Annotation preprocessing improves the detection of genetic effects on transcript usage

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6 December 2017

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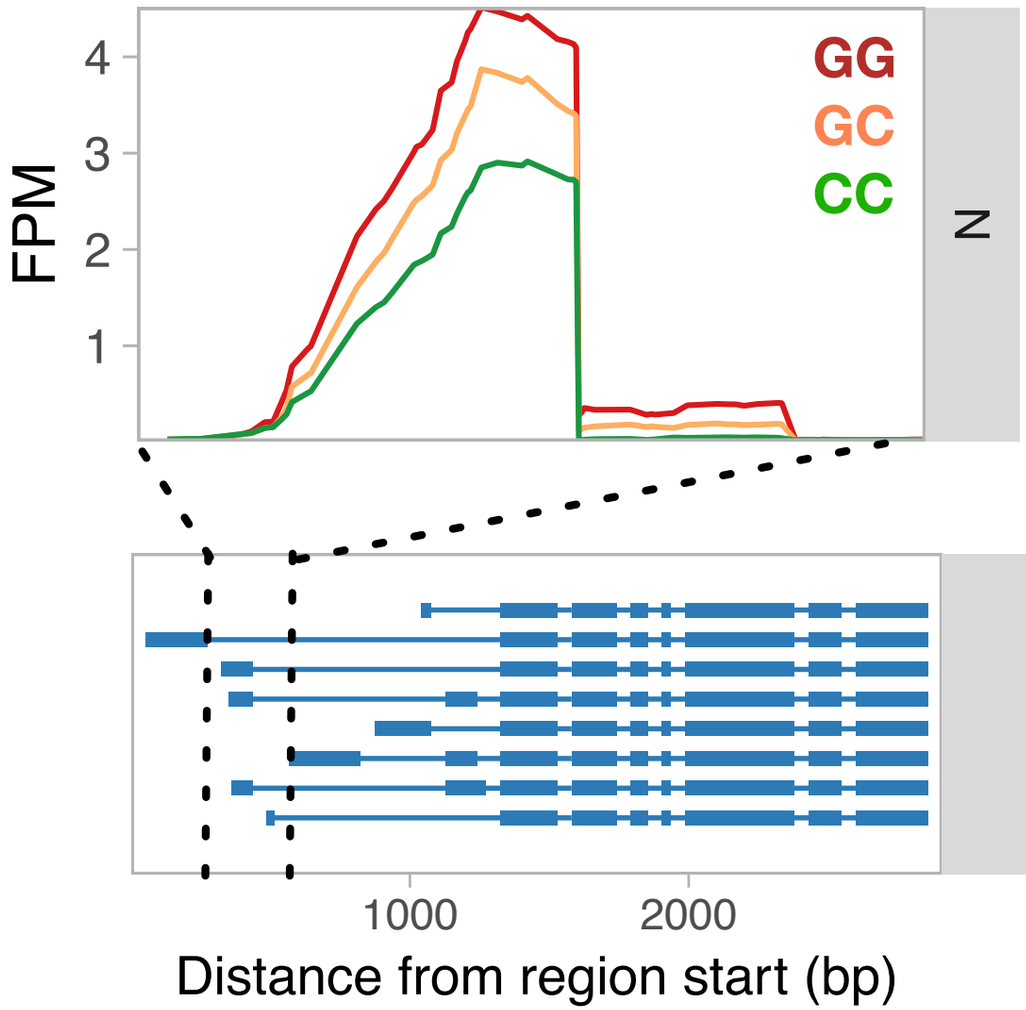
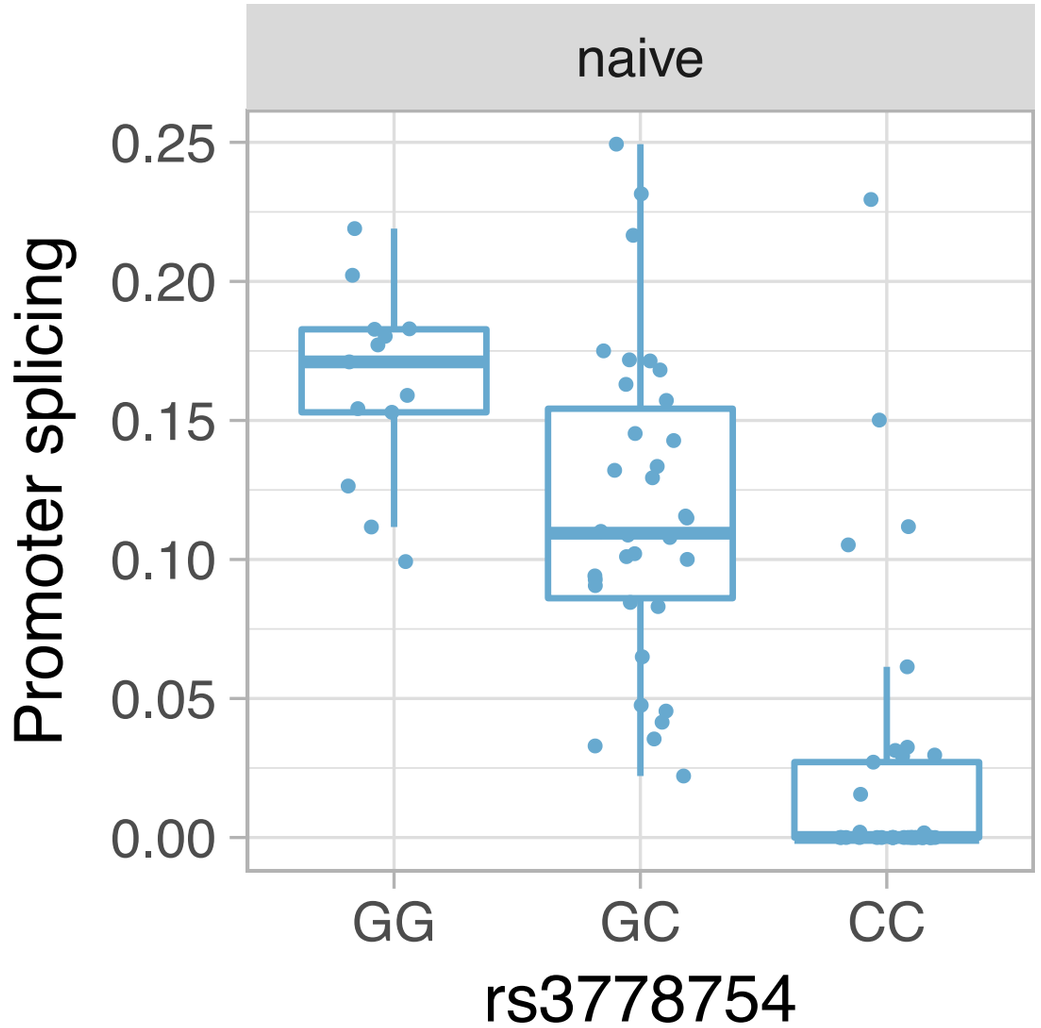
http://kauralasoo.net

Alternative 5’ splice site in the first exon of IRF5

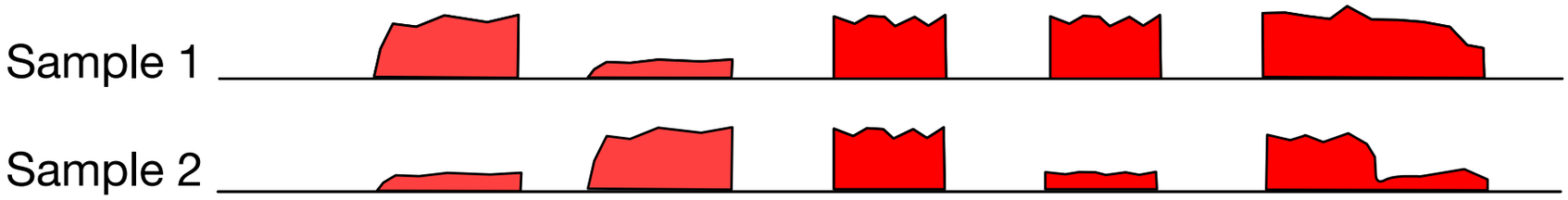




Transcript usage quantitative trait locus (QTL)

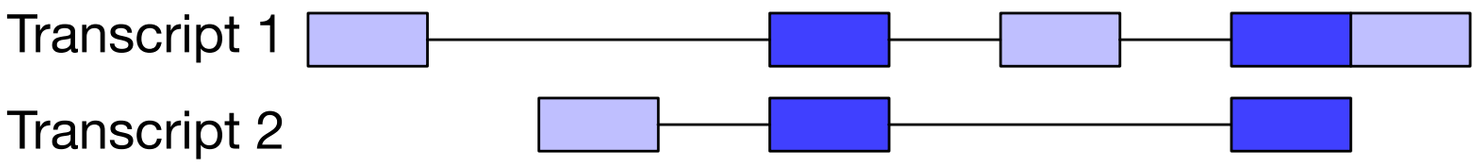
Ideal world

Scenario A: annotations match expressed transcripts



RNA-seq read coverage

Expressed transcripts

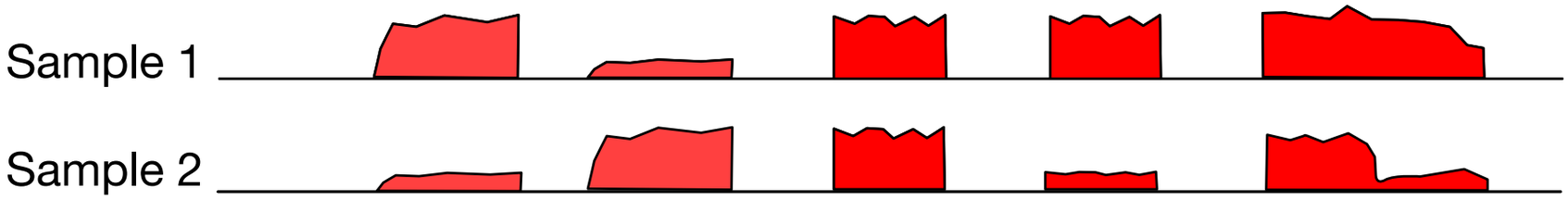


	Sample 1	Sample 2
Transcript 1	80%	20%
Transcript 2	20%	80%

True transcript usage

Ideal world

Scenario A: annotations match expressed transcripts



RNA-seq read coverage

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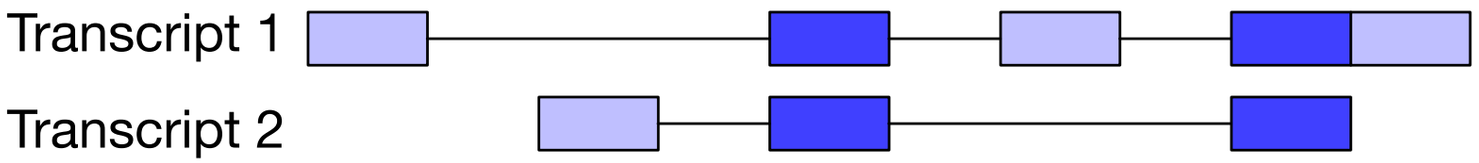

	Sample 1	Sample 2
Transcript 1	80%	20%
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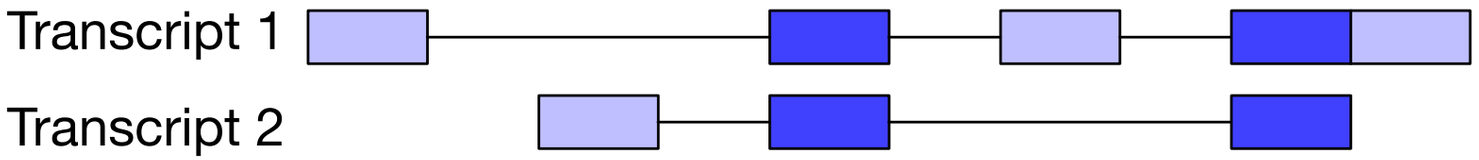
True transcript usage

Annotated transcripts



Transcript 1	80%	20%
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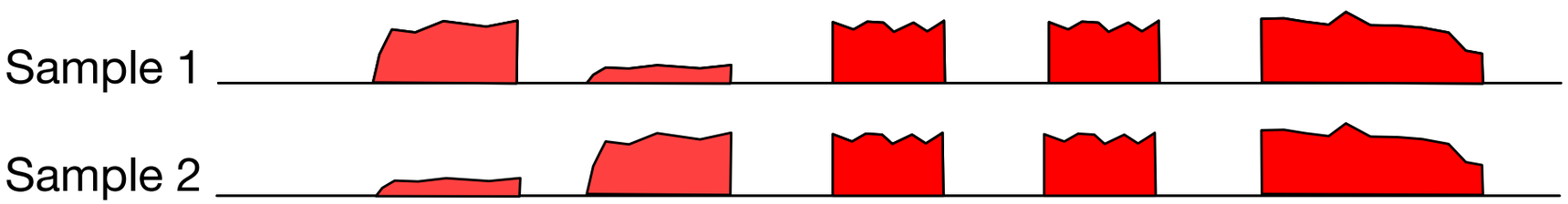
Estimated transcript usage

