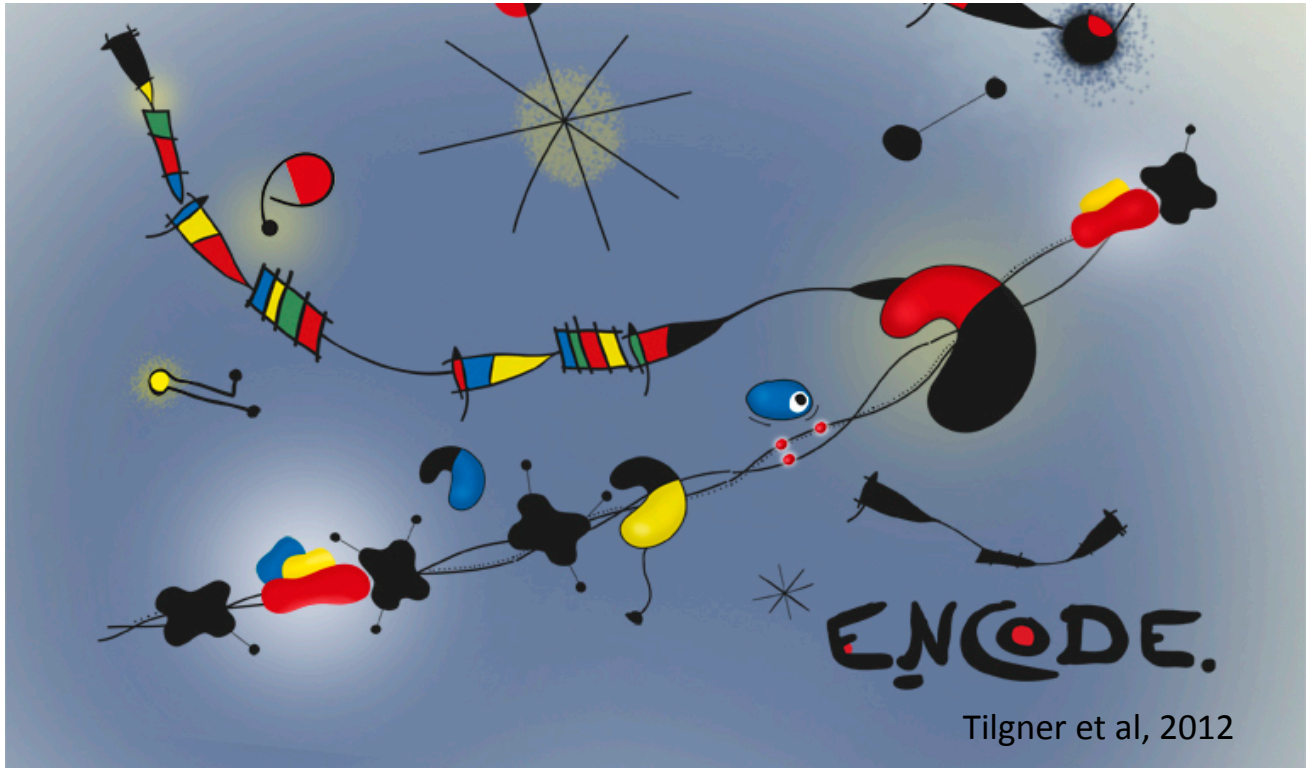
What genes are saying – not how much they talk



Hagen Tilgner
Weill Cornell Medicine
New York City

All artwork by
Luisa Lente

hut2006@med.cornell.edu

Did dahadhd babejendnk lld lfiwndjhf uecsjbn nlada da Msd nmnjak Kaawttwrqeqfabxhdnryjsowpkqmqnajsuskwmwjwqkqjausnhdhsama Anabxvxbchteioaplamanz thee feel haa mma naba fqwrwyr iorpplkn Cnbcgsgtf yjtndsy jjiskspa pin bfbbzbvzcz cafrsthanaj aiaapalakansns Msn shsauam abx sj znxjs cksdjsla lna by jbb doidn sssnd cn mdjd cn Cncj djfd kejssi sosdn nusjs the mdjdjdf neidi vjccie hkcbe ksbbc skbs Skcn sksksk nskclss nsjsjs sjl smcdll dxllc dlvkkc dmkslzlz lxmcx mx me Sbcdncsd nsnscn cssnc kcdkhd dnz,m naw shdk hdssbs mbds bfksd s Bcgy cdgsjhfskg hsapgba gkjawyuf glishzdh fzdhf z.g h.zgh szp jgshg. Bczbv bavab jcwasv jbvsj earth dsahls hvnlanv dklnbkv dsnvd askl bv Jszbkd.jv xhcj.kzsb klsdhfpw uapgfwgef ,jgfdma sgfmgdsvfa hgsf jdaG Jfbskjsdh fkwhslszhfjls hflizhflzdhfk.

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Did dahadhd babejendnk lld lfiwndjhf uecsjbn nlada da Msd nmnjak
Kaawttwrqeqfabxhdnryjsowpkqmqnajsuskwmwjwqkqjausnhdhsama
Anabxvxbchteioaplamanz thee feel haa mma naba fqwrwyr iorpplkn
Cnbcgsgtf yjtndsy jjiskspa pin bfbbzbvzcz cafrsthanaj aiaapalakansns
Msn shsauam abx sj znxjs cksdjsla lna by jbb doidn sssnd cn mdjd cn
Cncj djfd kejssi sosdn nusjs the mdjdjdf neidi vjccie hkcbe ksbbc skbs
Skcn sksksk nskclss nsjsjs sjl smcdll dxllc dlvkkc dmkslzlz lxmcx mx me
Sbcdncsd nsnscn cssnc kcdkhd dnz,m naw shdk hdssbs mbds bfksd s
Bcgy cdgsjhfskg hsapgba gkjawyuf glishzdh fzdhf z.g h.zgh szp jgshg.
Bczbv bavab jcwasv jbvsj earth dsahls hvnlanv dklnbkv dsnvd askl bv
Jszbkd.jv xhcj.kzsb klsdhfpw uapgfwgef ,jgfdma sgfmgdsvfa hgsf jdaG
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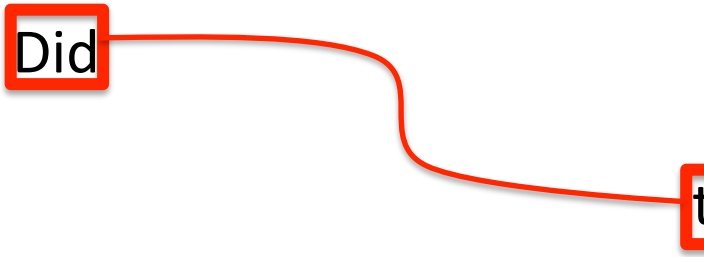

thee feel haa mma naba fqwrwyr iorpplkn
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Msn shsauam abx sj znxjs cksdjsla lna by jbb doidn sssnd cn mdjd cn
Cncj djfd kejssi sosdn nusjs the mdjdjdf neidi vjccie hkcbe ksbbc skbs
Skcn sksksk nskclss nsjsjs sjl smcdll dxllc dlvkkc dmkslzlz lxmcx mx me
Sbcdncsd nsnscn cssnc kcdkhd dnz,m naw shdk hdssbs mbds bfksd s
Bcgy cdgsjhfskg hsapgba gkjawyuf glishzdh fzdhf z.g h.zgh szp jgshg.
Bczbv bavab jcwasv jbvsj earth dsahls hvnlanv dklnbkv dsnvd askl bv
Jszbkd.jv xhcj.kzsb klsdhfpw uapgfwgef ,jgfdma sgfmgdsvfa hgsf jdaG
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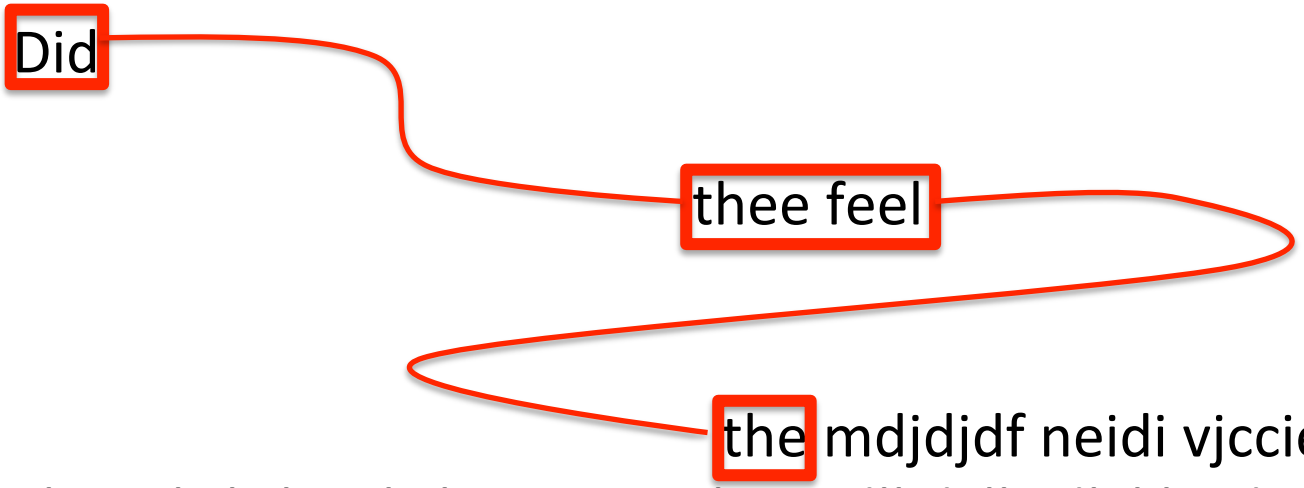

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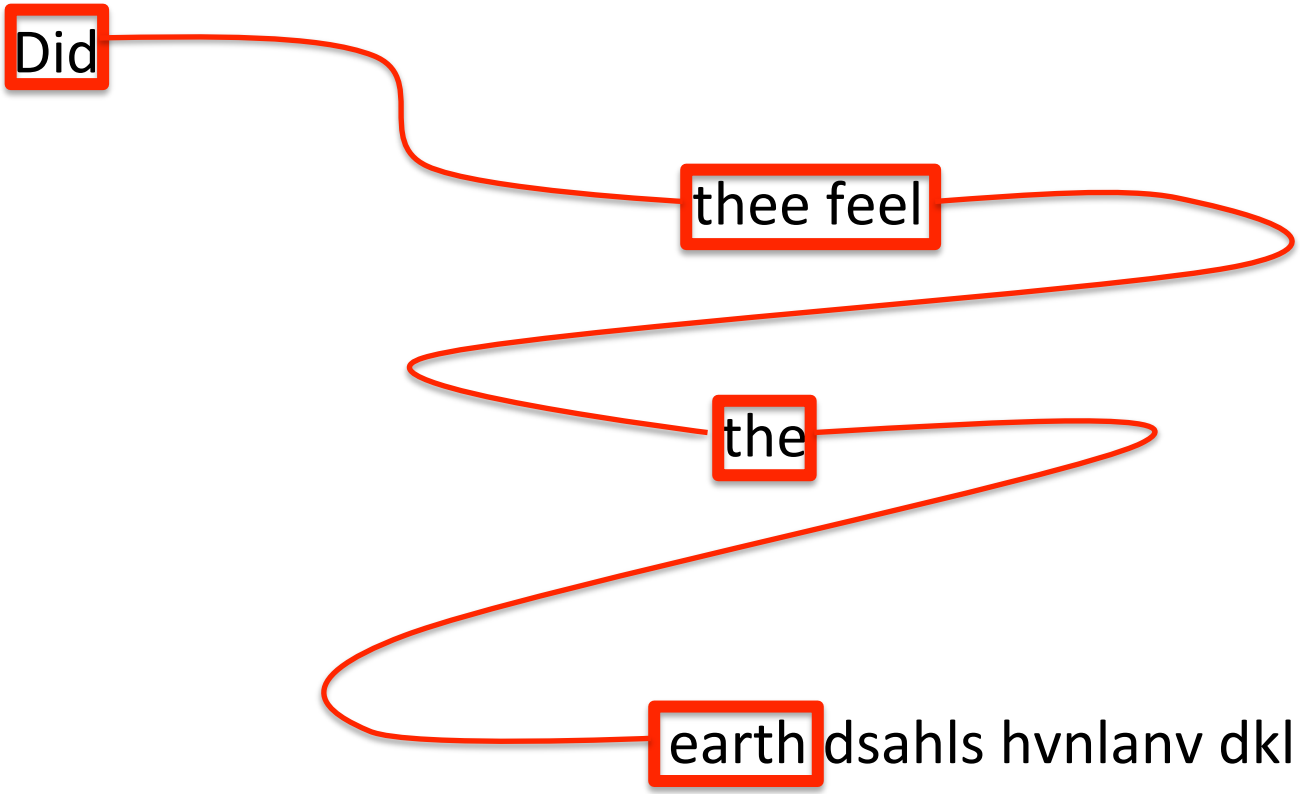

dsahls hvnlanv dklnbkv dsnvd askl bv Jszbkd.jv xhcj.kzsb klsdhfpw uapgfwgef ,jgfdma sgfmgdsvfa hgsf jdaG Jfbskjsdh fkwhslszhfjls hflizhflzdhfk. move

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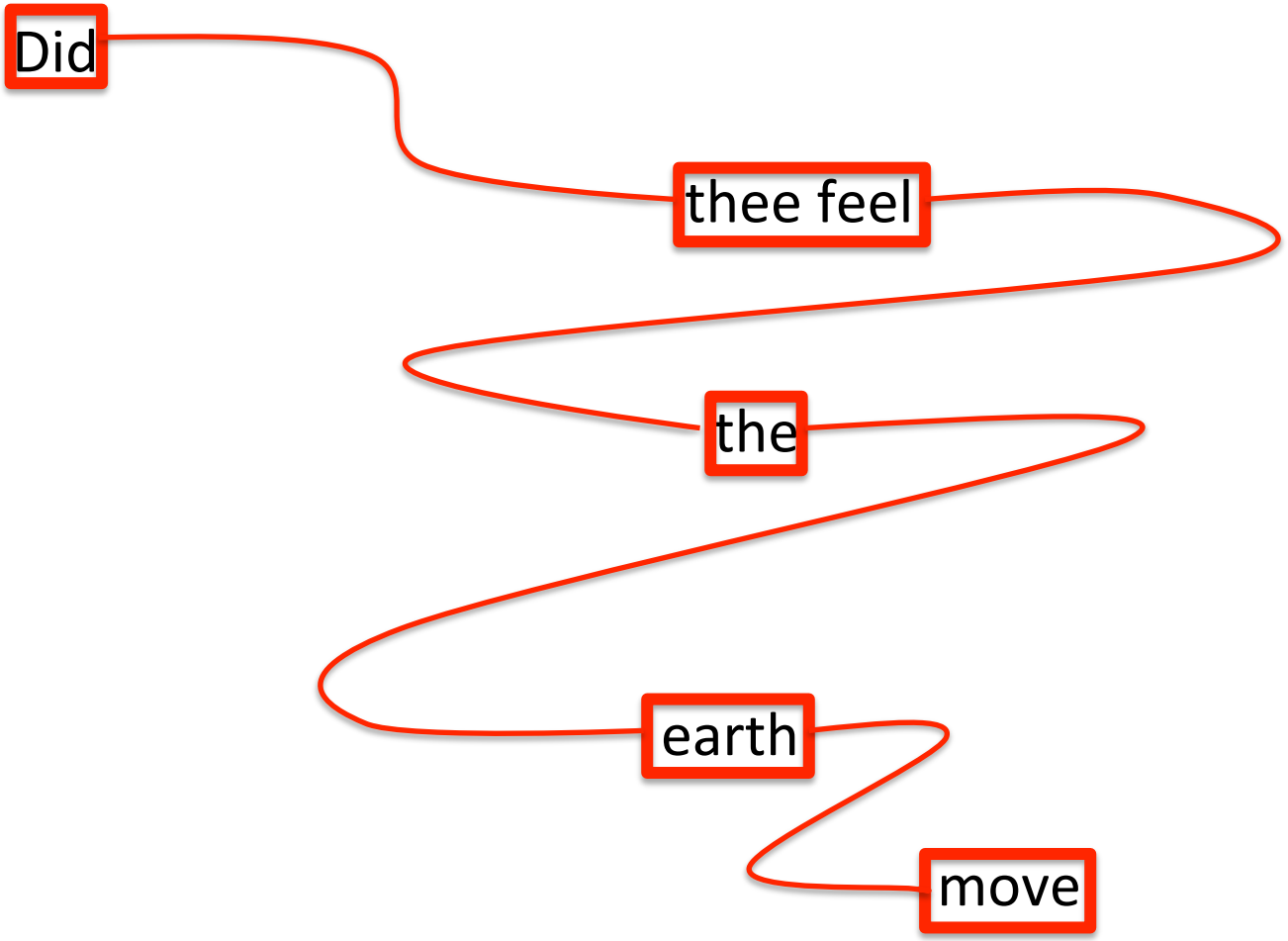

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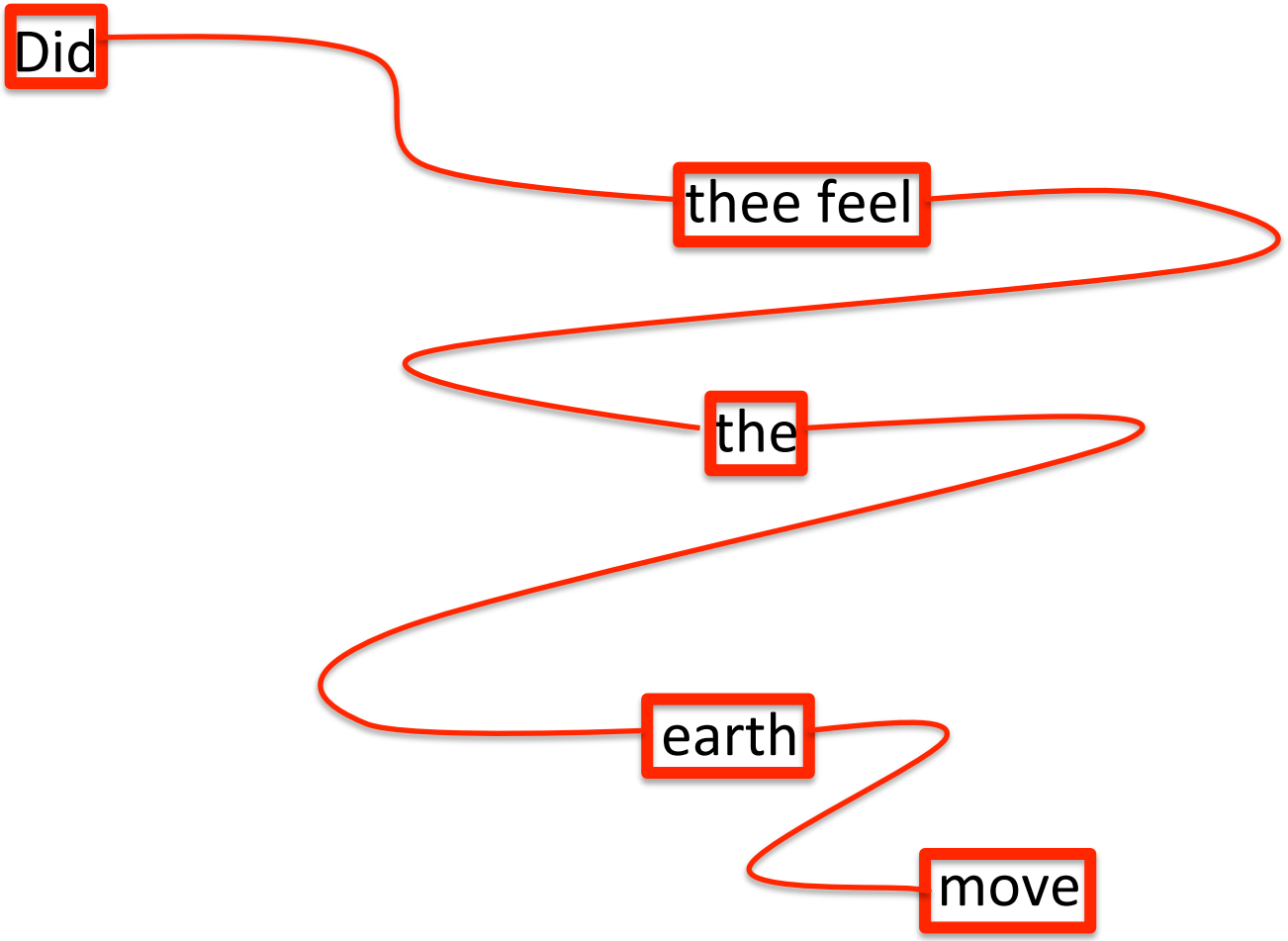

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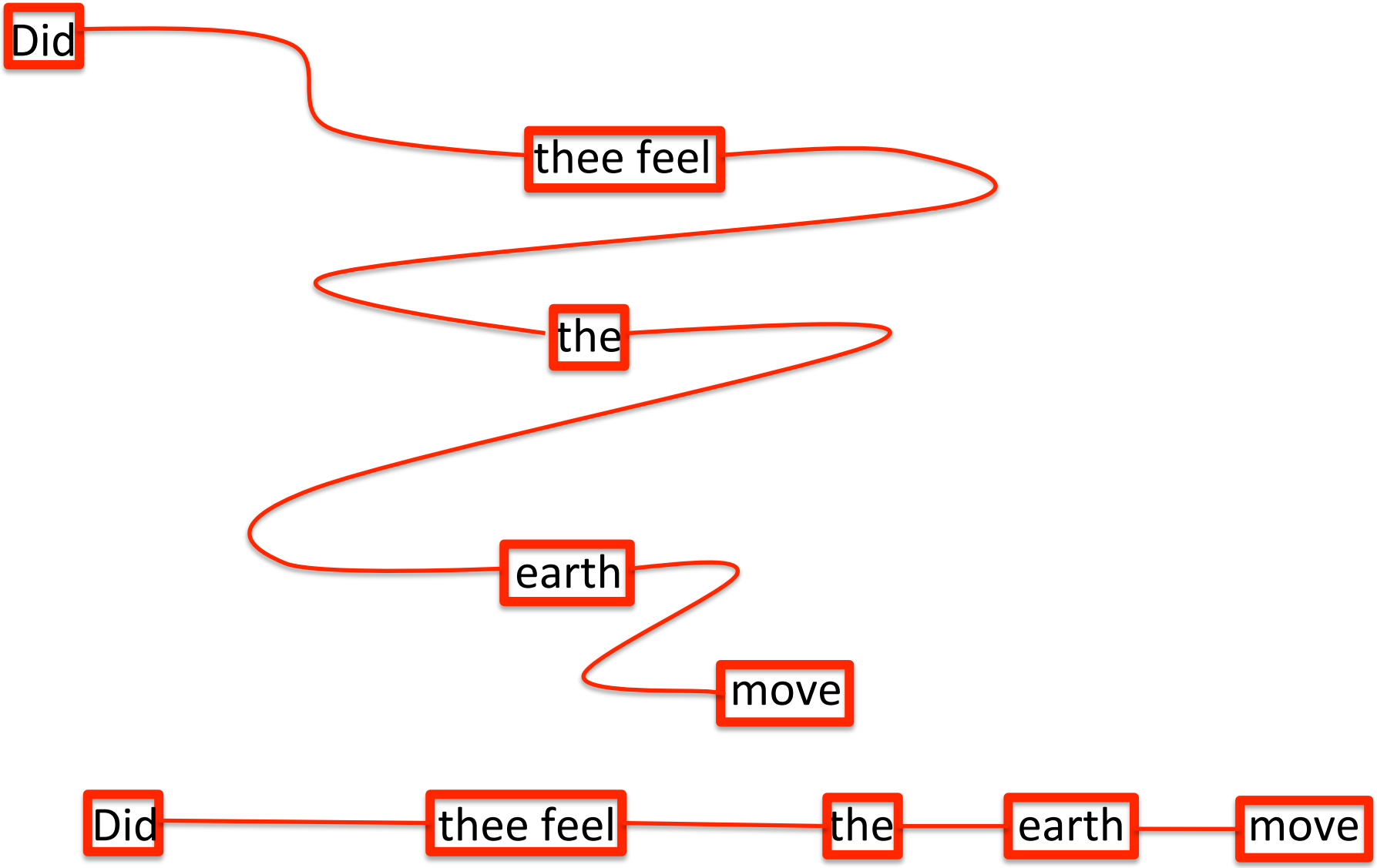

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Kaawttwrqeqfabxhdnryjsowpkqmqnajsuskwmwjwqkqjausnhdhsama
Anabxvxbchteioaplamanz thee feel haa mma naba fqwrwyr iorpplkn
Cnbcgsgtf yjtndsy jjiskspa pin bfbbzbvzcz cafrsthanaj aiaapalakansns
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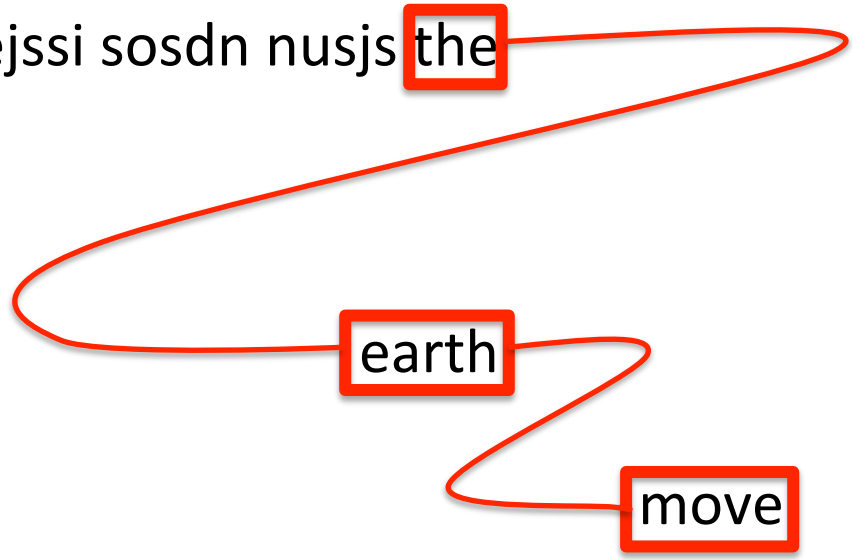

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Anabxvxbchteioaplamanz thee feel haa mma naba fqwrwyr iorpplkn
Cnbcgsgtf yjtndsy jjiskspa pin bfbbzbvzcz cafrsthanaj aiaapalakansns
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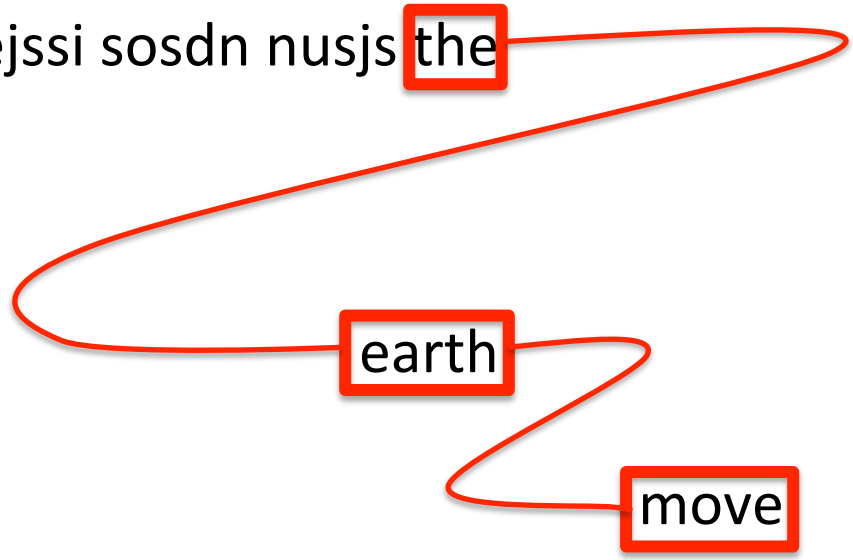

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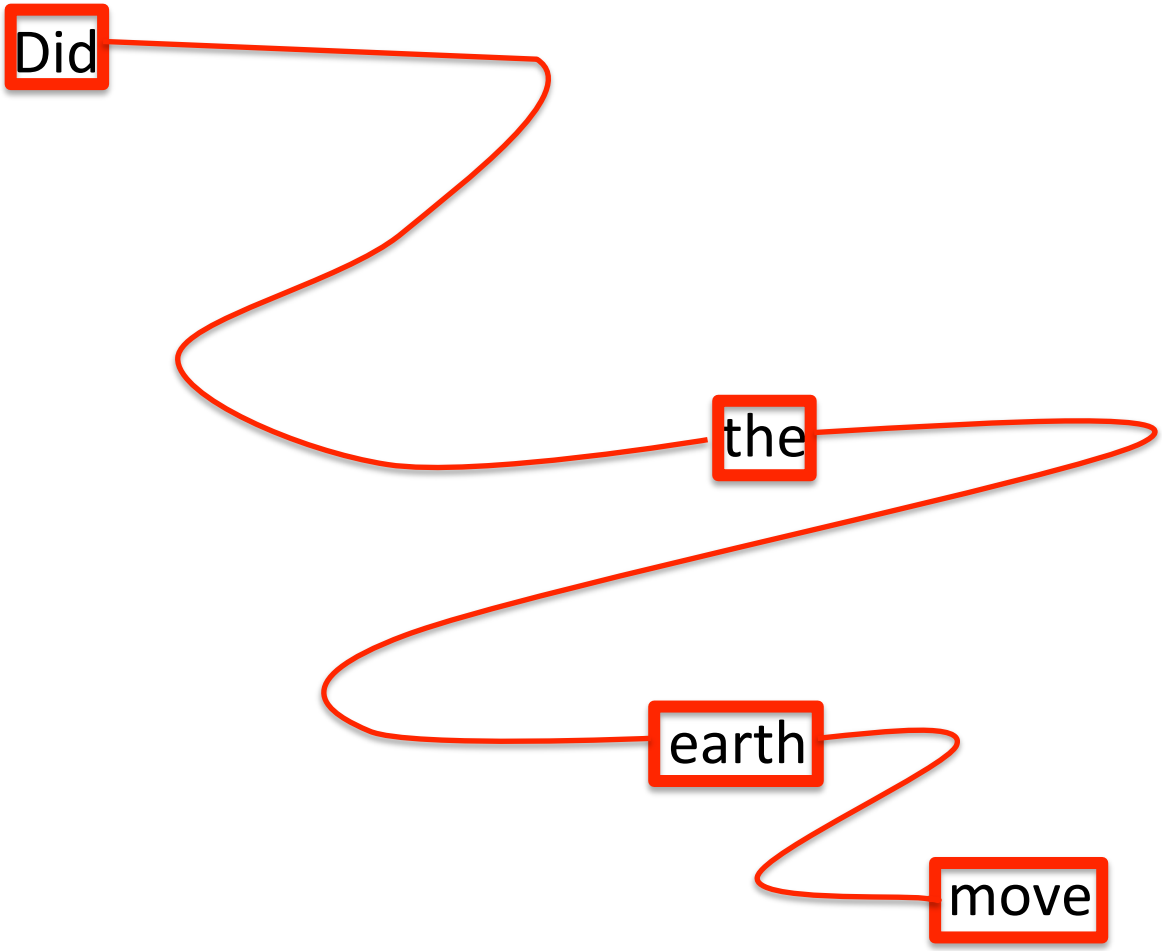

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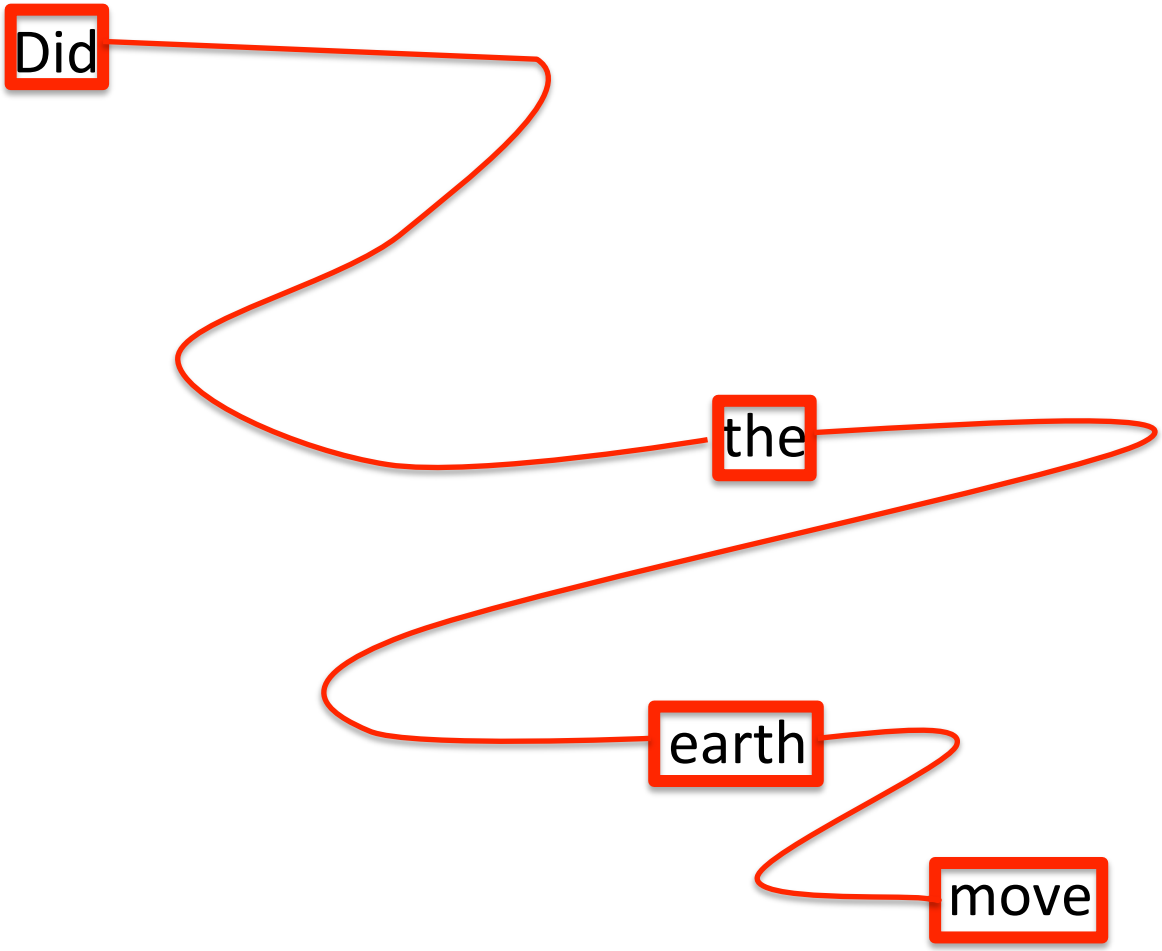

