Genomic Characterization of Human Long Noncoding RNAs

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PhD defense

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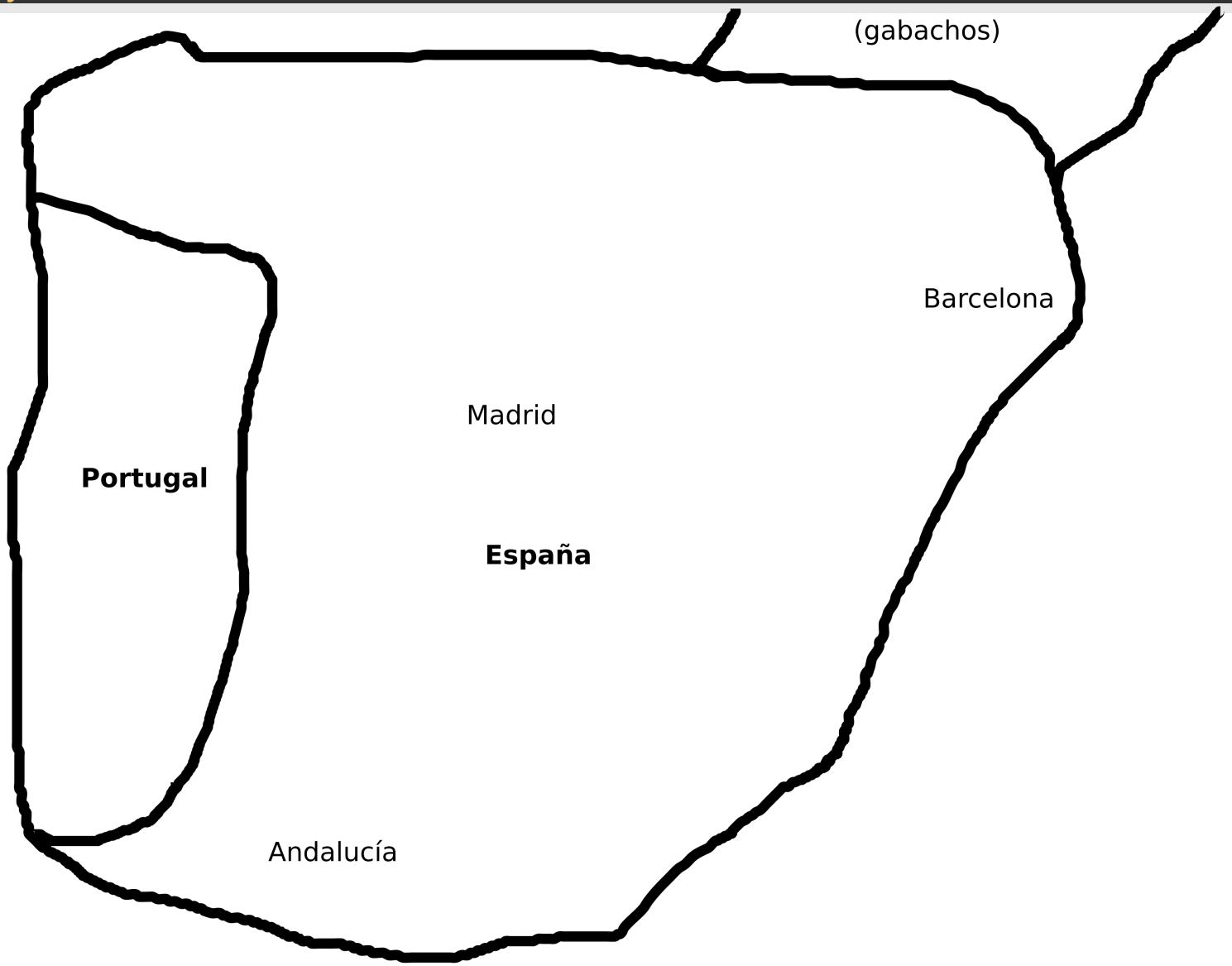
UNIVERSITAT DE BARCELONA

The current state of long noncoding RNA (lncRNA) annotation

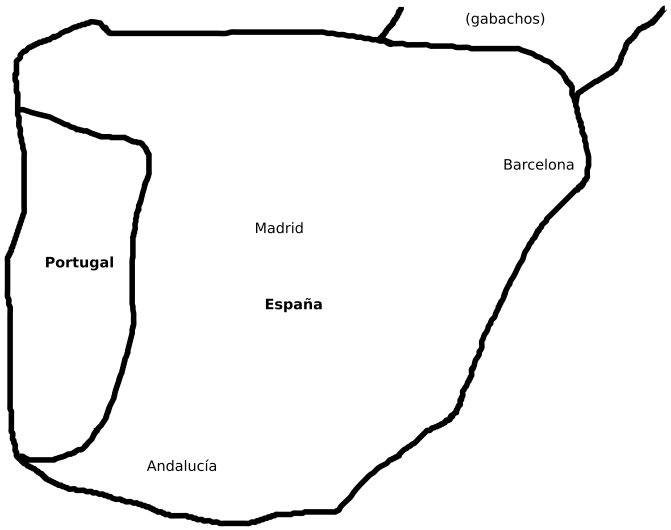
The goal

The current state of long noncoding RNA (lncRNA) annotation

The reality



What this work is about: improving lncRNA annotation



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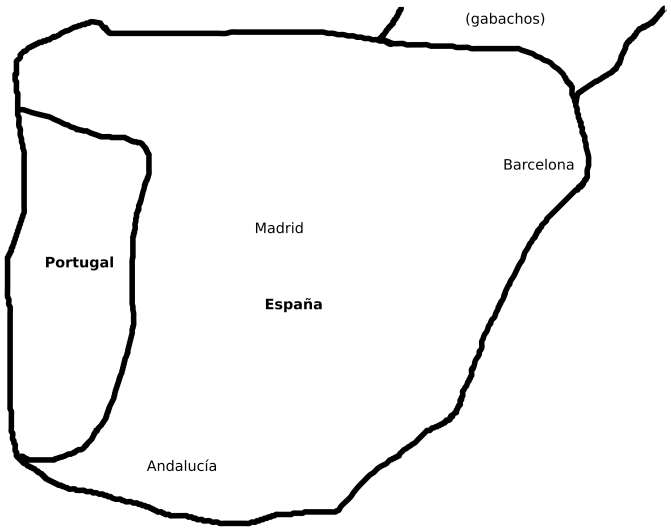
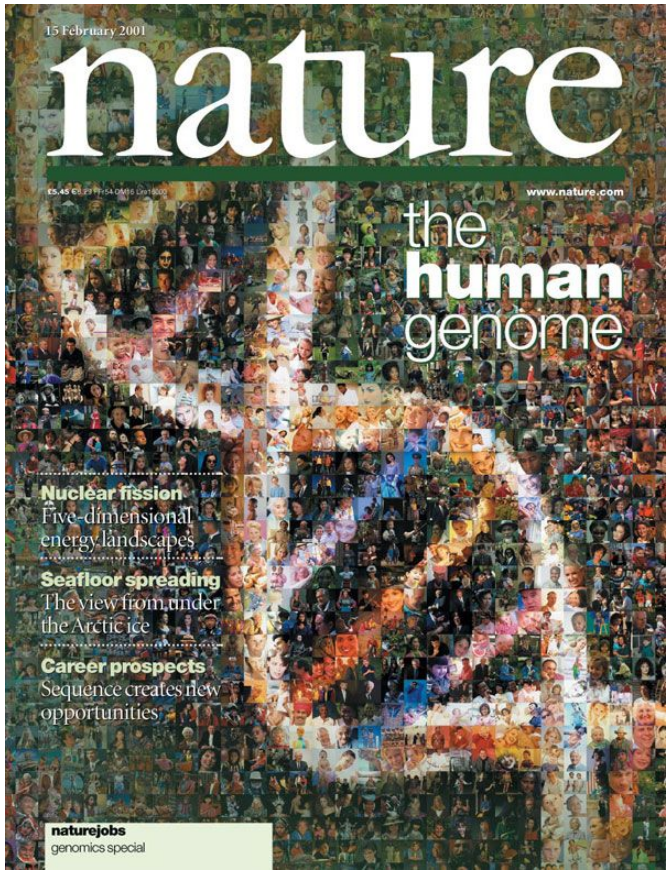


Sequencing the Human Genome

Venter *et al. Science*, 2001



Lander *et al. Nature*, 2001

The complete sequence of the human genome

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TCTCCTGTCTCCCTTTGCTCGCCCCTAAATCCTTCCTCGGACTCCAACTCCCGCCGTGCCTTGCGCTGAGATCCCTGCGGCAAAG
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AAGTTGGTCAACTTGGGGGAGTTTGGGTGTTTGCCCATGTGGGTTTCCTGAA.....

The complete (bare) sequence of the human genome

Annotating the human genome

The complete sequence of the human genome

.....GGCTTGTAGTCACCTCGGAATTGTTAGGCGACGAAAAAAACTACTCACCCCAGGTGTCCCTGCGTCAGAGGCGGGTTTC
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AAGTTGGTCAACTTGGGGGAGTTTGGGTGTTTGCCCATGTGGGTTTCCTGAA.....

The annotated human genome

.....GGCTTGTAGTCACCTCGGAATTGTTAGGCGACGAAAAAAACTACTCACCCCAGGTGTCCCTGCGTCAGAGGCGGGTTTC
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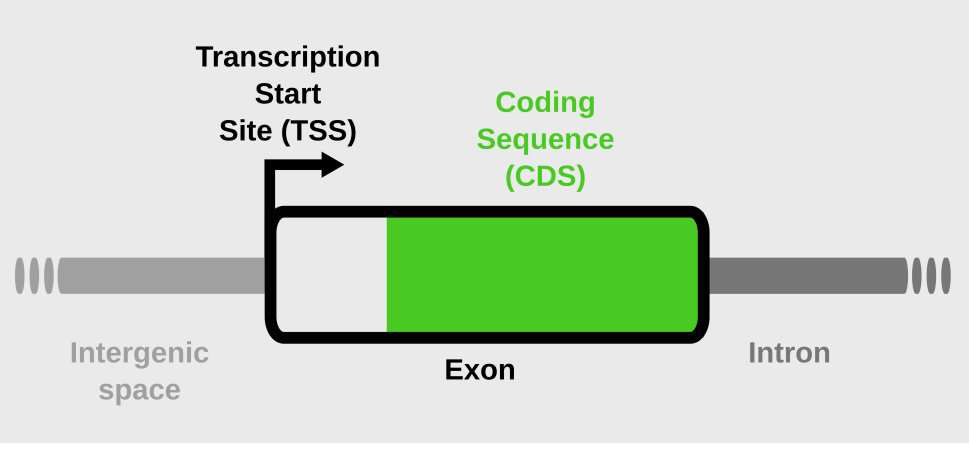

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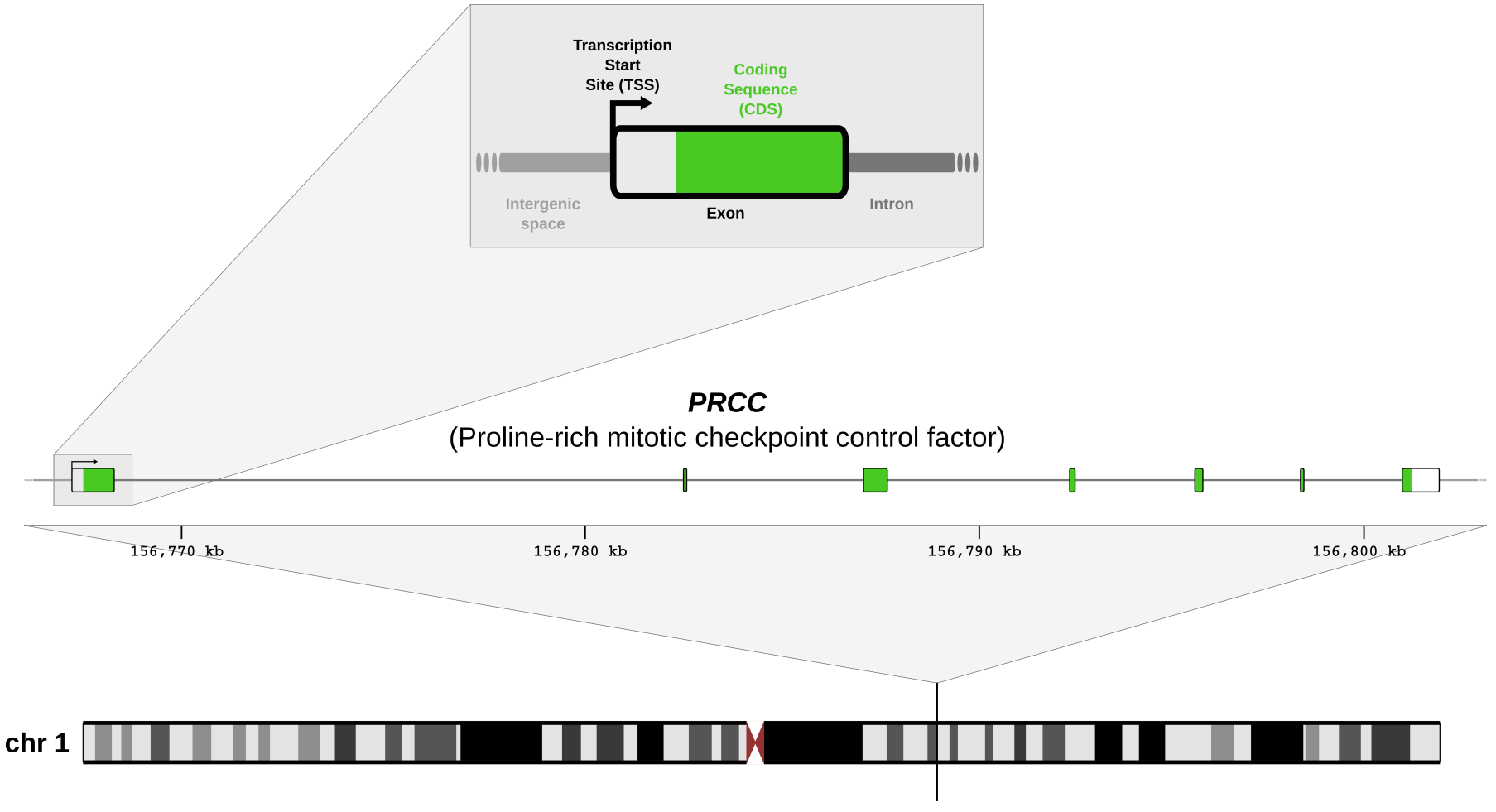

How to annotate genes

The gold standard: evidence-based, manually-curated gene annotation

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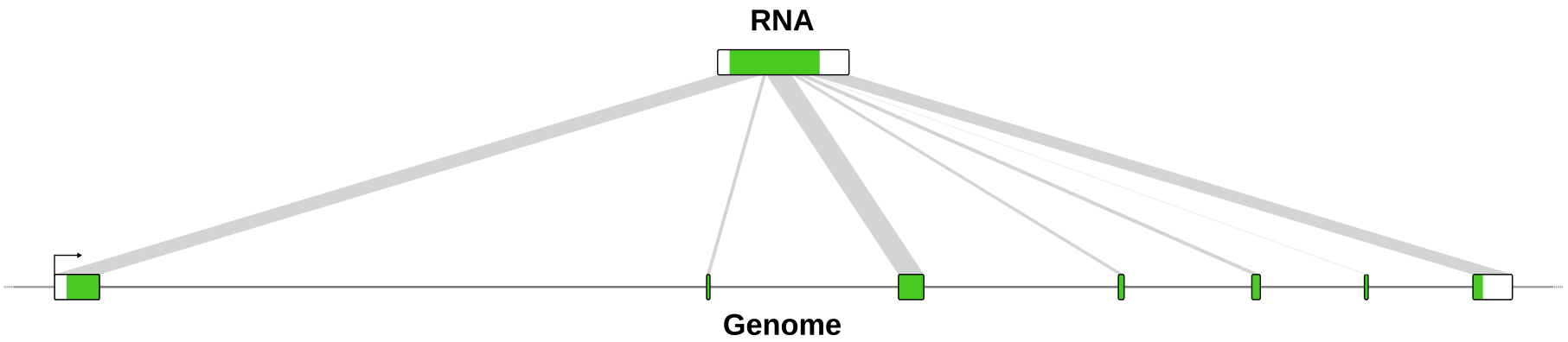

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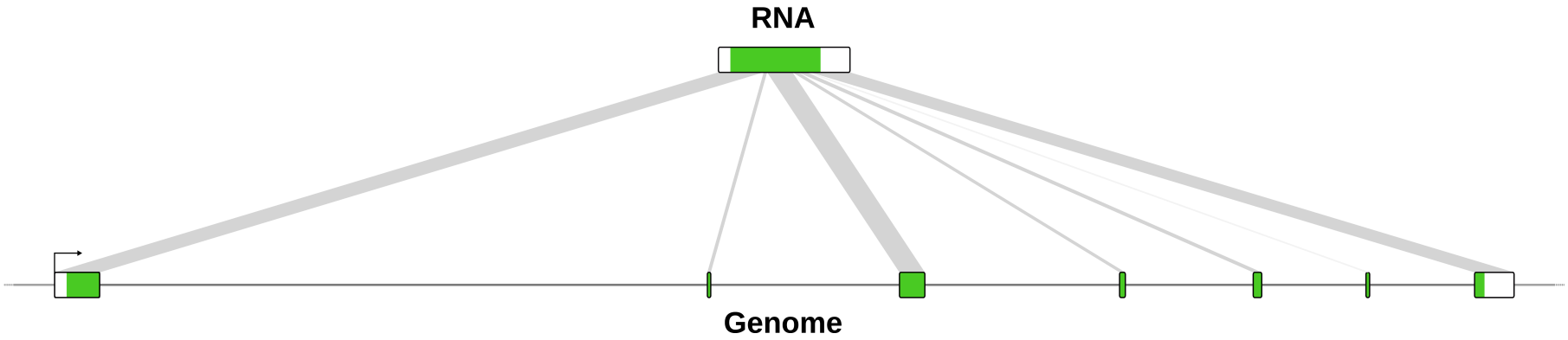

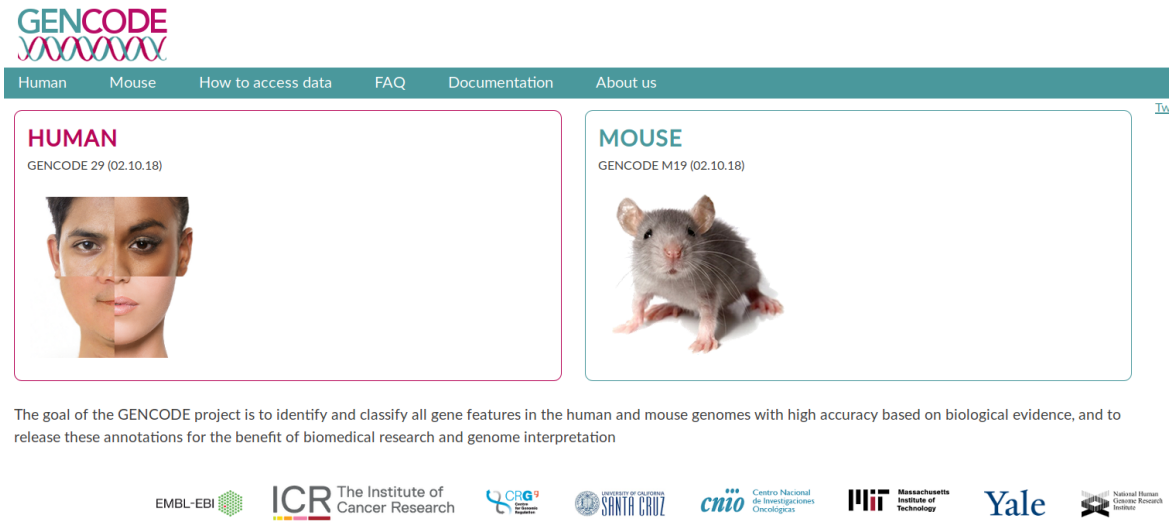

https://www.gencodegenes.org/

Sequencing RNA (cDNA)

Comparison of DNA sequencing platforms (*ca.* 2016)

Performance	poor					good

	1st-gen.
Technology	**Sanger**
Available	1977-
Read length (bases)	~800
Yield (# reads)	(low)
Base accuracy (%)	99.999

Goodwin, McPherson, and McCombie *Nature Reviews. Genetics*, 2016

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Read length (bases)	~800	150	<20k	<200k
Yield (# reads)	(low)	>200M	55k	100k
Base accuracy (%)	99.999	99.9	99	88

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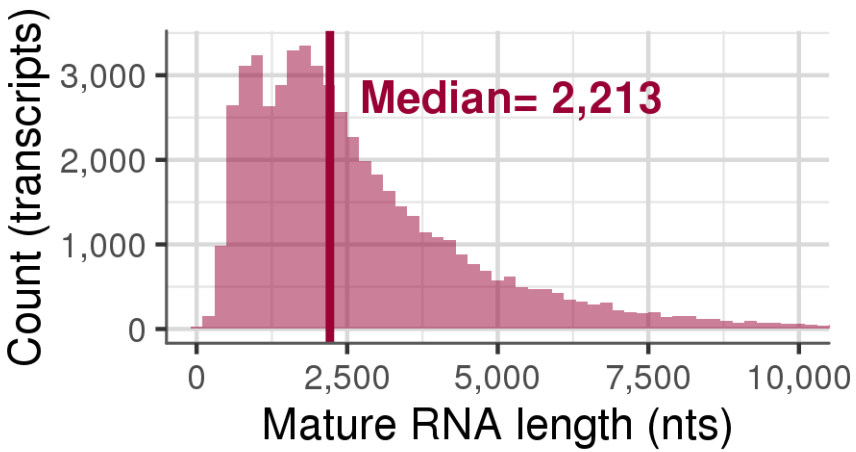

(GENCODE v21)

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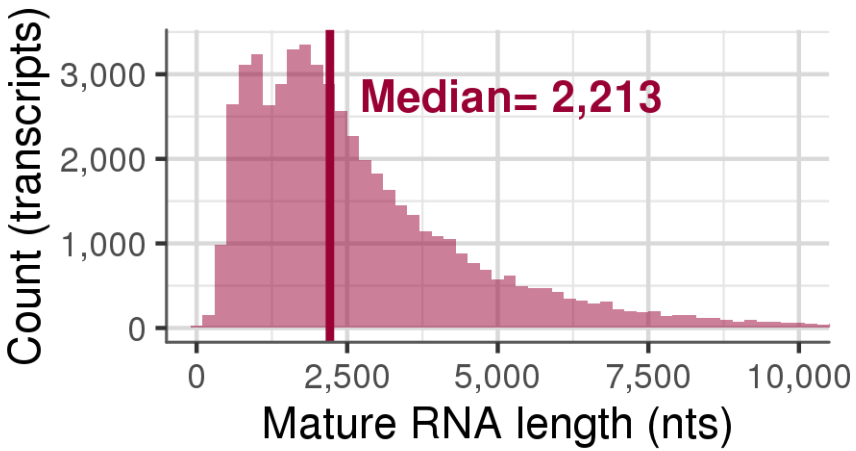
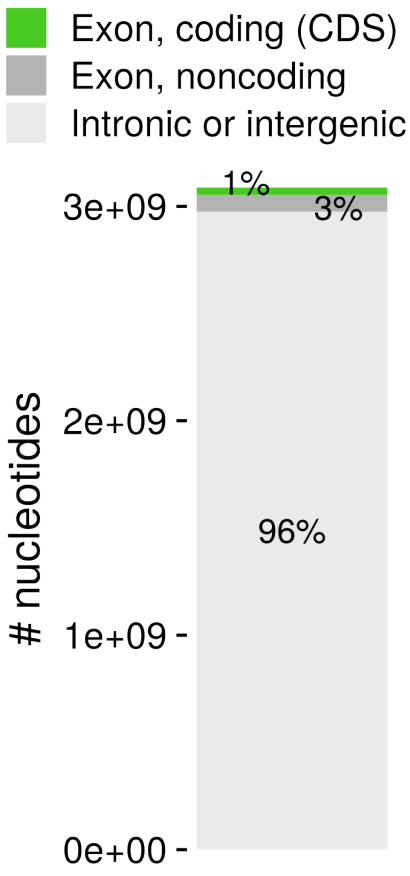


(GENCODE v21)

High-throughput, **end-to-end** transcript sequencing is now possible thanks to **TGS**

How complete is the human genome's annotation?



