Insights from within the ENCODE project

The interplay of small and long RNAs

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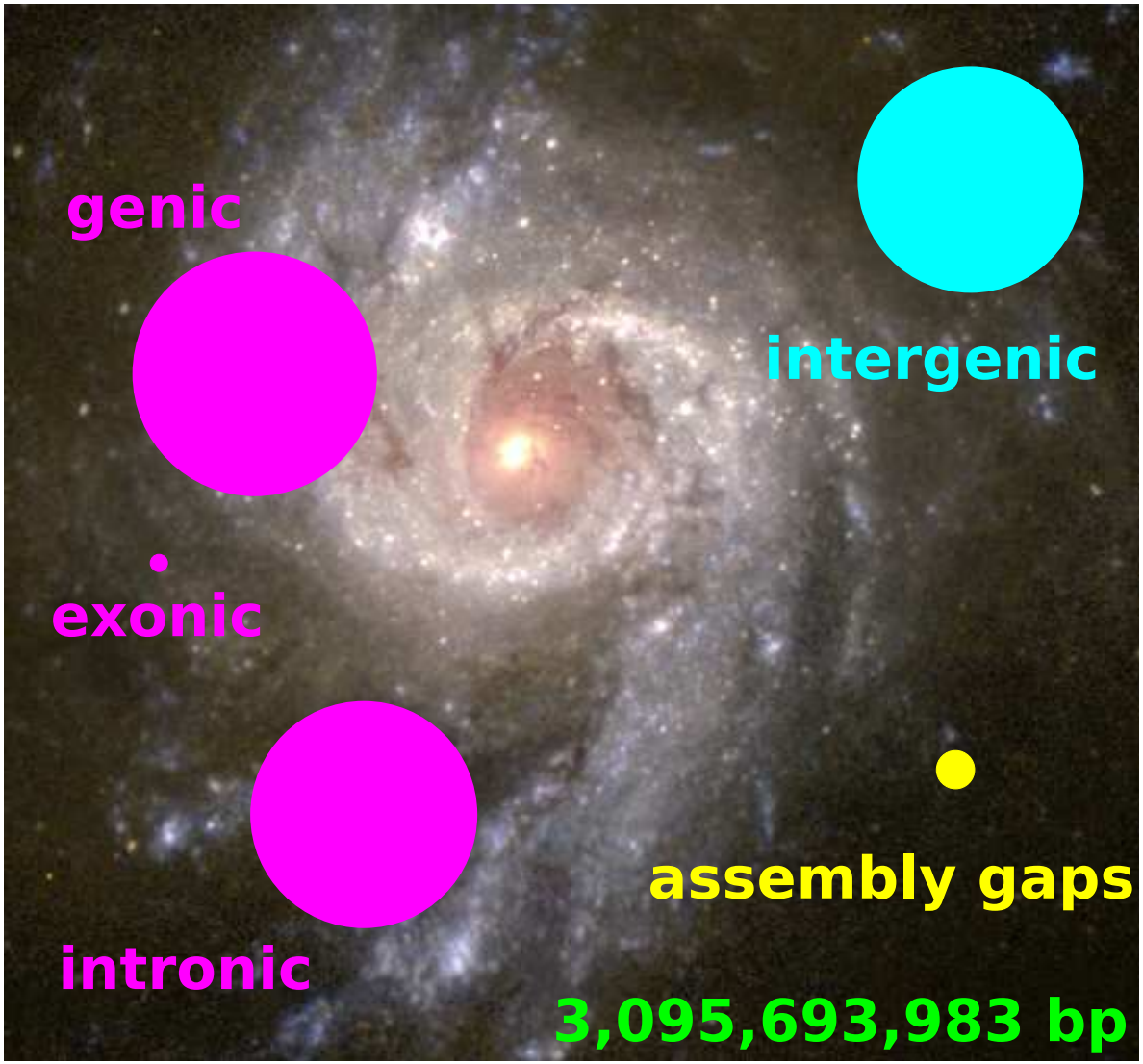
The human genome (GRCh37/hg19)

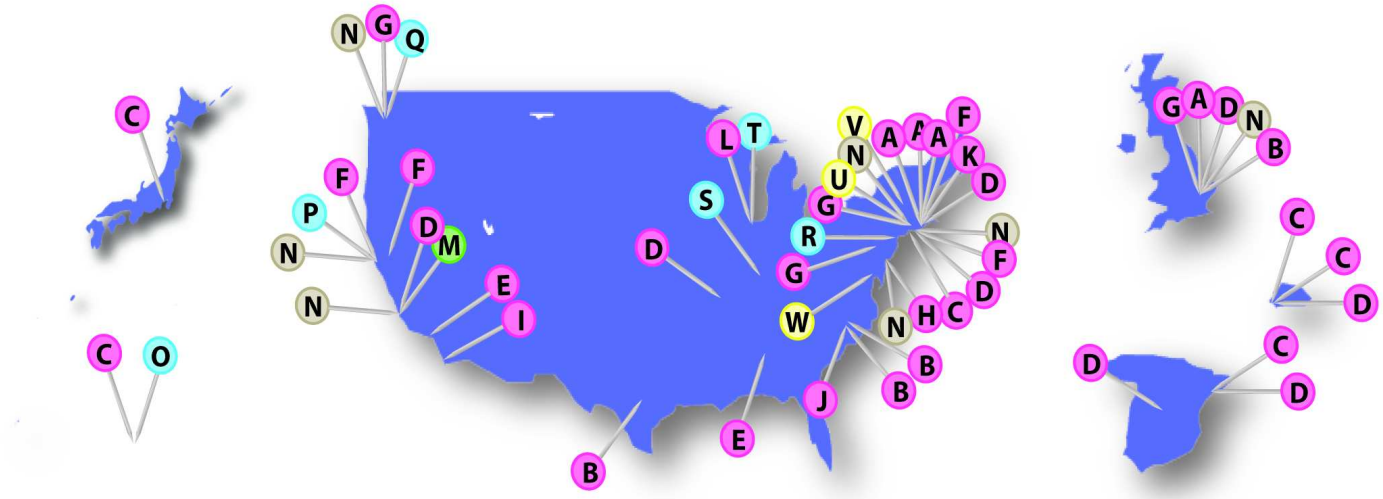
H. sapiens
(Symbolfoto)



ENCODE - What's this?

