

Retained introns are translated and contribute antigens to the MHC I immunopeptidome

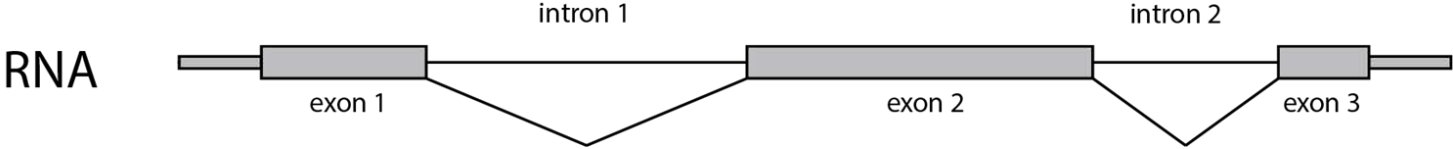
Sarah Chen

PRIMES Computational Biology 2019

Central Dogma



↓
Transcription

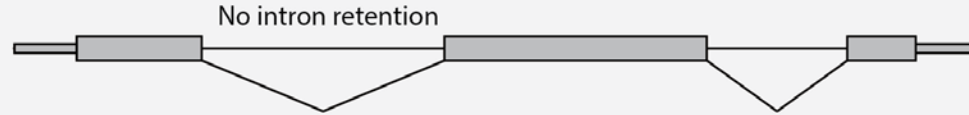


↓
Translation

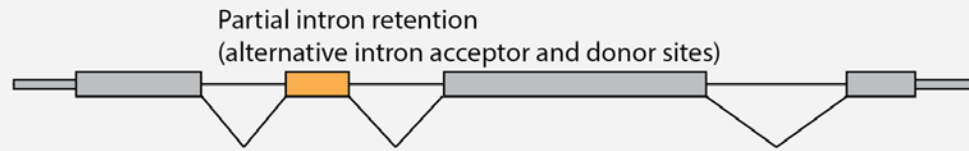
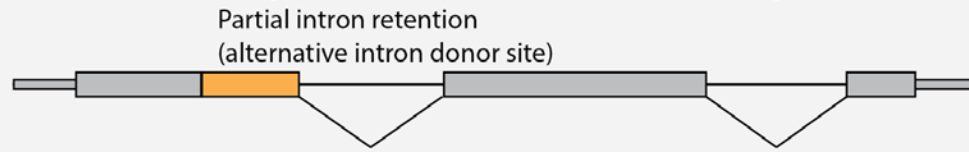
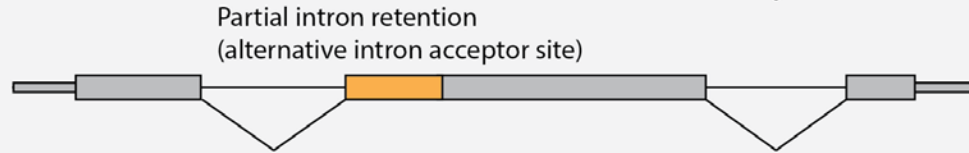
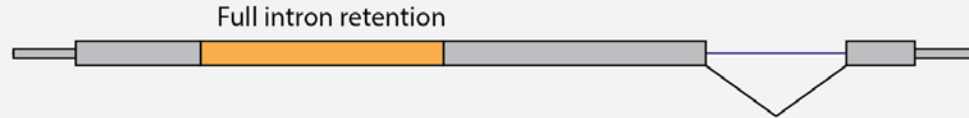


Types of Intron Retention

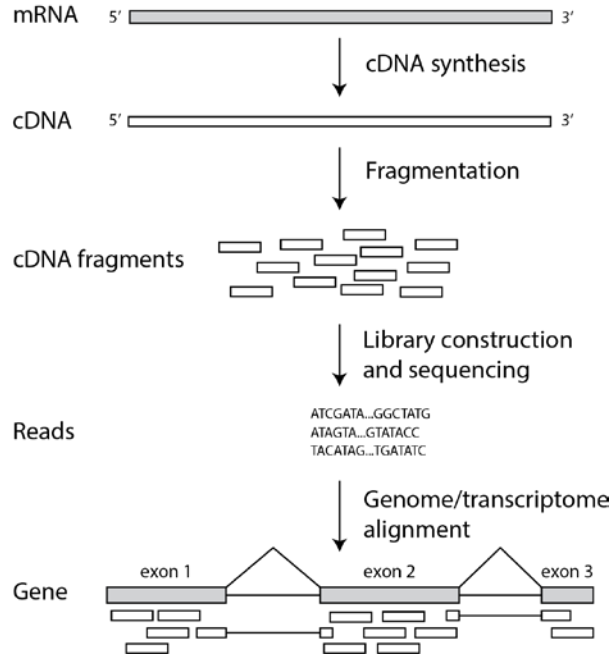
mRNA (canonically spliced):



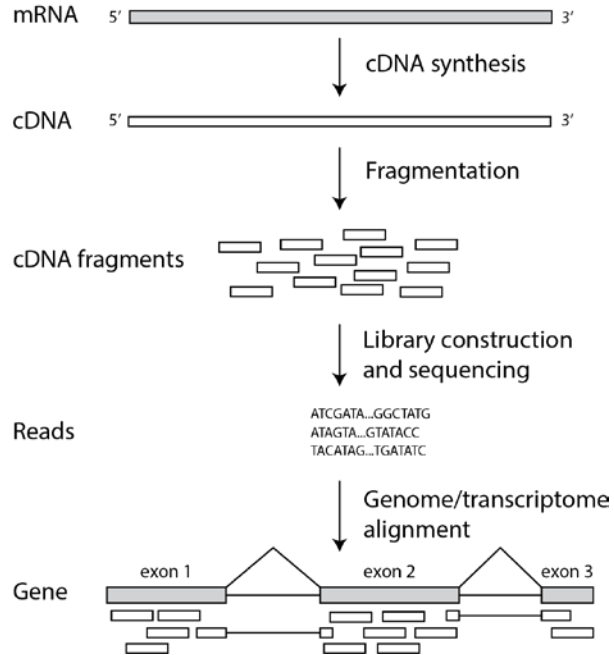
mRNA (with retained intron):