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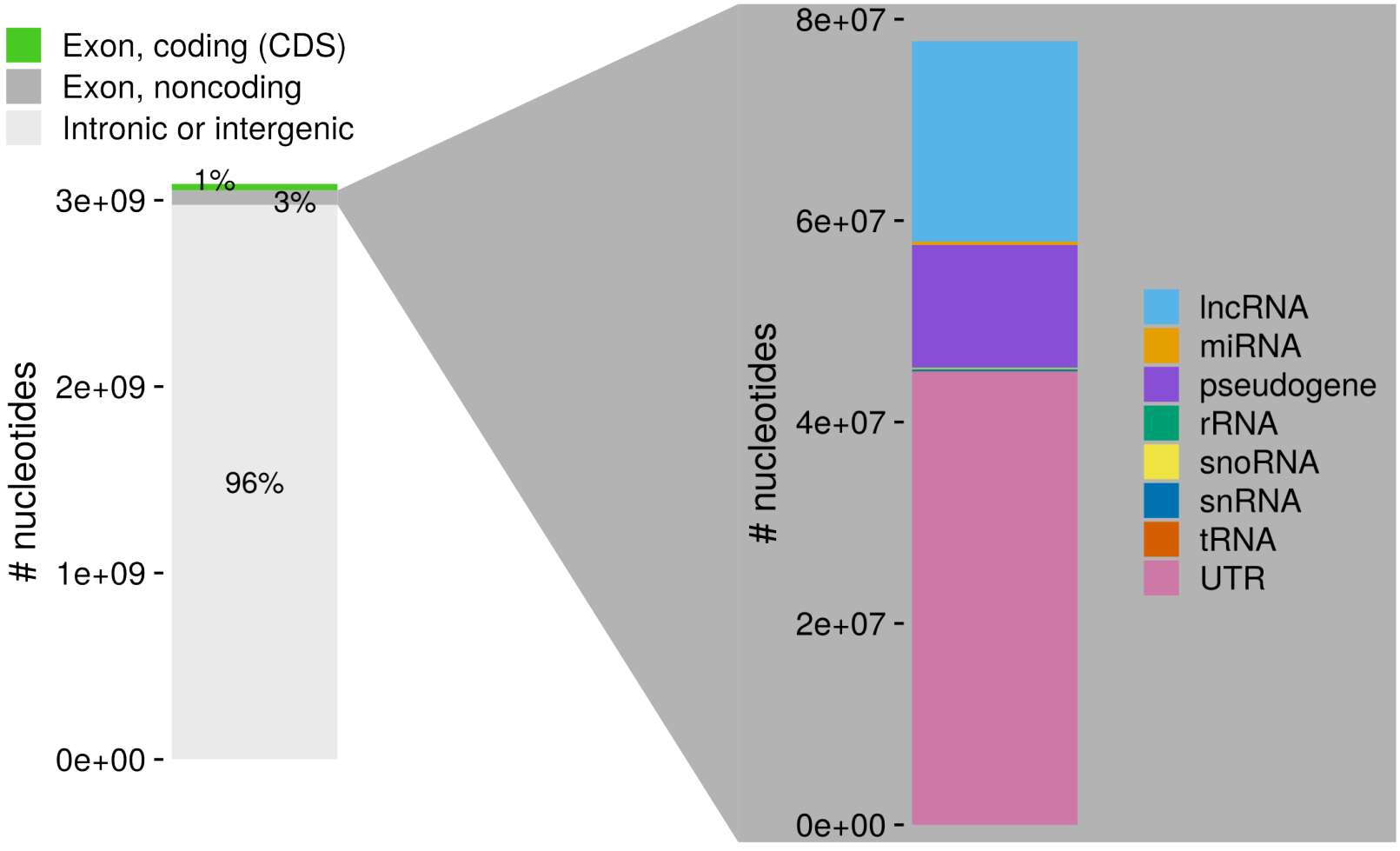

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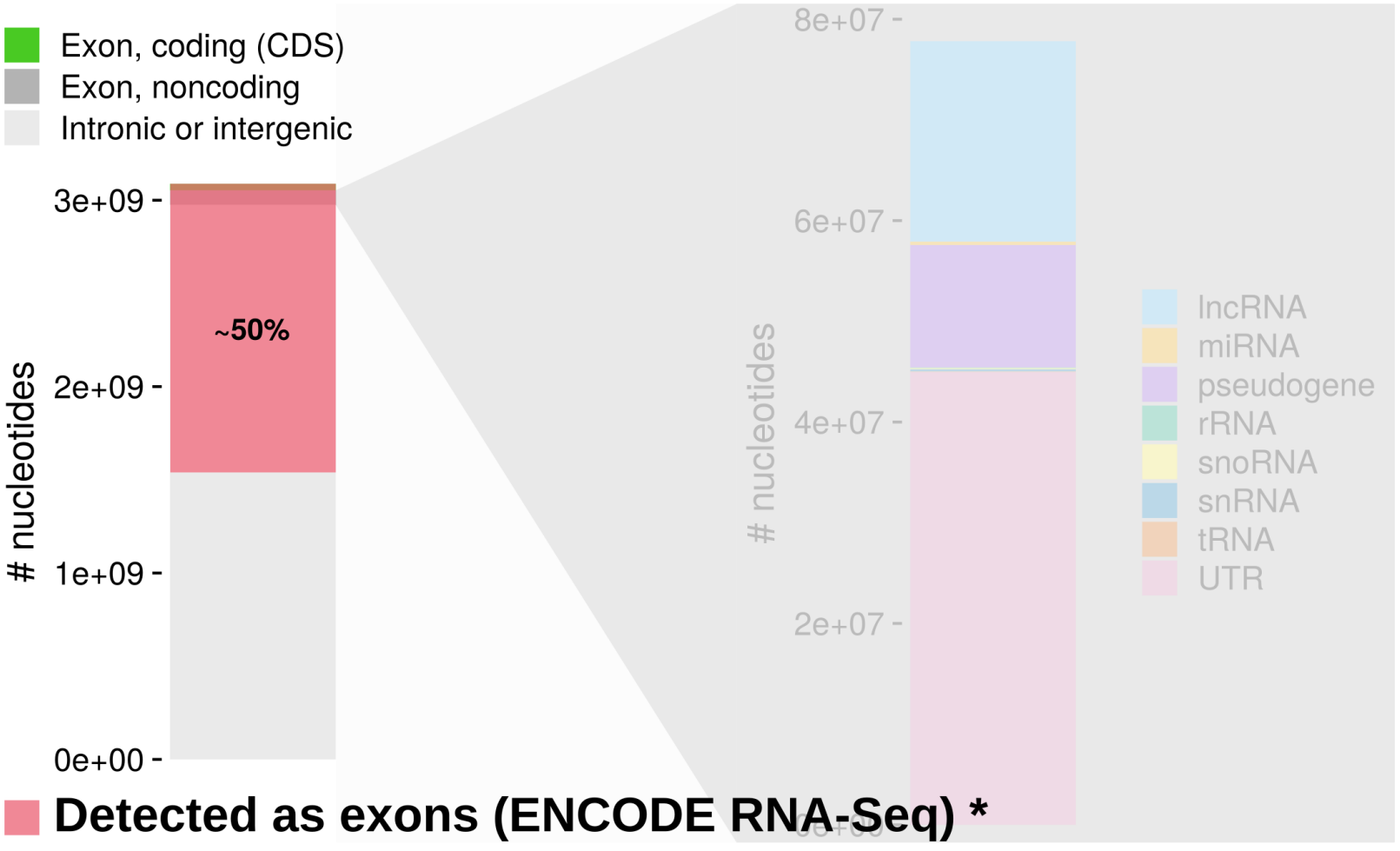

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* Djebali *et al.*
Nature, 2012

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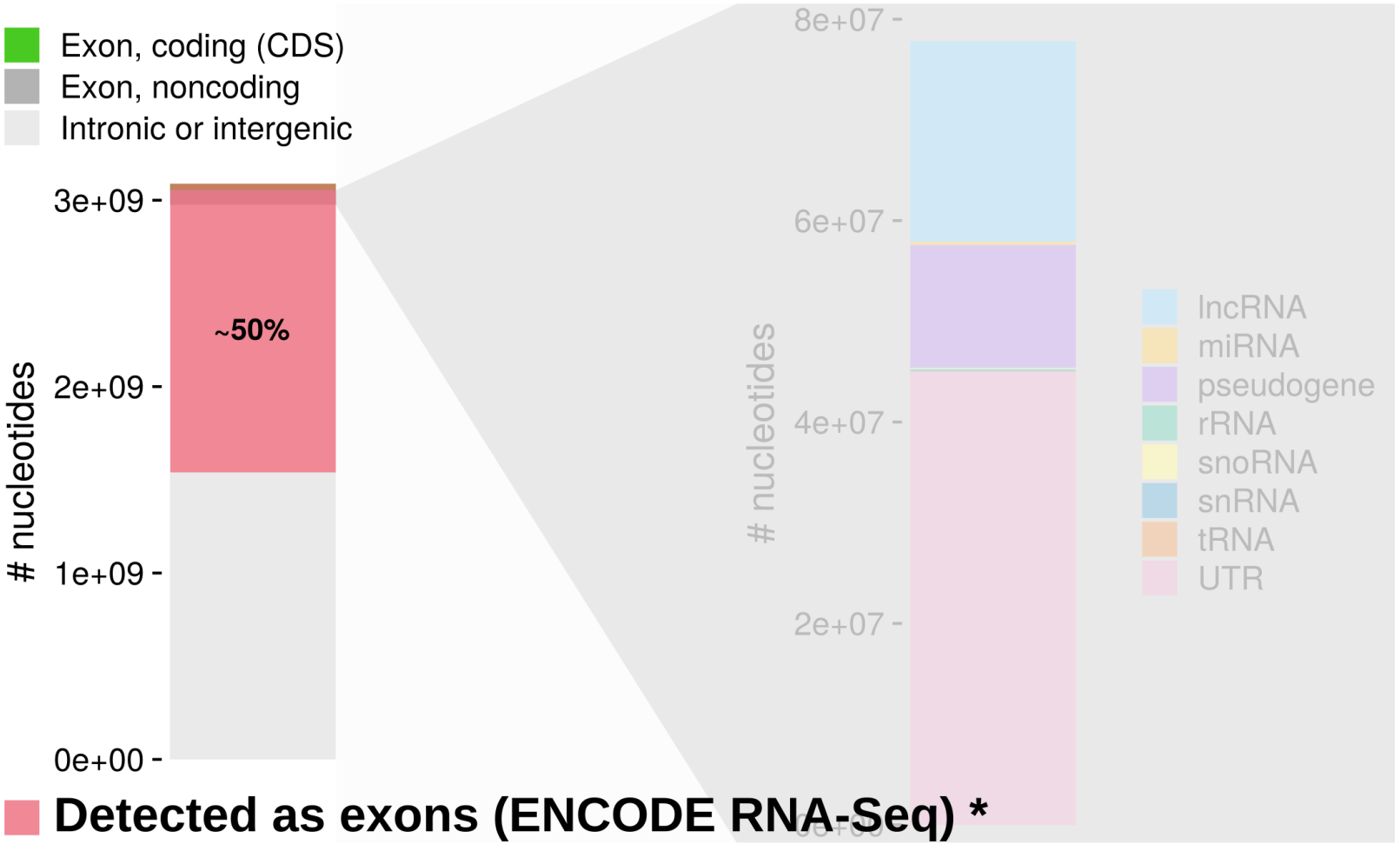

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- The human genome is overwhelmingly **noncoding**

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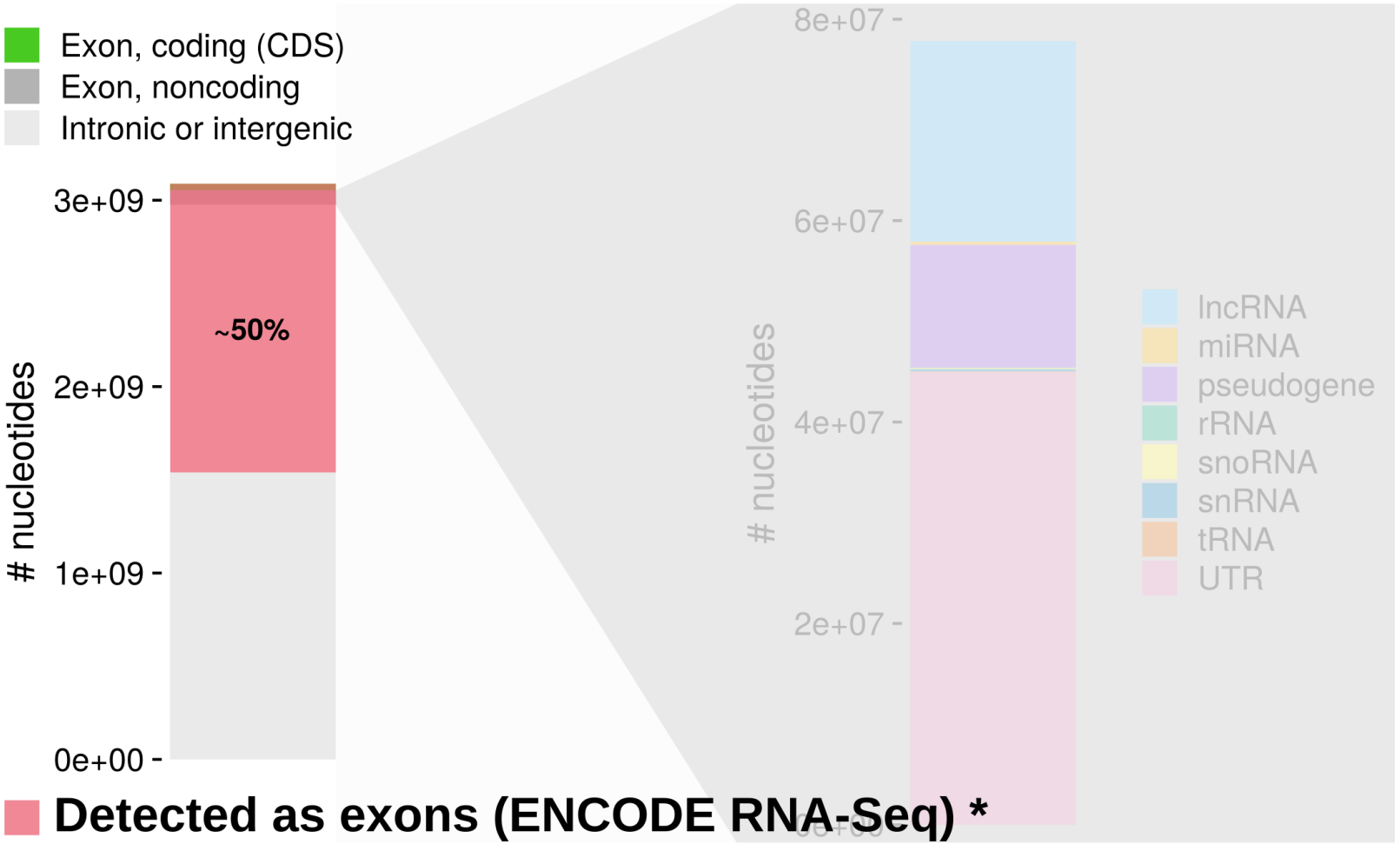
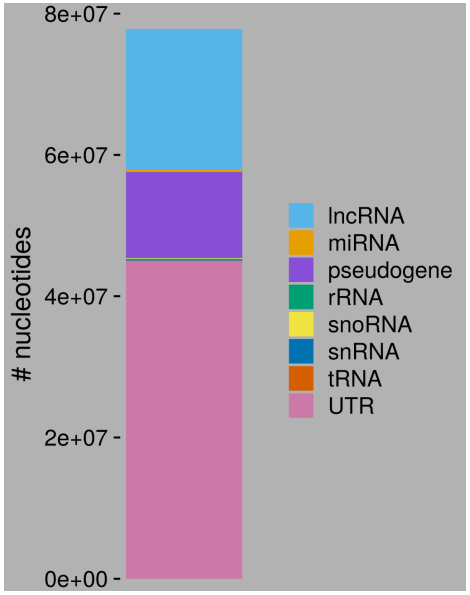


(GENCODE v21)
* Djebali *et al.* *Nature*, 2012

- The human genome is overwhelmingly **noncoding**
- Only a small fraction of the **noncoding "exome"** is annotated as such in **reference gene catalogs**

Long (intervening) noncoding RNAs



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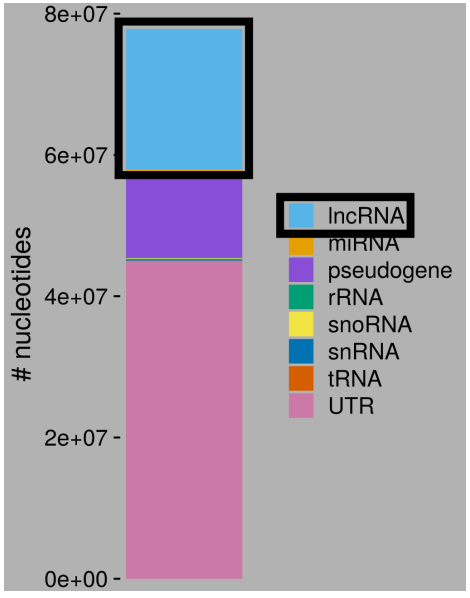
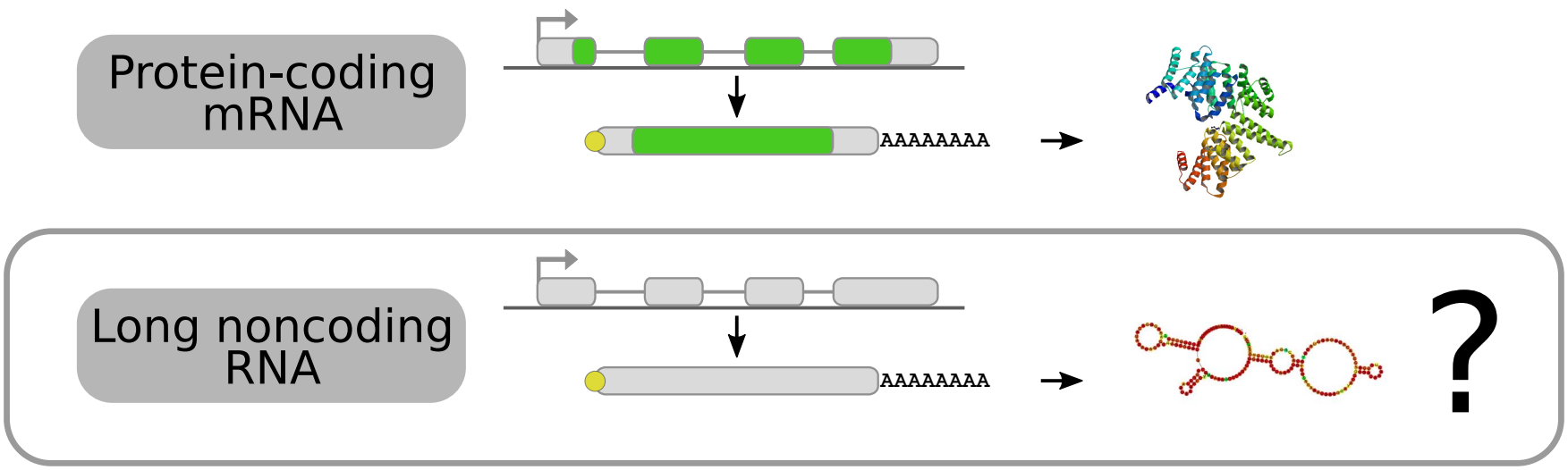


- **Long** (>200nts) and **non-coding**



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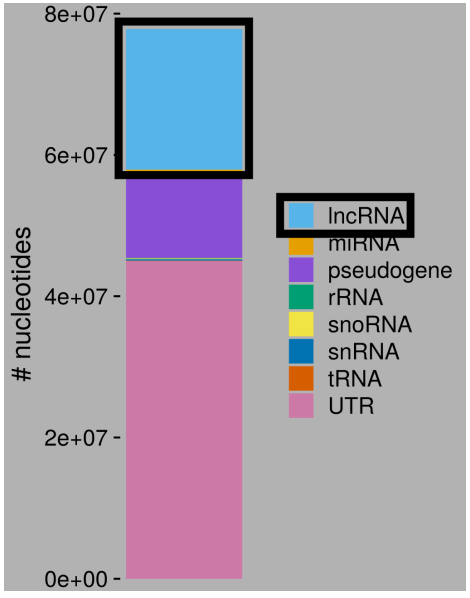
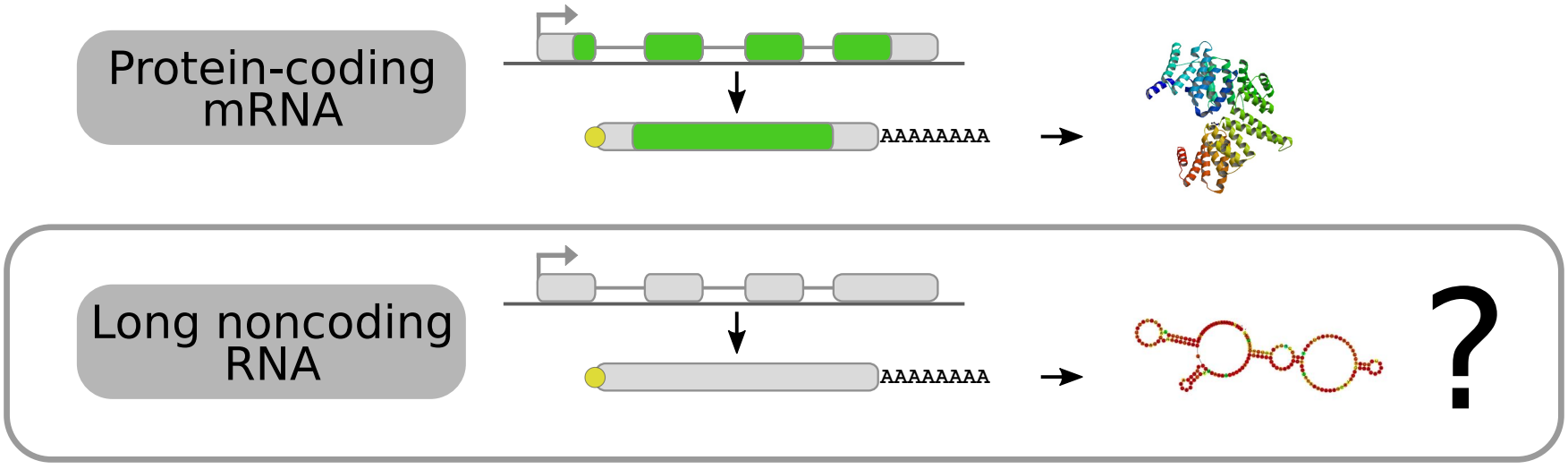


- **Long** (>200nts) and **non-coding**
- Most are **poorly conserved** at the sequence level



LncRNAs perform various functions in the cell

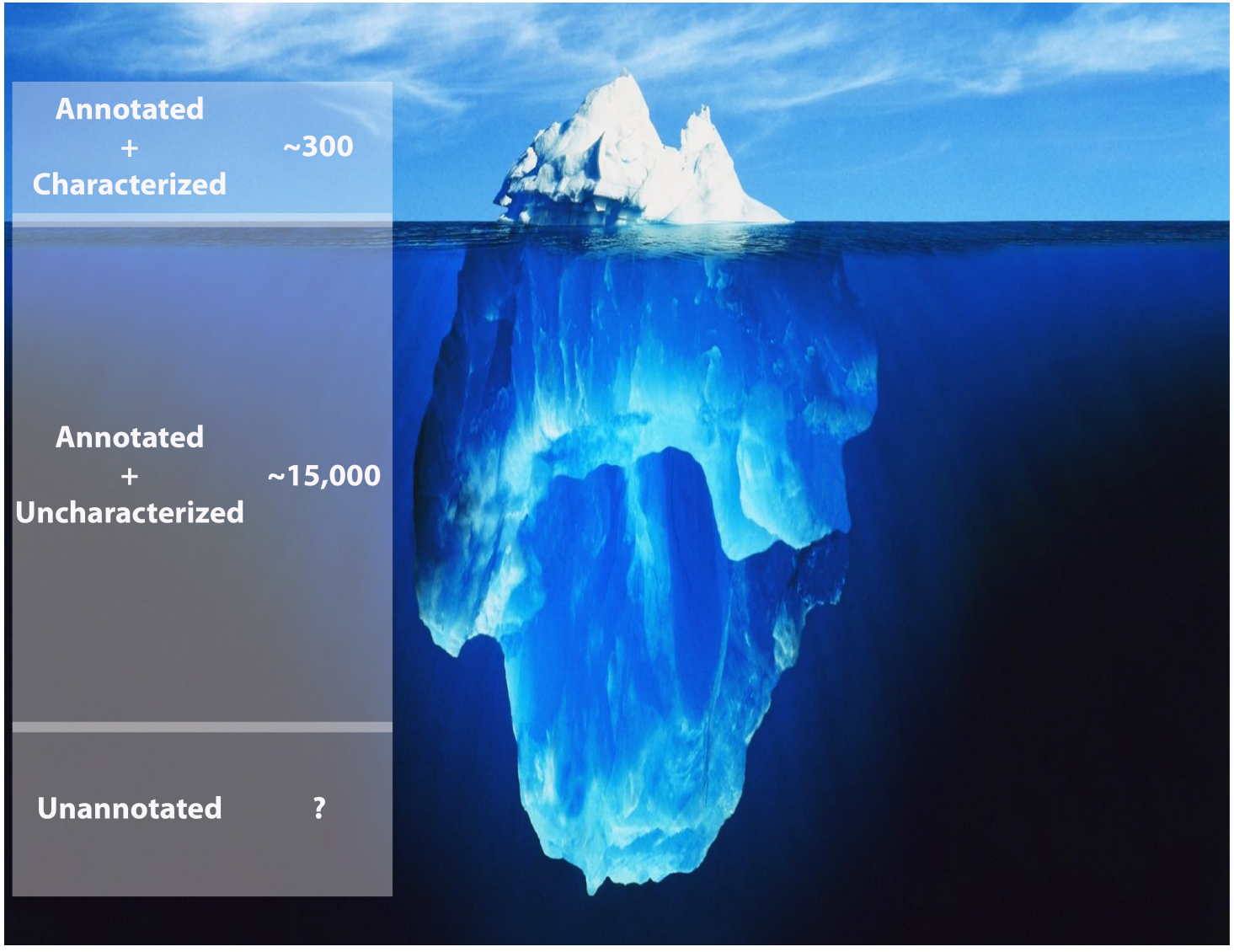
Some examples of functionally characterized lncRNAs:

- ***H19***: control of cell growth and proliferation - Brannan *et al. Molecular and cellular biology*, 1990
- ***Xist***: chromosome X inactivation - Brown *et al. Nature*, 1991
- ***Air***: *Ig2fr* gene silencing in *cis* - Sleutels, Zwart, and Barlow *Nature*, 2002
- ***MALAT1***: nuclear organization - Ji *et al. Oncogene*, 2003
- ***HOTAIR***: chromatin scaffolding - Rinn *et al. Cell*, 2007
- ***lincRNA-p21***: global gene repression - Huarte *et al. Cell*, 2010
- ***Upperhand***: *Hand2* gene regulation (heart development) - Anderson *et al. Nature*, 2016
- ***...***

(Low sequence conservation)

Functional characterization of lincRNAs

A Genetic *Terra Incognita*



(Human)
(lincRNAdb – GENCODE v21)

LincRNAs and mRNAs show distinct genomic properties

(GENCODE v7, Derrien *et al. Genome Research*, 2012)

LncRNA 5' ends are depleted in markers of transcription initiation (**15% less CAGE coverage**)

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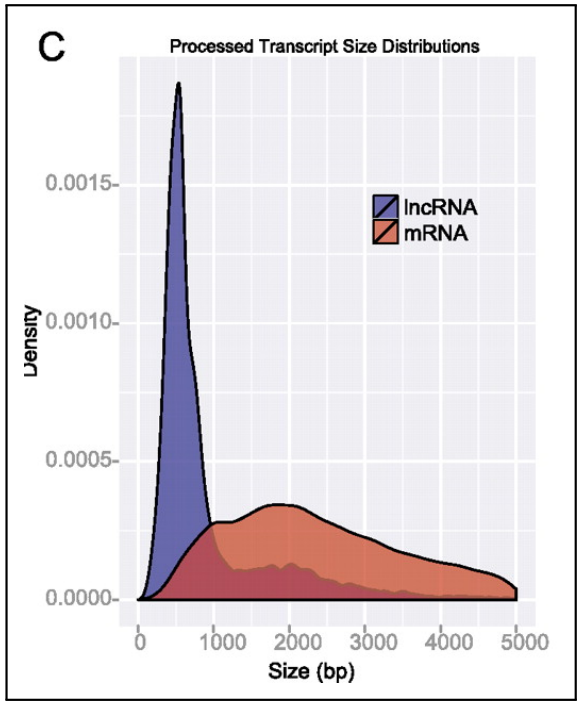

LncRNAs are shorter than mRNAs

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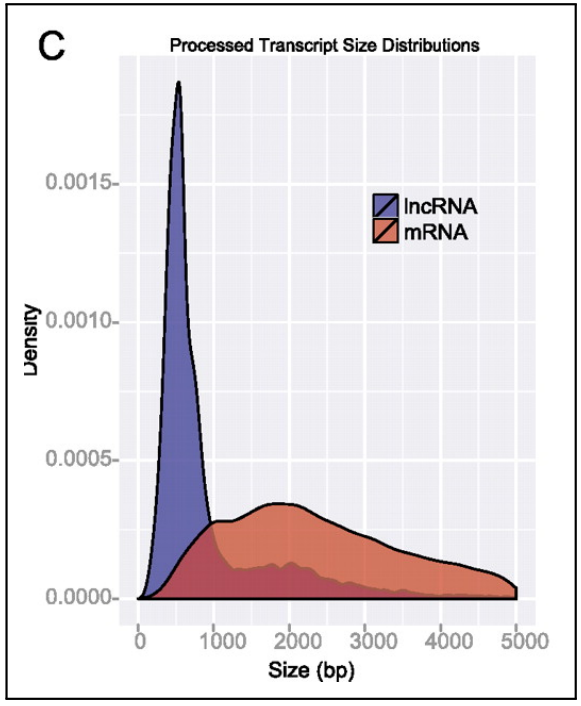

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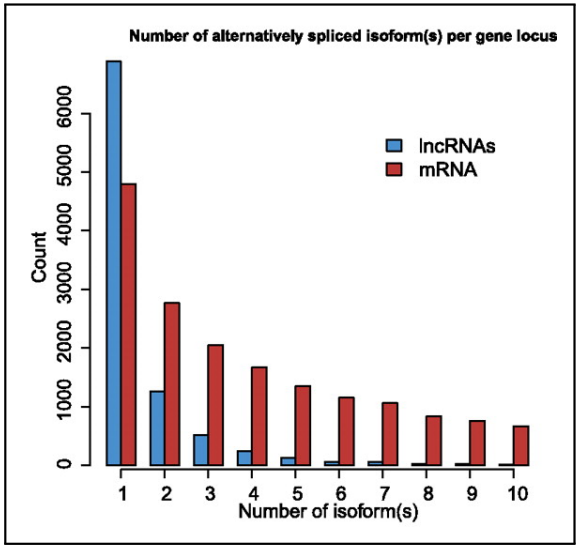
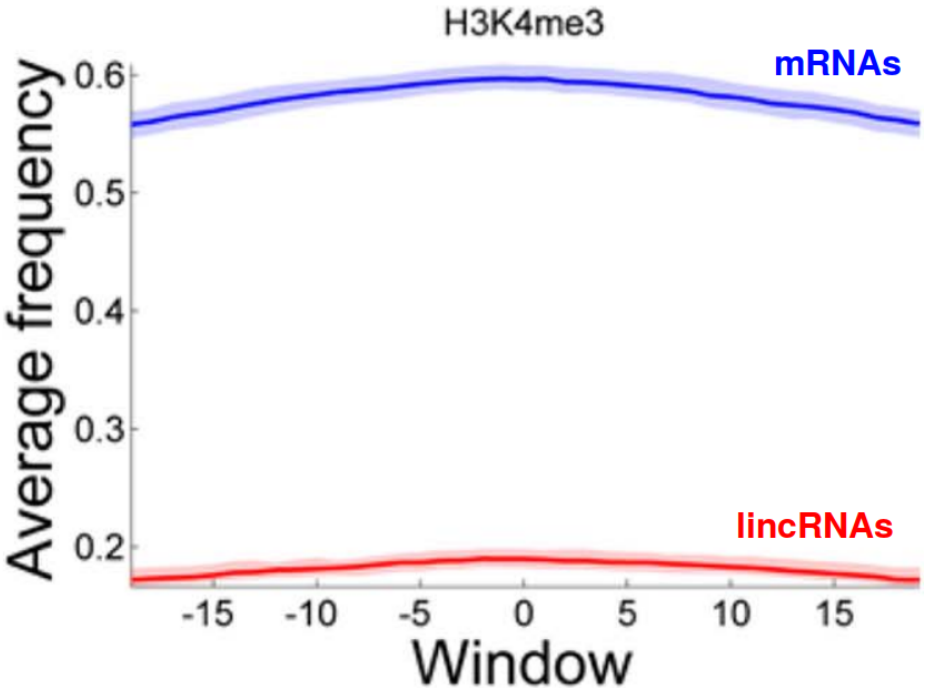


LncRNAs are less alternatively spliced than mRNAs

LincRNA promoter environment

"Promoter Analysis Reveals **Globally Differential Regulation** of Human Long Non-Coding RNA and Protein-Coding Genes"
(Alam *et al. PLoS ONE*, 2014)



LincRNA promoters are **depleted in active chromatin marks**

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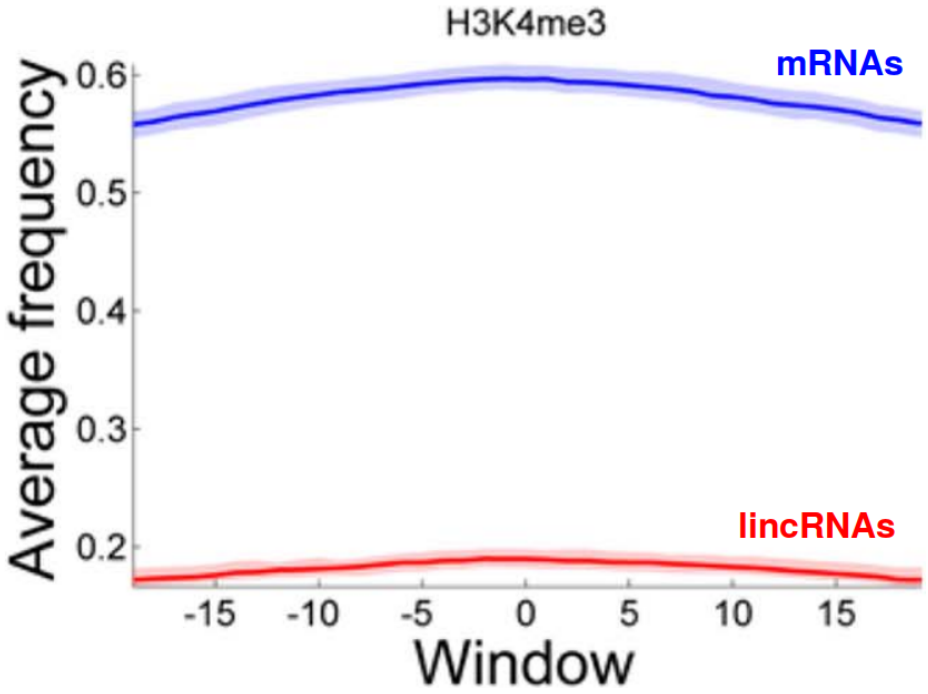

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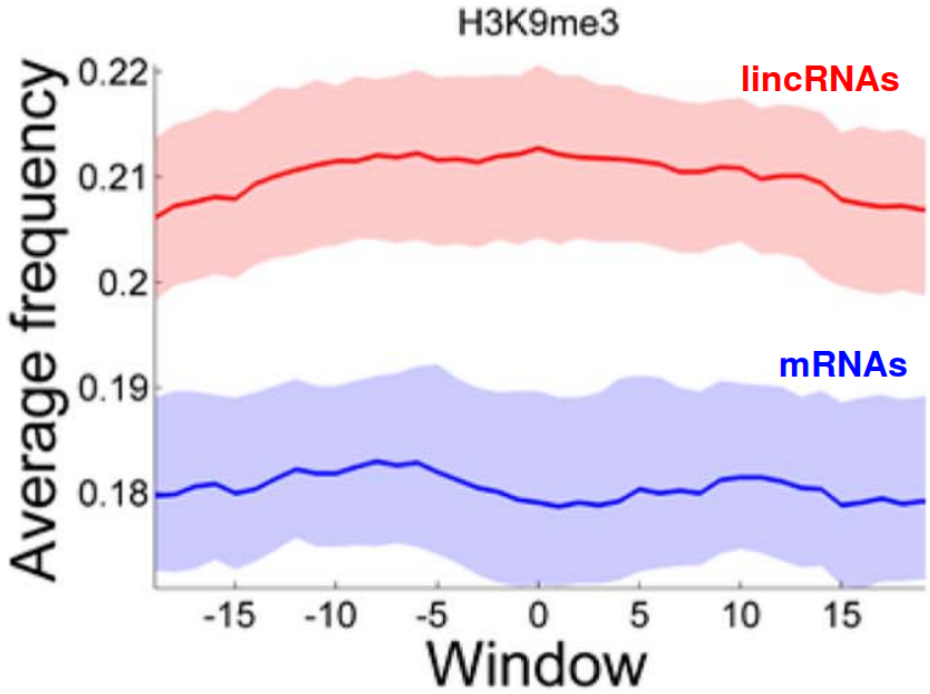

LincRNA promoters are **enriched in repressive chromatin marks**

... or are they, really?

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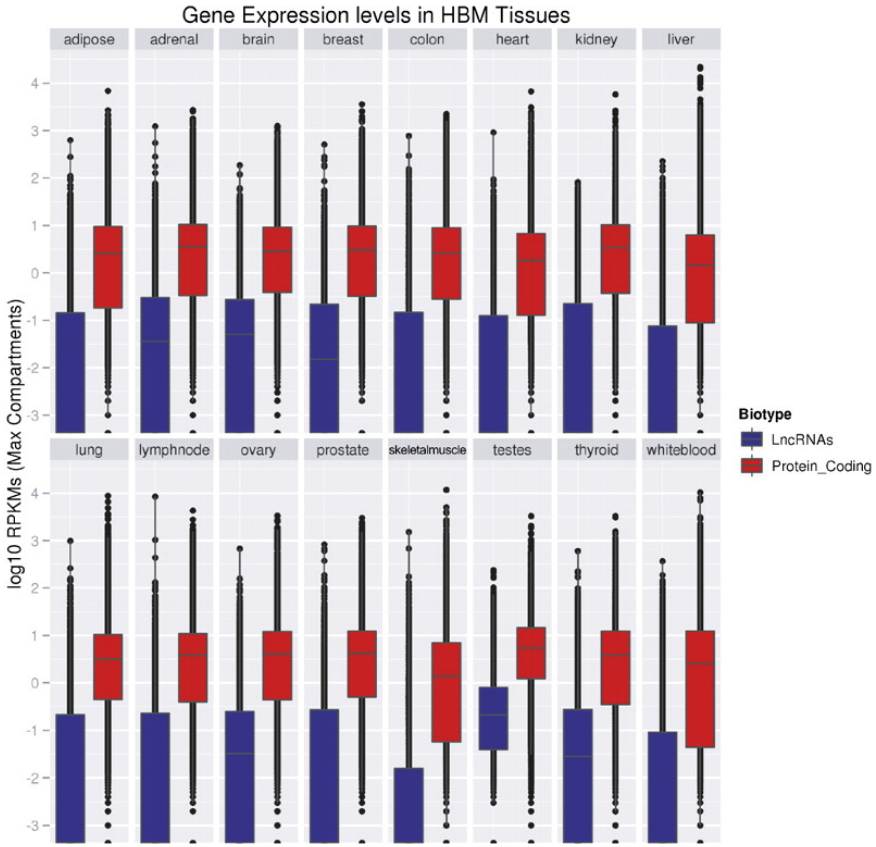
LincRNAs have low expression levels

(GENCODE v7, Derrien *et al. Genome Research*, 2012)



In a typical (polyA+) RNA-Seq experiment, lincRNAs account for **<3% of the reads**

(Bakel *et al. PLoS biology*, 2010)

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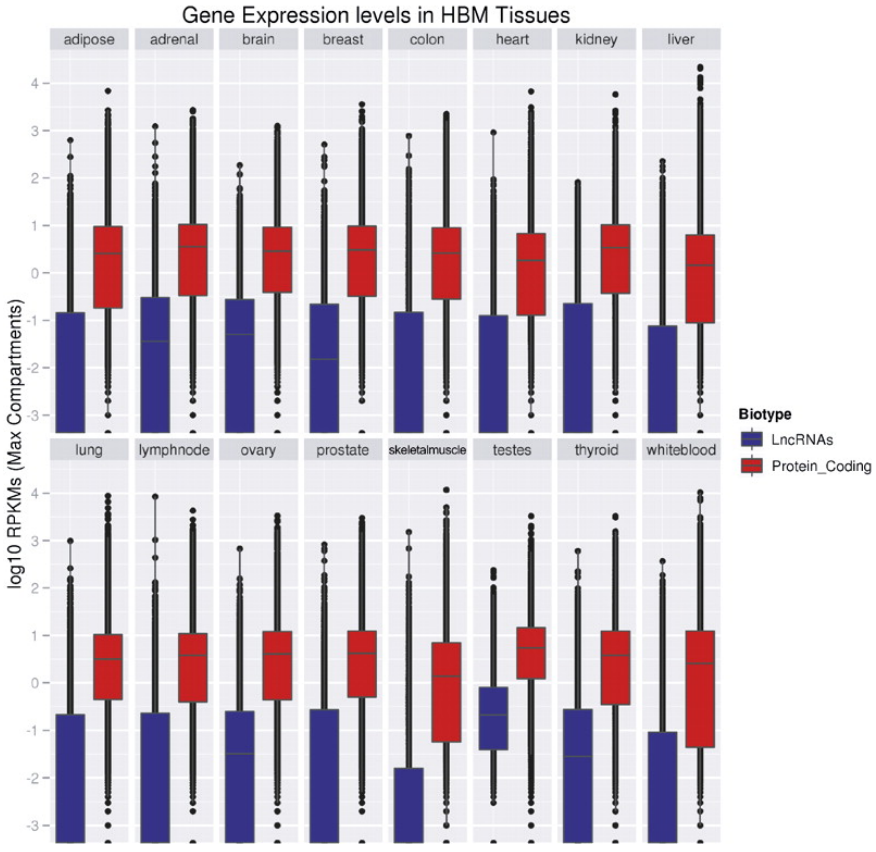

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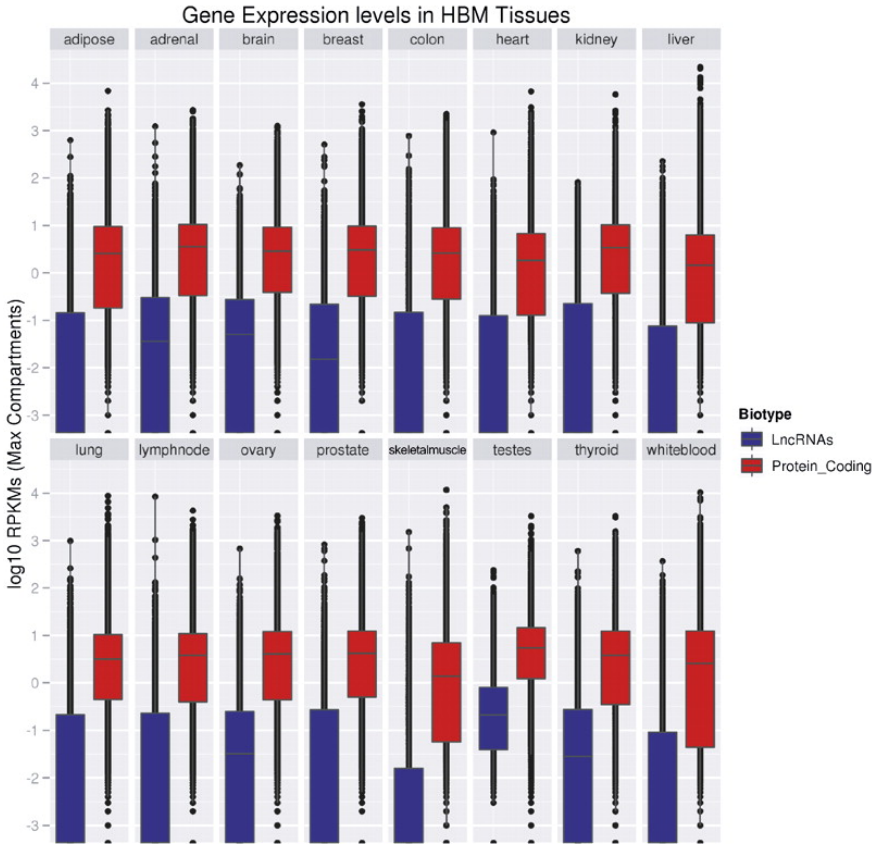

Low expression

⇓

Under-representation of intact lncRNA transcripts in cDNA libraries

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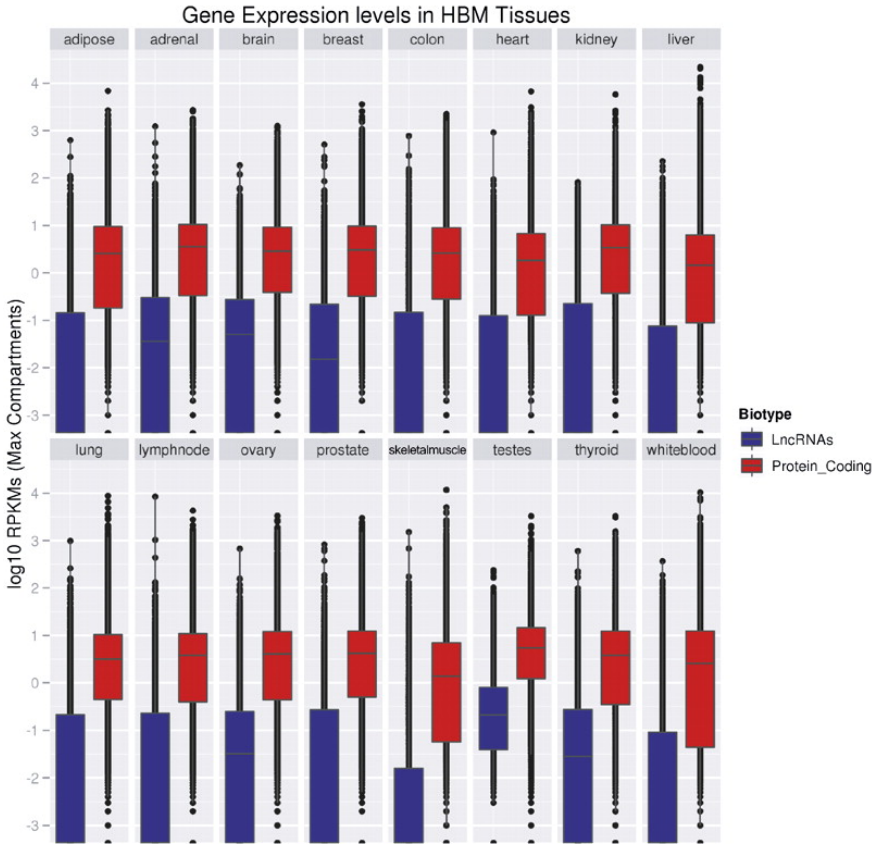

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⇓

Annotation artifacts

(truncated lncRNA transcript models)

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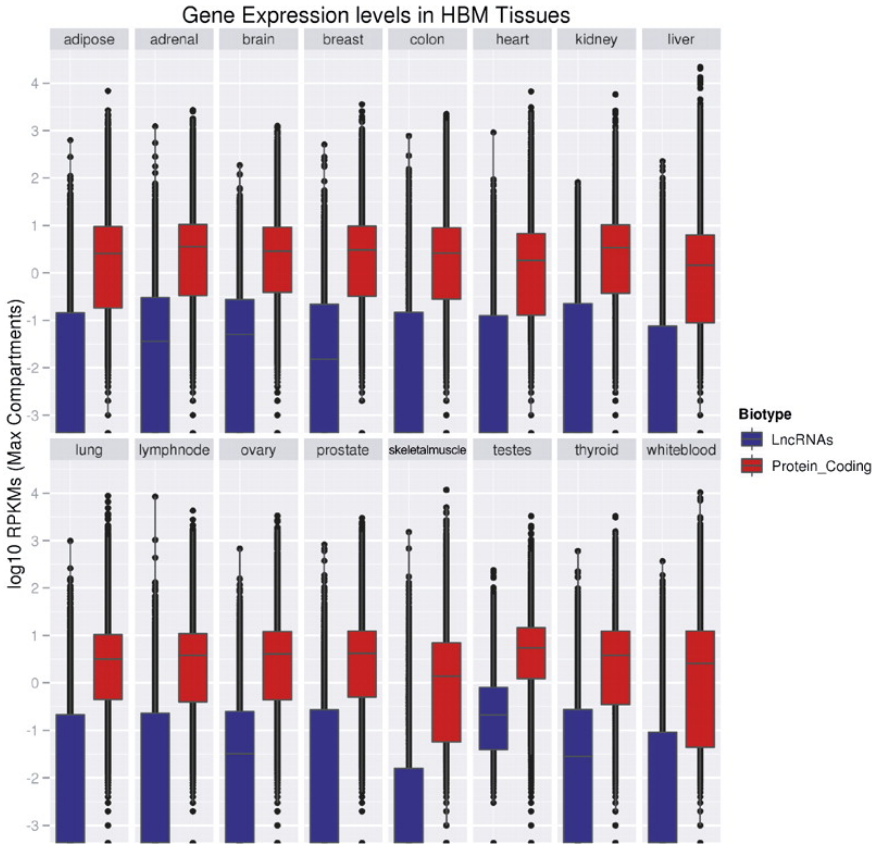
... or are they, really?