- **ENC**yclopedia **O**f **D**NA **E**lements
- mission: "enable the scientific and medical communities to interpret the human genome sequence"
- discover and define functional elements (genes, transcripts, transcriptional regulatory regions, chromatin states, DNA methylation patterns)
- introduce standards to ensure high-quality data
- develop novel algorithms
- data are public (with some restrictions)
- collective effort

ENCODE groups around the globe



Production Groups

- A **Broad Institute**, Harvard University, MIT, Cambridge University
- B **Duke University**, University of Texas, Austin, UNC-Chapel Hill, EBI
- C **CSHL**, University of Geneva, Centre for Genomic Regulation, RIKEN, University of Lausanne, Genome Institute of Singapore
- D **Sanger Institute** Washington University, Yale University, Centre for Genomic Regulation, UCSC, MIT, University of Lausanne, CNIO
- E **HudsonAlpha**, Caltech
- F **Stanford University**, Yale University, UC Davis, Harvard University
- G **University of Washington**, U Mass Med School, EBI, Princeton University
- H **Penn State**
- I **UC - San Diego**
- J **UNC - Chapel Hill**
- K **Boston University**
- L **University of Chicago**

Data Coordination Center

- M **UCSC**

Data Analysis Center

- N **EBI**, UC Berkeley, Yale University Penn State, UCSC, University of Washington, U Mass Med School

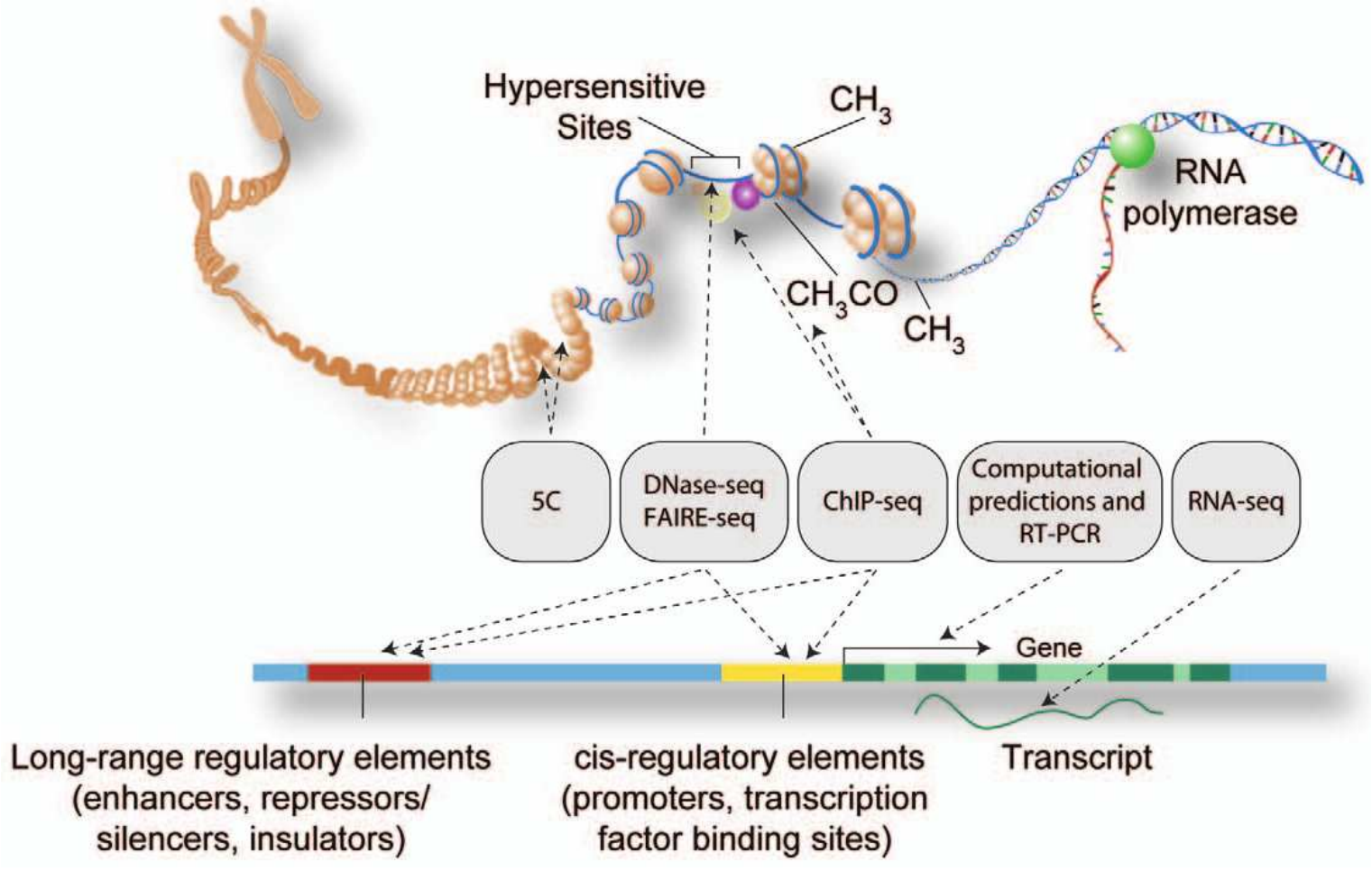
Technology Groups

- O **Genome Institute of Singapore**
- P **Stanford University**
- Q **University of Washington**
- R **Albert Einstein College of Med**
- S **Indiana University**
- T **University of Chicago**