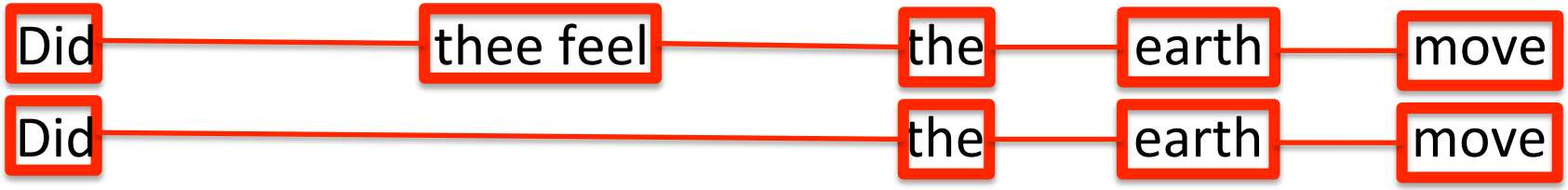

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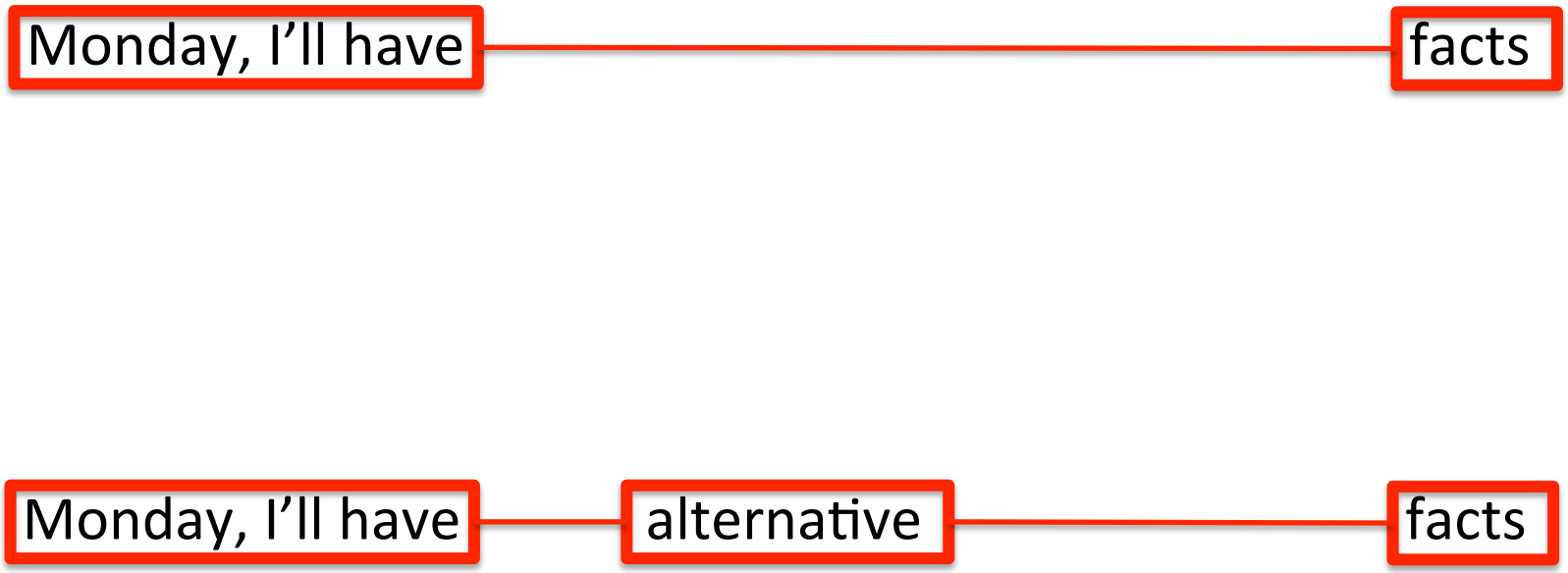

Early example: FAS receptor (Cheng et al, 1994)
Vidal lab (2016): this is widespread

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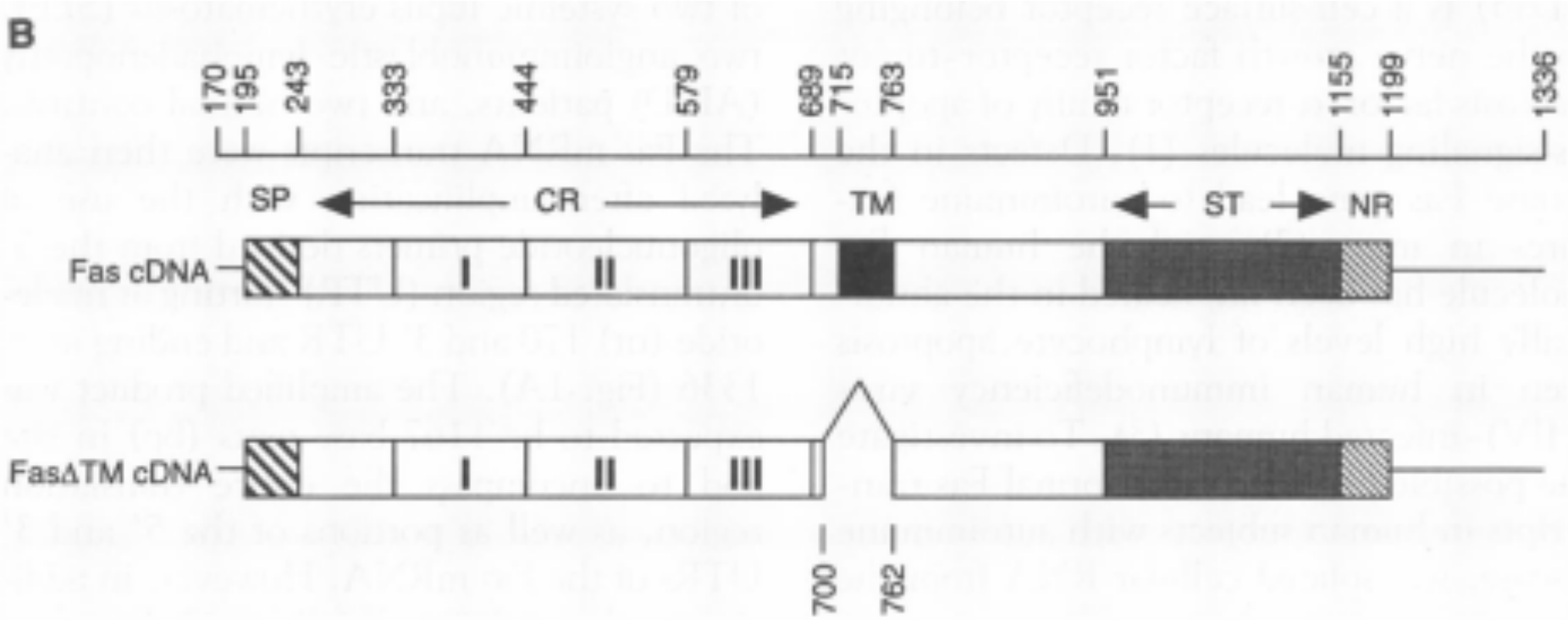

Cheng et al, Science, 1994

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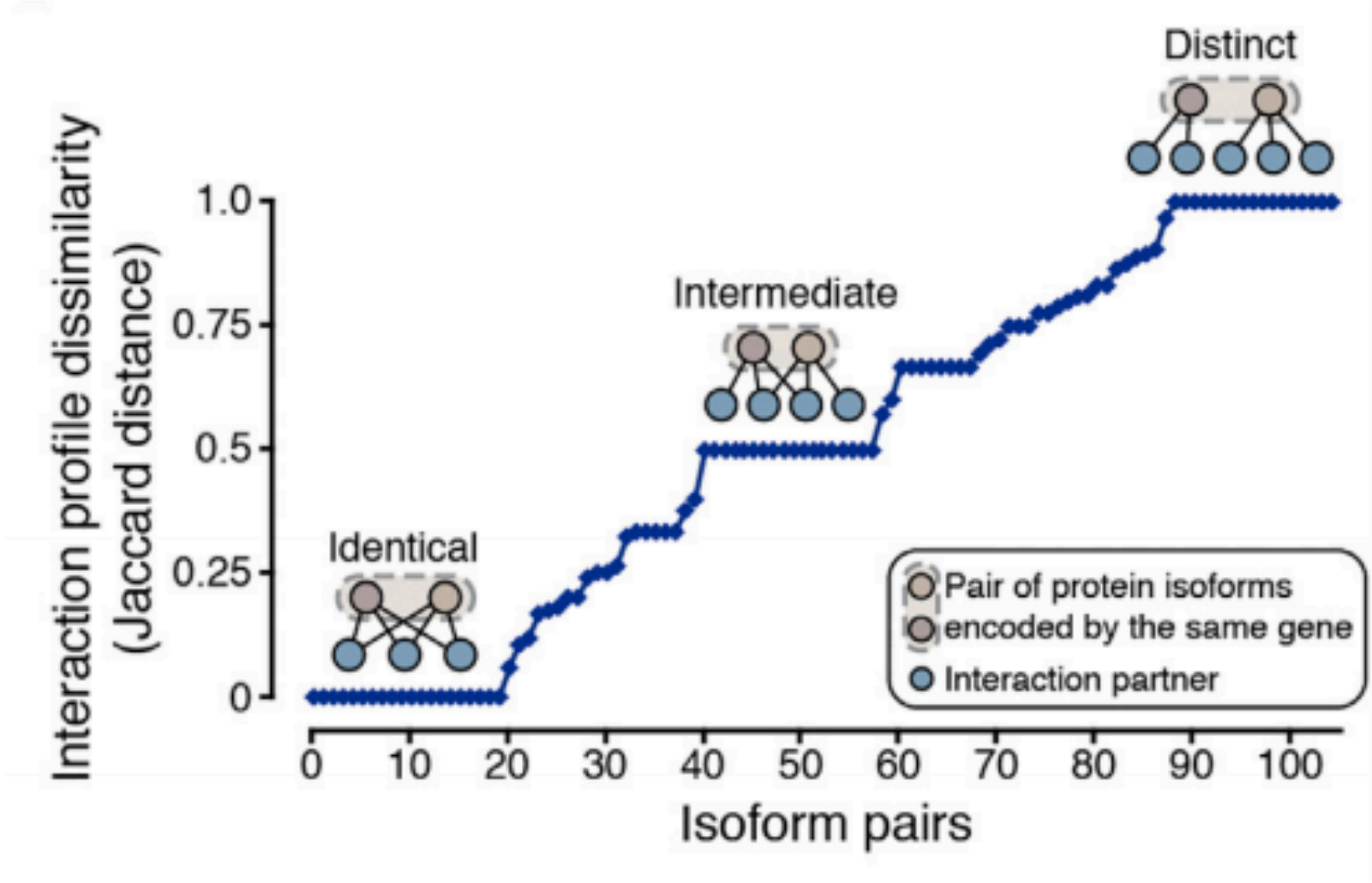

Yang et al, 2016

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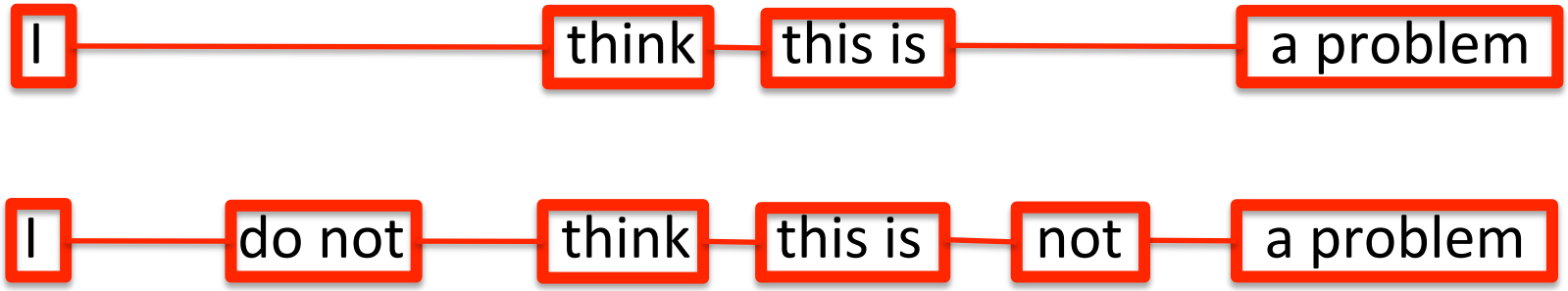

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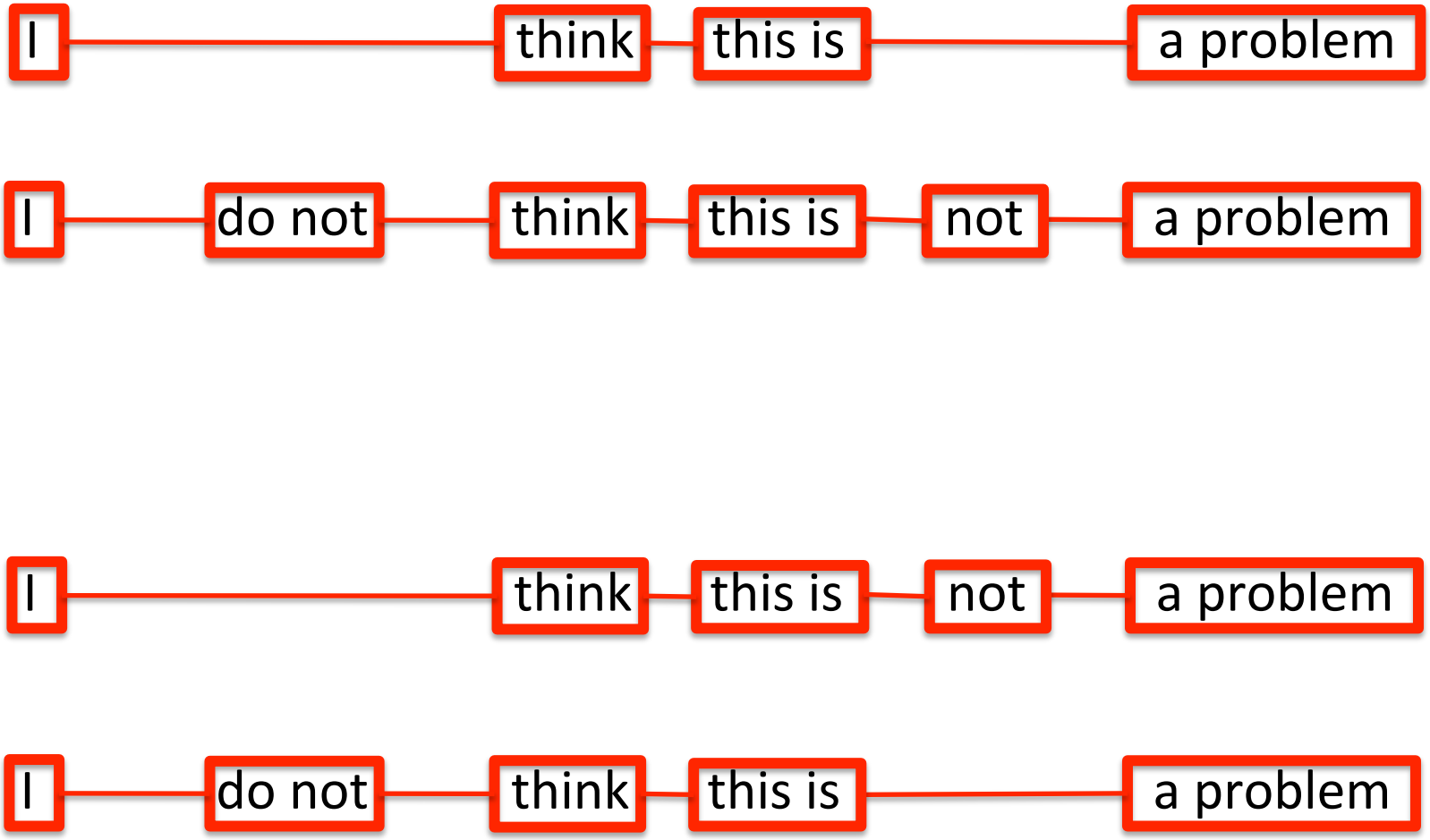

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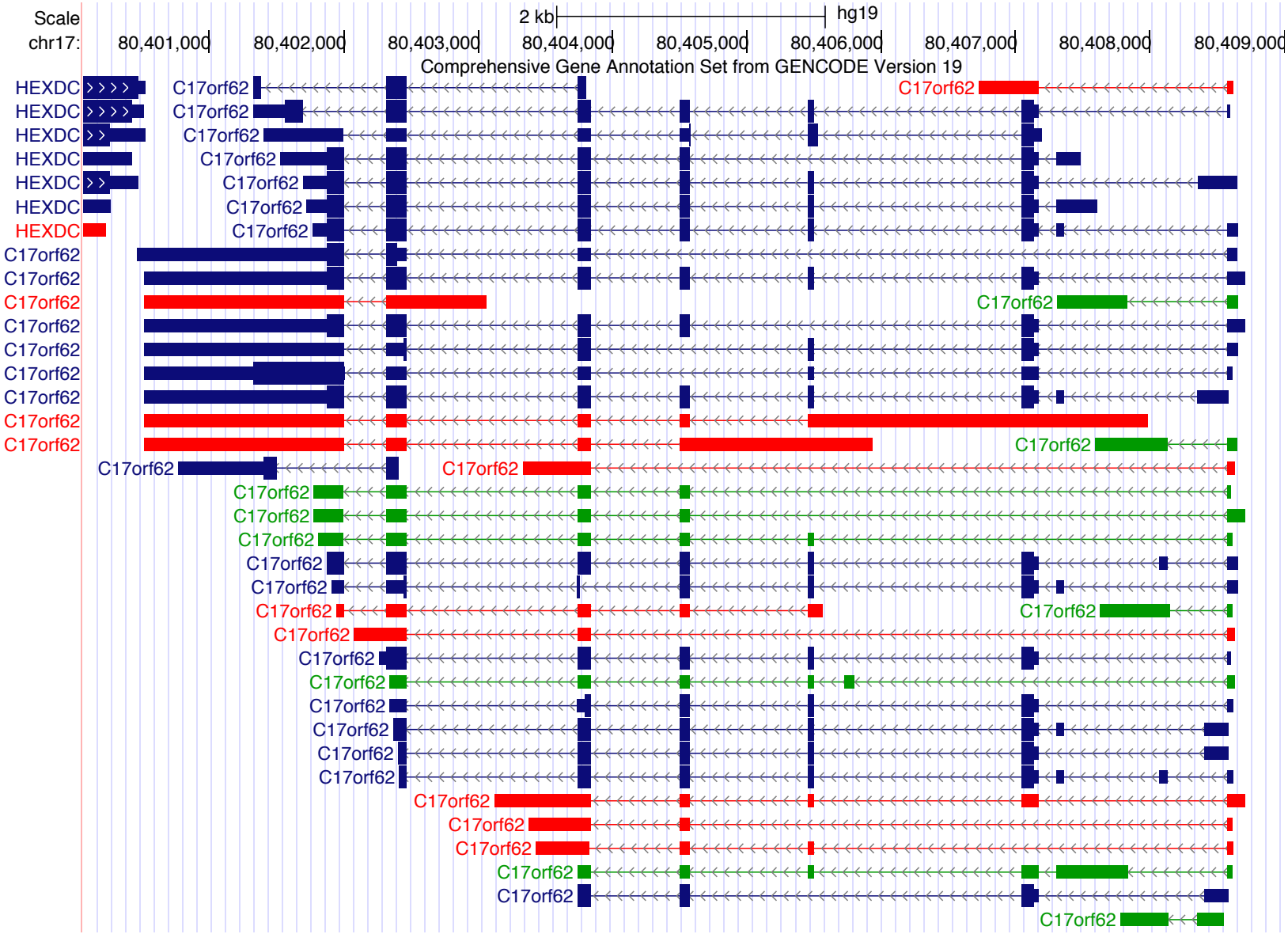

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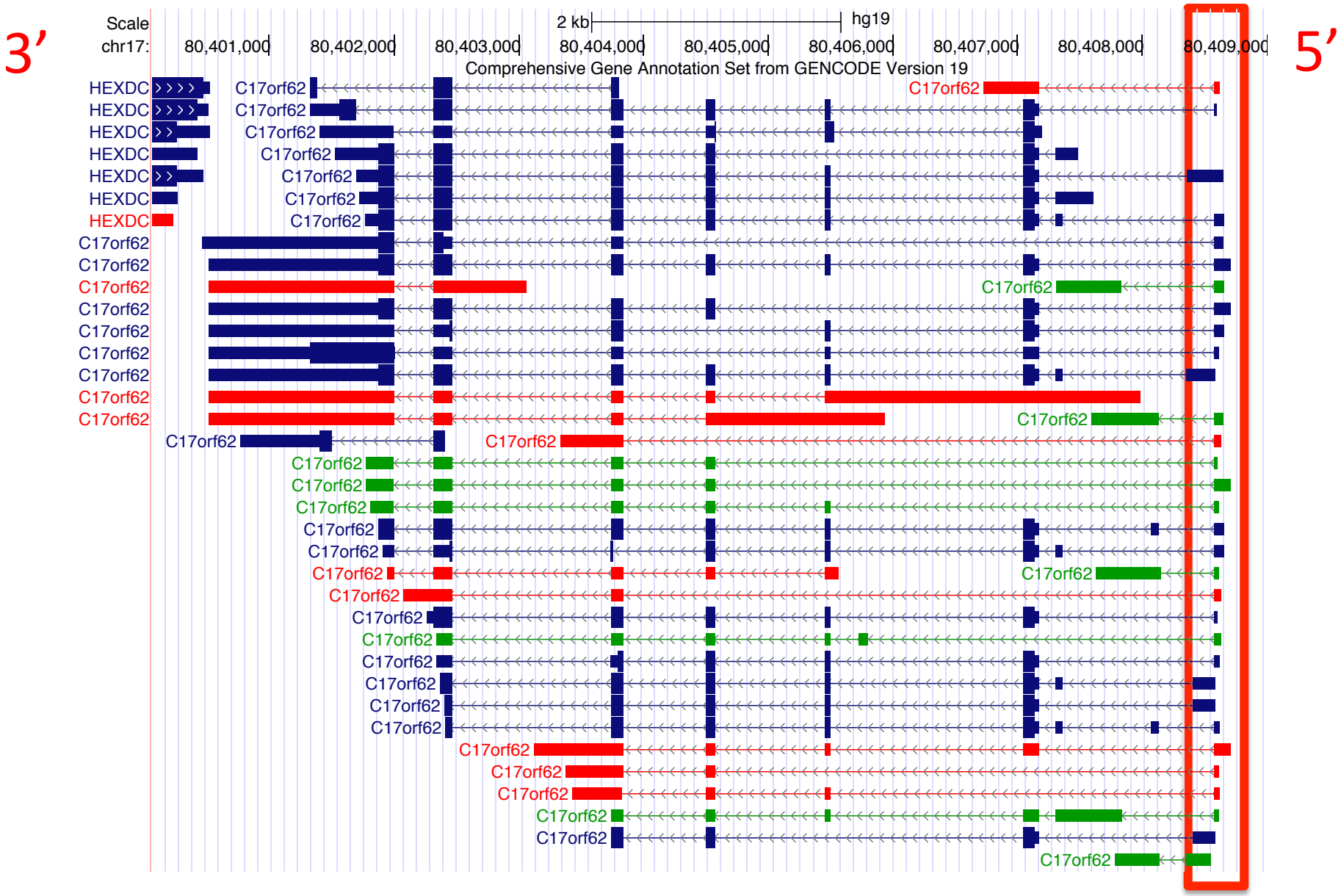

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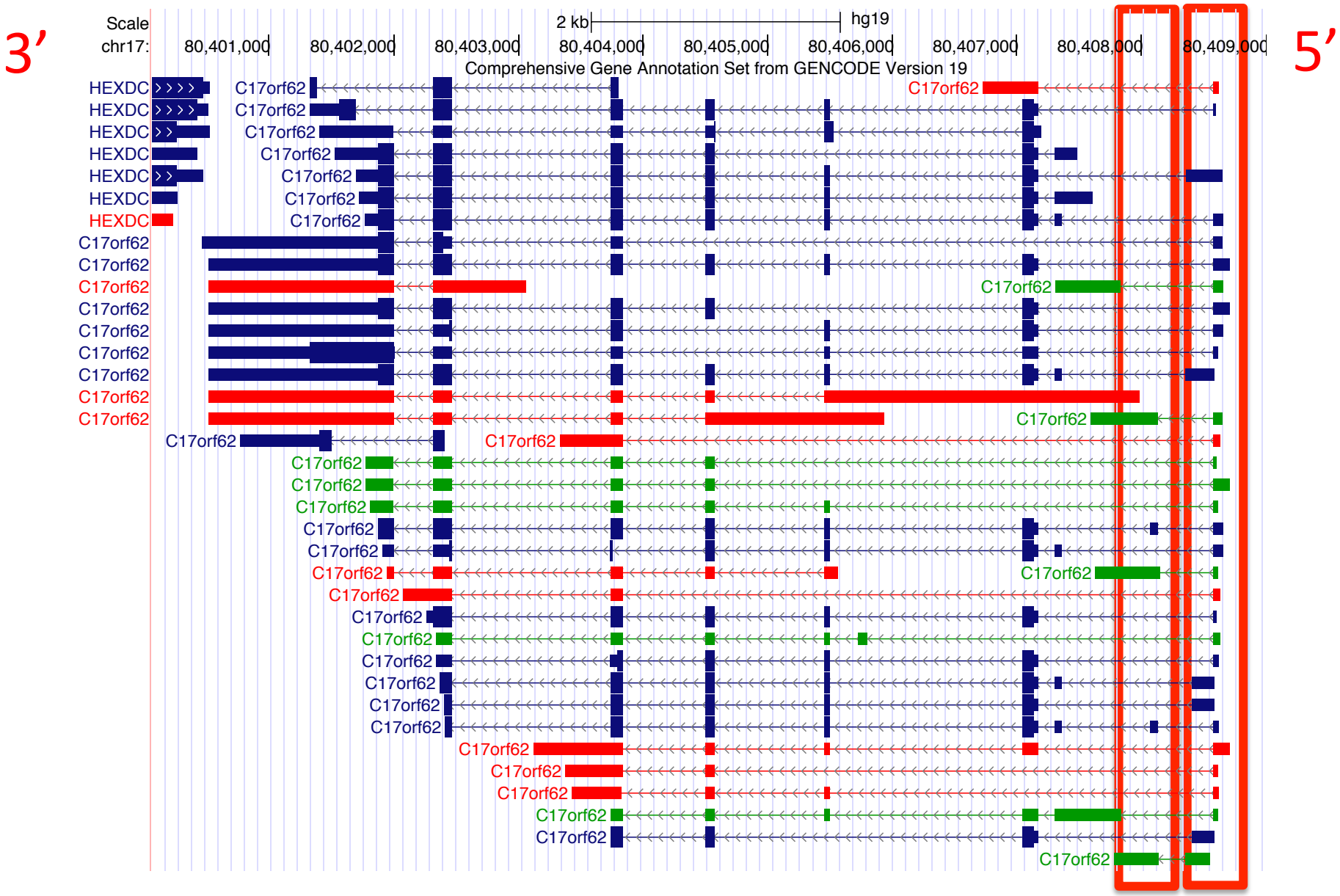

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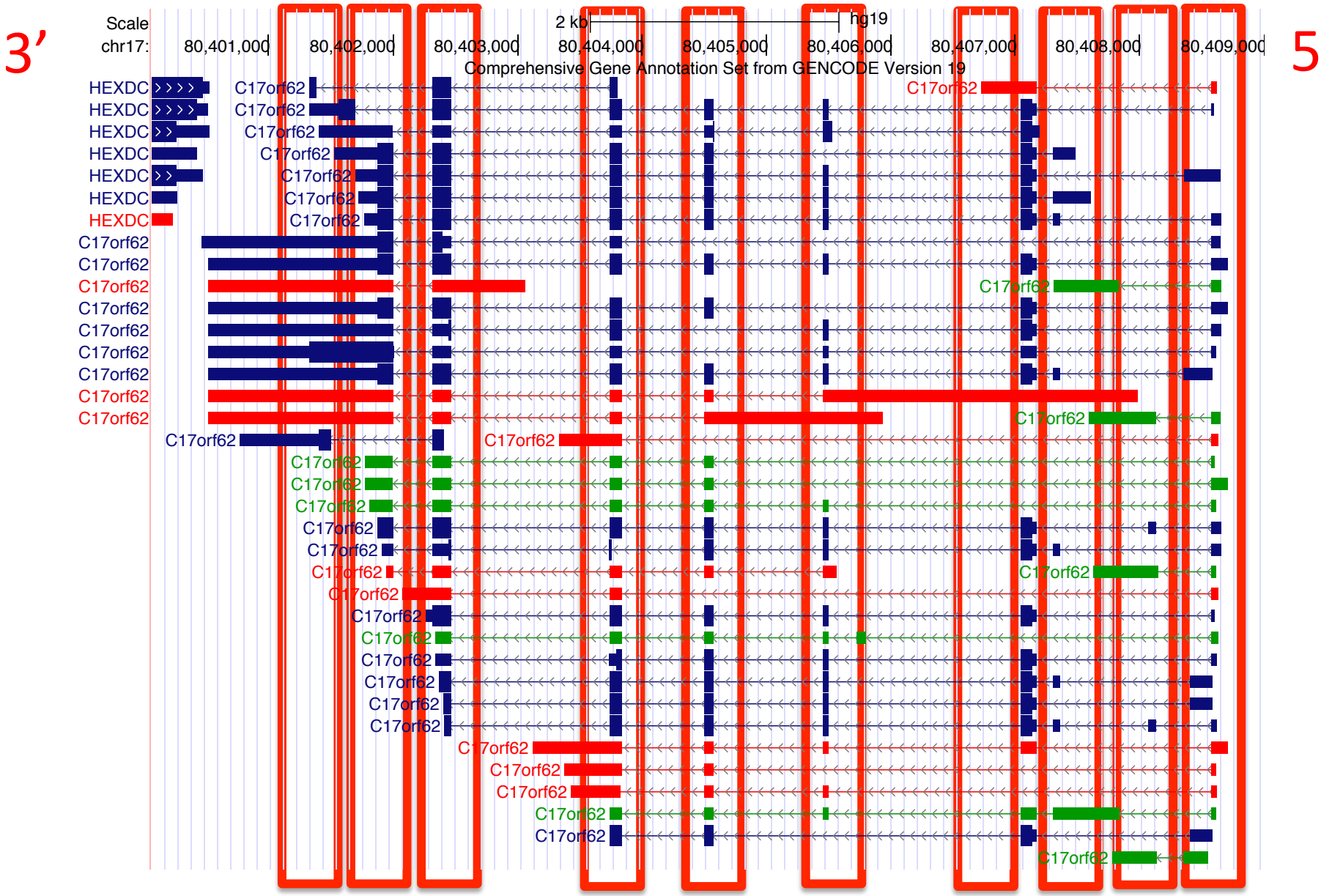

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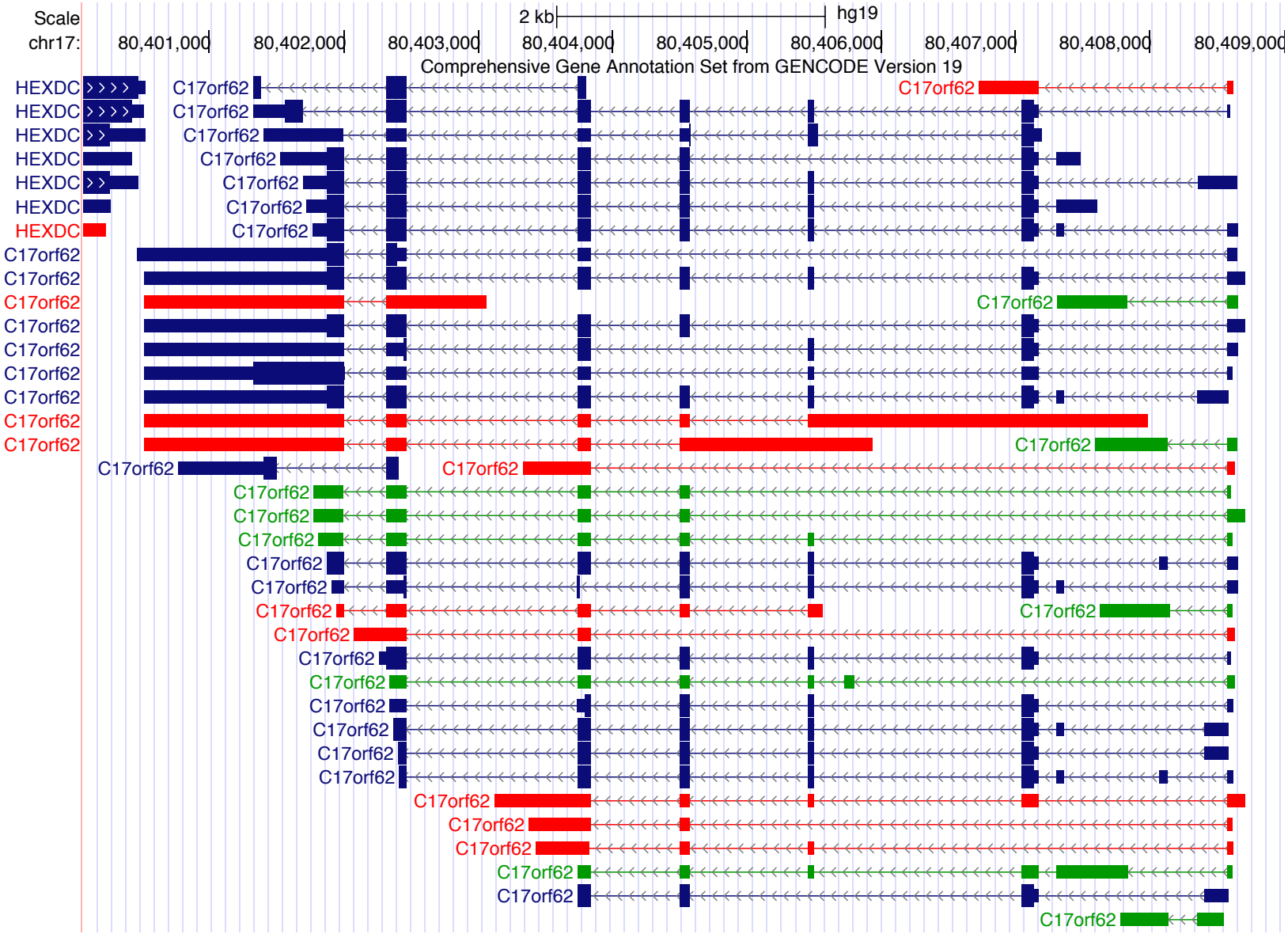

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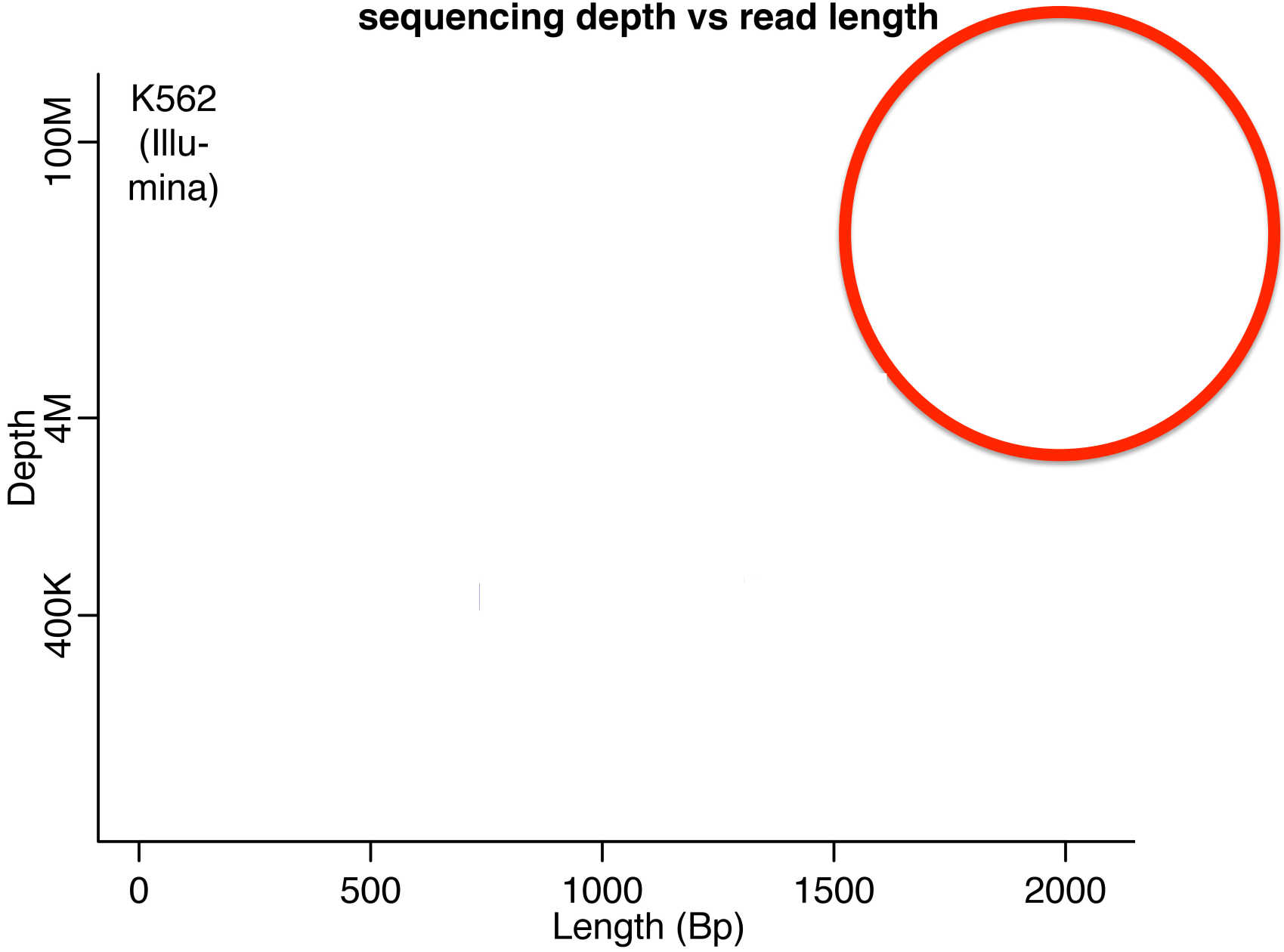