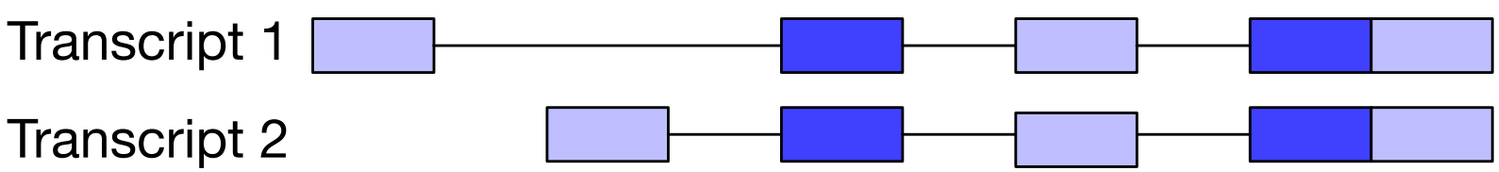
Real world

Scenario B: annotations differ from expressed transcripts



RNA-seq read coverage

Expressed transcripts



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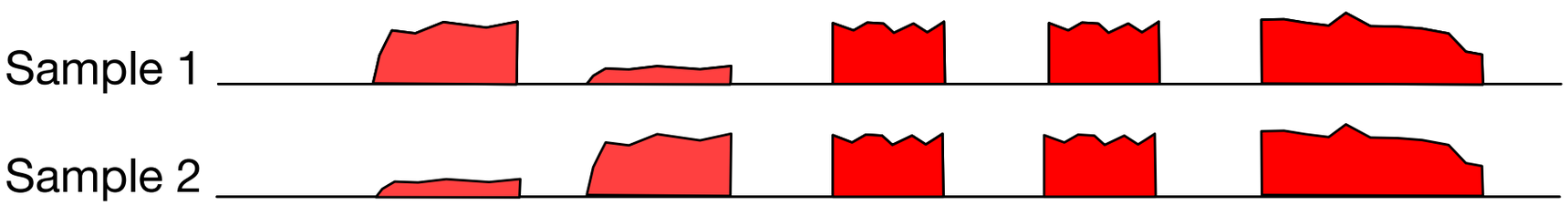
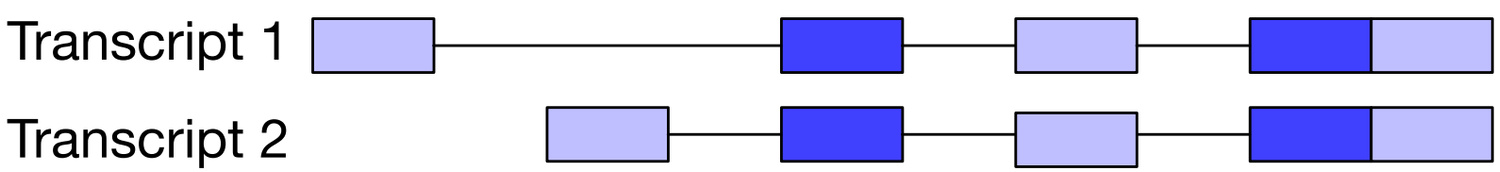


RNA-seq read coverage

Expressed transcripts



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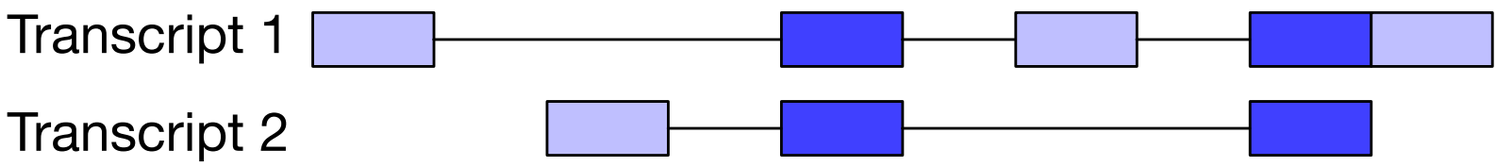
True transcript usage

Annotated transcripts

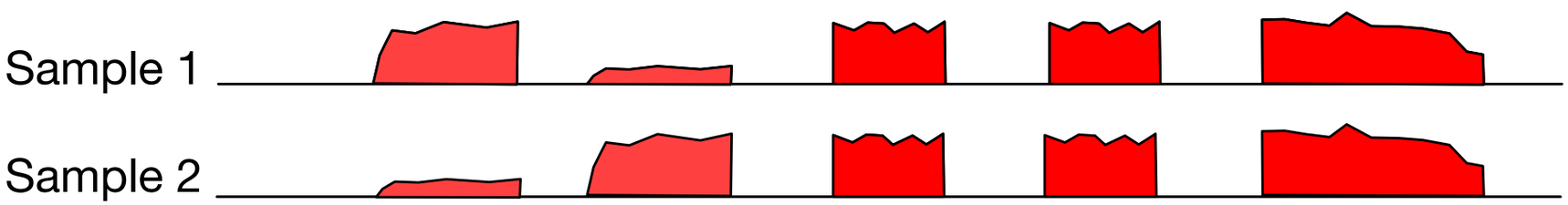


	Sample 1	Sample 2
Transcript 1	93%	73%
Transcript 2	7%	27%

Estimated transcript usage

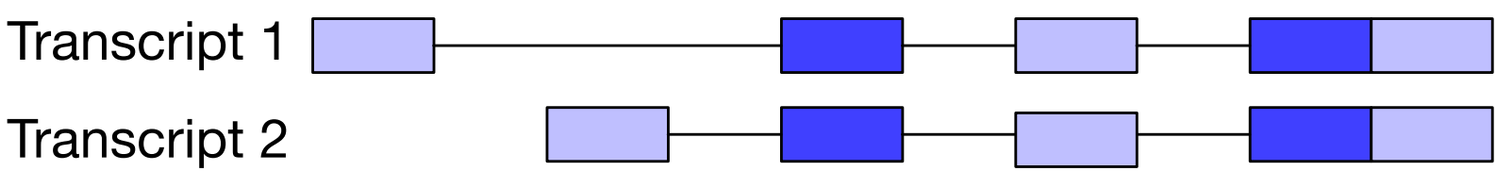
Real world

Scenario B: annotations differ from expressed transcripts



RNA-seq read coverage

Expressed transcripts



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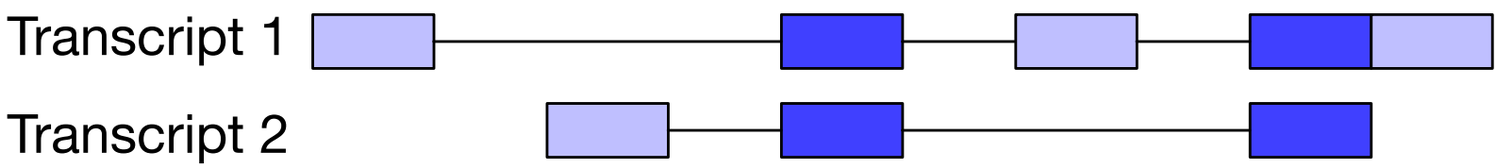
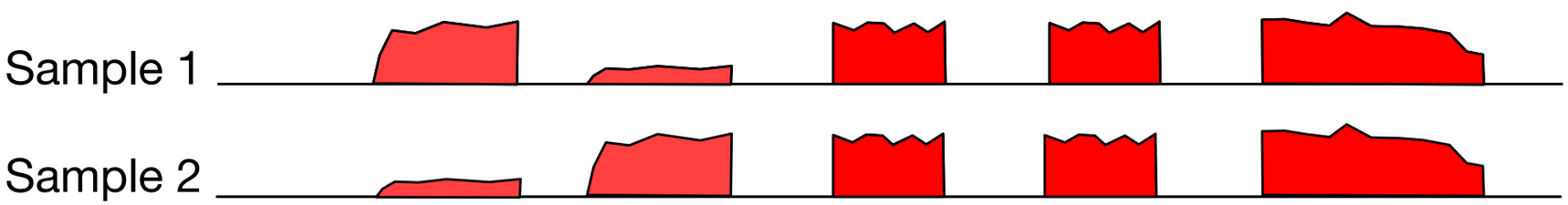


93%	73%	**Estimated transcript usage**
7%	27%	

Biased estimates!

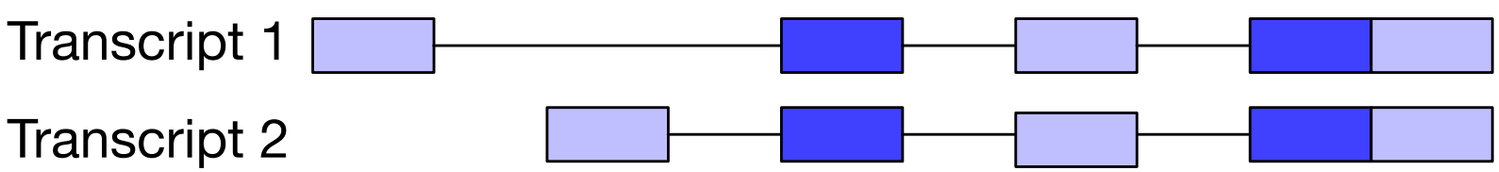
Real world

Scenario B: annotations differ from expressed transcripts



RNA-seq read coverage

Expressed transcripts



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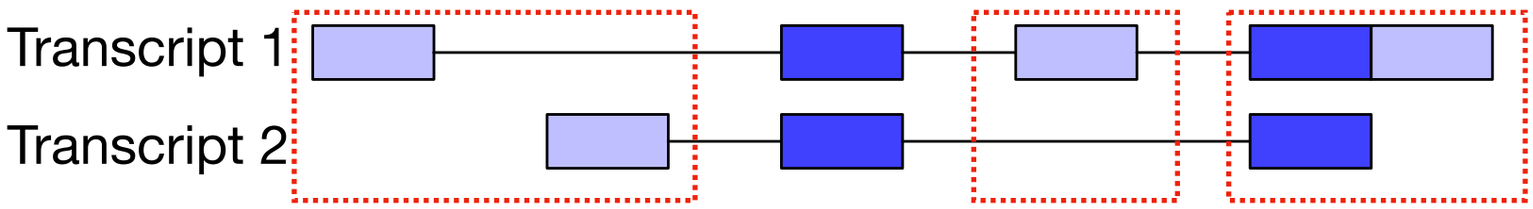
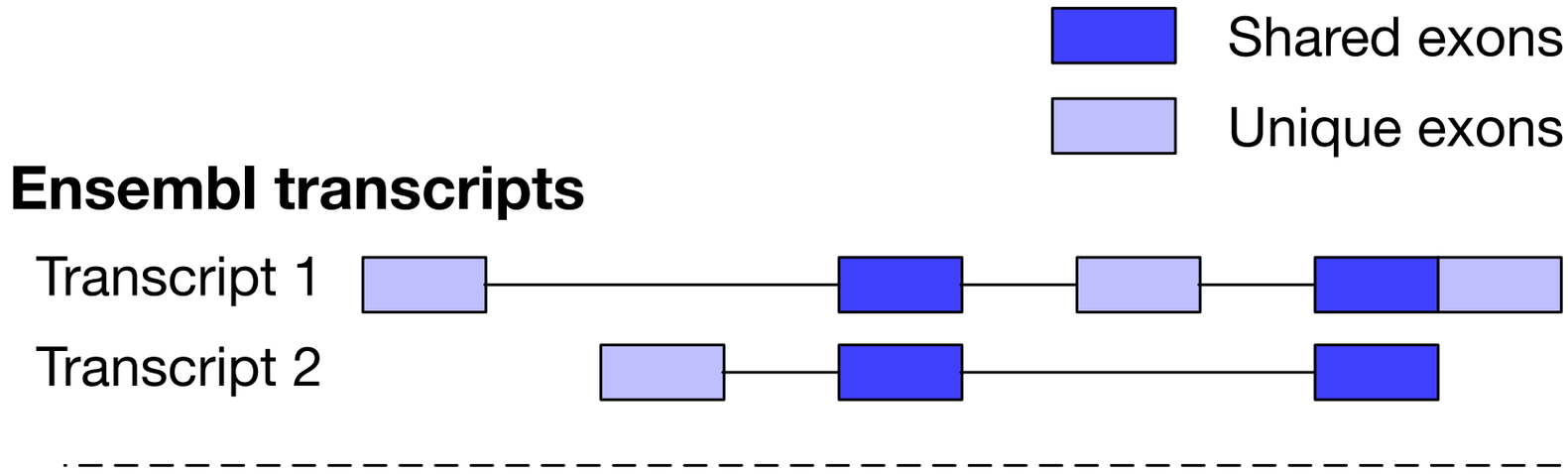
Annotated transcripts