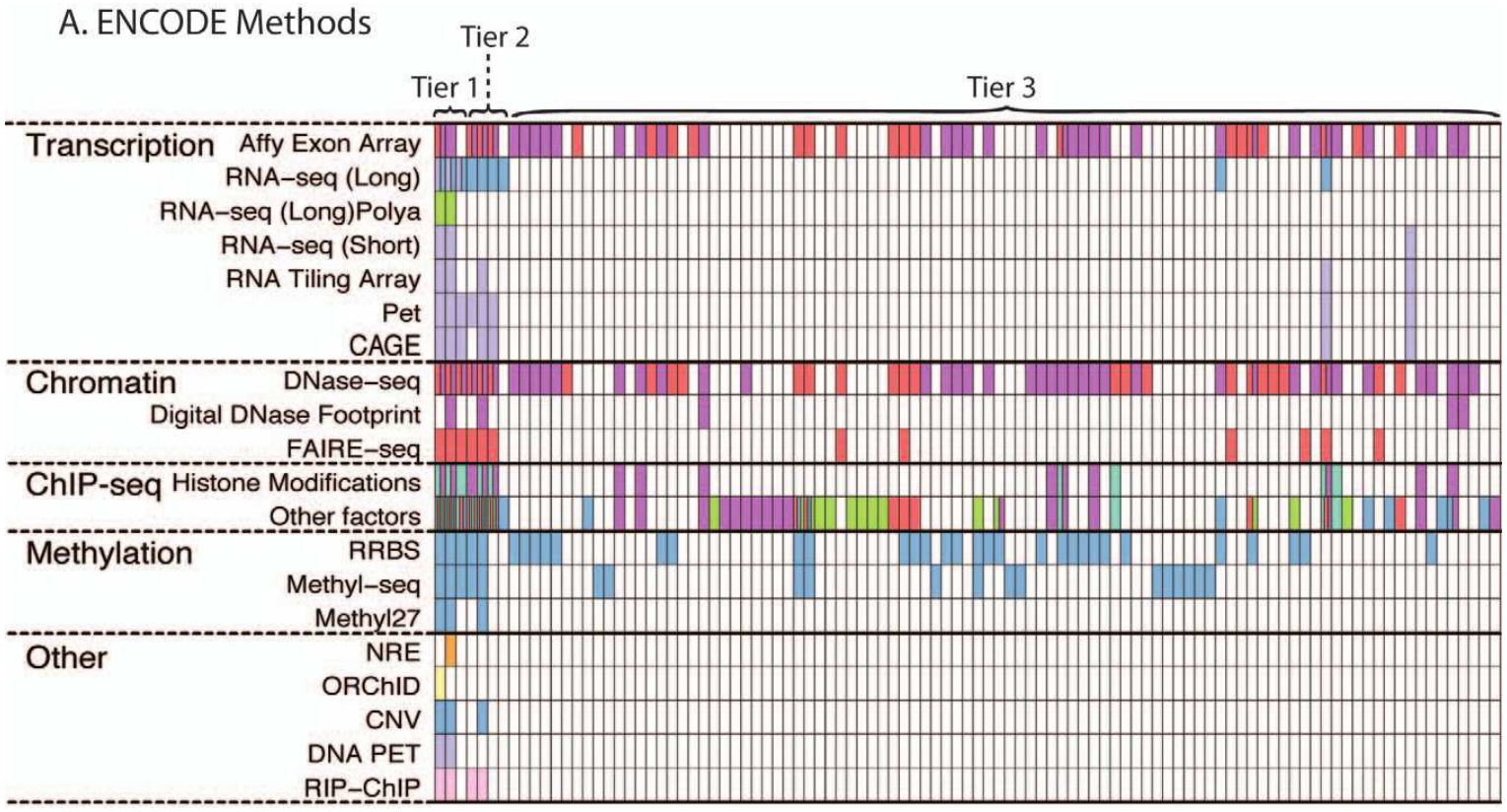
Other Groups

- U **U Mass Med School**
- V **SUNY Albany**
- W **NHGRI**

Organization

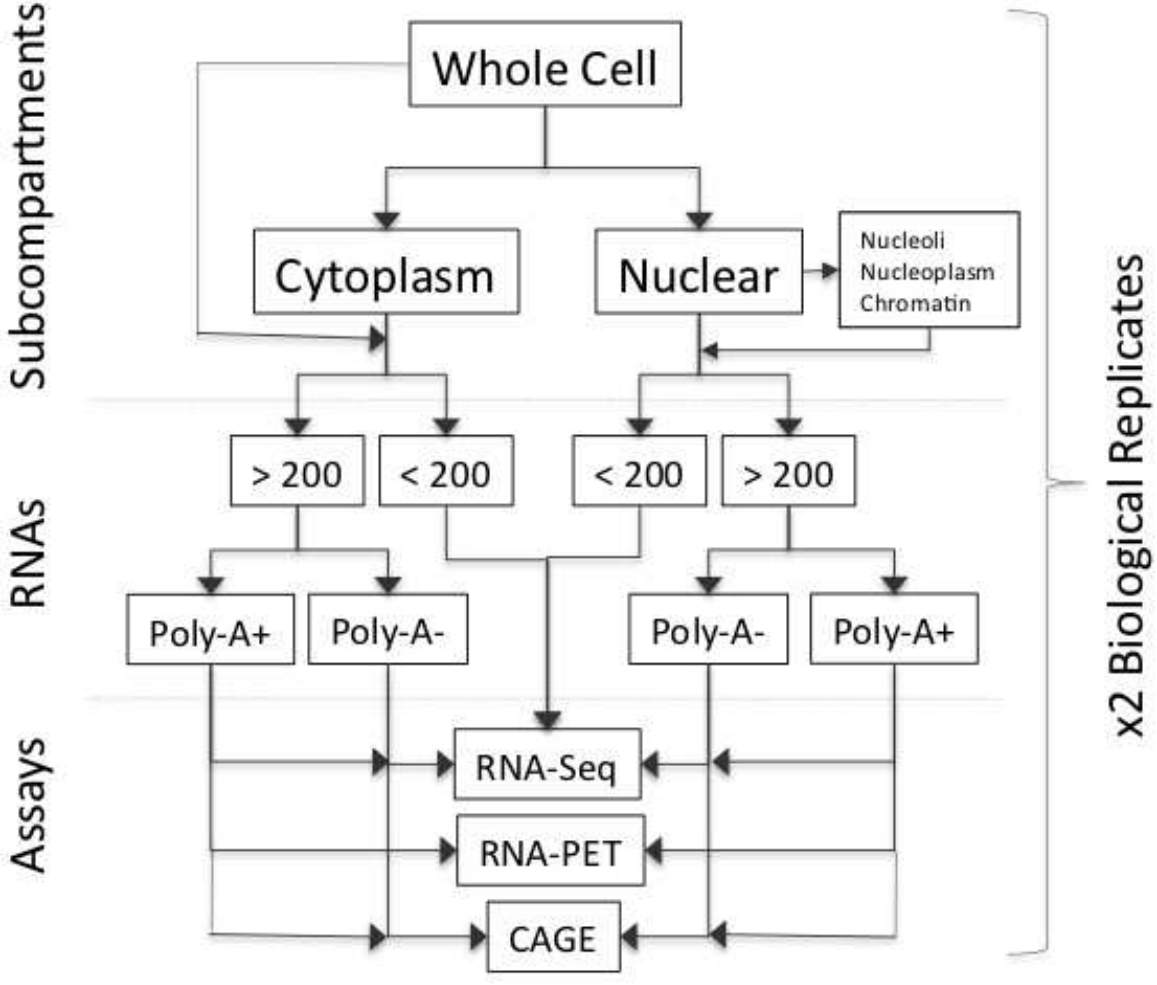


Cell lines used for RNAseq

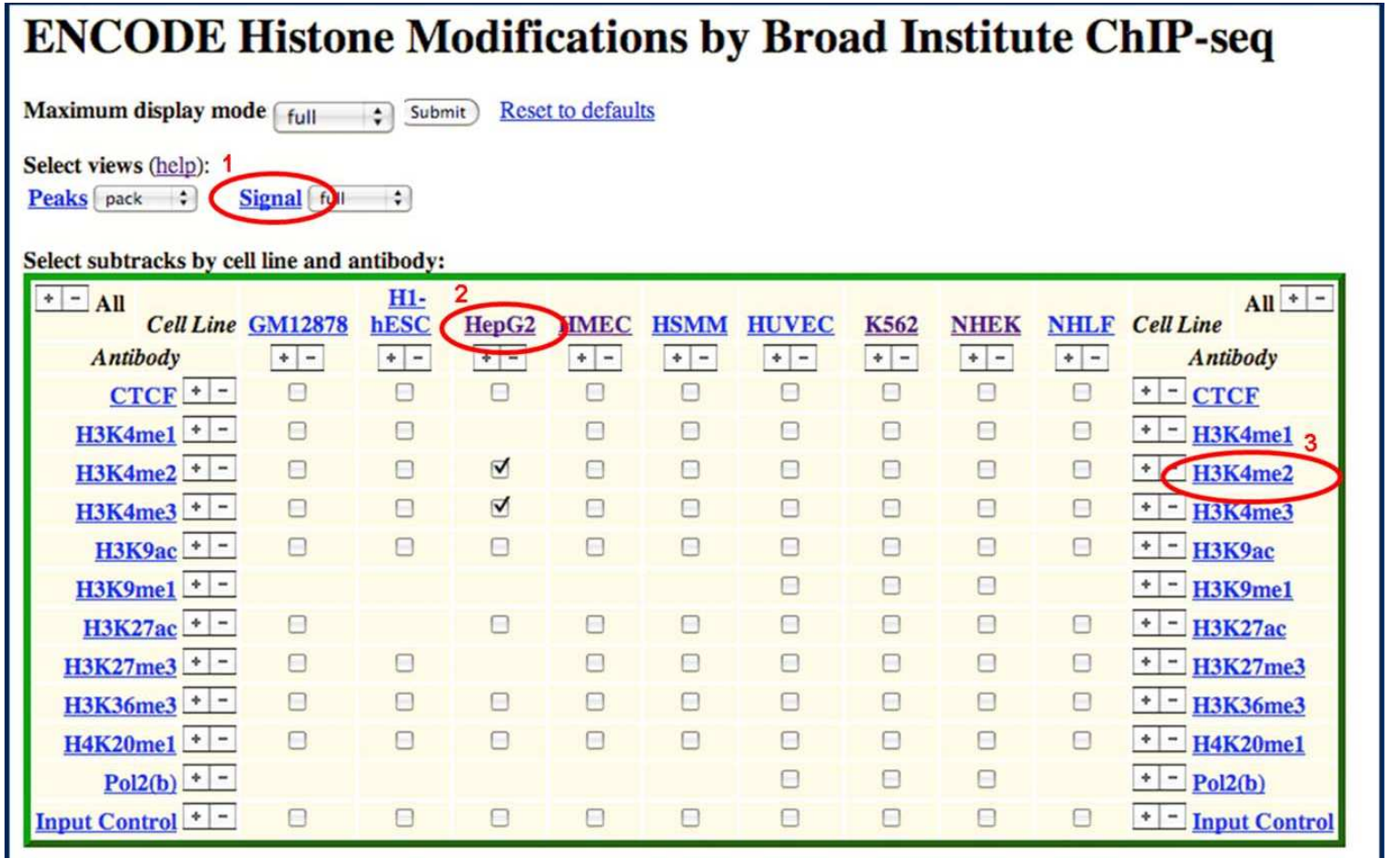


http://genome.ucsc.edu/ENCODE/cellTypes.html

RNA fractionation



Data Repository



ENCODE Histone Modifications by Broad Institute ChIP-seq

Maximum display mode: full — Submit — Reset to defaults

Select views (help): 1

Peaks: pack — Signal: full

Select subtracks by cell line and antibody:

All	Cell Line / Antibody	GM12878	H1-hESC	HepG2	HMEC	HSMM	HUVEC	K562	NHEK	NHLF	Cell Line / Antibody	All