LincRNAs have low expression levels

(GENCODE v7, Derrien *et al. Genome Research*, 2012)



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Low expression

⇓

Under-representation of intact lncRNA transcripts in cDNA libraries

⇓

Annotation artifacts

(truncated lncRNA transcript models)

⇒ **Need for targeted sequencing techniques**

Other lncRNA catalogs

Catalog	# lincRNA loci	Short-read assembly?	Ref.
NONCODE	96,000	**Yes (partially)**	*Fang et al.*, 2017
MiTranscriptome	63,000	**Yes**	Iyer *et al.*, 2015
FANTOM CAT	28,000	**Yes**	Hon *et al.*, 2017
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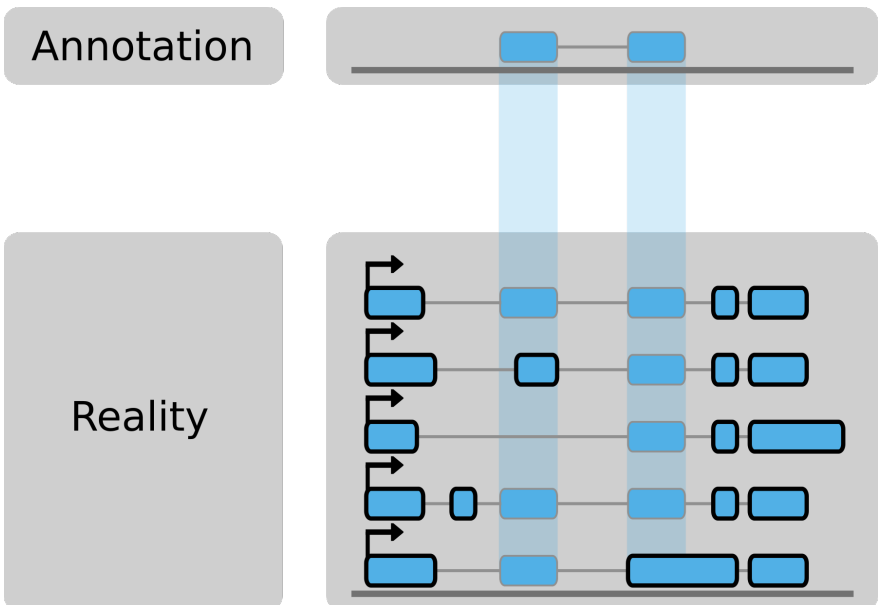
Short-read-based assembly leads to **inaccurate transcript models**, especially:

- Wrong 5'/3' boundaries
- Wrong lncRNA models

(Steijger *et al. Nature Methods*, 2013)

Accurate gene annotations: a foundation for lncRNA functional characterization



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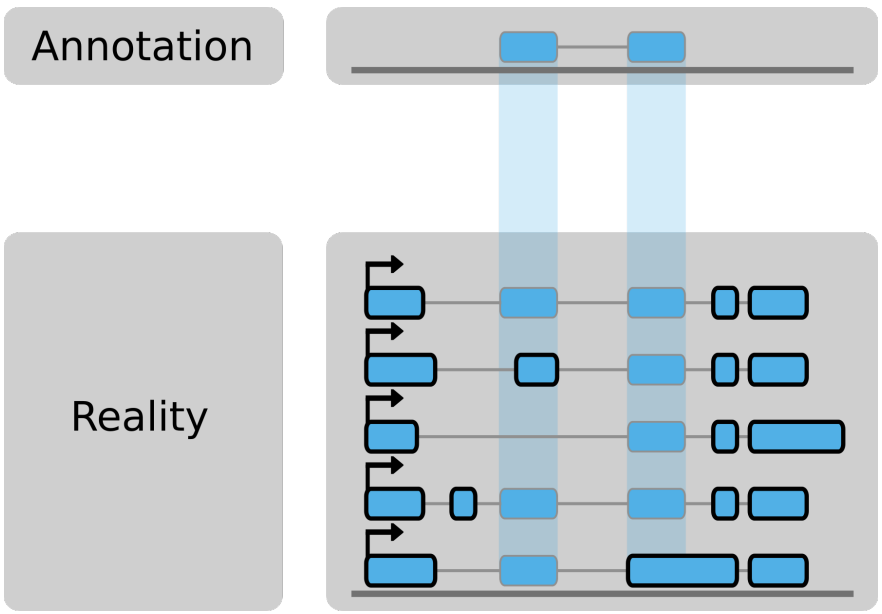

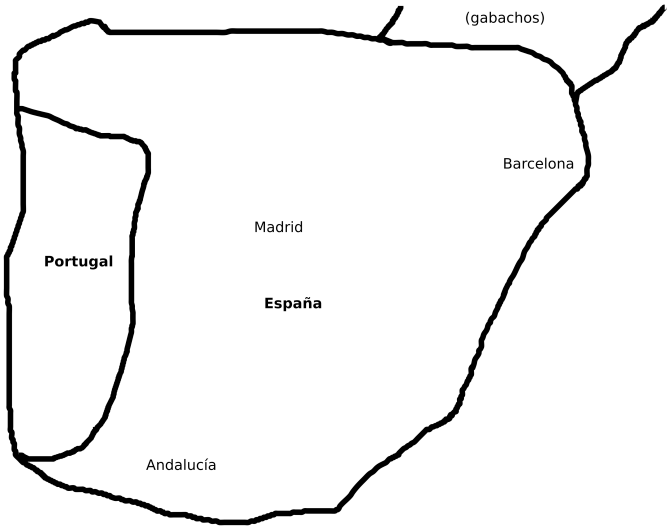

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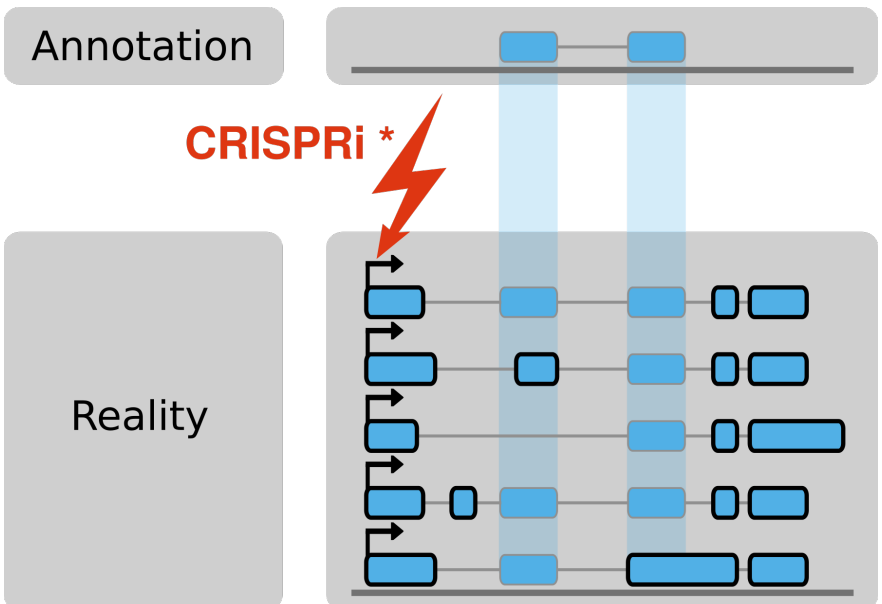
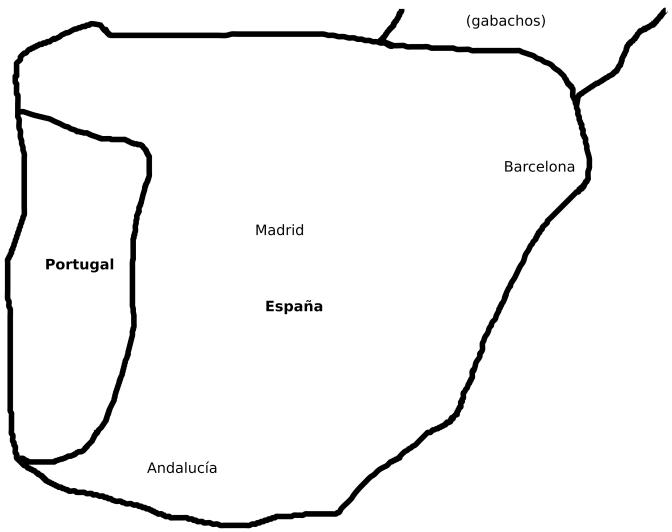


(* *e.g.* Liu *et al. Science*, 2017)



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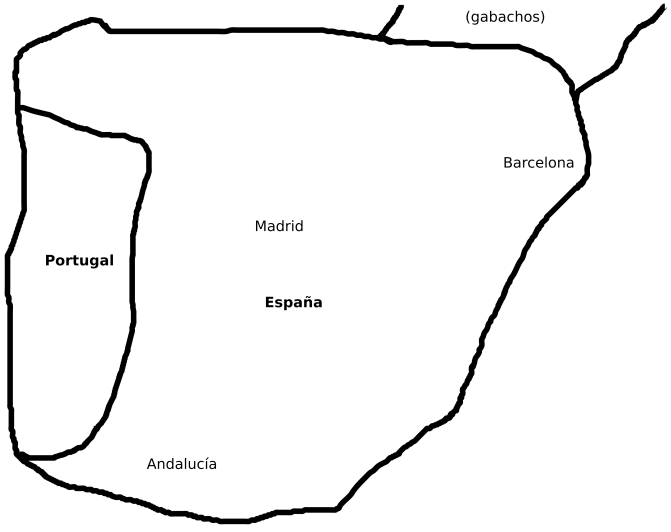
- Currently, reference lncRNA catalogs are **incomplete/inaccurate**
- LncRNAs are expressed at **low levels**
- Long-read (TGS) sequencing (**PacBio**) promises to revolutionize gene annotation:
  - Full-length, high-quality transcript sequencing
  - High-throughput
  - Lower depth than short-read SGS (Illumina)

Objectives



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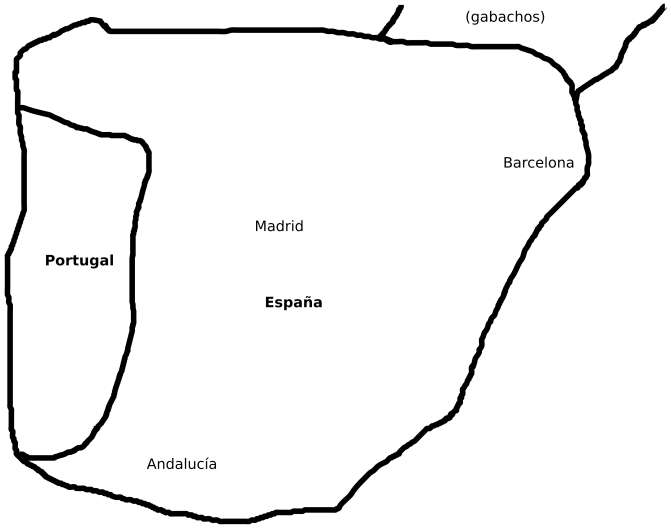

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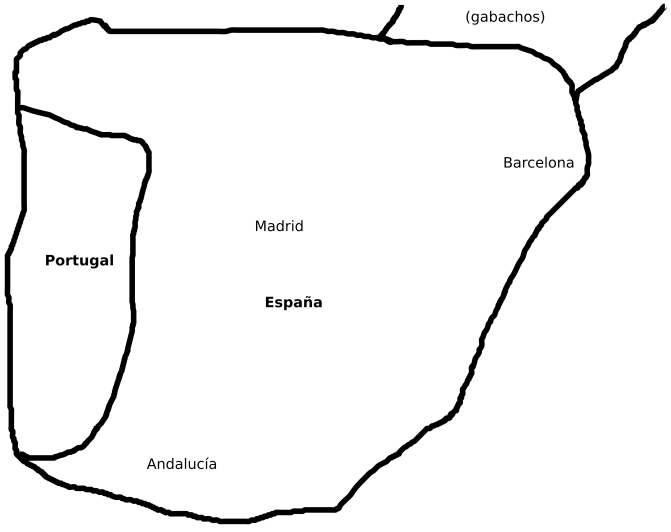

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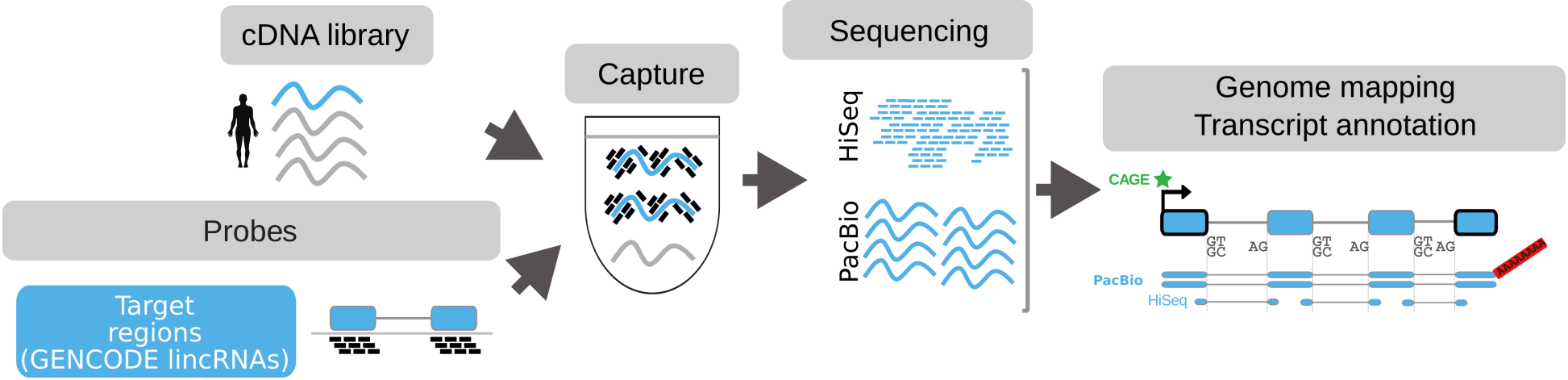
- Using this enhanced map, re-evaluate some of the **genomic properties of lncRNAs**

The CLS method: Capture Long-read Sequencing

High-throughput lncRNA annotation



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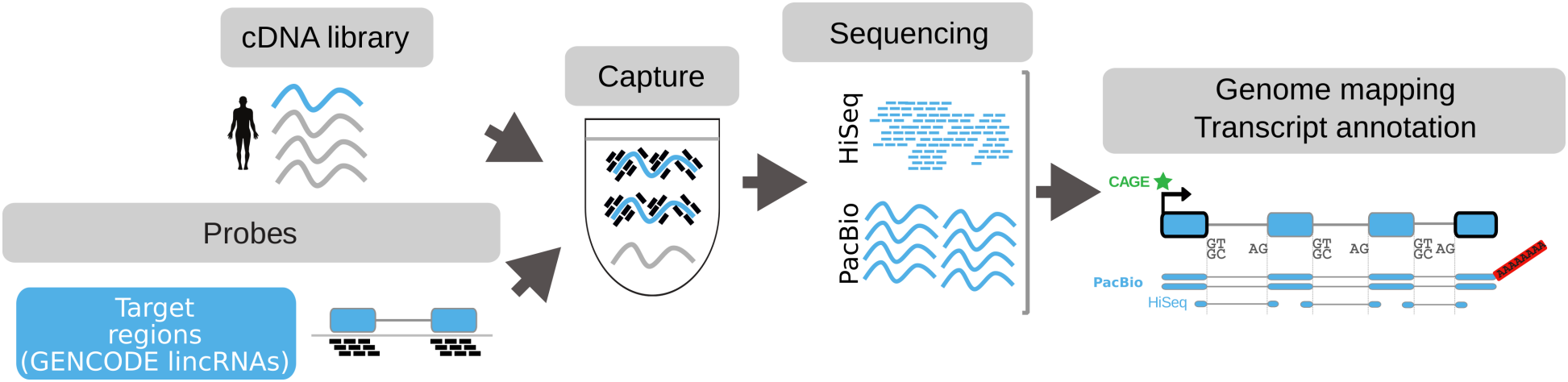

CLS targets:

- ~6,000 human lincRNA loci
- ~10 Mb (~50% of total annotated)

Lagarde *et al. Nature Genetics*, 2017

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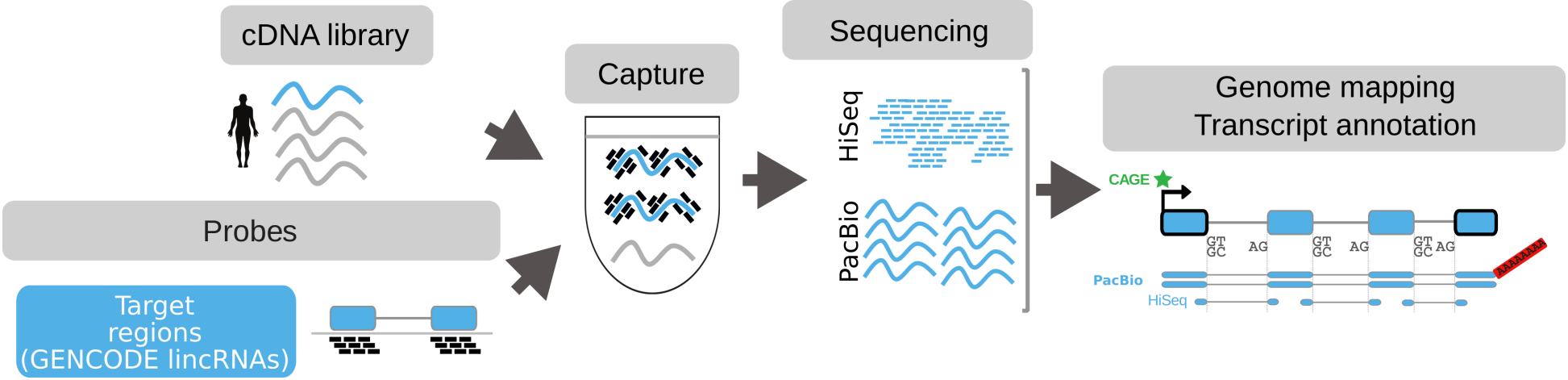

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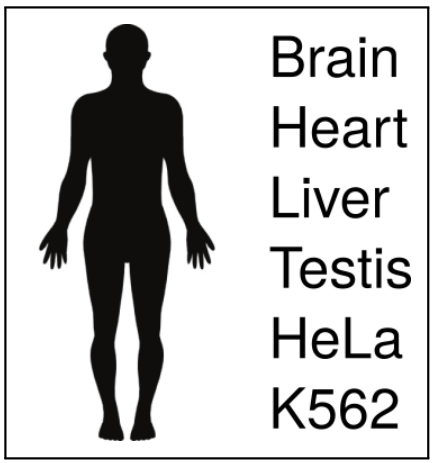
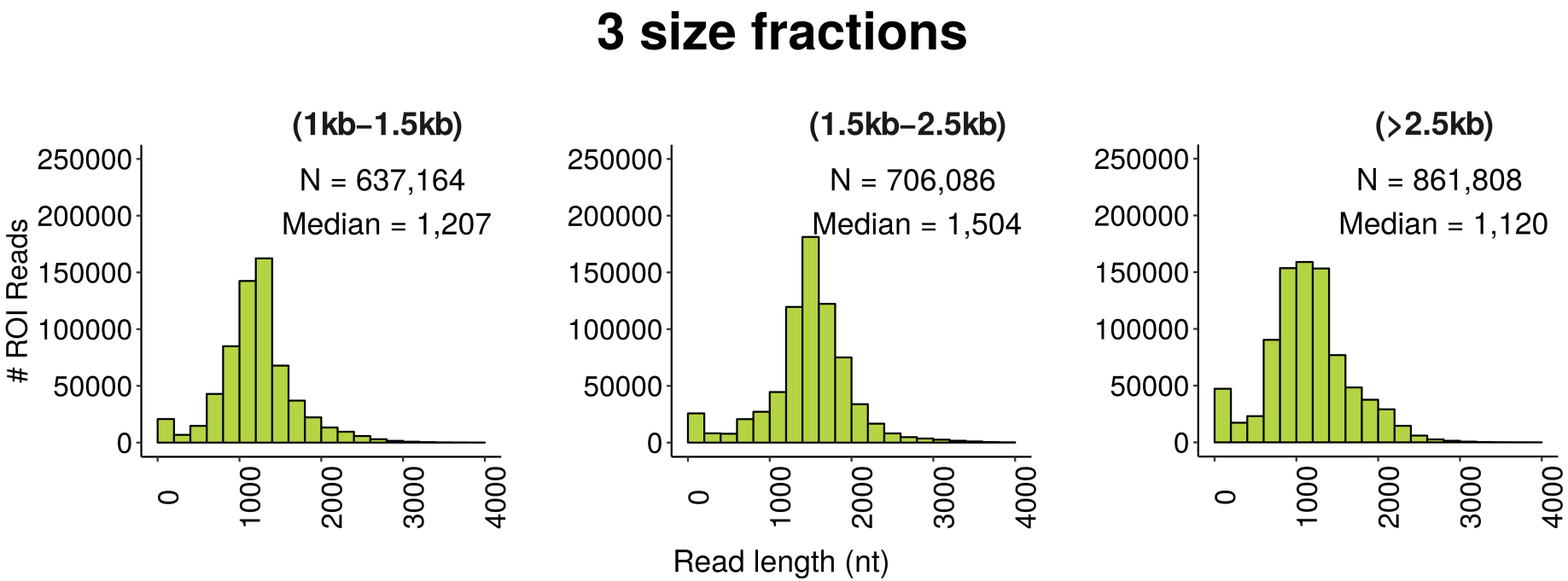


CLS sequencing output

PacBio RSII:



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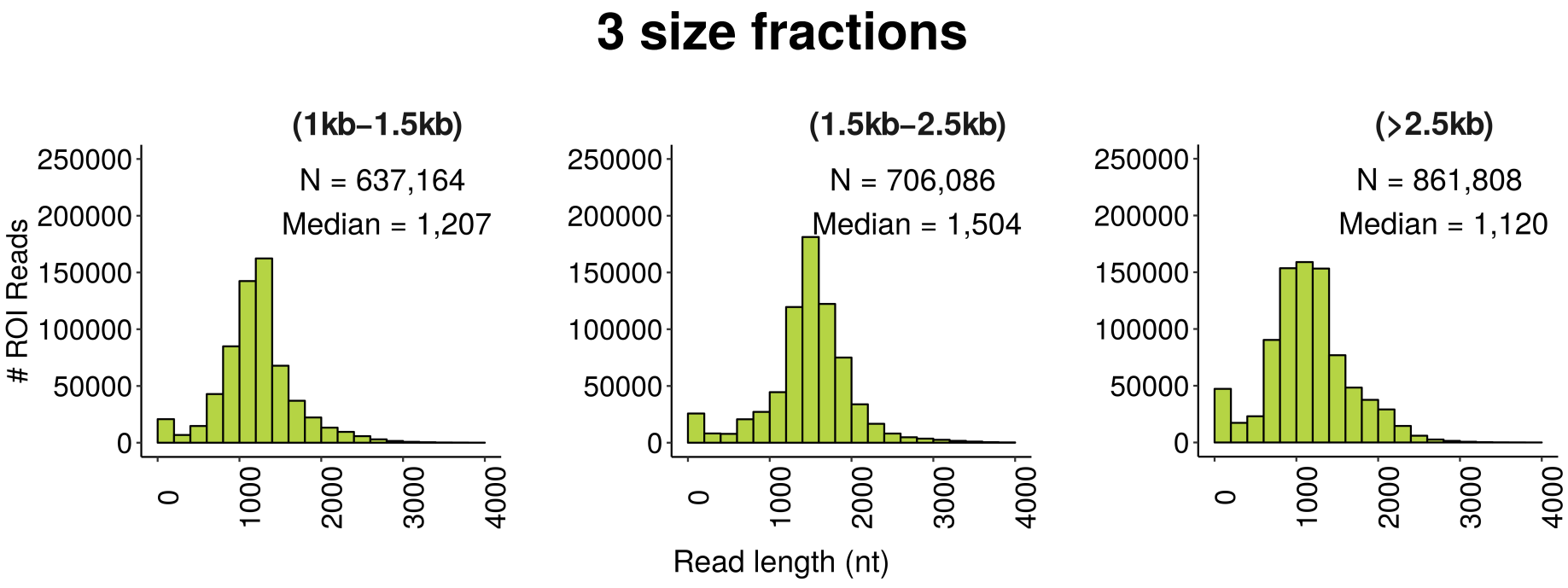

