An analysis of splicing variation across SRA with Rail-RNA

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Johns Hopkins University
Genome Informatics 2015

in genomics

use prior knowledge

study data *ab initio*

in RNA-seq analysis

use gene annotation:

quantify with/ align to known transcripts

avoid gene annotation:

observe alternative splicings

in RNA-seq analysis

use gene annotation:
quantify with/ align to known transcripts

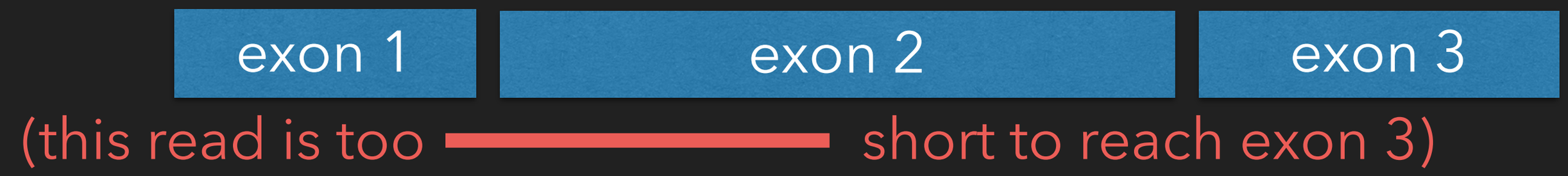
avoid gene annotation:
alternative splicings?

study merits/drawbacks
with many RNA-seq samples

Study many RNA-seq samples

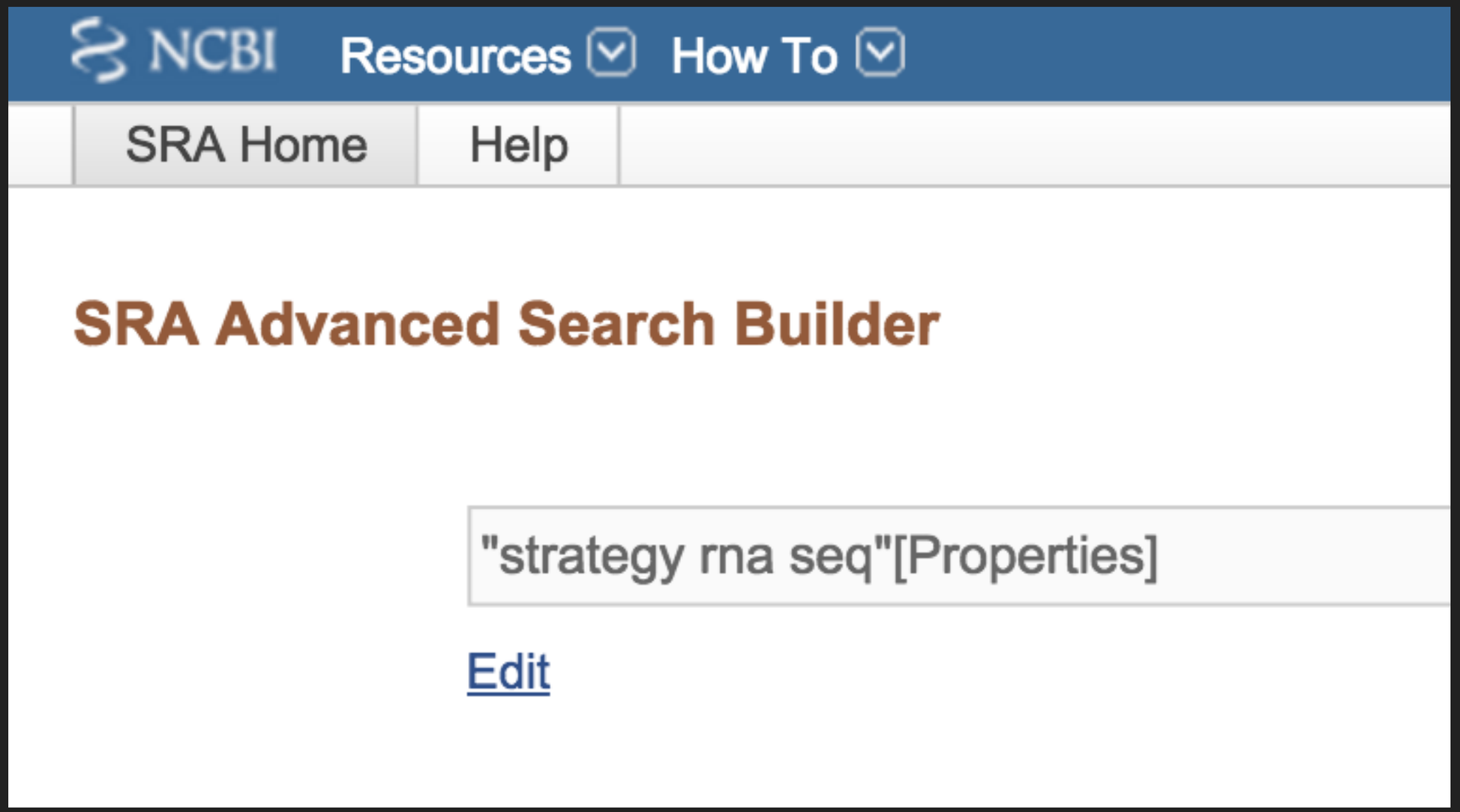
SRA (> 4 PB): short reads hard to assemble; missing exons in **60%** of transcripts

(RGASP 2013 doi:10.1038/nmeth.2714)