	CTCF	☐	☐	☐	☐	☐	☐	☐	☐	☐	CTCF	
	H3K4me1	☐	☐		☐	☐	☐	☐	☐	☐	H3K4me1	
	H3K4me2	☐	☐	☑	☐	☐	☐	☐	☐	☐	H3K4me2	
	H3K4me3	☐	☐	☑	☐	☐	☐	☐	☐	☐	H3K4me3	
	H3K9ac	☐	☐	☐	☐	☐	☐	☐	☐	☐	H3K9ac	
	H3K9me1						☐	☐	☐		H3K9me1	
	H3K27ac	☐		☐	☐	☐	☐	☐	☐	☐	H3K27ac	
	H3K27me3	☐	☐		☐	☐	☐	☐	☐	☐	H3K27me3	
	H3K36me3	☐	☐	☐	☐	☐	☐	☐	☐	☐	H3K36me3	
	H4K20me1	☐	☐	☐	☐	☐	☐	☐	☐	☐	H4K20me1	
	Pol2(b)						☐	☐	☐		Pol2(b)	
	Input Control	☐	☐	☐	☐	☐	☐	☐	☐	☐	Input Control	

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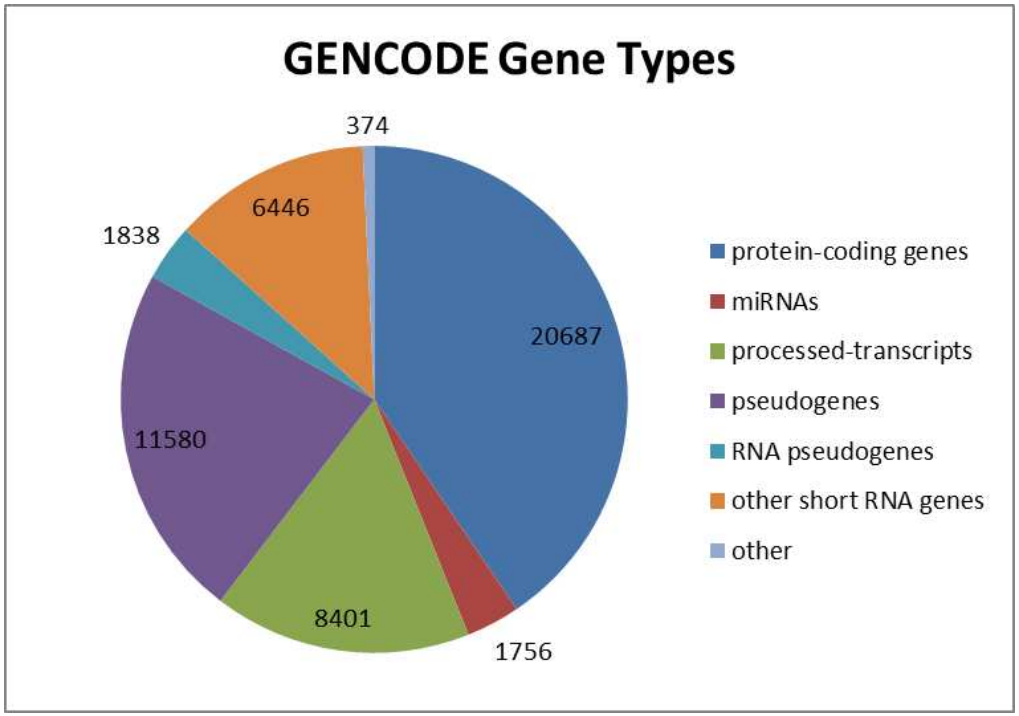
data release policy:
`http://www.encodeproject.org/ENCODE/terms.html`

