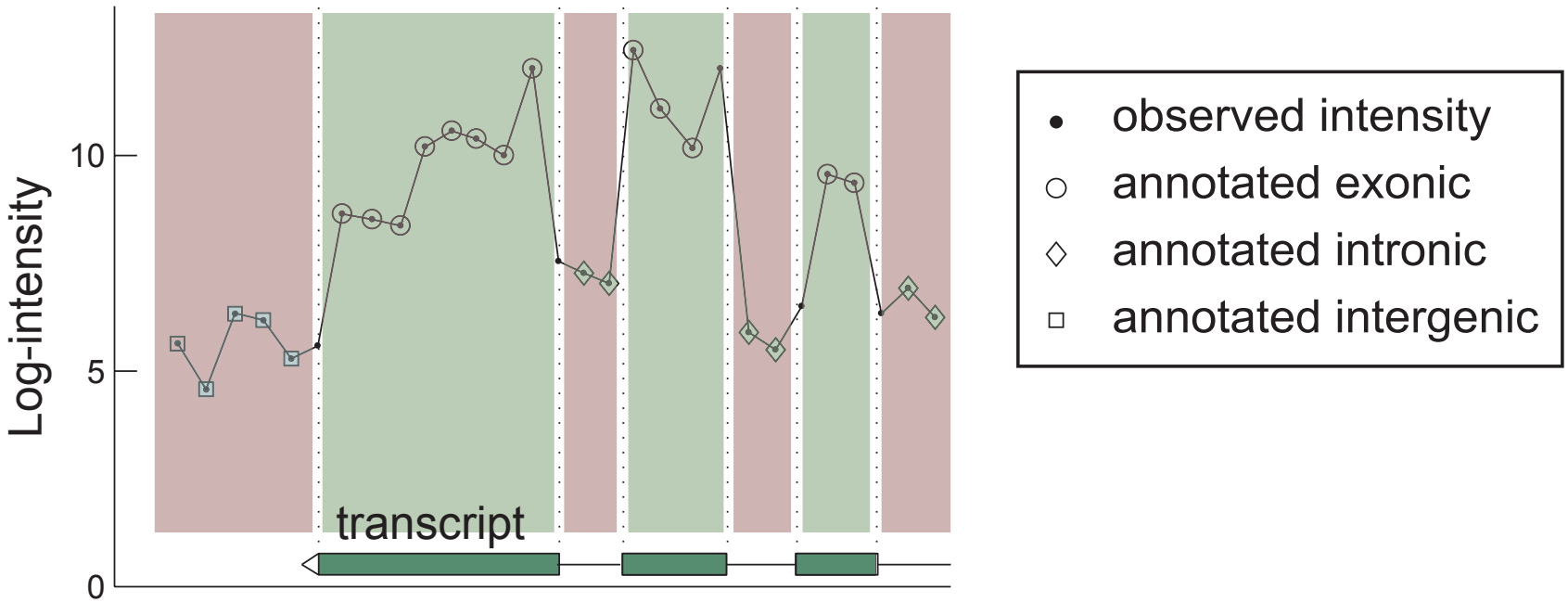
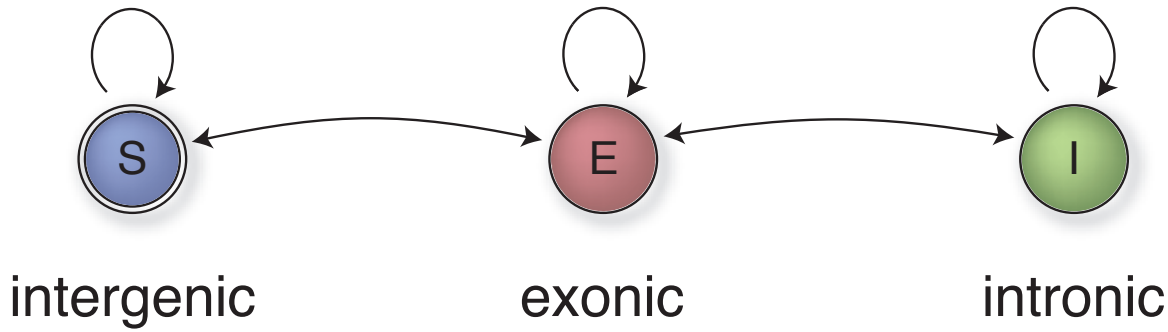


Novel segmentation method ("mSTAD"/"mTIM")

- accounts for spliced transcripts
- provides very accurate predictions

(Zeller et al., 2008a)

The mSTAD/mTIM Approach



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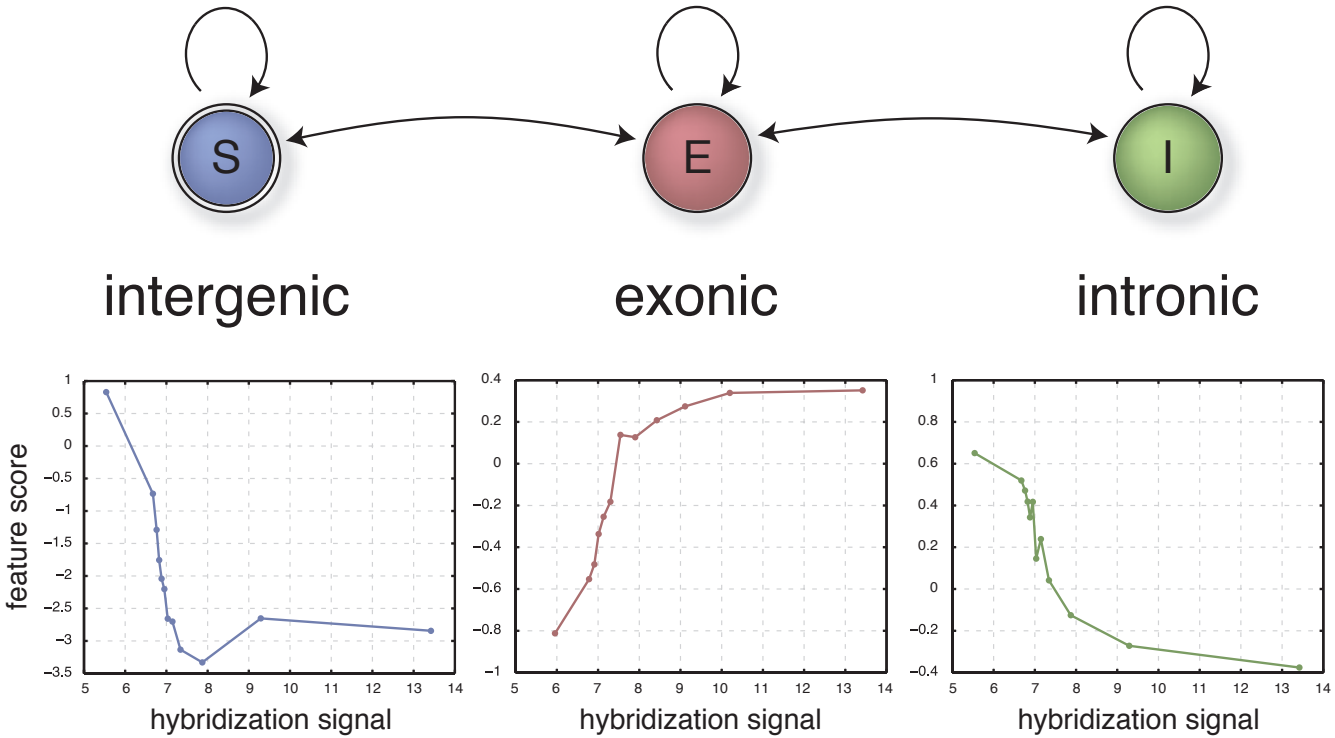

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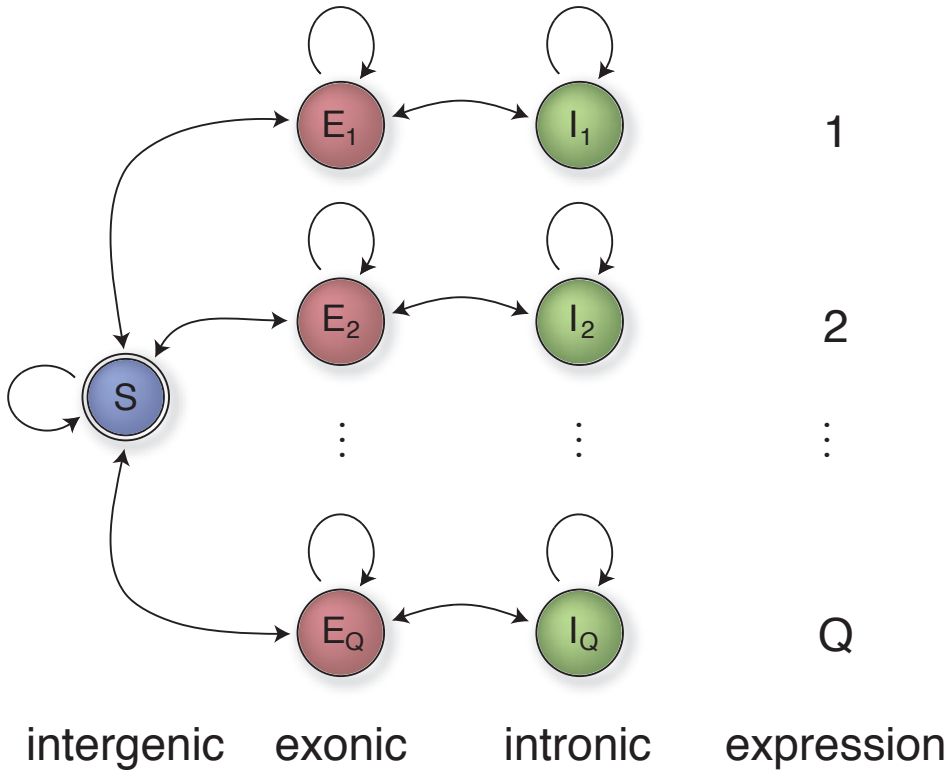

- Learn to associate a state with each probe given its hybridization signal and local context
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- HM-SVM training: Optimize transformations: signal $\longrightarrow$ score

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Digestion ...

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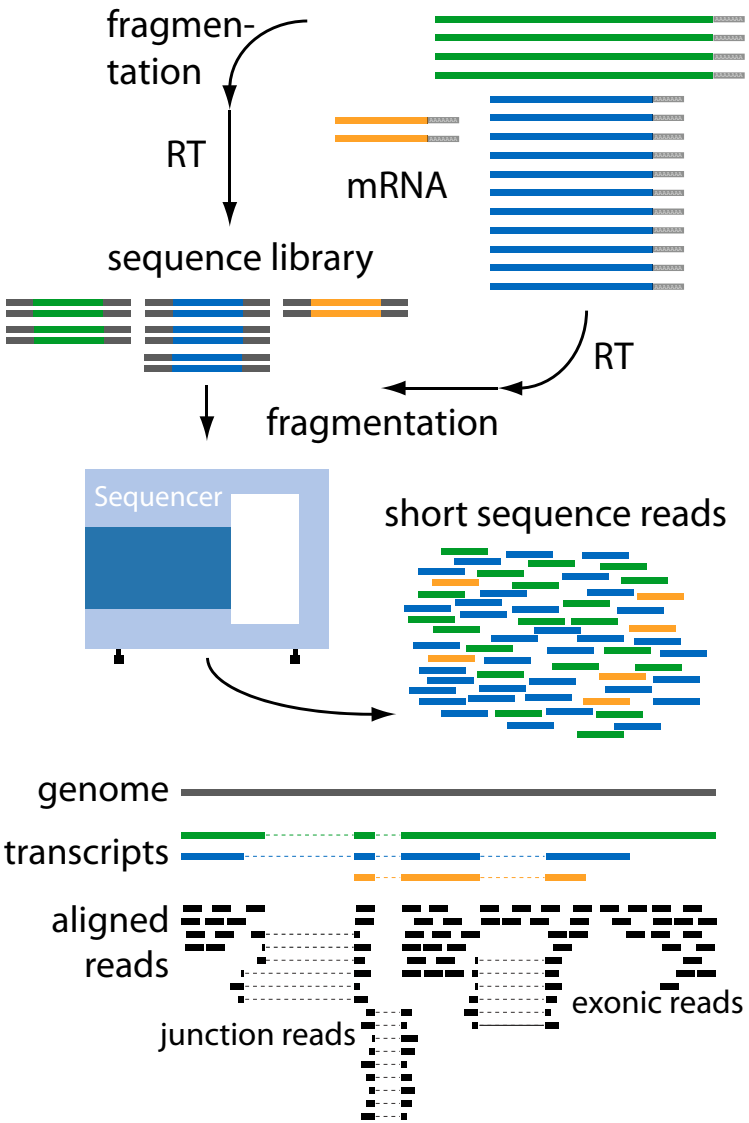
- Spliced reads used to generate a more complete splicing graph
- Paths through splicing graph define transcripts for quantitation

RNA-Seq Biases and Quantitation



Biases due to ...