=> Compare exon-exon junctions found across SRA RNA-seq with annotated junctions

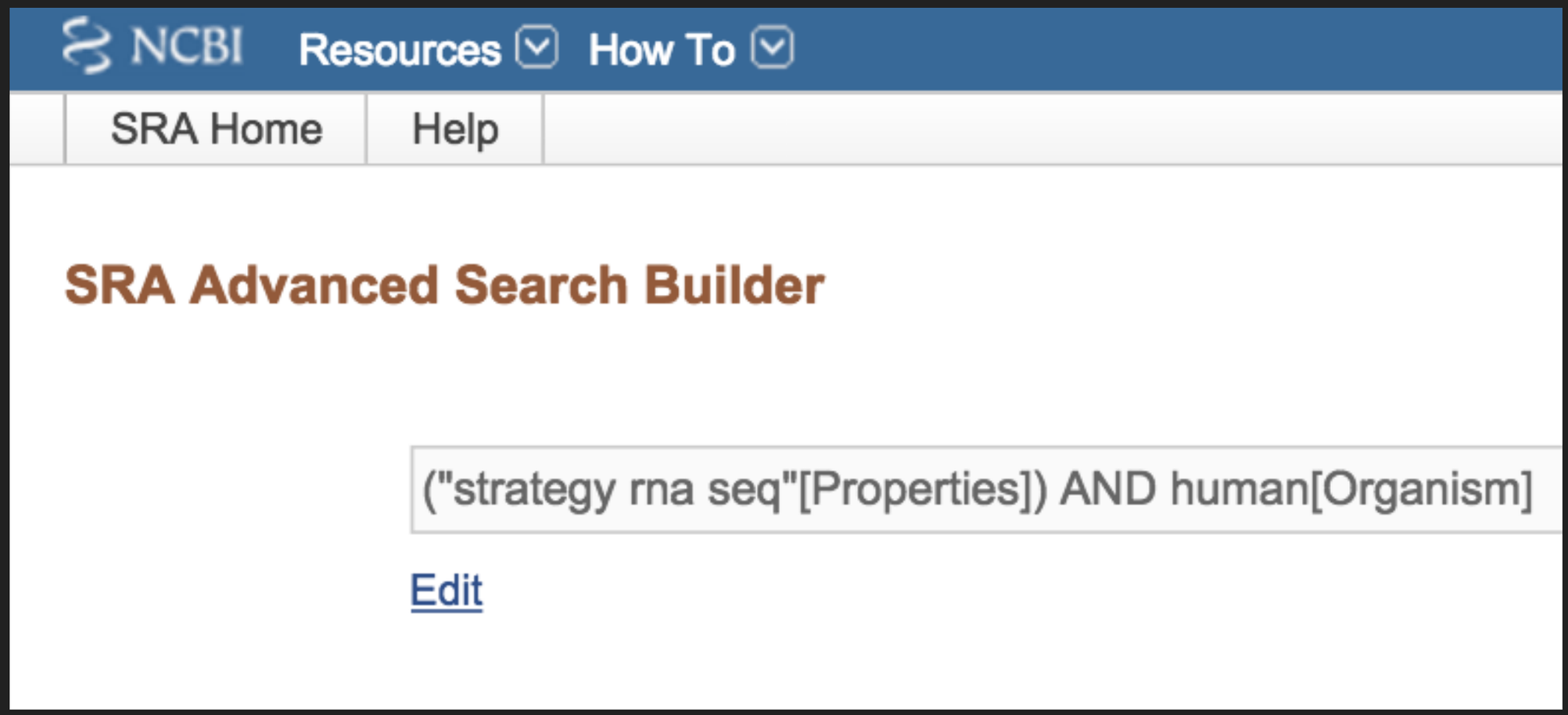
Filtering SRA



(from http://www.ncbi.nlm.nih.gov/sra/advanced)

→ ≈180k publicly available runs

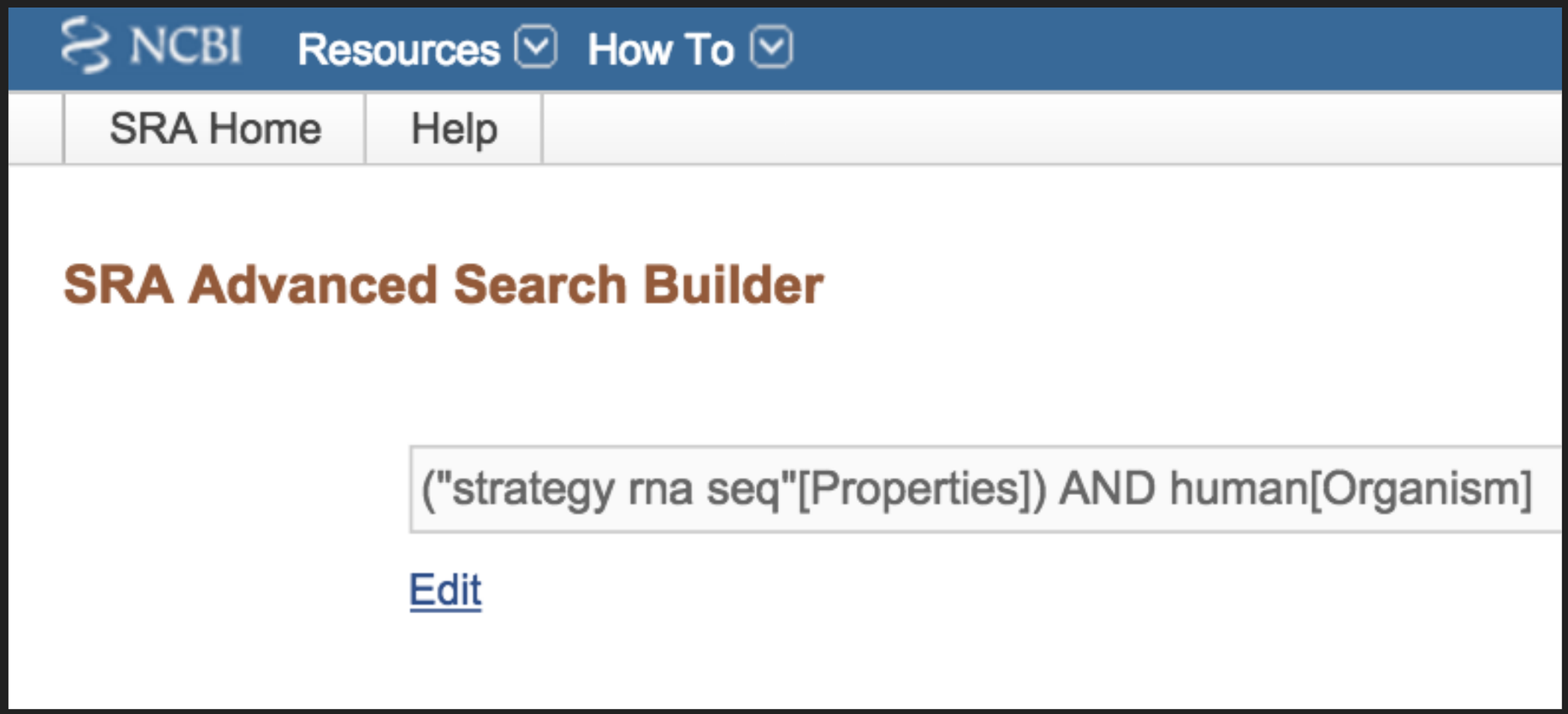
Filtering SRA



(from http://www.ncbi.nlm.nih.gov/sra/advanced)

→ ≈36k publicly available runs

Filtering SRA



(from http://www.ncbi.nlm.nih.gov/sra/advanced)

+ Illumina instruments[Properties]

→ ≈22k runs as of late May ‘15

How to find junctions across

21,504 RNA-seq runs?

(62 terabases of reads)

annotation-agnostic pipeline

Rail-RNA

http://rail.bio

`derfinder`

`biocLite("derfinder")`

Leo
Collado-Torres

Alyssa
Frazee

sidesteps assembly & annotation limitations

resolves isoform-level features

`derfinder` finds unannotated (D)ERs

8.3% of age-associated DERs outside annotated genes across 72 prefrontal cortex samples:

Jaffe et al. (Nat Neuro, doi:10.1038/nn.3898)

6.9% of ERs outside annotated genes across 465 GEUVADIS LCLs:

Nellore et al. (j.mp/rail-pre)

Rail-RNA + amazon web services™

- No competition for compute
- Rapid: 8 days to results
- Repeatable:

  http://github.com/nellore/gi2015

  for commands and data
- Inexpensive: ~$0.70/sample

2 commands x 43 batches
gave, across 21,504 samples

One 7-GB `tsv.gz`

42,882,032

junctions

What gene annotation says

For *hg19*,

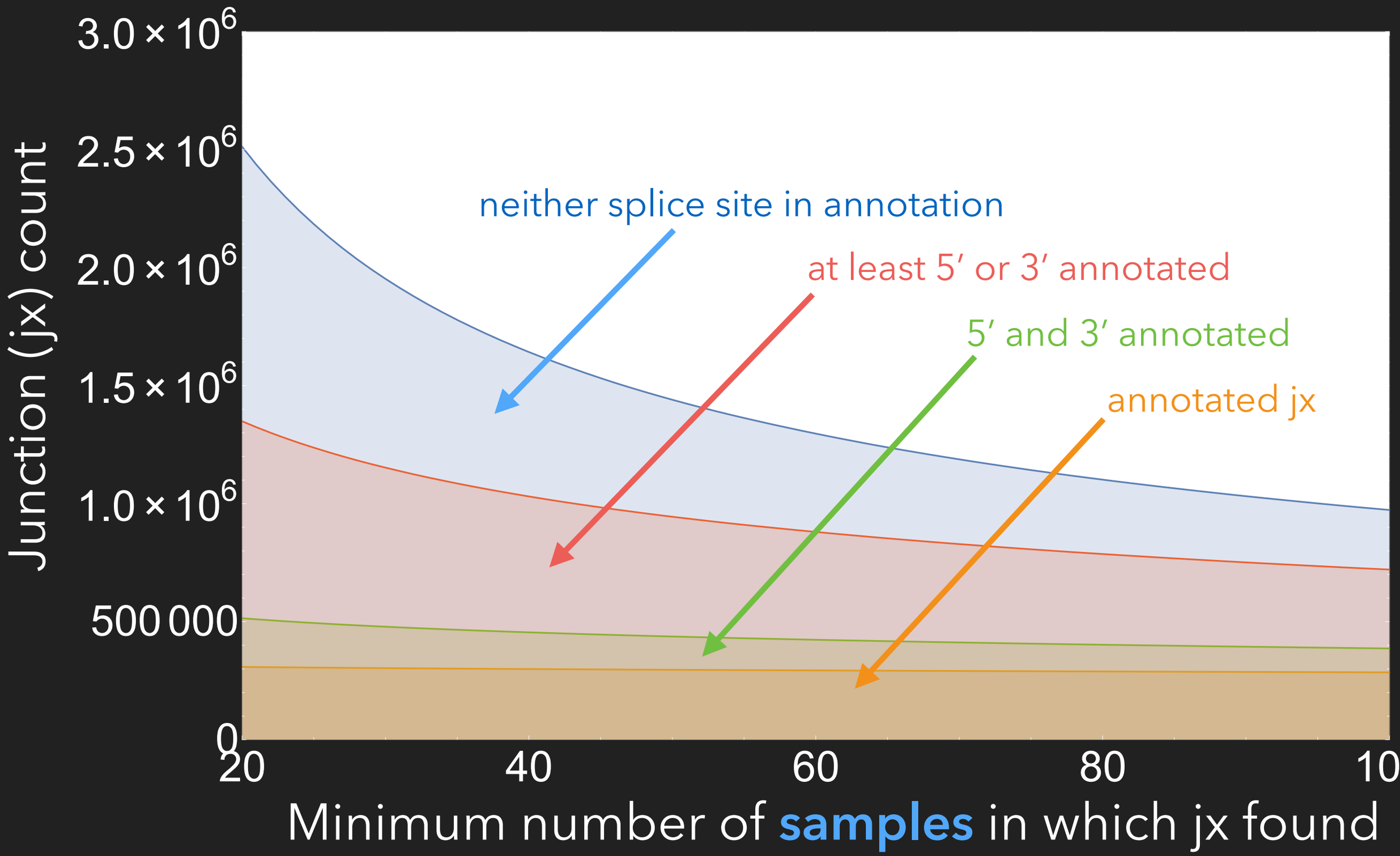
Ensembl v75 ∪ GENCODE v19 ∪ RefSeq

(almost subsumed
by Ensembl v75)