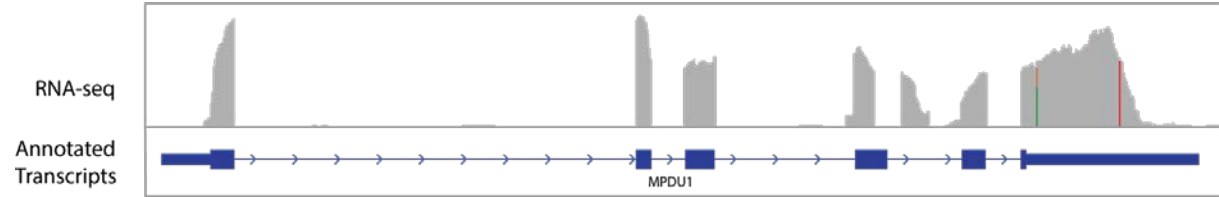
RNA-seq data can distinguish exons and introns



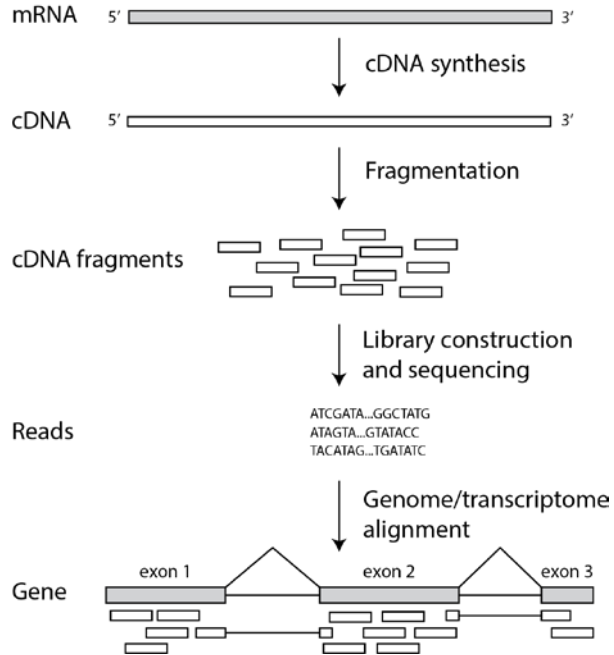
RNA-seq data can distinguish exons and introns



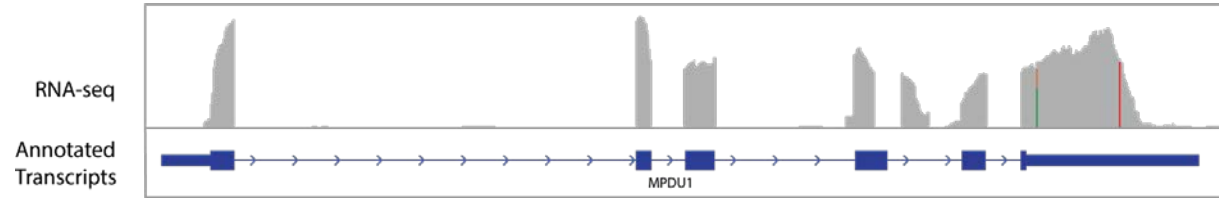
Canonical splicing:



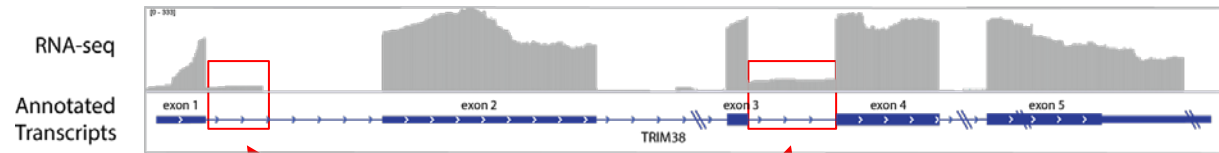
RNA-seq data can distinguish exons and introns



Canonical splicing:

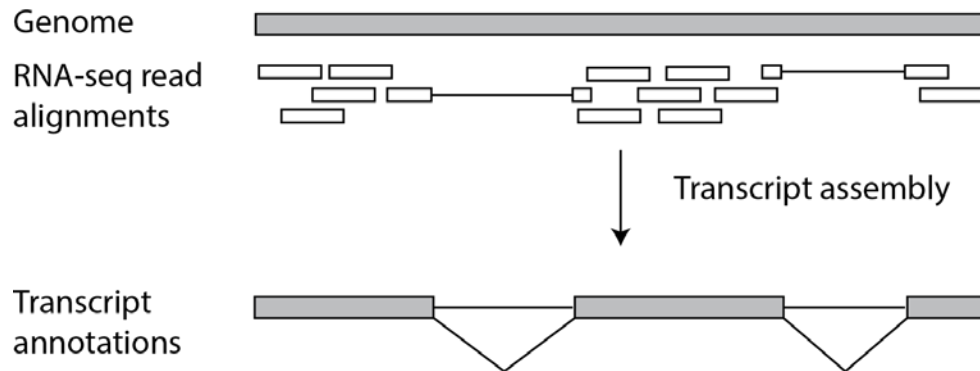


Potential Intron Retention:

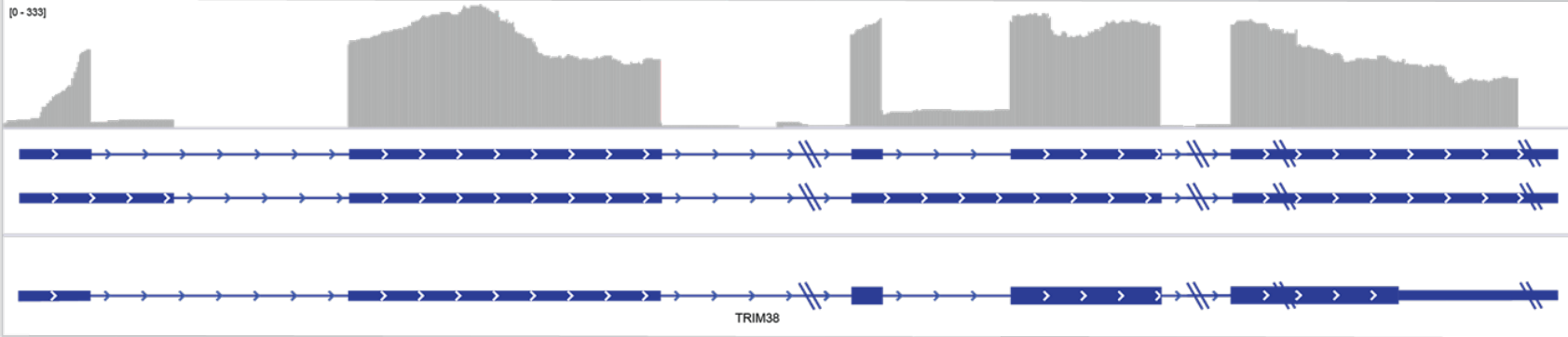
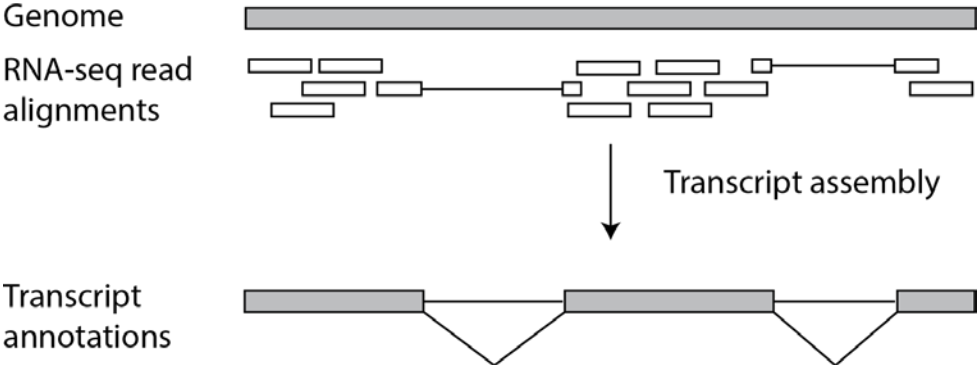


Intronic RNA-seq read support

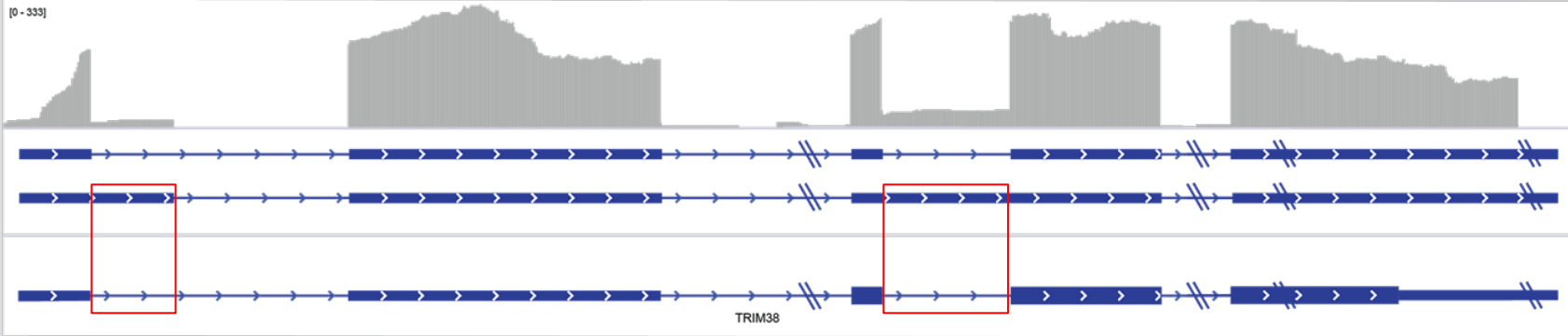
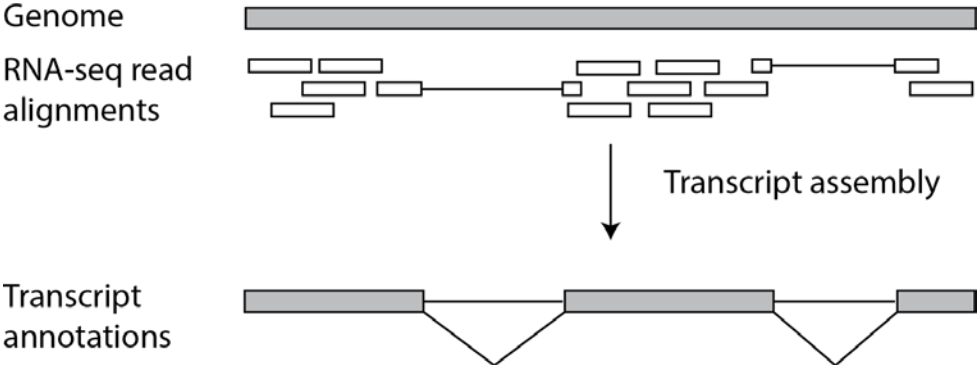
StringTie Transcript Assembly Can Predict Retained Introns