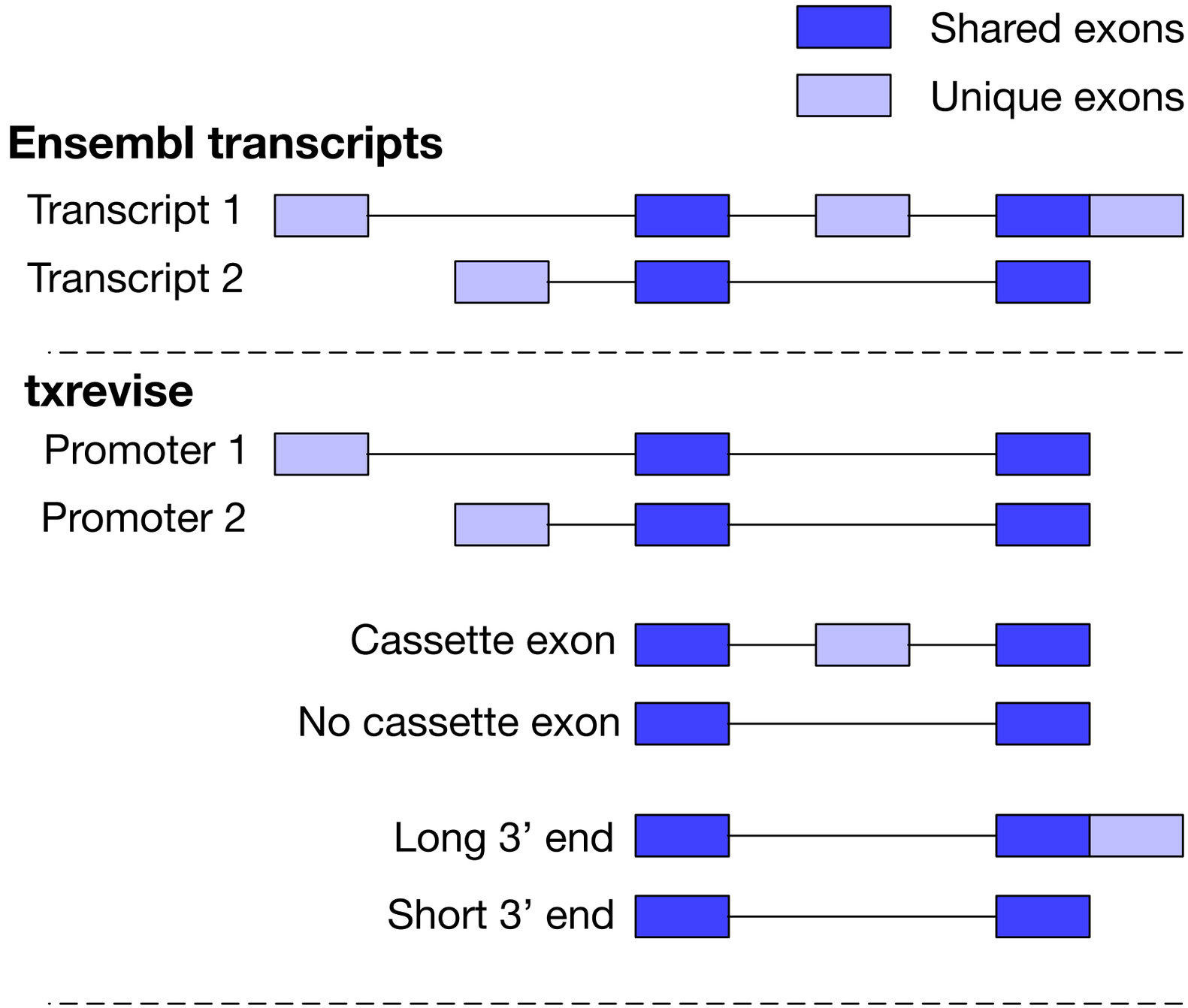


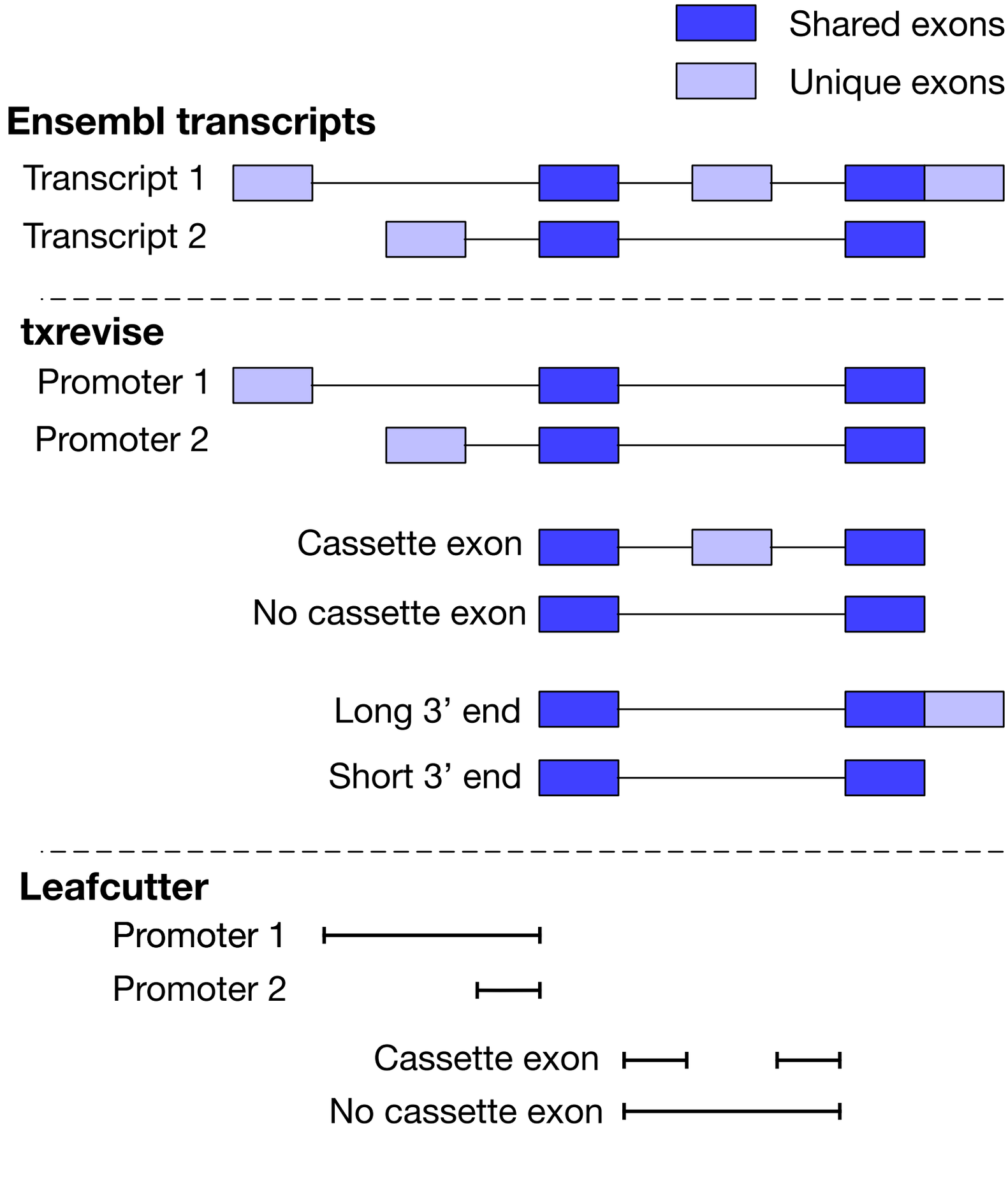
93%	73%	**Estimated transcript usage**
7%	27%	

Which event is driving the signal?

Biased estimates!


Shared exons
Unique exons
Ensembl transcripts
Transcript 1
Transcript 2


Shared exons
Unique exons
Ensembl transcripts
Transcript 1
Transcript 2
txrevise
Promoter 1
Promoter 2
Cassette exon
No cassette exon
Long 3’ end
Short 3’ end


Shared exons
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Promoter 2
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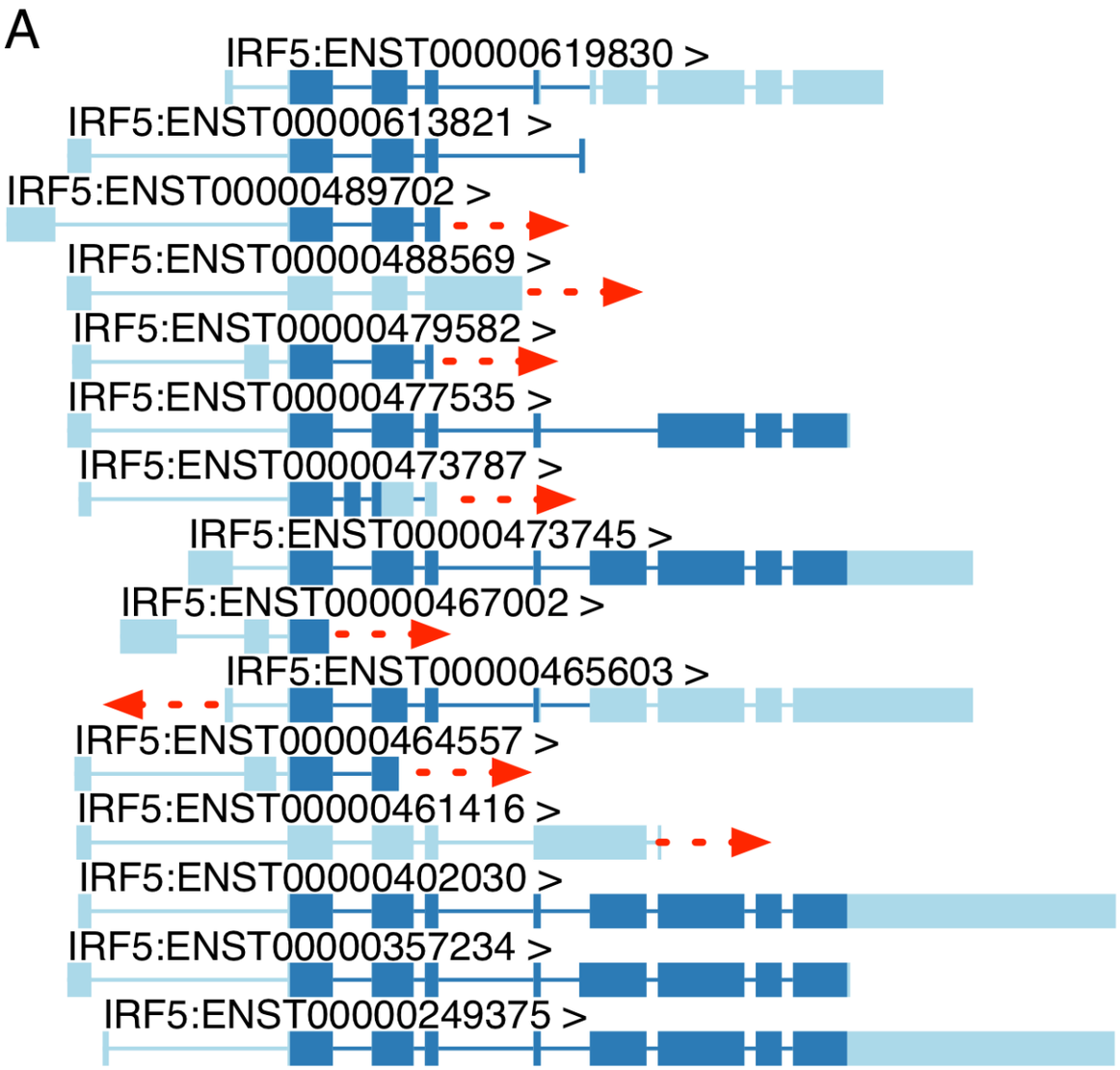
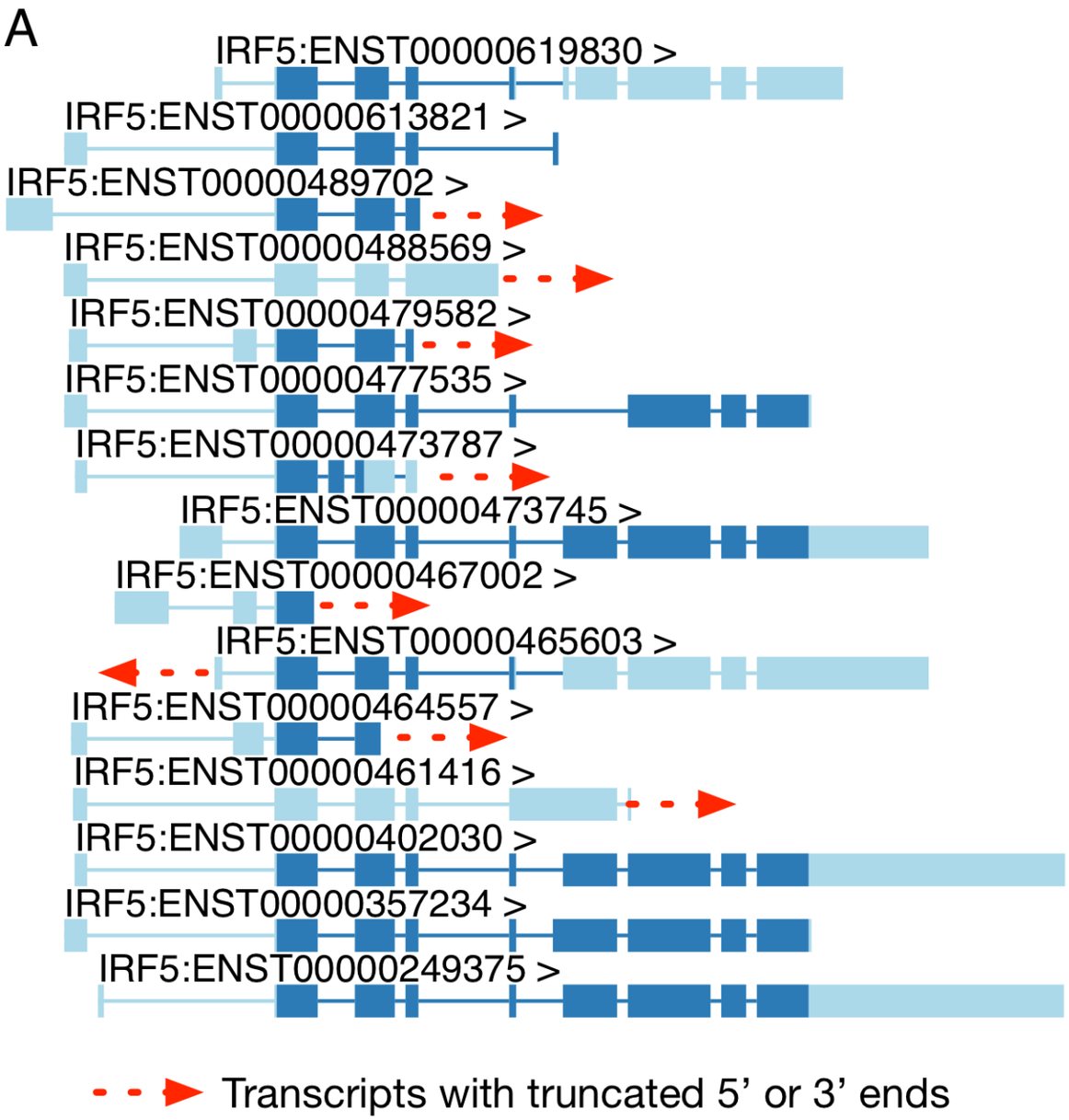
Long 3’ end
Short 3’ end
Leafcutter
Promoter 1
Promoter 2
Cassette exon
No cassette exon

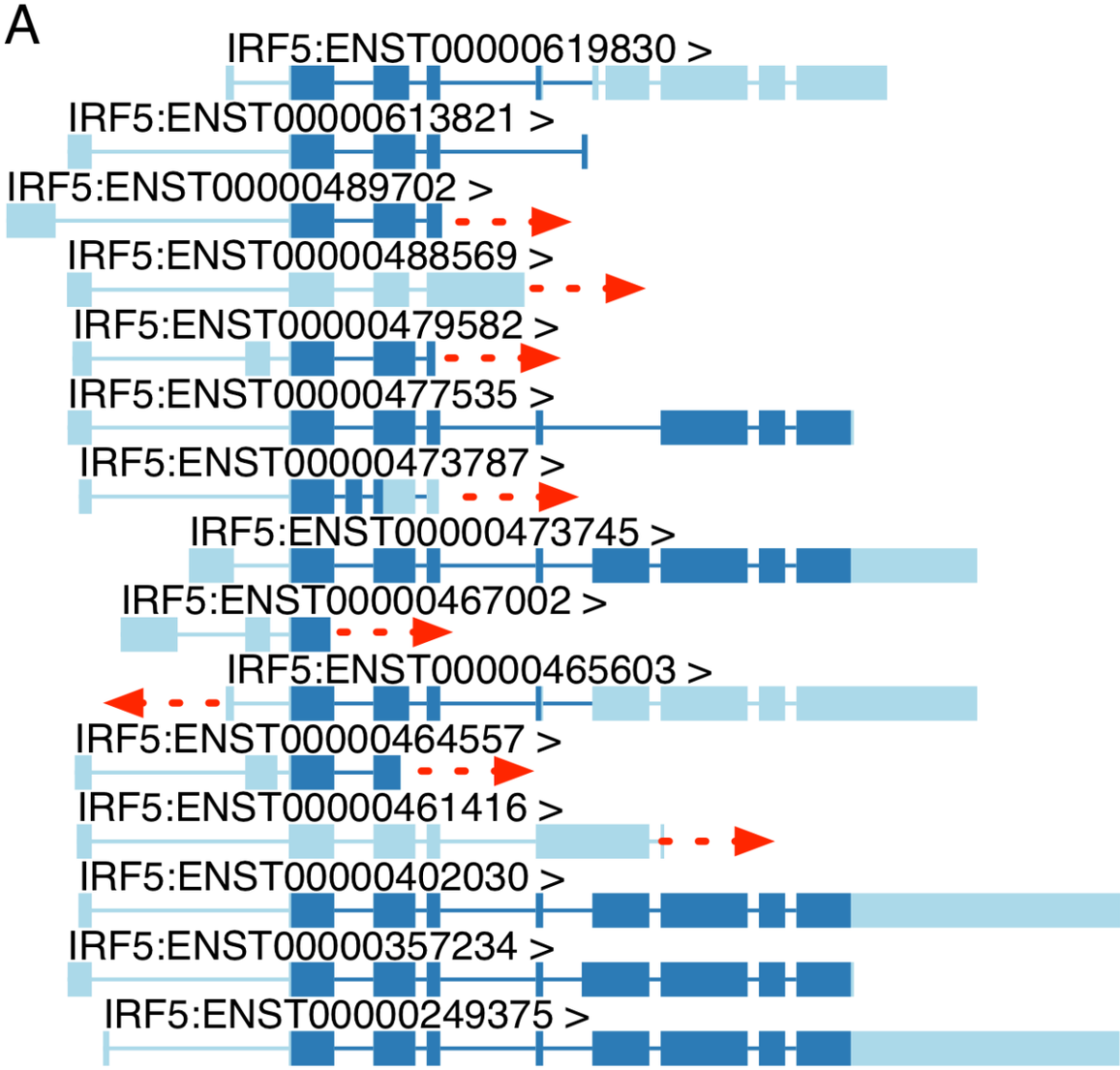
A


IRF5:ENST00000619830 >
IRF5:ENST00000613821 >
IRF5:ENST00000489702 >
IRF5:ENST00000488569 >
IRF5:ENST00000479582 >
IRF5:ENST00000477535 >
IRF5:ENST00000473787 >
IRF5:ENST00000473745 >
IRF5:ENST00000467002 >
IRF5:ENST00000465603 >
IRF5:ENST00000464557 >
IRF5:ENST00000461416 >
IRF5:ENST00000402030 >
IRF5:ENST00000357234 >
IRF5:ENST00000249375 >
Transcripts with truncated 5’ or 3’ ends