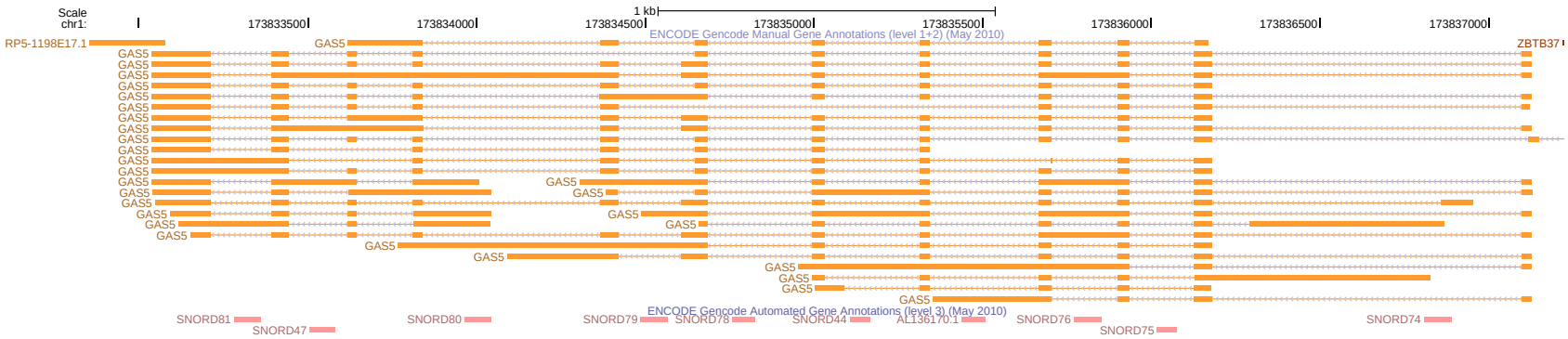
GENCODE Annotation



- provided by Sanger center
- integration of ENSEMBL and HAVANA
- manually curated annotation
- 51,082 genes
- 161,375 transcripts
- majority of tx is noncoding

Host Genes

- several small noncoding RNAs (ncRNAs) not transcribed as individual genes
- processed from introns of longer, multi-exonic, and often non protein coding polII transcripts
- $\rightarrow$ 'polycistronic transcripts' driven by a single promoter, several genes (i.e. small RNAs) are transcribed and subsequently processed into the functional products by nucleases.
- examples: snoRNA host genes GAS5 and RPL7a



Processing of small ncRNAs from host genes

snoRNAs:

- processed from introns (lariat debranching)
- or exonucleolytic cleavage

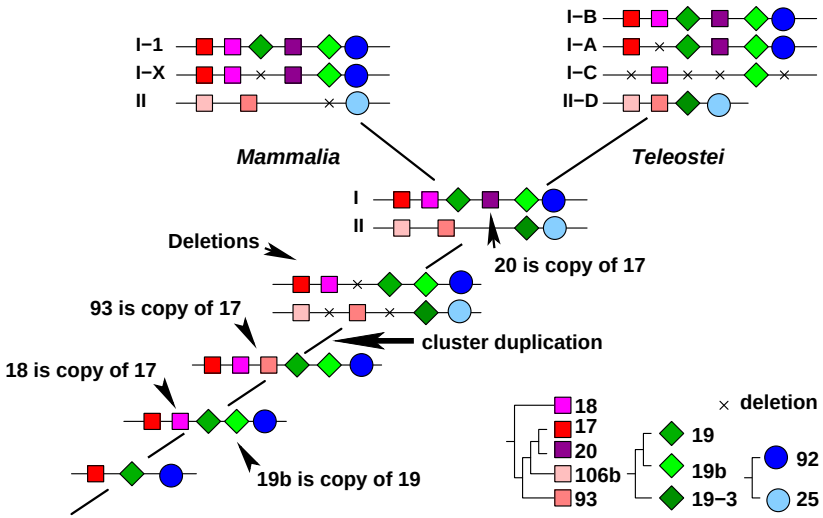
miRNAs

- processed post- or co-transcriptional
- processing does not interfere with splicing