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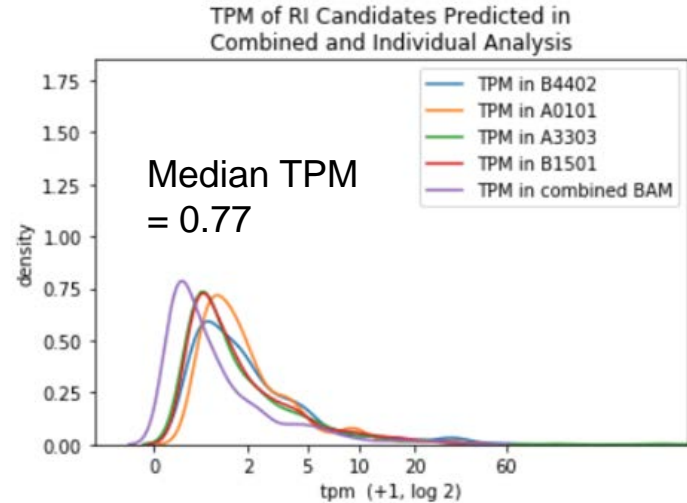
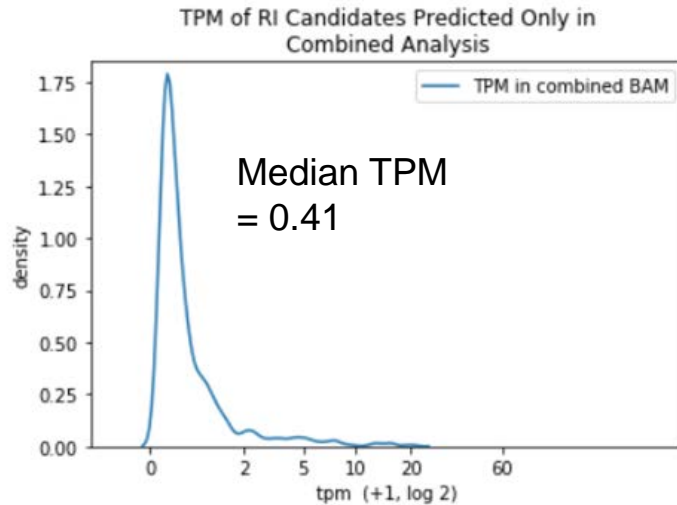


StringTie Transcript Assembly Can Predict Retained Introns



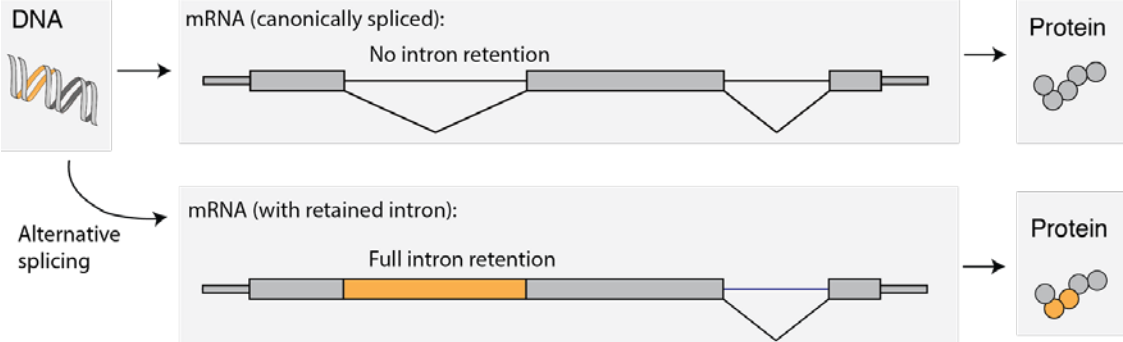
Retained Intron Prediction

- Method applied to 4 B721 data samples treated as replicates, evaluated individually and in combination - **1799** retained introns predicted overall



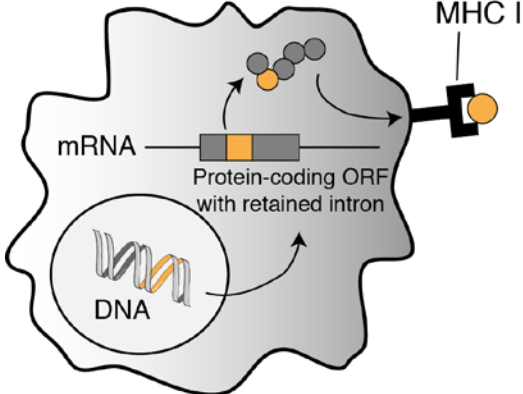
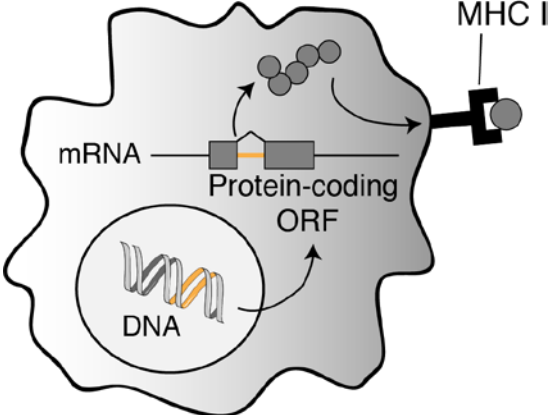
- Combined analysis increases sensitivity of retained intron prediction to lowly expressed candidates