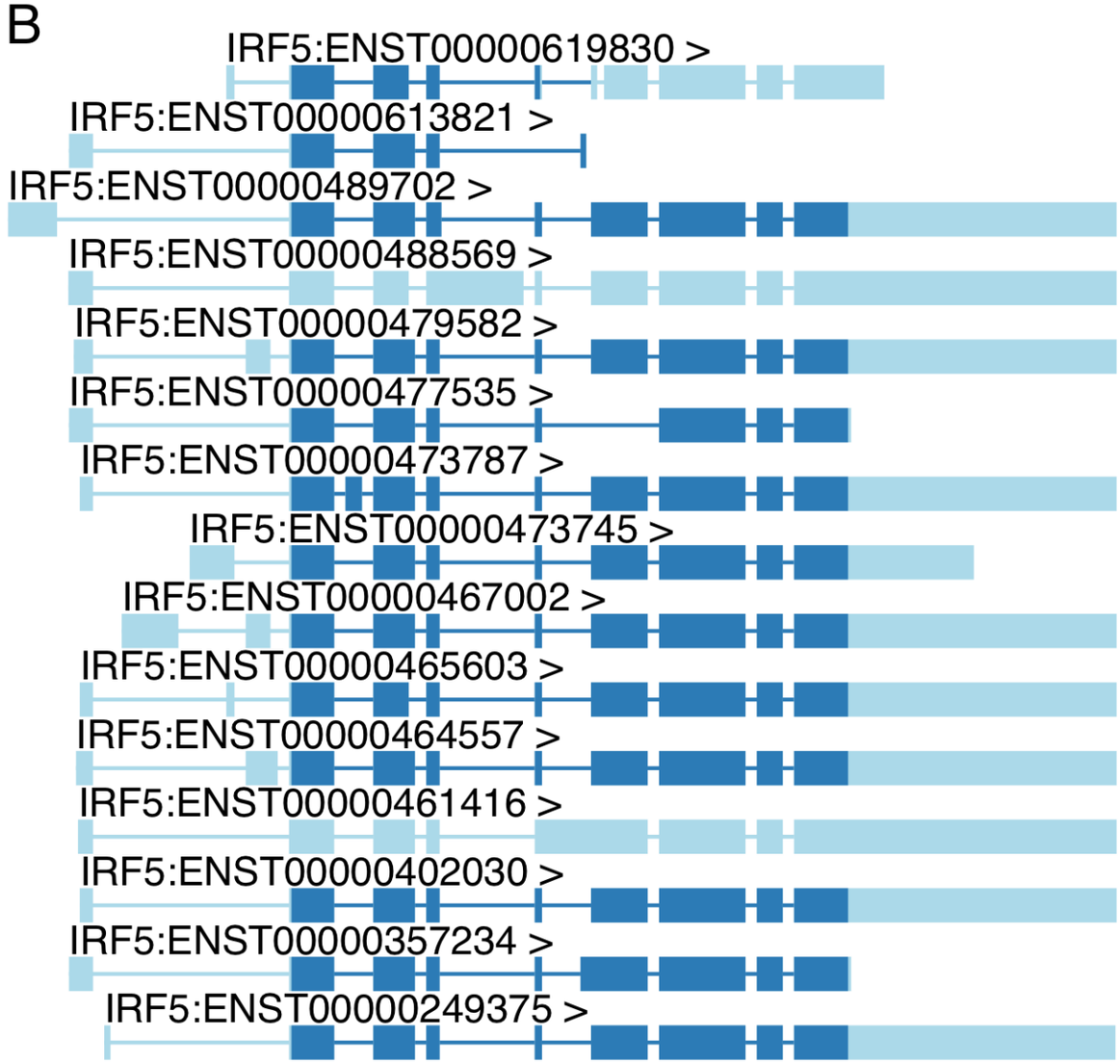


58% of the transcripts are truncated!

A

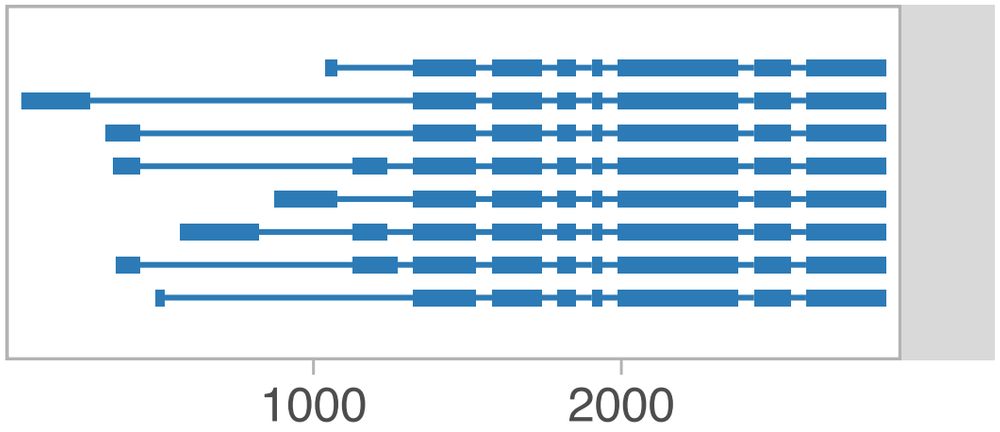


B

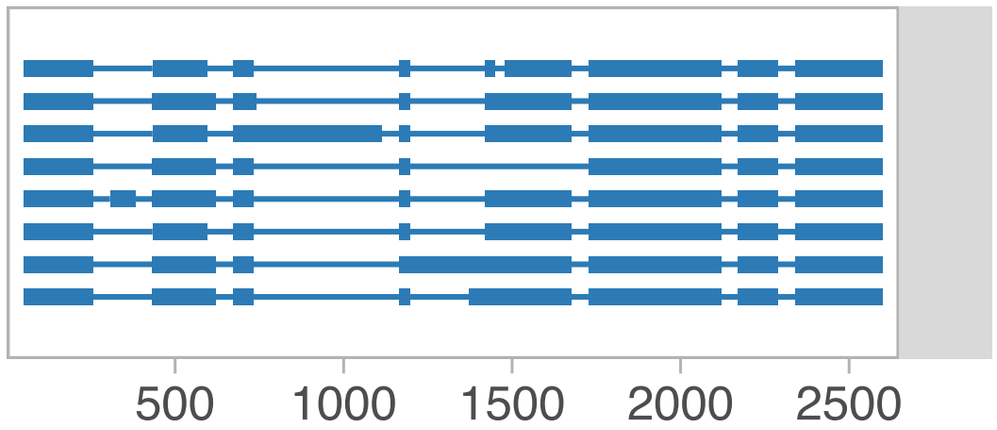


58% of the transcripts are truncated!