small RNAs often functionally related to host genes

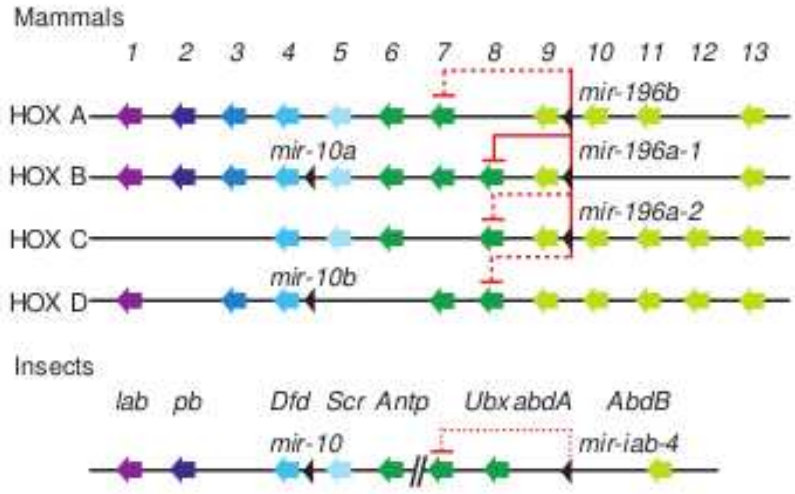
Evolution of miRNAs

miRNA-17 cluster

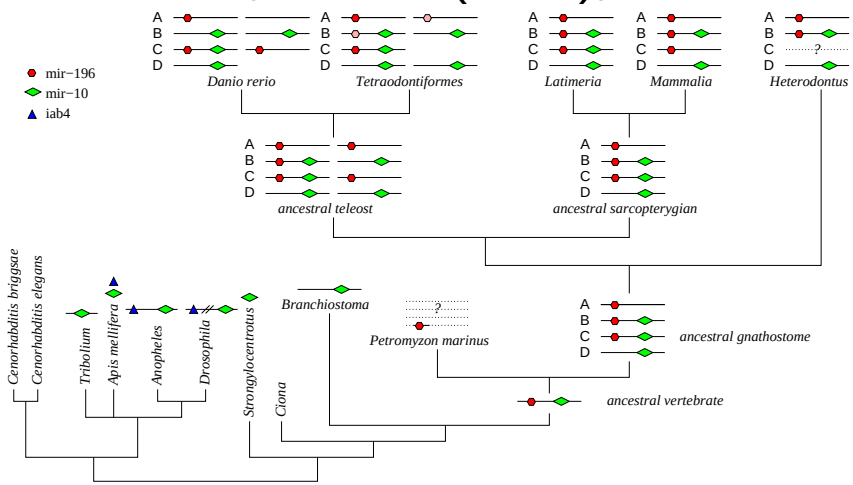


Tanzer and Stadler. J Mol Biol. 2004; 339:327-35

miRNA 10 & 196



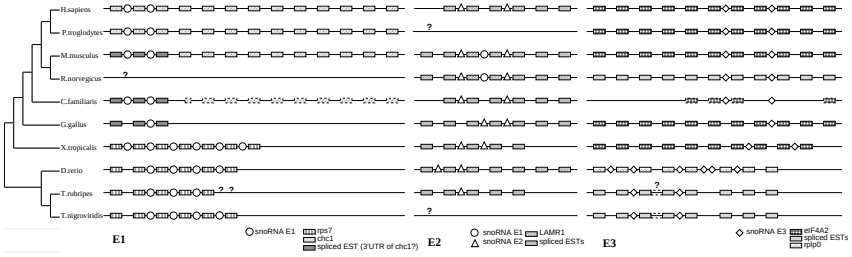
Yekta *et al.*, Science(2004), 304:594-6



Tanzer *et al.* J Exp Zoolog B Mol Dev Evol. 2005; 304:75-85

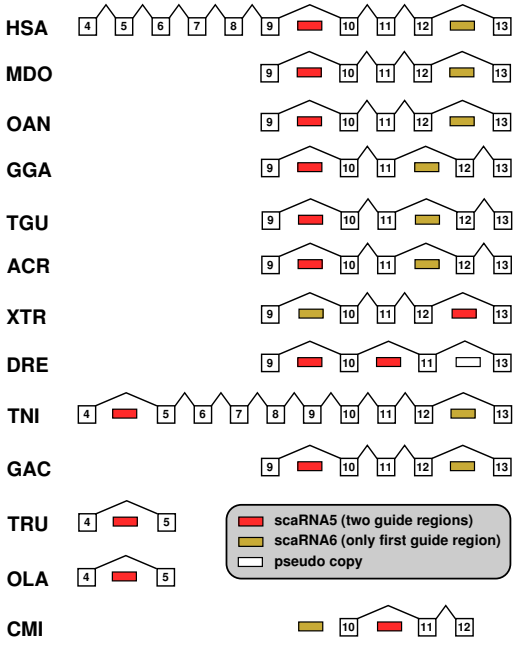
snoRNAs and their host genes

snoRNAs E1, E2, E3



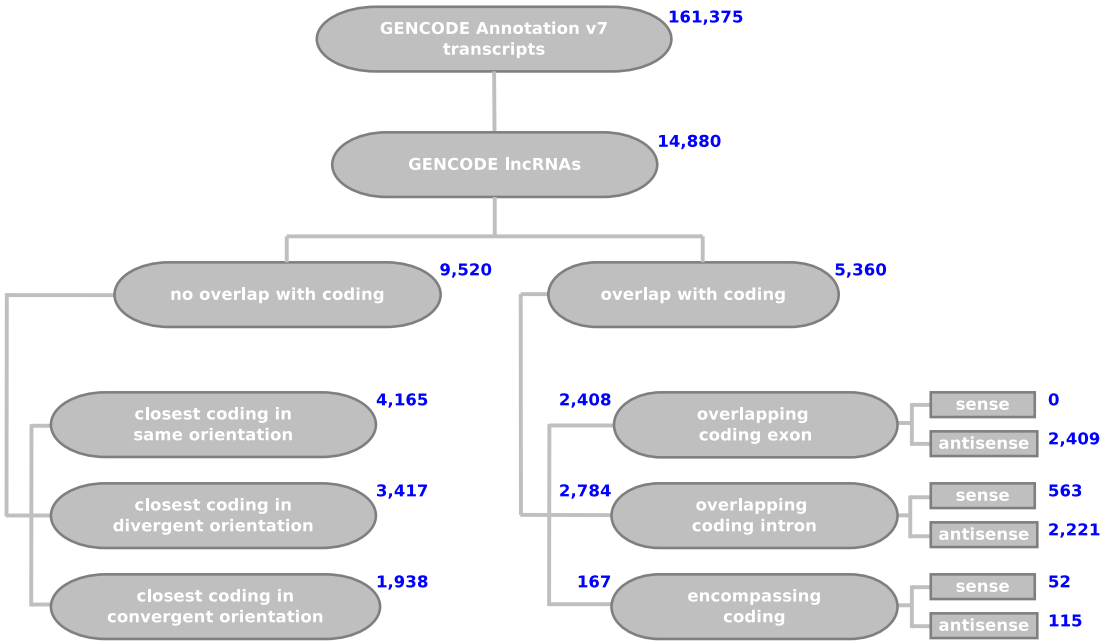
Bompfünewerer *et al.* Th. Biosci. 2005; 123:301-369