Total:

- ~2.2 million PacBio reads
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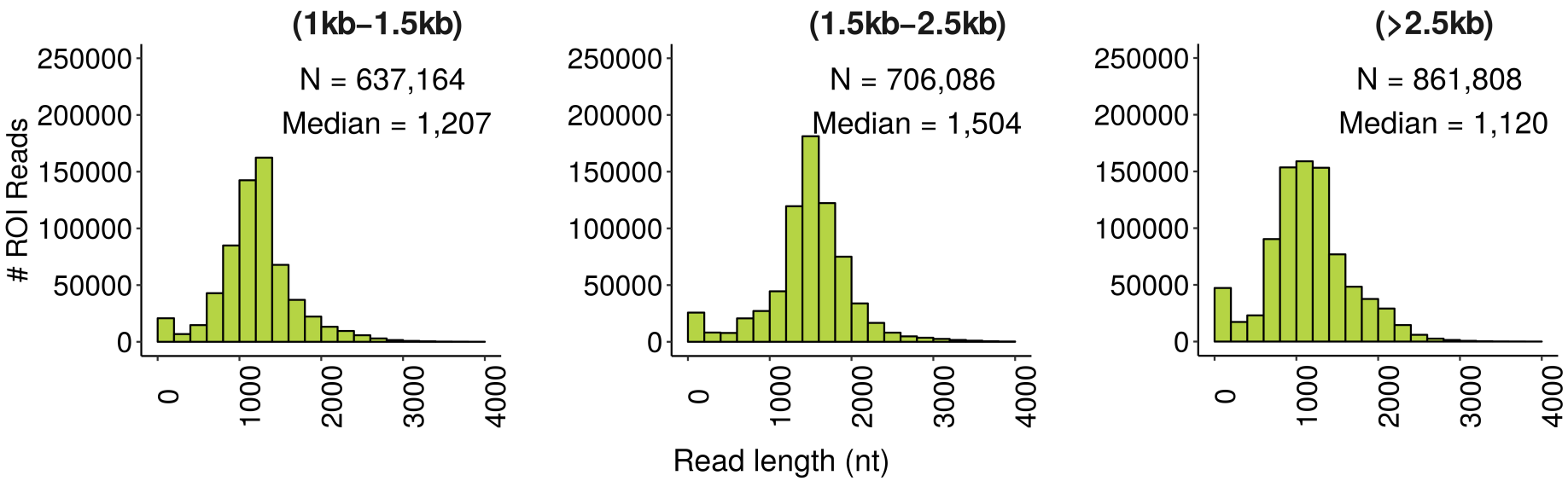
3 size fractions



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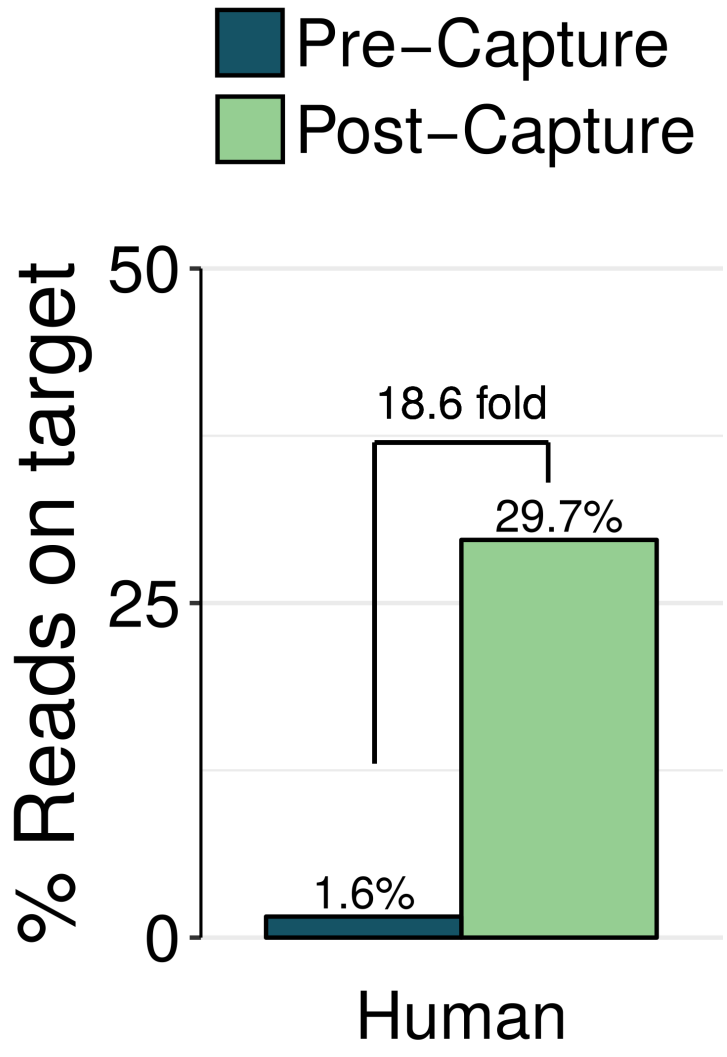
+ **HiSeq** 2500: 212 million PE stranded reads

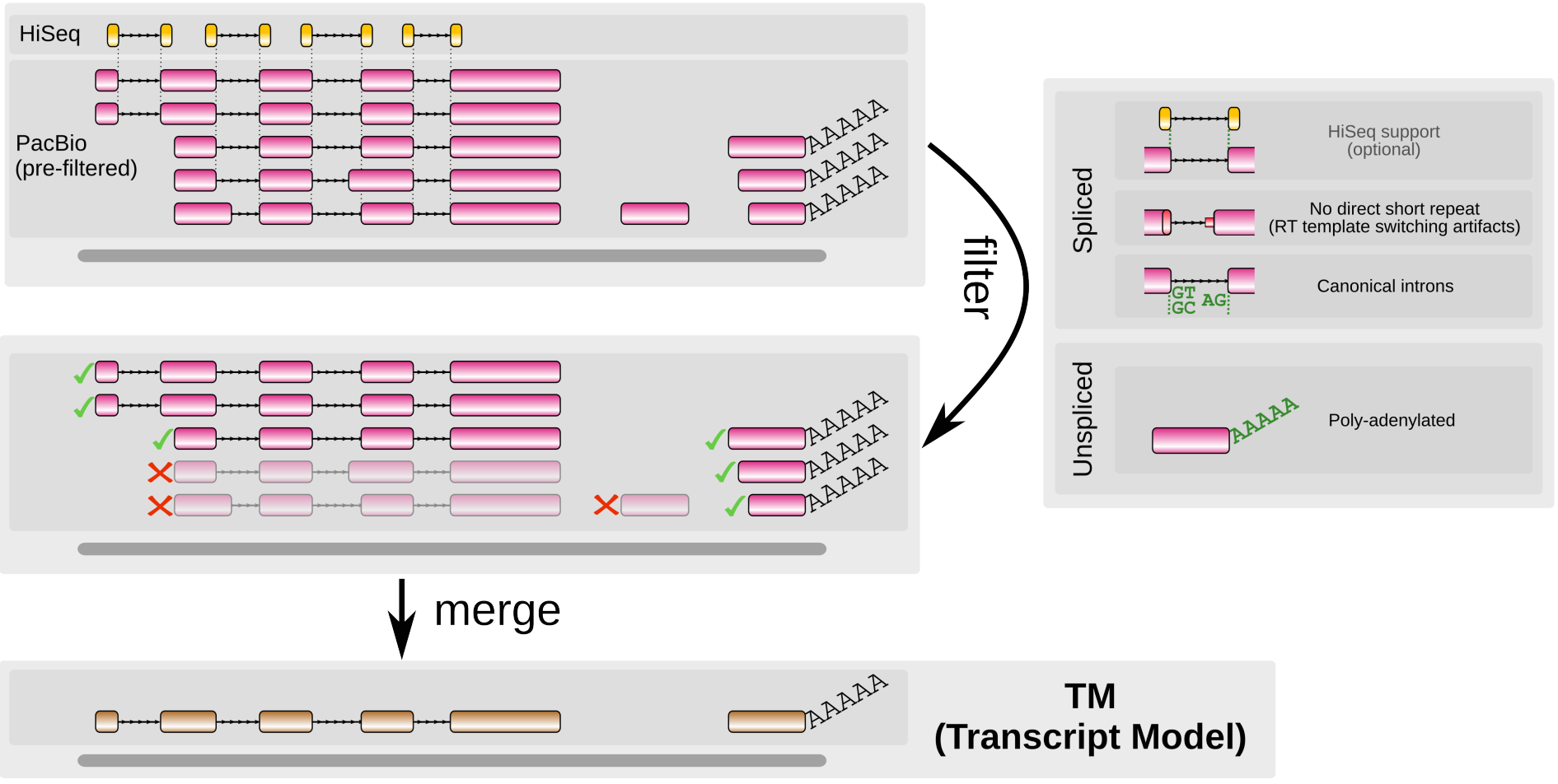
Lagarde *et al. Nature Genetics*, 2017

CLS libraries are highly enriched for targets



Lagarde *et al. Nature Genetics*, 2017

Read quality filtering and merging



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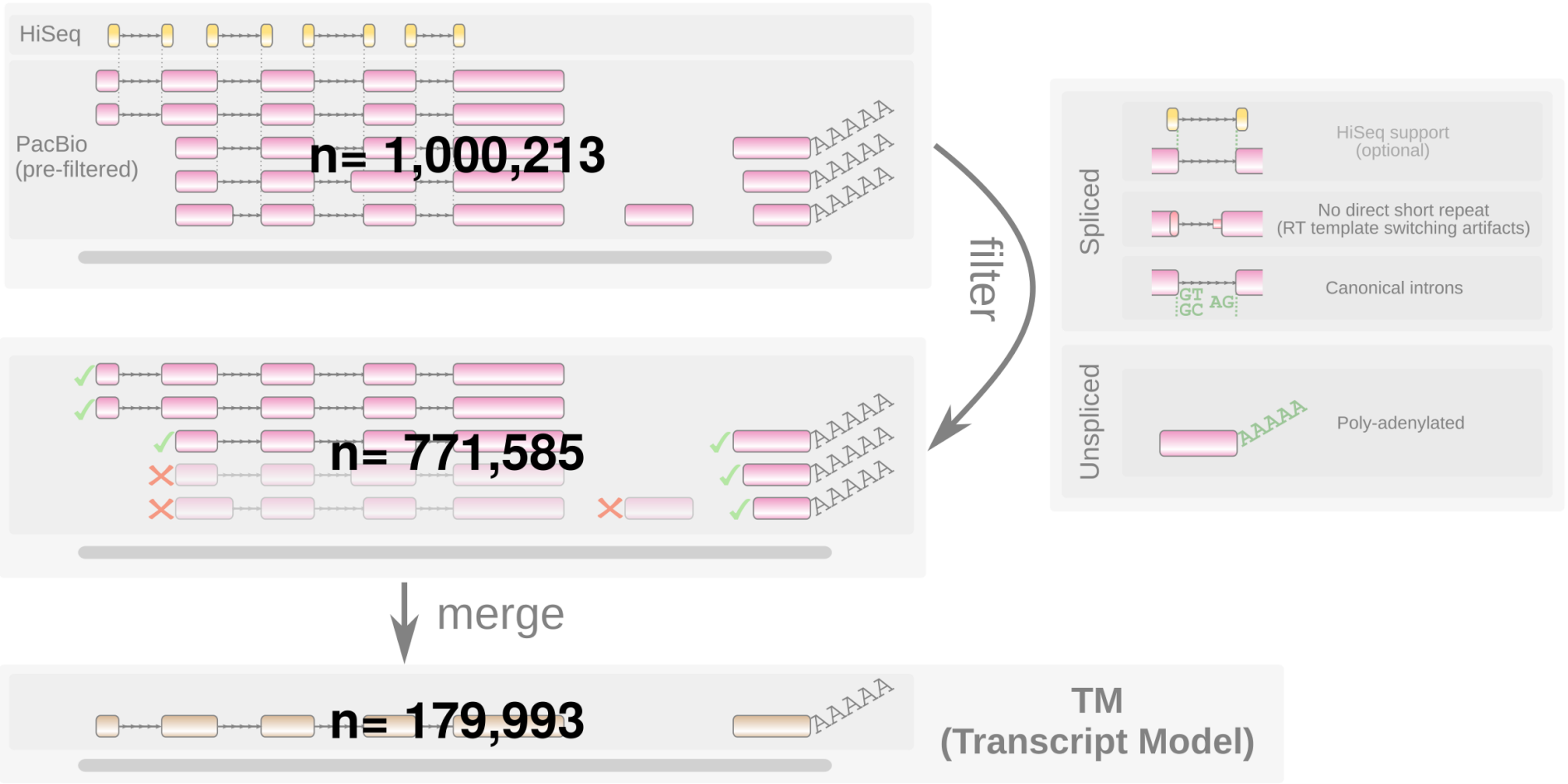
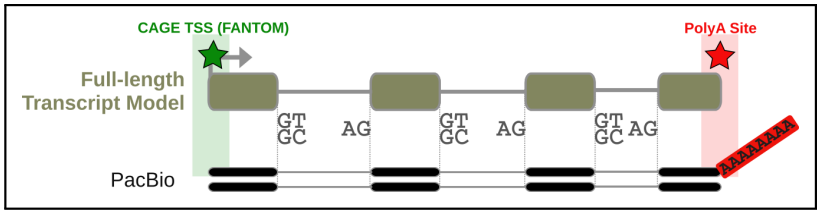


A full-length transcript catalog



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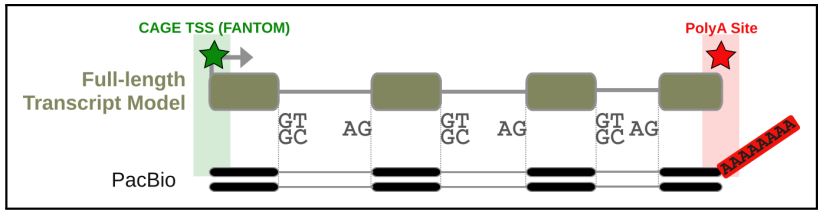

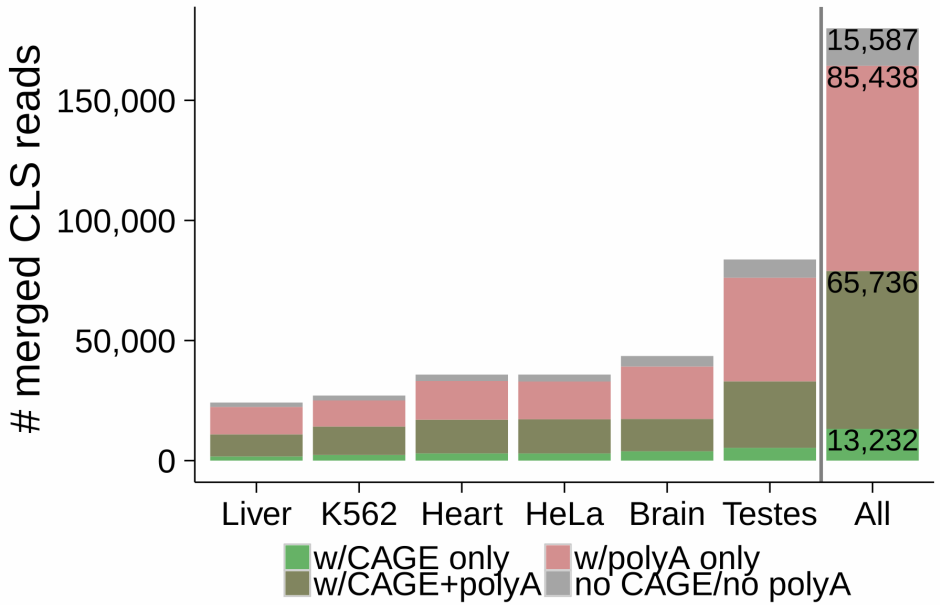

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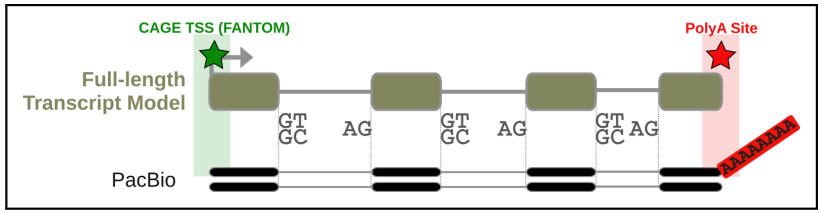

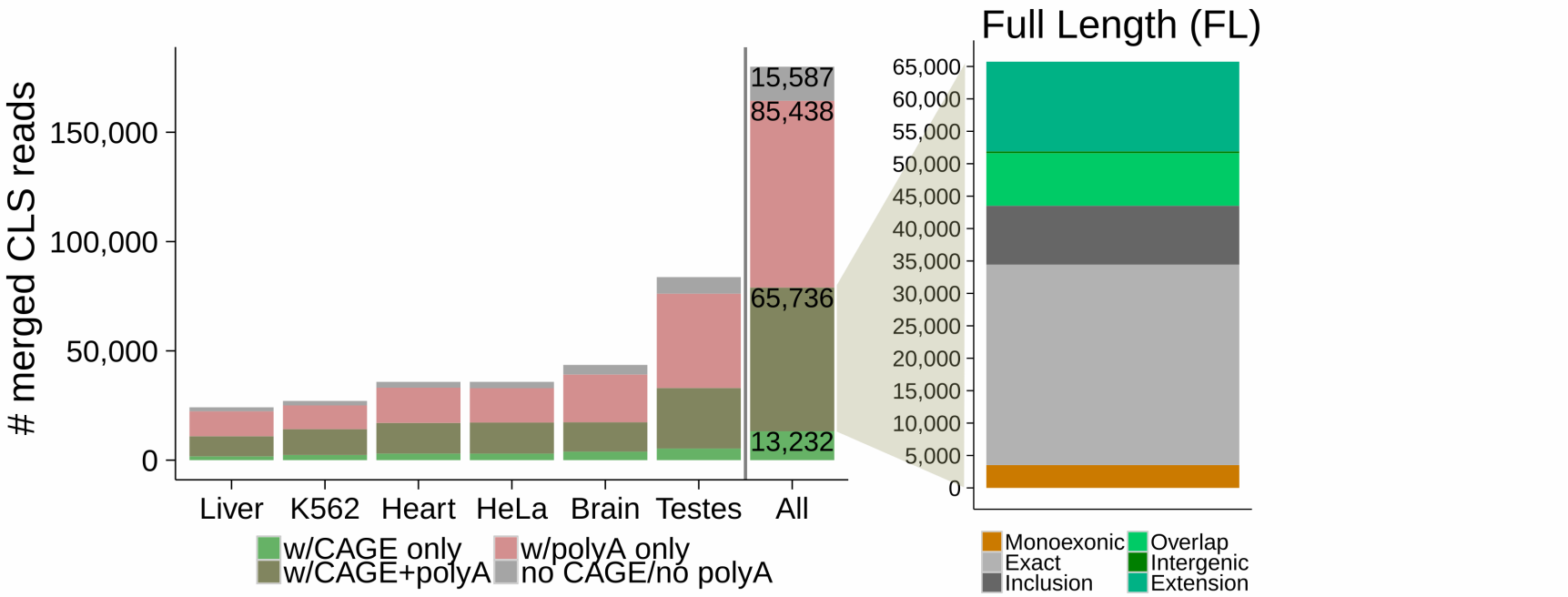

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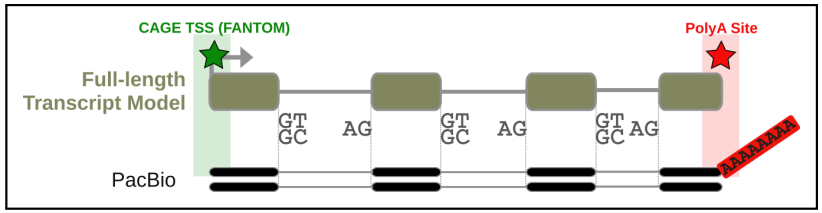
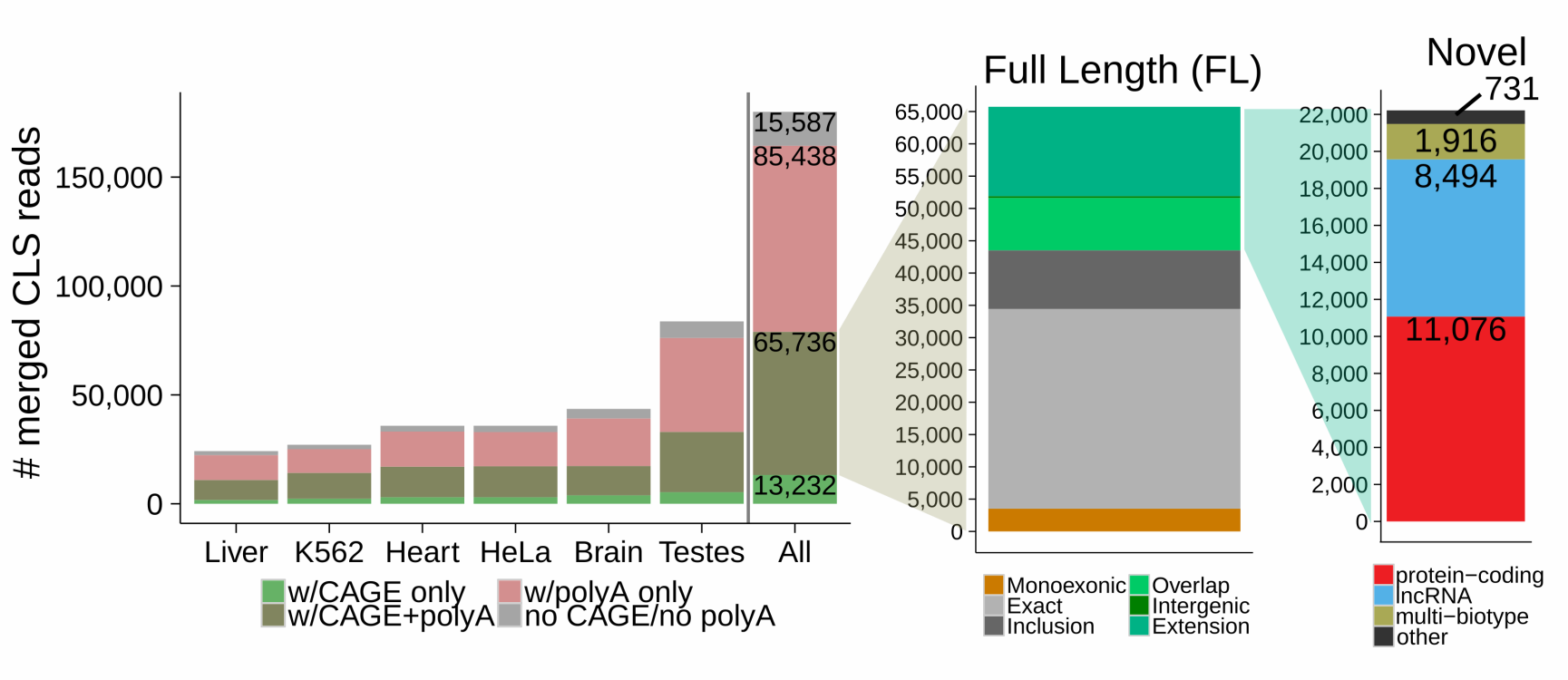
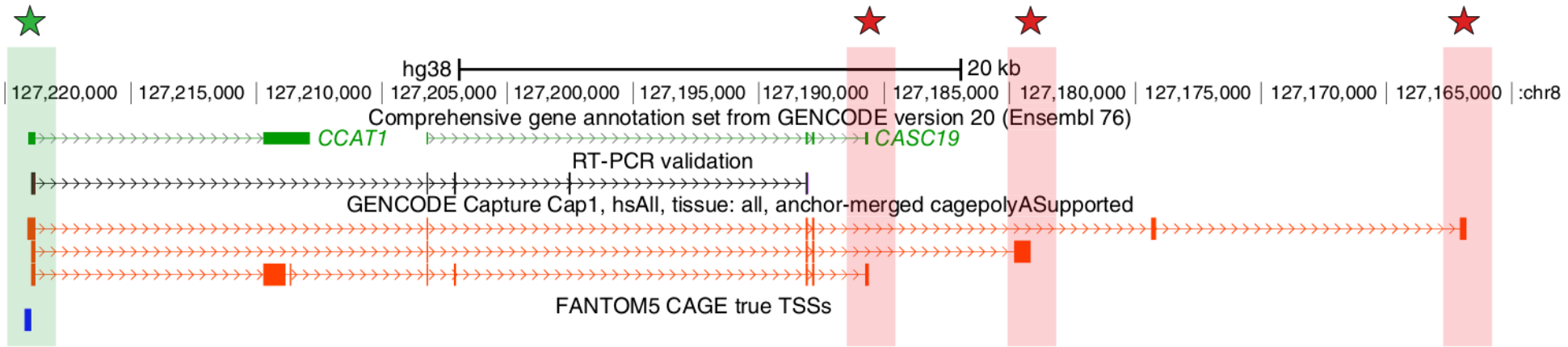