- cDNA library construction
- Sequencing
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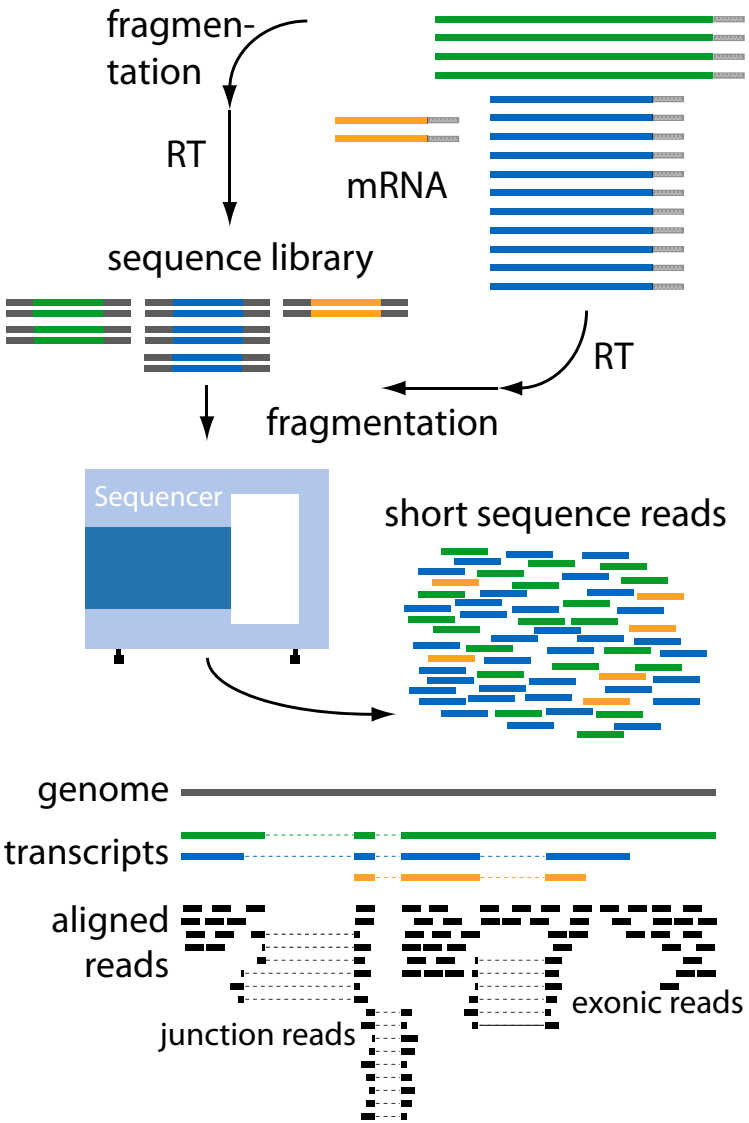

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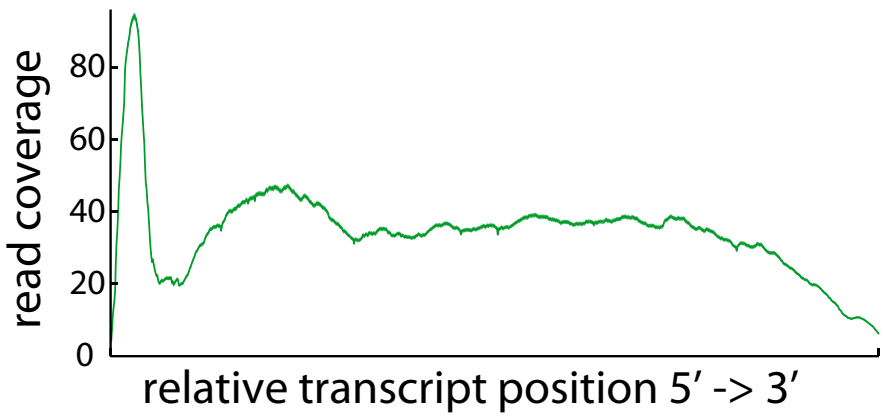
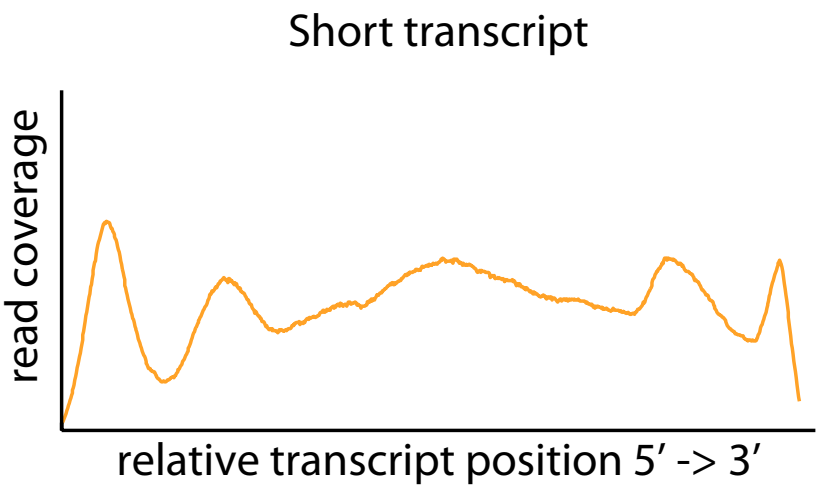


(average over annotated transcripts of length ≈1kb for the *C. elegans* SRX001872 dataset)

rQuant – Basic Idea



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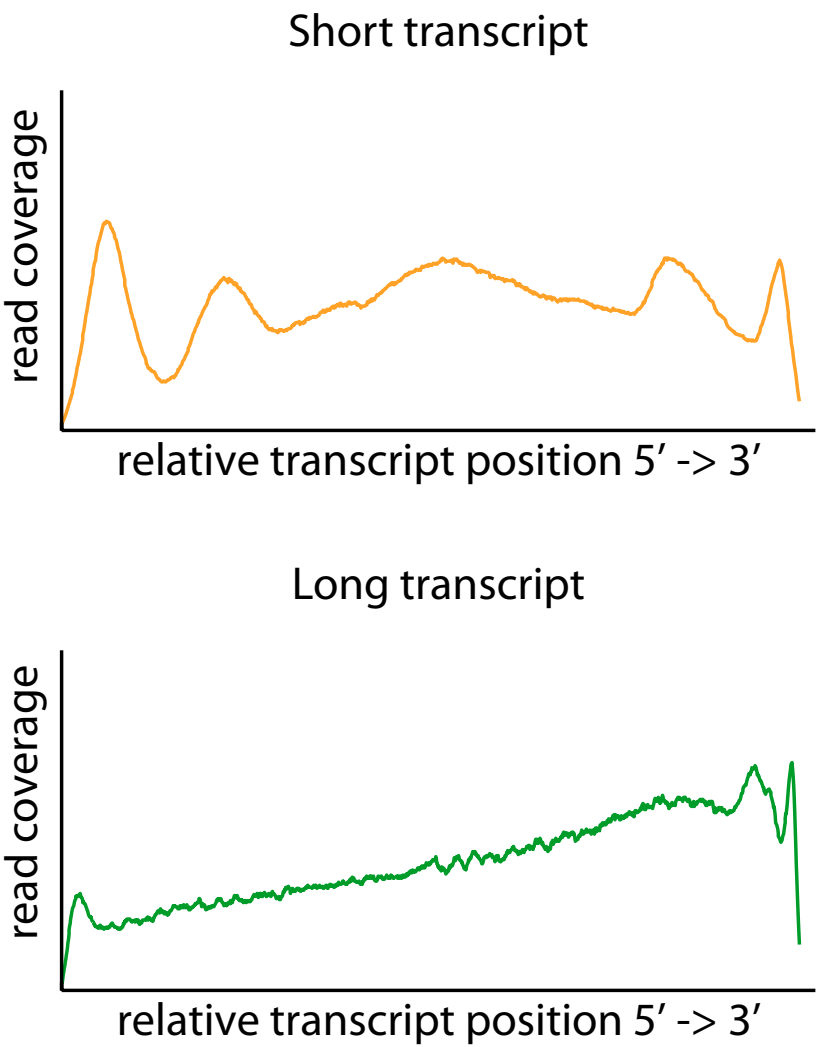

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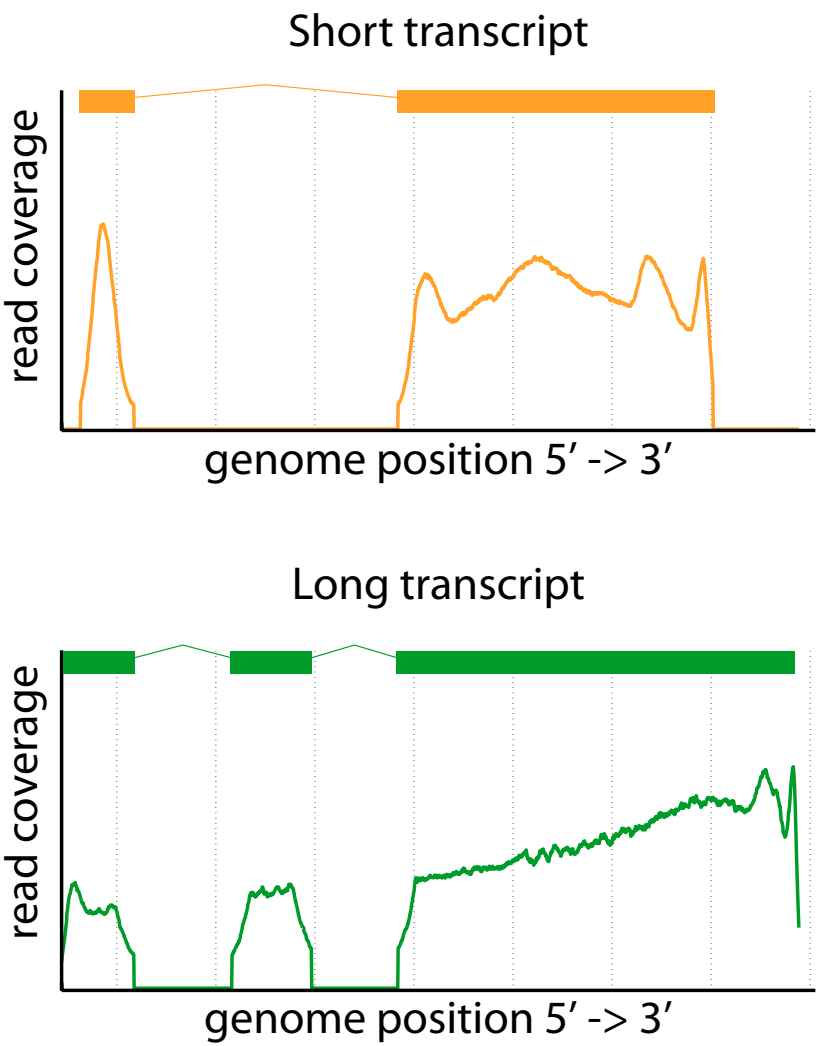