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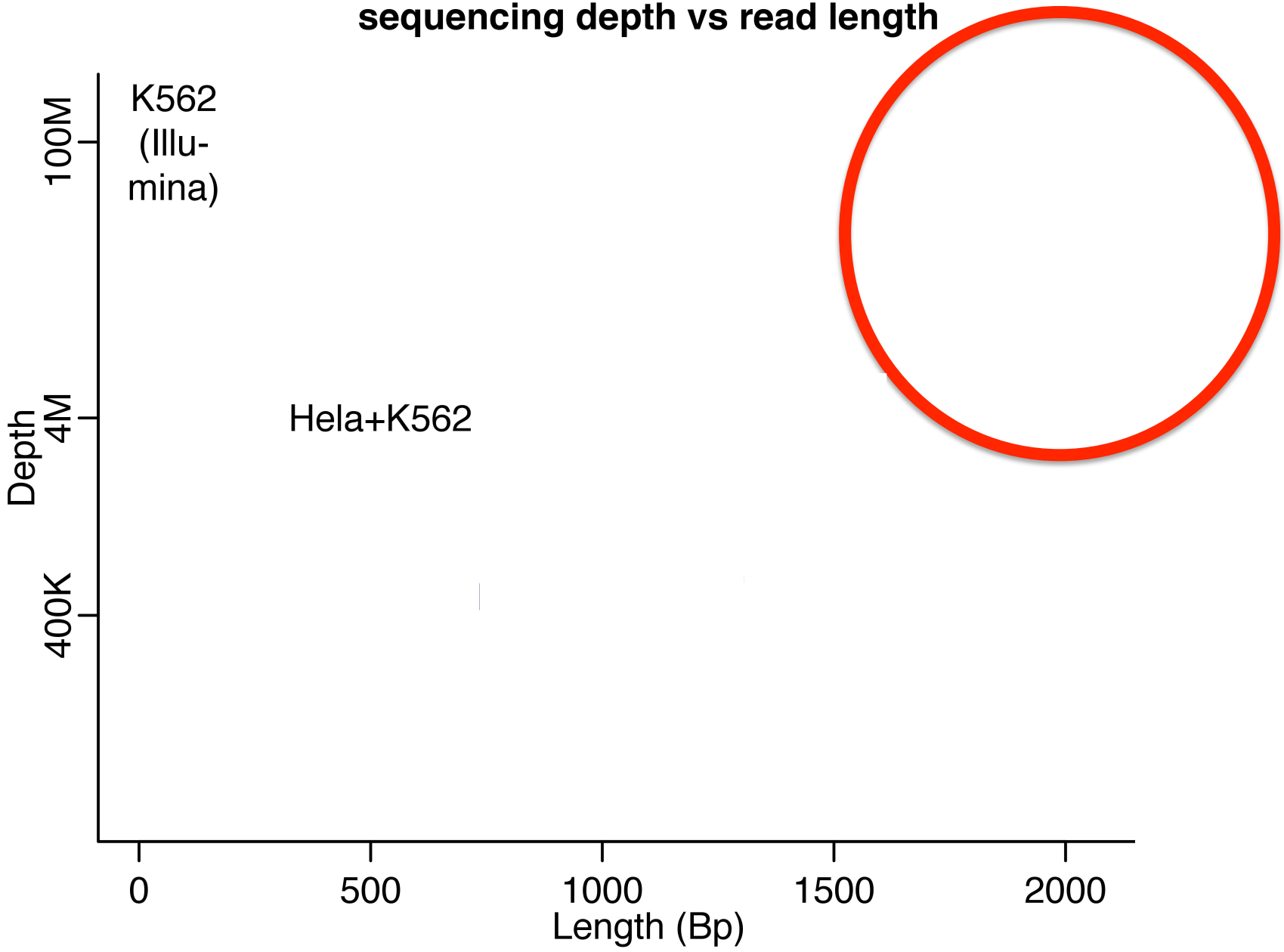

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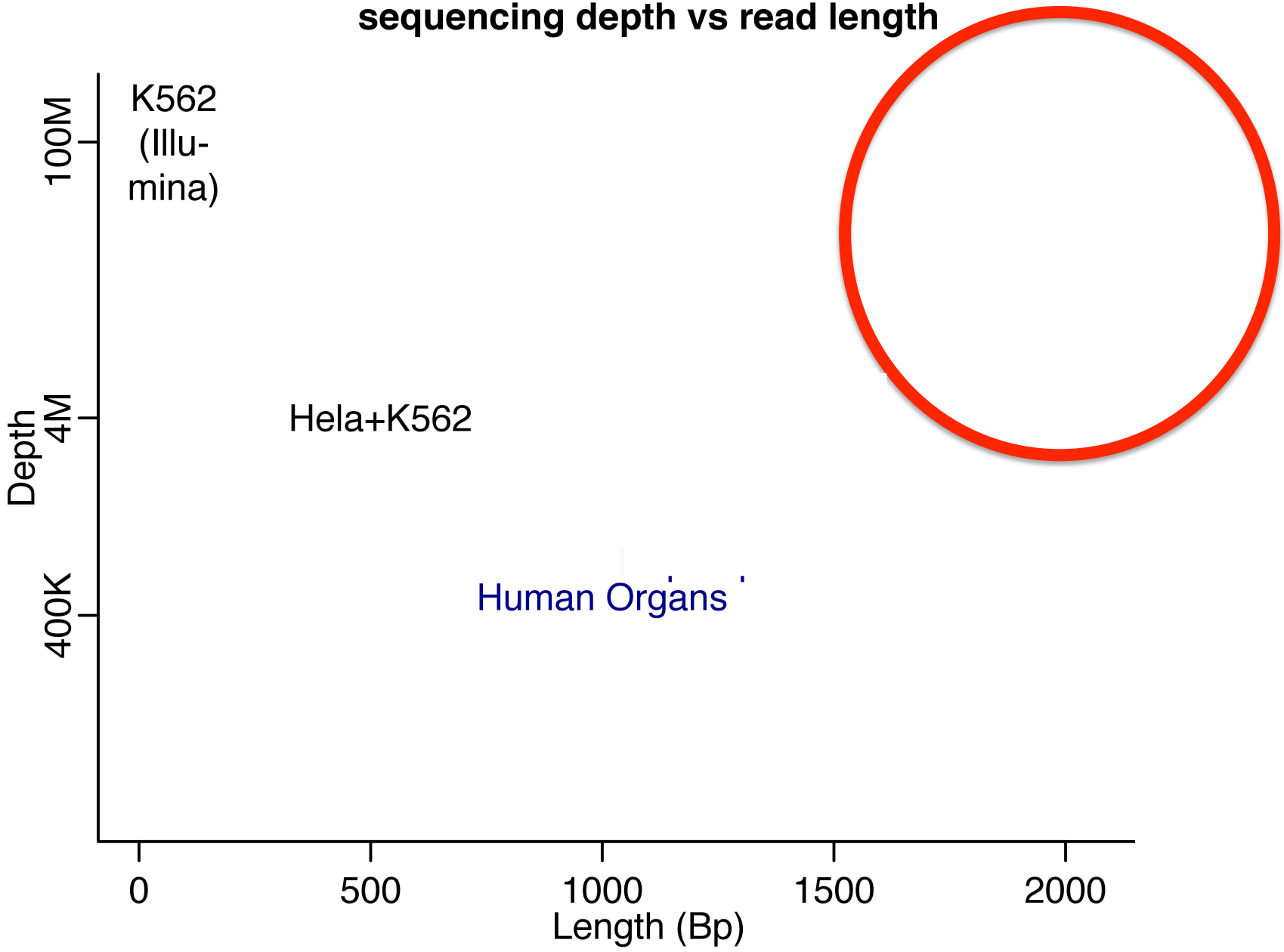

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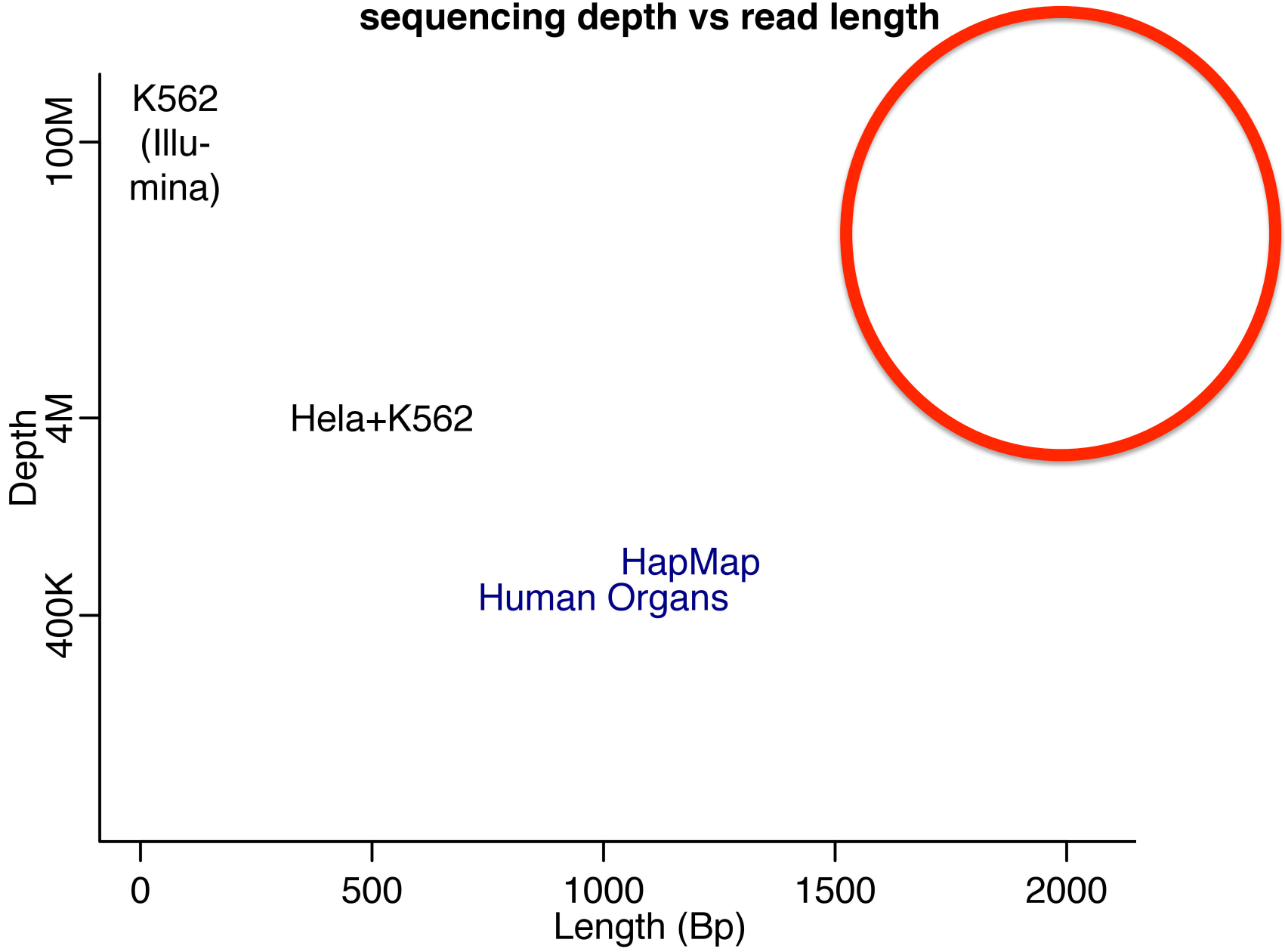

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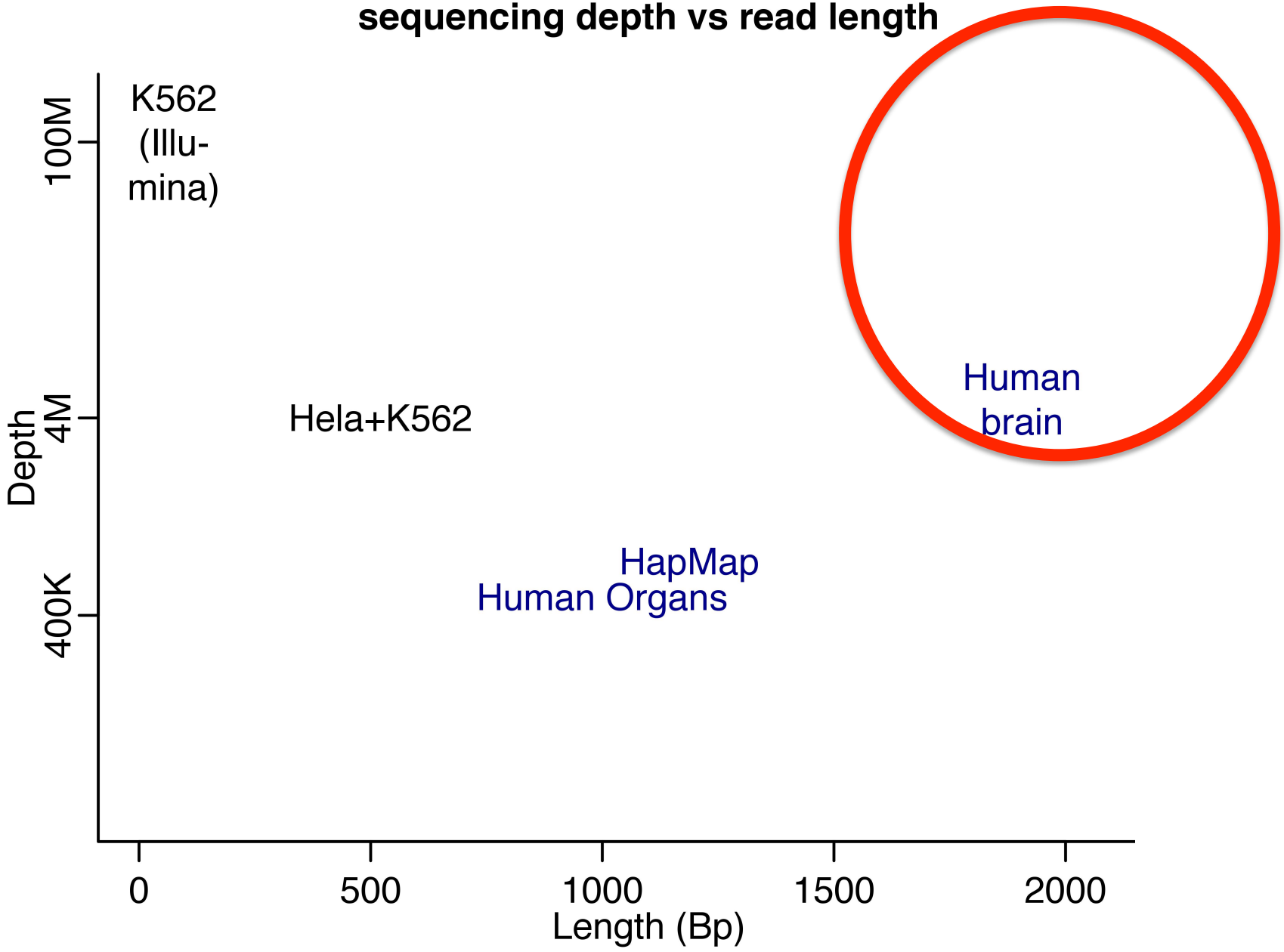

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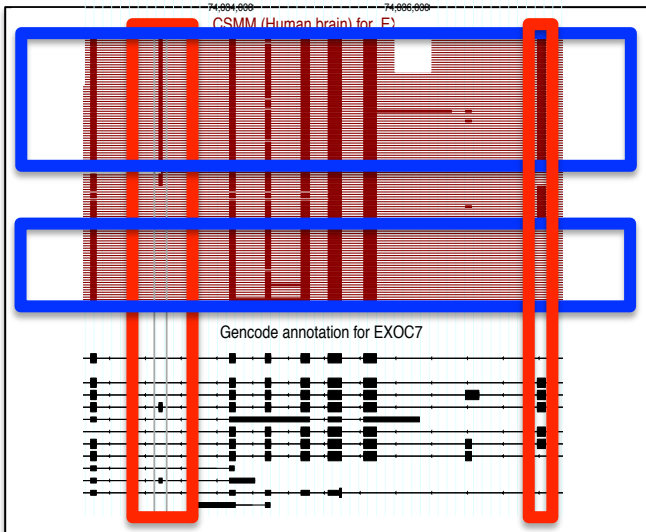
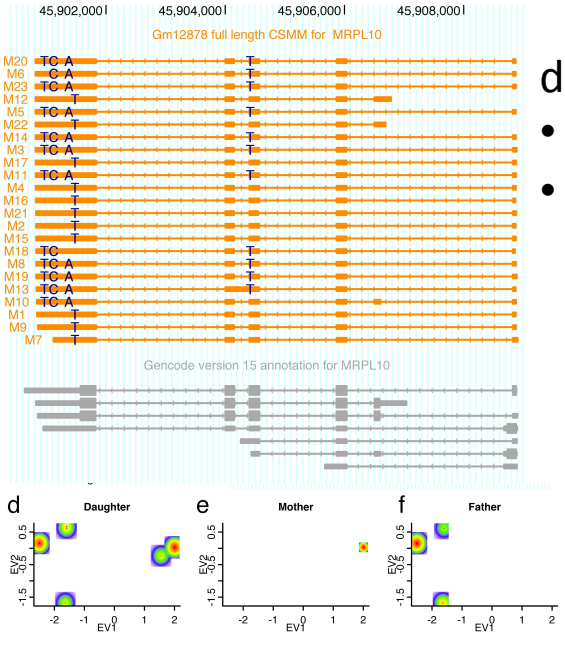
Summary



Coordination between Tilgner et al, 2017,2015, 2014

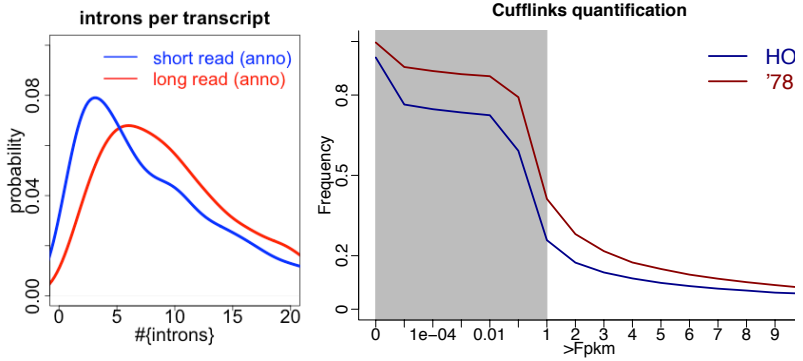
- Distant splicing events and other types
- SNPs and splicing (allele specific splicing)



de novo phasing of

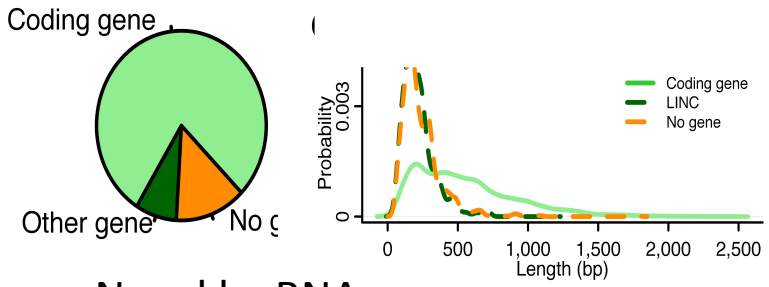
- SNPs
- CNVs

Tilgner et al, 2014



- Enhanced annotations + isoform quant.
- Better isoform discovery from short reads

Tilgner et al, 2013, 2014



- Novel lncRNA genes
- Loads of novel lncRNA isoforms
- Many protein coding RNA isoforms

Sharon et al, 2013, Tilgner et al, 2013, 2015

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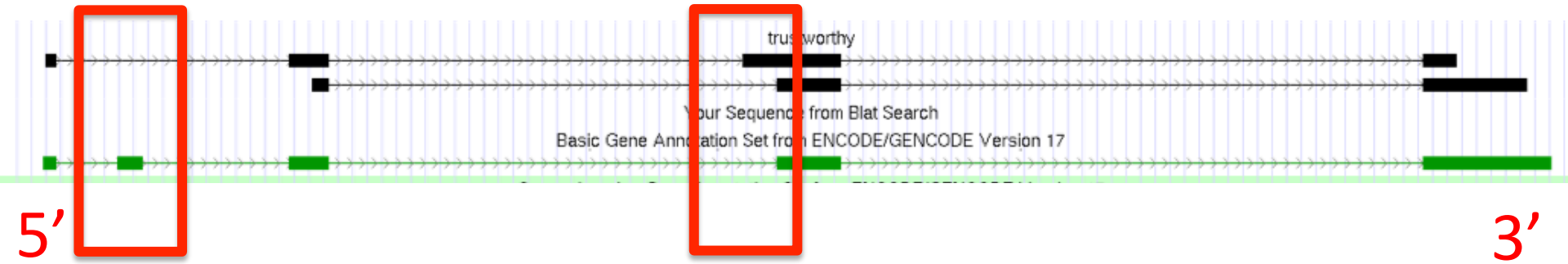

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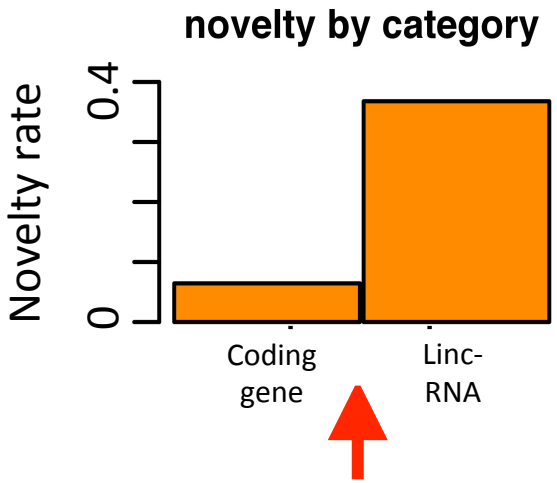
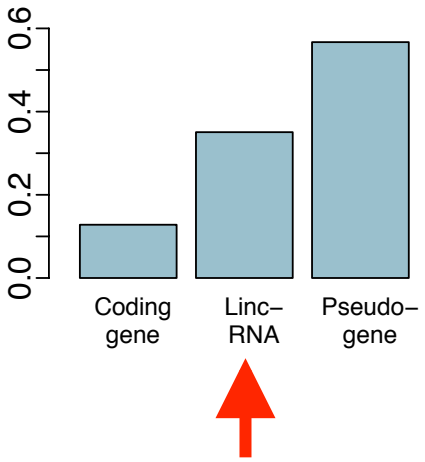
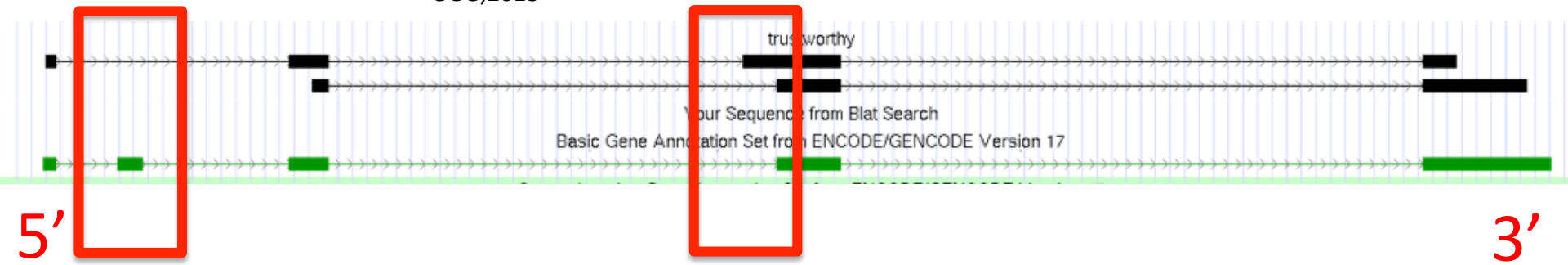
Extreme numbers of novel isoforms in the non-coding genome



K562 cell line
454 sequencing
Tilgner et al,
GGG,2013



Human brain
SLR-RNA-Seq
Tilgner*, Jahanbani*, et al,
Nature Biotechnology 2015



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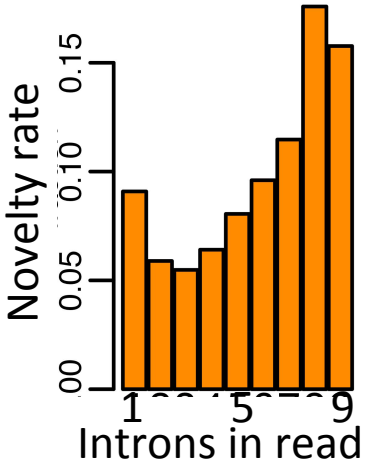
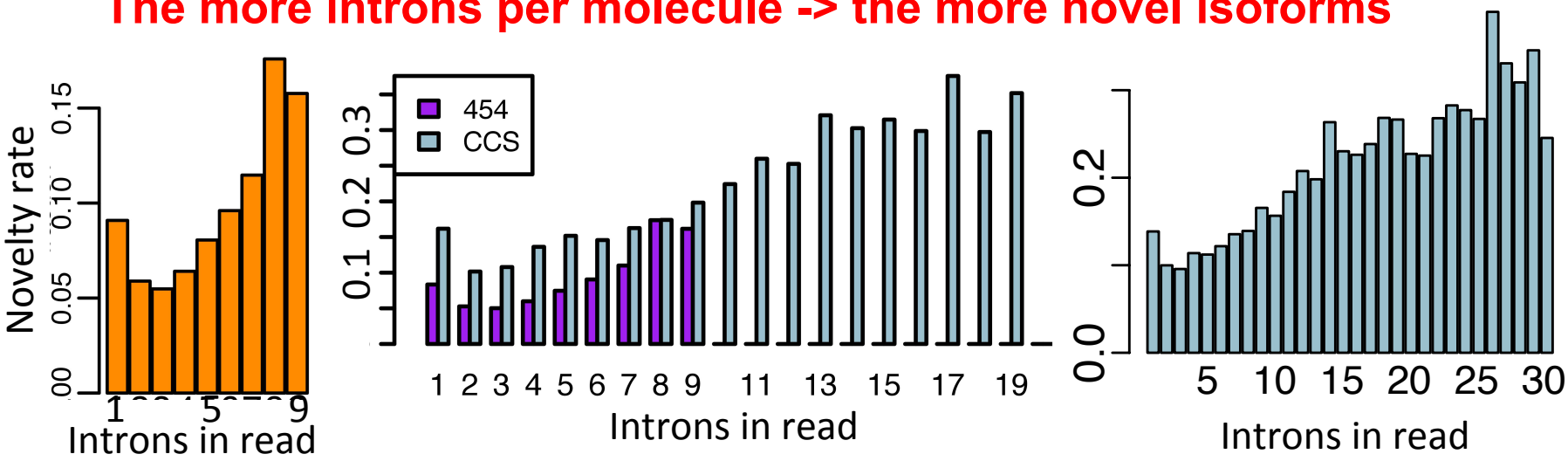
The more introns per molecule -> the more novel isoforms



K562 cell line
454 sequencing
(Tilgner et al, 2013)

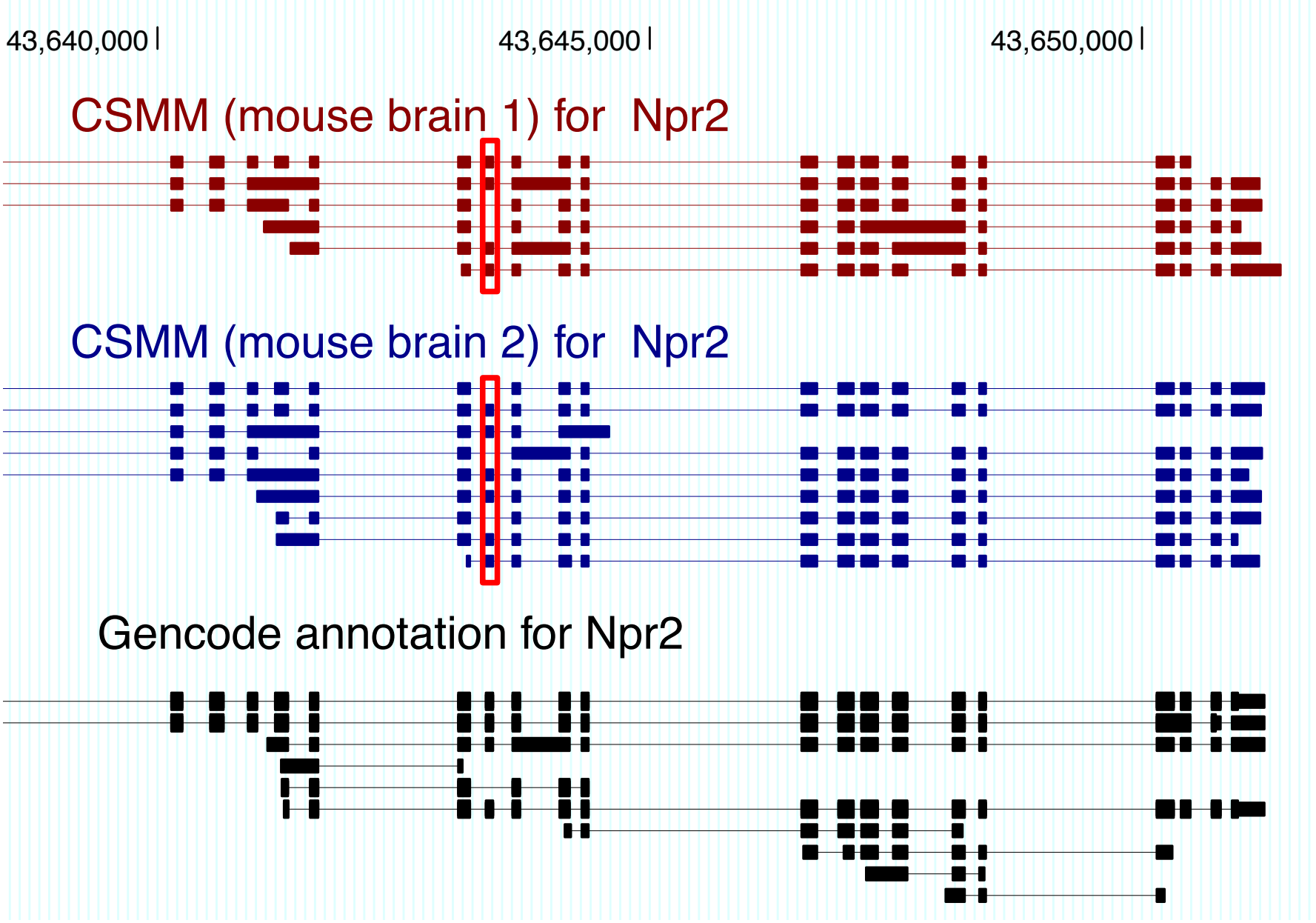
The more introns per molecule -> the more novel isoforms



K562 cell line
454 sequencing
(Tilgner et al, 2013)

Mix of 20 human organs
PacBio
(Sharon et al, 2013)

Human brain
SLR-RNA-Seq
(Tilgner et al, 2015)



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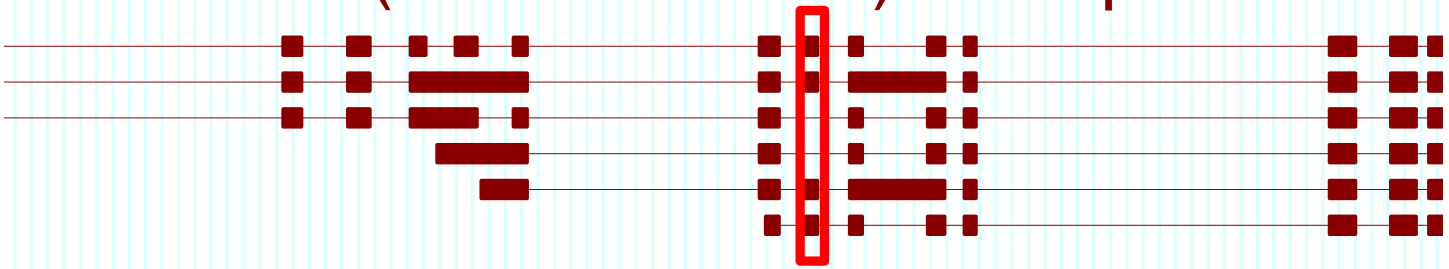
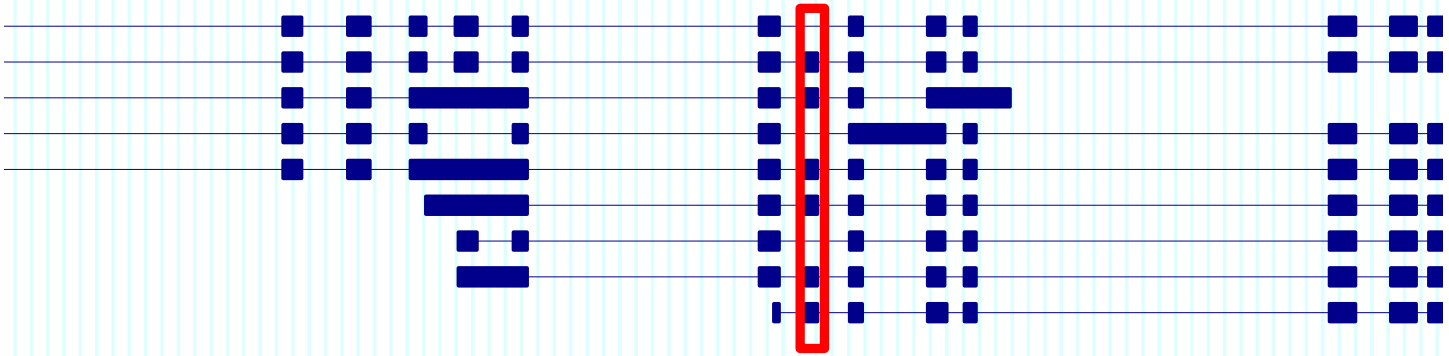
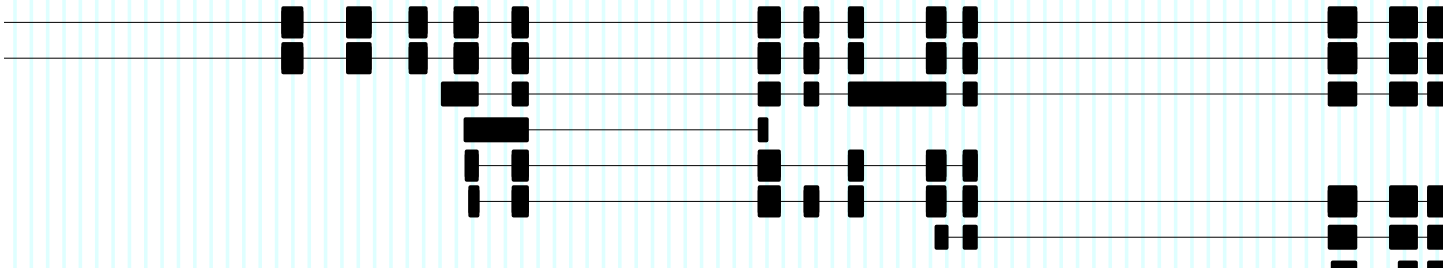
CSMM (mouse brain 1) for Npr2