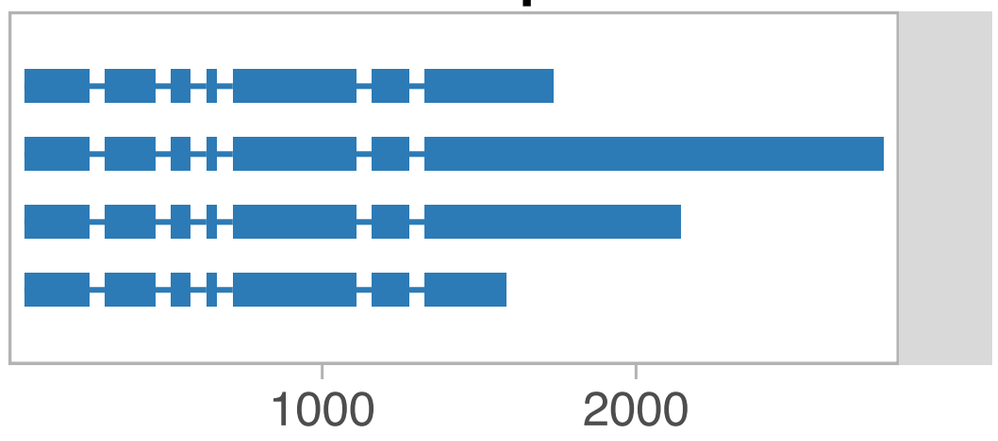
Alternative transcript starts



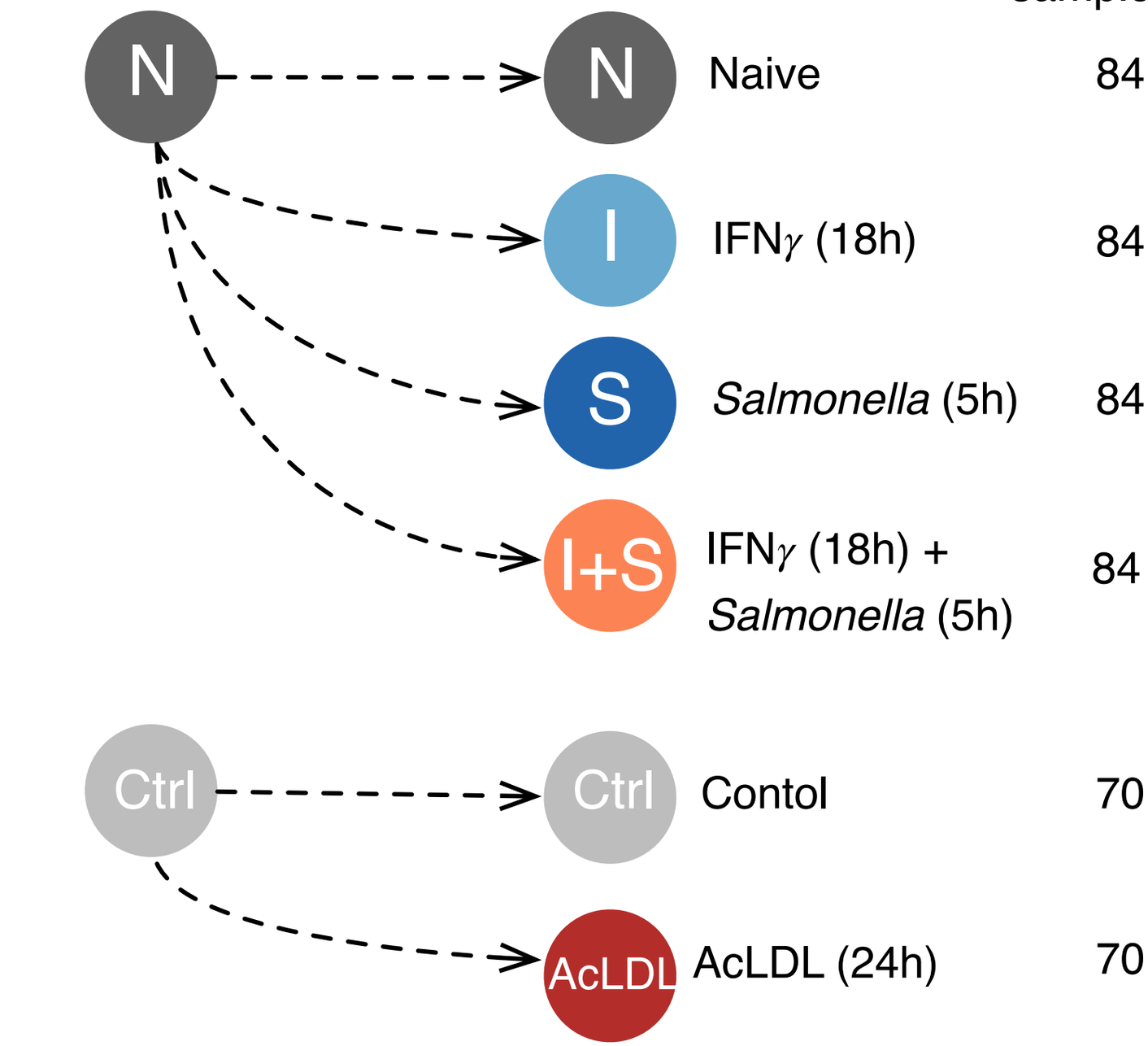
Alternative middle sections



Alternative transcript ends

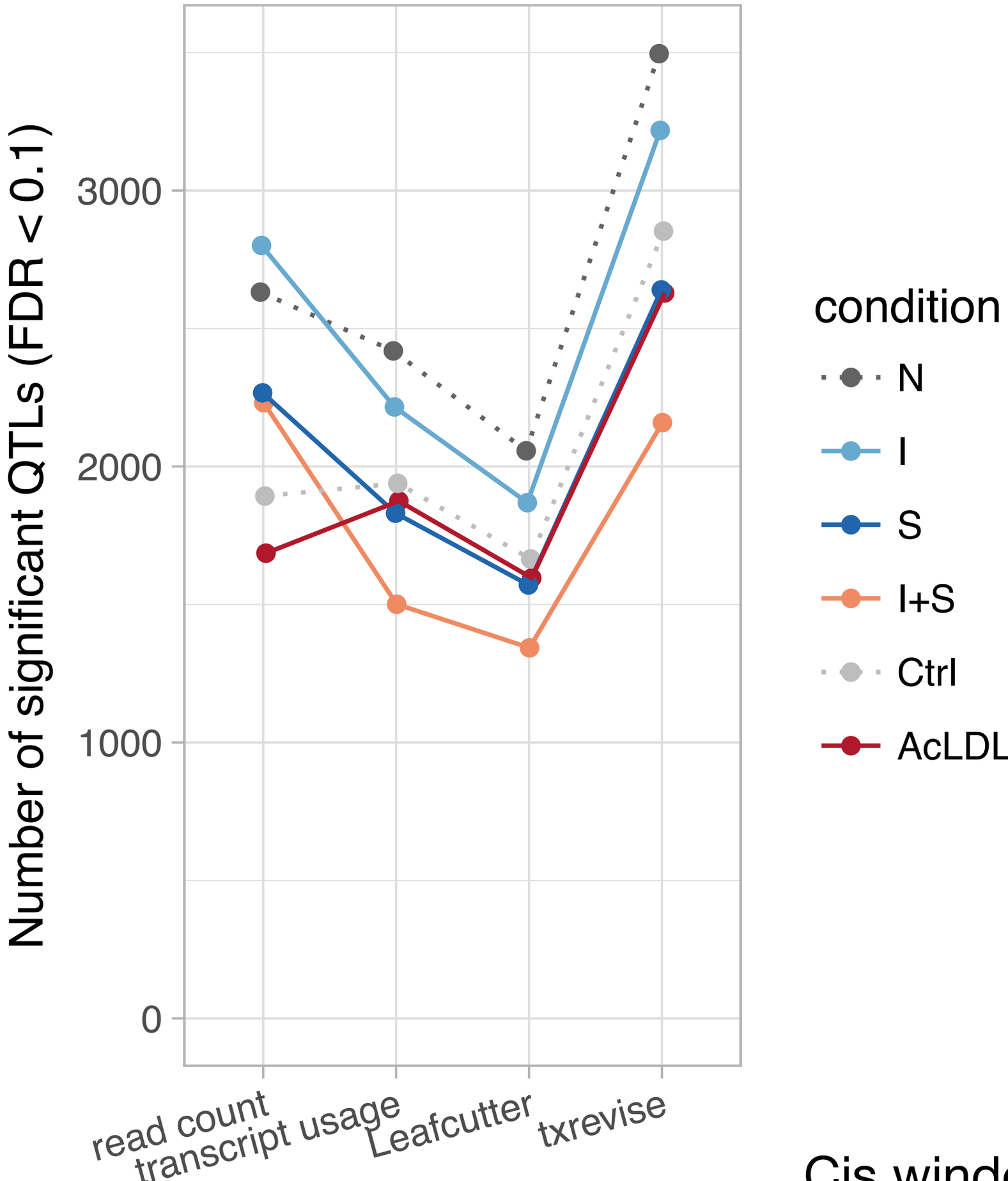


Human macrophages



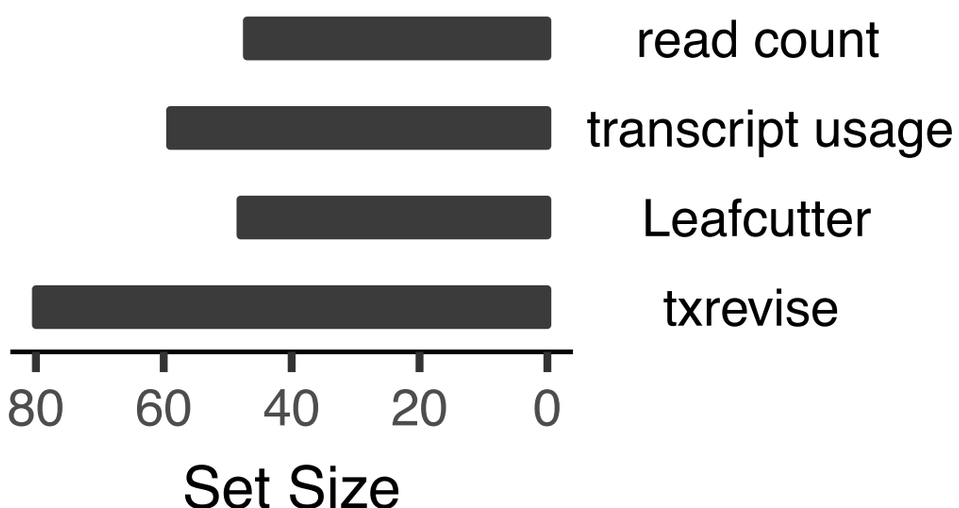
Quantification strategies

- **read count** - STAR alignment + featureCounts
- **transcript usage** - Ensembl transcripts + Salmon
- **txrevise** - transcript events + Salmon
- **Leafcutter** - exon-exon junction read counts



Cis window: +/- 100kb

Overlap with associations for 33 complex traits



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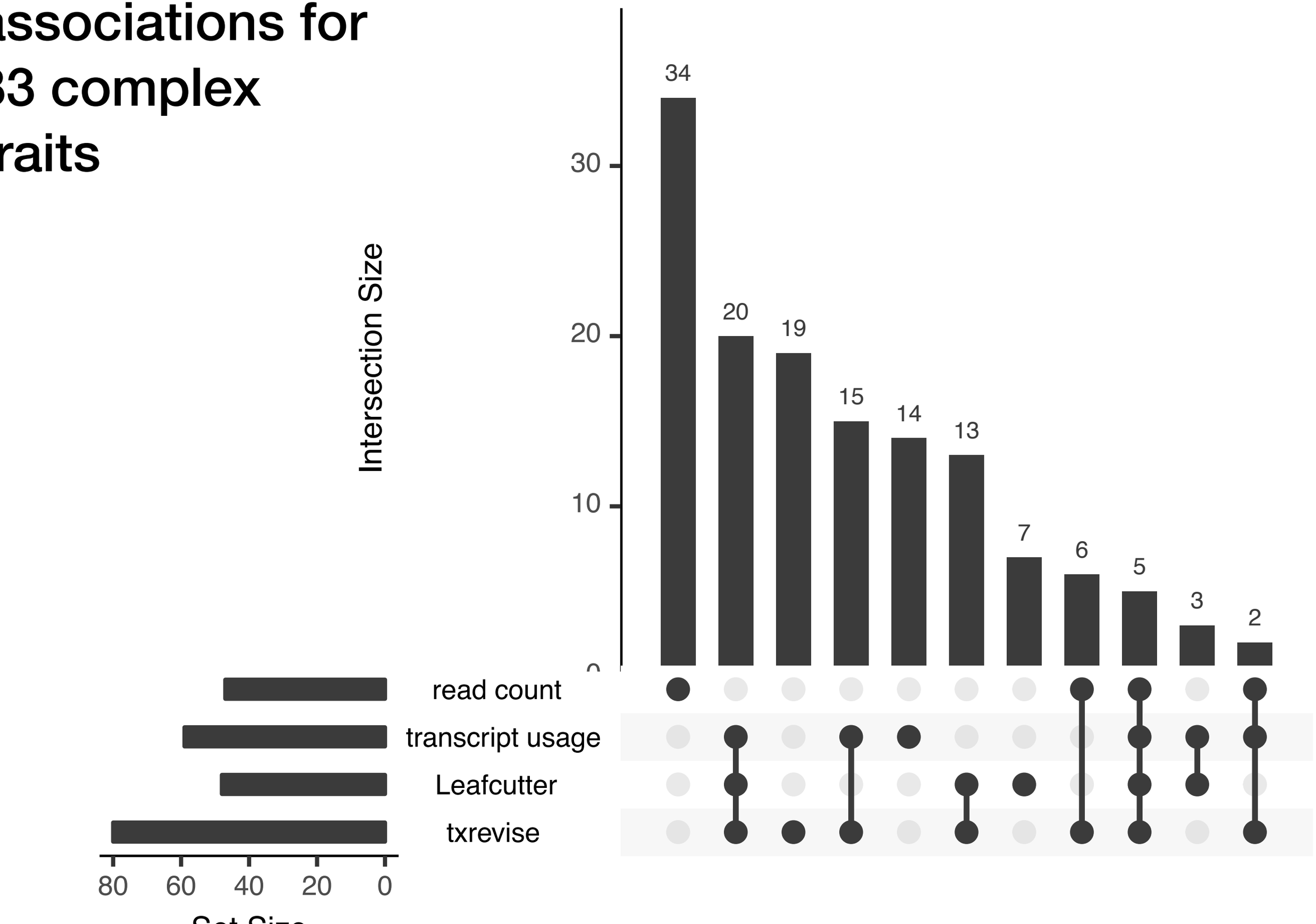
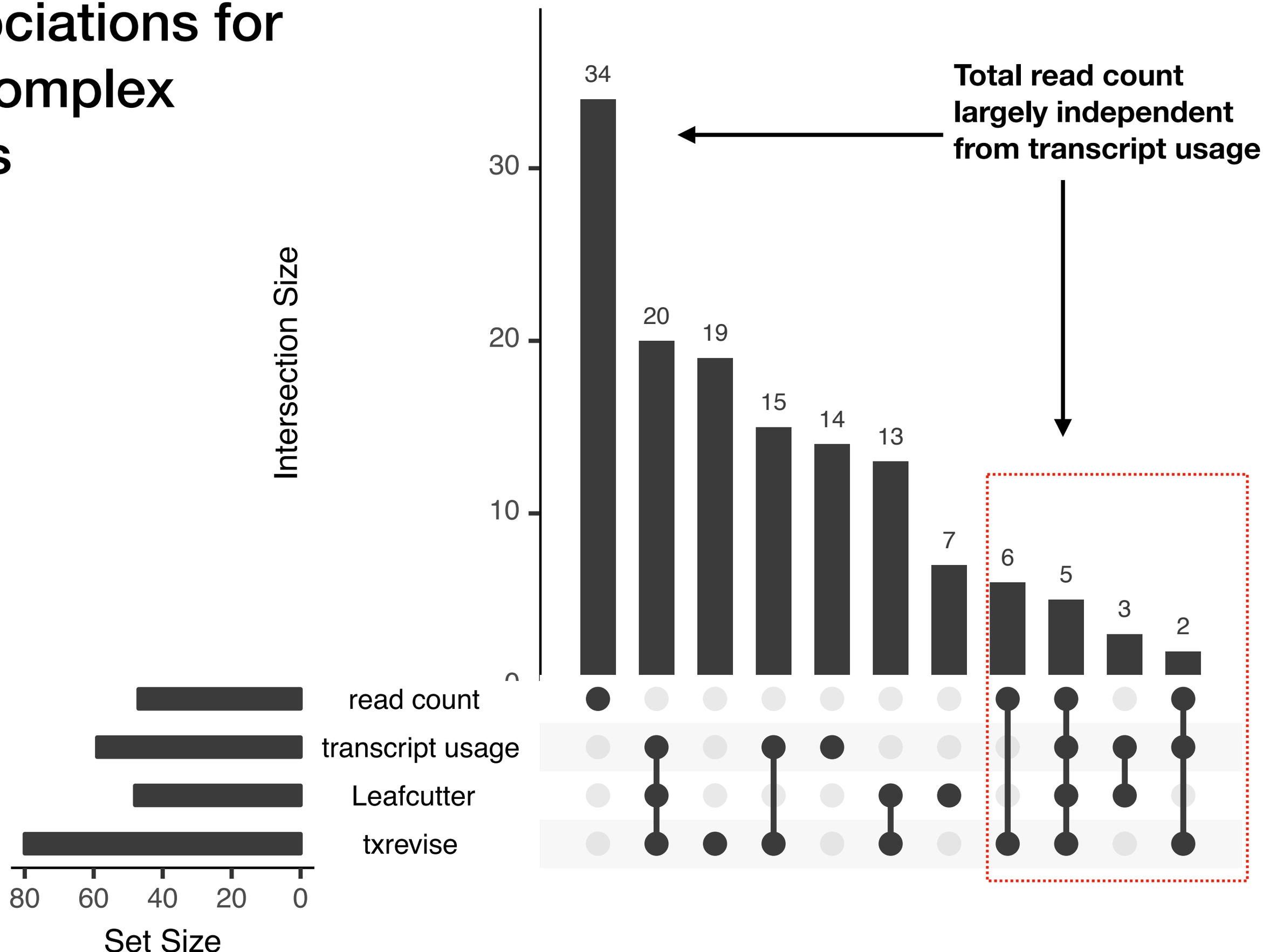