Retained Introns Can Be Translated and Presented by MHC I

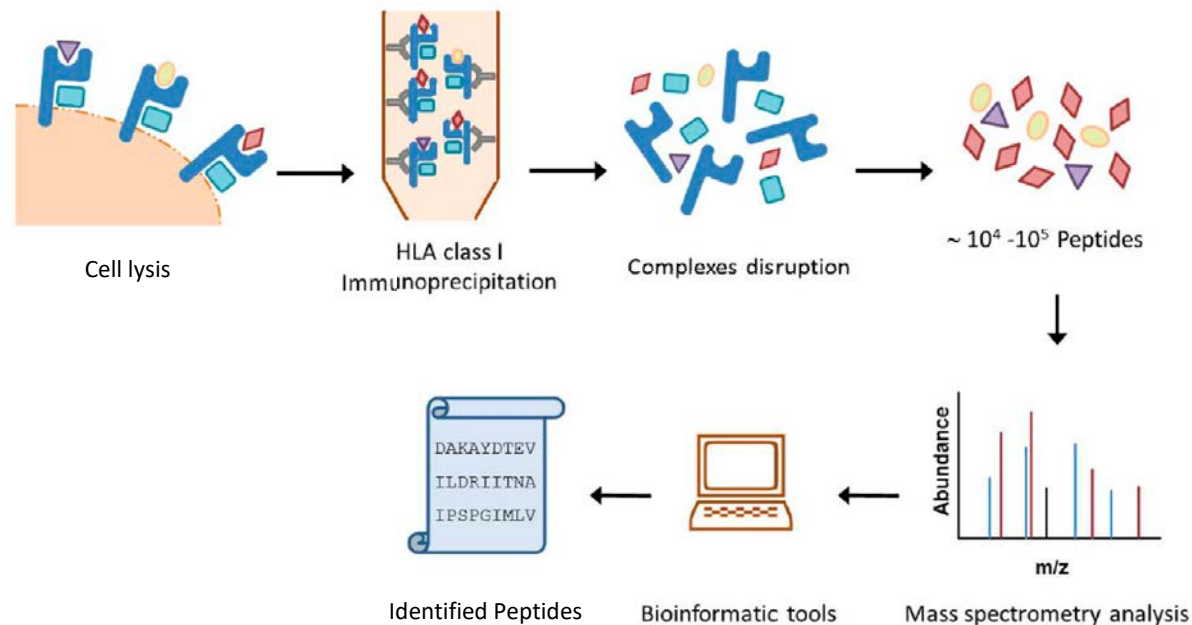


Canonical Splicing

Retained Intron

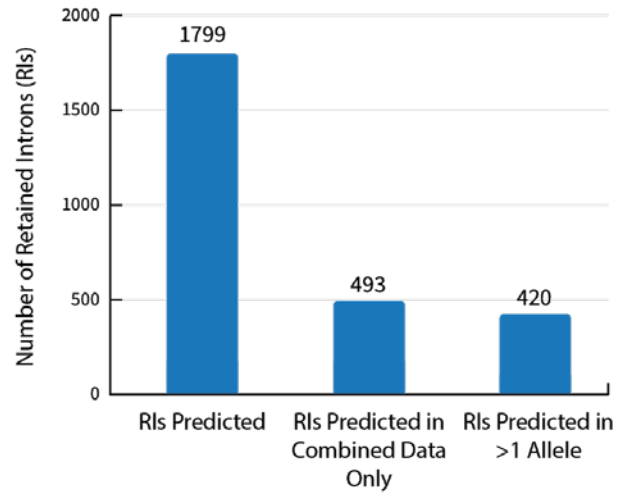


Validating Retained Intron Candidates with Mass Spectrometry (MS)