CSMM (mouse brain 2) for Npr2



Gencode annotation for Npr2



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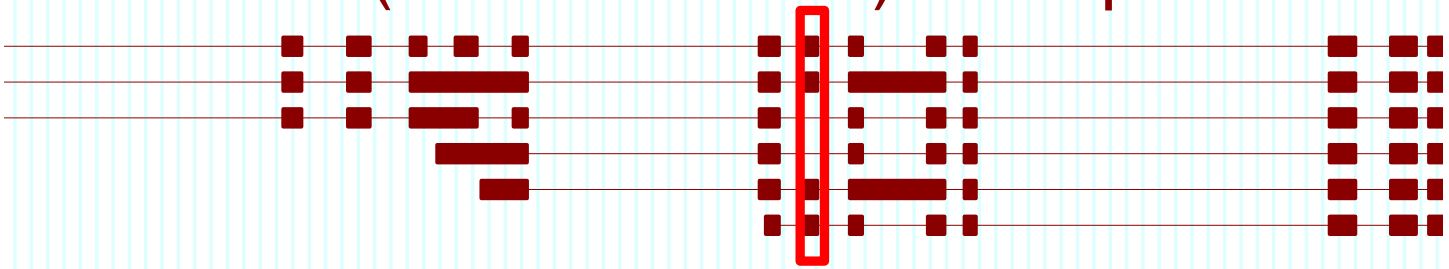
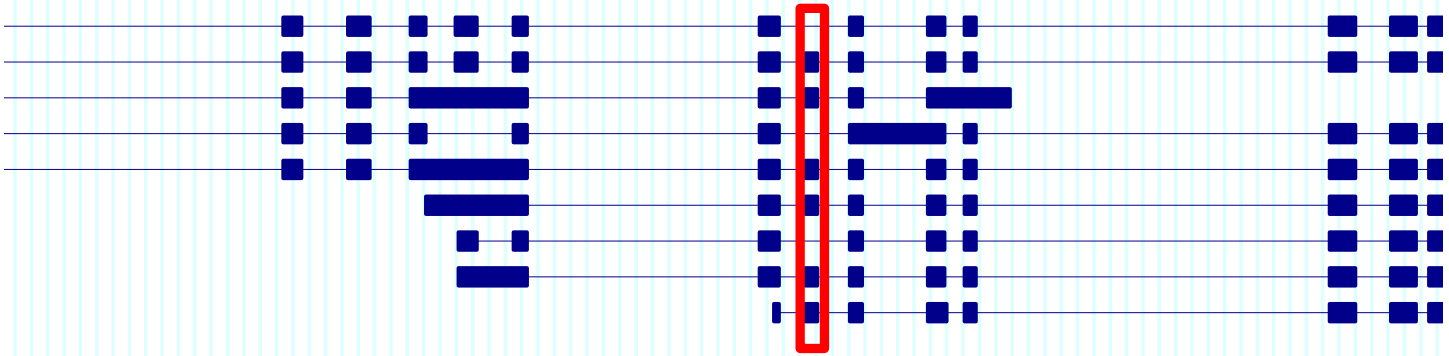
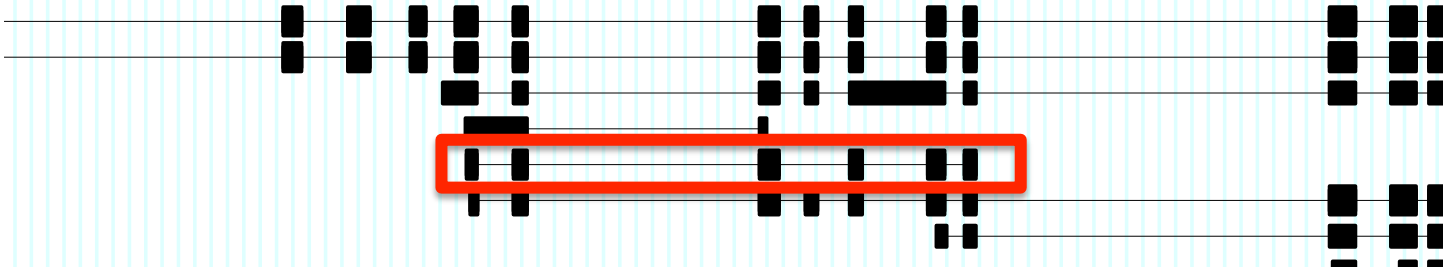
CSMM (mouse brain 1) for Npr2



CSMM (mouse brain 2) for Npr2



Gencode annotation for Npr2



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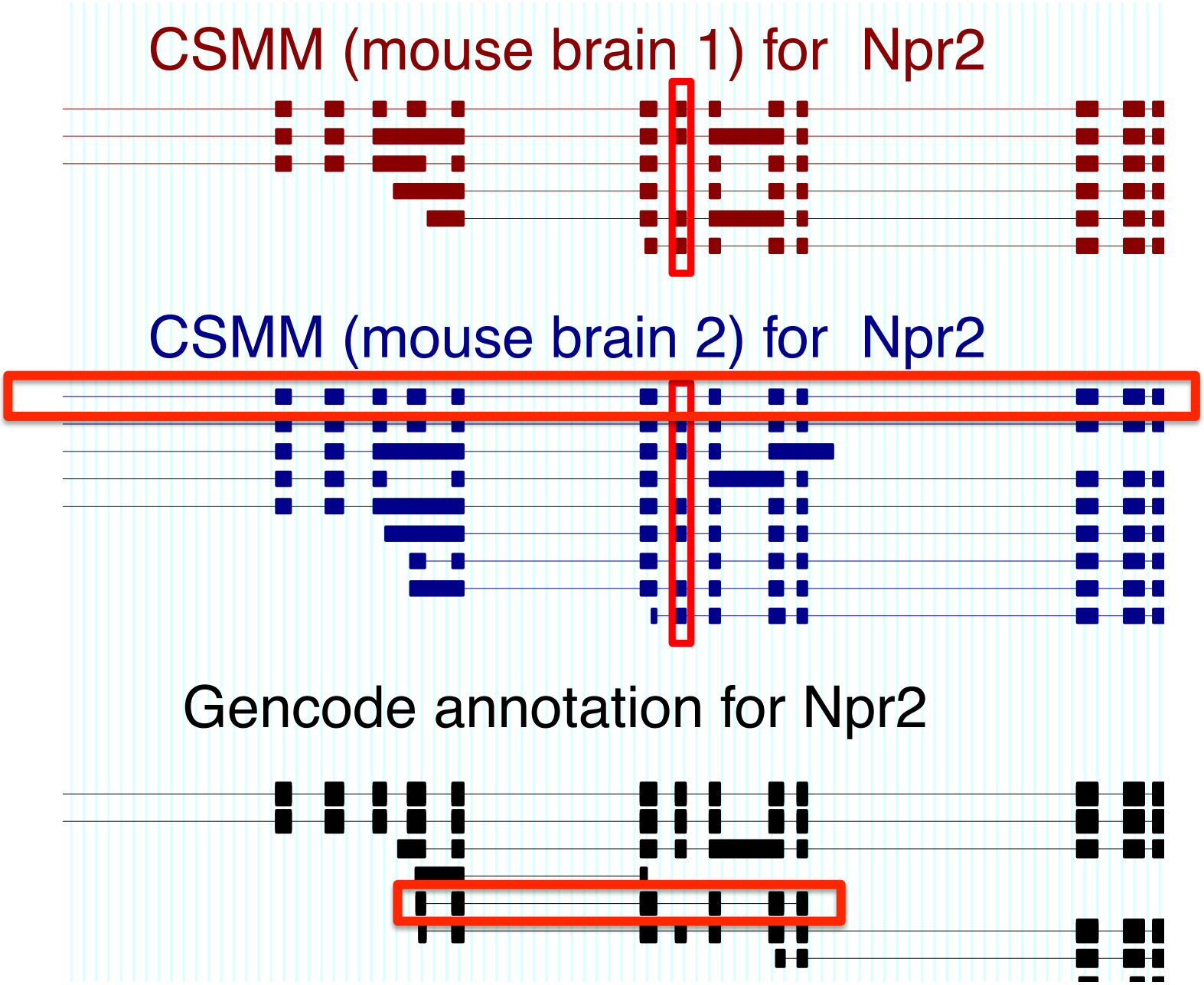

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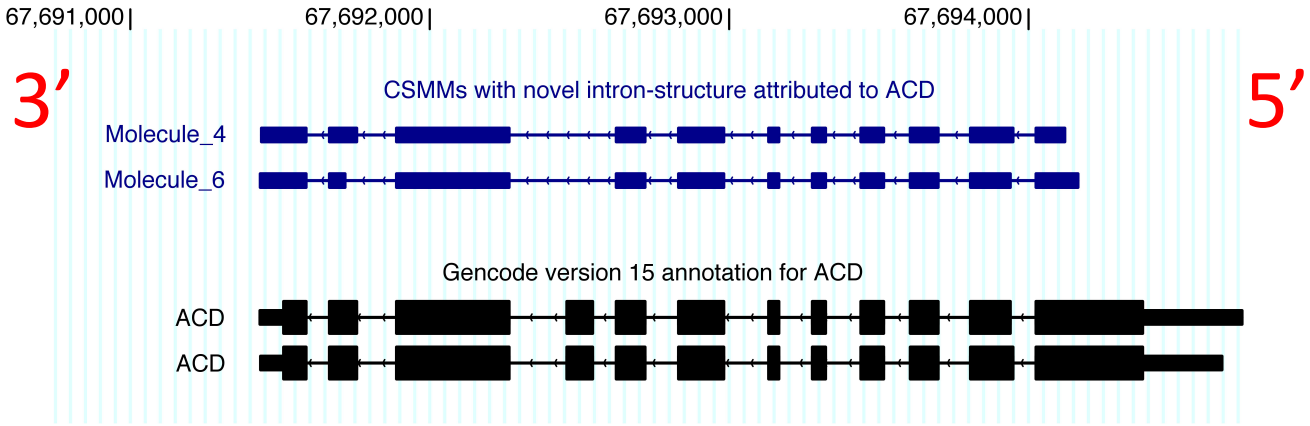
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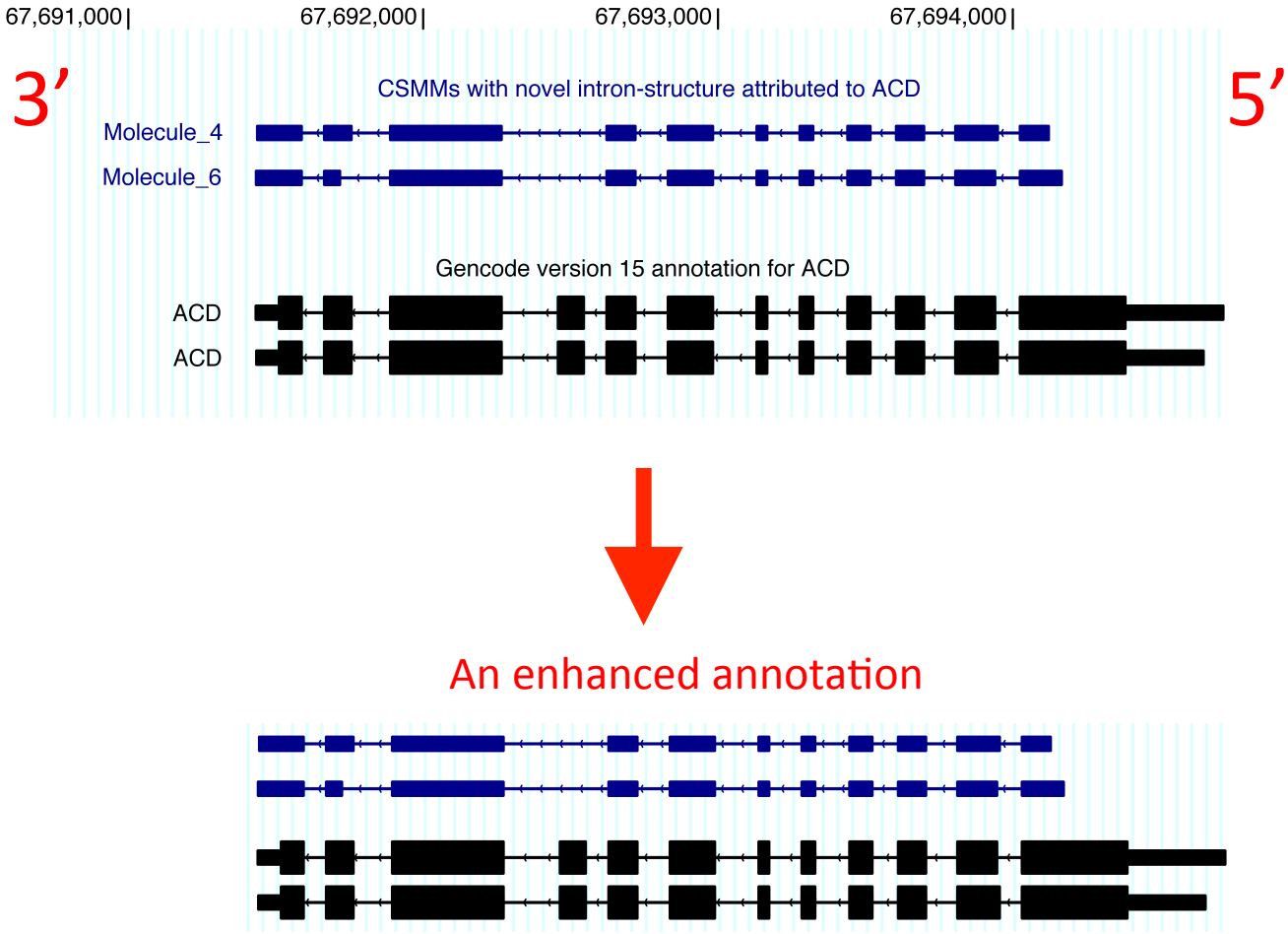
3’
5’
CSMMs with novel intron-structure attributed to ACD
Molecule_4
Molecule_6
Gencode version 15 annotation for ACD
ACD
ACD


67,691,000|
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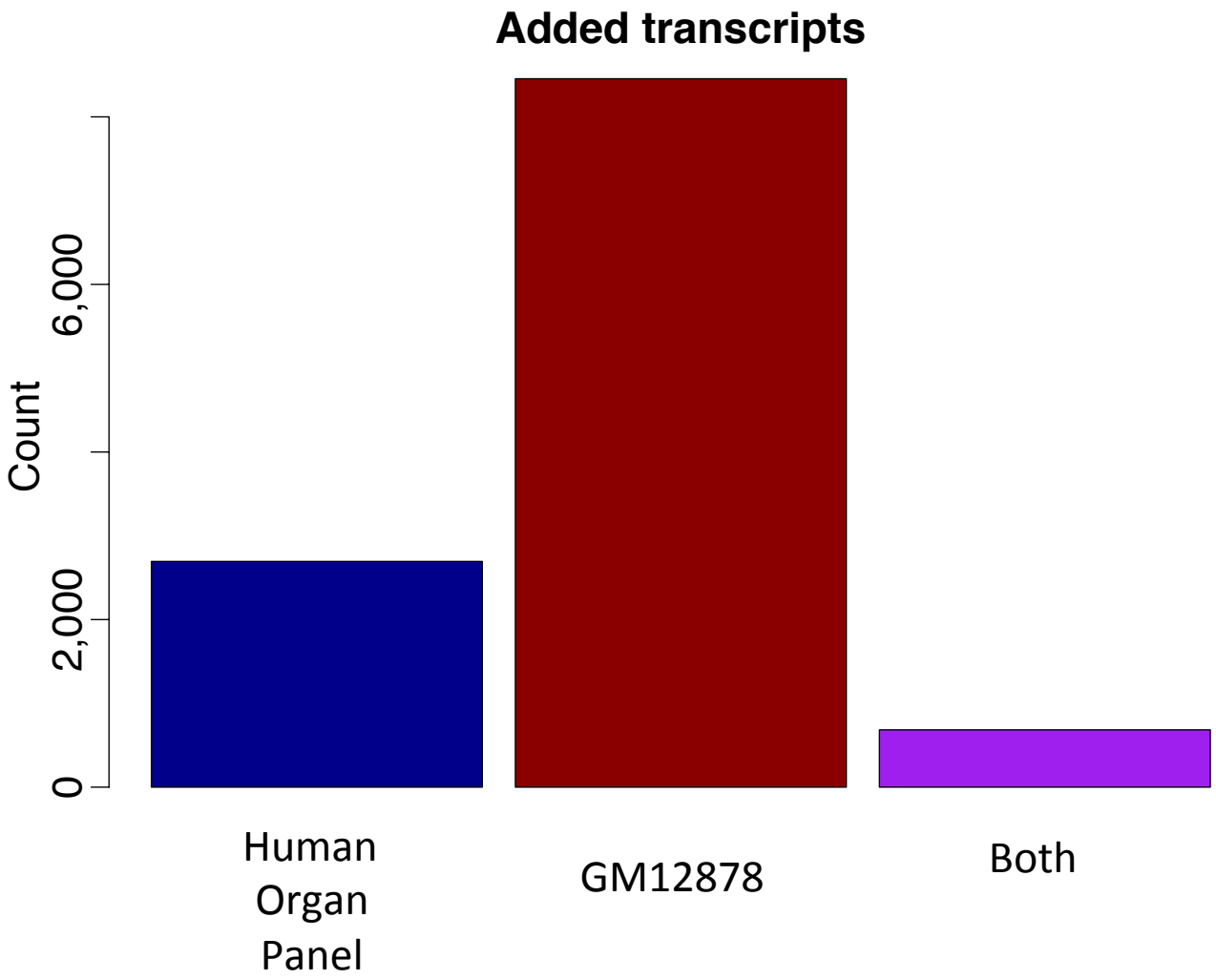
3’
5’
CSMMs with novel intron-structure attributed to ACD
Molecule_4
Molecule_6
Gencode version 15 annotation for ACD
ACD
ACD
An enhanced annotation


Added transcripts
Count
0
2,000
6,000
Human Organ Panel
GM12878
Both

New isoforms from

- **GM12878**
- **Human Organ Panel**

Illumina data from

- **GM12878**

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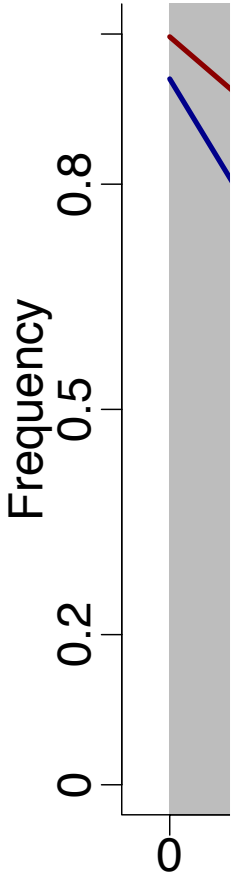
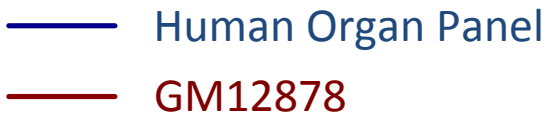
New isoforms from
- **GM12878**
- **Human Organ Panel**

Illumina data from
- **GM12878**

Cufflinks quantification





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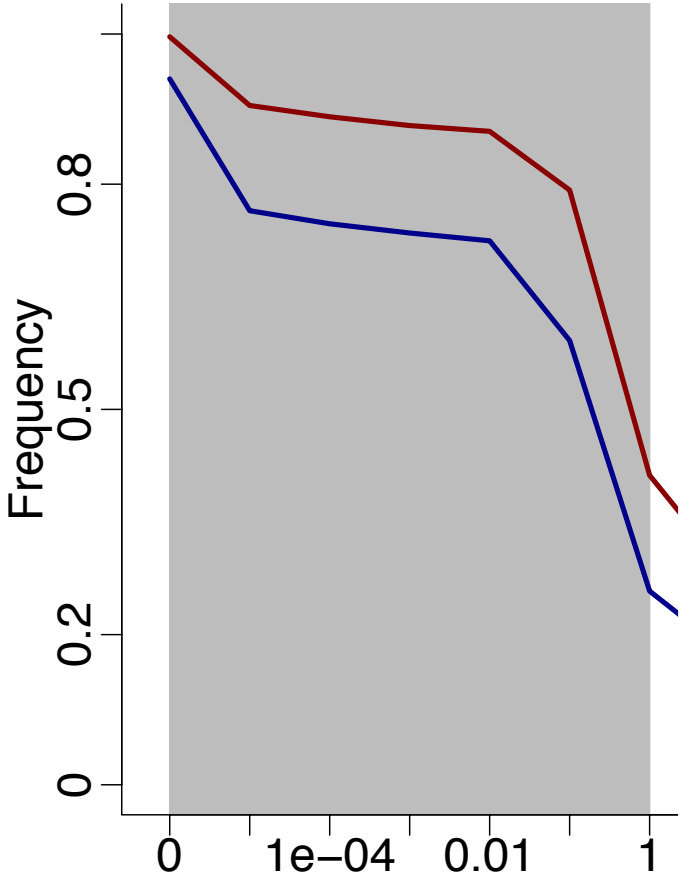
New isoforms from
- **GM12878**
- **Human Organ Panel**

Illumina data from
- **GM12878**

Cufflinks quantification



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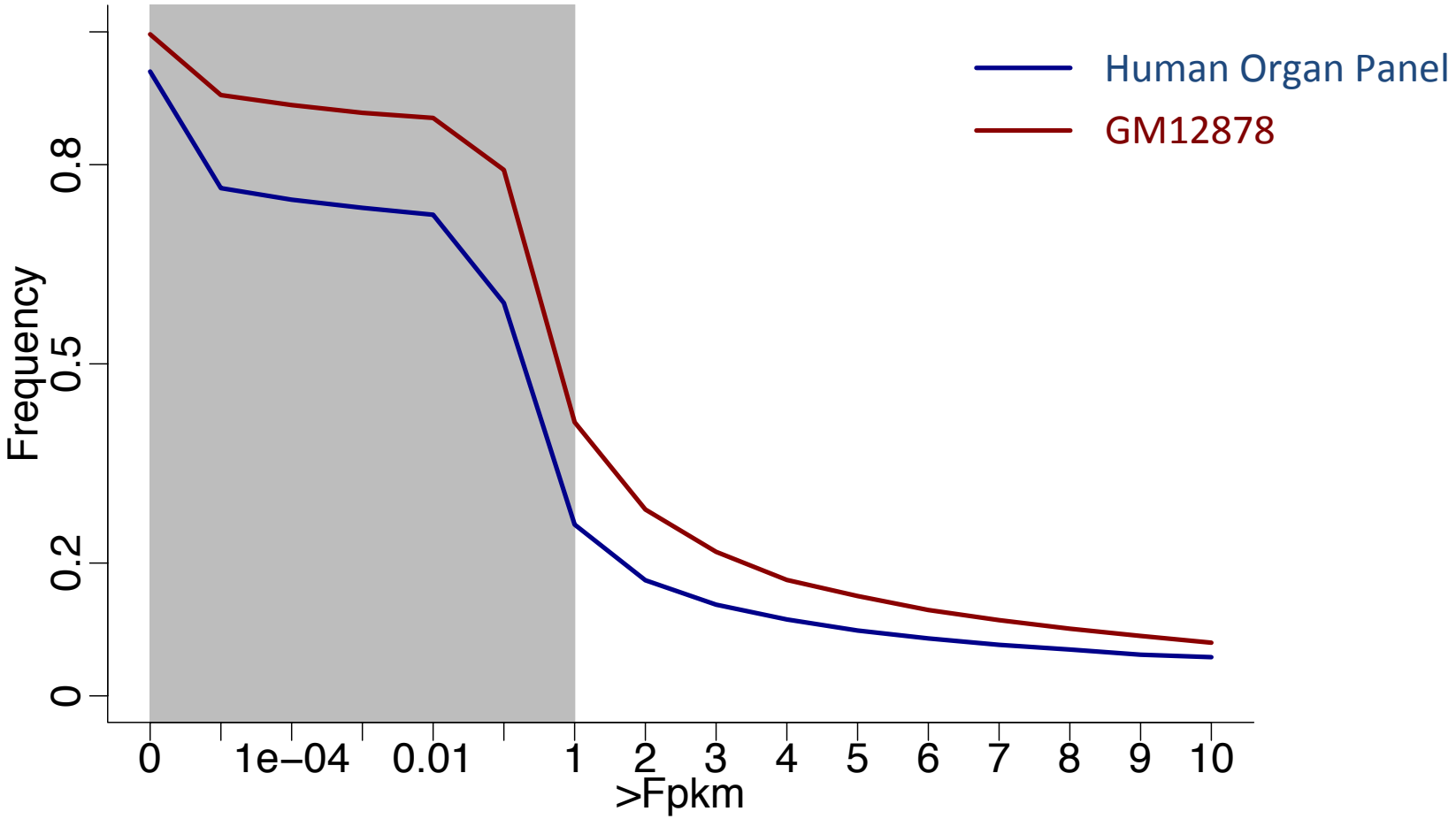
New isoforms from
- **GM12878**
- **Human Organ Panel**

Illumina data from
- **GM12878**

Cufflinks quantification



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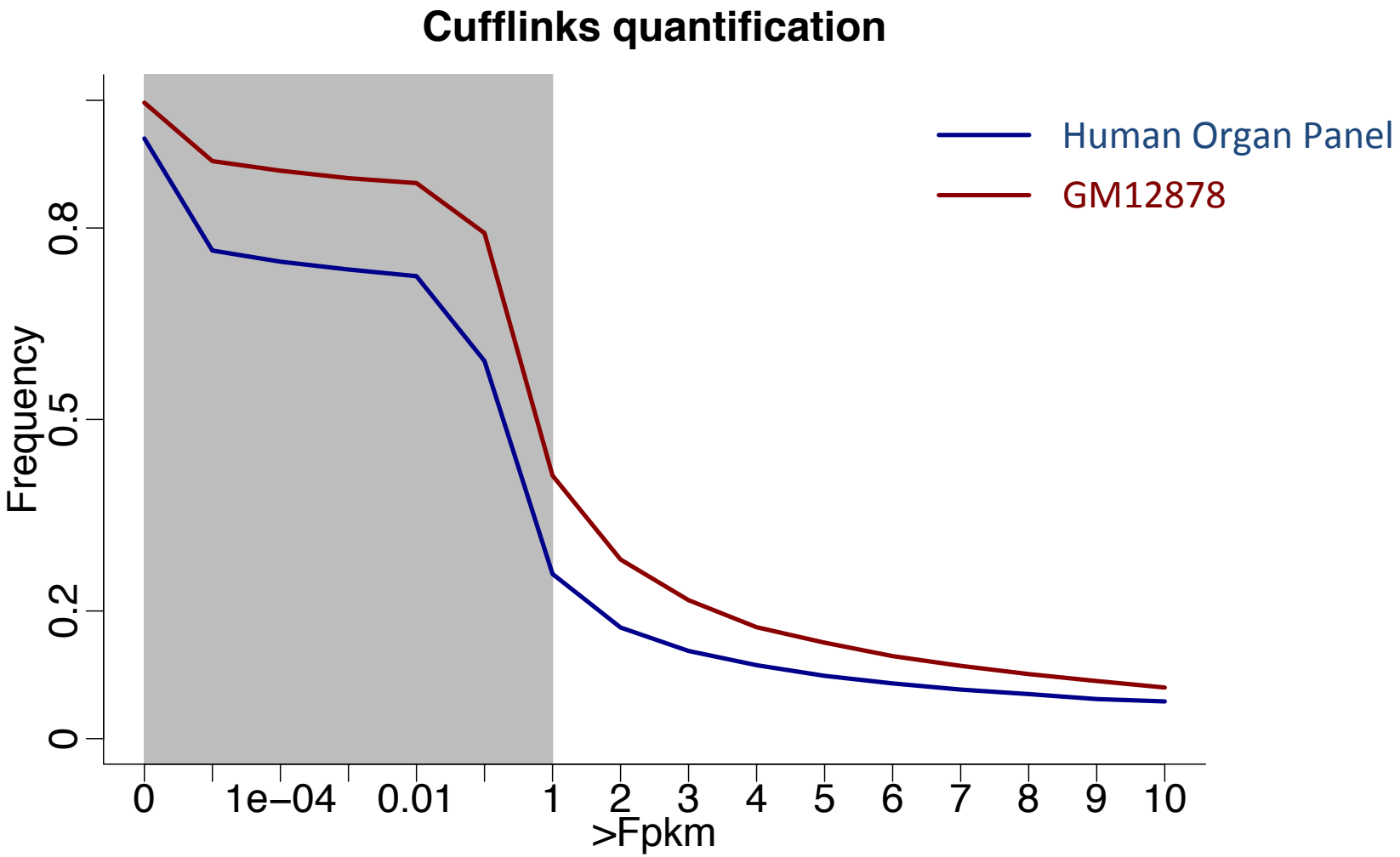
Enhanced annotation improves isoform quantification



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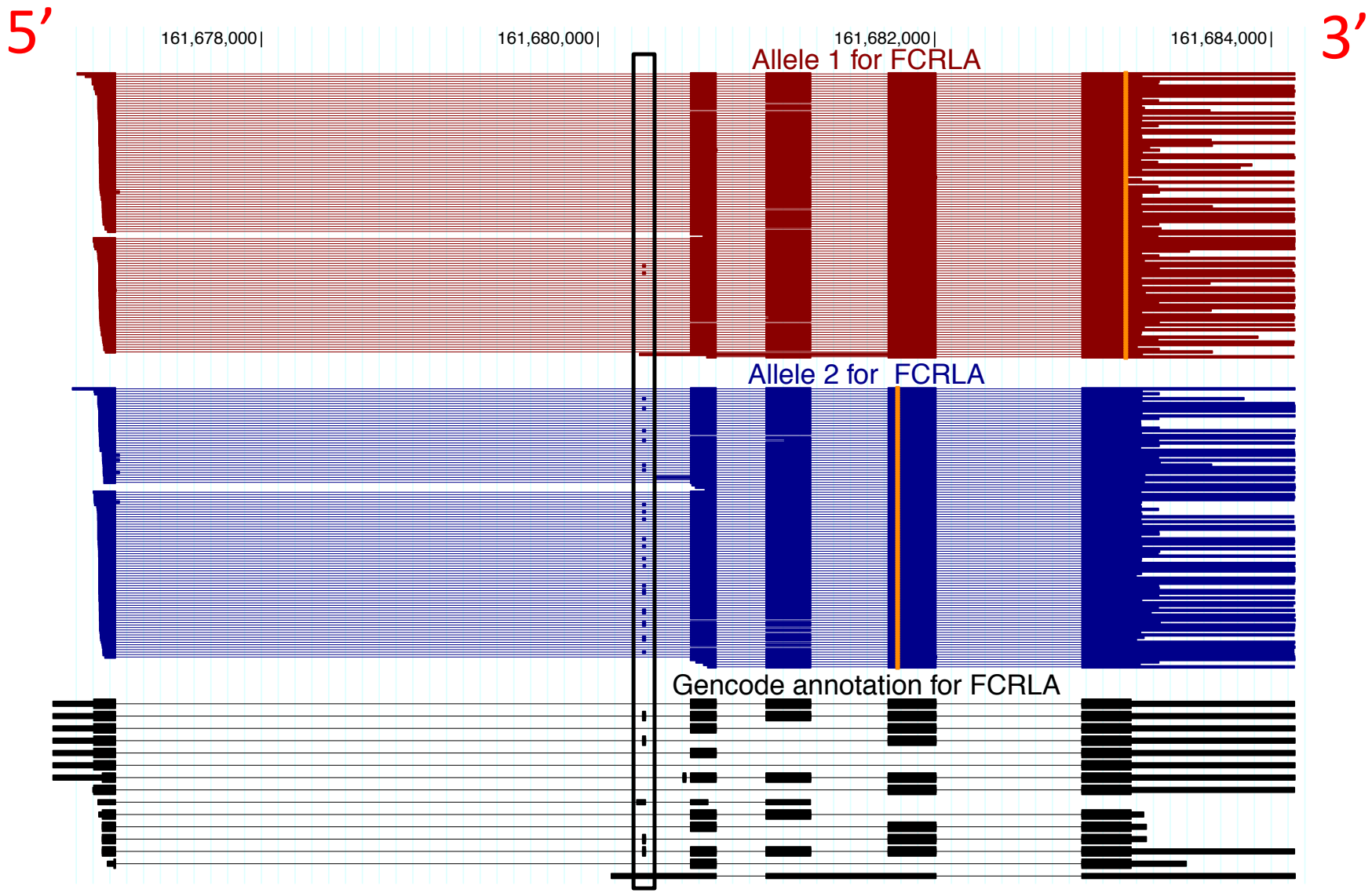

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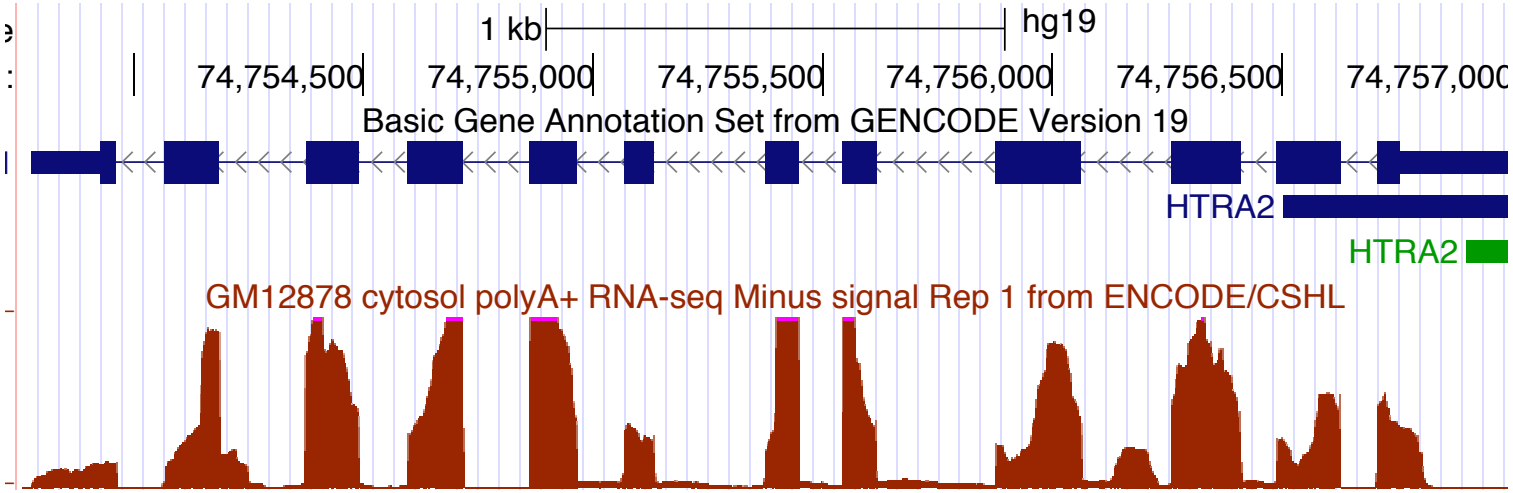