Overlap with associations for 33 complex traits



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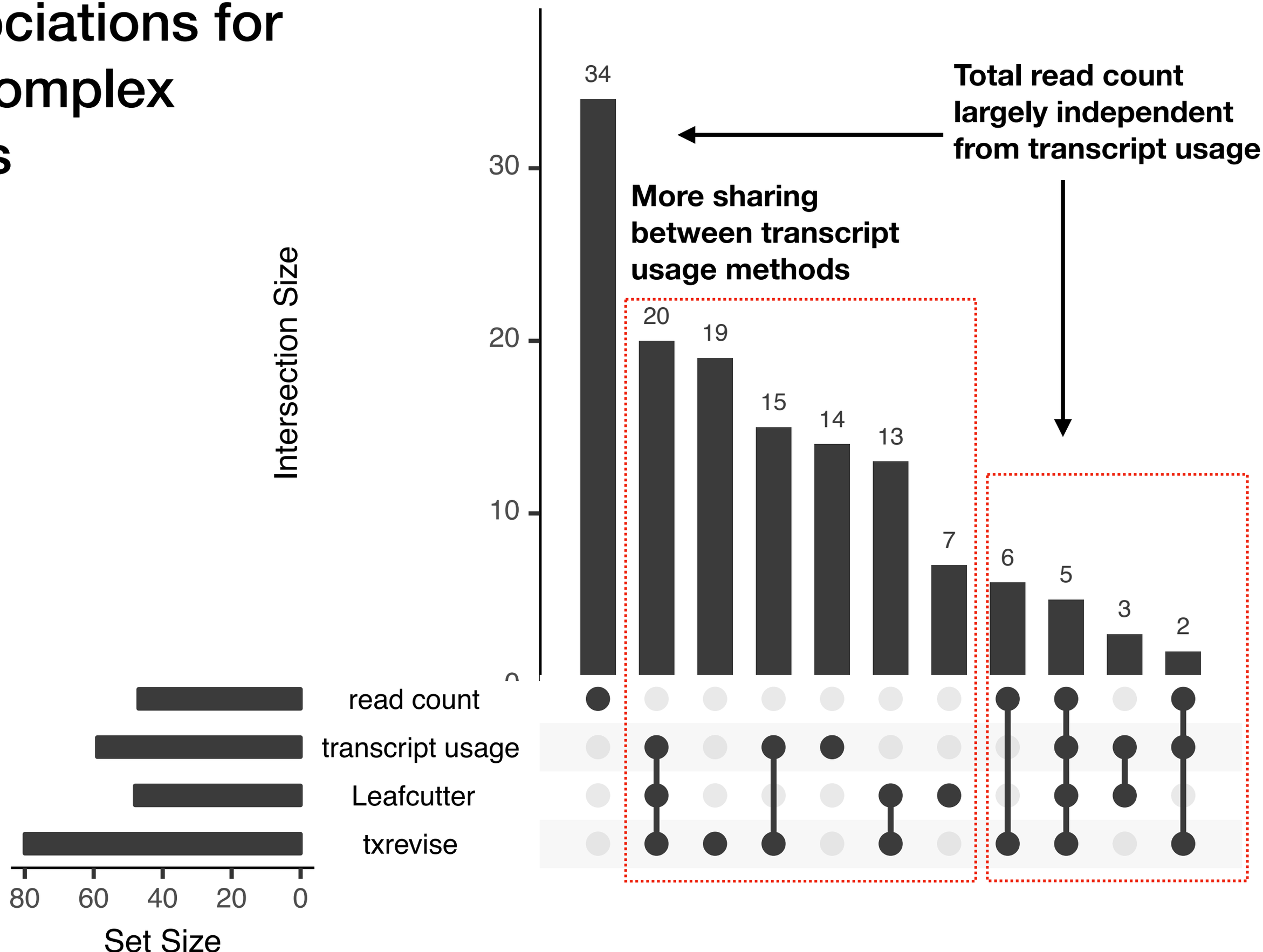
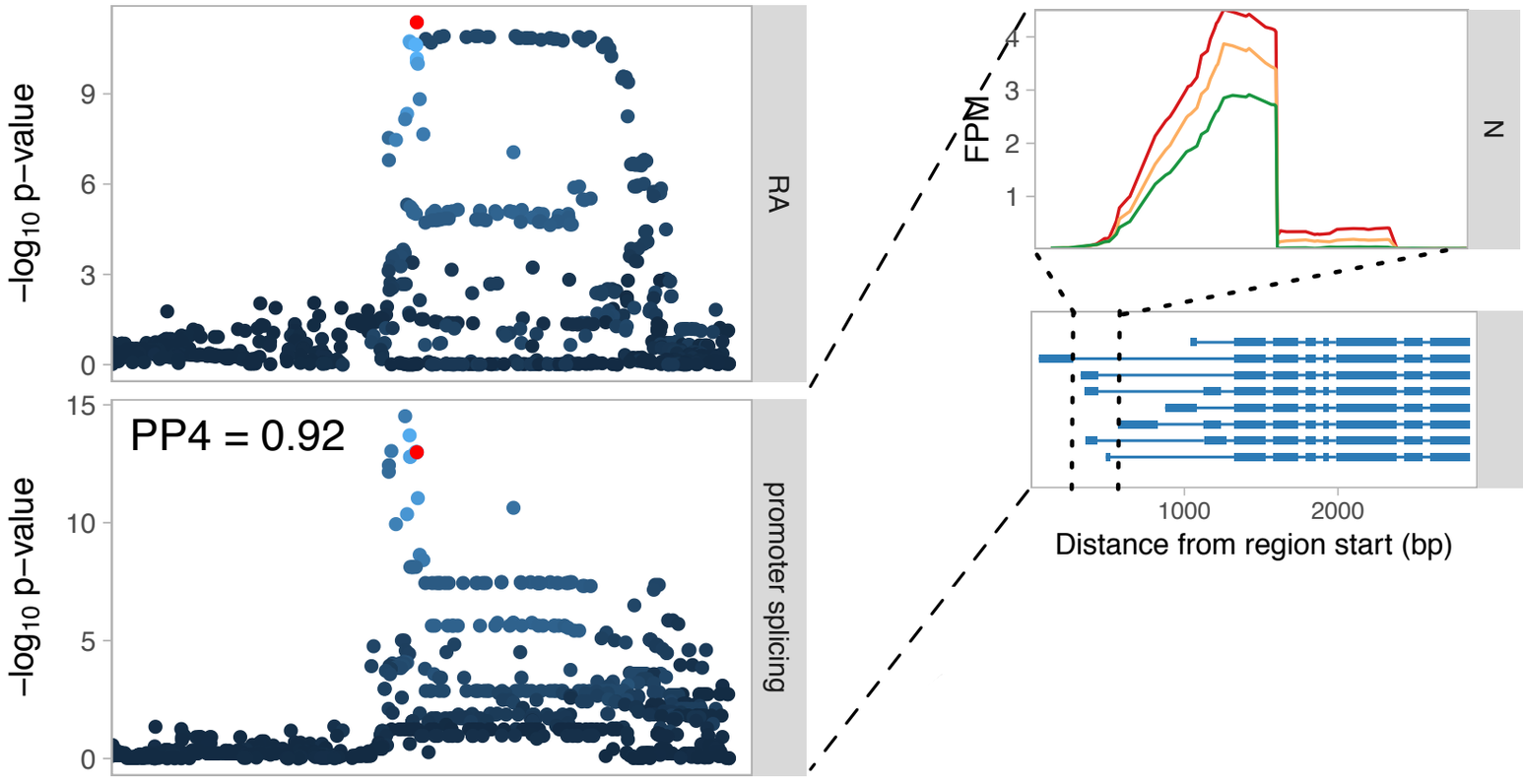
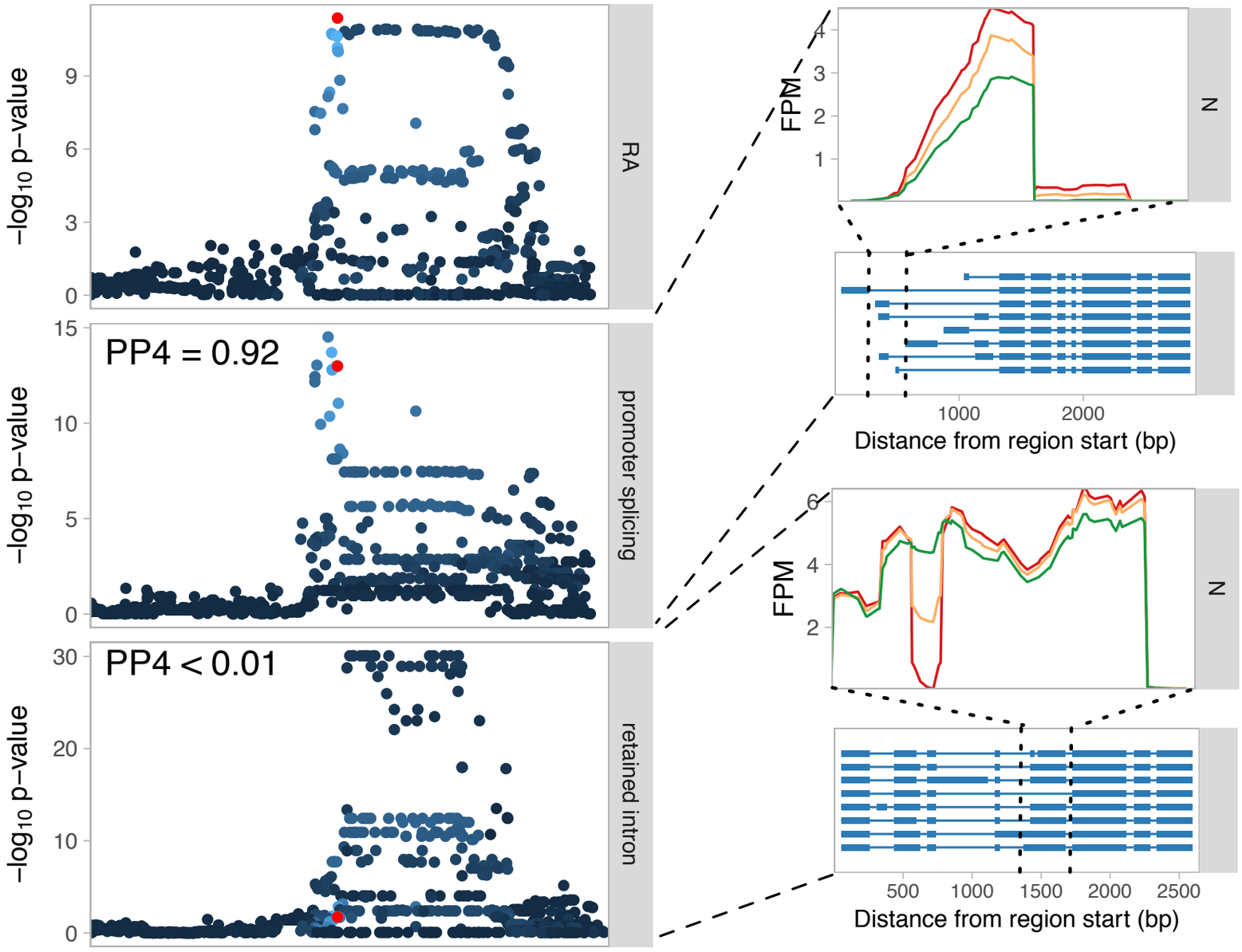




Detected by:

- ✗ read count
- ✗ transcript usage
- ✓ Leafcutter
- ✓ txrevise



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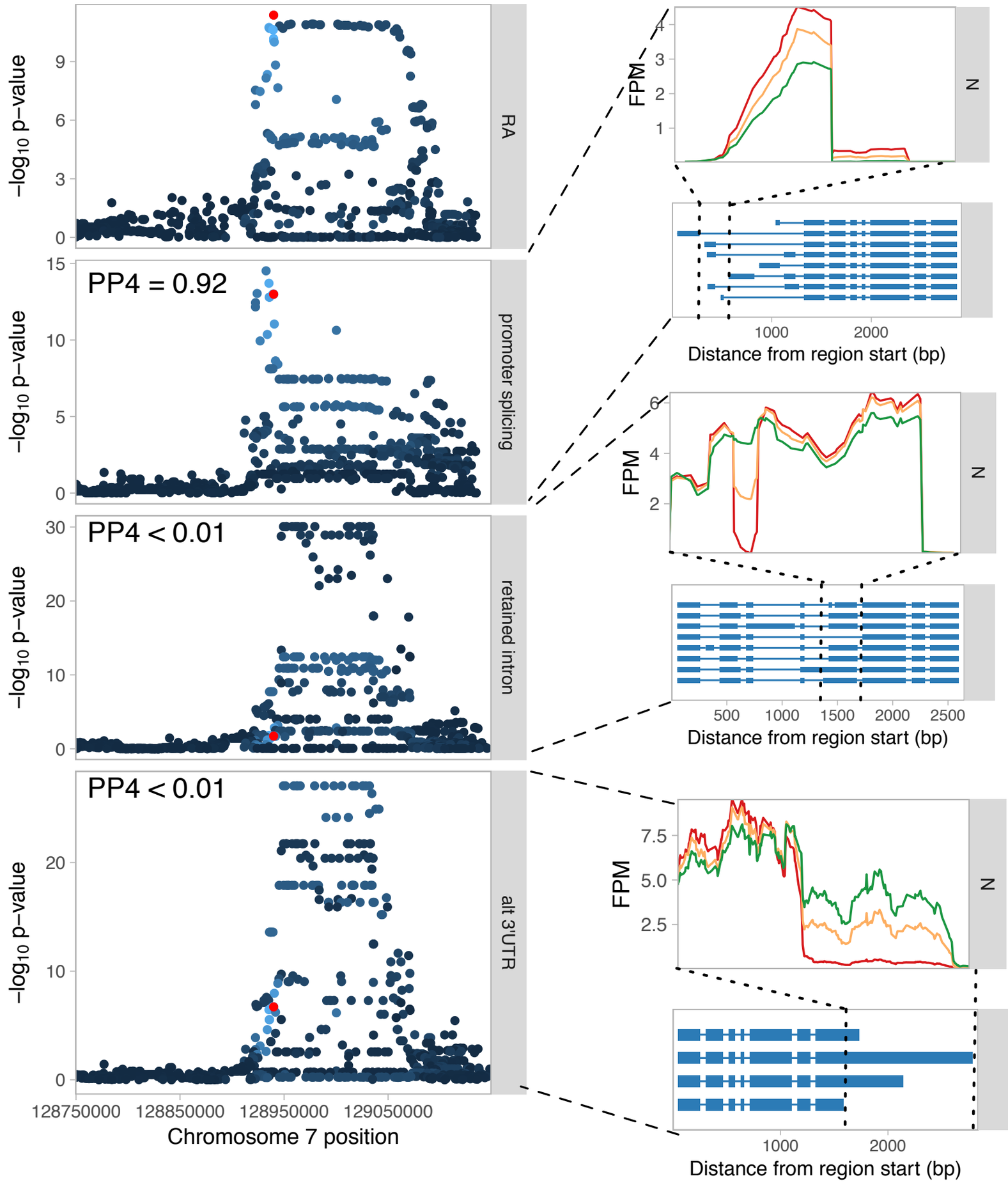