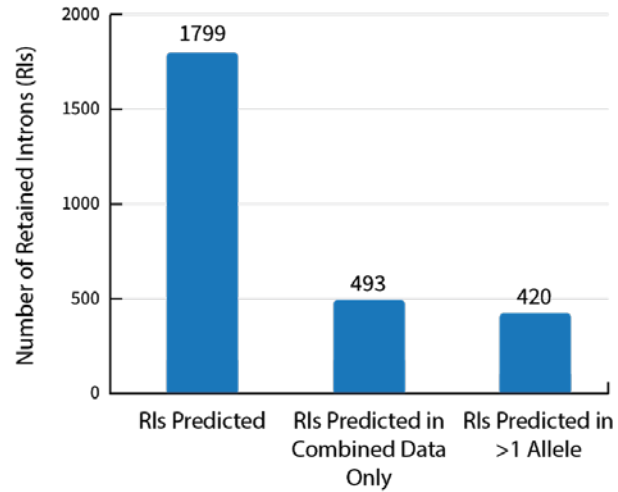
Retained Introns Validated by MS

Retained Introns Predicted Across Replicates

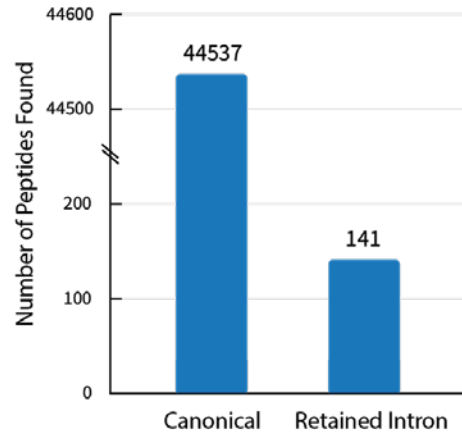


Retained Introns Validated by MS

Retained Introns Predicted Across Replicates

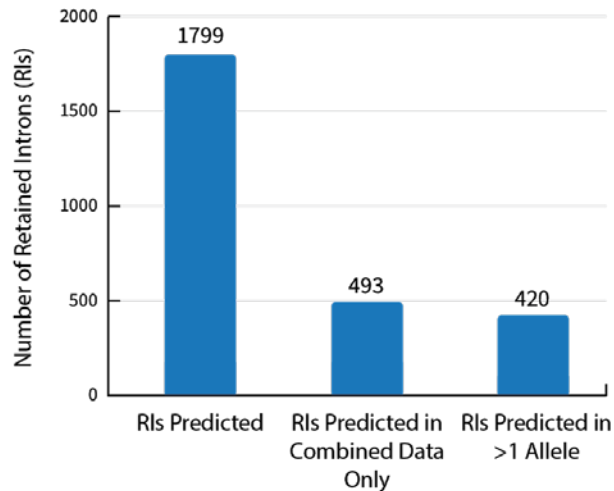


Number of Peptides Found by MS Matching Canonical and Retained Intron Sequences

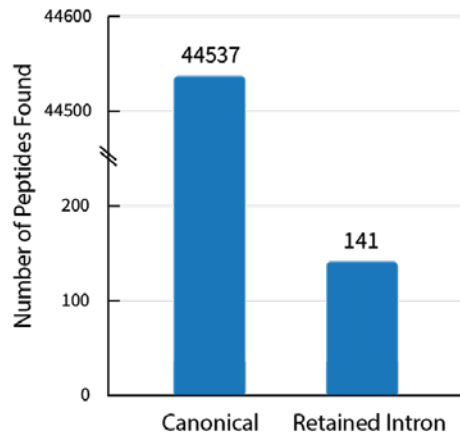


Retained Introns Validated by MS

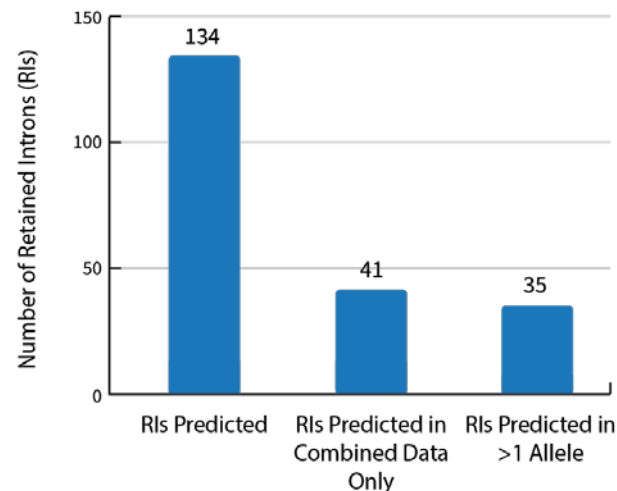
Retained Introns Predicted Across Replicates



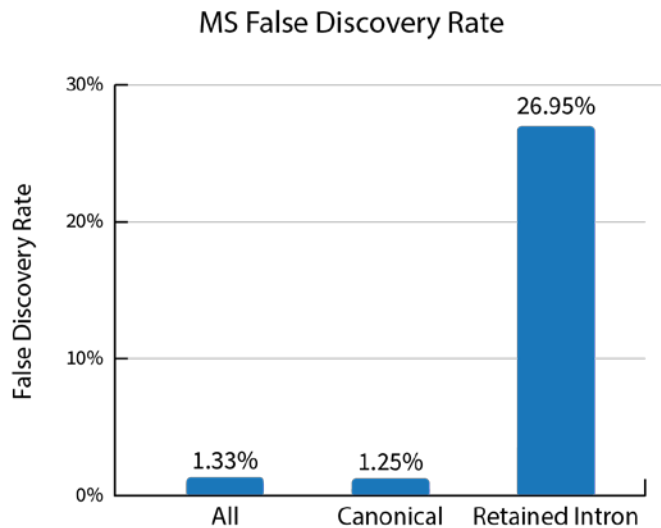
Number of Peptides Found by MS Matching Canonical and Retained Intron Sequences