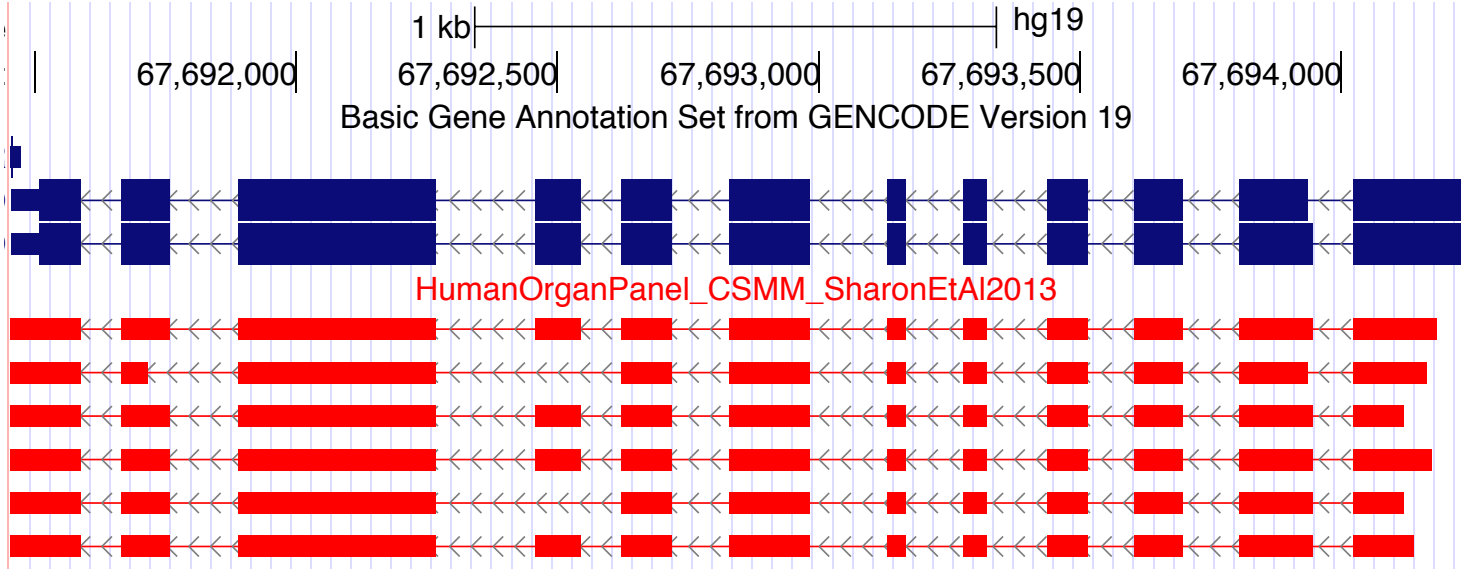

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They took the paper but not the cover ☹

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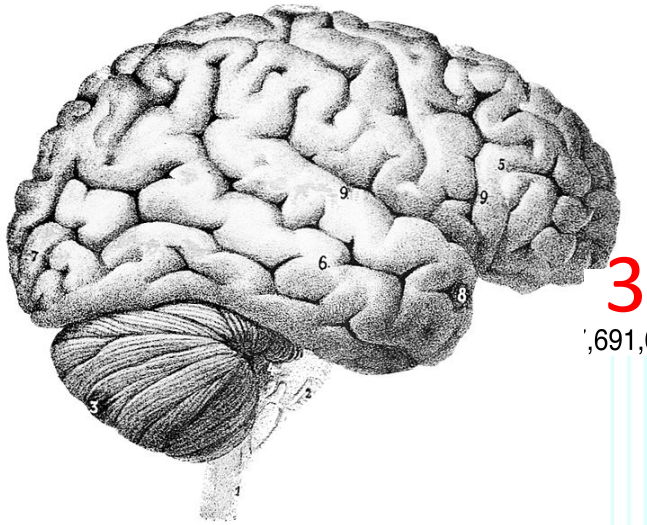

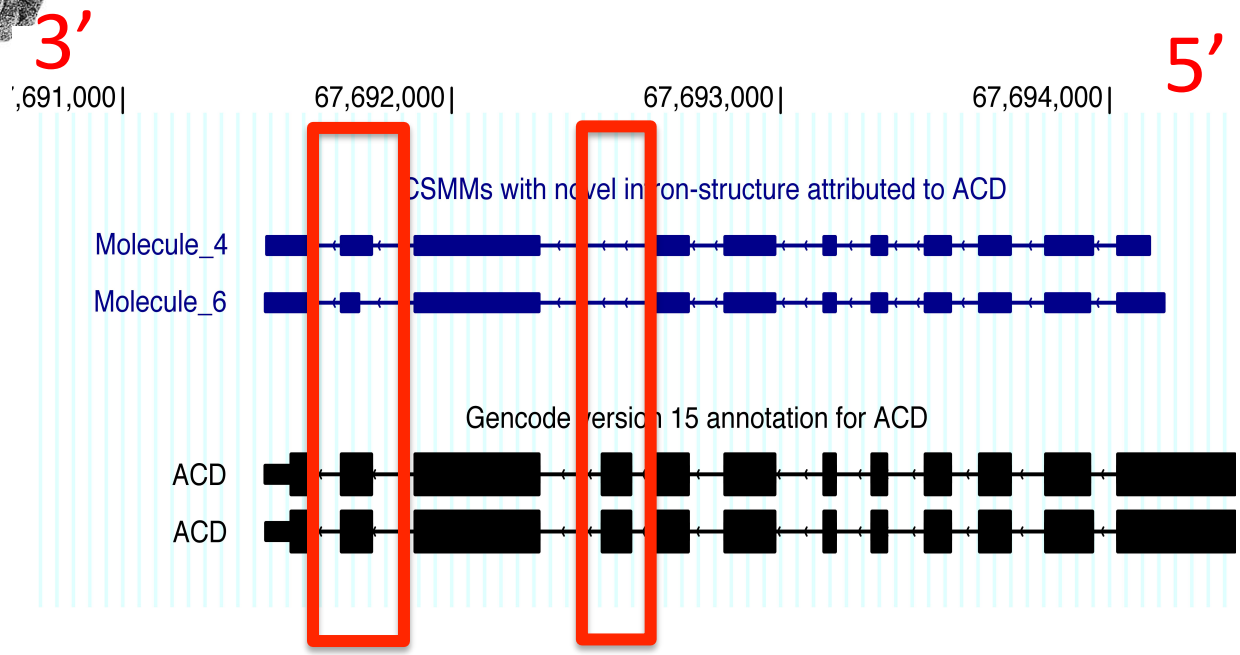

- Keep 1-to-1 correspondence of reads and RNA molecules
- Get LOTS of reads with low error-rate

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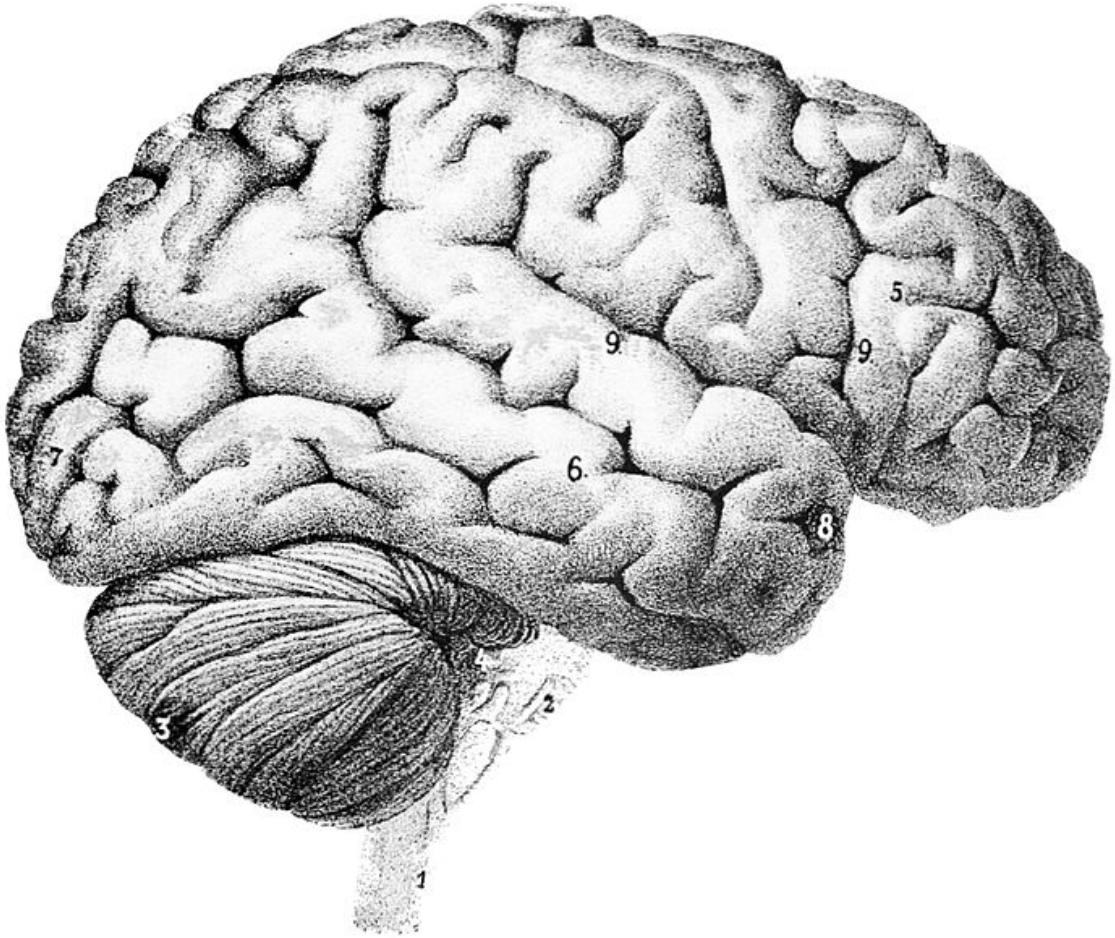

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Understanding brain isoforms through quantitative long-read sequencing

- SLR-RNA-Seq, Nature. Biotech 2015
- spISO-Seq, Genome Res. 2017

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SLR–RNA–Seq

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SLR–RNA–Seq

polyA–selected RNA

DNA–RNA hybrid with primers

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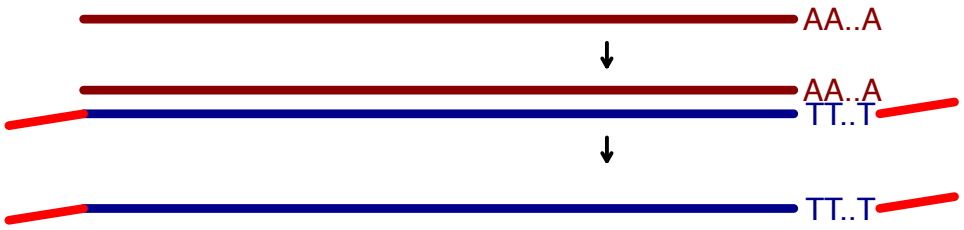
SLR–RNA–Seq



polyA–selected RNA

DNA–RNA hybrid with primers

sscDNA with primers

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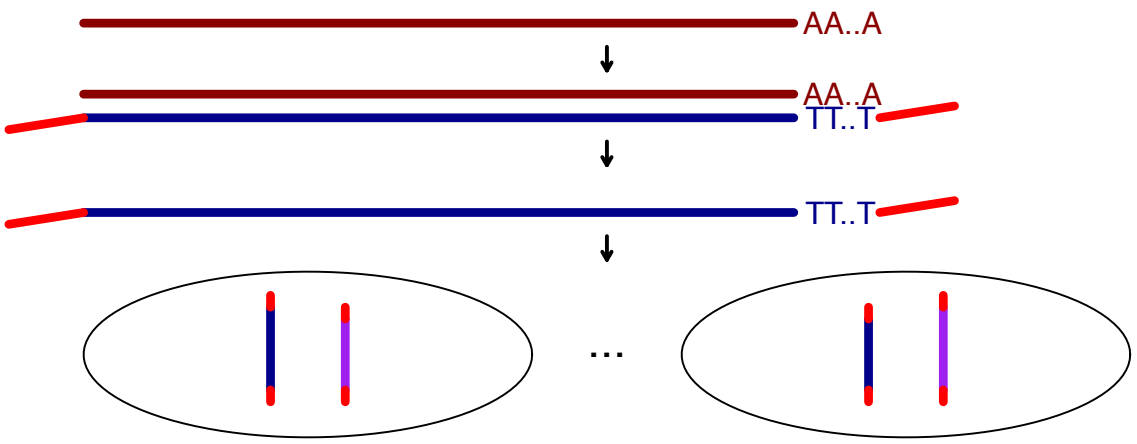
SLR–RNA–Seq



polyA–selected RNA

DNA–RNA hybrid with primers

sscDNA with primers

pools of few sscDNA–molecules per well

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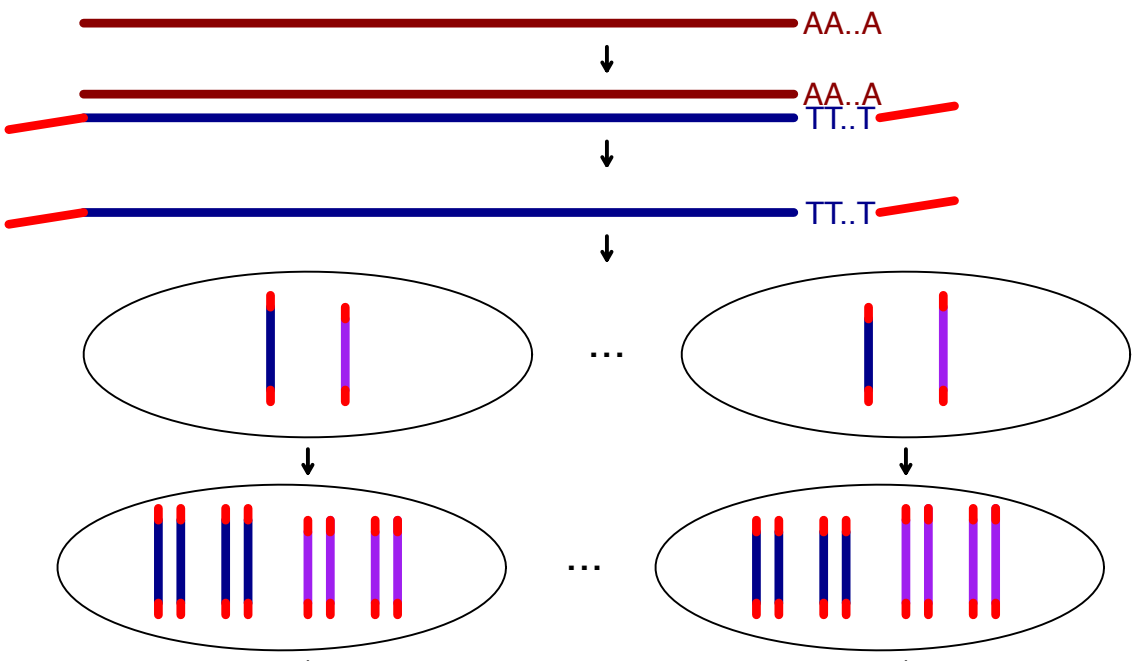
SLR–RNA–Seq



polyA–selected RNA

DNA–RNA hybrid with primers

sscDNA with primers

pools of few sscDNA–molecules per well

amplified dscDNA–pools (per well)

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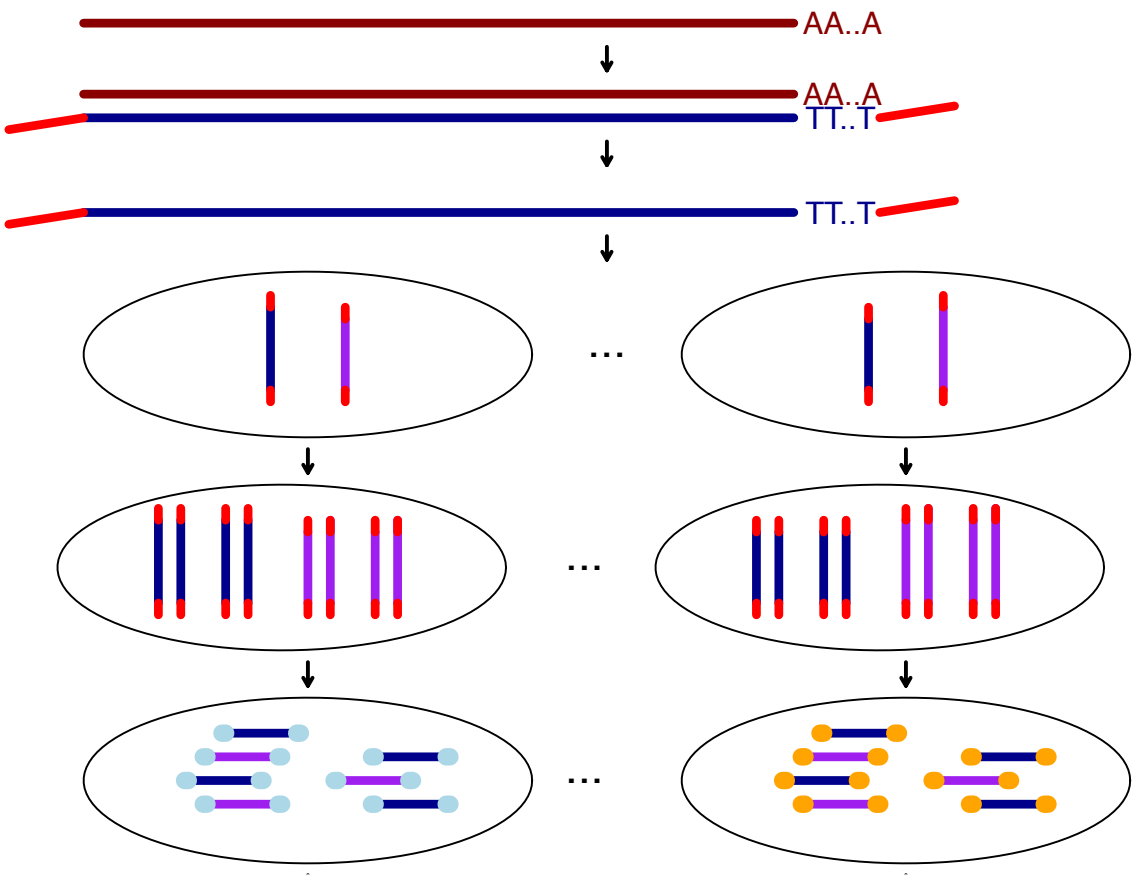
SLR–RNA–Seq



polyA–selected RNA

DNA–RNA hybrid with primers

sscDNA with primers

pools of few sscDNA–molecules per well

amplified dscDNA–pools (per well)

well–specific (barcoded) fragments

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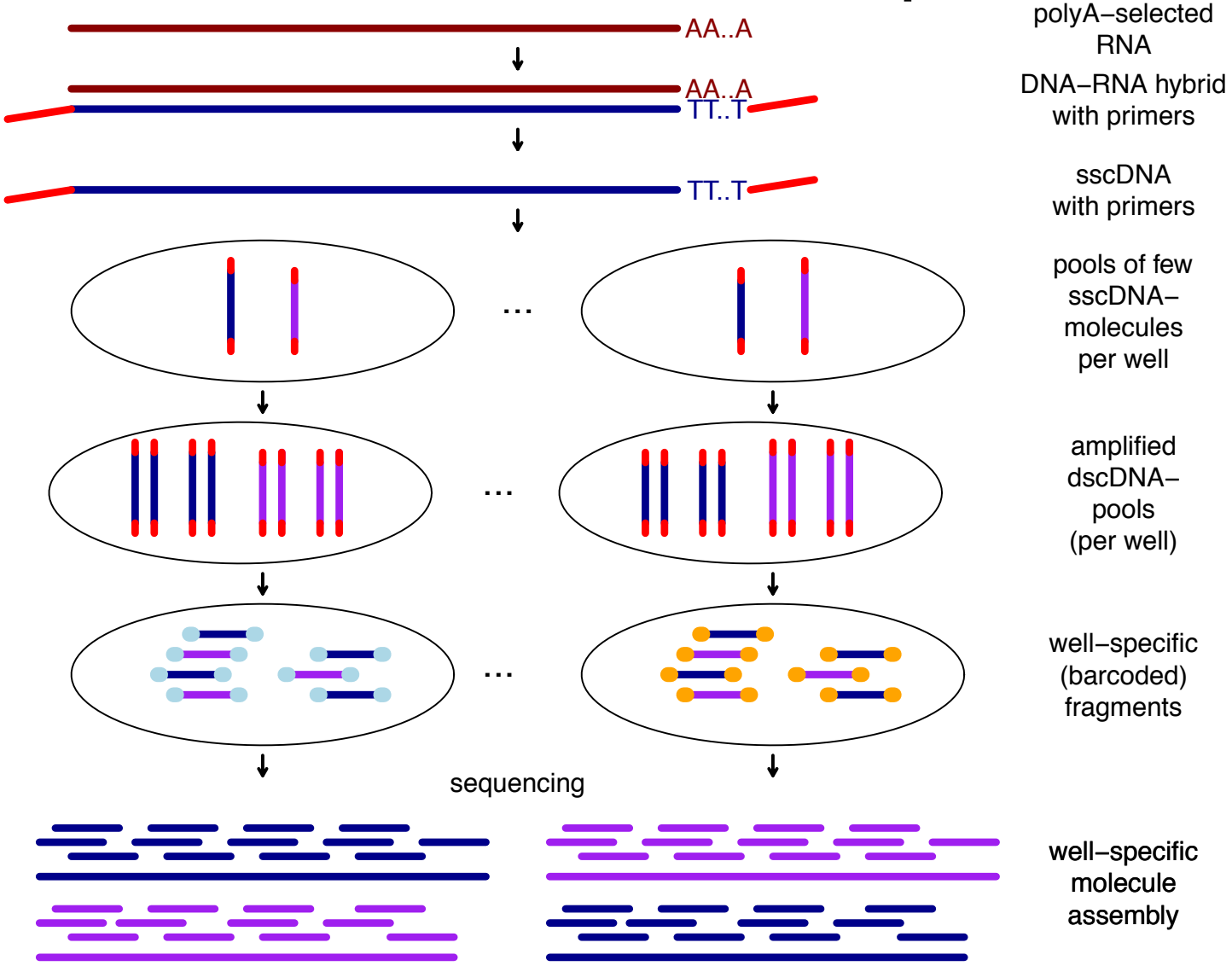
SLR–RNA–Seq



polyA–selected RNA

DNA–RNA hybrid with primers

sscDNA with primers

pools of few sscDNA–molecules per well

amplified dscDNA–pools (per well)

well–specific (barcoded) fragments

well–specific molecule assembly

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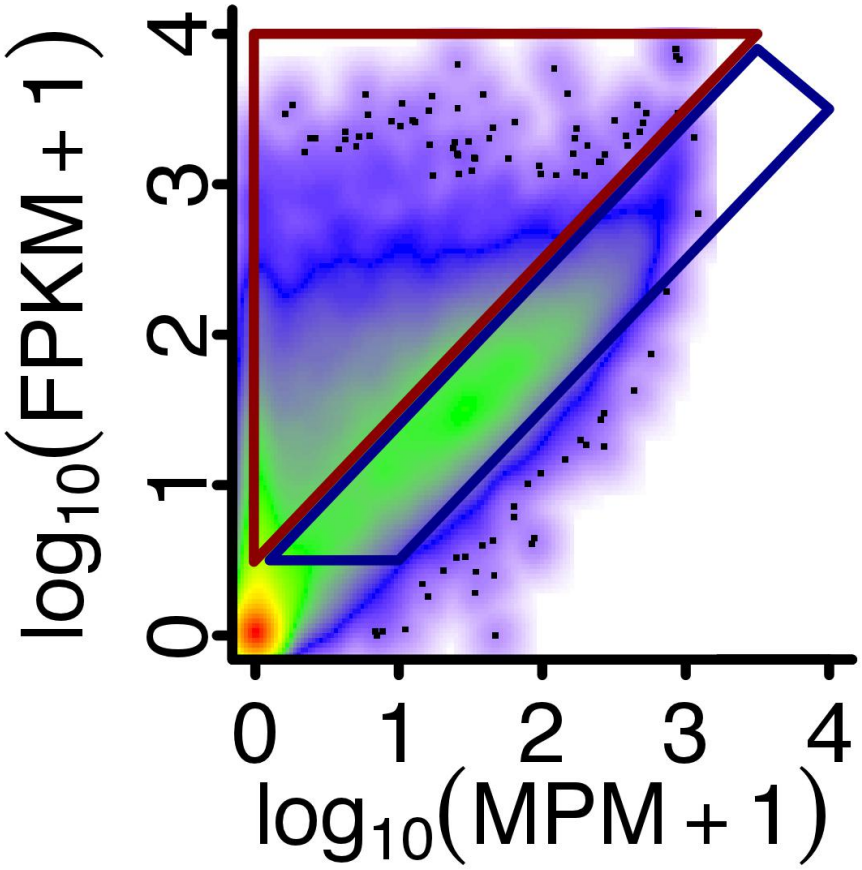
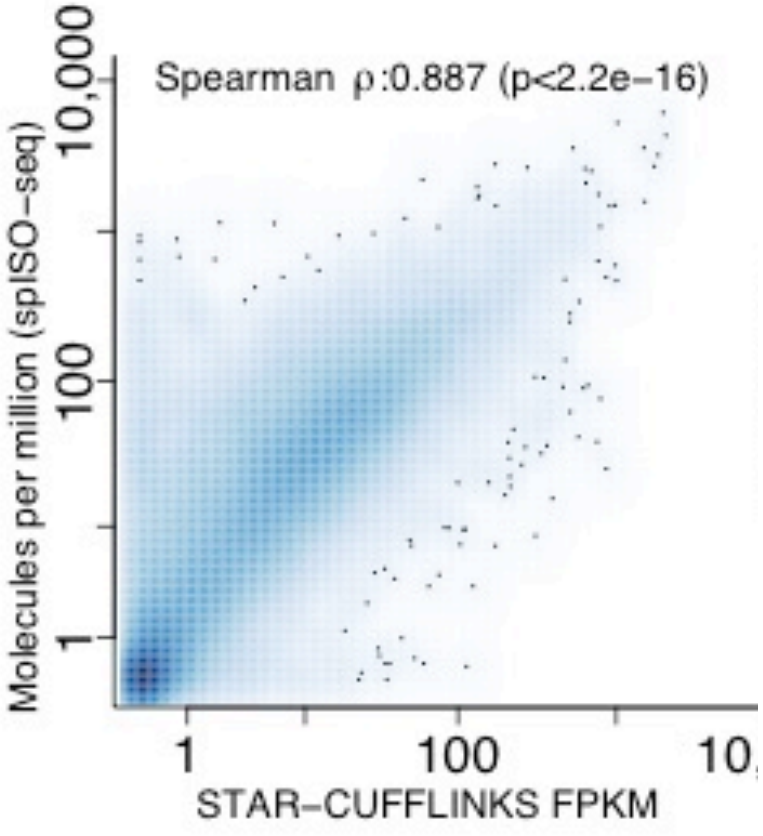


SLR-RNA-Seq vs. short reads
Tilgner et al,
Nature Biotech 2015



spISO-Seq vs. short reads
Tilgner et al,
Genome Research, 2017

MPM: molecules per million (expression measurement for long reads)

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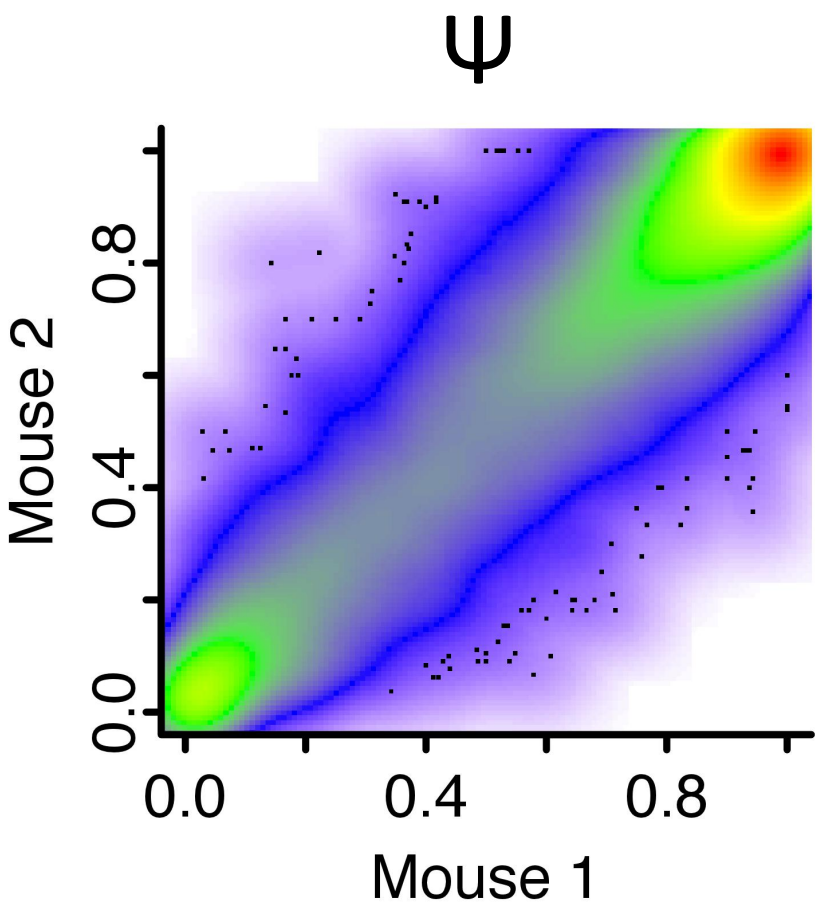

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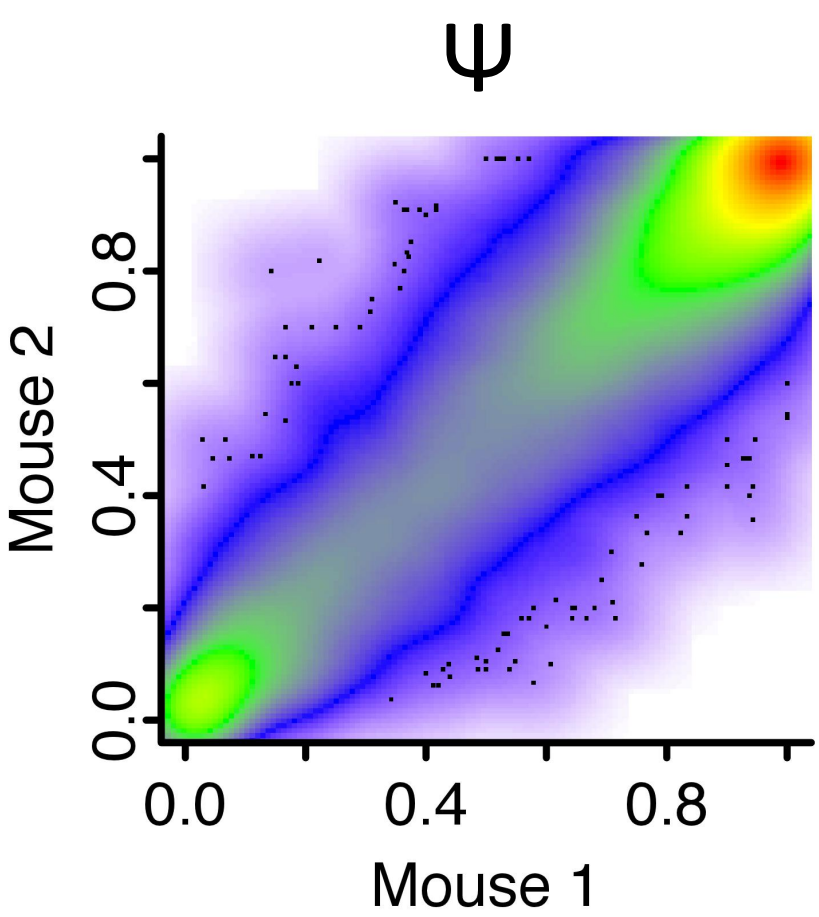

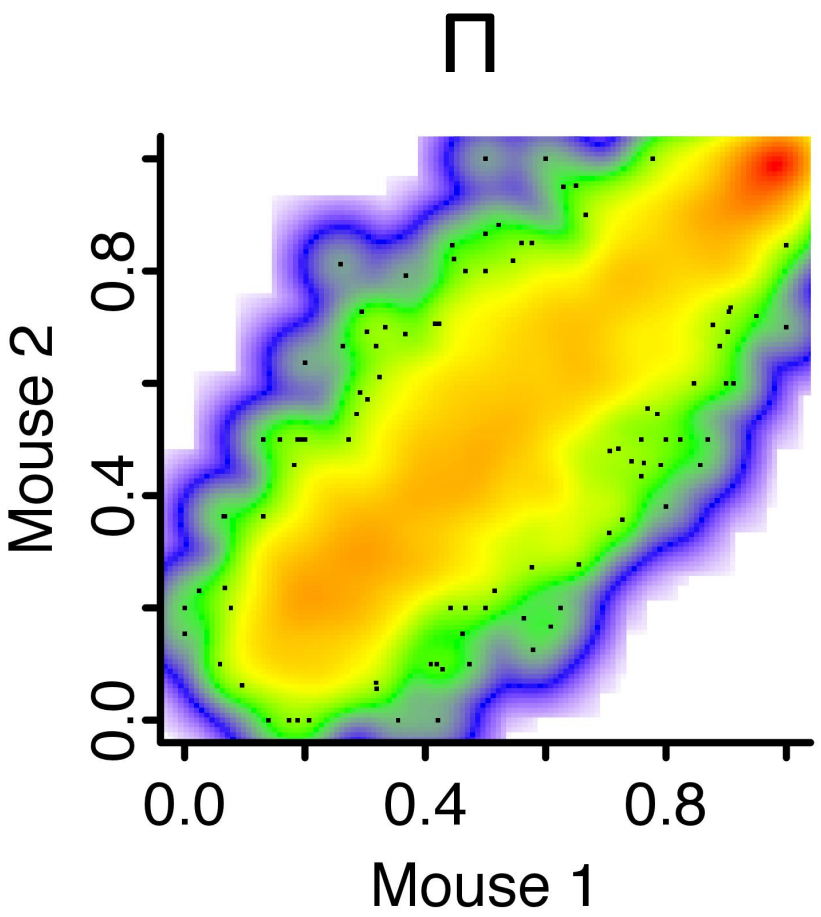

PSI: Percent Spliced In for exons and splice sites (Wang et al, 2008)

PI: Percent Isoform values (for long reads)

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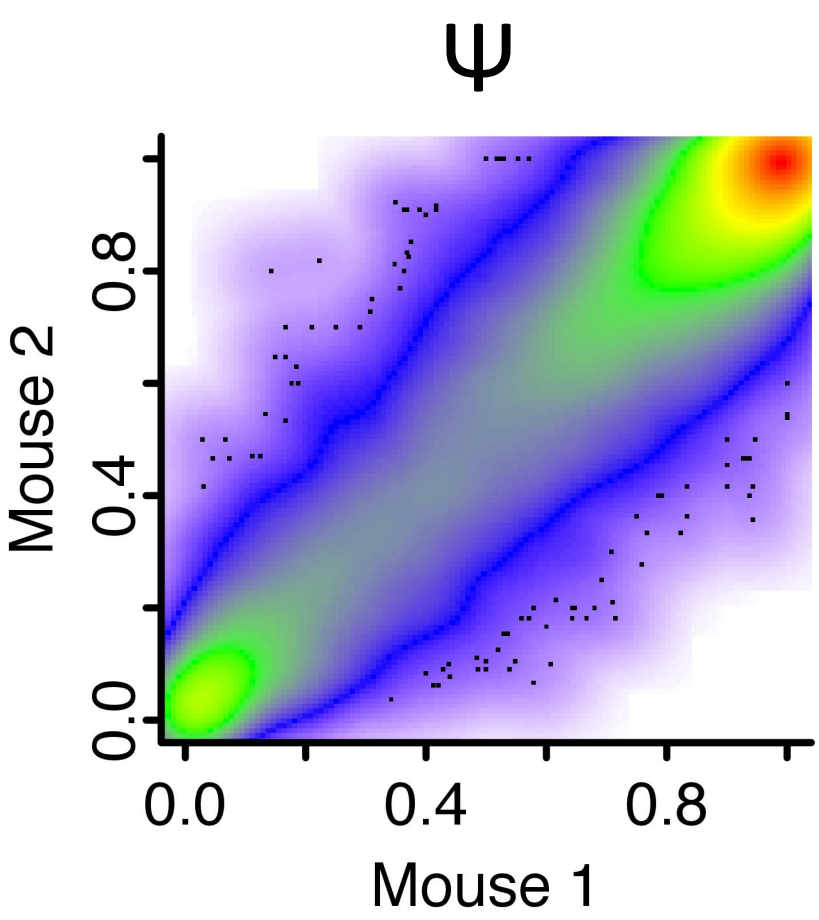