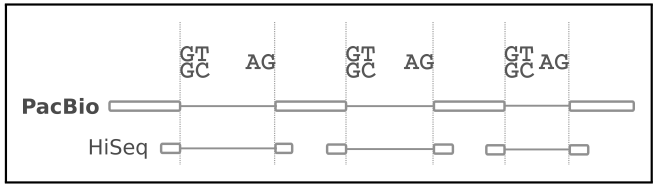
Lagarde *et al. Nature Genetics*, 2017

Novel full-length transcript structures in the *CCAT1* lincRNA locus



Lagarde *et al. Nature Genetics*, 2017

CLS splice junctions match the quality of GENCODE-annotated ones



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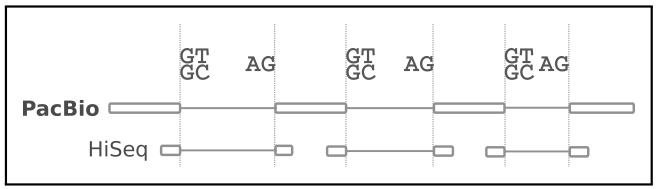

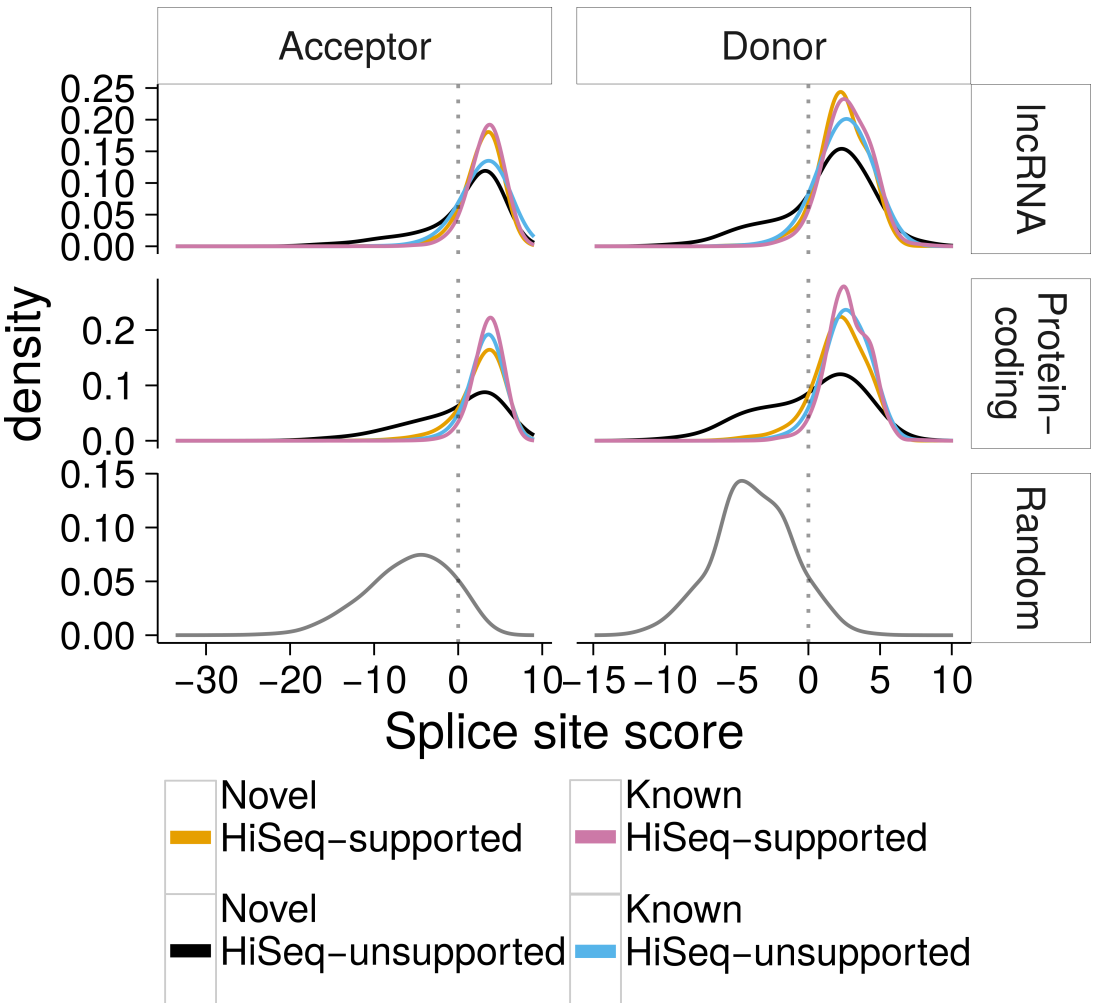

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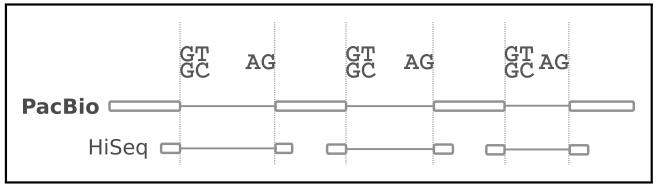
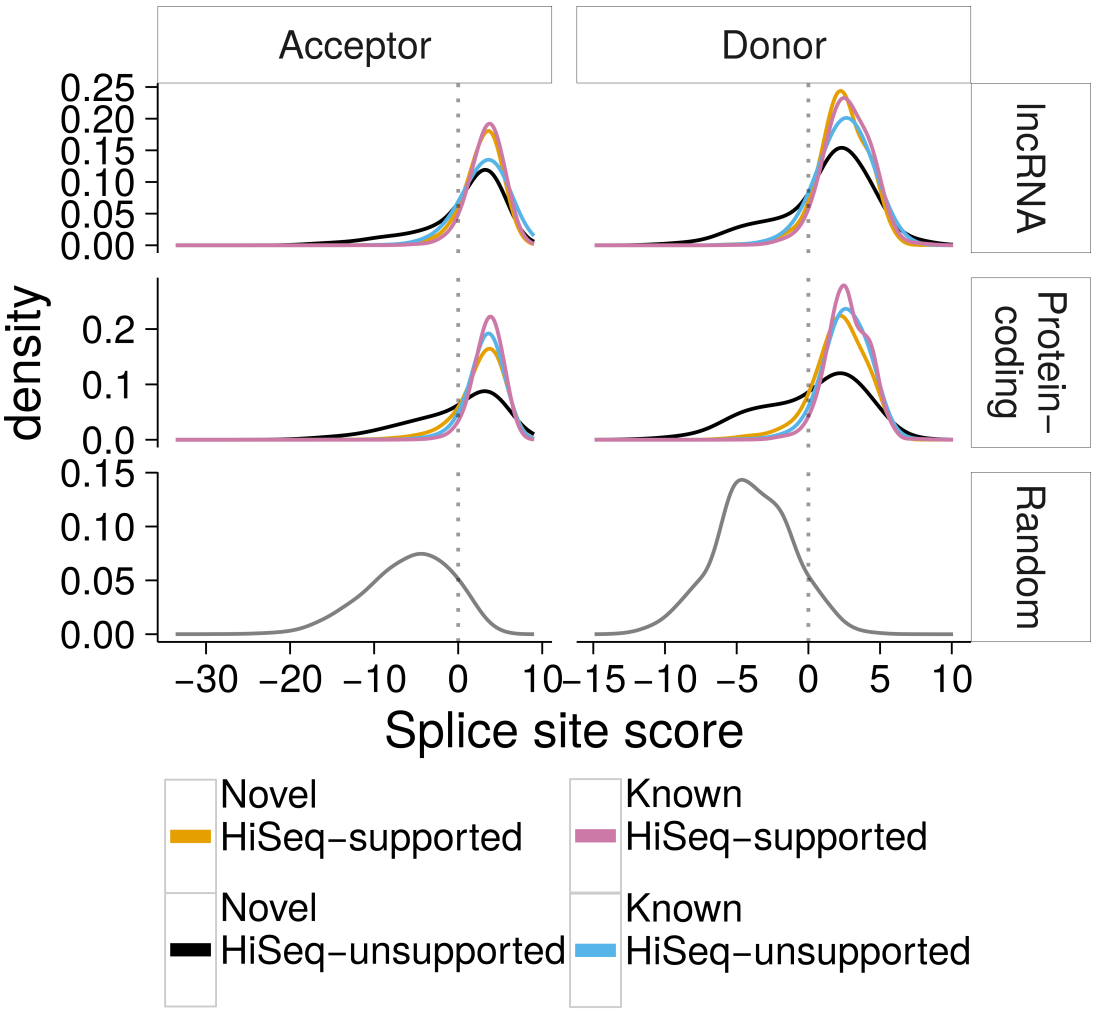
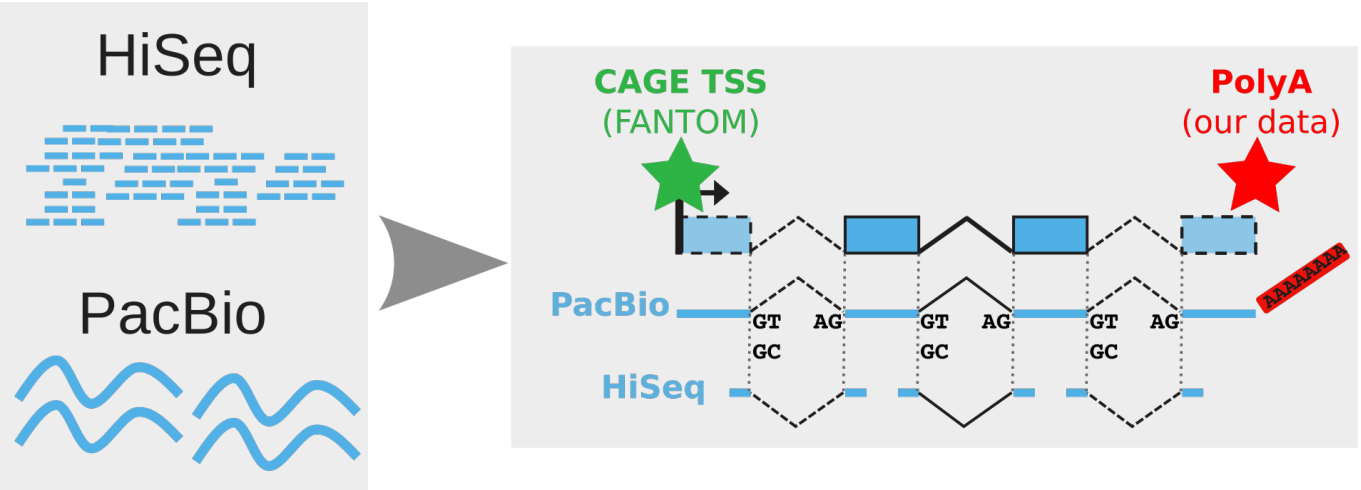




86% of CLS TMs are fully HiSeq-supported

Lagarde *et al. Nature Genetics*, 2017

Short- *vs* long-read sequencing



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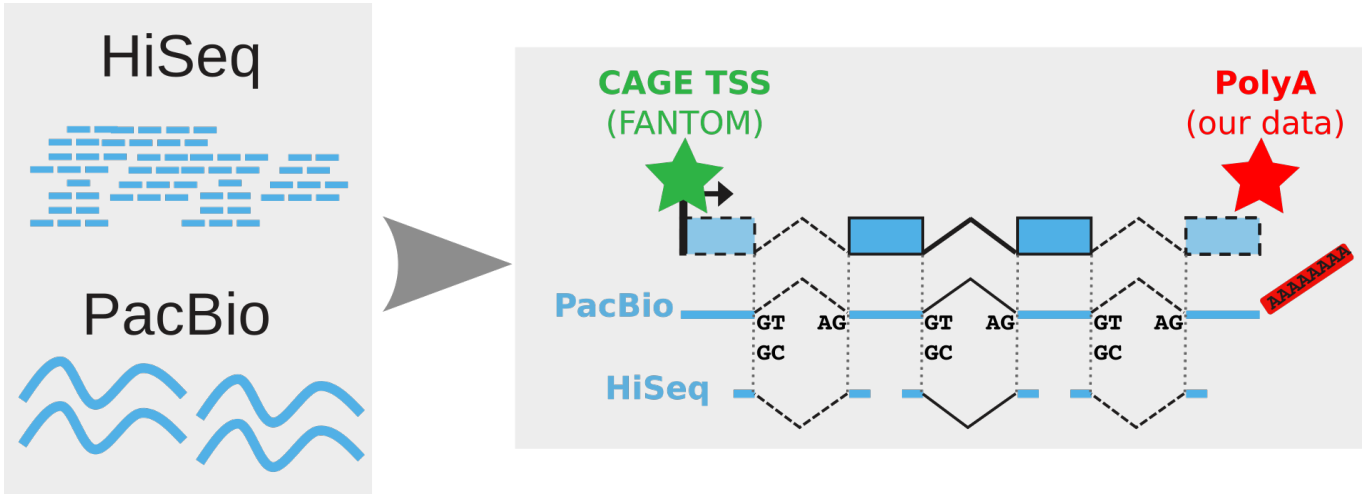


CLS

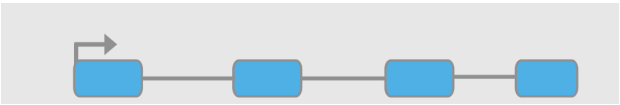
vs

Transcript reconstruction software *

(* Pertea *et al. Nature Biotechnology*, 2015)

CLS discovers a wealth of novel lincRNA transcript structures...

... and vastly outperforms StringTie



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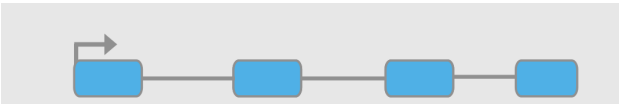

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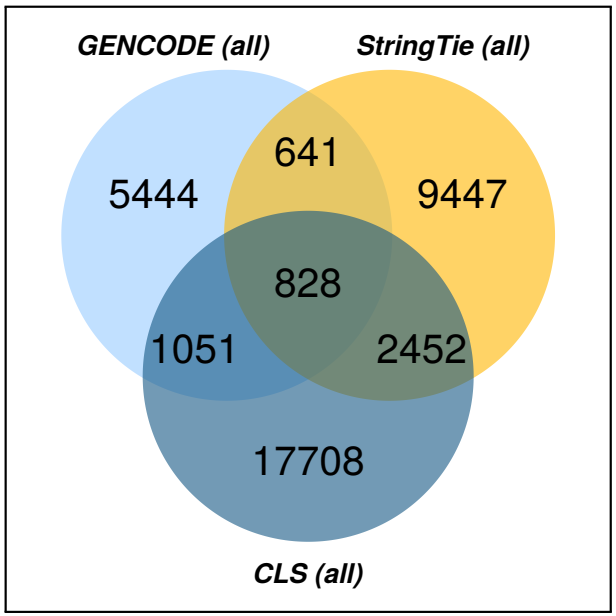

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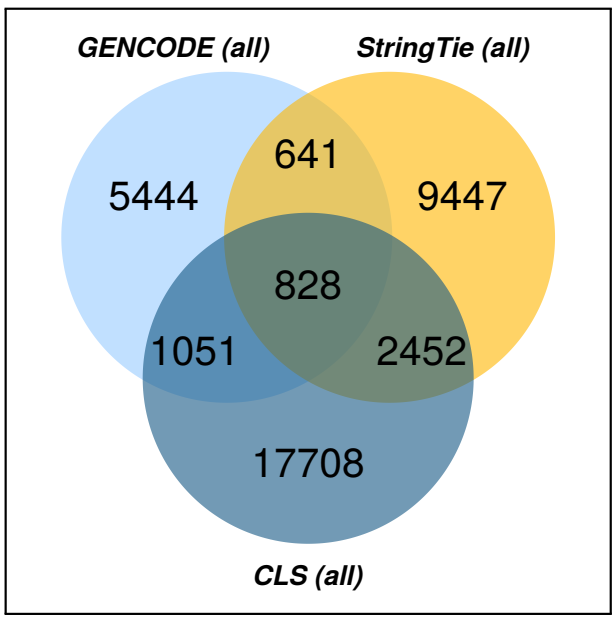

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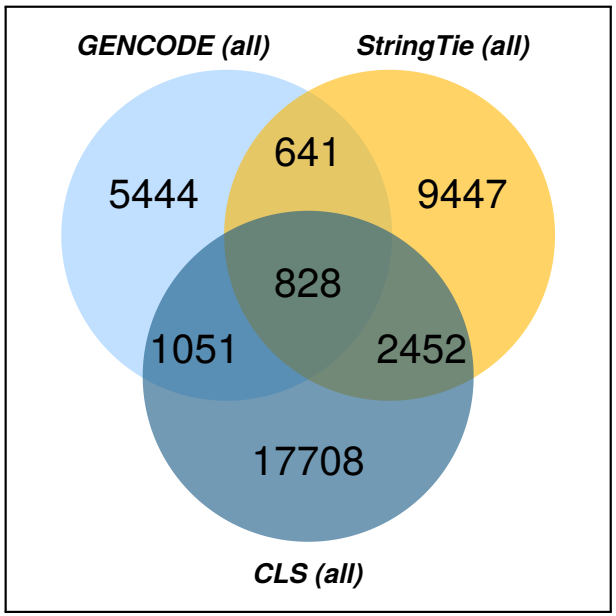

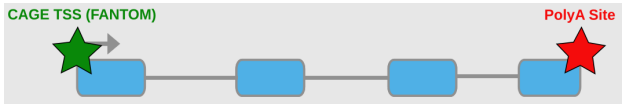

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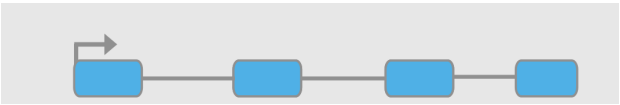

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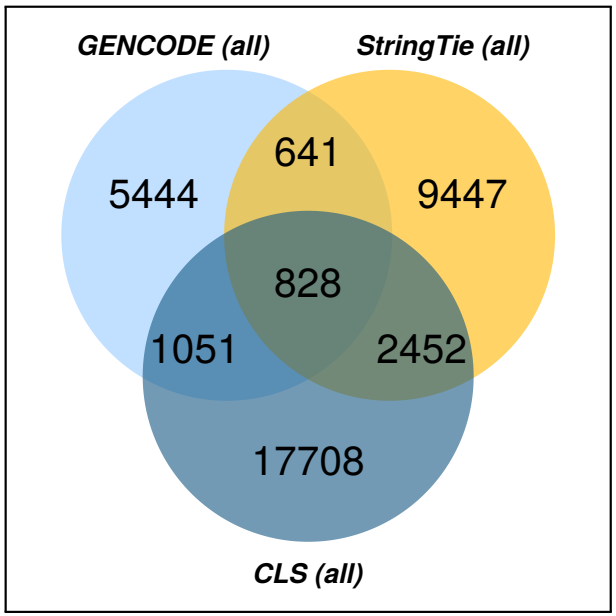

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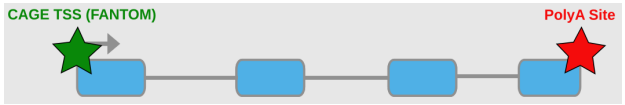

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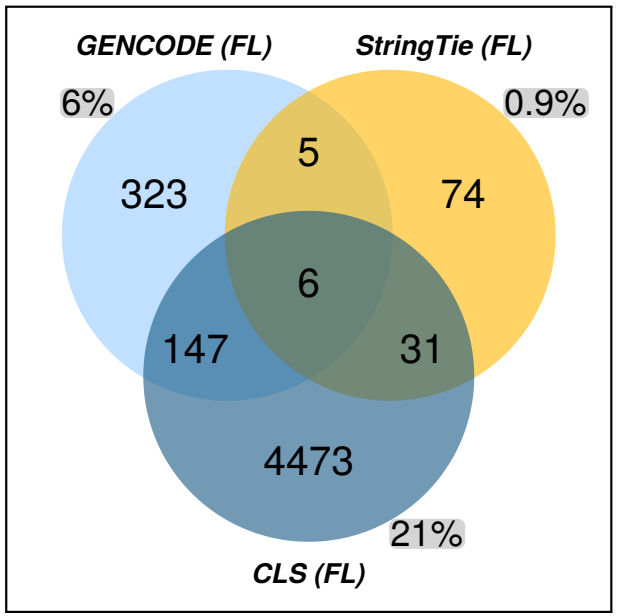
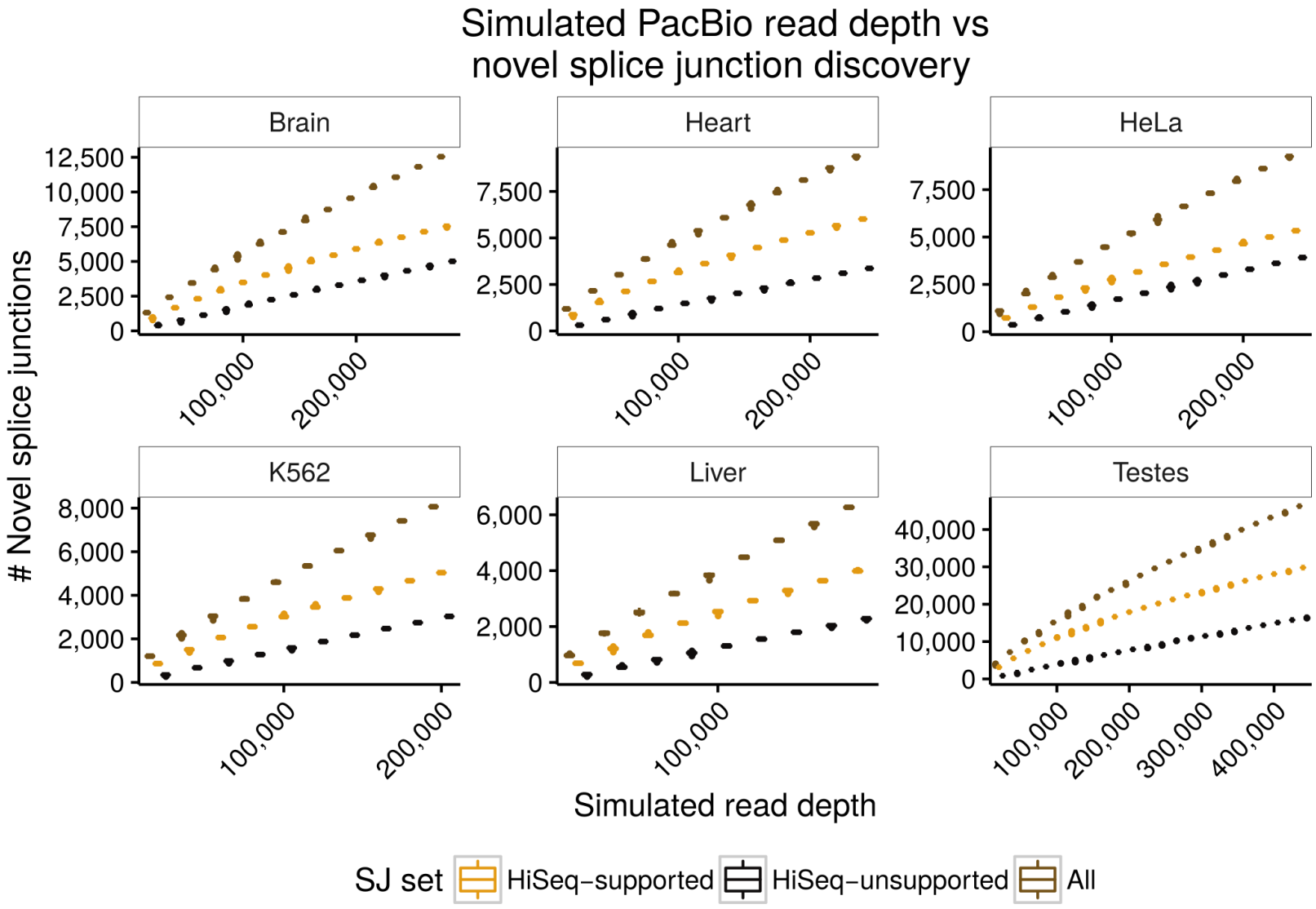