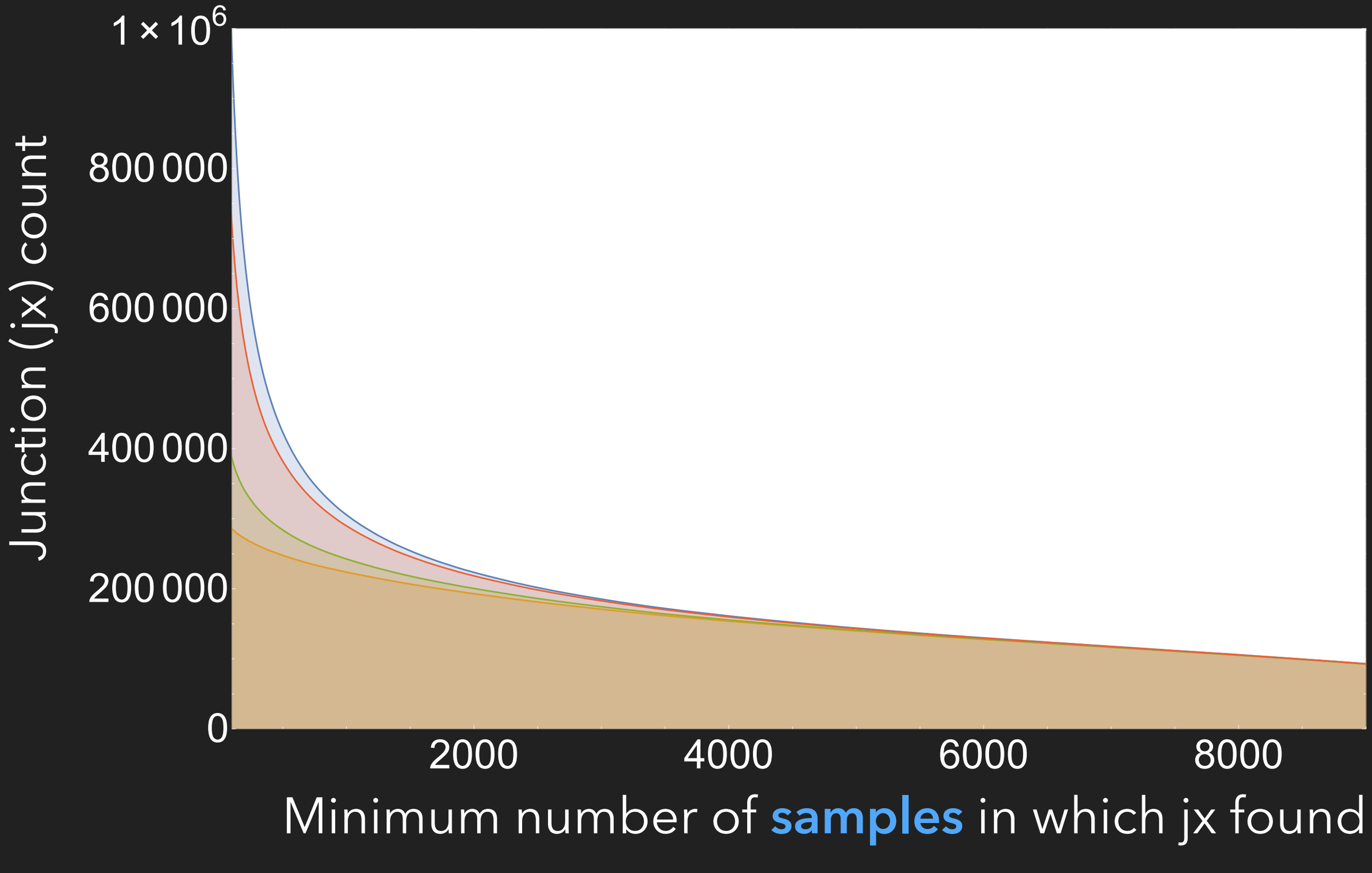
≈350,000

junctions

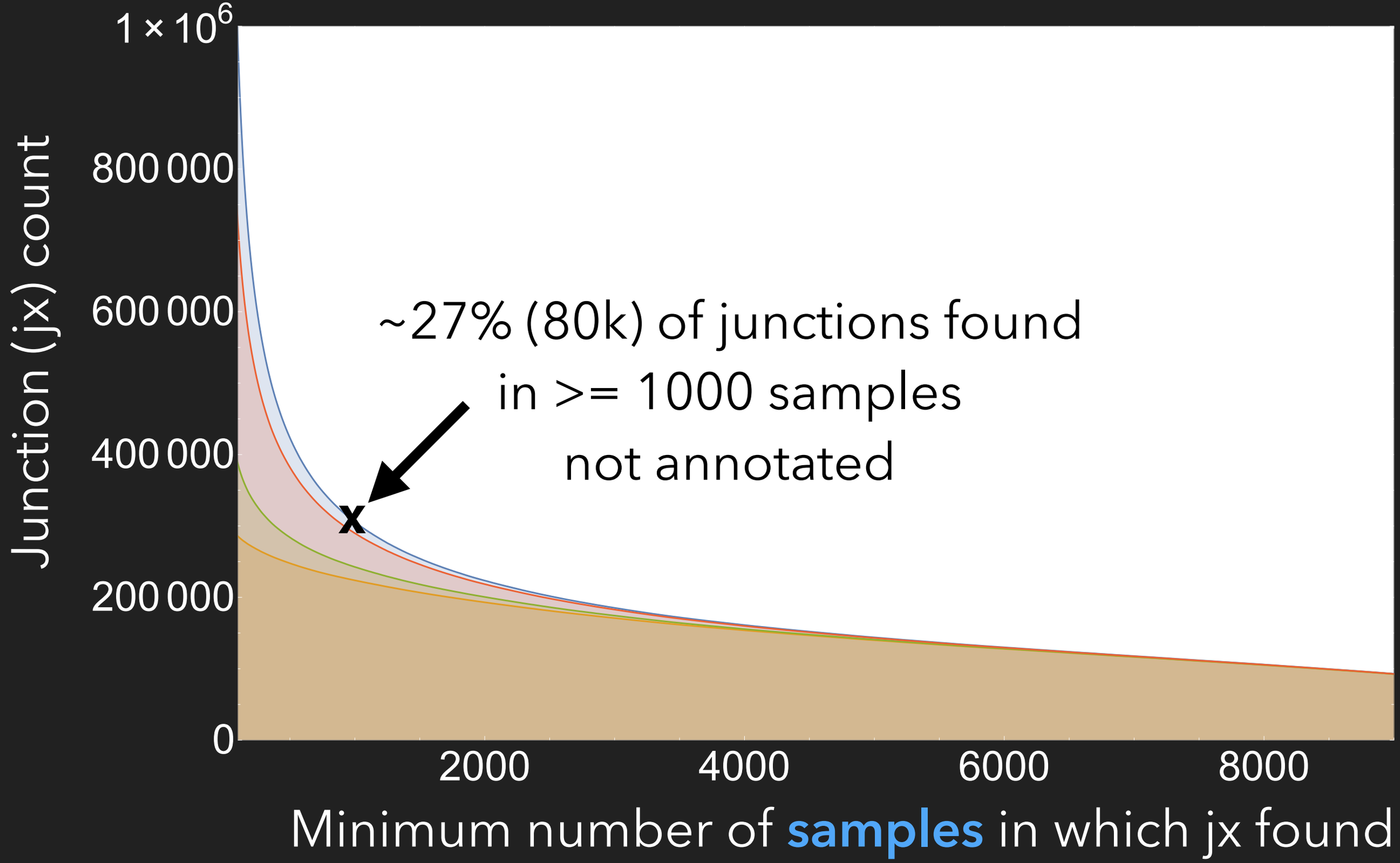
Increasing evidence in annotation



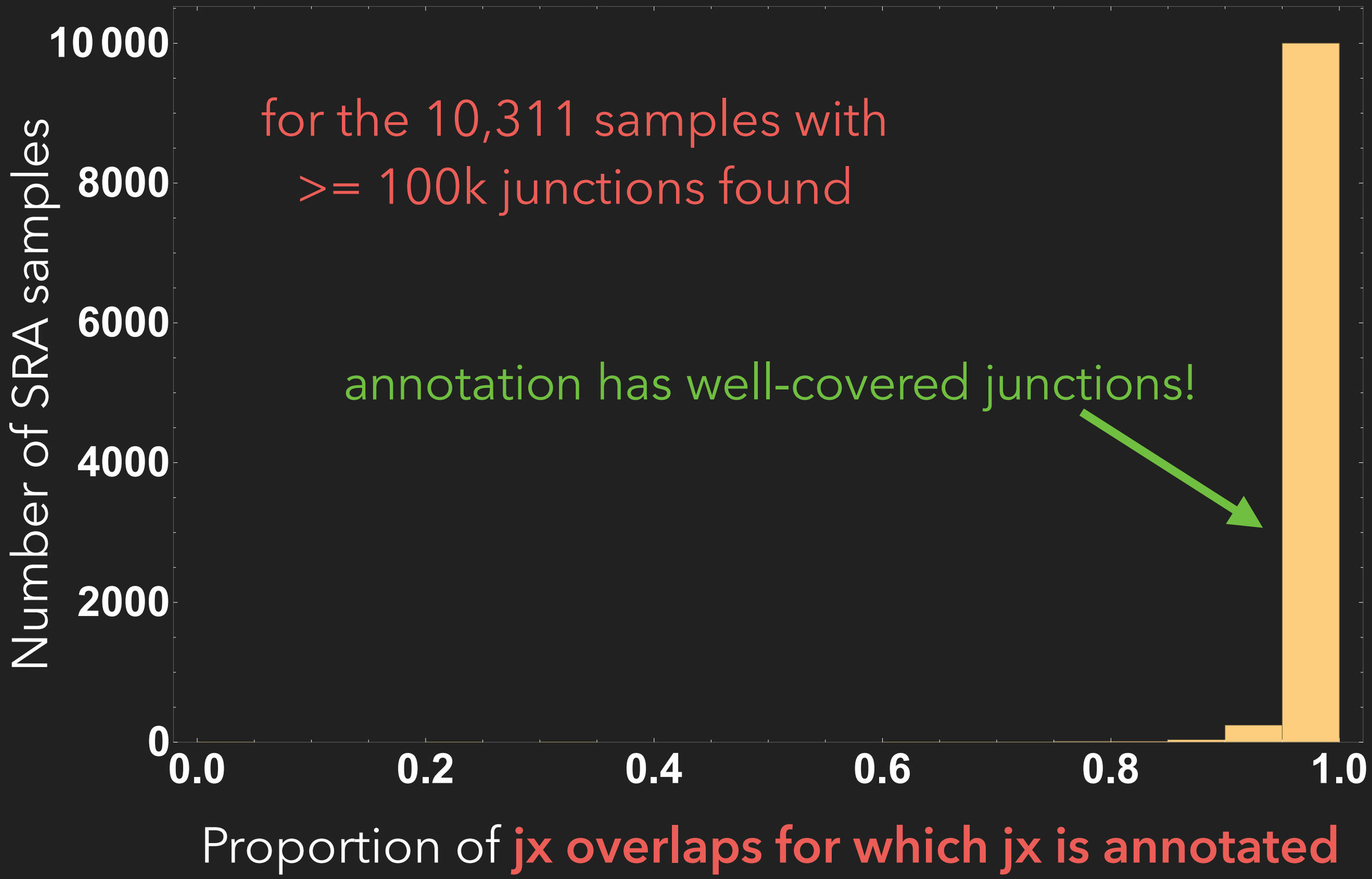
Asymptote to annotation



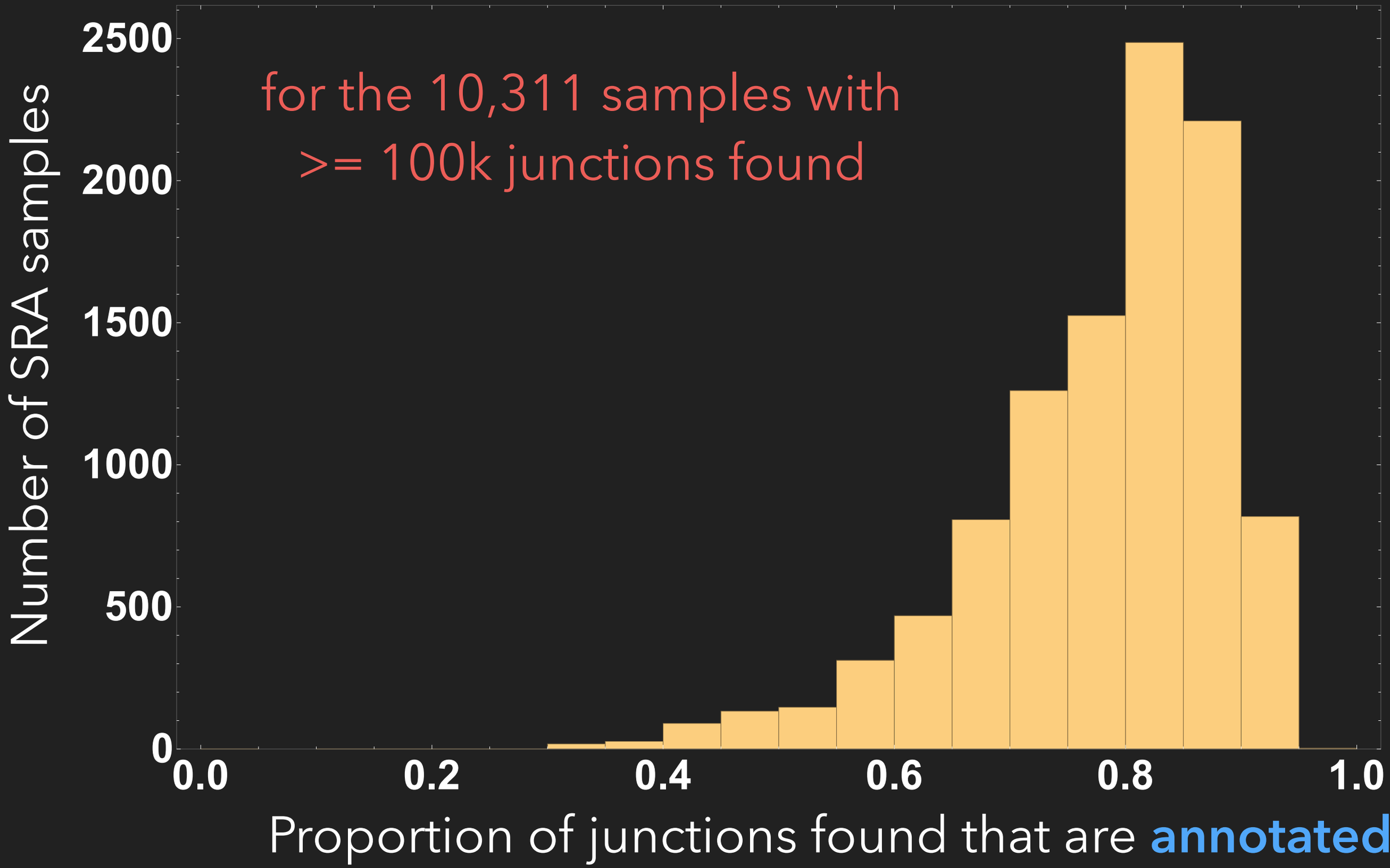
Asymptote to annotation



Junction (jx) overlaps by sample



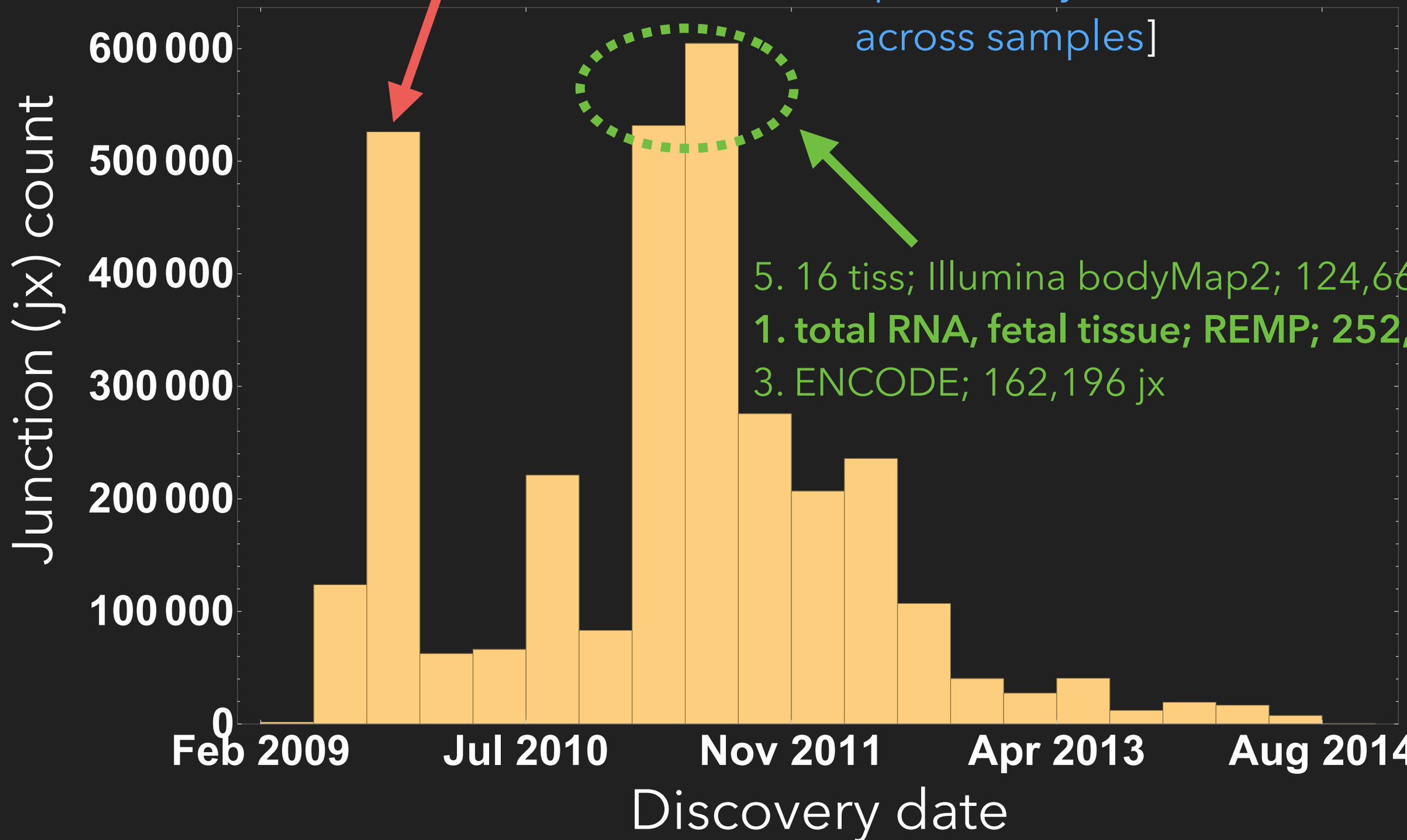
Annotated junctions by sample



Are we still finding new junctions?

4. 69 LCLs; Pickrell et al.; 155,069 jx
2. 41 Coriell CLs; Cheung et al.; 163,007 jx

[Considers only the 3,211,228 jxns spanned by at least 20 reads across samples]



5. 16 tiss; Illumina bodyMap2; 124,664 jx
1. total RNA, fetal tissue; REMP; 252,628 jx
3. ENCODE; 162,196 jx