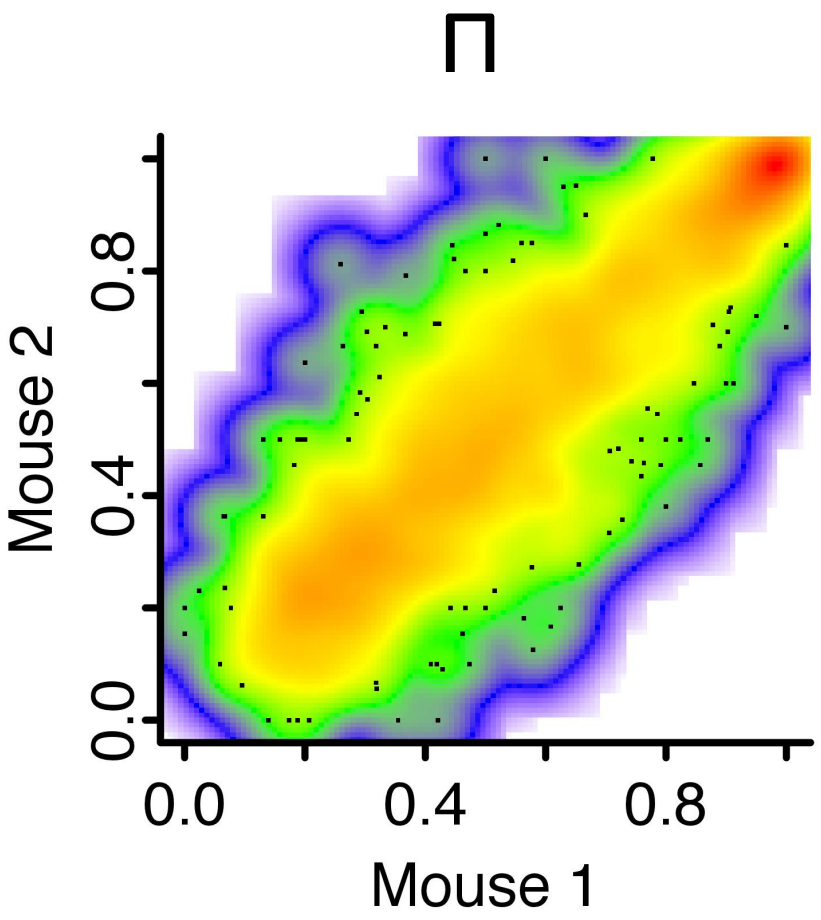

Data at: http://stanford.edu/~htilgner/2014_humanMouseBrain_SLR_RNA_Seq/index_SLRseq.html

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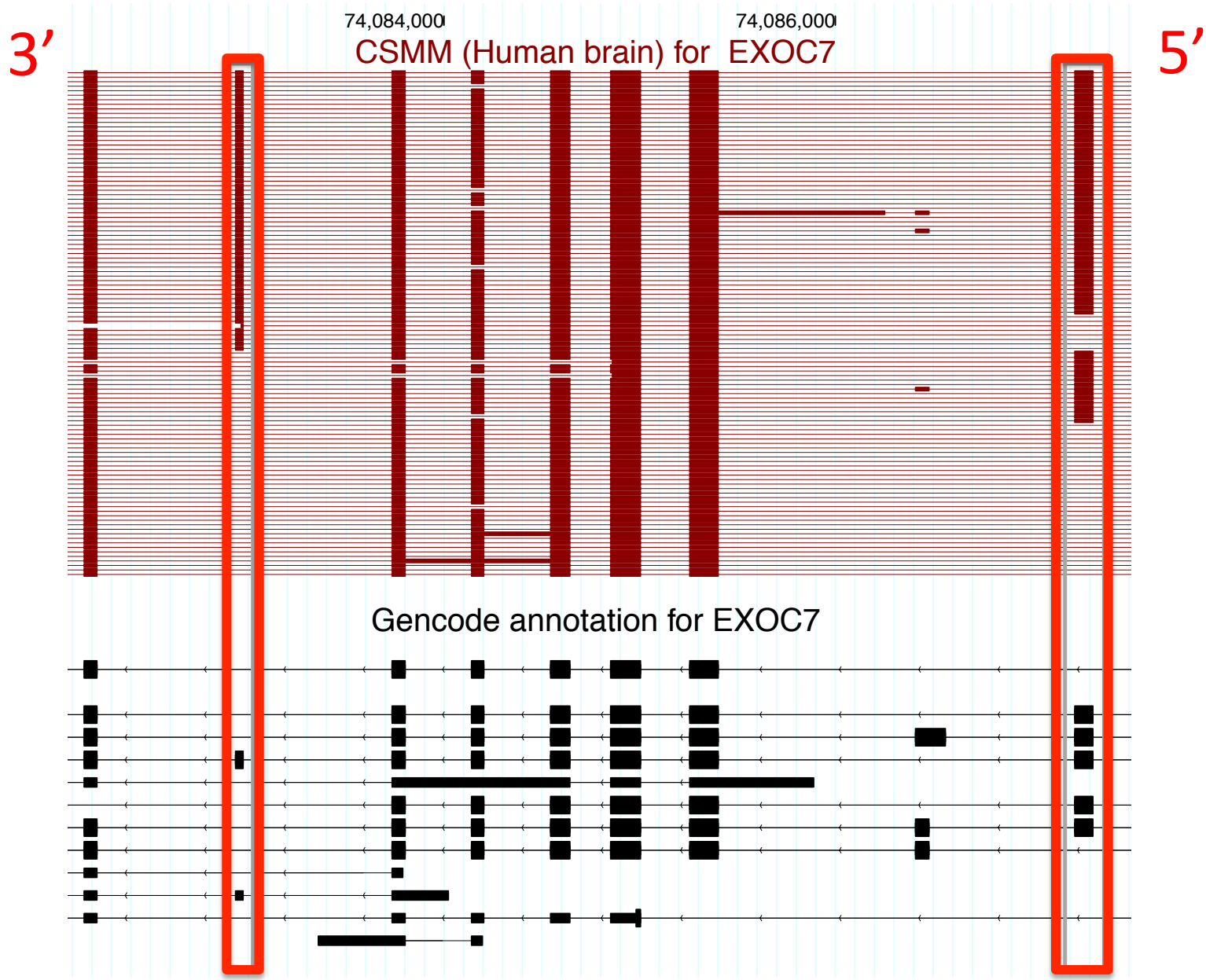

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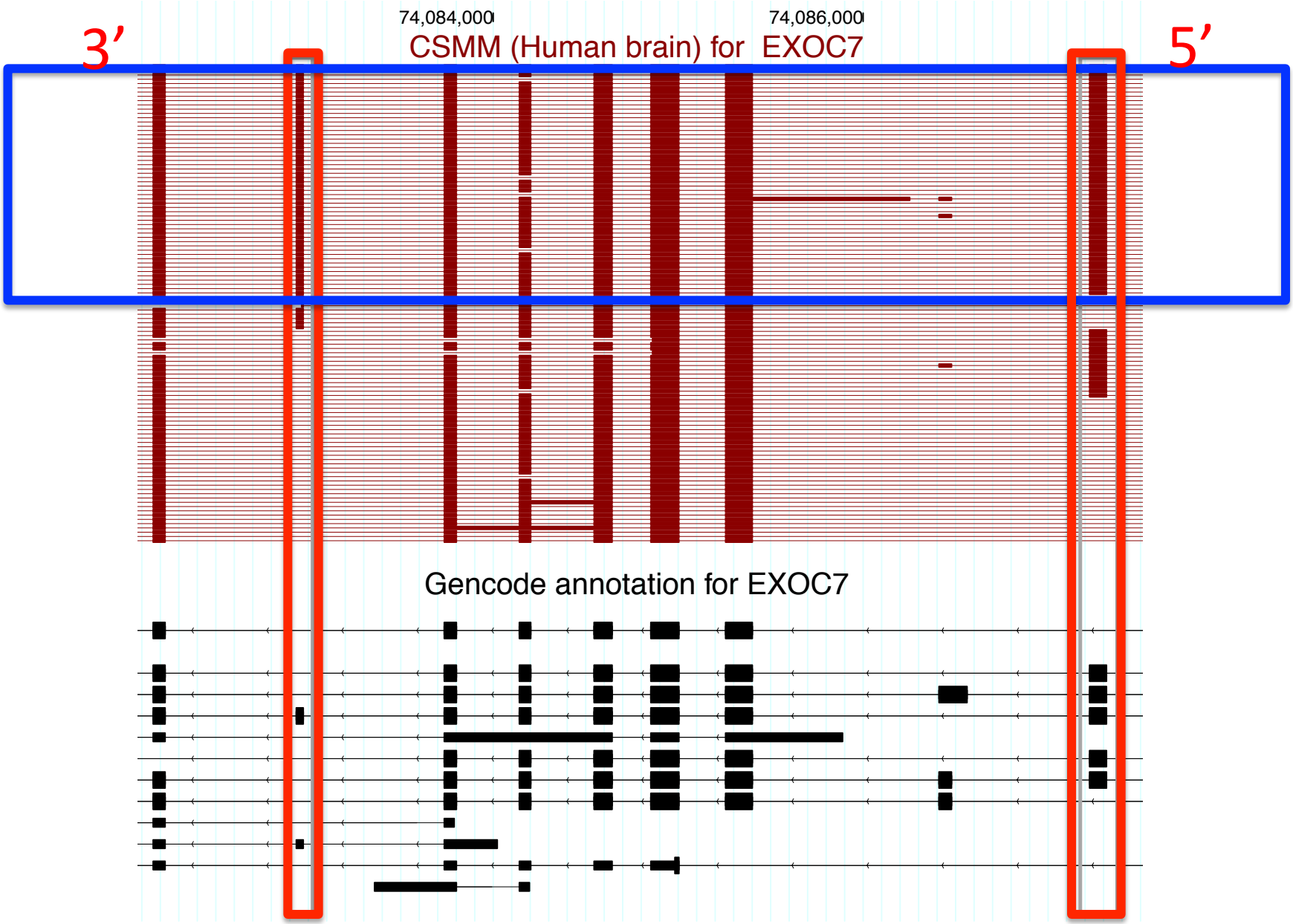

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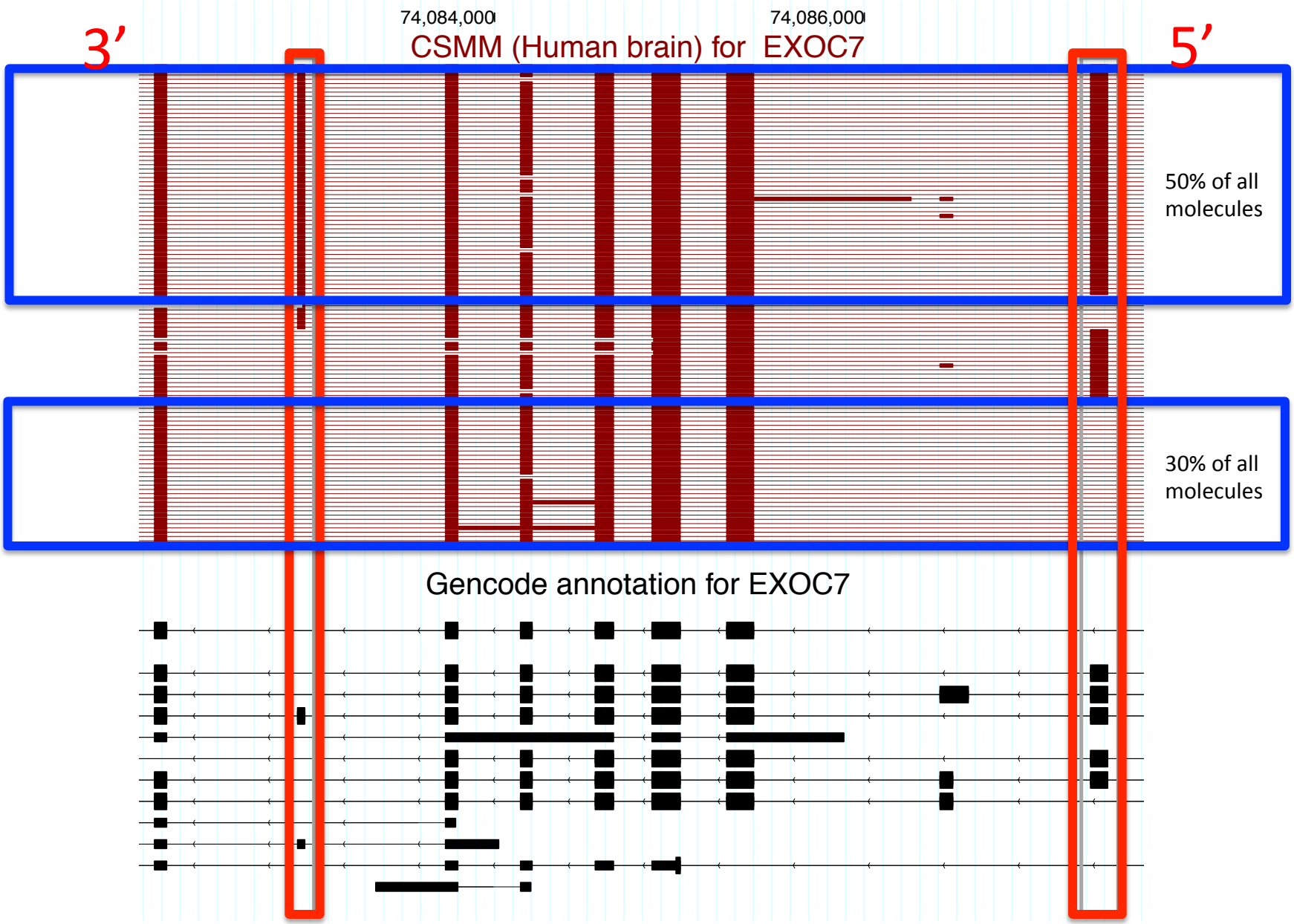

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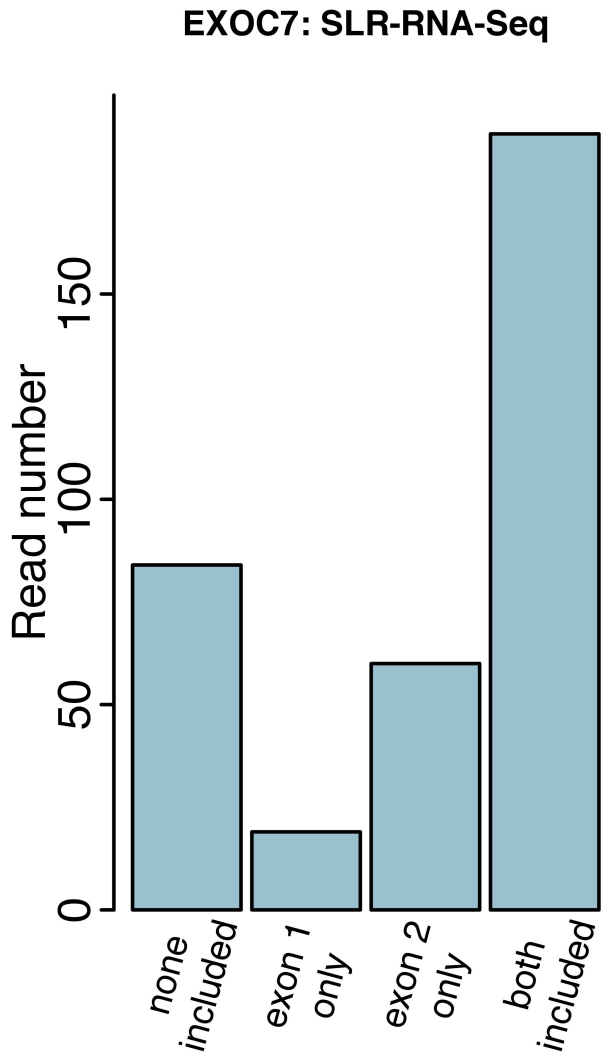

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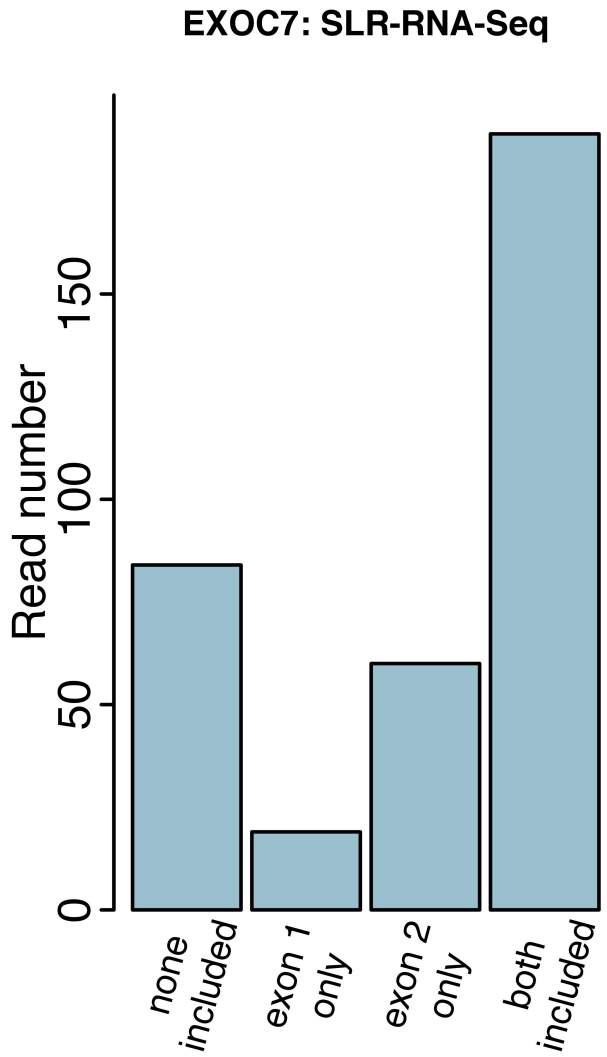
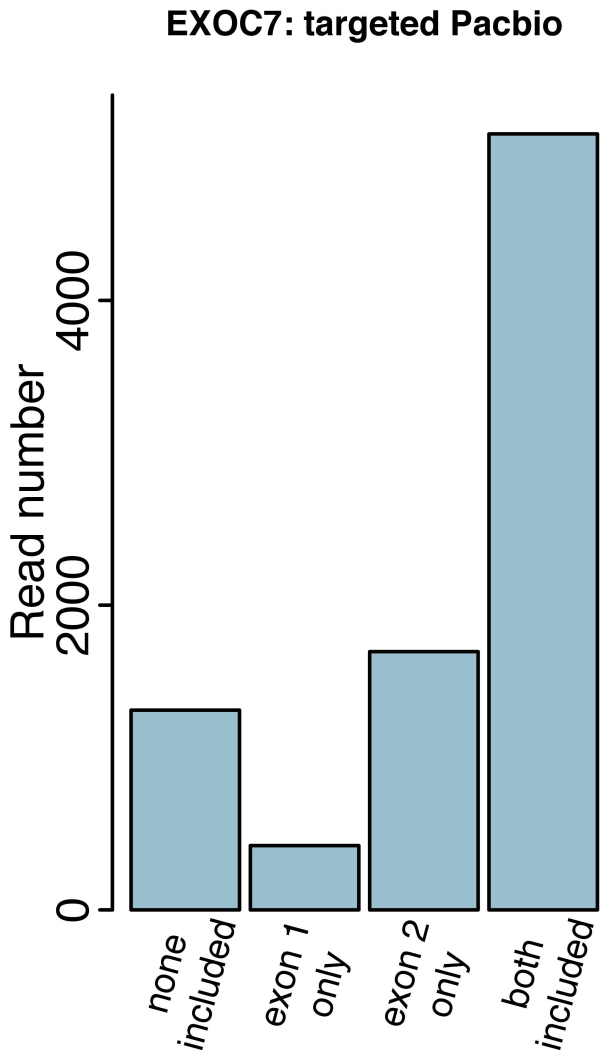
Coordination is (largely) reproducible in targeted experiment





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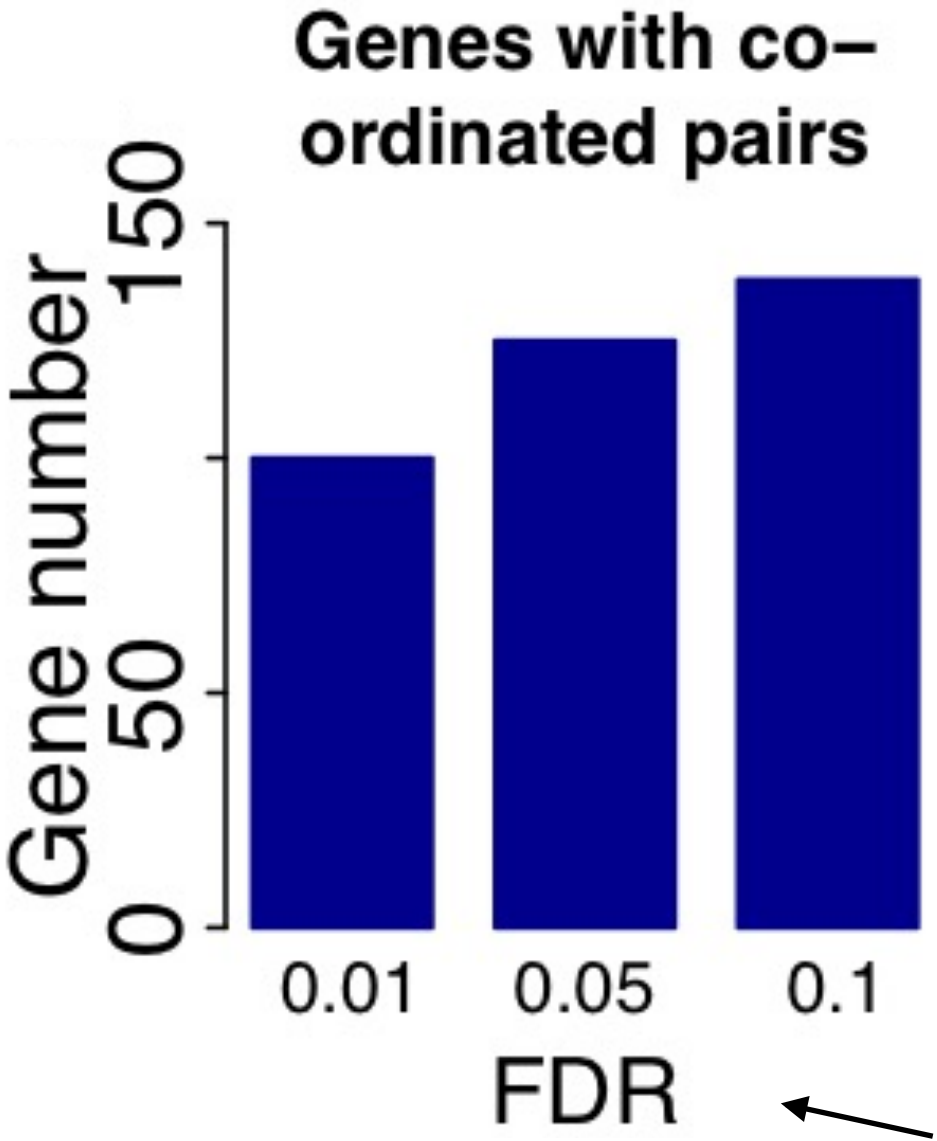

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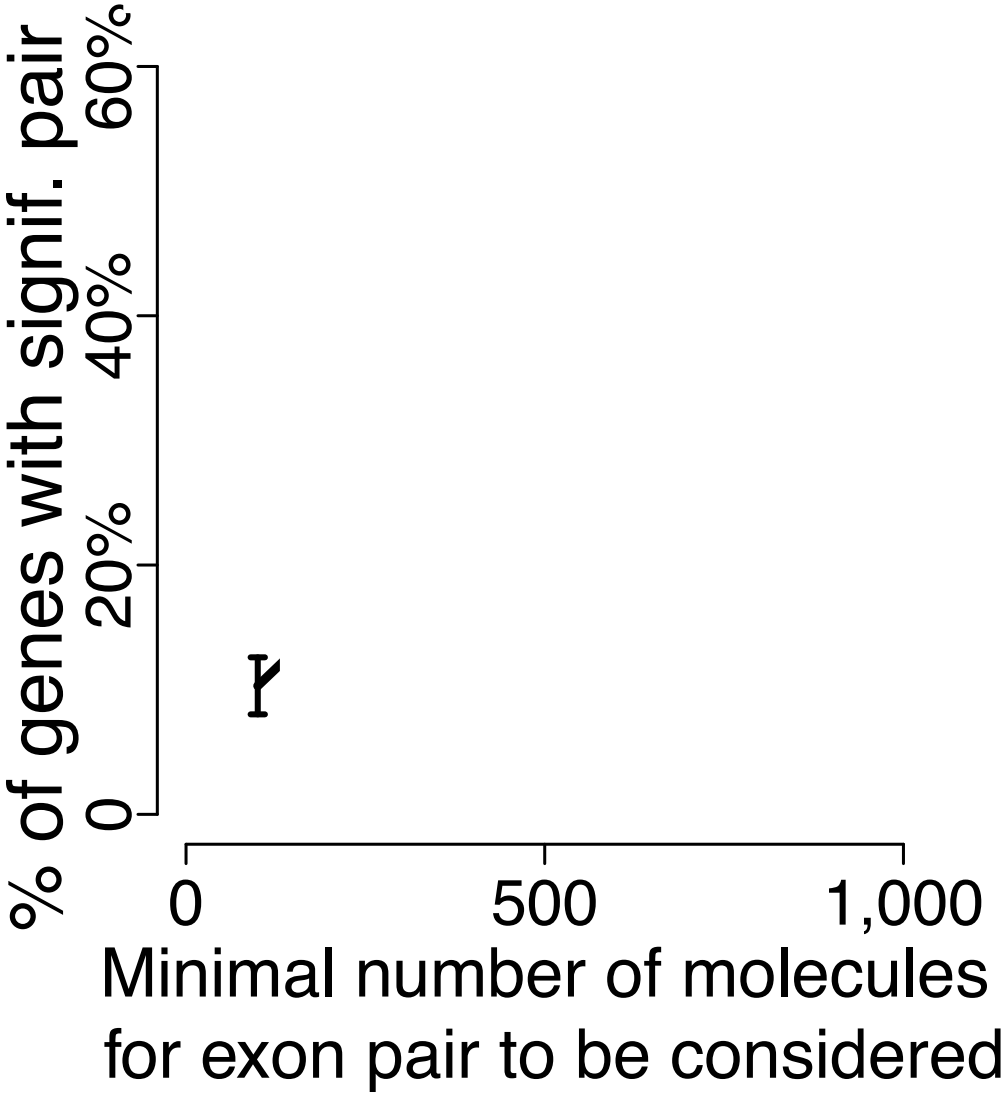

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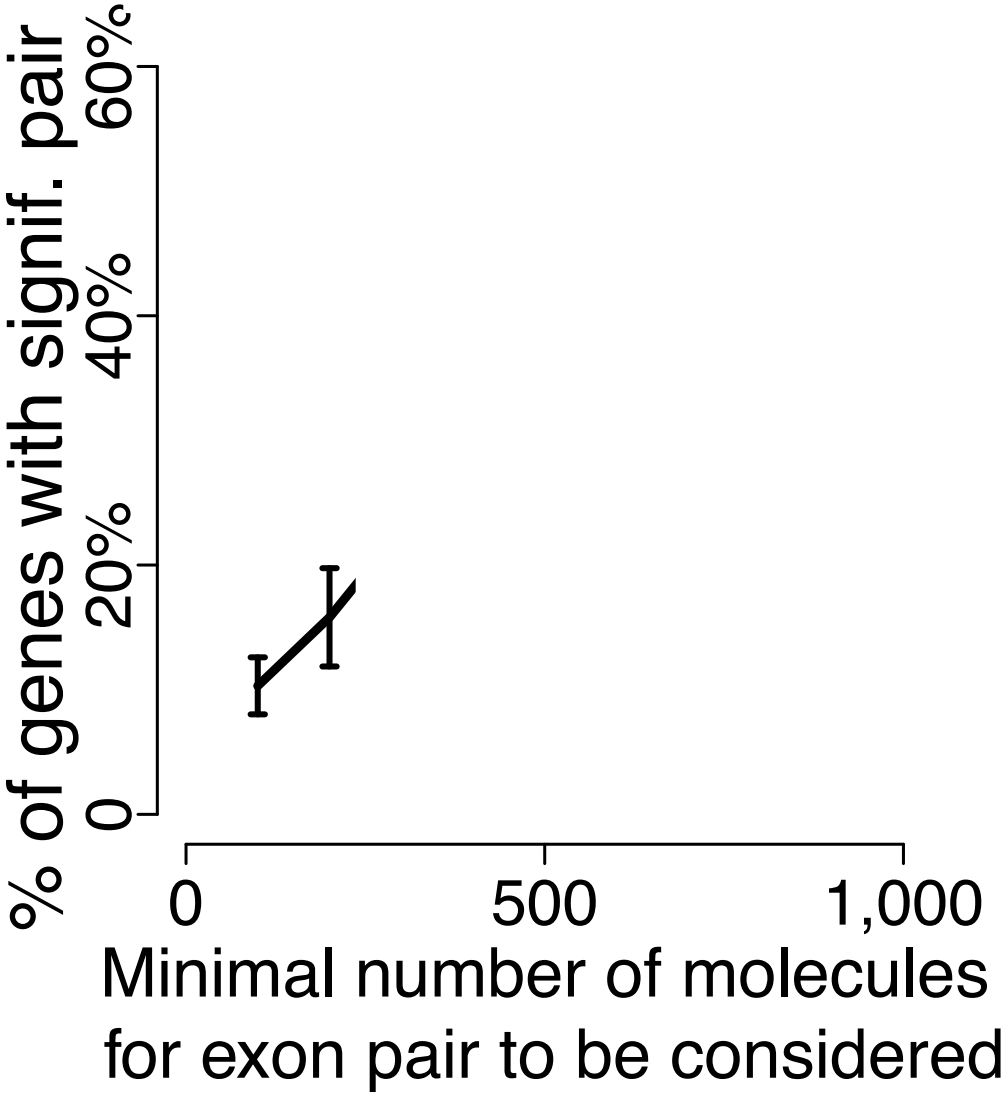

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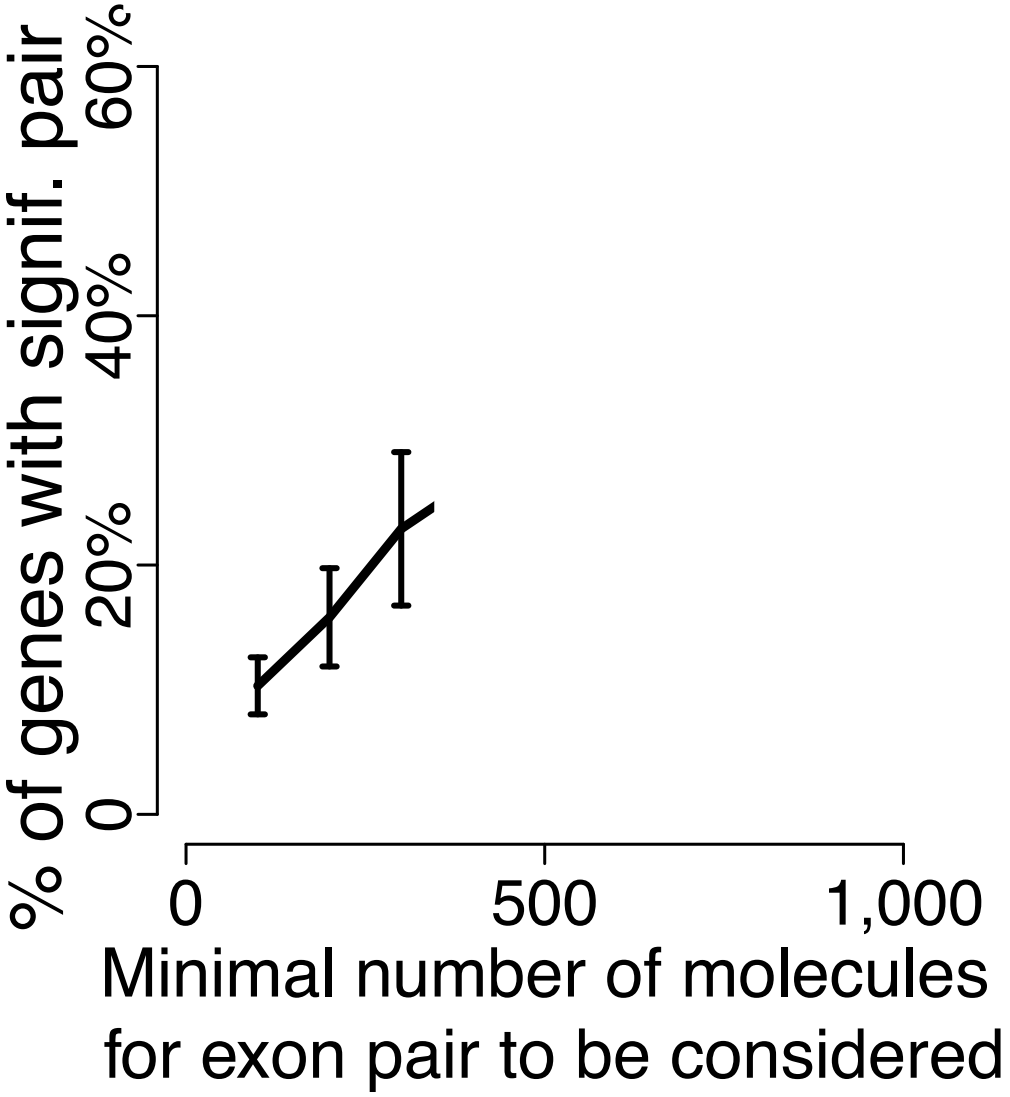

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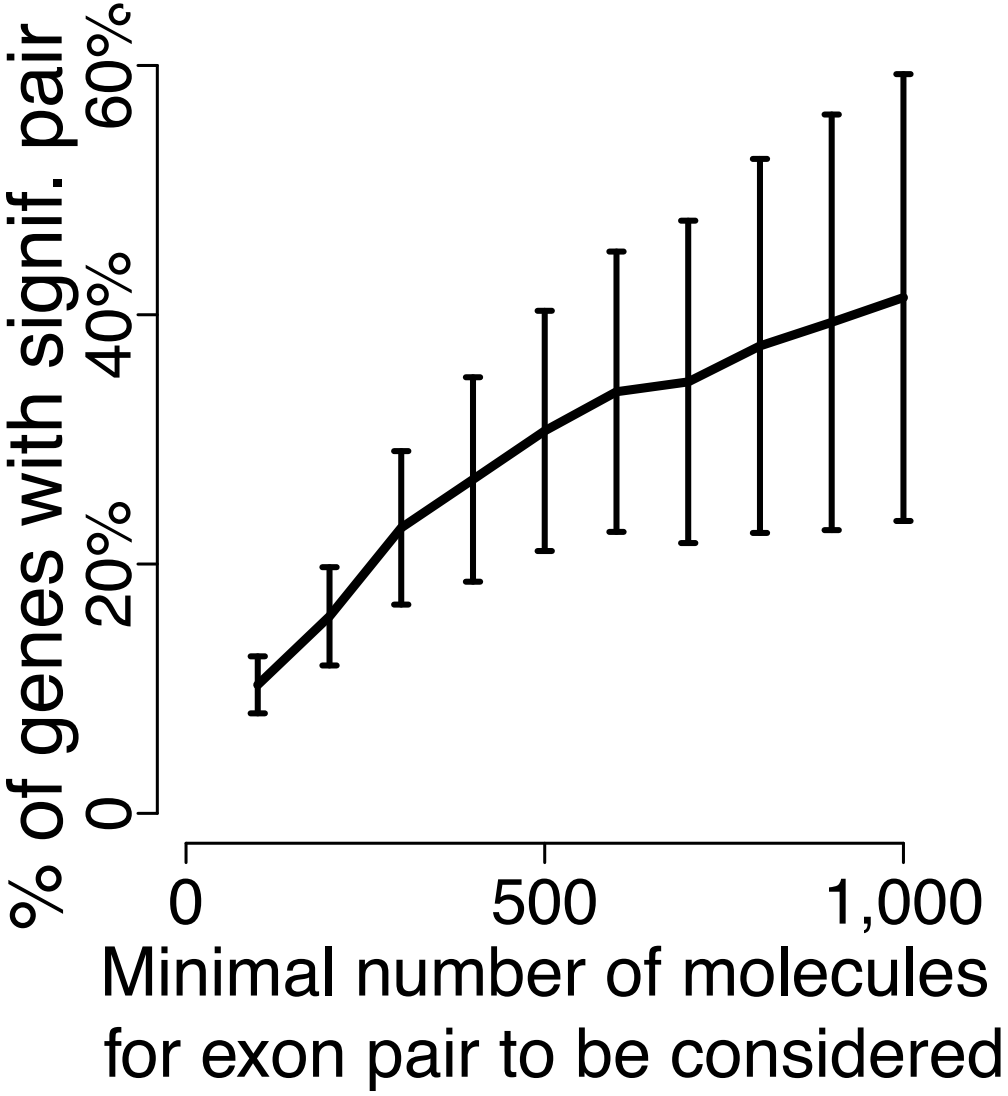

23.5%-59.3% (95% CI) of genes with multiple distant alternative exons are expected to show coordination at a log-odds-ratio of 0.5 or above.