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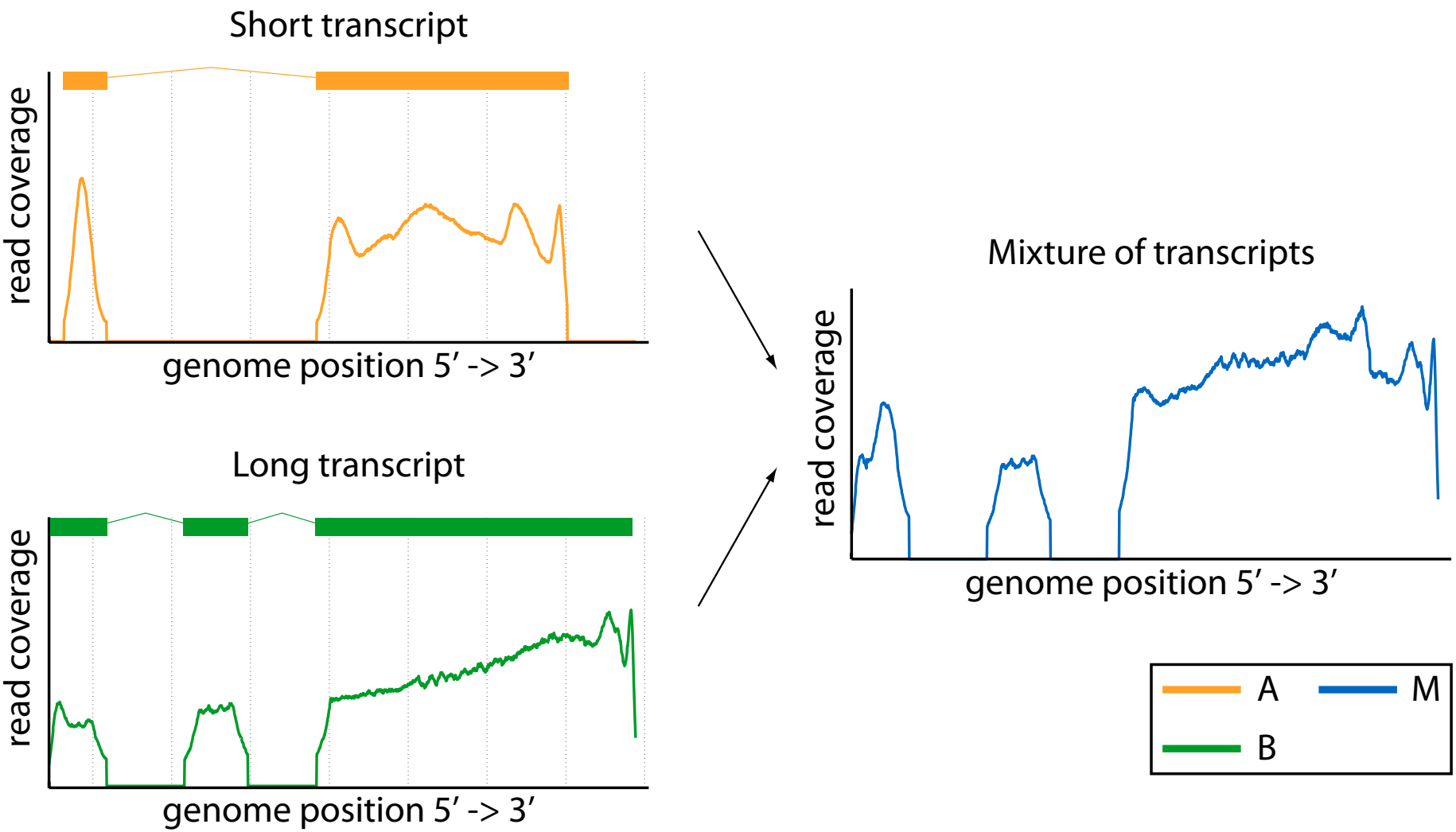

$$M_i = w_A A_i + w_B B_i$$

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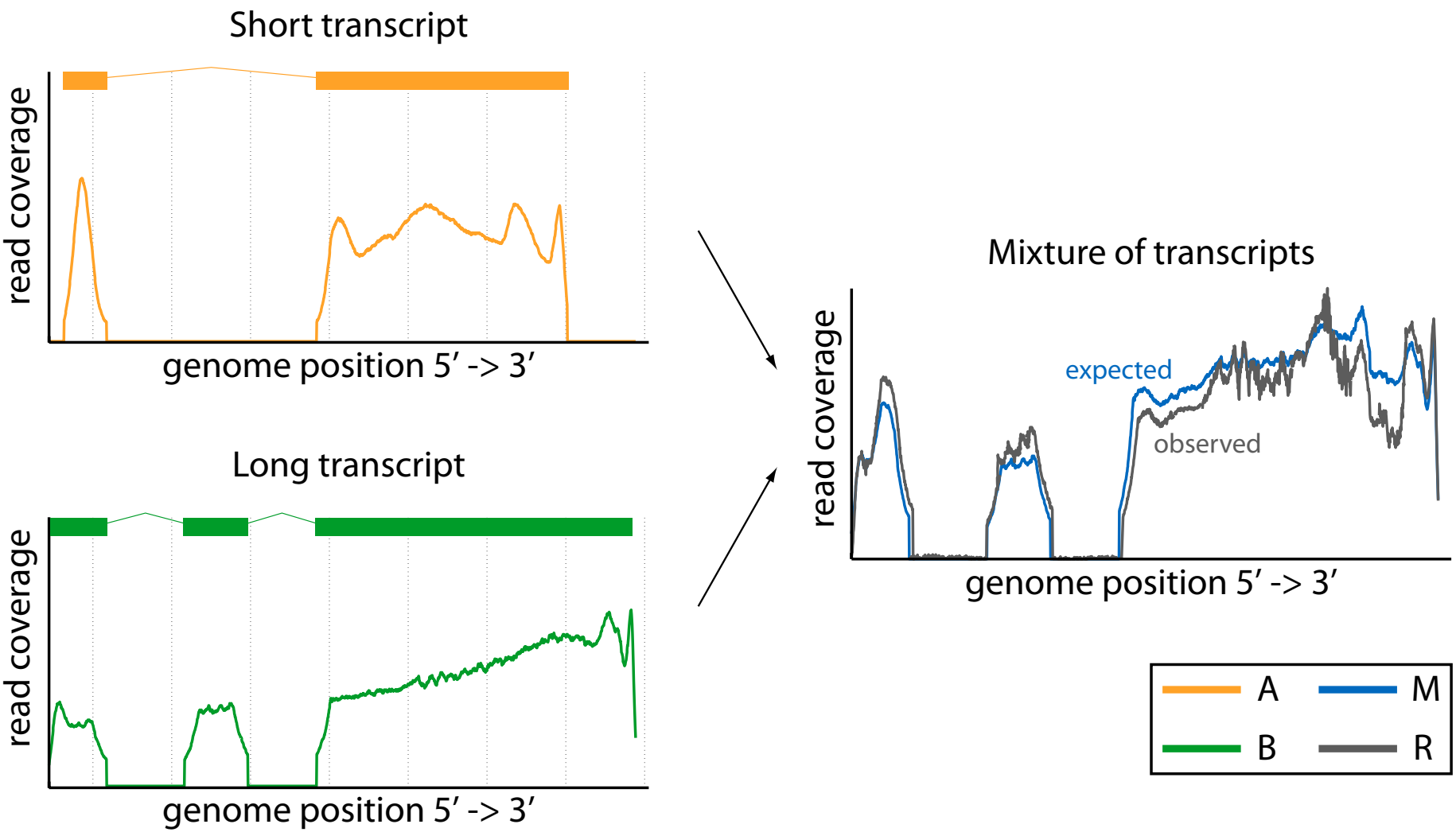

$$M_i = w_A A_i + w_B B_i \quad \Rightarrow \quad \min_{w_A, w_B} \sum_i \ell(M_i, R_i)$$

rQuant – Iterative Algorithm

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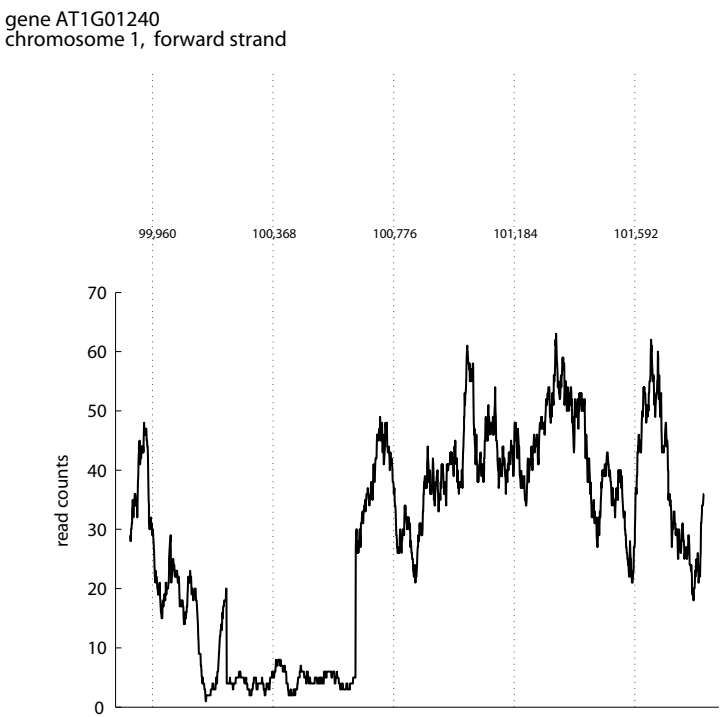

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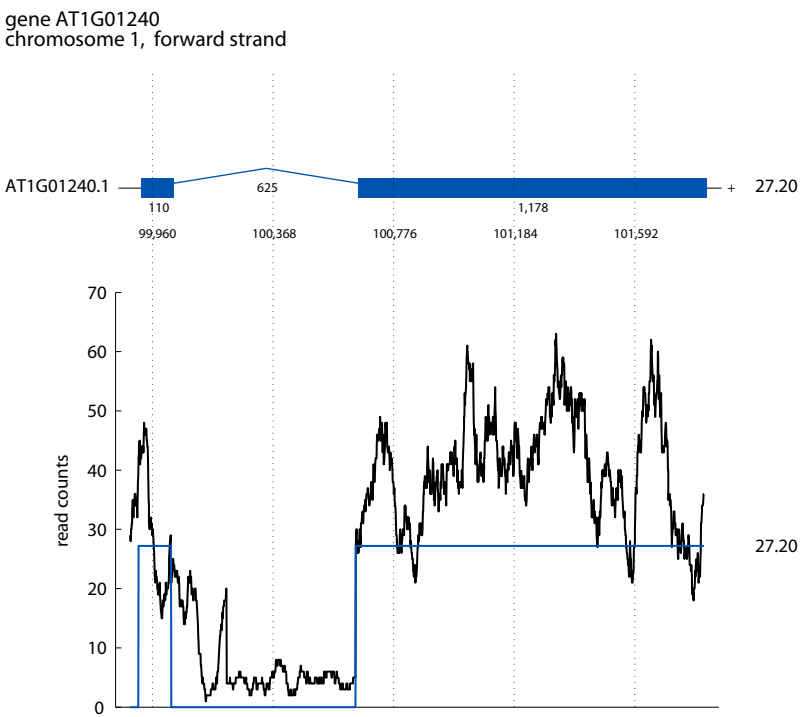

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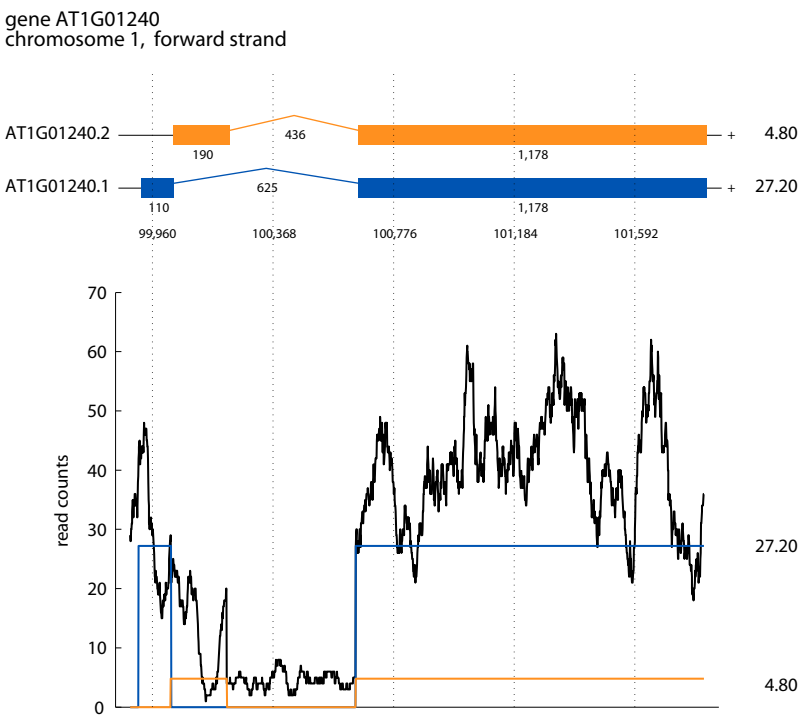