Mixed news

Junctions in annotation are widespread and well-covered

Junctions in annotation don’t capture splicing diversity for many SRA samples

annotation-agnostic pipeline

Rail-RNA

http://rail.bio

bigWigs: order of magnitude
smaller than BAMs

`derfinder`

```
biocLite("derfinder")
```

scripts for recovering junctions

&

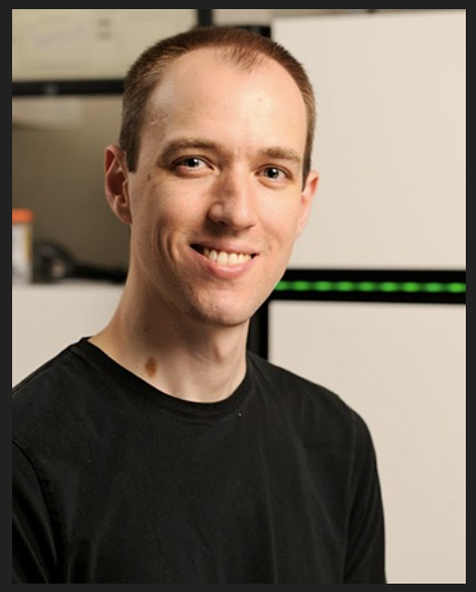
processed data

@

http://github.com/nellore/gi2015

Collaborators

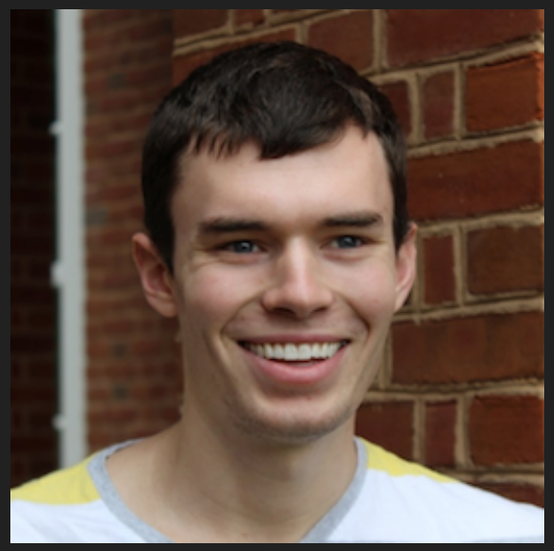
Jeff Leek



Ben Langmead

Leo Collado-Torres

Andrew Jaffe



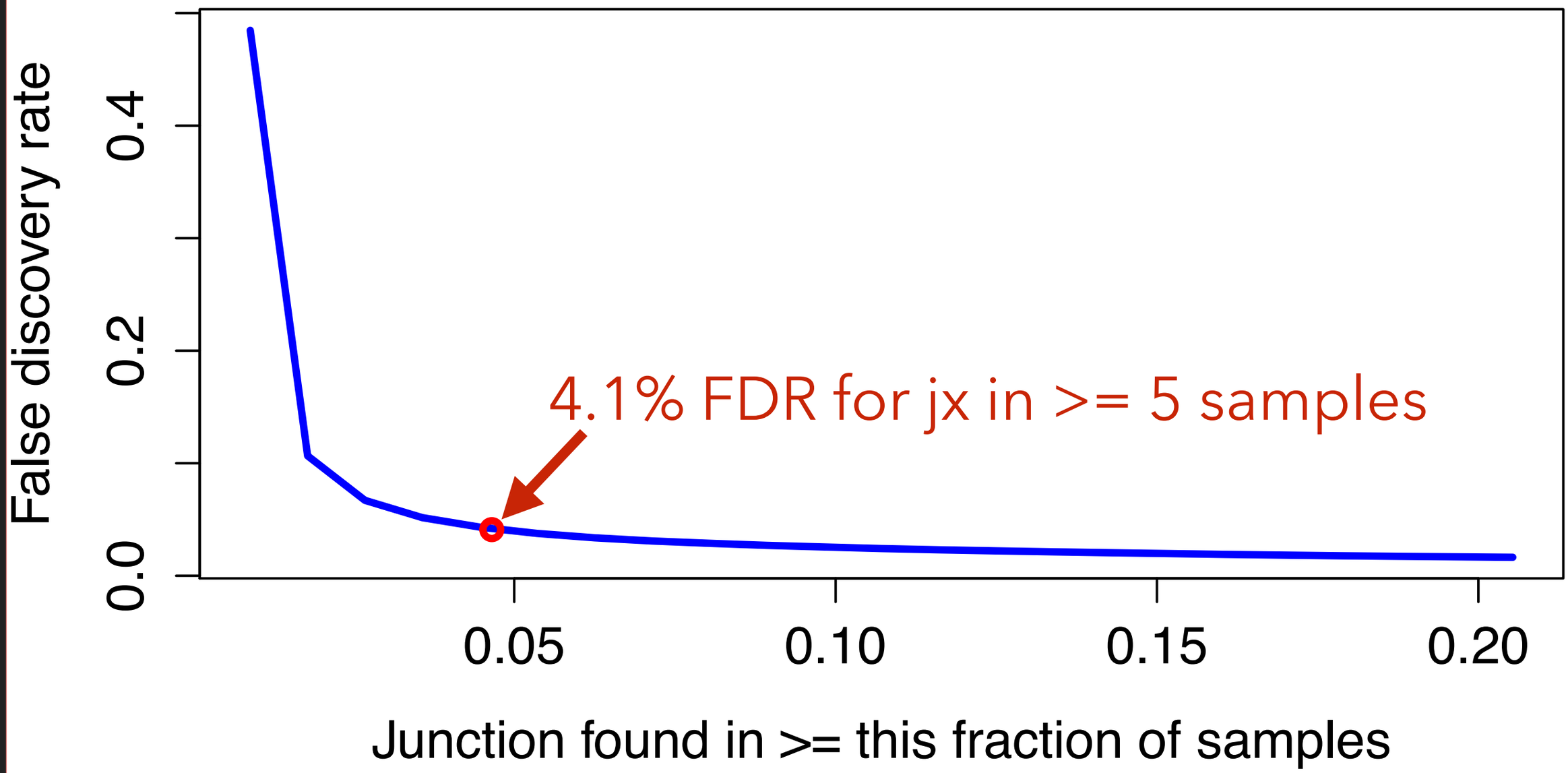
Jacob Pritt

Chris Wilks

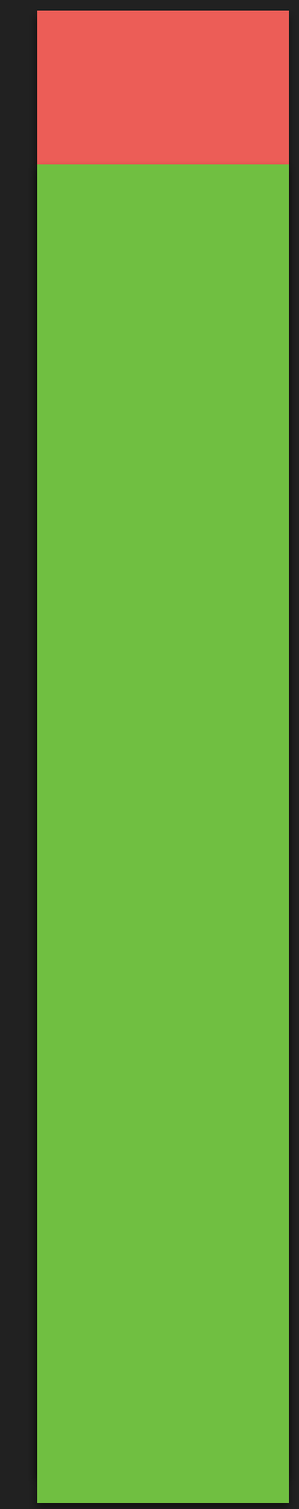
José Alquicira Hernández

Summer interns: Nishika Karbhari, James Morton, Robert Phillips, Sara Wang

First–pass junction–call FDR estimated from 112 GEUVADIS–like simulations



Why so many junctions?



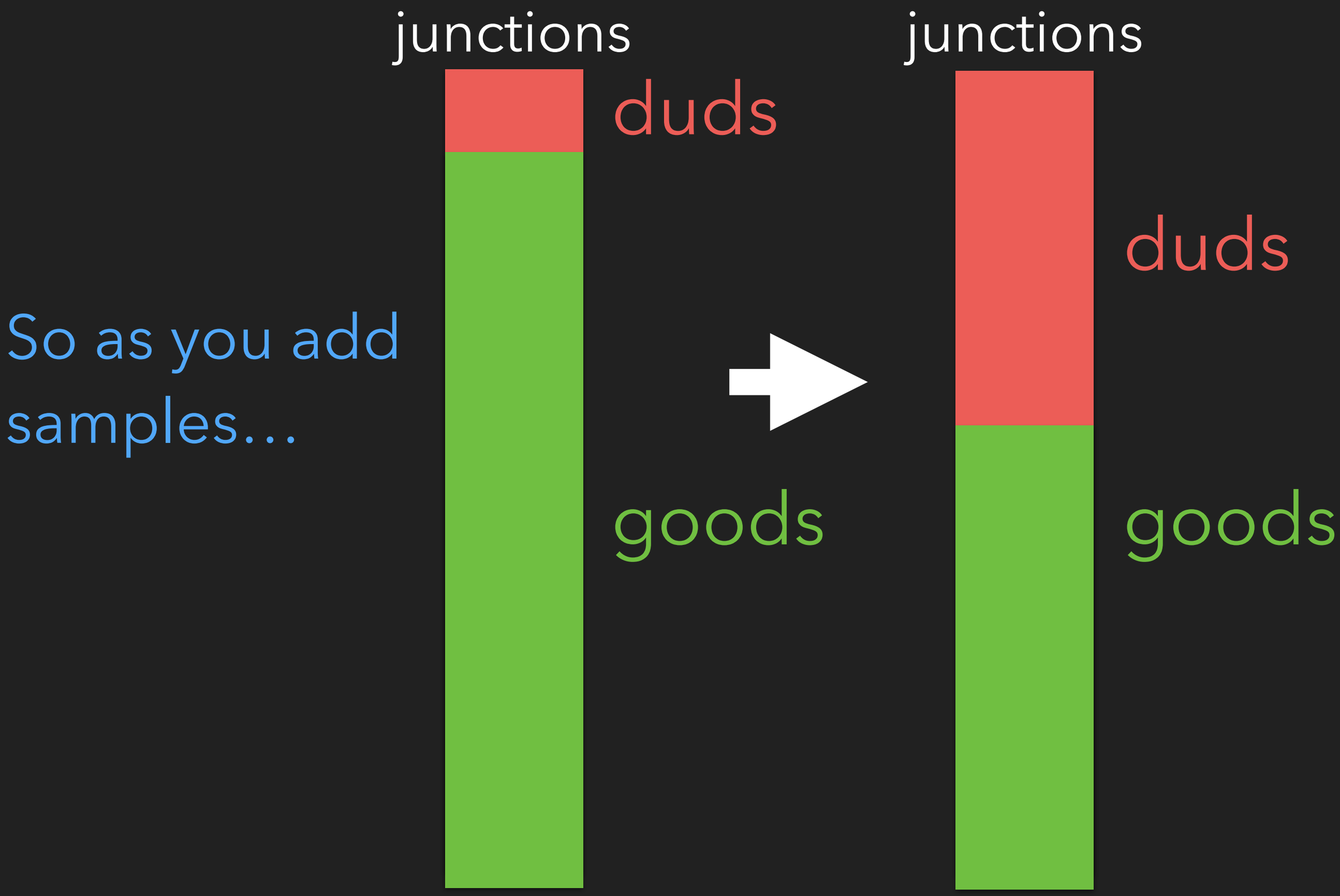
On a single sample, *every* aligner will find some good junctions and some duds (or very rare junctions).