THE justification for isoform sequencing (otherwise no long reads needed … just get probability of isoform from product of probabilities of variables sites)

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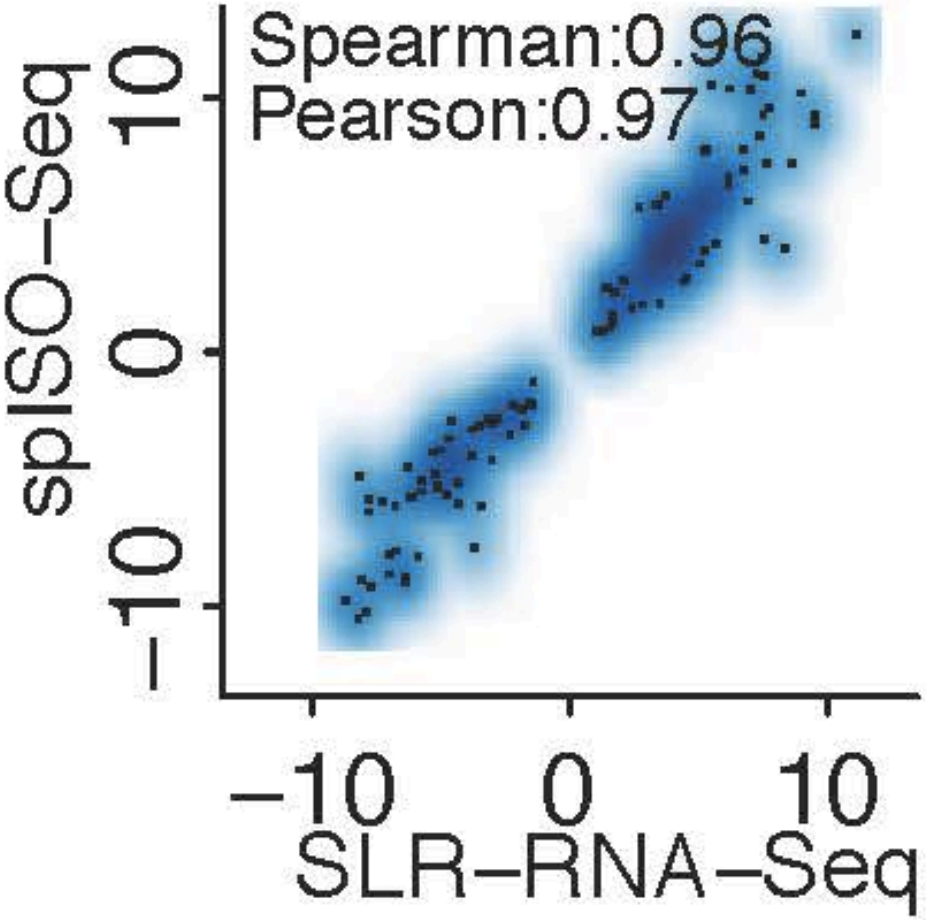
Comparability of coordination quantification



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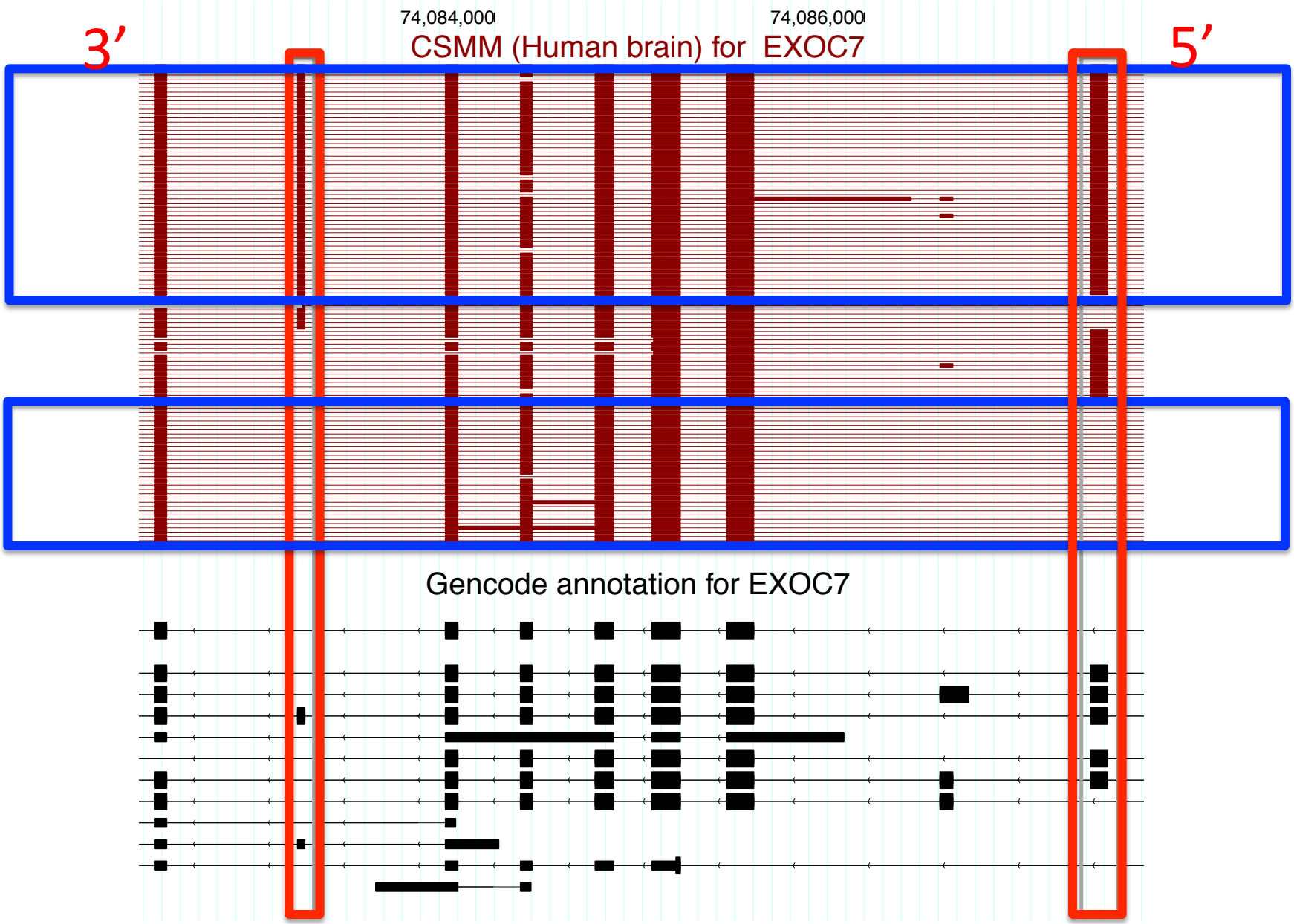

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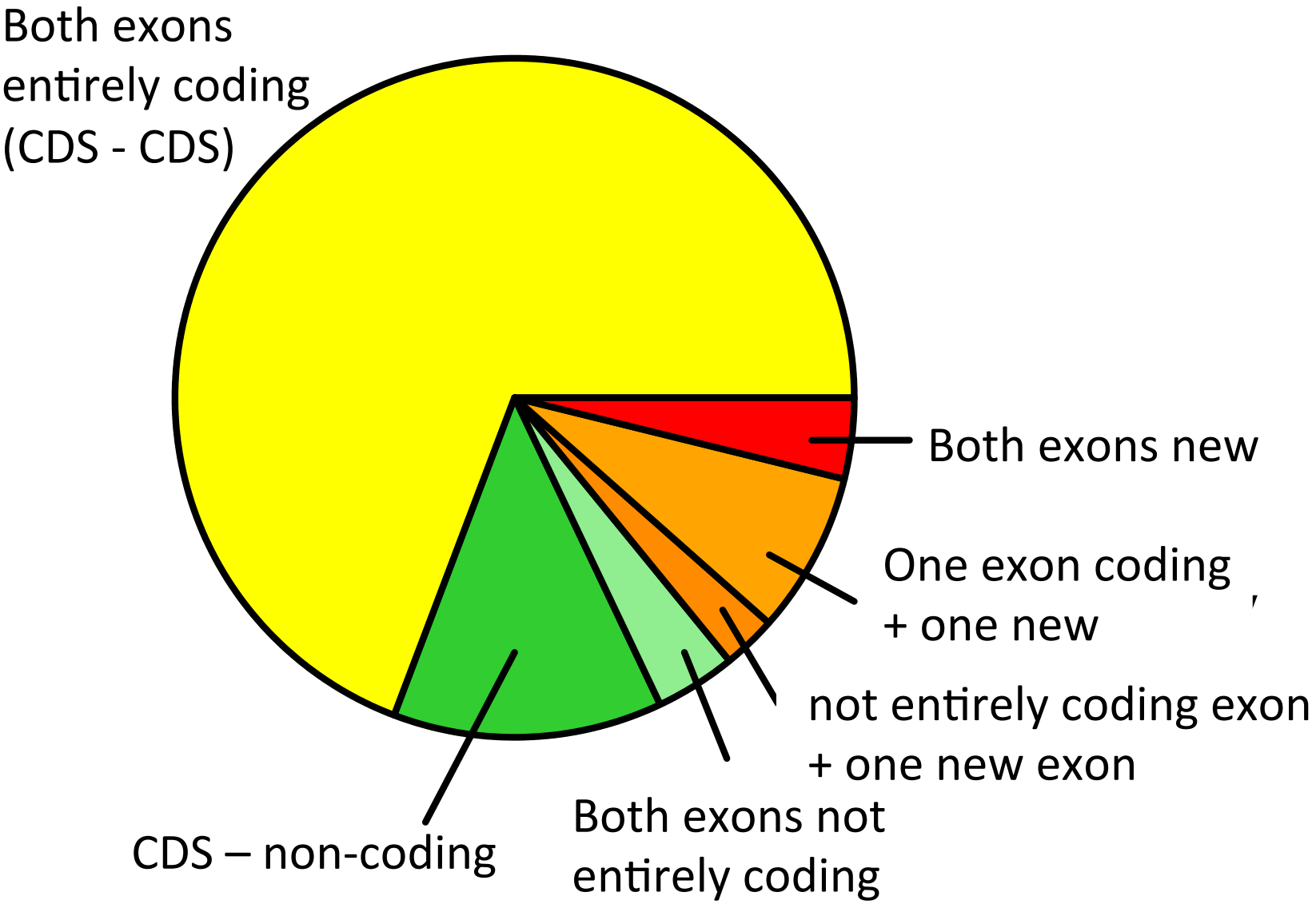

A phased brain proteome ?

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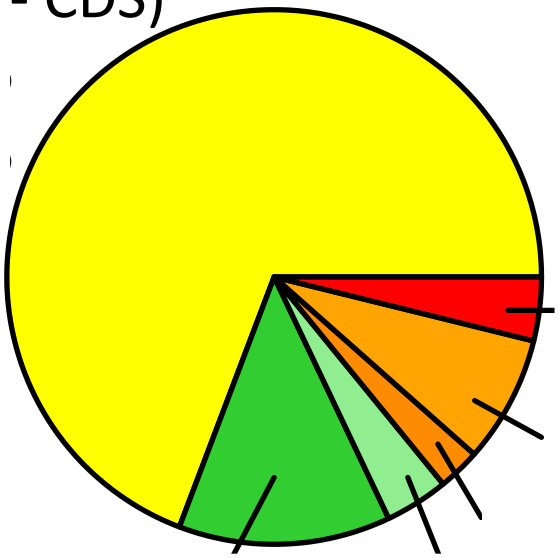
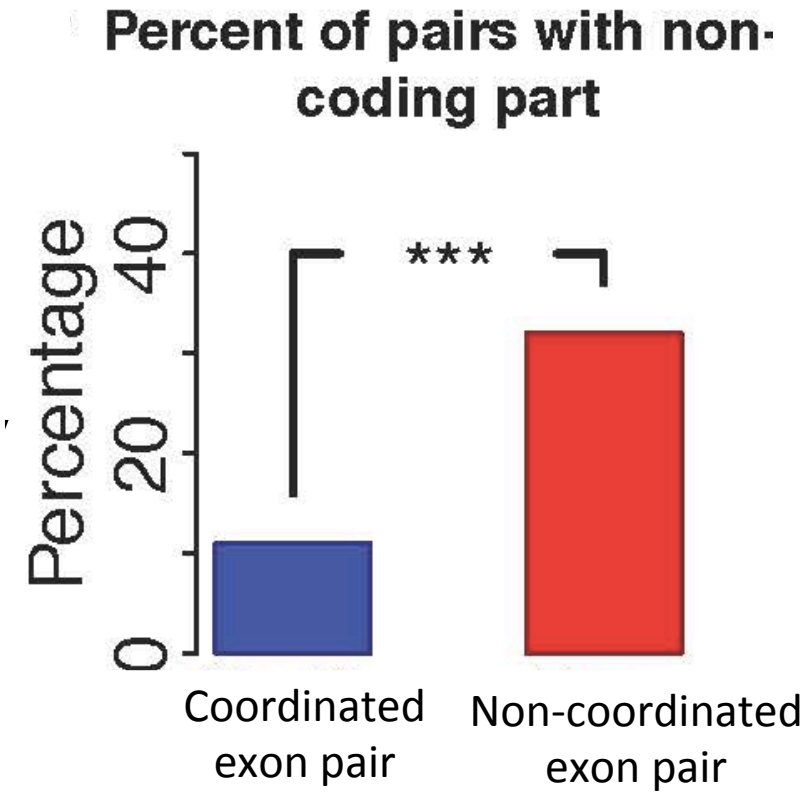
Both exons entirely coding (CDS - CDS)





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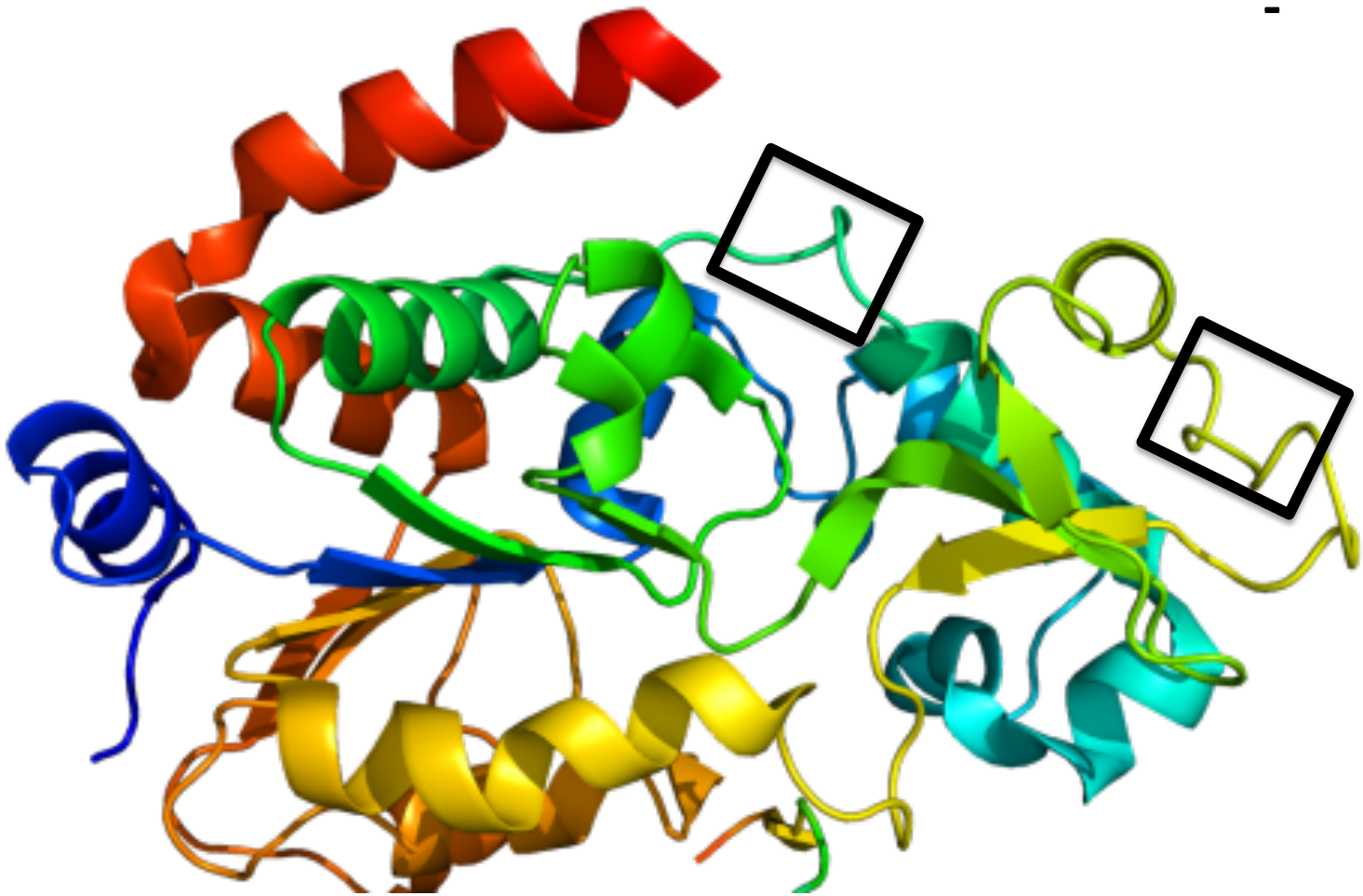

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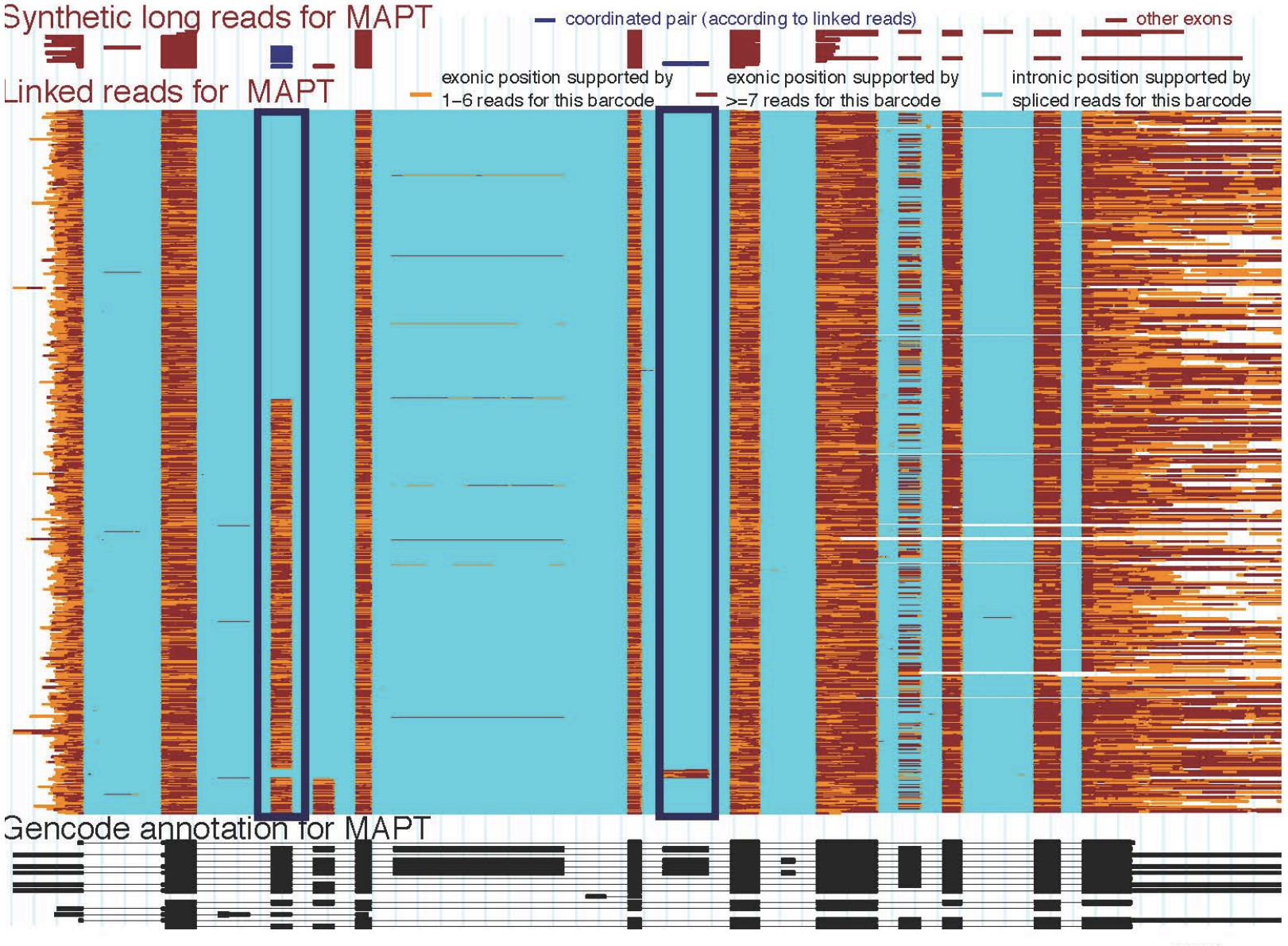

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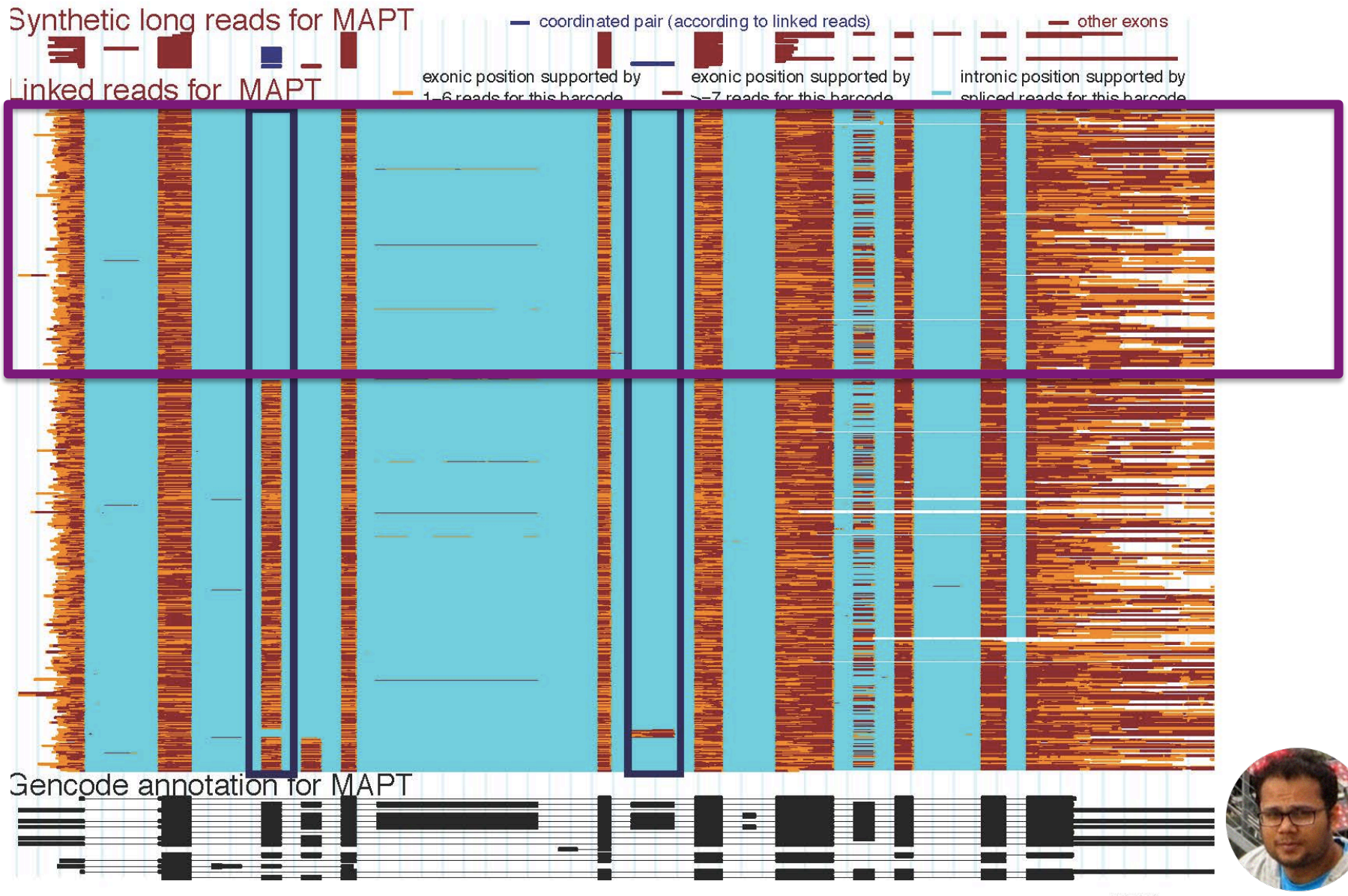

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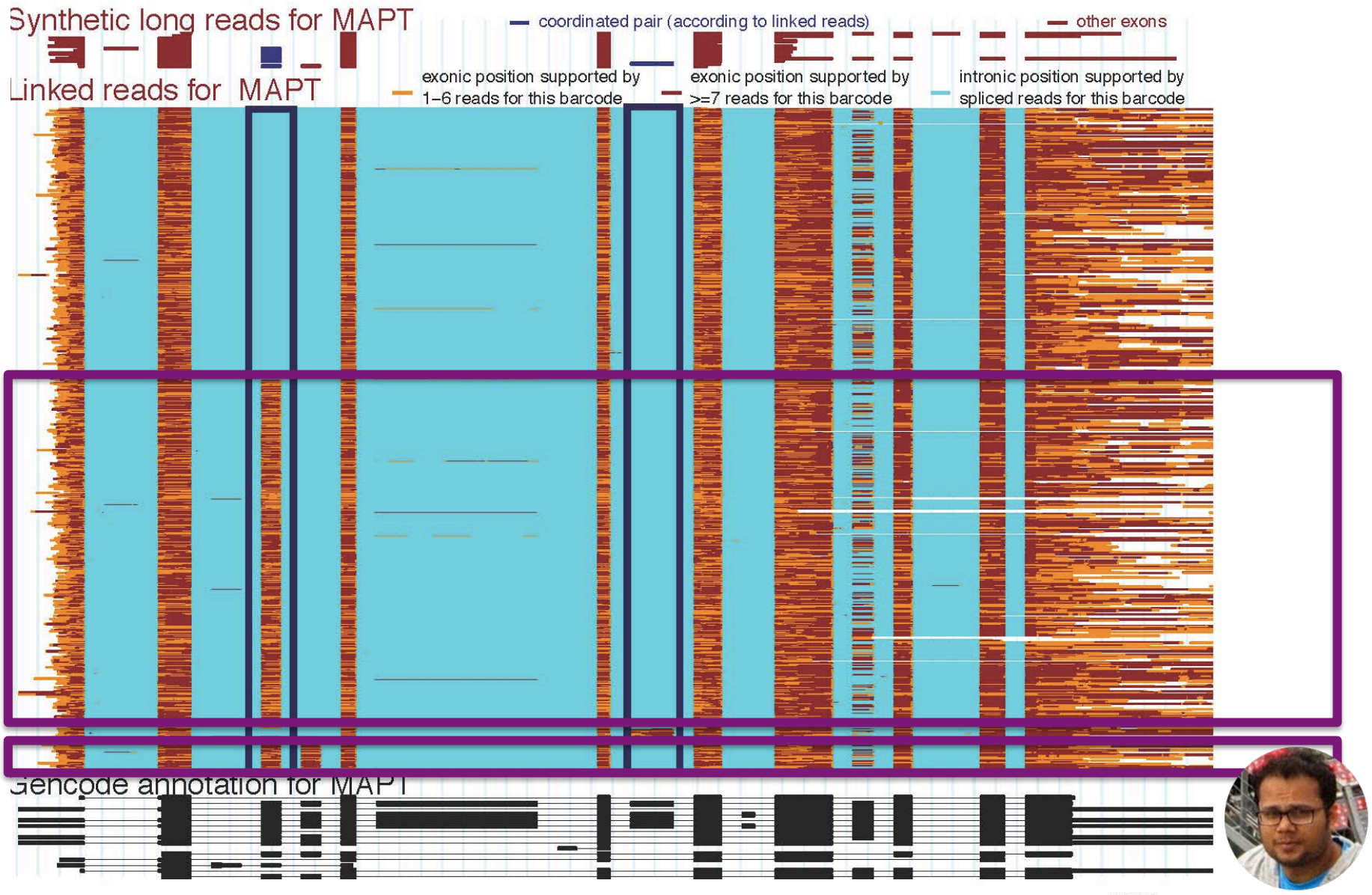

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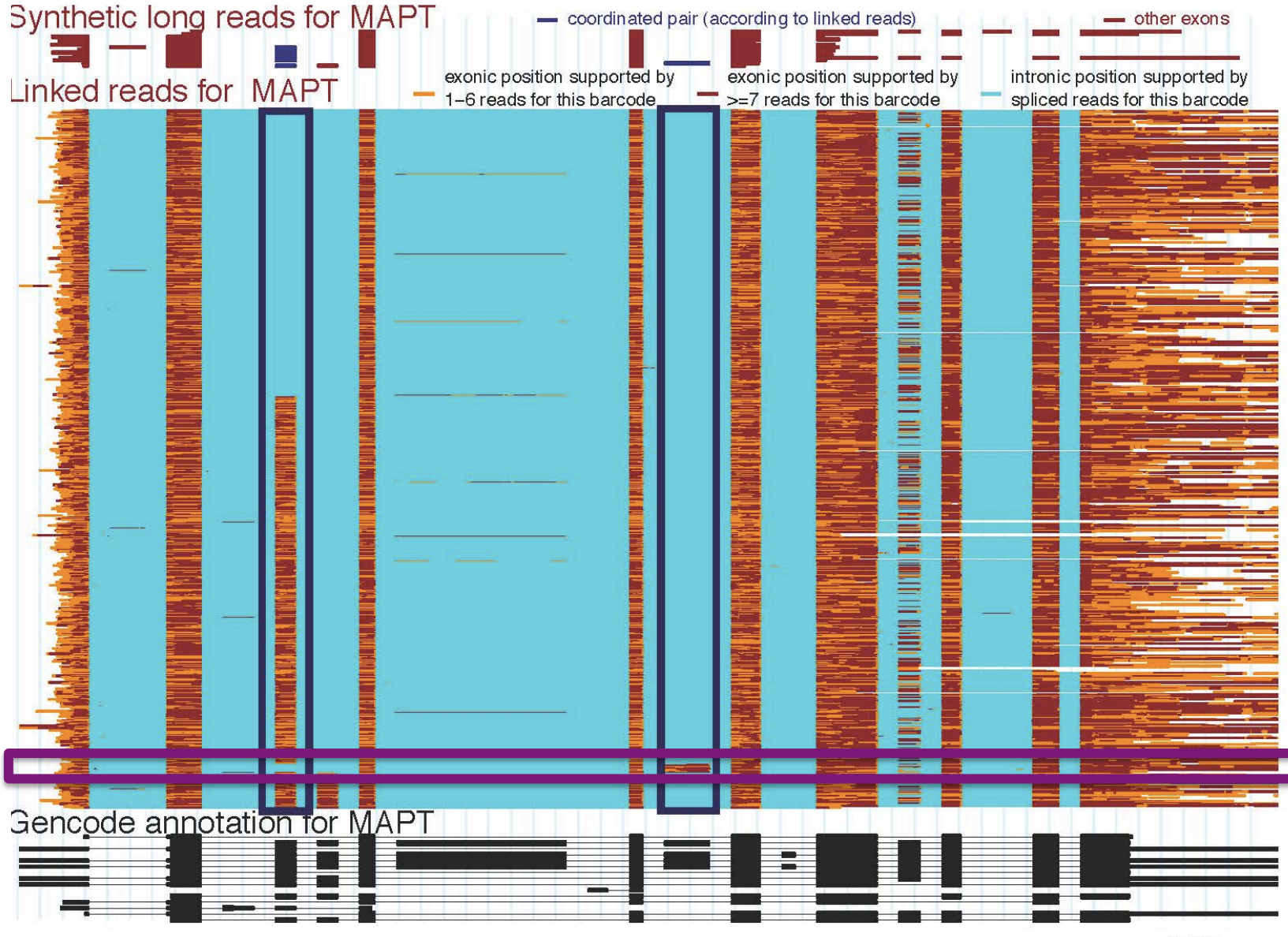
MAPT (tau) splicing is governed by coordination



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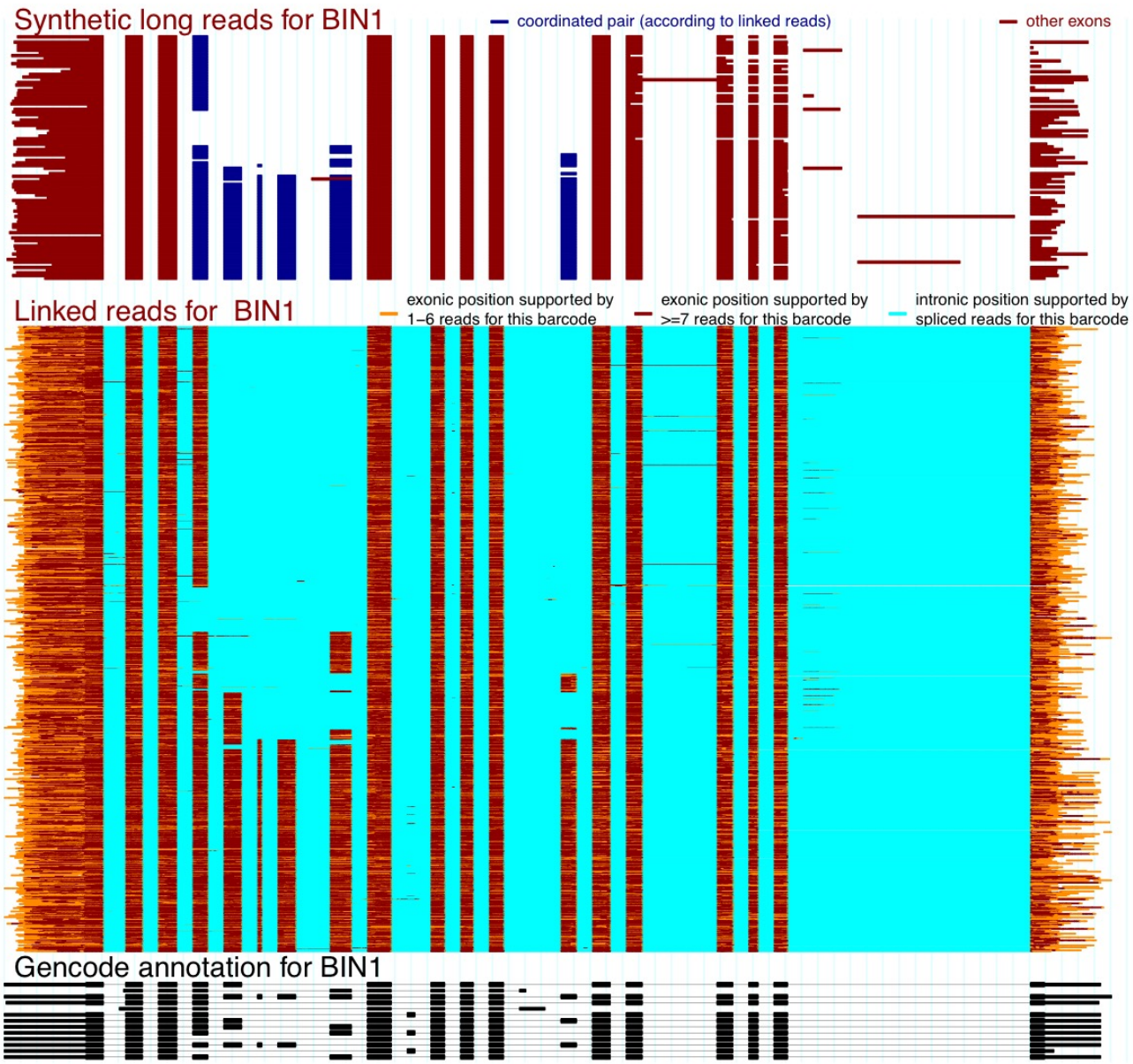