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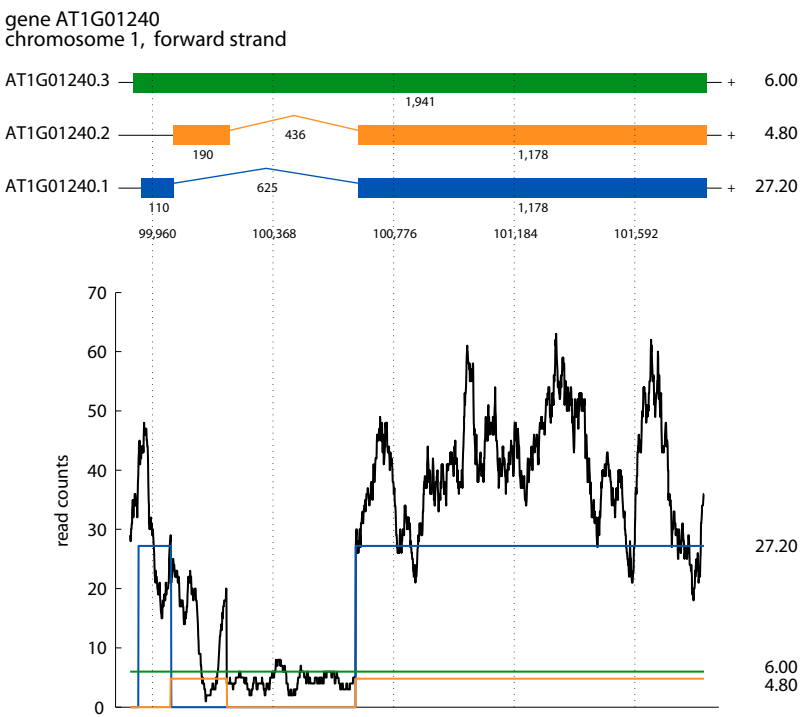

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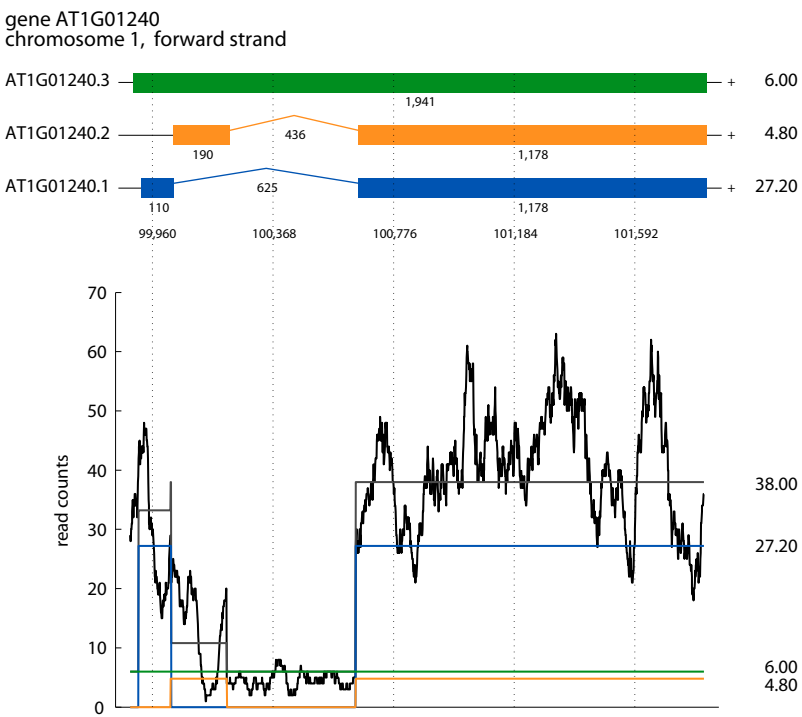

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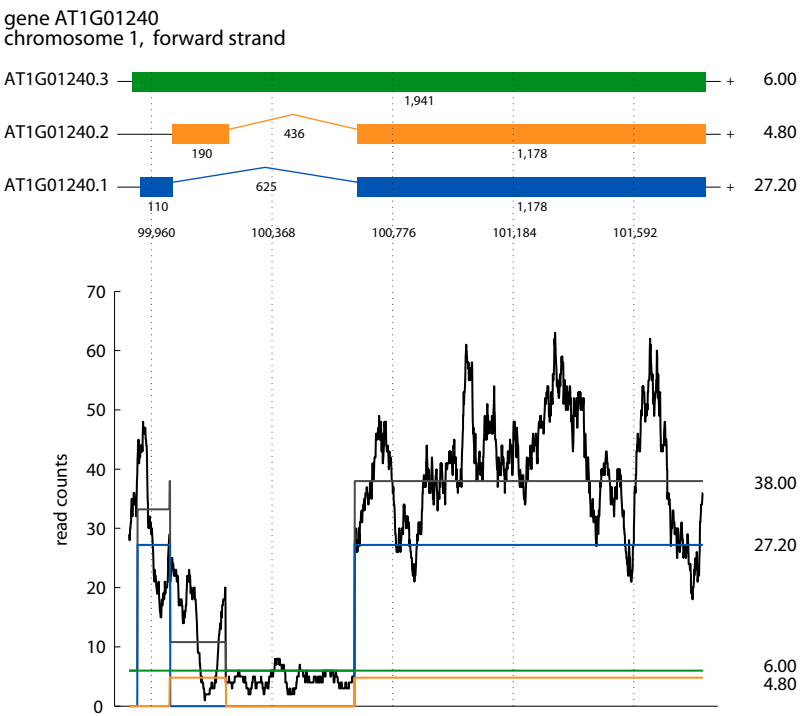

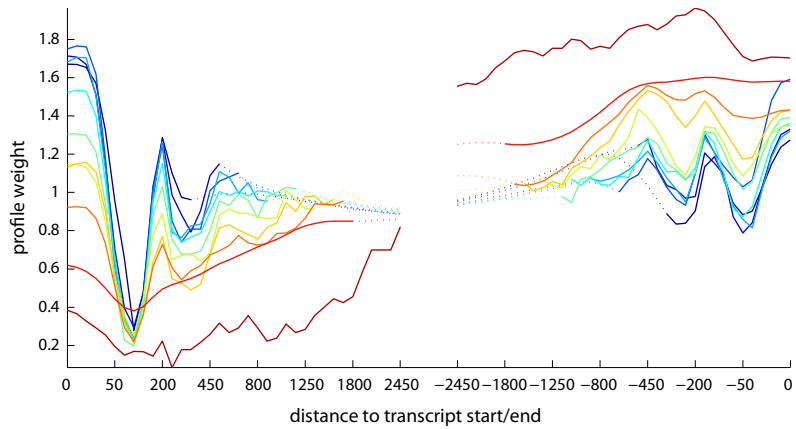

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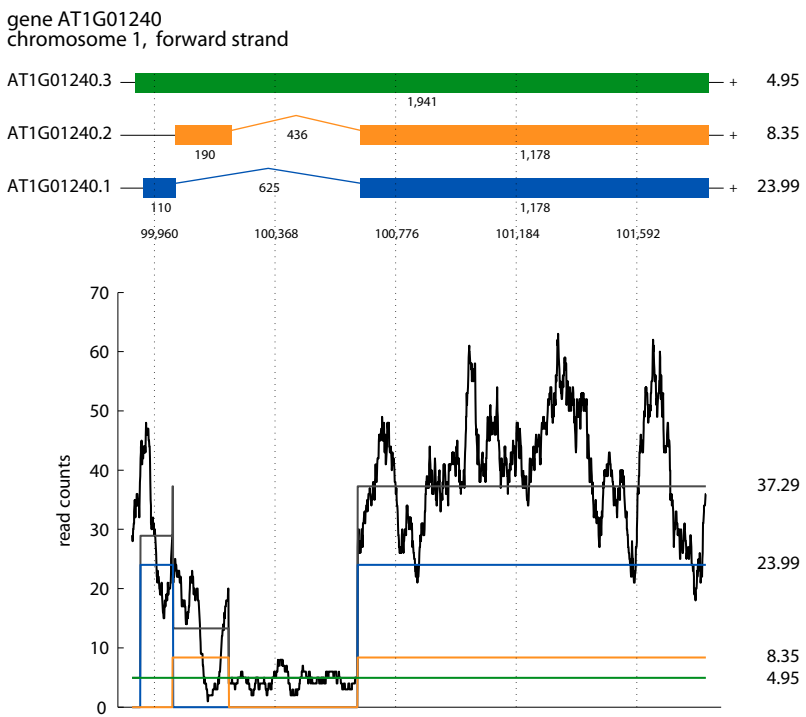
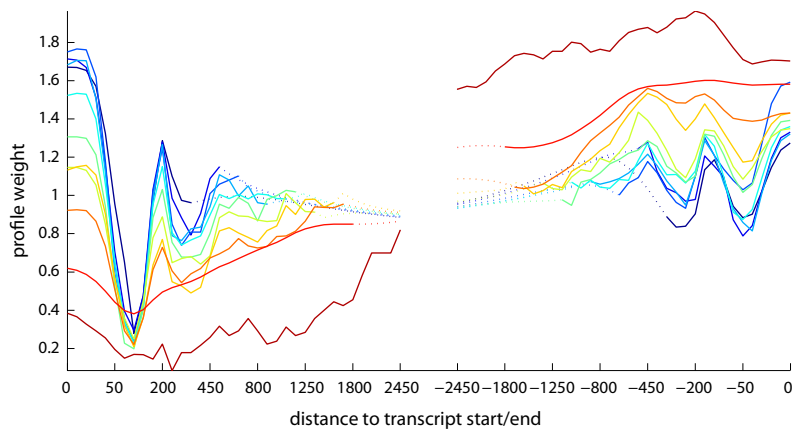
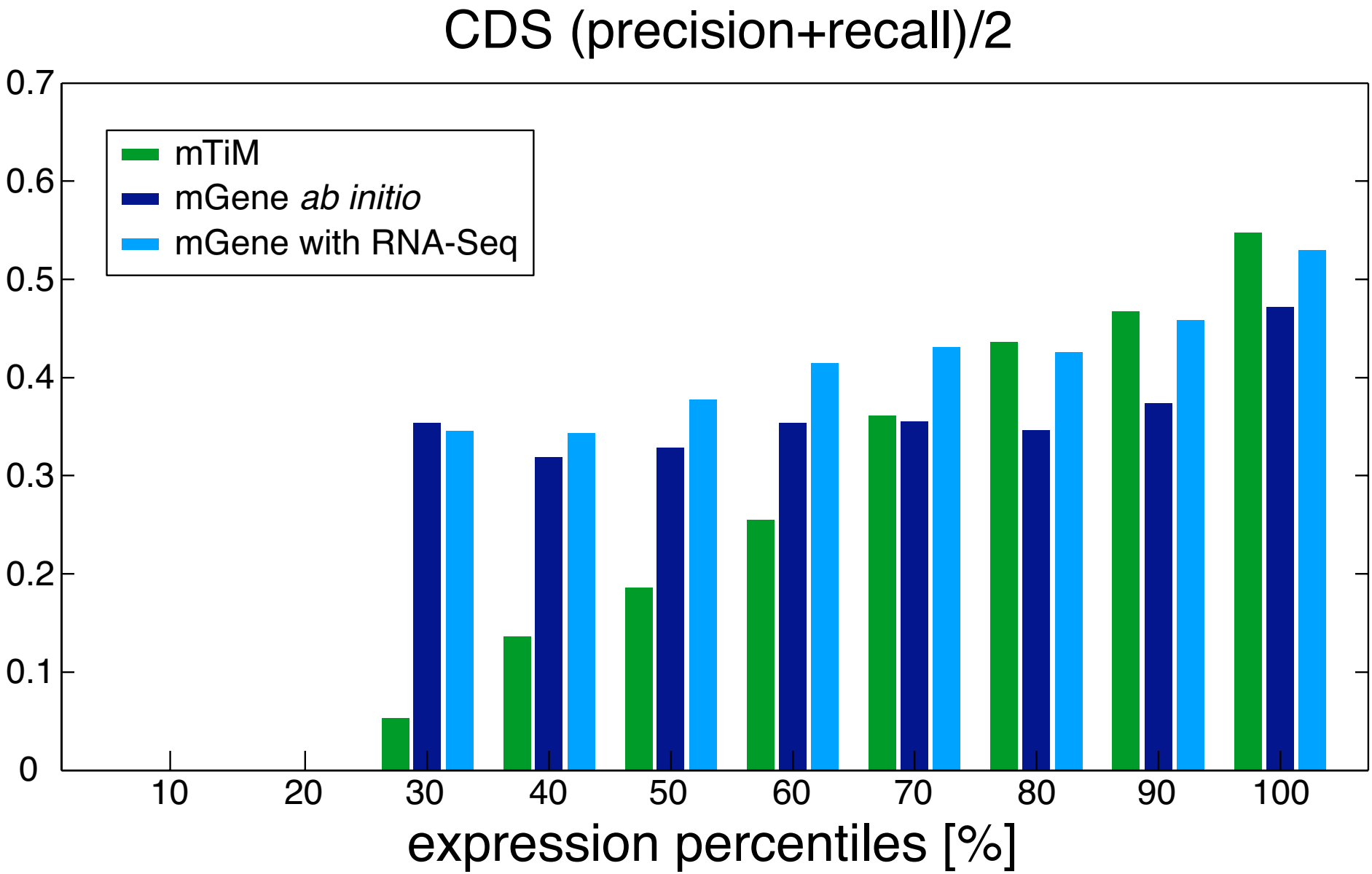
3. Repeat 1. and 2. until convergence.