- CLS expands **FL** lincRNA **transcript** annotation ~**10x** (481 → 4,985)
- Novel CLS **FL** transcript structures are found in **947 lincRNA loci**

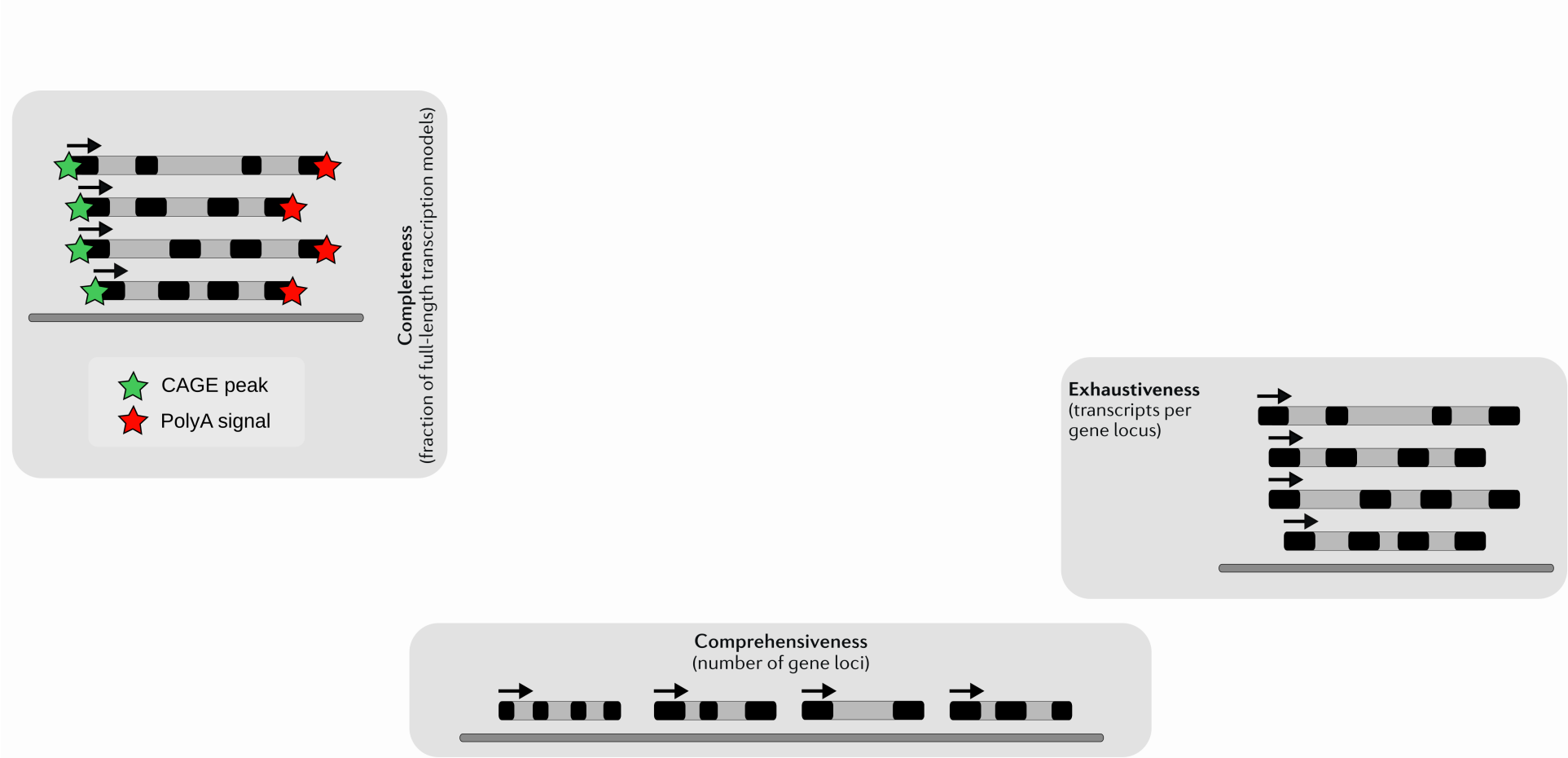
(Lagarde *et al. Nature Genetics*, 2017)

We’re still far from annotation completeness



(Lagarde *et al. Nature Genetics*, 2017)

Comparison of current lncRNA gene catalogs



(Uszczynska-Ratajczak *et al. Nature Reviews Genetics*, 2018)

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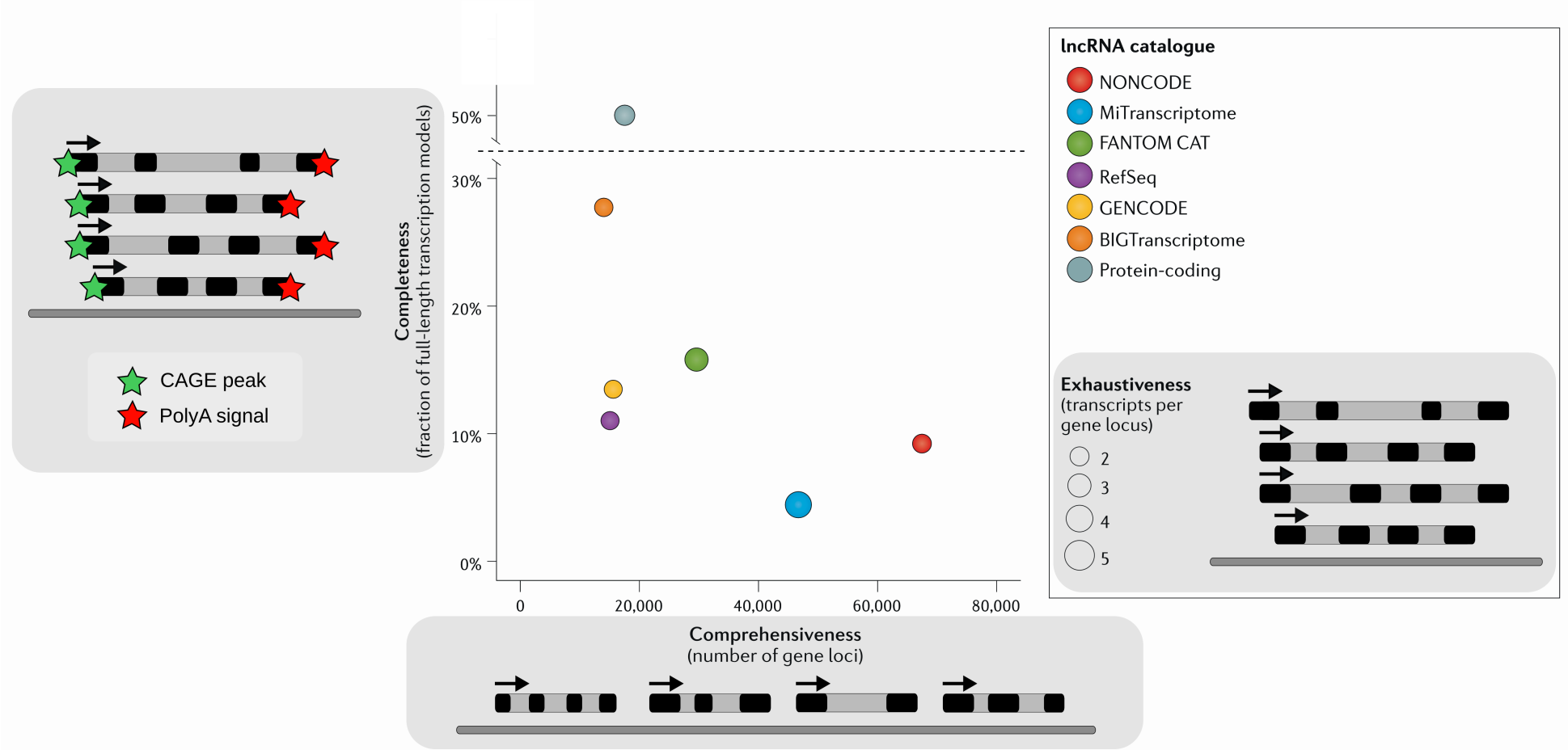

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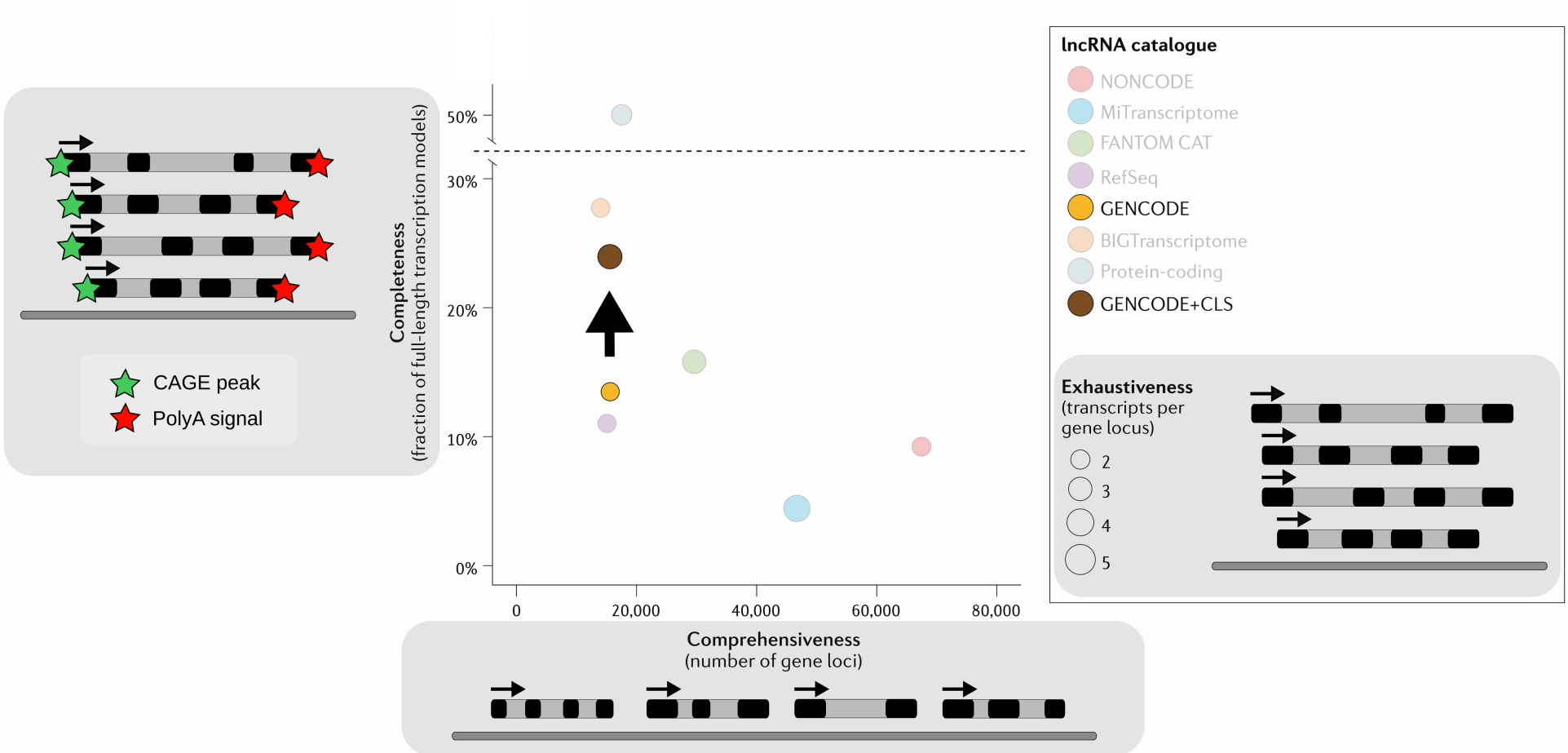
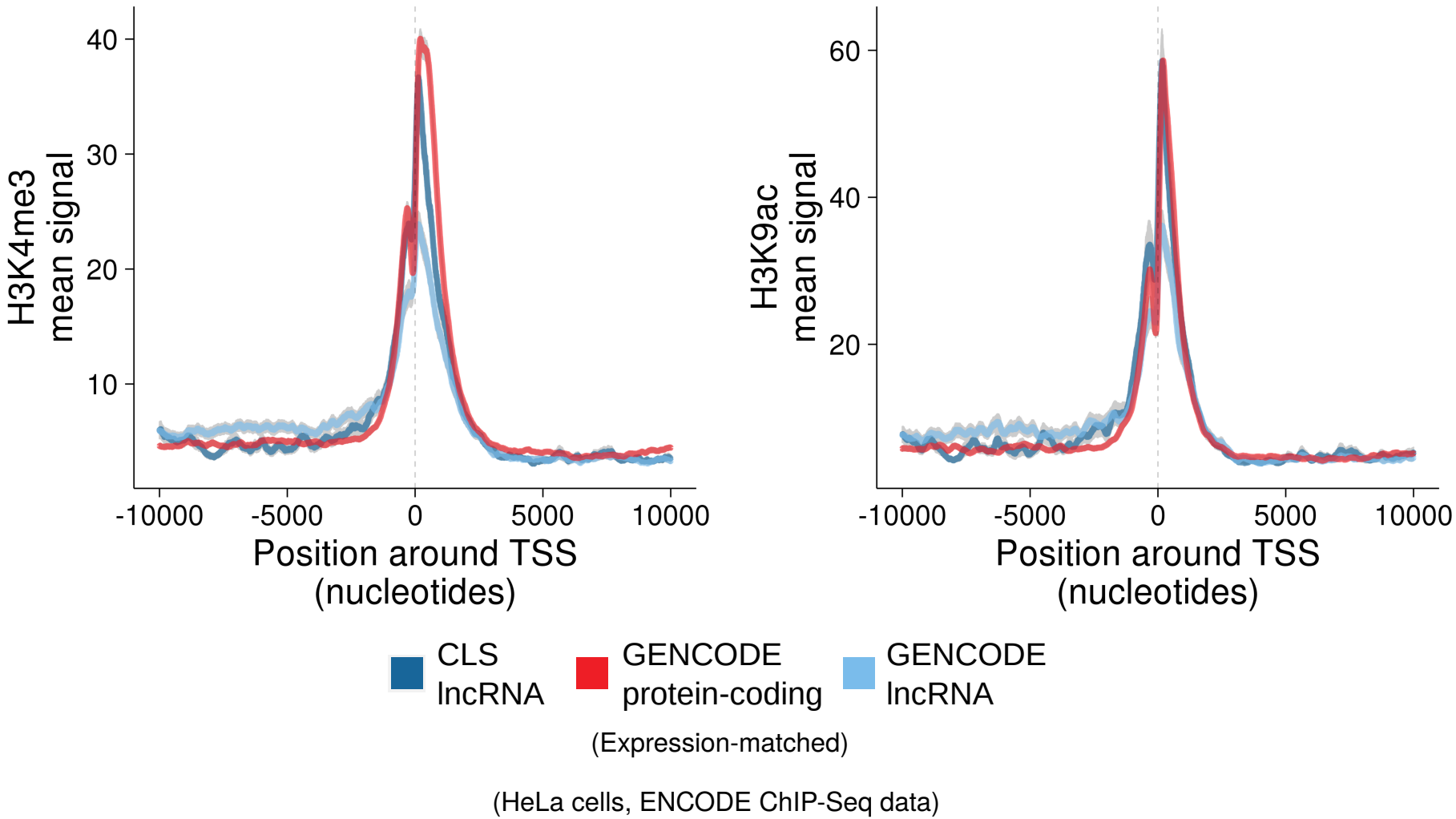


(Uszczynska-Ratajczak *et al. Nature Reviews Genetics*, 2018)

CLS improves GENCODE's

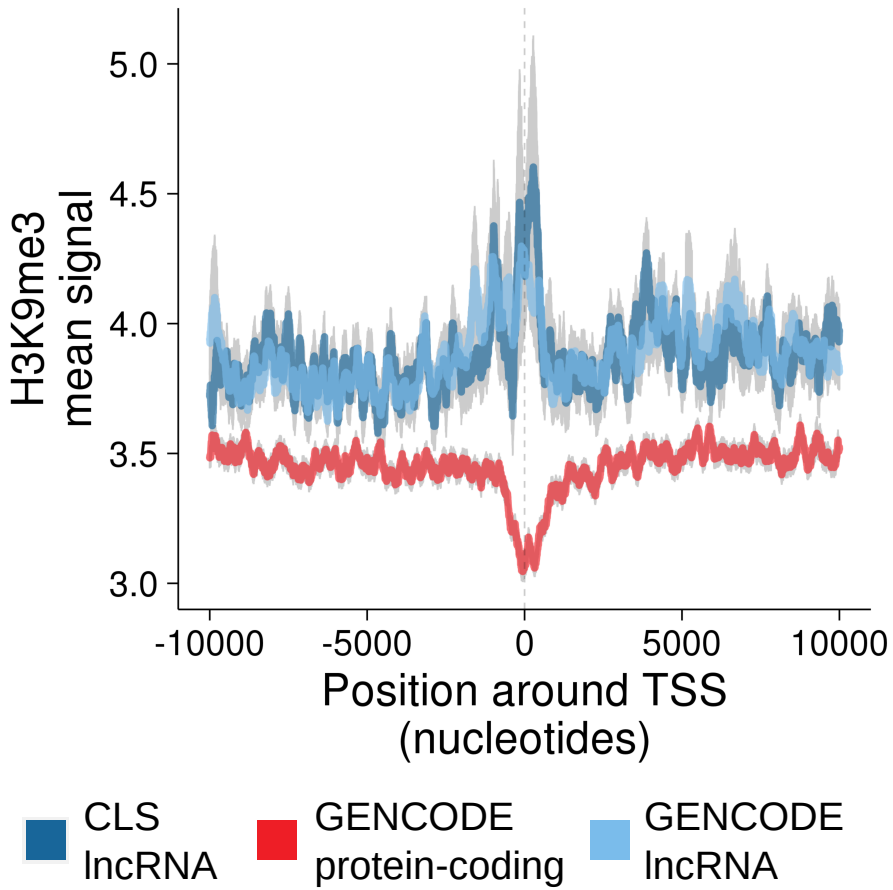
- **exhaustiveness** (1.9 → 3.3 transcripts/locus)
- **completeness** (13.5% → 24%)

LincRNA promoters share **similar levels** of **active** chromatin marks as protein-coding genes



(Lagarde *et al. Nature Genetics*, 2017)

LincRNA promoters show **higher levels** of **repressive** marks than protein-coding genes

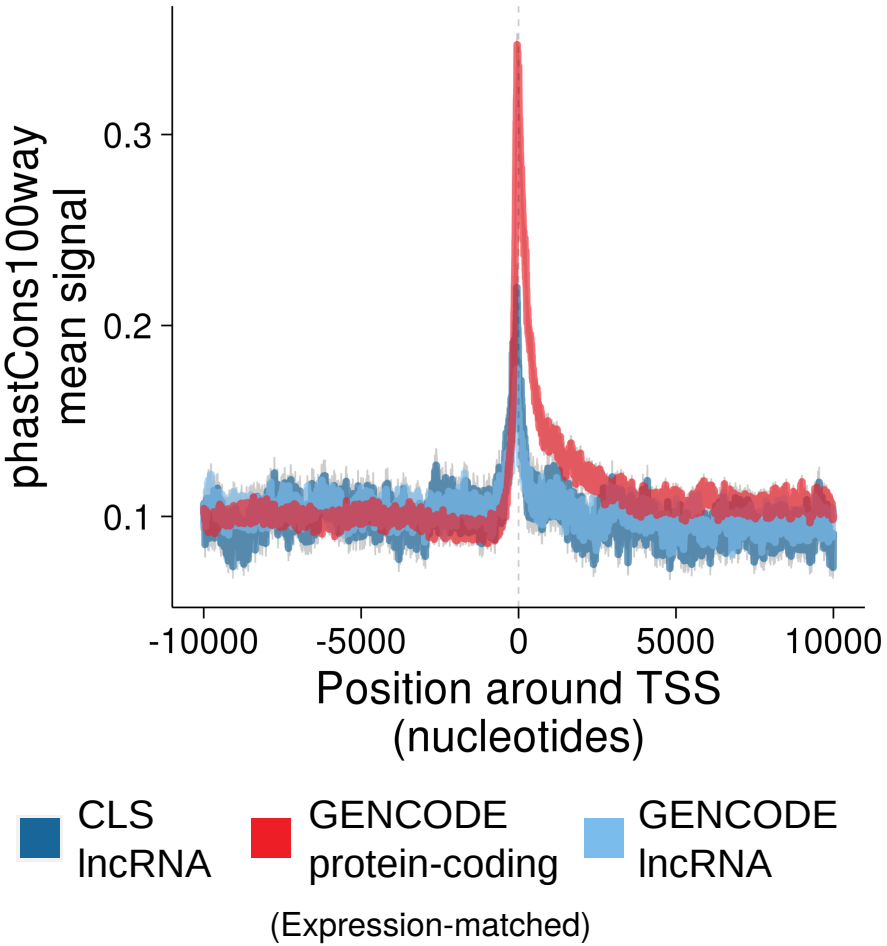


(HeLa cells, ENCODE ChIP-Seq data)

(Lagarde *et al. Nature Genetics*, 2017)

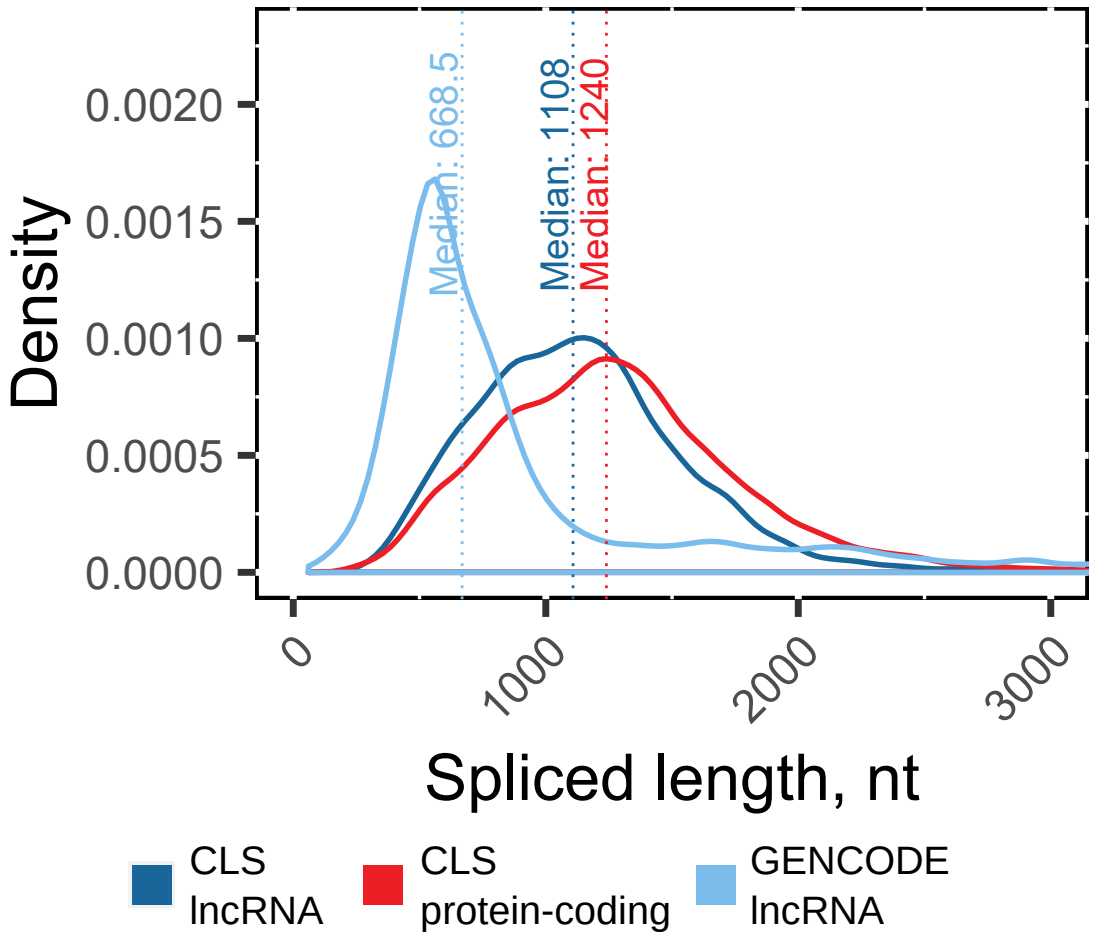
LincRNA promoters are evolutionarily conserved...