Retained Introns Supported by MS Predicted Across Replicates

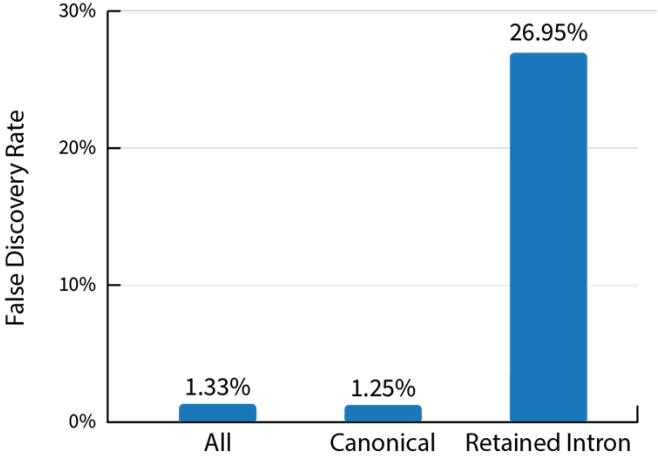


MS False Discovery Rate and Search Space Size

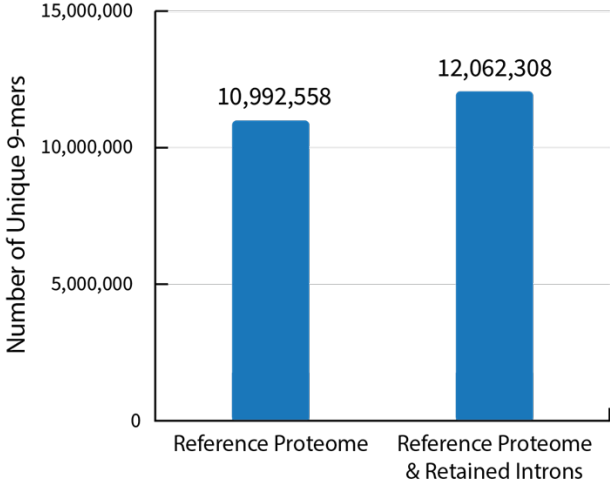


MS False Discovery Rate and Search Space Size

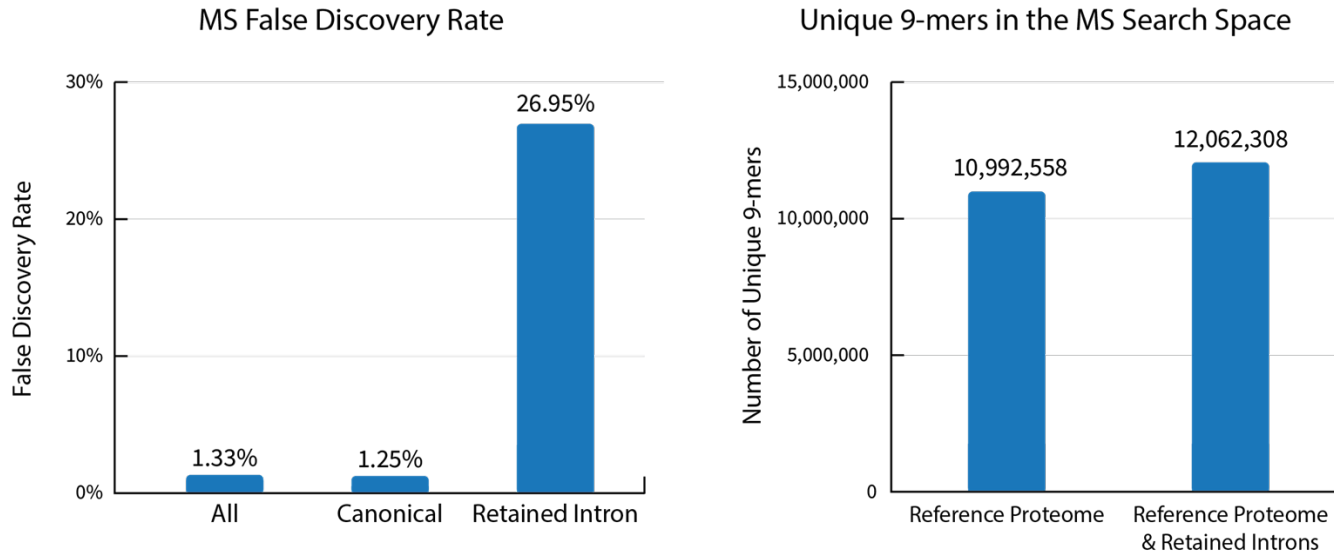
MS False Discovery Rate



Unique 9-mers in the MS Search Space



MS False Discovery Rate and Search Space Size

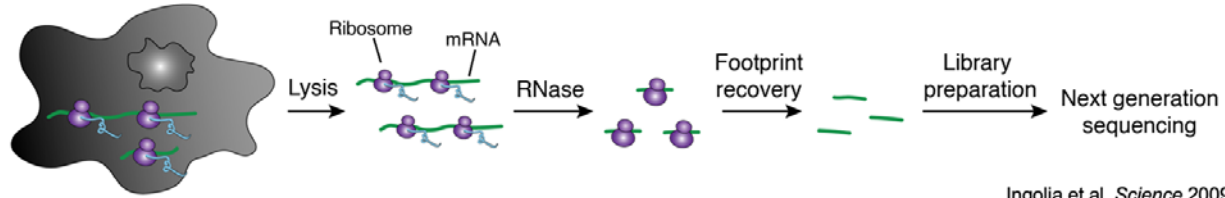


Ribo-seq has the potential to improve the retained intron identification process

- Reducing the number of predictions → higher % of validated predictions
- Decreasing the search space → decreasing FDR.

Seeking better predictions with Ribo-seq

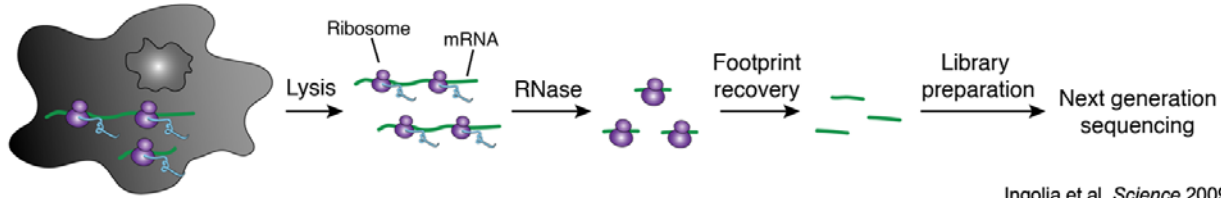
Ribo-seq provides additional information about translation of genome sequences



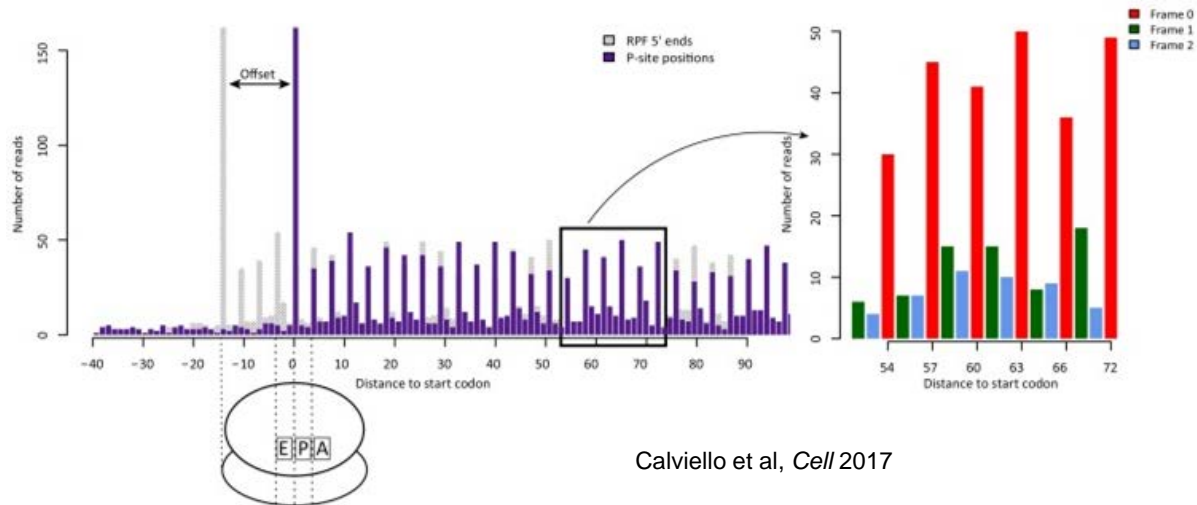
[Ingolia et al, Science 2009](#)