Preliminary Evaluation I



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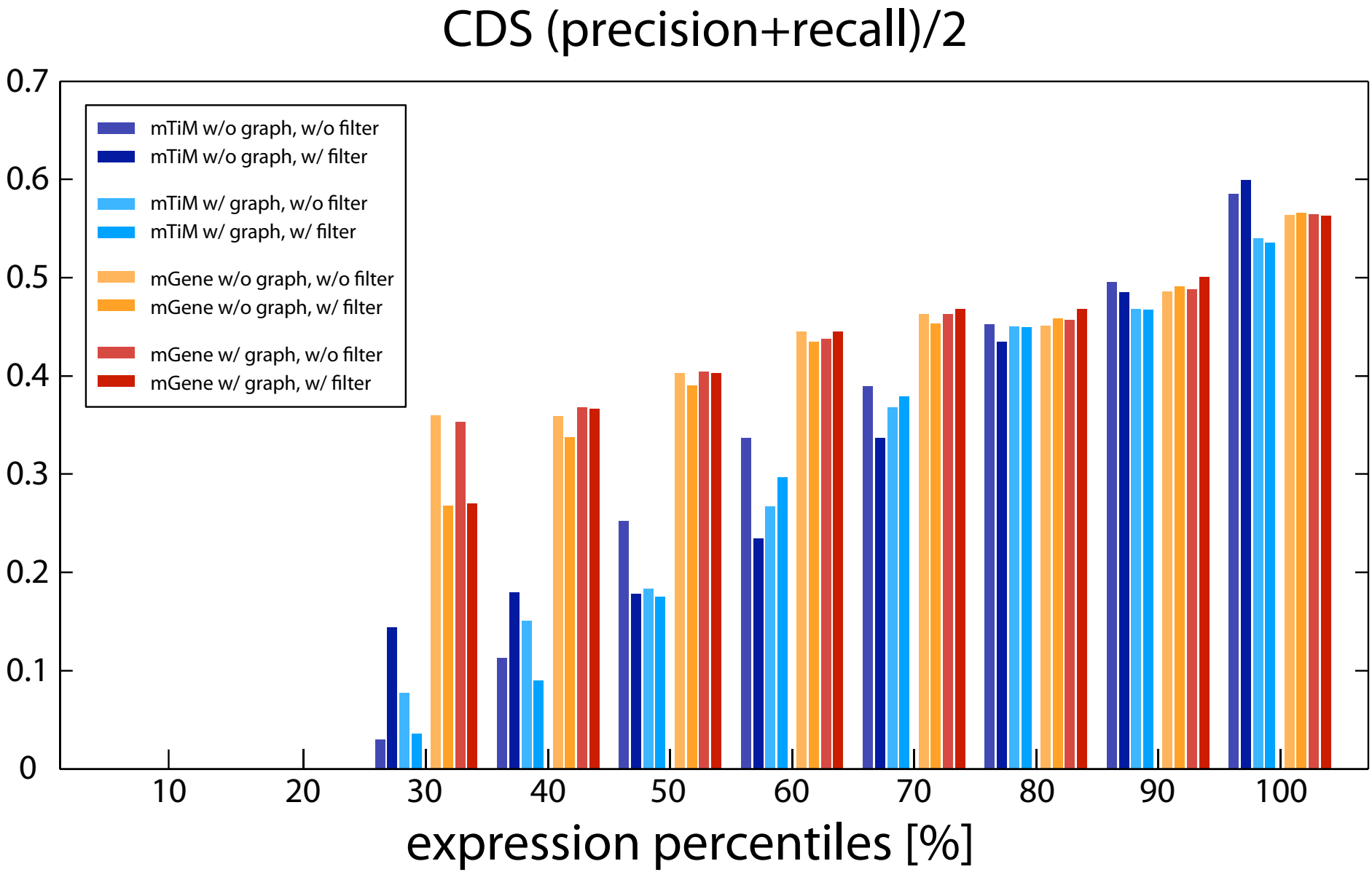

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Acknowledgements

RGASP Team

- Jonas Behr (FML)
- Georg Zeller (FML & MPI)
- Regina Bohnert (FML)

Funding by DFG &
Max Planck Society.

Thank you for your attention.

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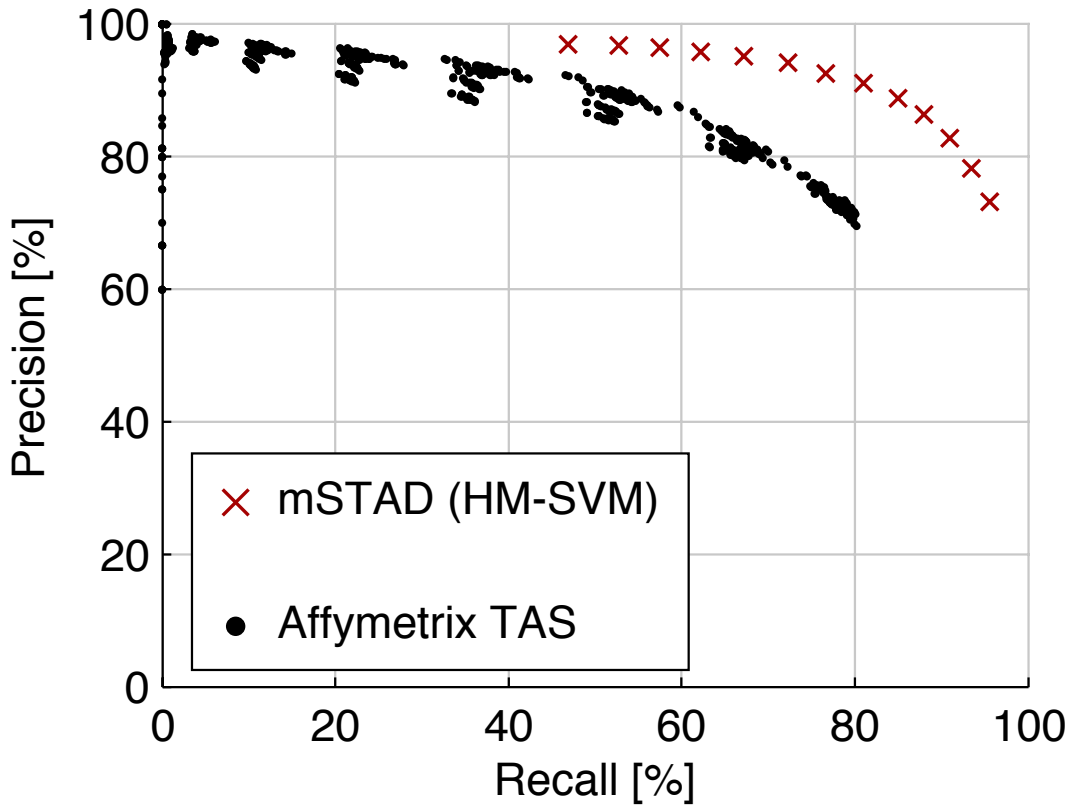
Method Comparison



Substantially improved exon probe recognition over the most widely used "transfrag" method

www.fml.mpg.de/raetsch/research/tiling/

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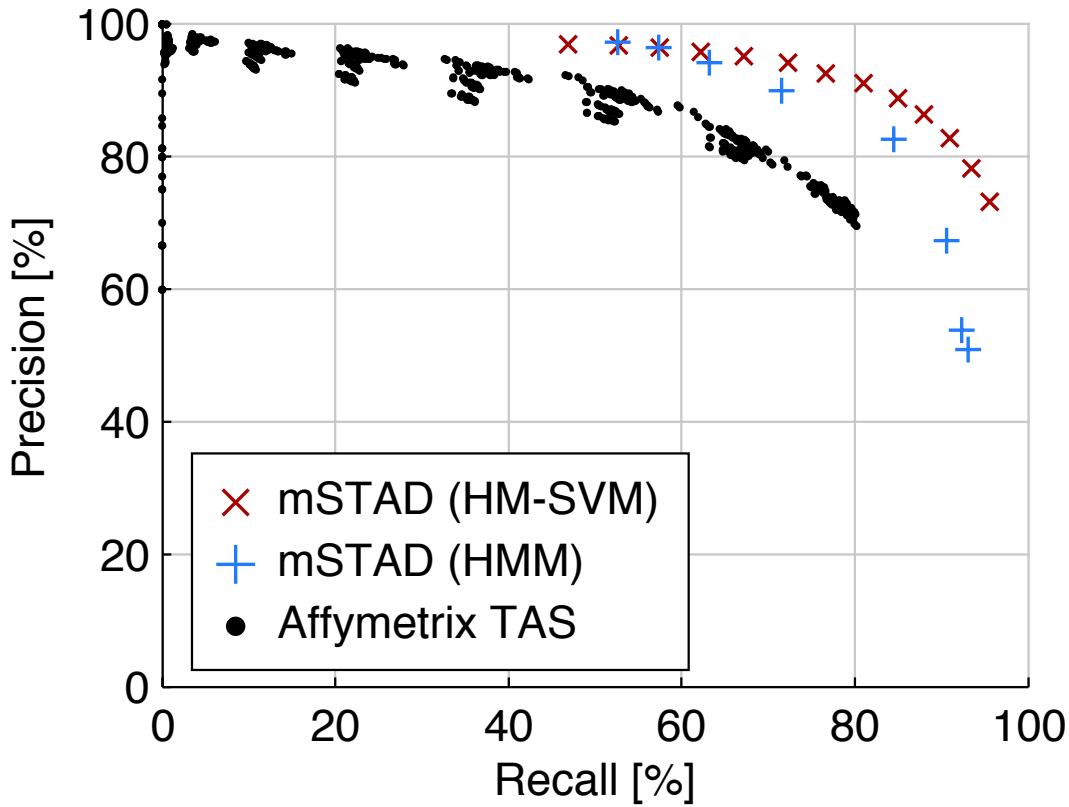

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Priming and Fragmentation Biases

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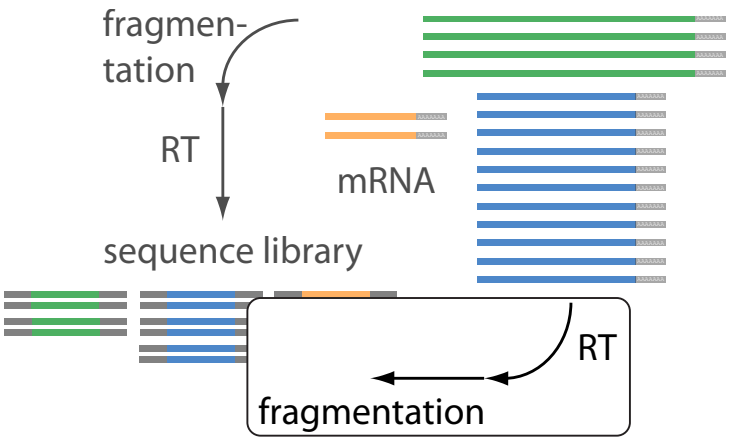