... although less than their protein-coding counterparts



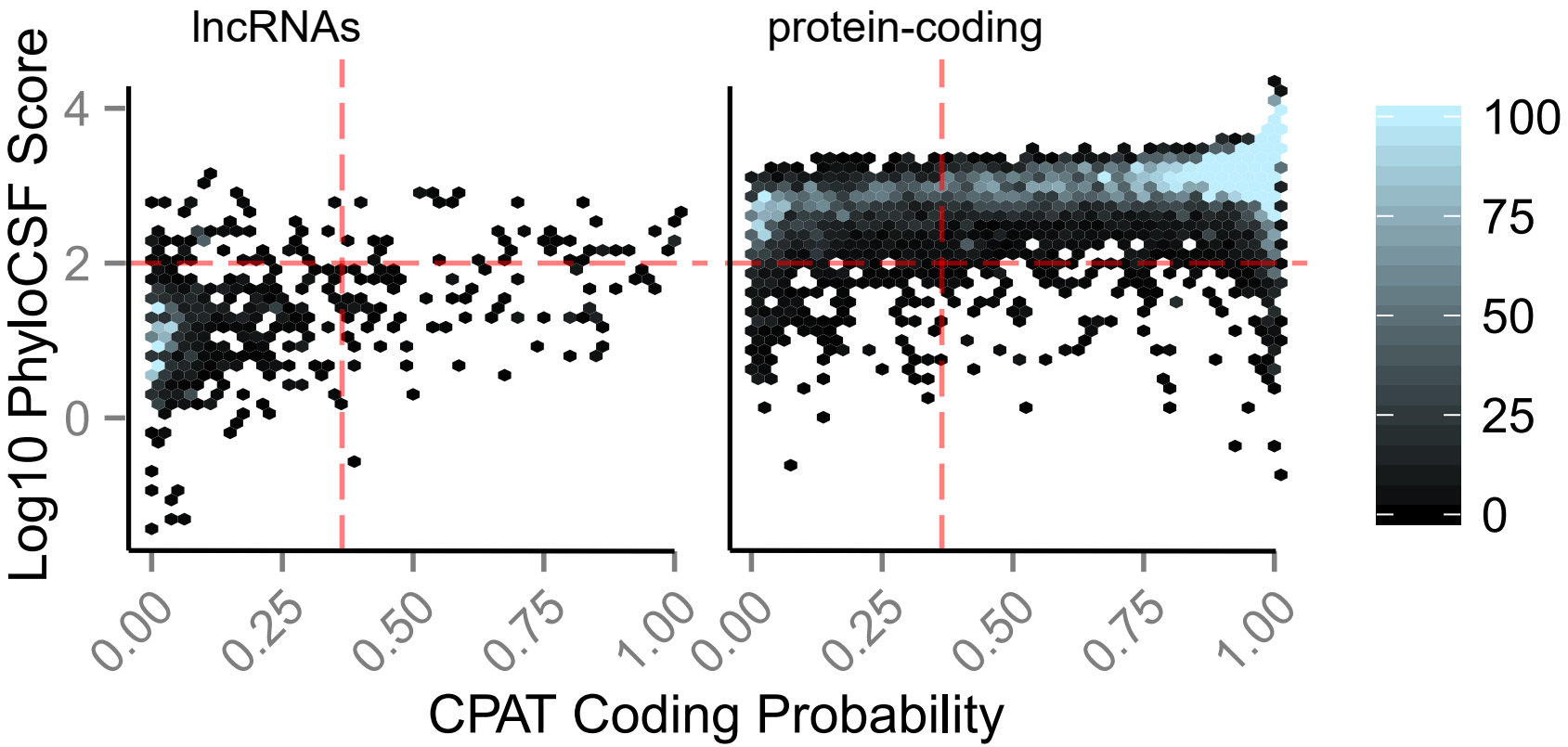
(Lagarde *et al. Nature Genetics*, 2017)

LincRNAs are not much shorter than mRNAs



(Lagarde *et al. Nature Genetics*, 2017)

LincRNAs show low coding potential, even after re-annotation



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Thanks

- **CRG**
  - **Rory Johnson** (University of Bern)
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  - James Wright
- **MIT** (Boston)
  - Irwin Jungreis

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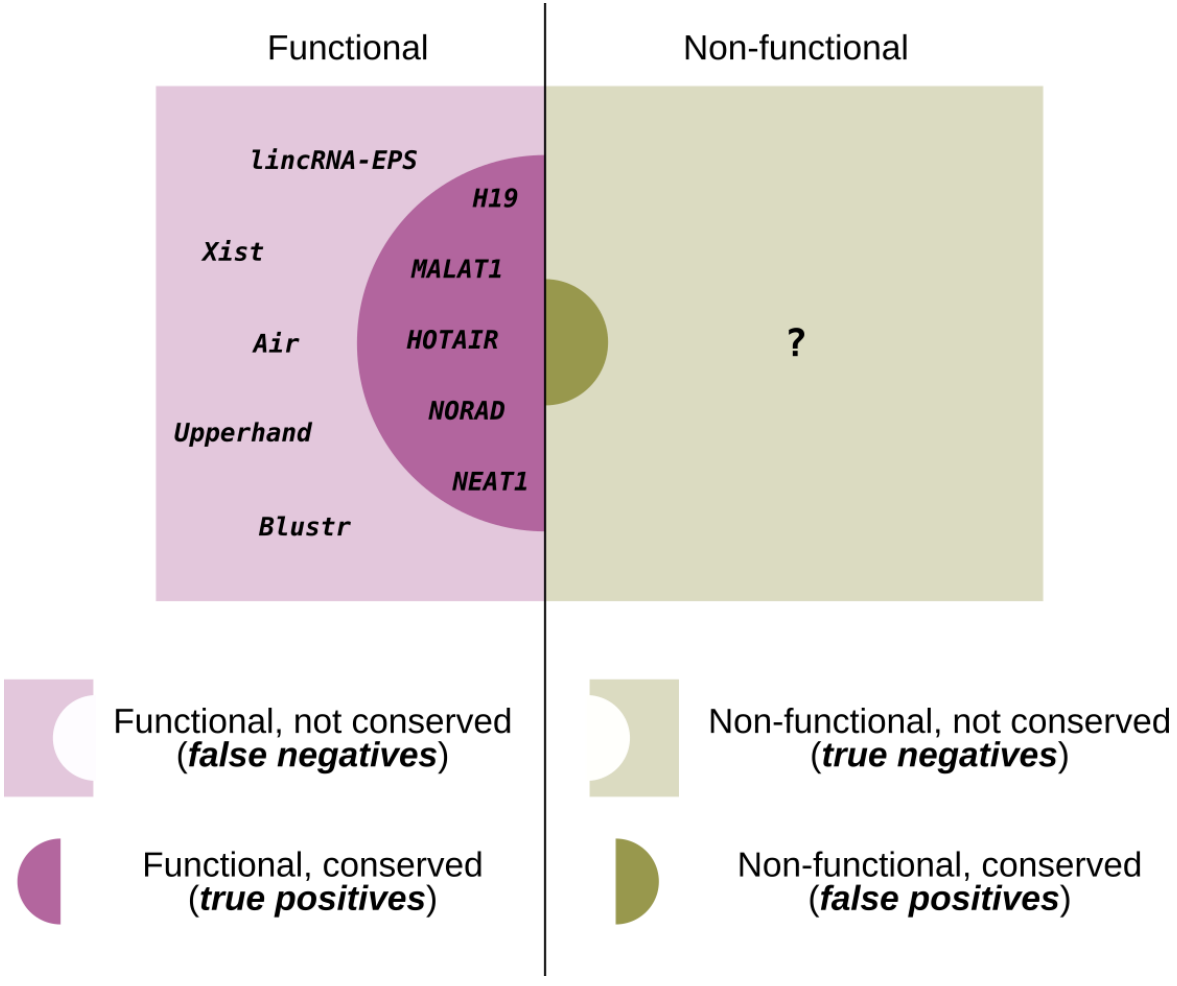
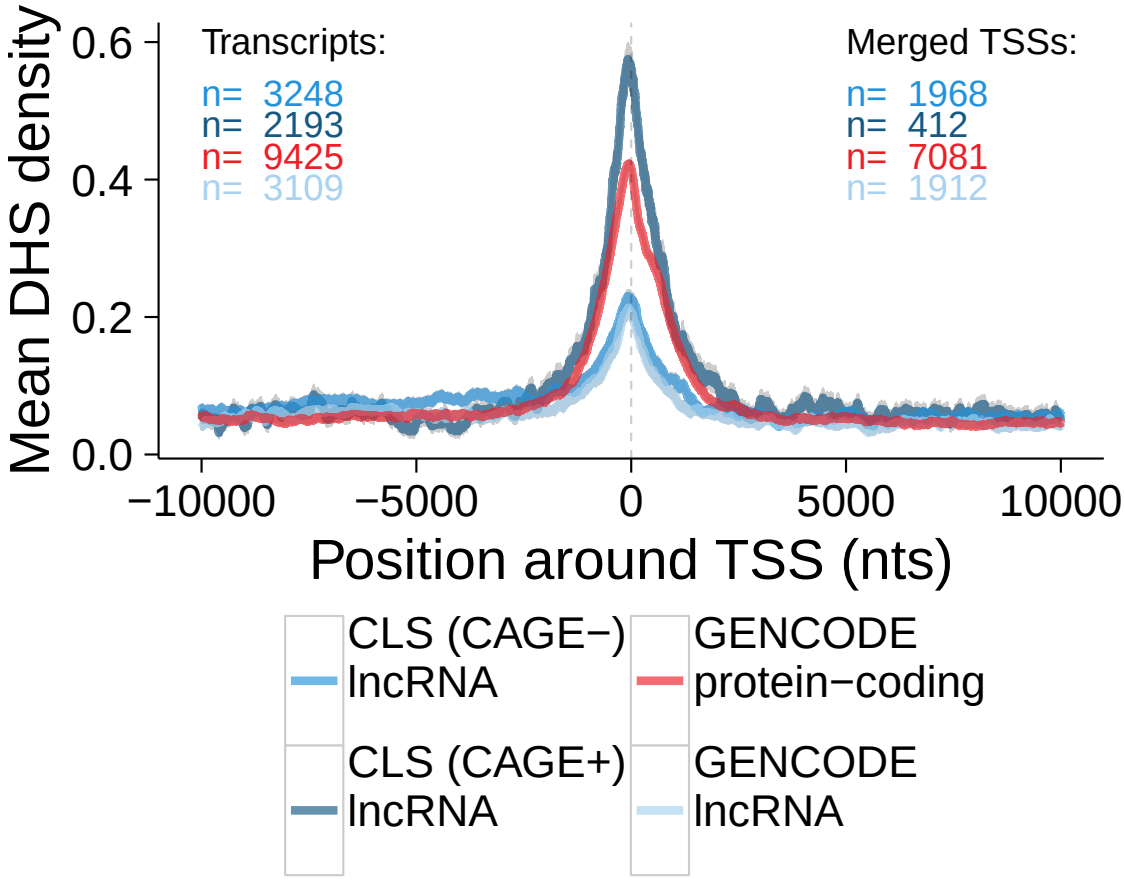
National Human Genome Research Institute

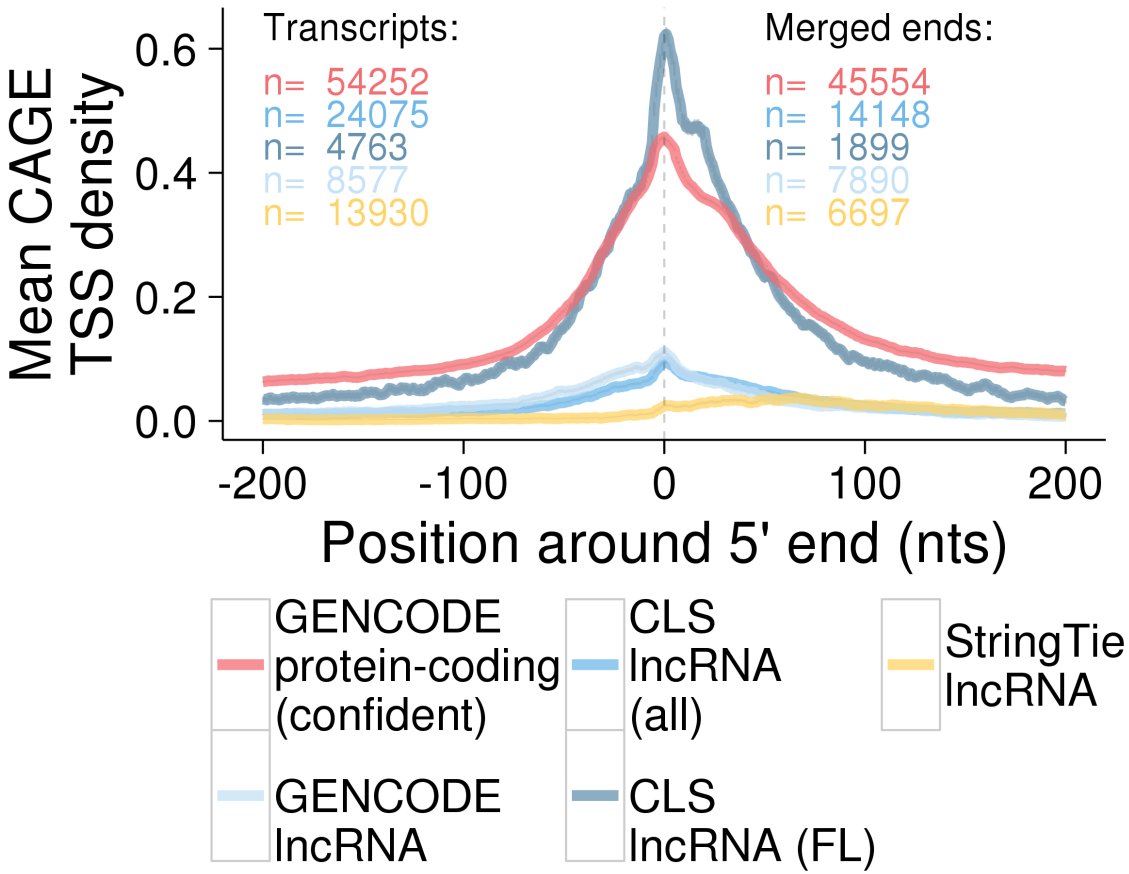
Evolutionary conservation as a predictor of lncRNA function



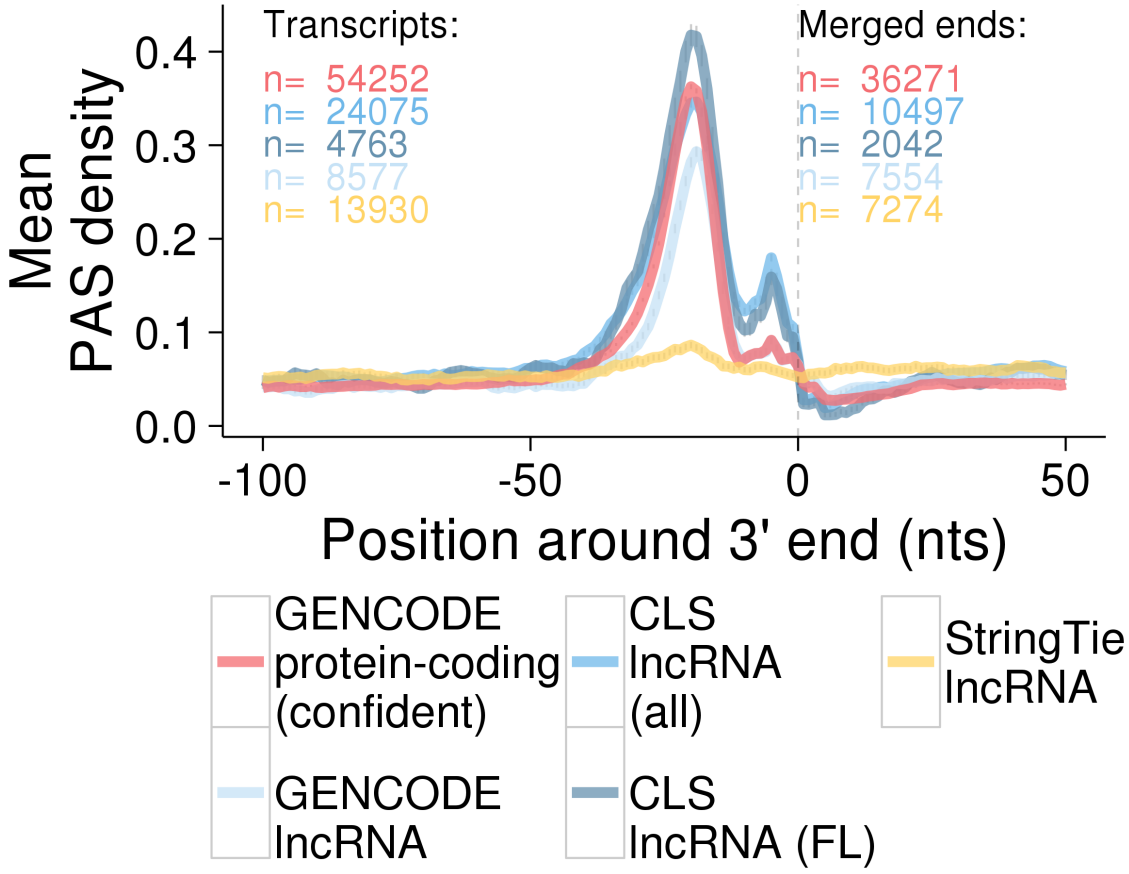
DNAse Hypersensitive sites



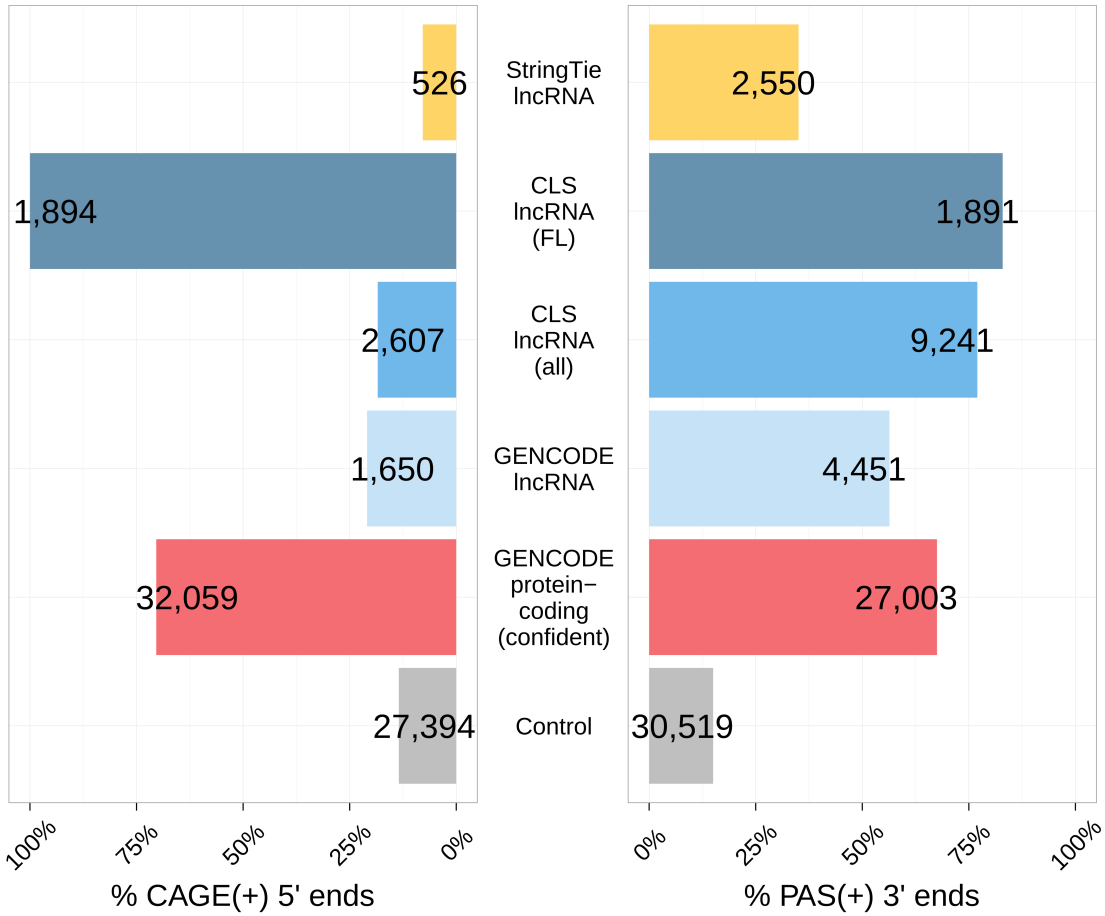
CLS/GENCODE/StringTie vs CAGE



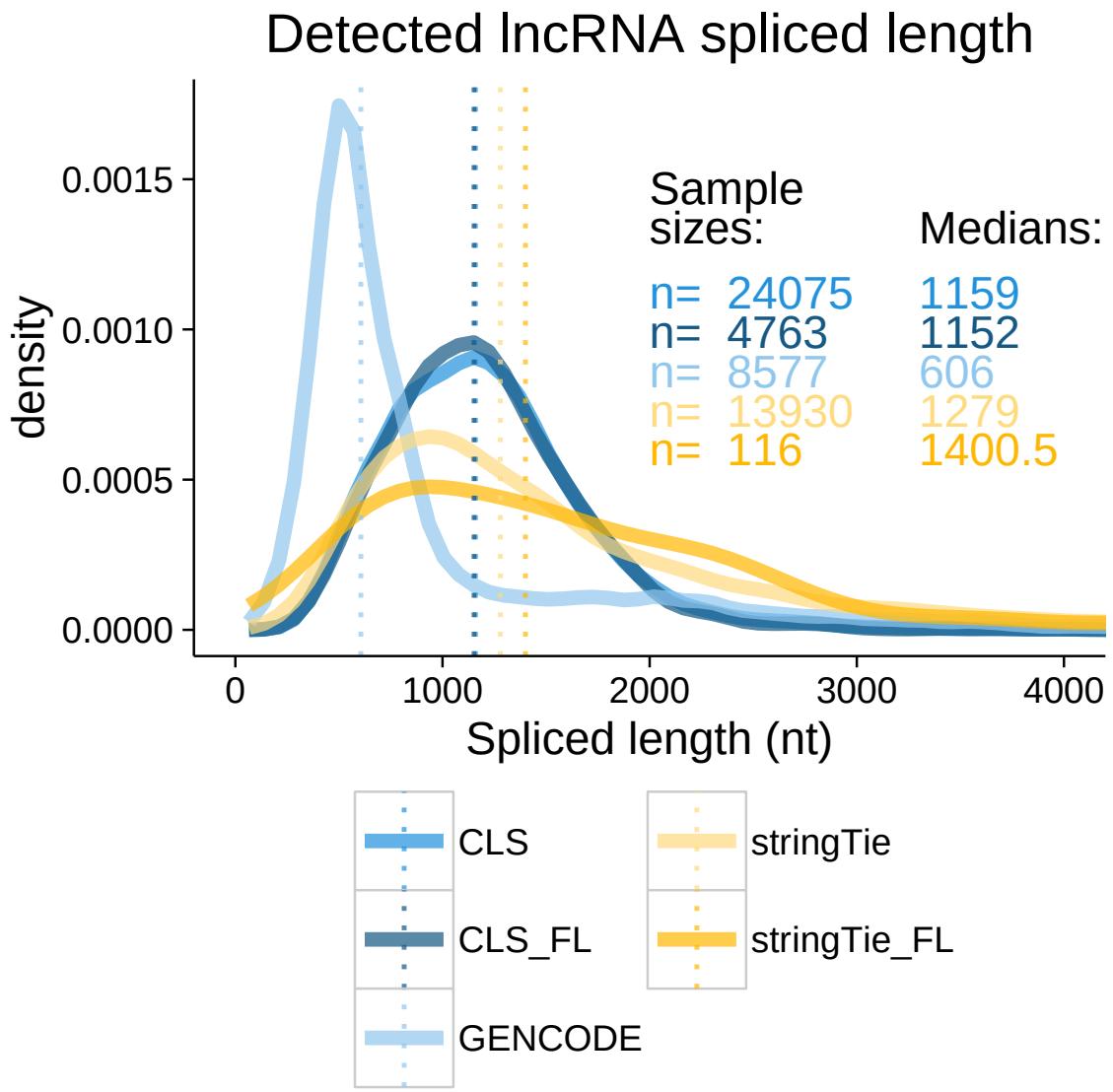
CLS/GENCODE/StringTie vs PAS



CLS/GENCODE/StringTie vs CAGE+PAS



CLS/GENCODE/StringTie TM length



HiSeq Discovery/Saturation