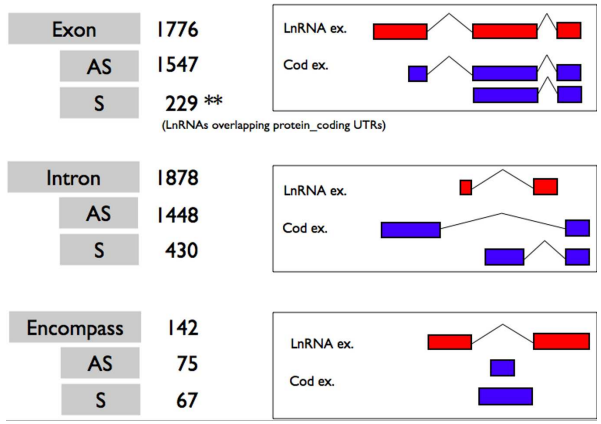
scaRNA5&6



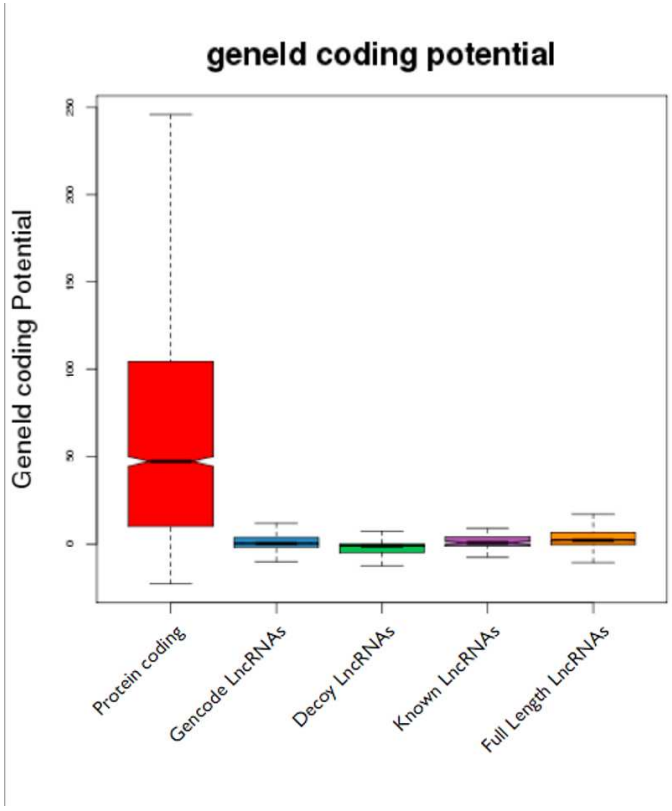
Marz et al., RNA Biol.(2011), 8:938-46

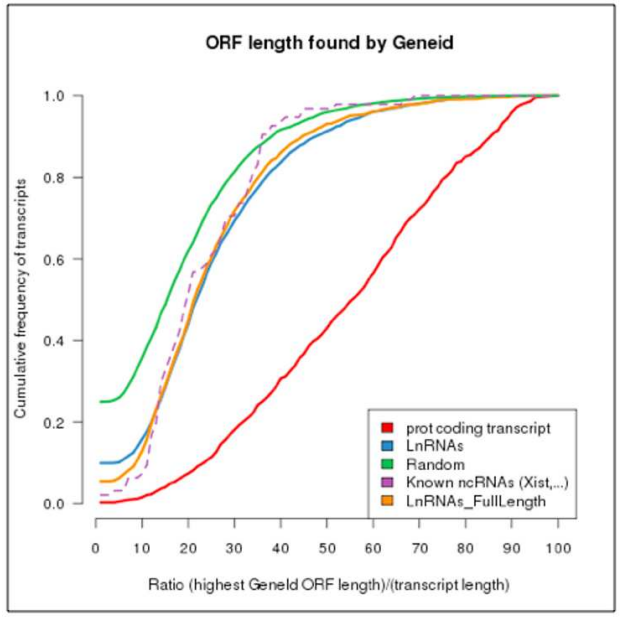
Long noncoding RNAs - genomic context





Are long noncoding RNAs non coding?

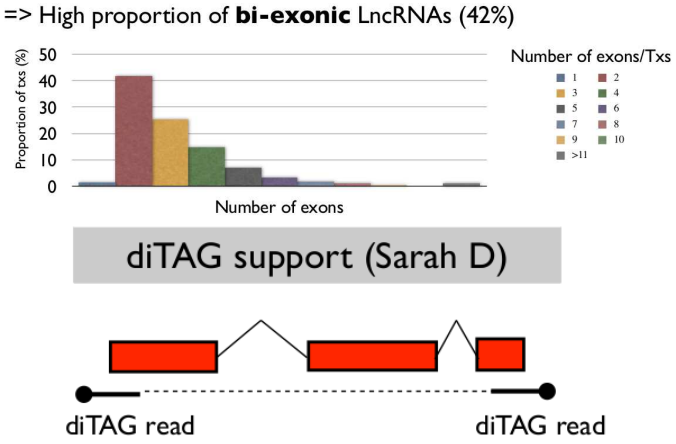




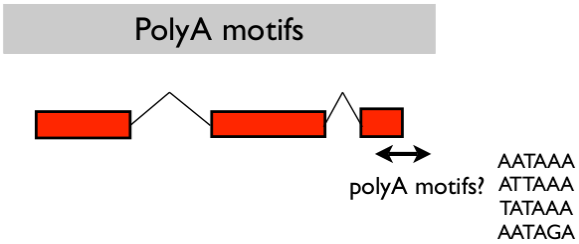
- coding potential in the range of other known ncRNAs
- ORF length: 20% of sequence (protein coding: 60%)