Why so many junctions?

Comparing the junctions found in many simulated samples, there is *much more overlap* between goods than between duds. (See http://j.mp/rail-pre .)

vs.

Why so many junctions?



We ran

(~500 runs)

```
rail-rna prep elastic
--manifest batch_X.tsv
--core-instance-count 20
--output s3://bucket/batch_X_prepped
--core-instance-bid-price 0.13
--master-instance-bid-price 0.13
--core-instance-type c3.2xlarge
--master-instance-type c3.2xlarge
```

for $X \in \{0, \ldots, 42\}$

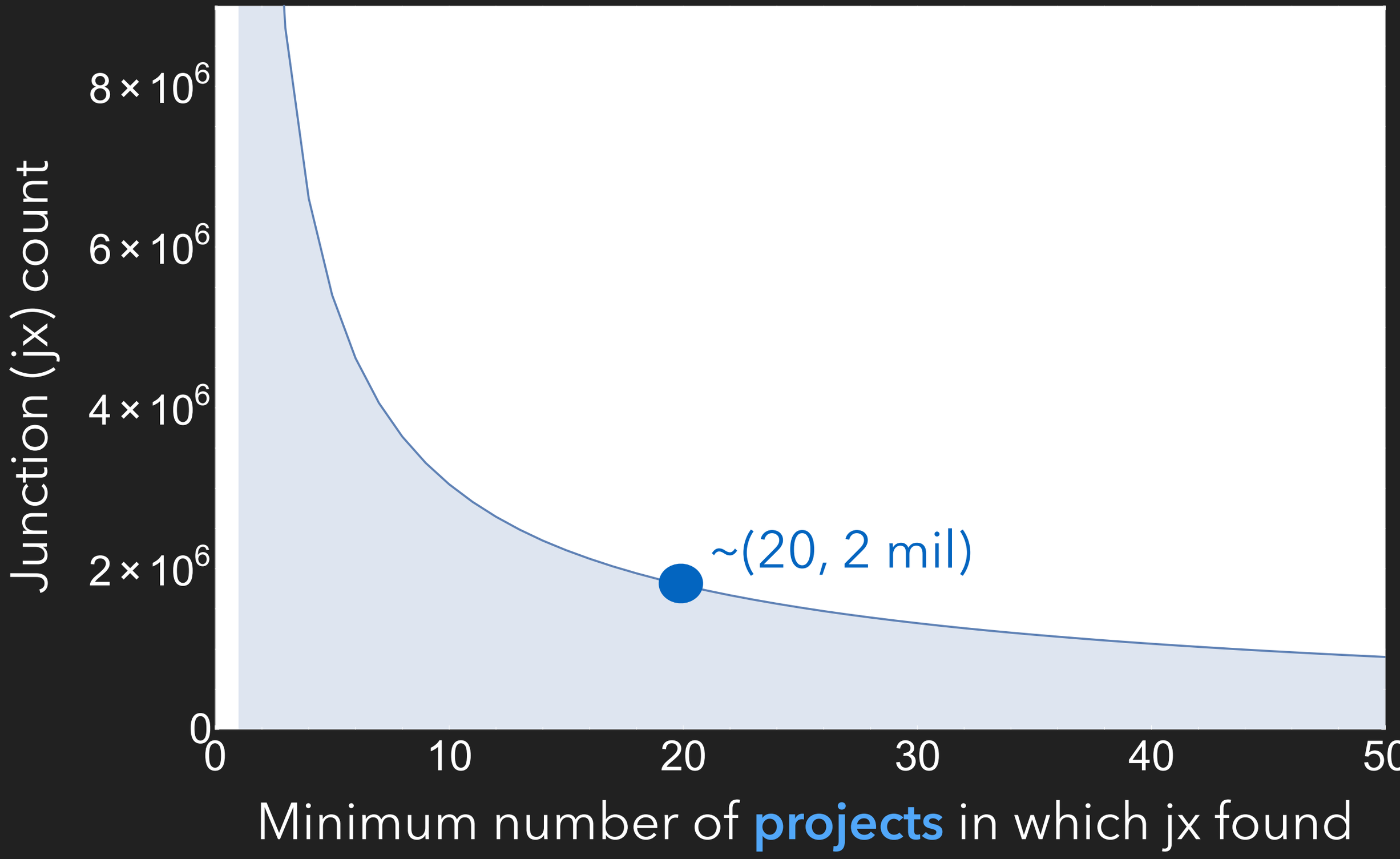
to download/preprocess data, copy to S3

We ran

```
rail-rna align elastic
—-manifest batch_X.tsv
—-input s3://bucket/batch_X_prepped
—-output s3://bucket/batch_X_itn
--core-instance-bid-price 0.60
--master-instance-bid-price 0.60
—-core-instance-count 60
—-core-instance-type c3.8xlarge
—-master-instance-type c3.8xlarge
—-deliverables itn
```