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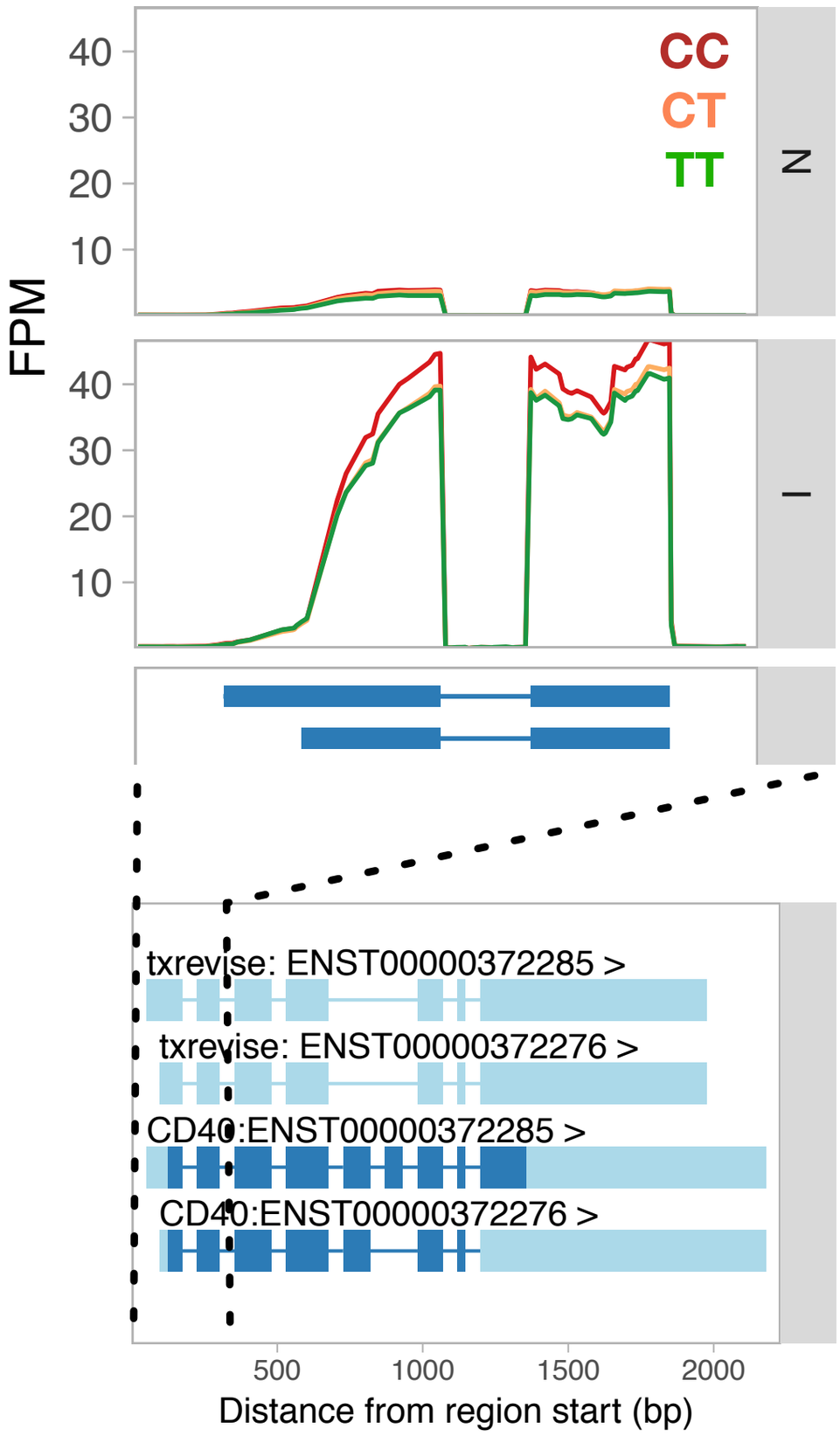
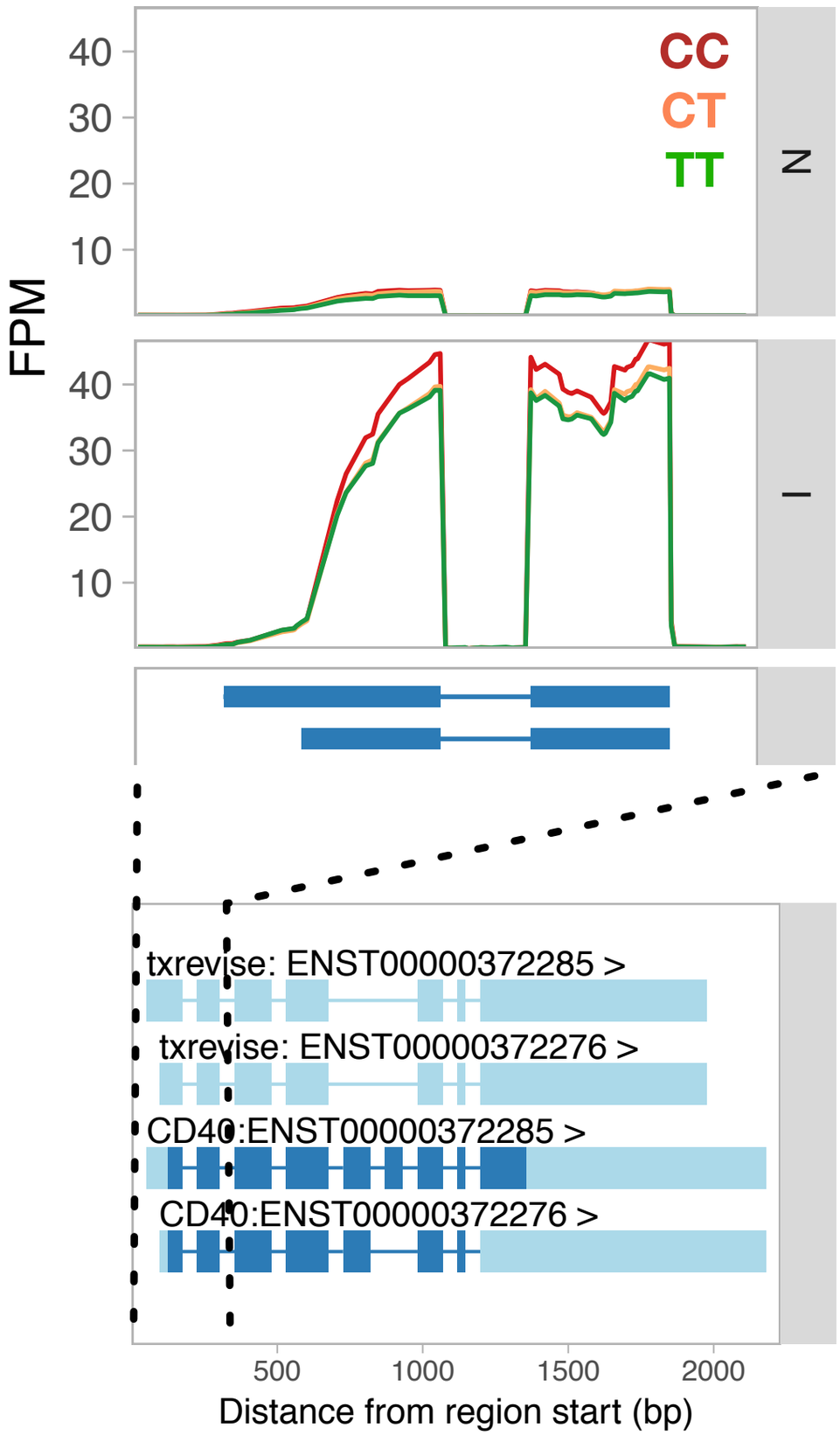
Response QTLs: genetic effects that appear after stimulation

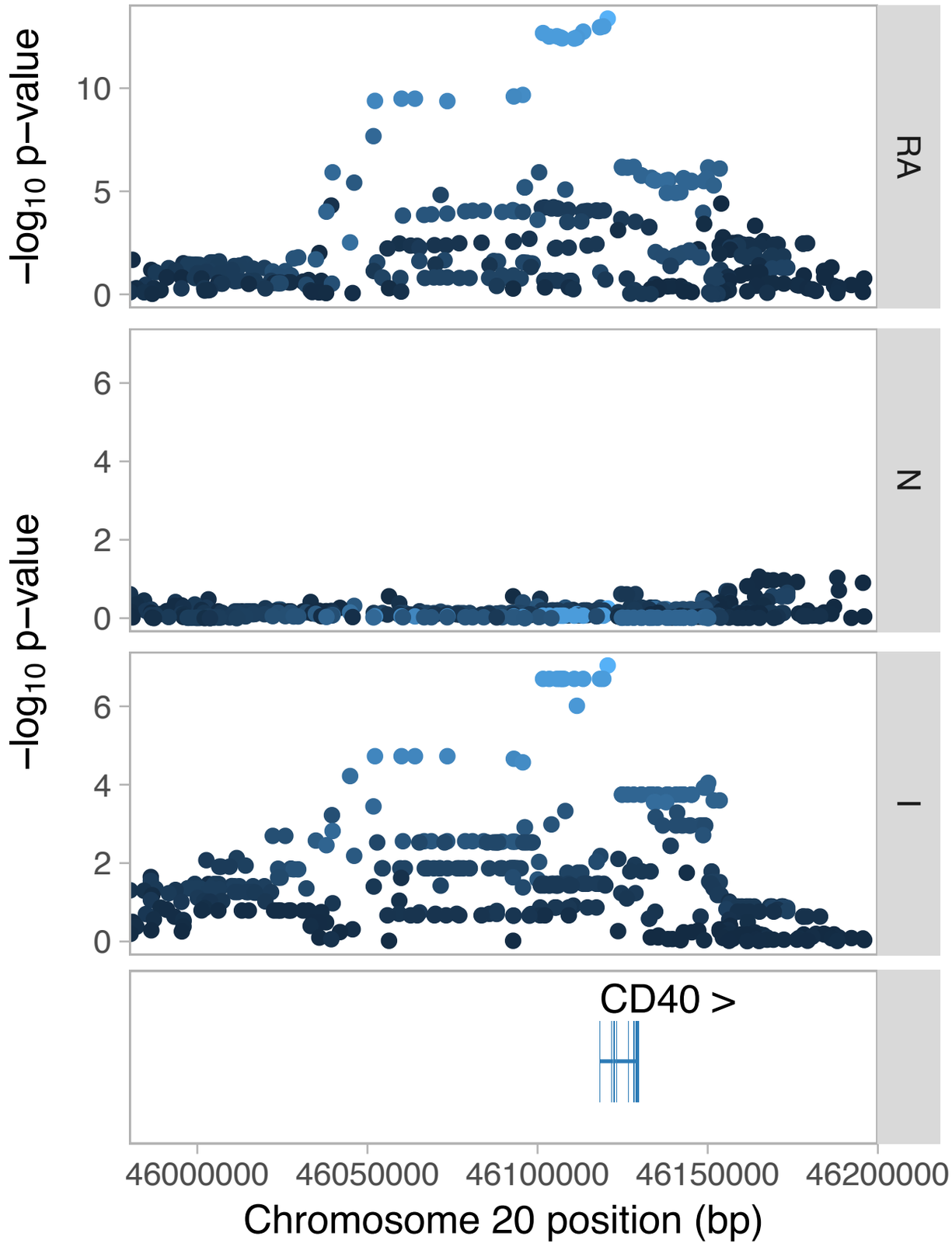
IFNg-specific response QTL at the 5'UTR of CD40

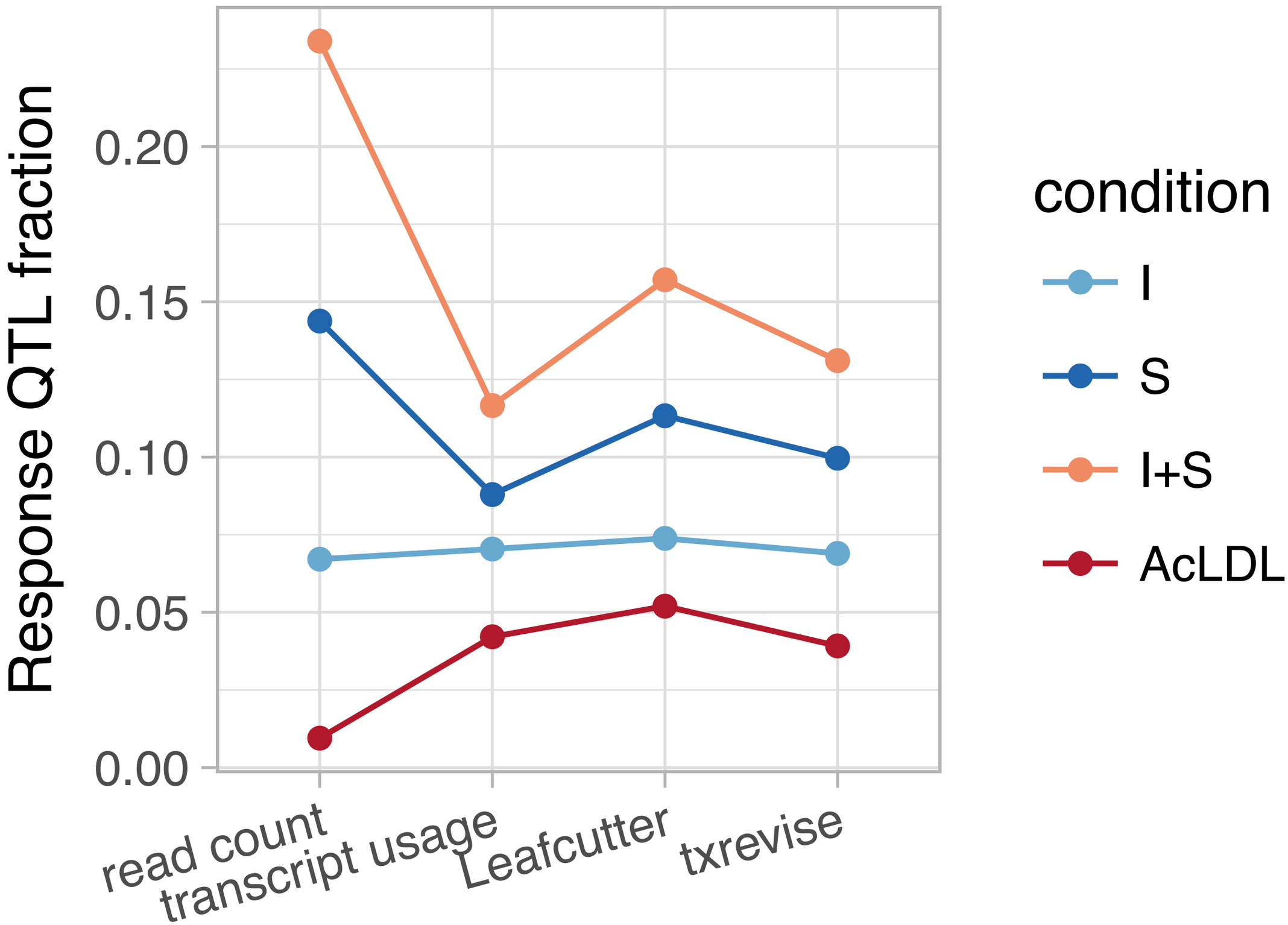


IFNg-specific response QTL at the 5'UTR of CD40



Colocalisation with rheumatoid arthritis (RA) GWAS hit



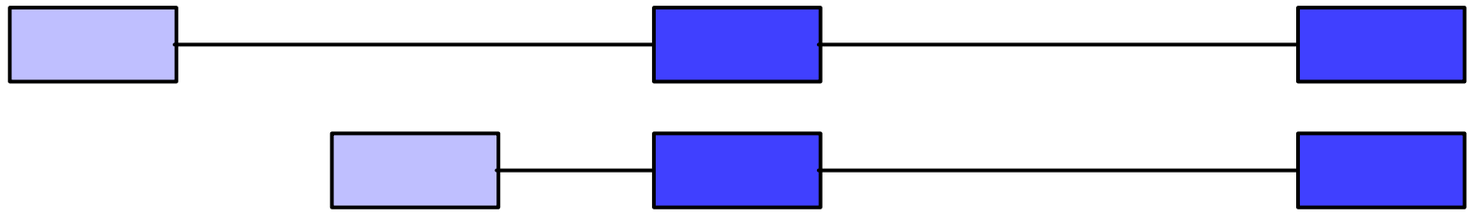

Response QTL fraction
0.20
0.15
0.10
0.05
0.00
read count
transcript usage
Leafcutter
txrevise
condition
I
S
I+S
AcLDL