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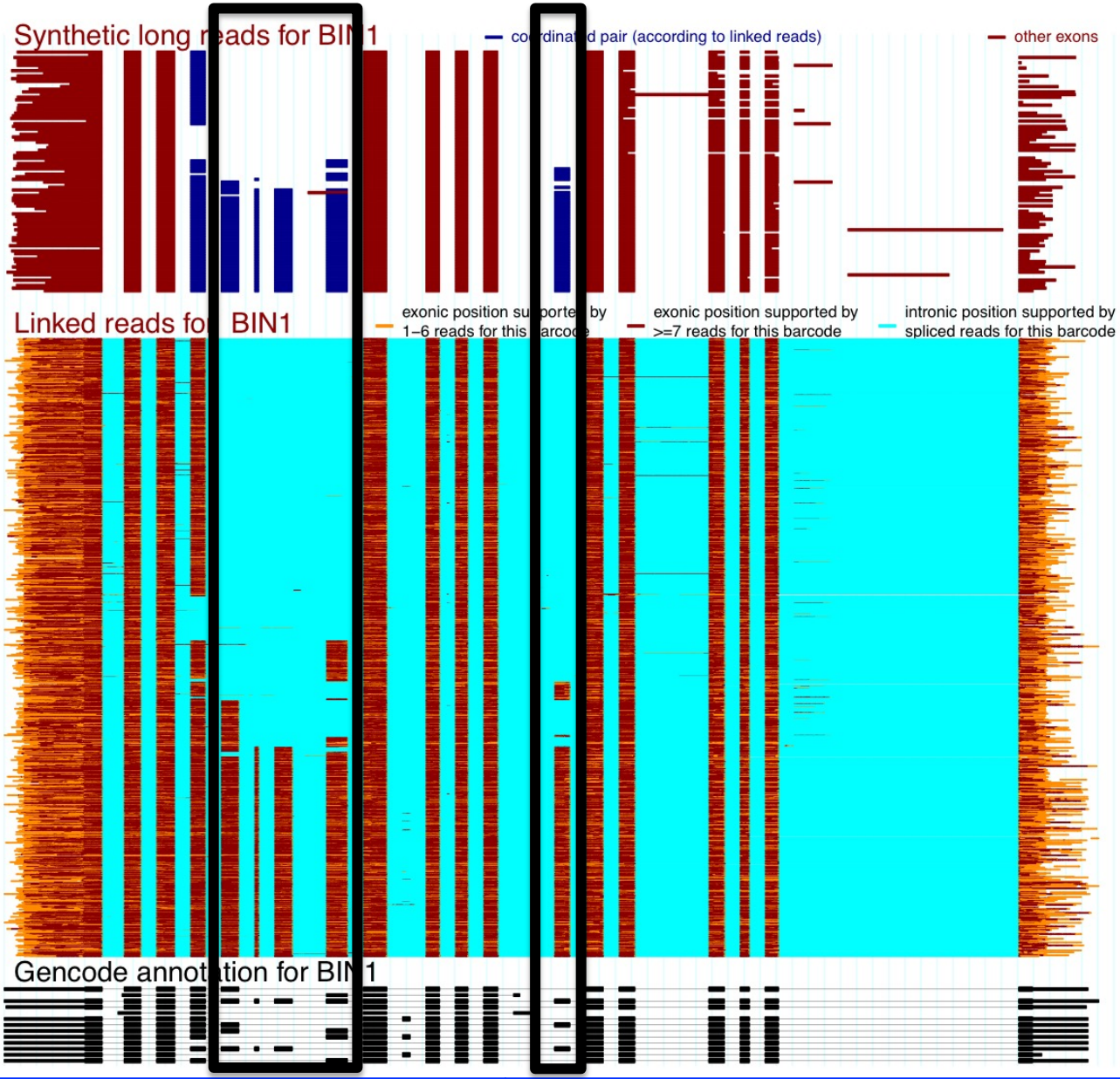

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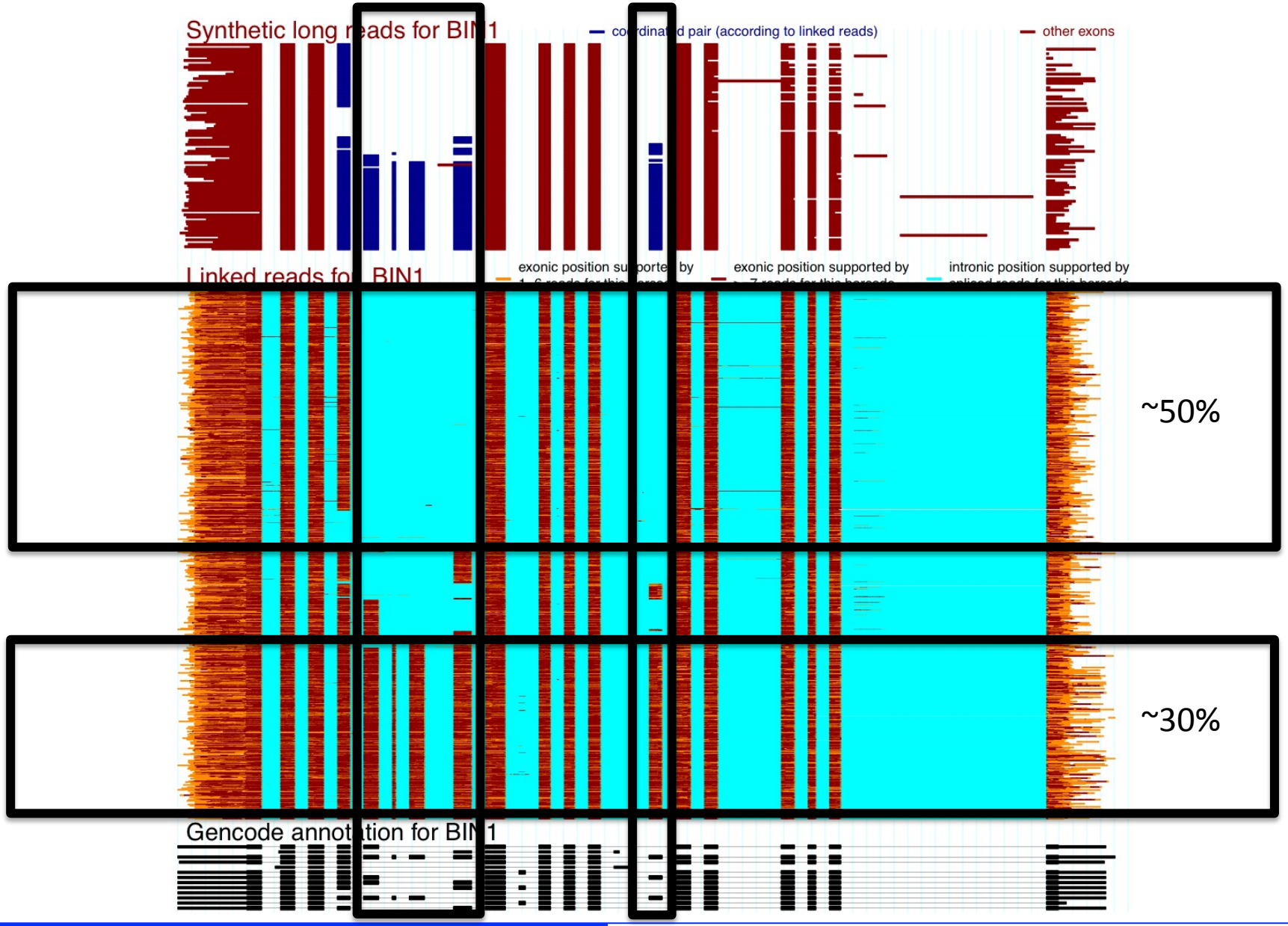
BIN1 (strongly AD associated) splicing is governed by coordination



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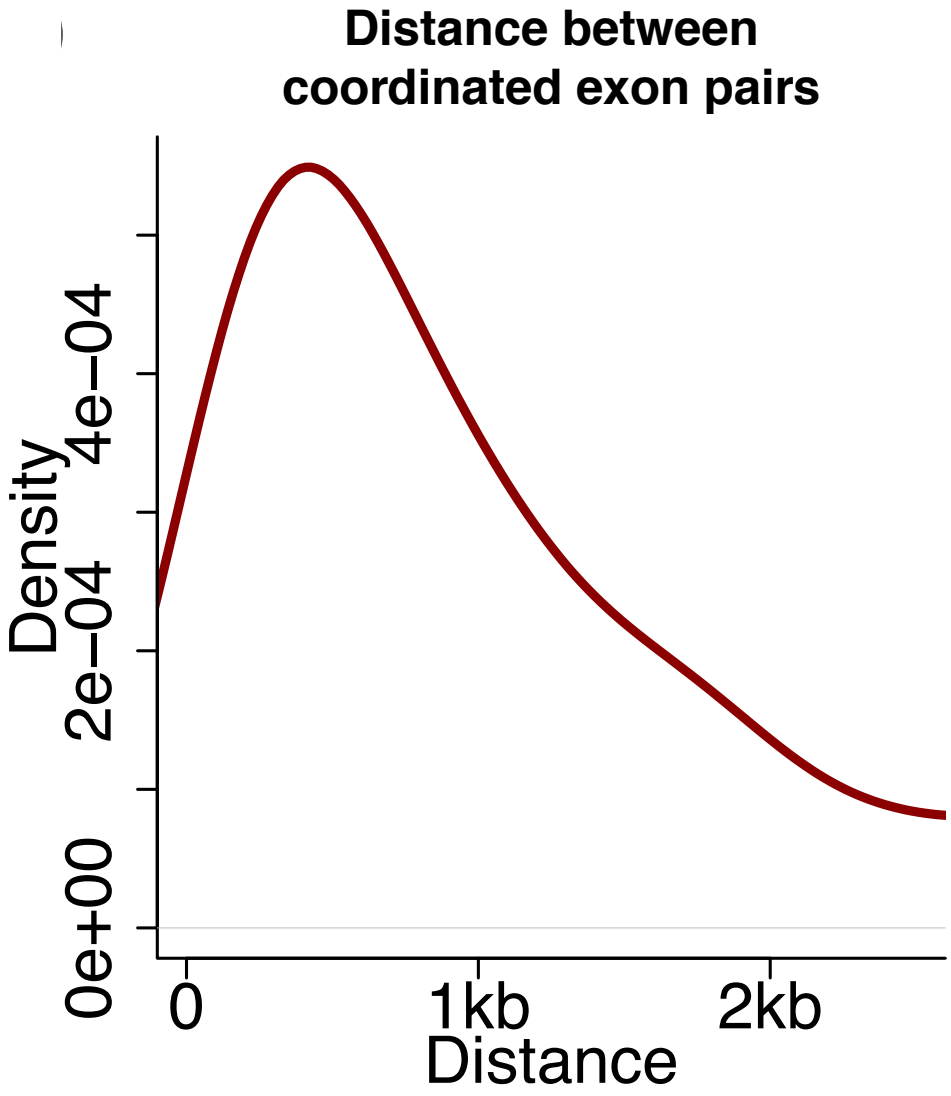

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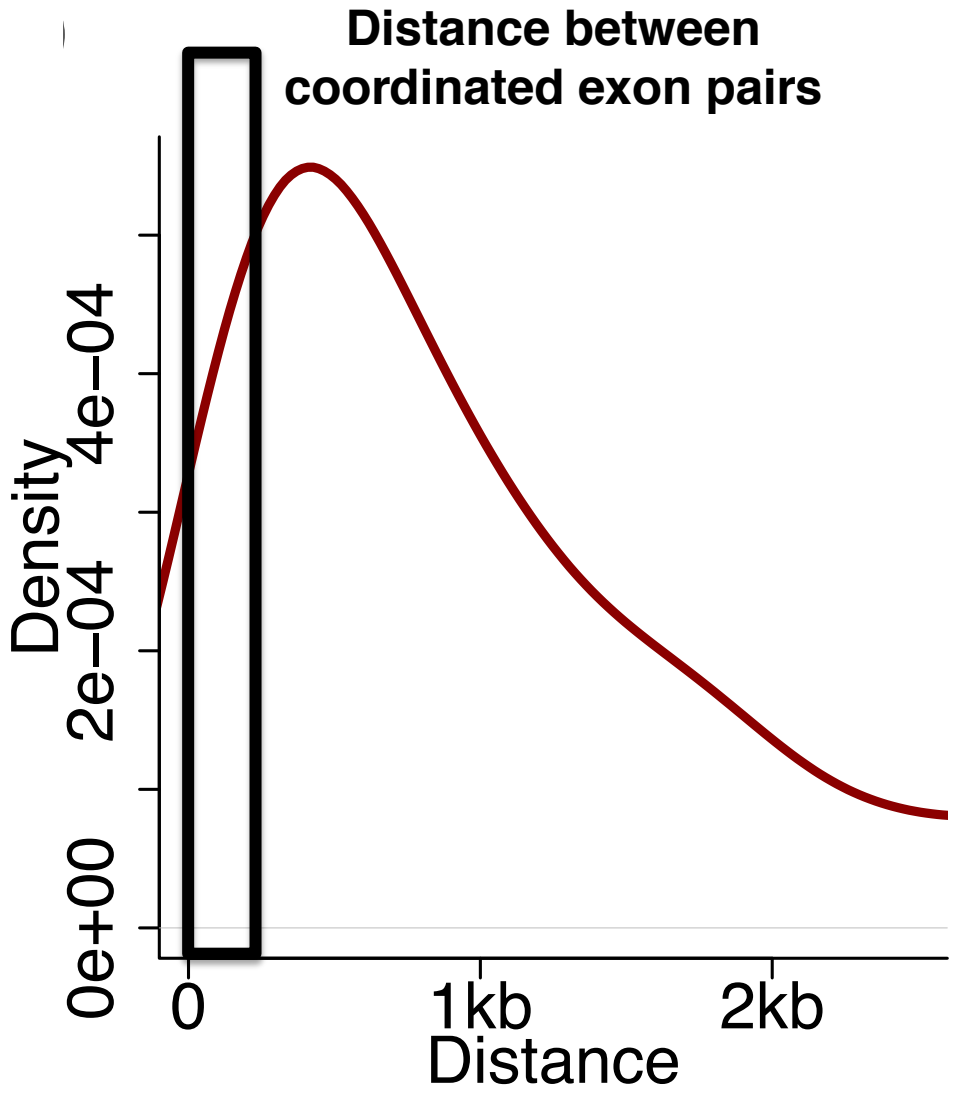

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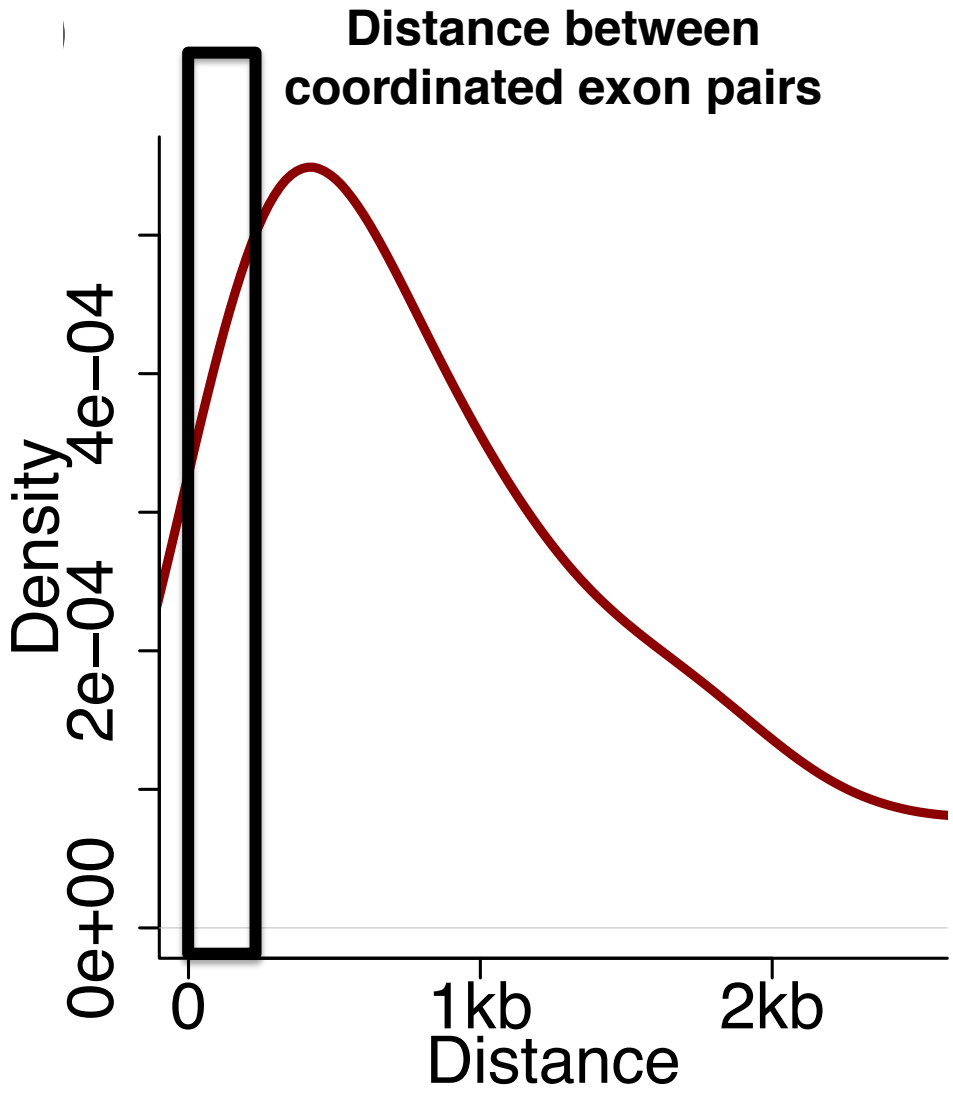
Discovery with Illumina -> limited by fragment size (possibly deeper)



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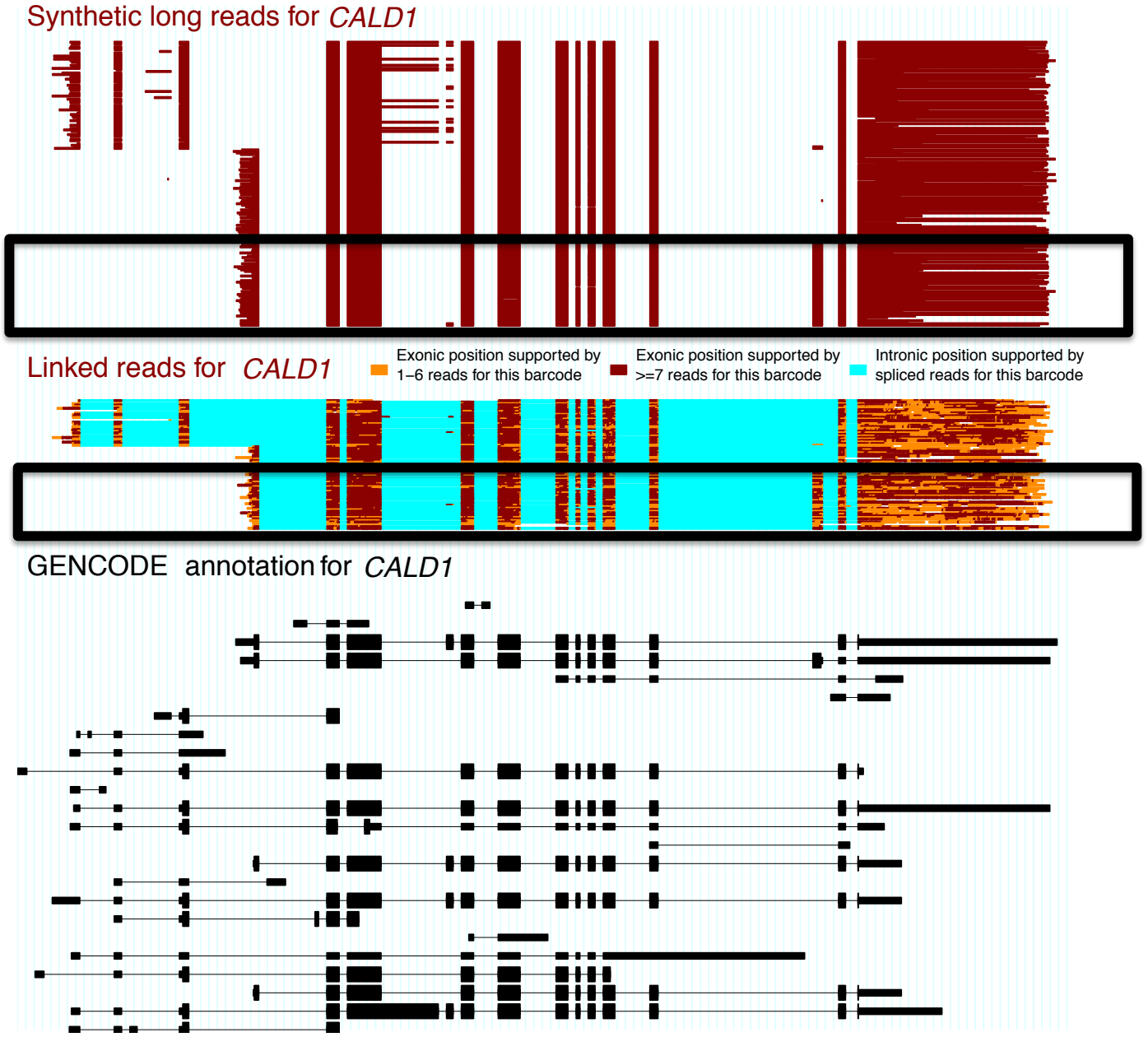

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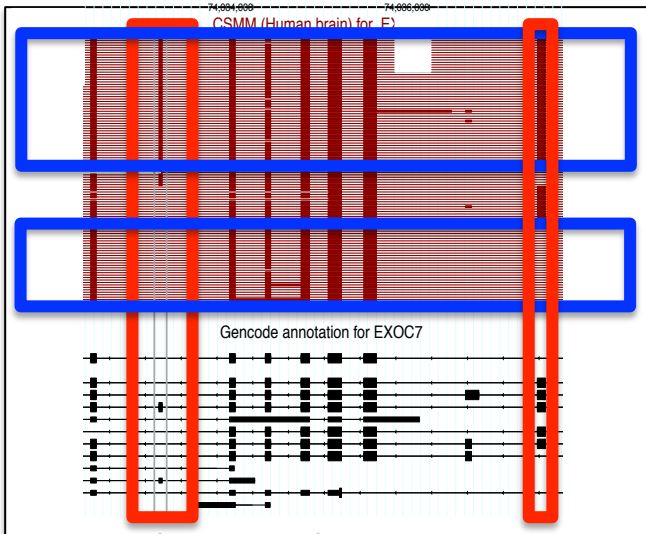
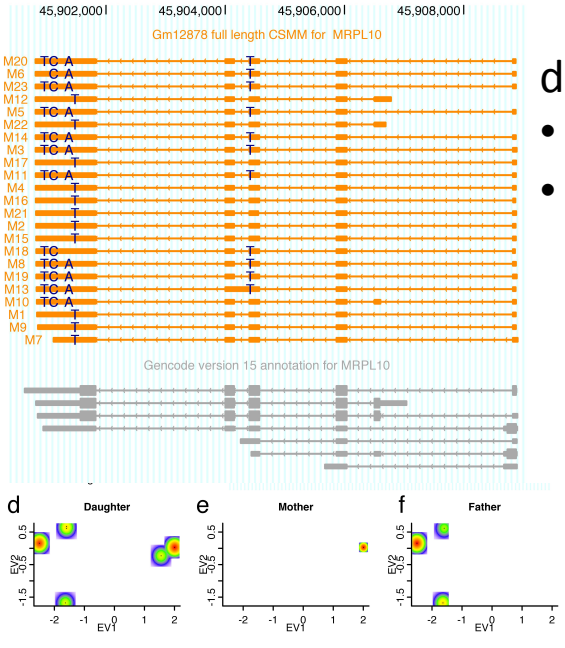
Summary



Coordination between Tilgner et al, 2017,2015,2014

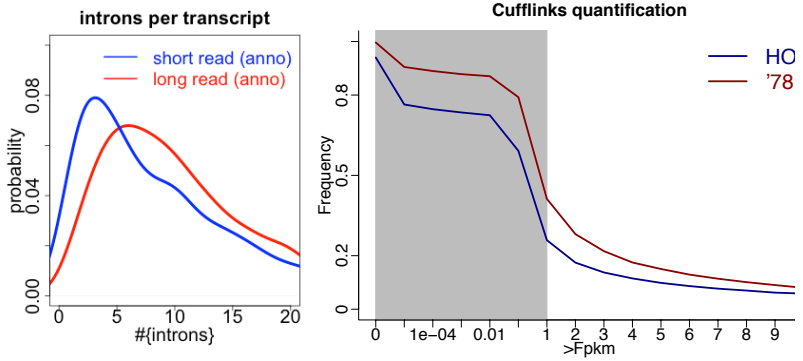
- Distant splicing events and other types
- SNPs and splicing (allele specific splicing)



de novo phasing of

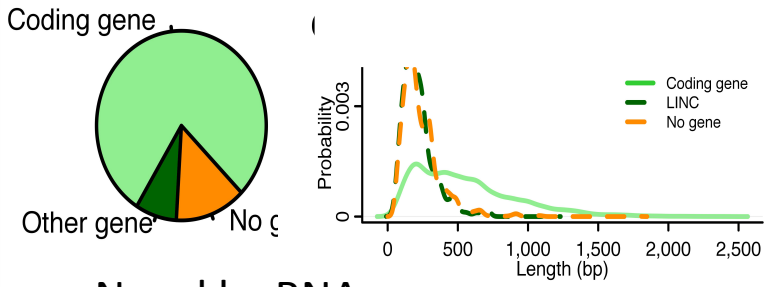
- SNPs
- CNVs

Tilgner et al, 2014



- Enhanced annotations + isoform quant.
- Better isoform discovery from short reads

Tilgner et al, 2013, 2014



- Novel lncRNA genes
- Loads of novel lncRNA isoforms
- Many protein coding RNA isoforms

Sharon et al, 2013, Tilgner et al, 2013, 2015

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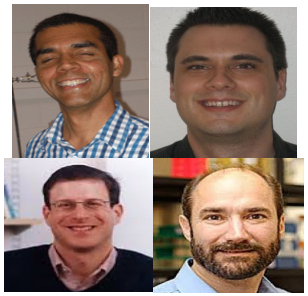
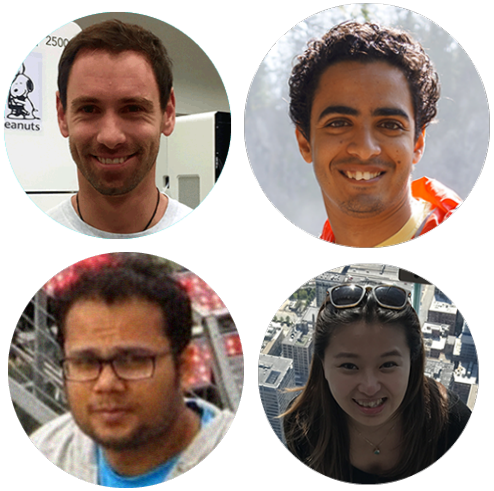
You for listening



K562 + Hela

Human organs + lymphoblastoid



SLRs

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Costantino Iadecola
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Luisa Lente

Funding:

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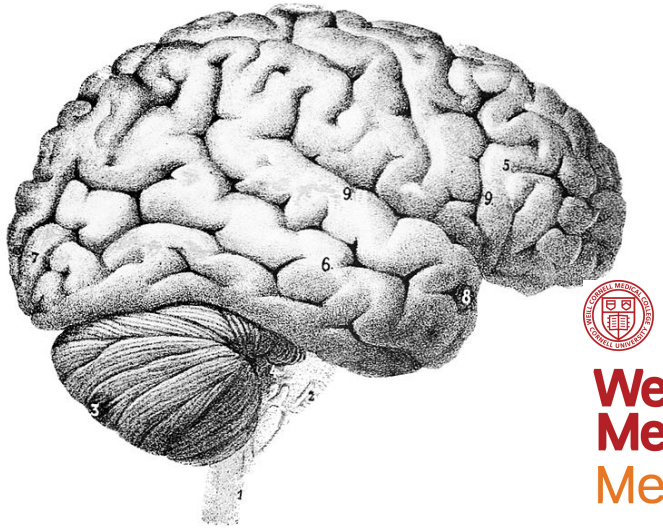

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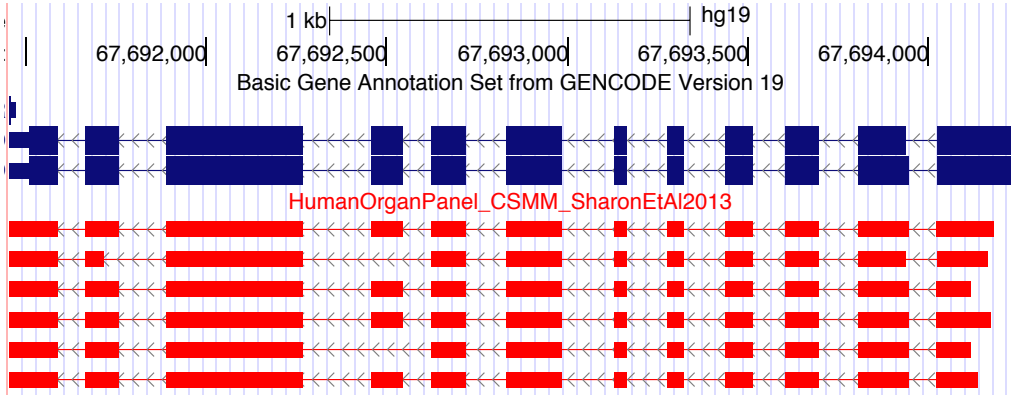

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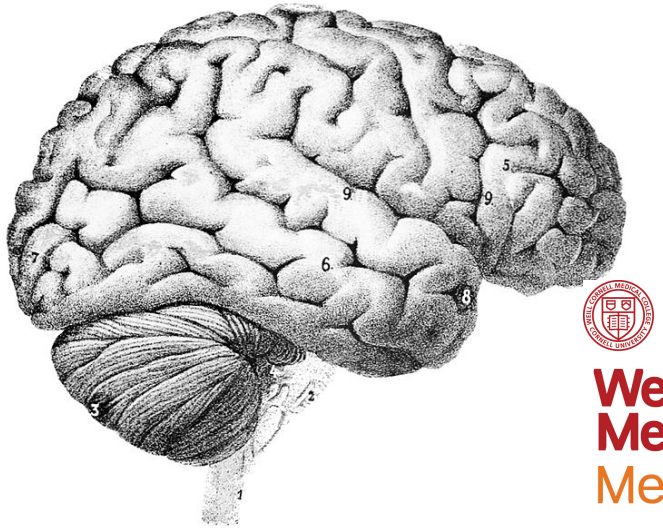

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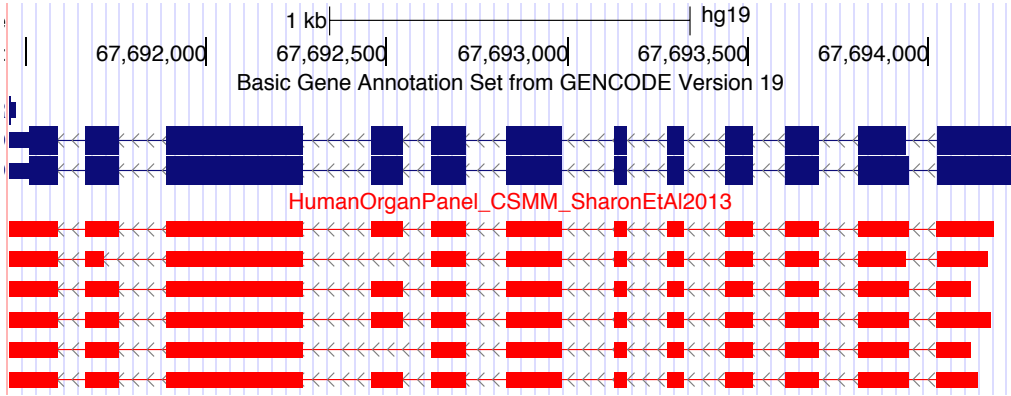
Looking for postdocs

- Algorithms/software
- Data analysis



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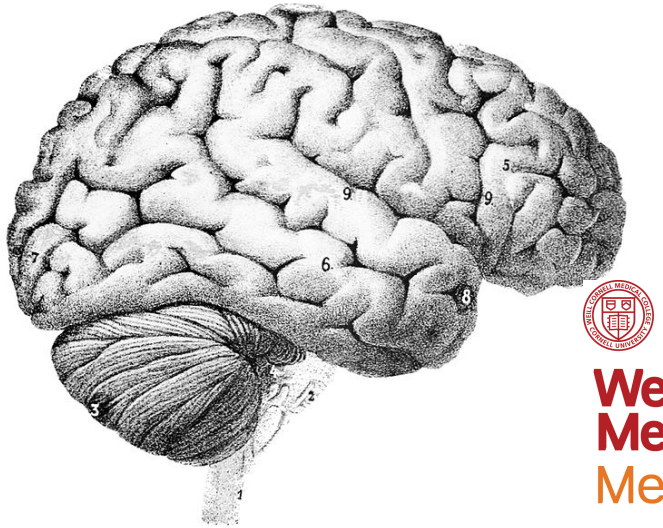

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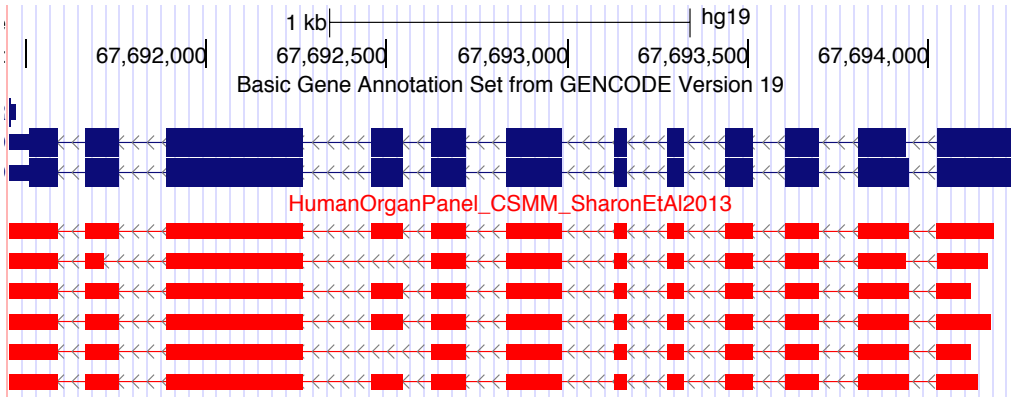
Collaborations welcome



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