to detect junctions from one pass of alignment

A steep dropoff: project-level



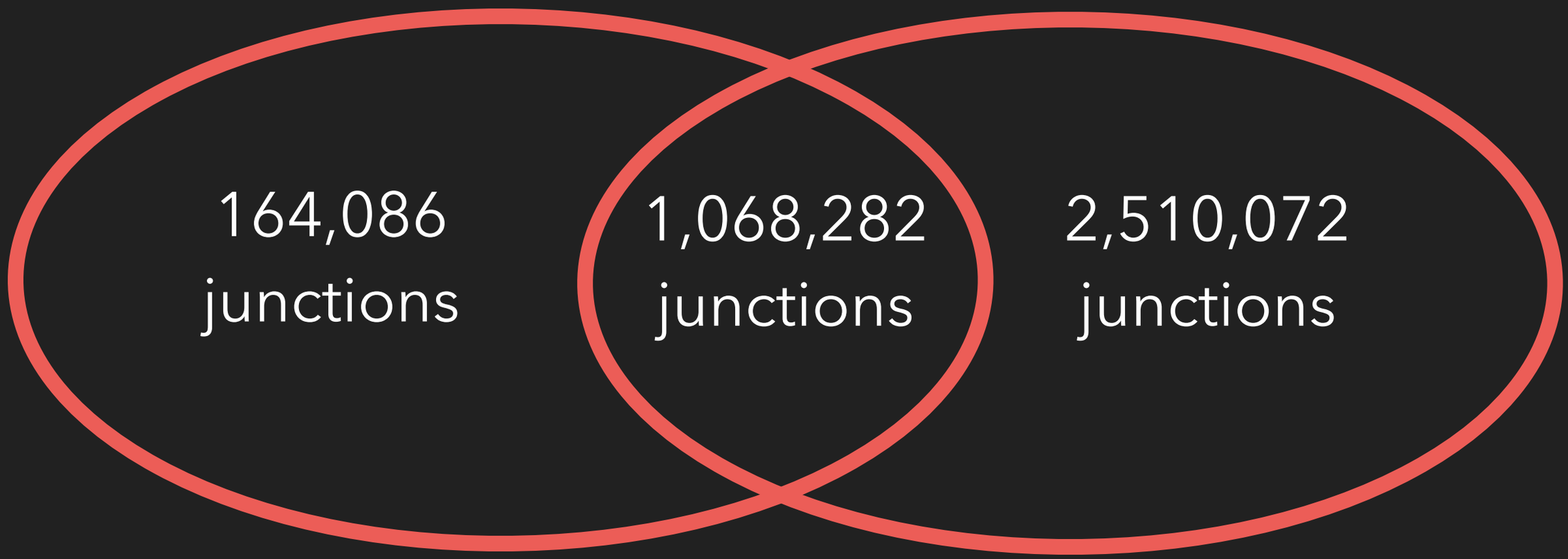
Comparison with SEQC

SEQC/MAQC-III (Nat Biotech, doi:10.1038/nbt.2957)

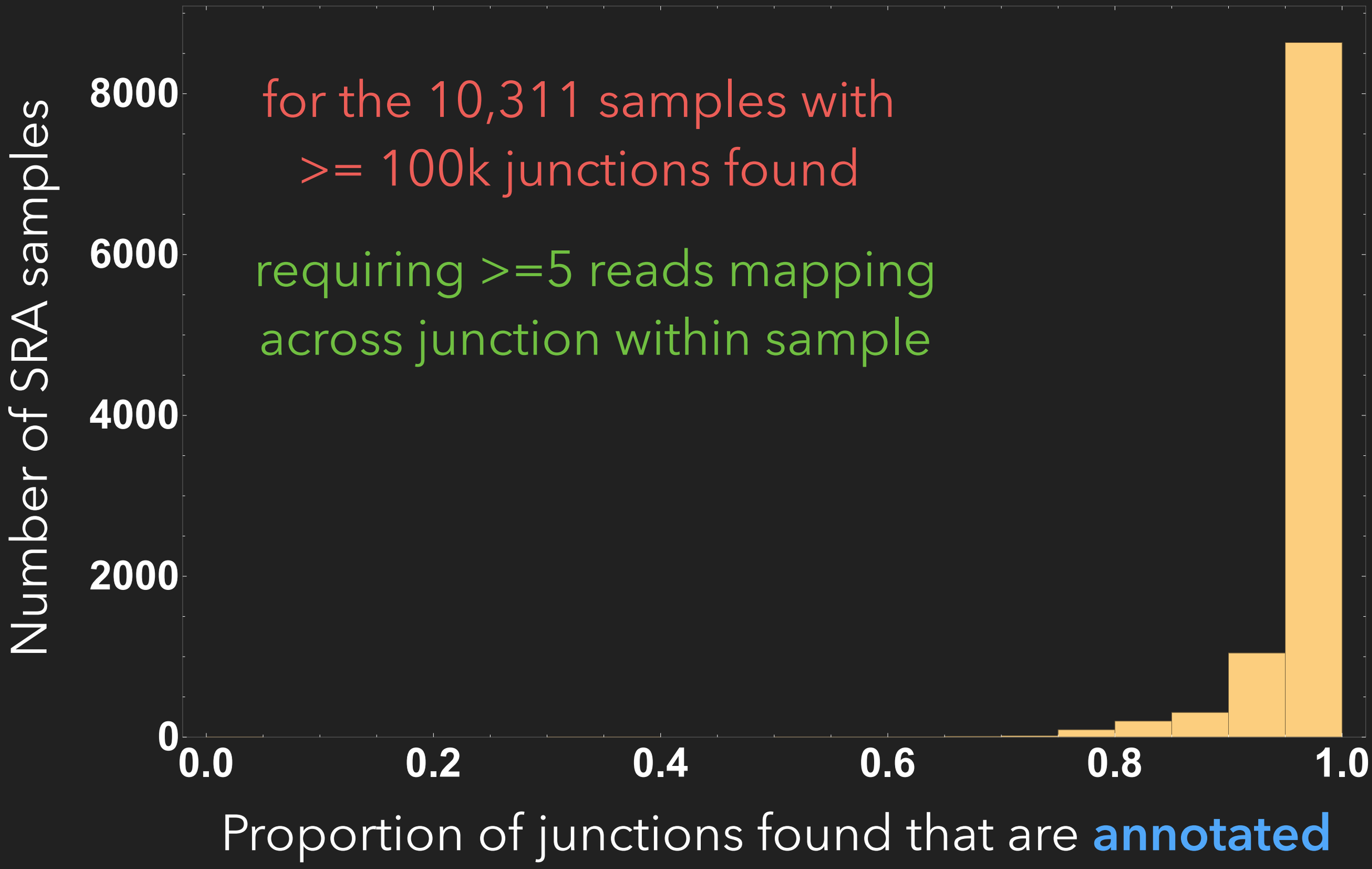
1720 samples in common with Rail;
universal human & brain reference samples

Rail-RNA
in at least
5 SEQC samples

One of rmake,
magic,
and subread



Annotated junctions by sample



Gedankenexperiments

More complete annotation **= better!**

Increased sensitivity

(Can detect isoform 2 now!)

More complete annotation **= worse!**

Decreased specificity

(What if isoform 2 is really rare?)

Actual experiment

RGASP simulated sample 1
(40 mil read pairs)

HISAT2 2.0.0-beta

		junctions	junction overlaps
fed true jx	prec:	**0.94**	0.98
	rec:	0.99	0.93
fed union of annotated jx	prec:	**0.80**	0.97
	rec:	0.95	0.92

Actual experiment

RGASP simulated sample 1
(40 mil read pairs)

STAR 2.4.2a

		junctions	junction overlaps
fed true jx	prec:	**0.98**	0.995
	rec:	0.99	0.91
fed union of annotated jx	prec:	**0.90**	0.98
	rec:	0.97	0.87

Rail-RNA's approach

Realign after collecting and filtering a list of junctions across **SIMILAR** samples.

sample 1 ← *read 1*

ATACATCAGACTAGACCGTACCACACAGCATGACAGTCATTCGACGTACT

intron

...ATACATCAGACTAGACCGTACCACAGTAGTTCATGACCCTCAGCAGCATGACAGTCATTCGACGTACTCGTATCGATACAGTACAGTAGCC...

chr1

CATAGCATGACAGTCATTCGACGTACTCGTATCGATACAGTACAGTAGCC

read 2 found to overlap junction on realignment

→ *sample 2*

similar: same feature to which you want to be sensitive

cell line, tissue type, population, experimental condition…

Junction (jx) filter

Keep a junction if and only if it's initially detected in:

(1) 5% of samples

OR

(2) at least 5 reads in any one sample

grabs common jx

so we don't miss jx that are probably there but unique to a sample

Comparison

Simulate from annotation,
then give competitors annotation

112 simulated LCLs (based on GEUVADIS)

mean overlap accuracy value | mean junction accuracy value

	Precisions	Recalls	F-scores
TopHat 2 ann	.815 | .947	.839 | **.982**	.826 | **.964**
STAR ann	.882 | **.977**	**.874** | .980	.878 | **.979**
HISAT ann	.895 | .922	.857 | **.982**	.875 | .951
Rail	**.969** | **.976**	.858 | .939	**.910** | .957

(http://j.mp/rail-pre)

A steep dropoff