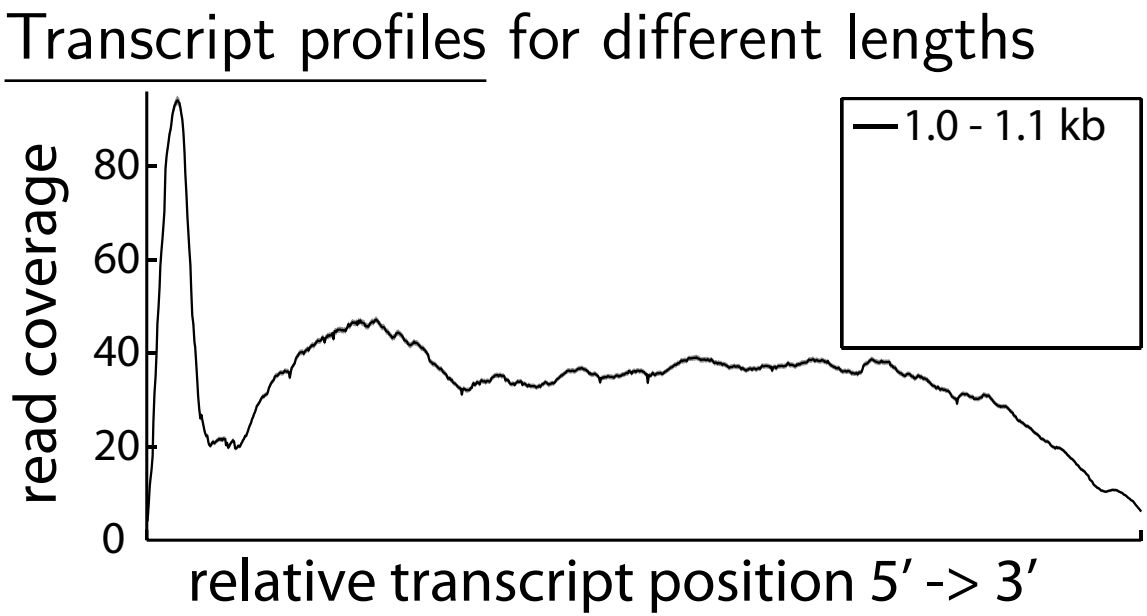

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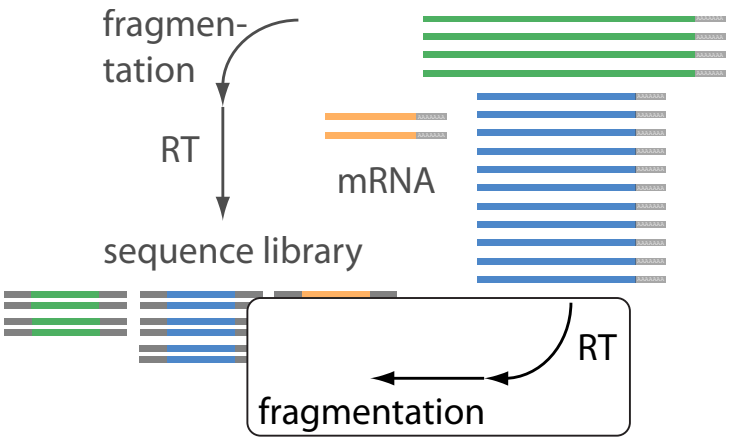

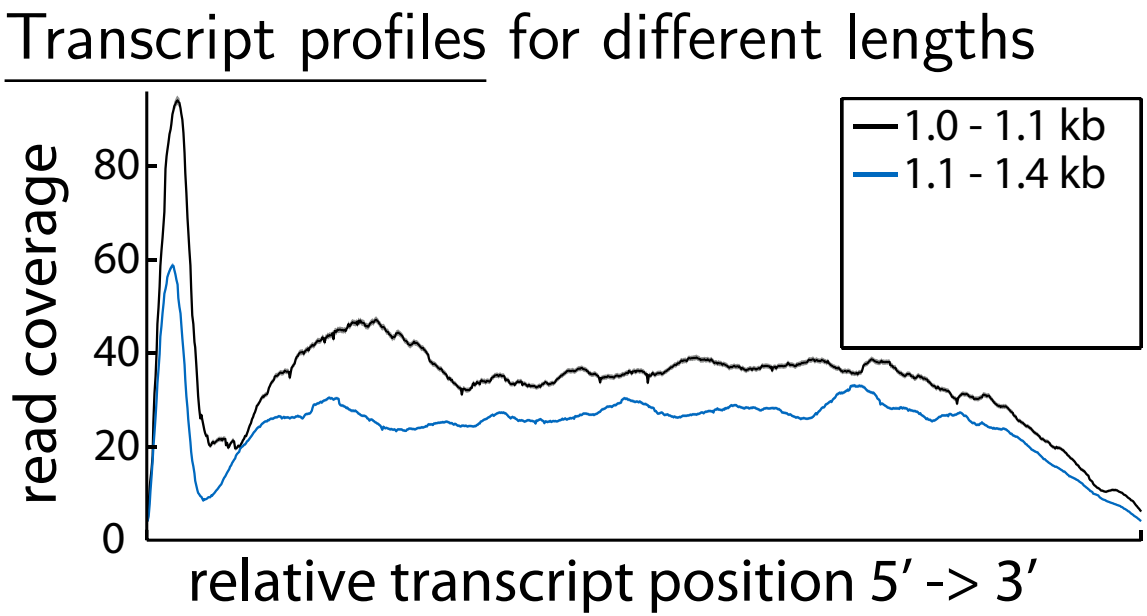

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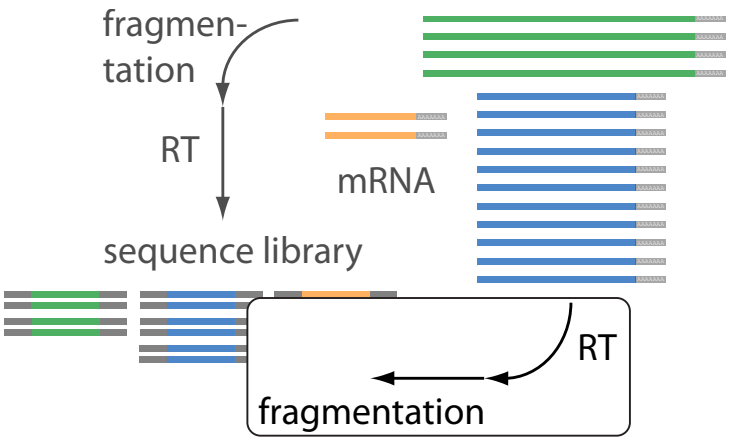

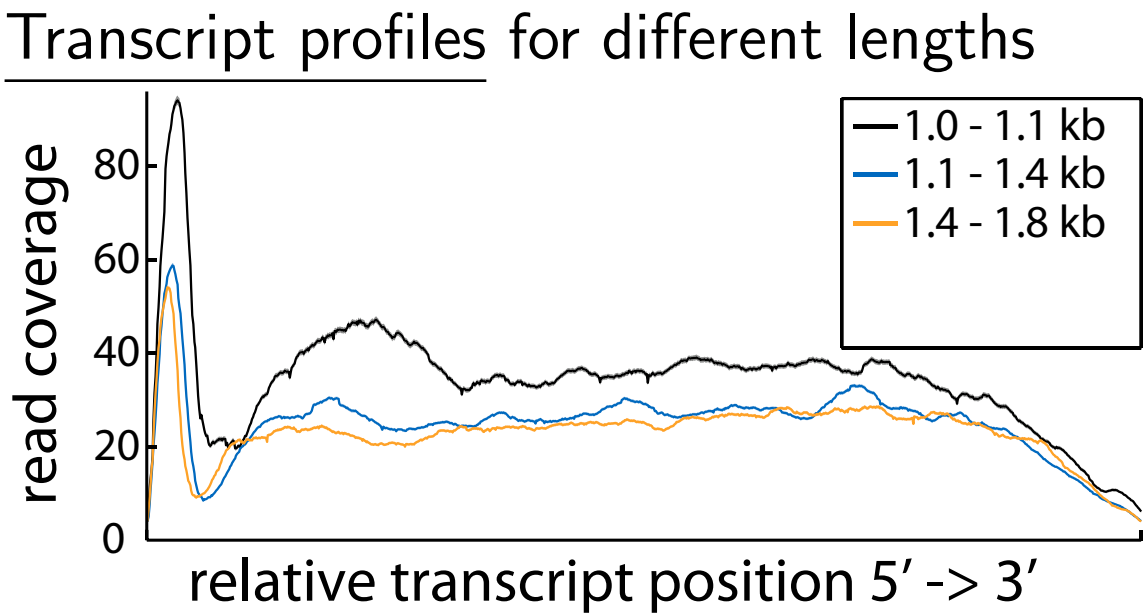

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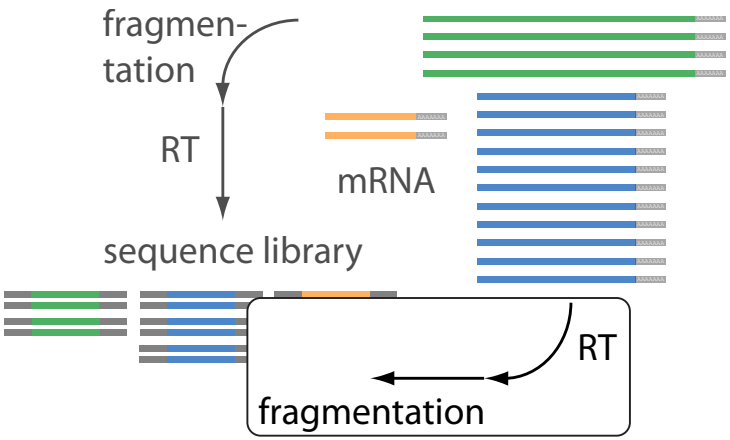

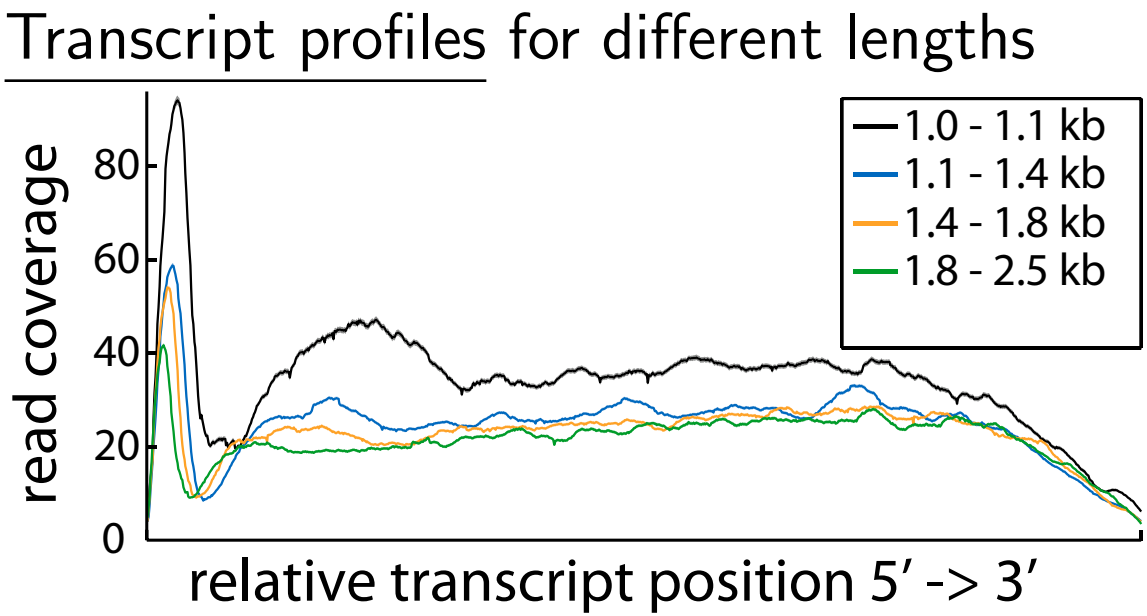

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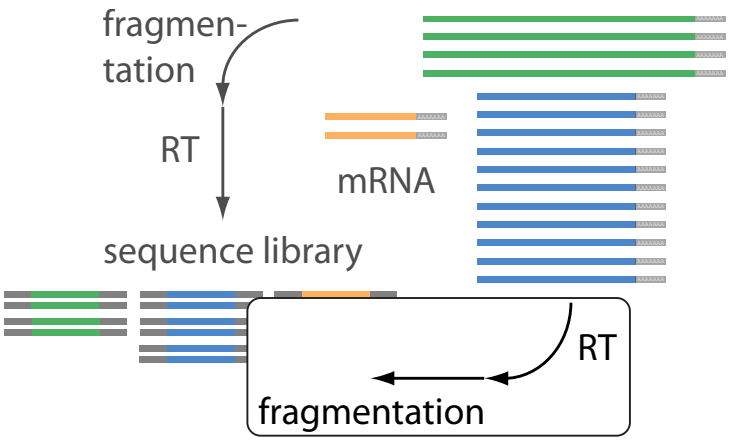