txrevise

Start

Promoter 1

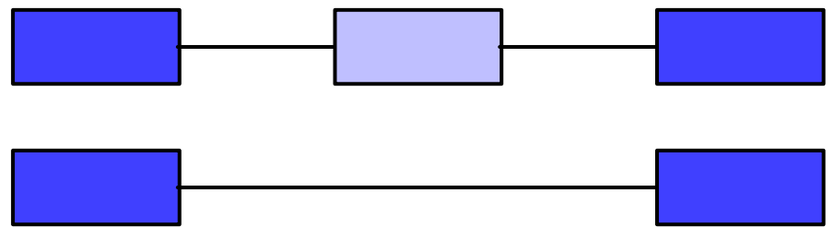
Promoter 2



Middle

Cassette exon

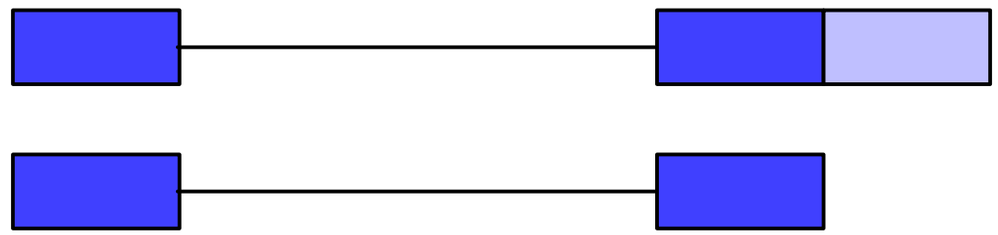
No cassette exon



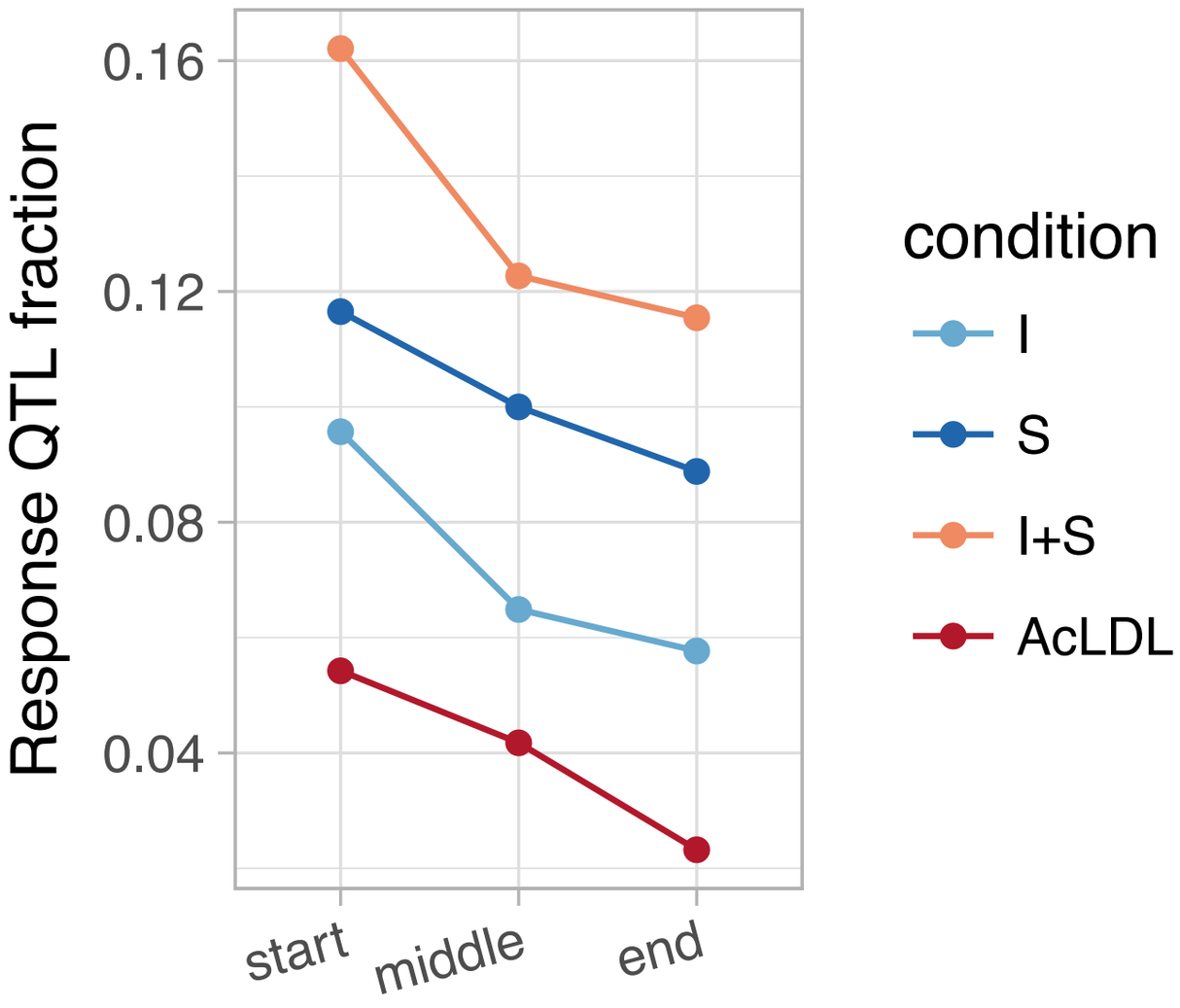
End

Long 3’ end

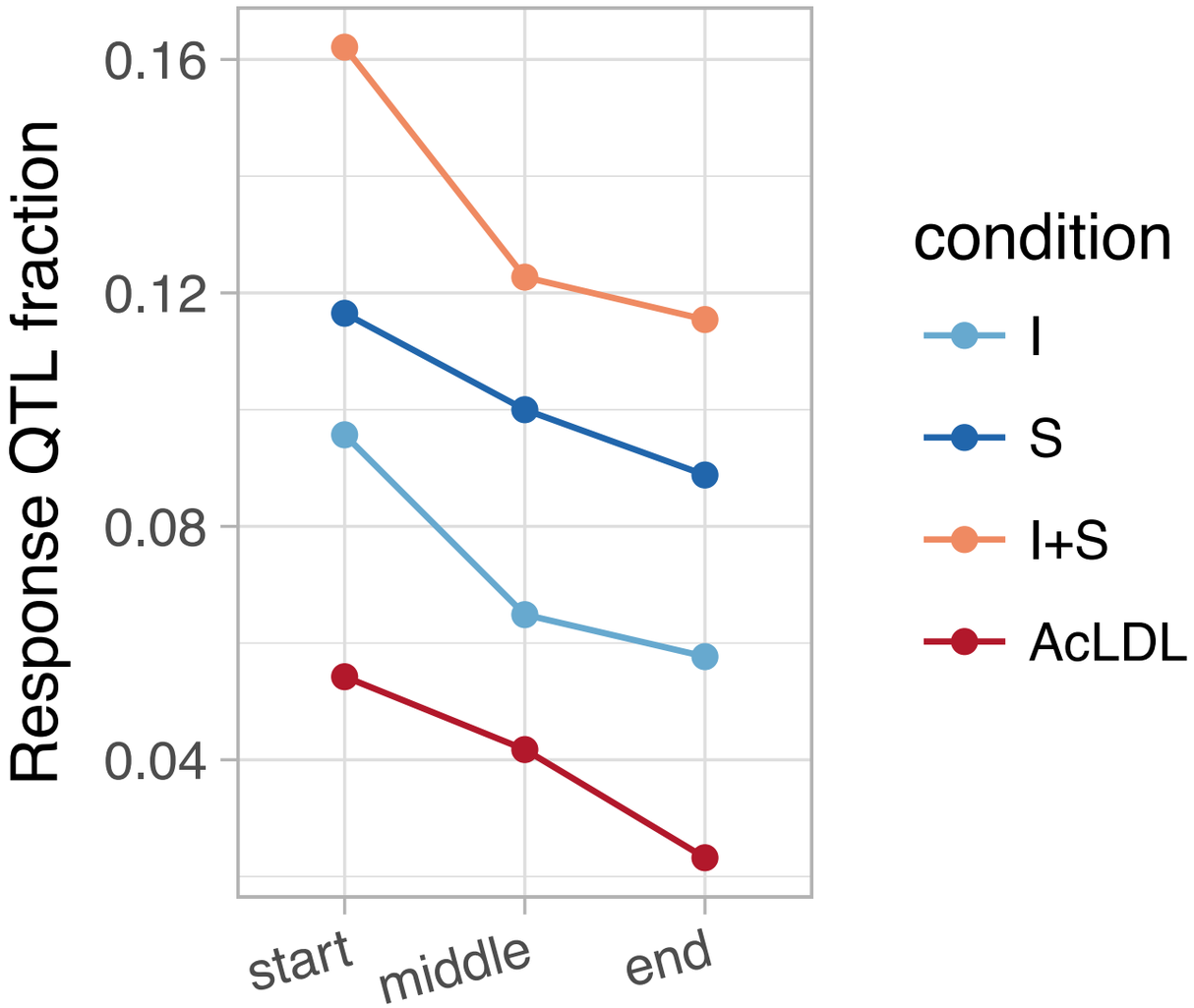
Short 3’ end

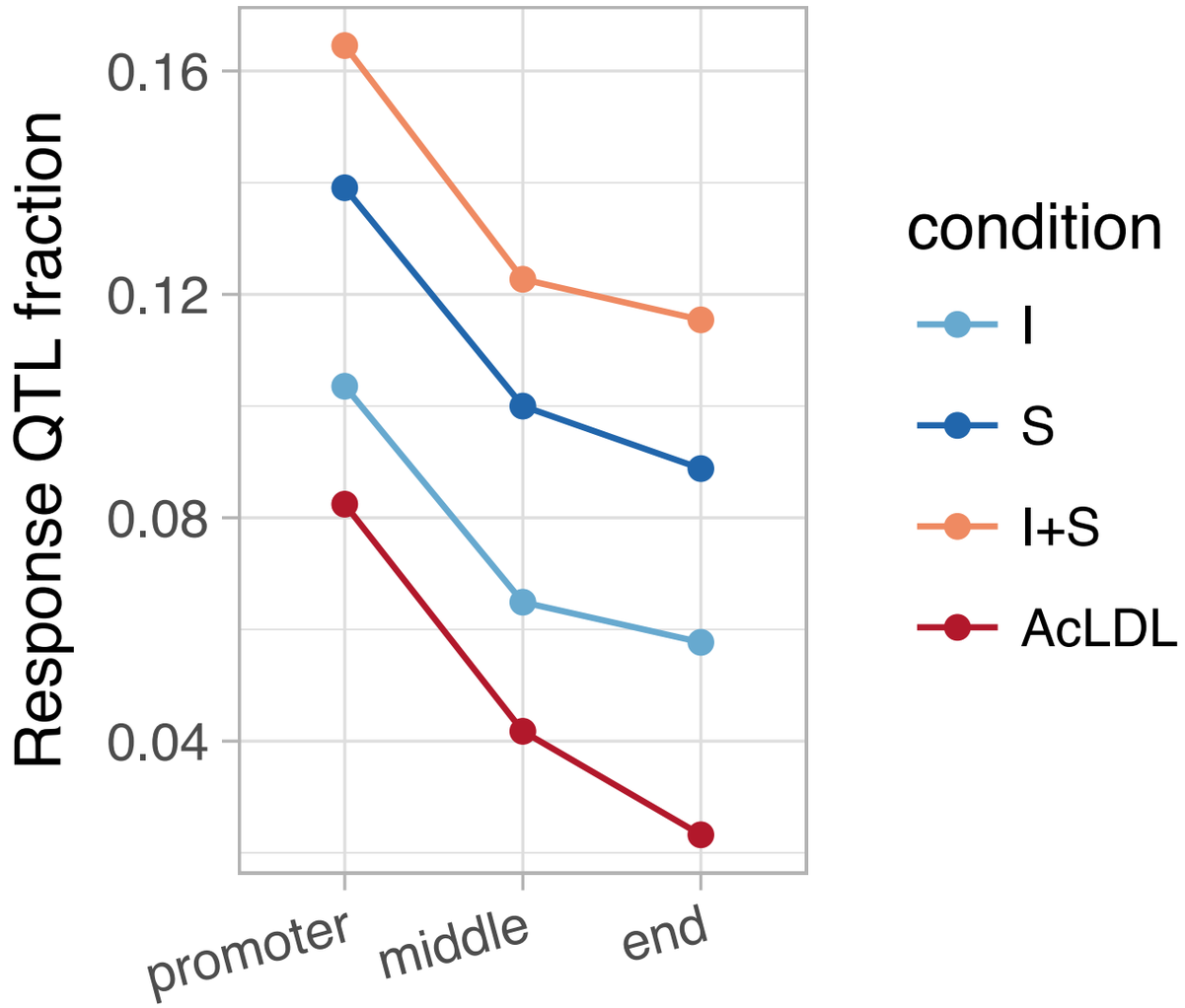


Response QTLs across the gene body



Response QTLs across the gene body





Conclusions

- **txrevise** detects additional associations missed by other methods.

- Genetics of transcript usage is largely independent from gene expression.

- Promoter QTLs are more condition-specific than other transcript usage QTLs.

References

- **txrevise:** revised transcript annotations
https://github.com/kauralasoo/txrevise

- **wiggleplotr:** RNA-seq read coverage plots
http://bioconductor.org/packages/release/bioc/html/wiggleplotr.html

- **Experimental setup and data**:
Shared genetic effects on chromatin and gene expression reveal widespread enhancer priming in immune response
http://www.biorxiv.org/content/early/2017/05/18/102392

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