Seeking better predictions with Ribo-seq

Ribo-seq provides additional information about translation of genome sequences

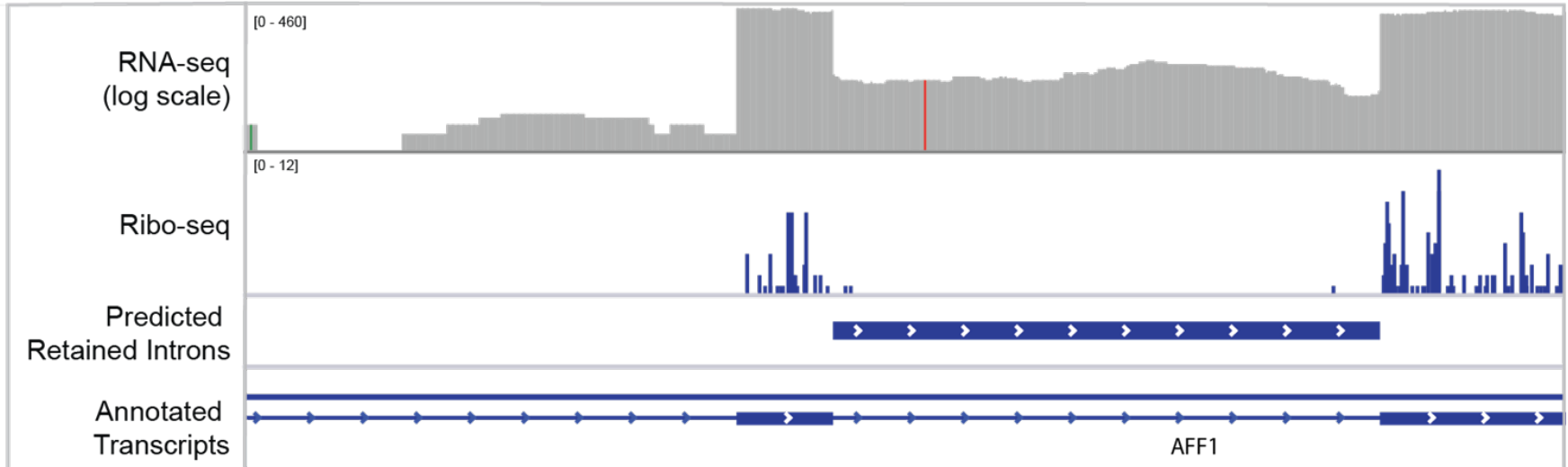


Schematic of aligned Ribo-seq reads:



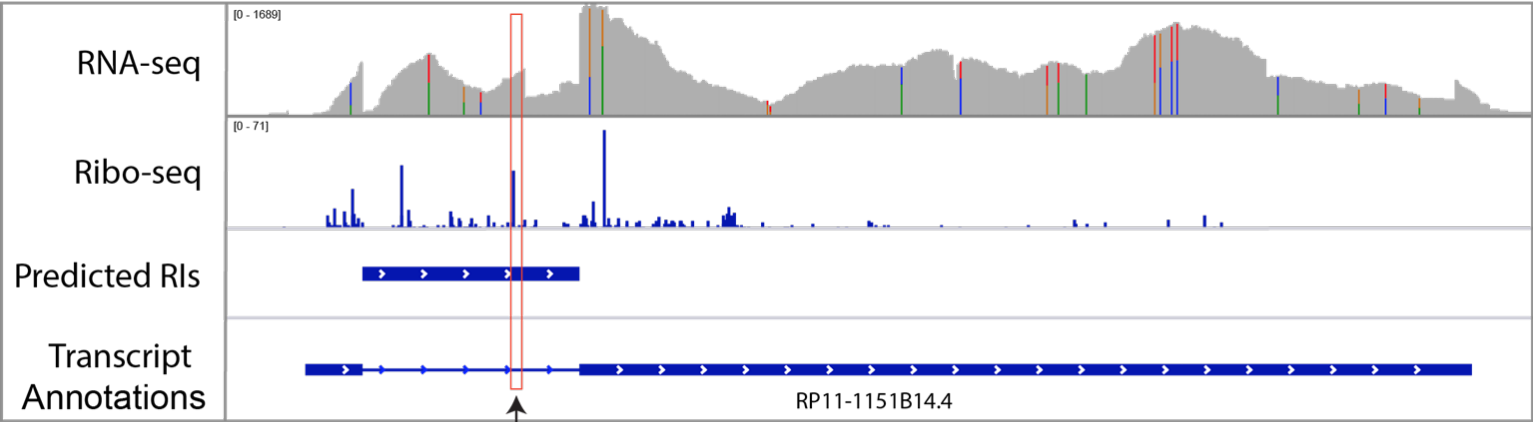
Ribo-seq can distinguish translated and untranslated transcripts

A retained intron supported by RNA-seq but not by Ribo-seq or mass spectrometry:

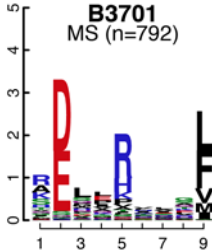


Ribo-seq can distinguish translated and untranslated transcripts

A retained intron supported by RNA-seq, Ribo-seq, and mass spectrometry:



Found Peptide: WDLQRTLGE



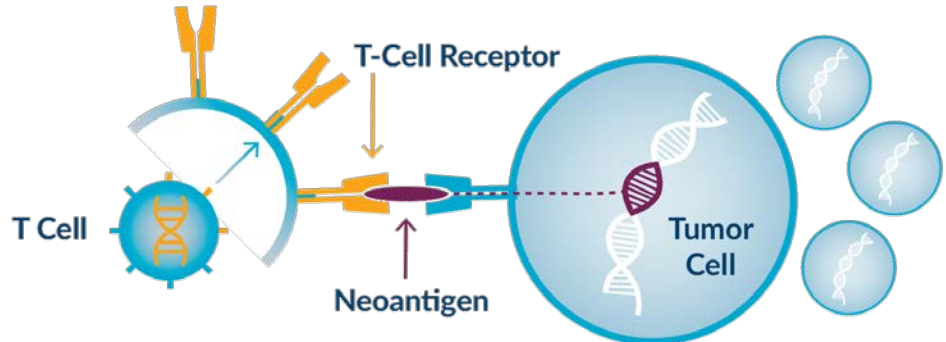
Summary & Future Directions

What we have done:

- *De novo* assembly from RNA-seq → 1799 retained introns predicted
- 141 peptides found by MS that support 134 retained introns
- Compared RNA-seq and Ribo-seq support for retained introns

Next steps:

- Continuing to explore potential of Ribo-seq for RI prediction
- Application to cancer data



Acknowledgements



Aviv Regev



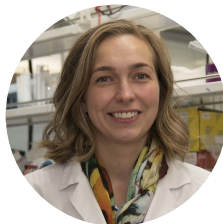
Slava Gerovitch



Brian Haas



Karl Clauser



Tamara Ouspenskaia



Travis Law



Phillips Academy
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