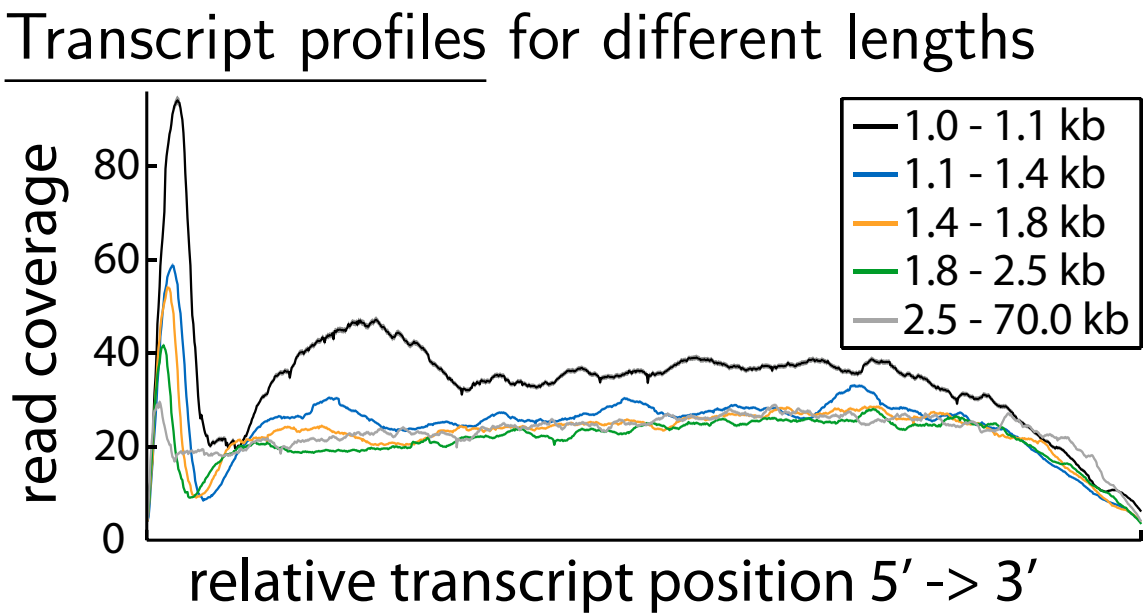

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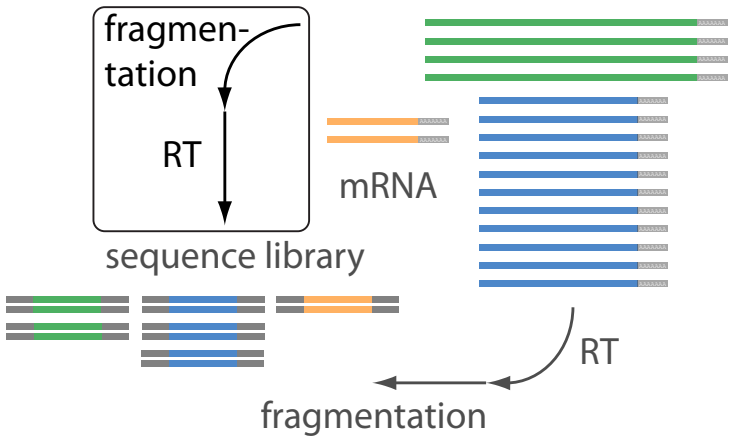

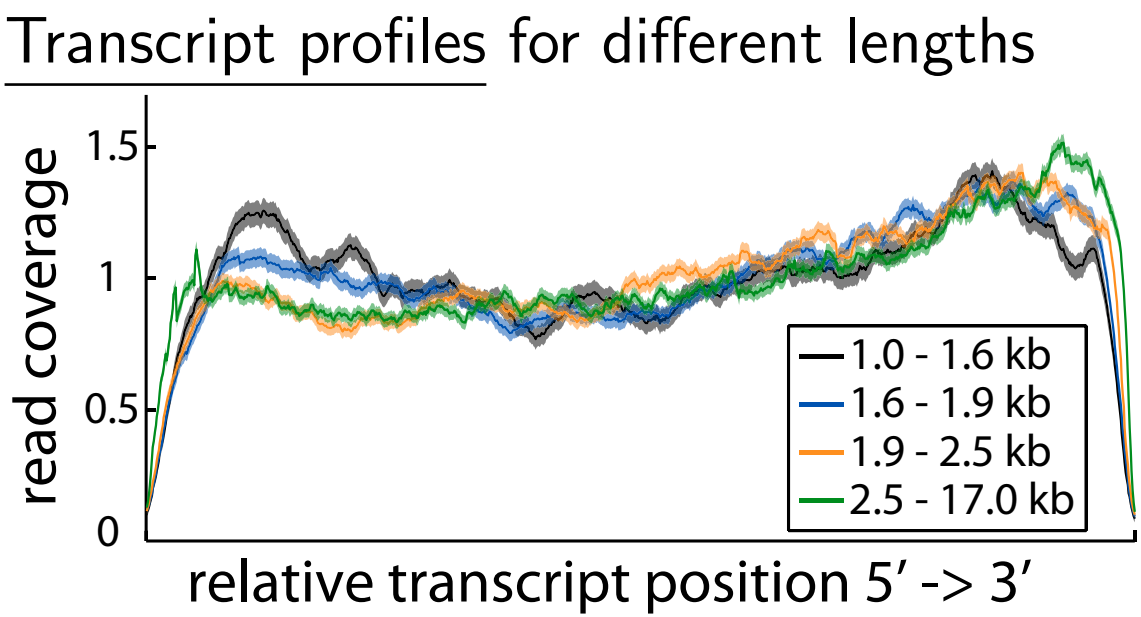

RNA-Seq data (*A. thaliana*, D. Weigel's Lab, MPI Tübingen)

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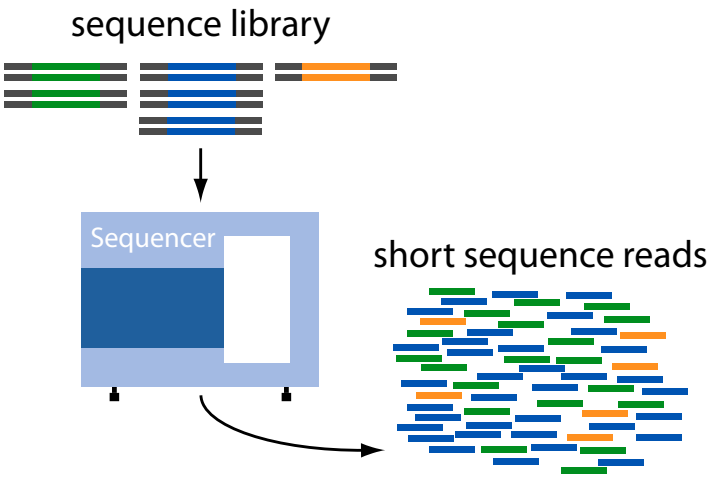
Sequence Bias



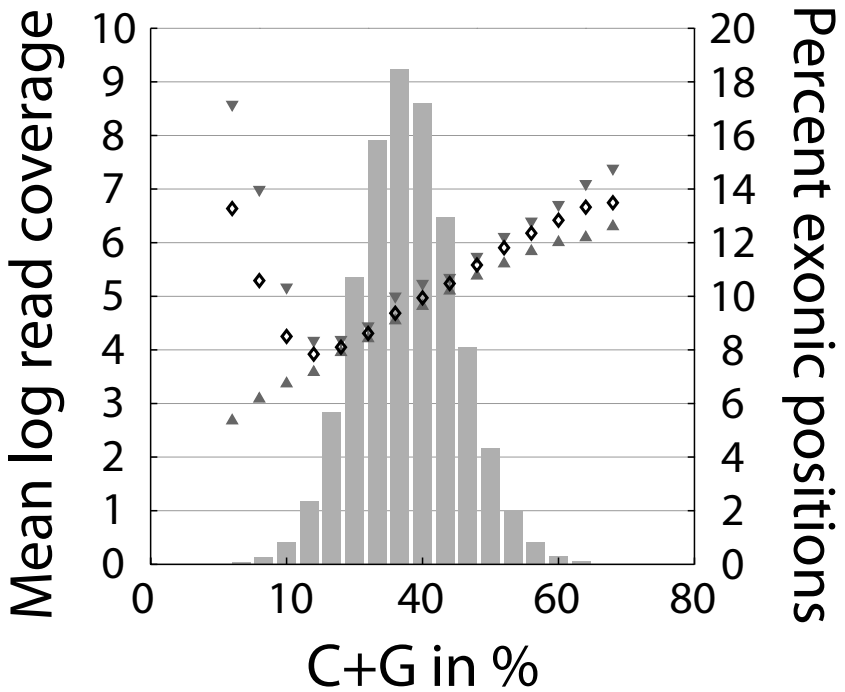
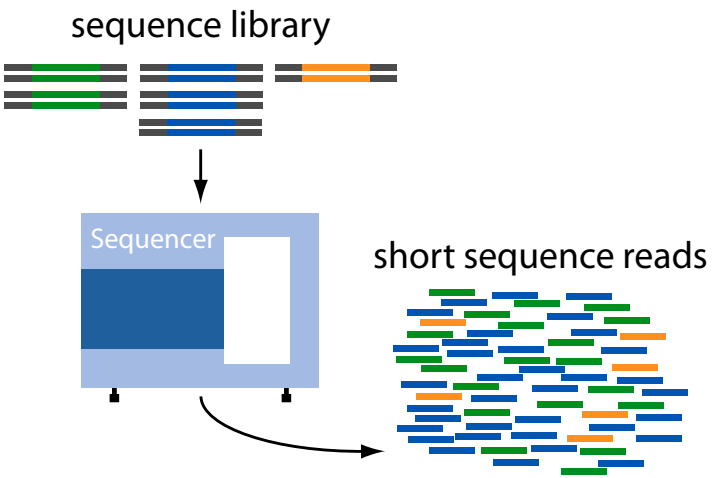


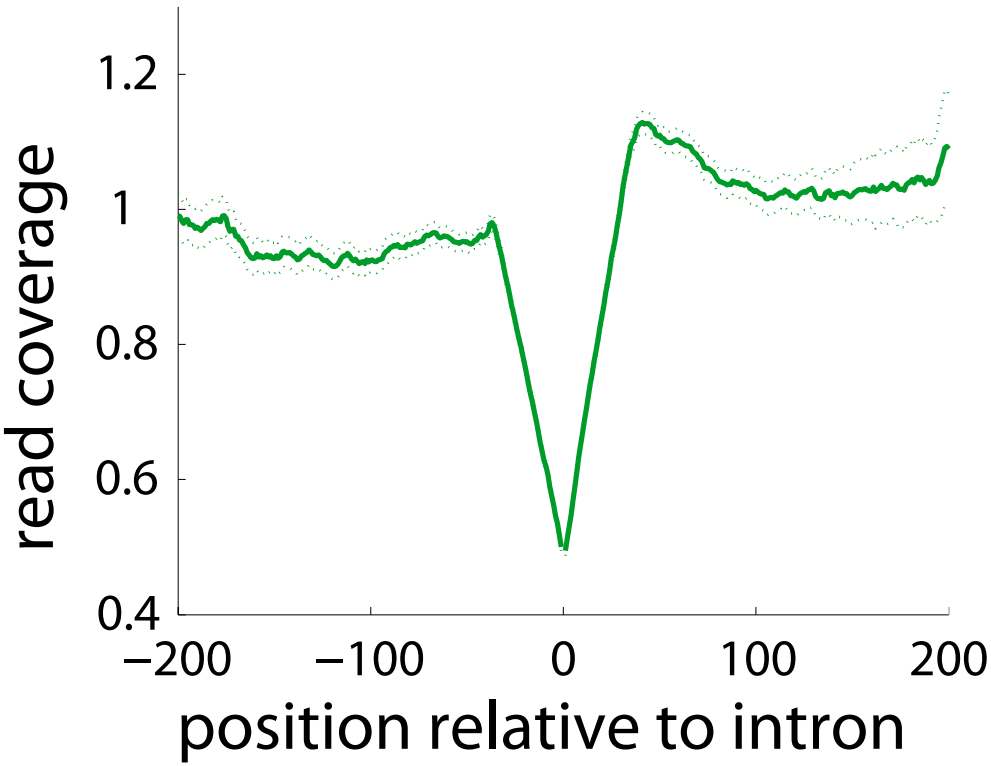
Figure provided by Georg Zeller

RNA-Seq data (*A. thaliana*, D. Weigel's Lab, MPI Tübingen)

- Exonic GC content
- Dinucleotides at the boundaries (Linsen et al., 2009)

Read Mapping Bias





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Our method rQuant: Position-wise, with profiles

(estimating library and mapping bias)

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Evaluation: Spearman correlation between

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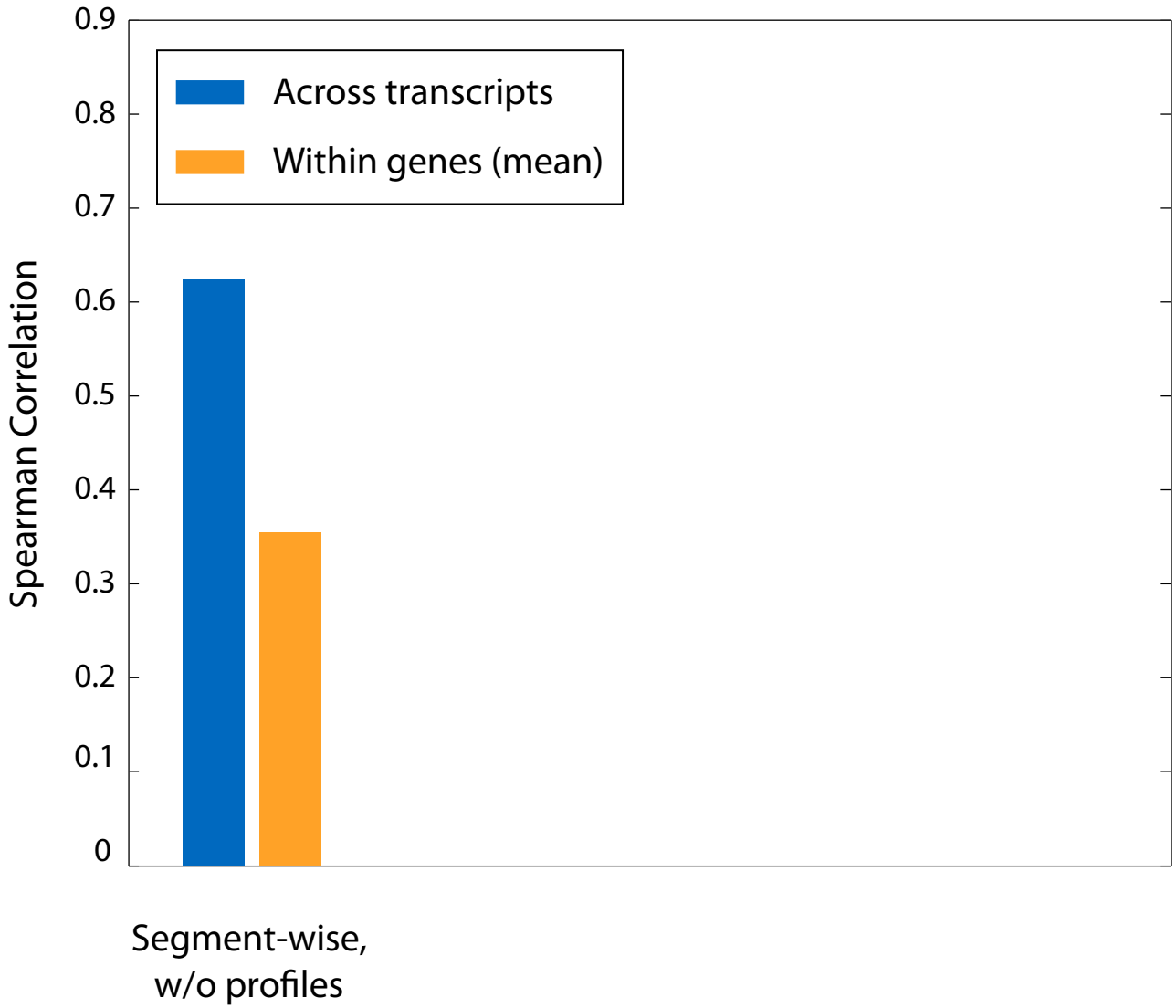

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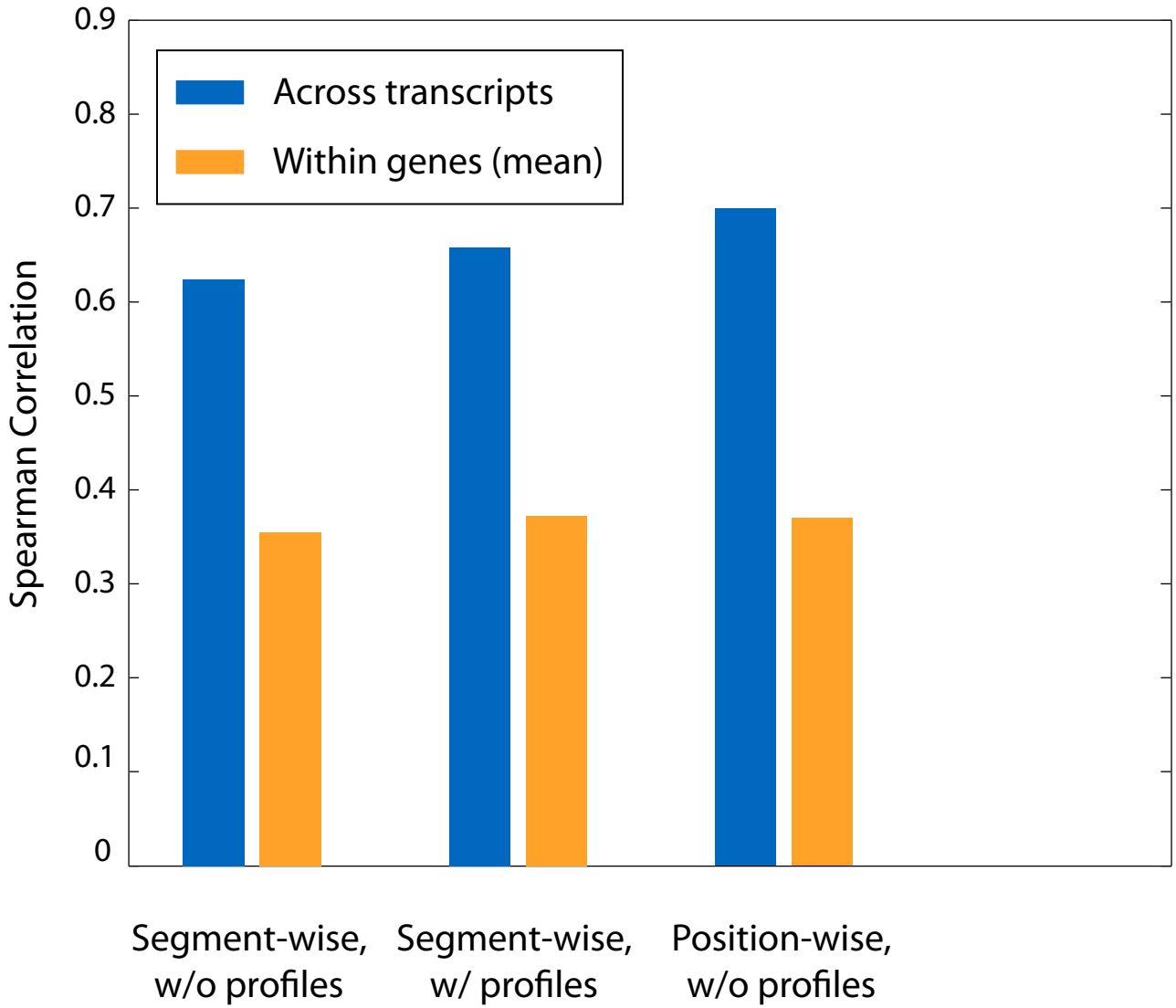

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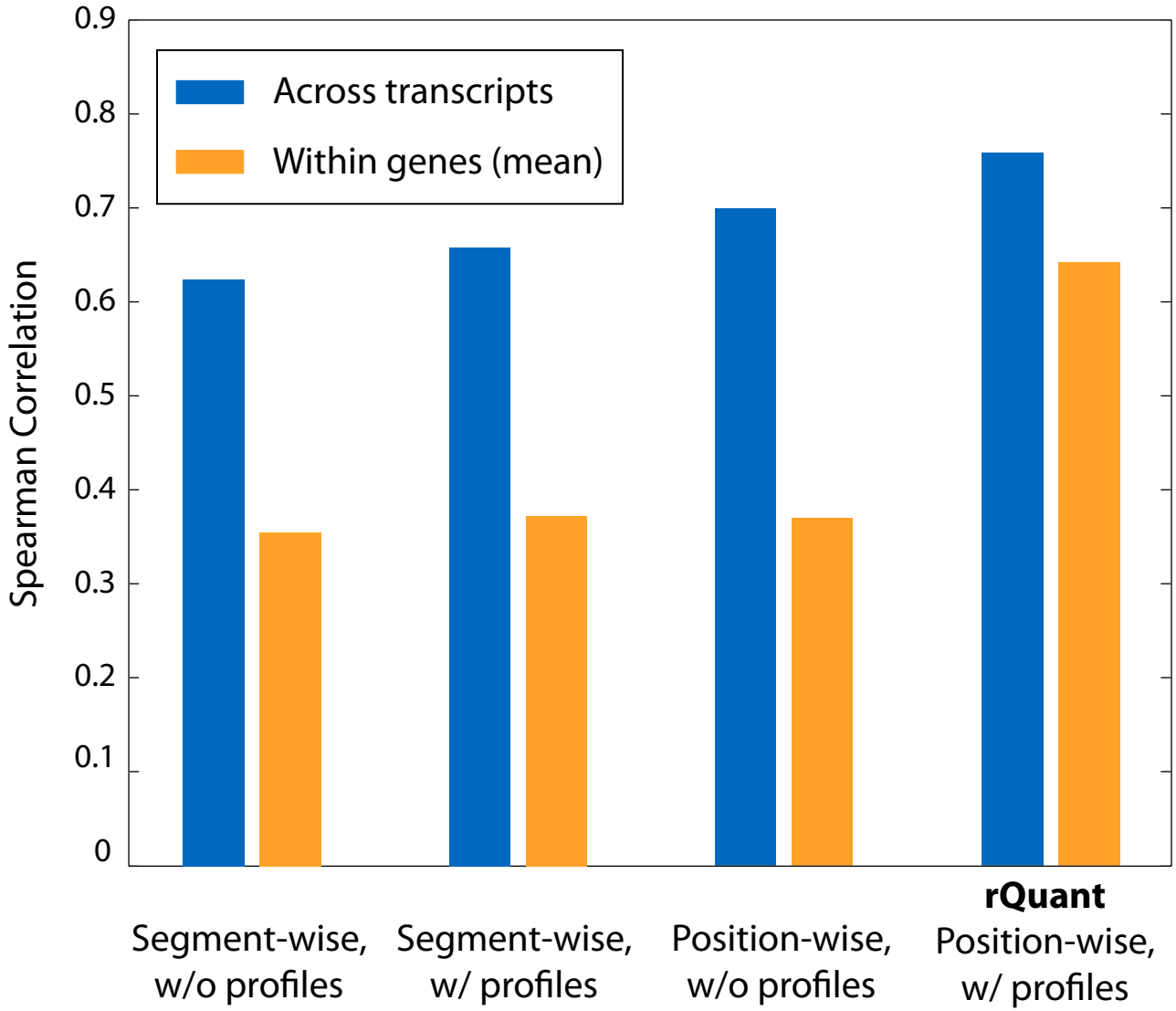

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