lncRNAs share mRNA like features

exon structure and number

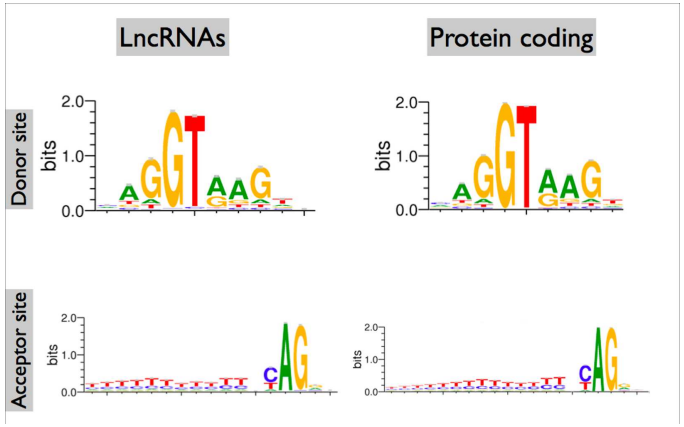


polyA sites



Category	% transcripts 100 bp TTS	200 bp TTS
Coding	54.7	80.3
LncRNAs	53.5	81.7

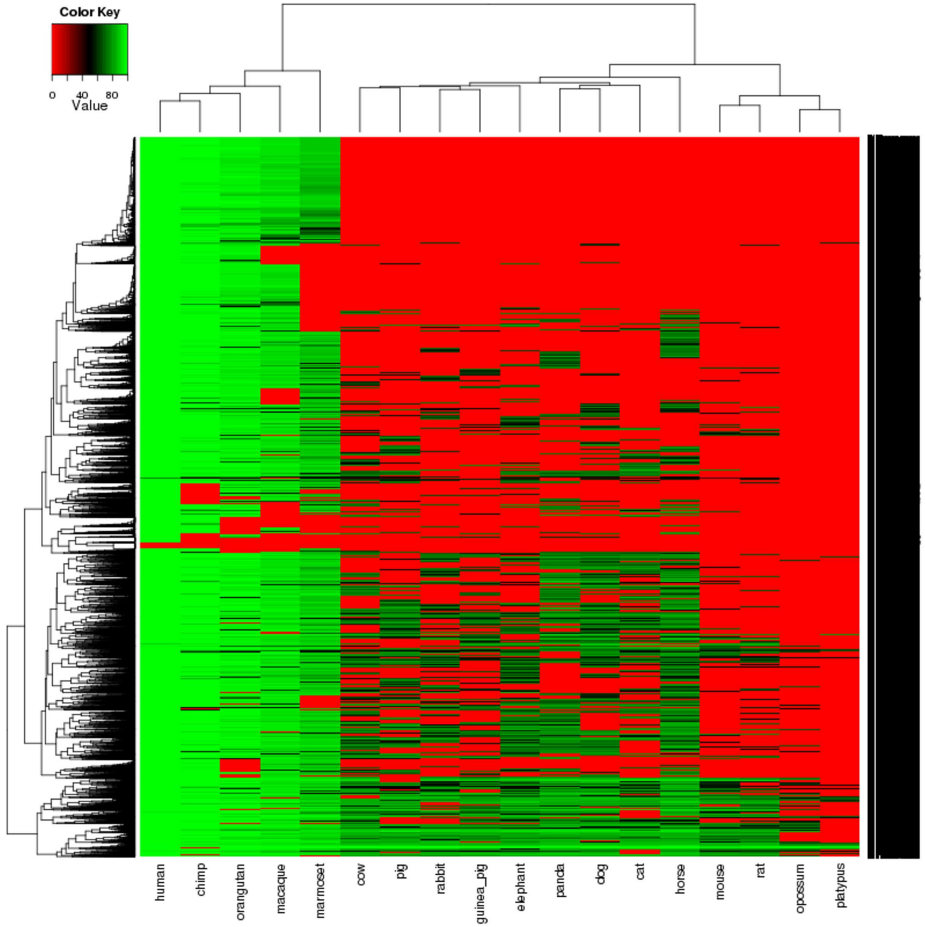
splice sites



	% introns lncRNA	prot. cod.
Donor site		
GT	95.046	99.027
GC	2.271	0.797
AT	0.993	0.016
Acceptor site		
AG	97.087	99.796
AC	1.088	0.070
GG	0.308	0.015

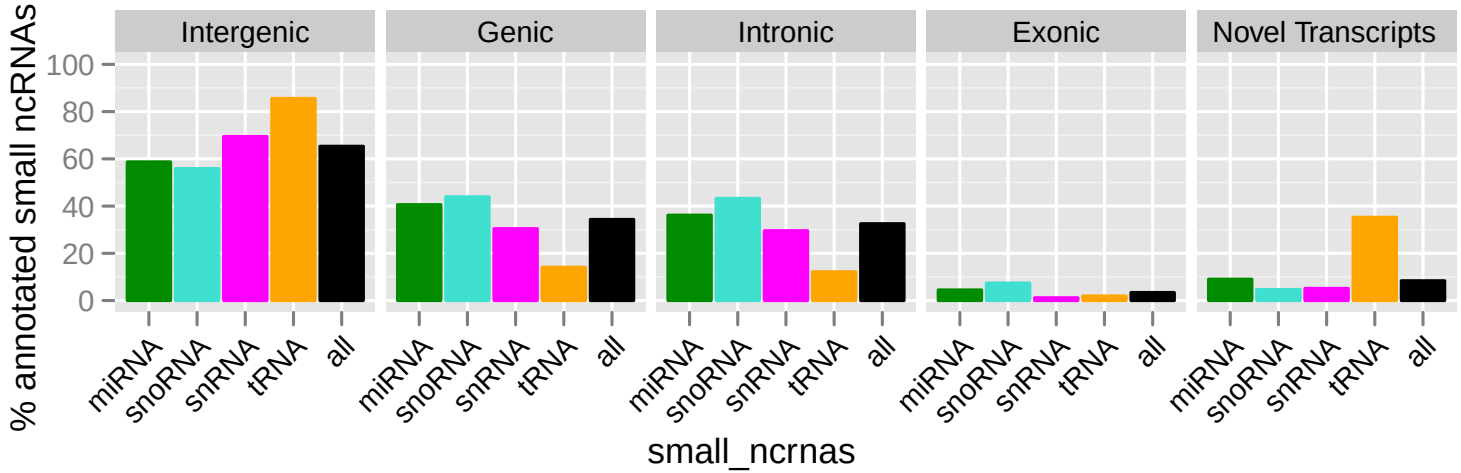
- mean transcript length: 938bp
- mRNA like poly A sites
- 42% bi-exonic transcripts
- full length: only 10% supported by ditags ← low expression?

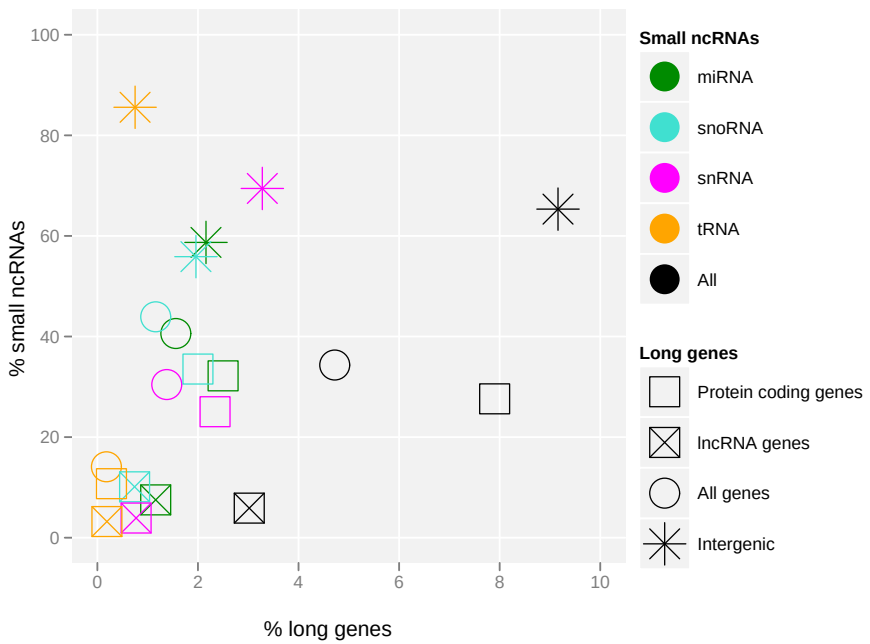
Conservation of lncRNAs



- blast/exonerate
- 31% primate specific
- 4% conserved acroos all species
- 194 families

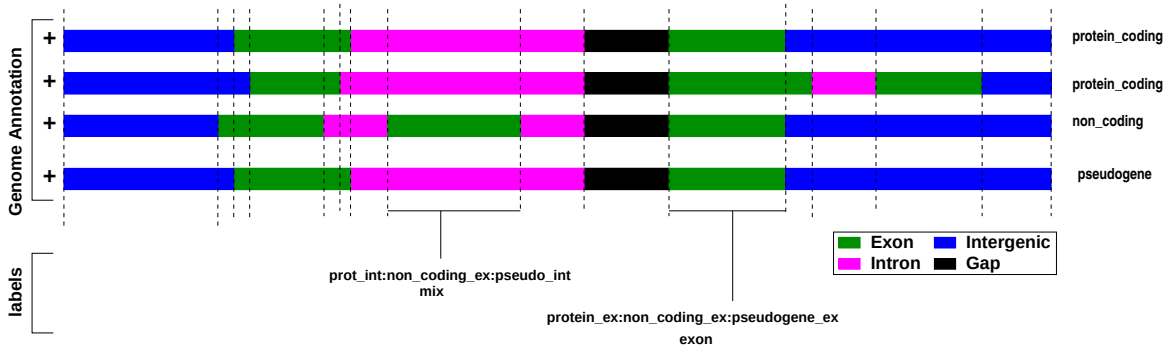
Small ncRNAs in GENCODE annotations

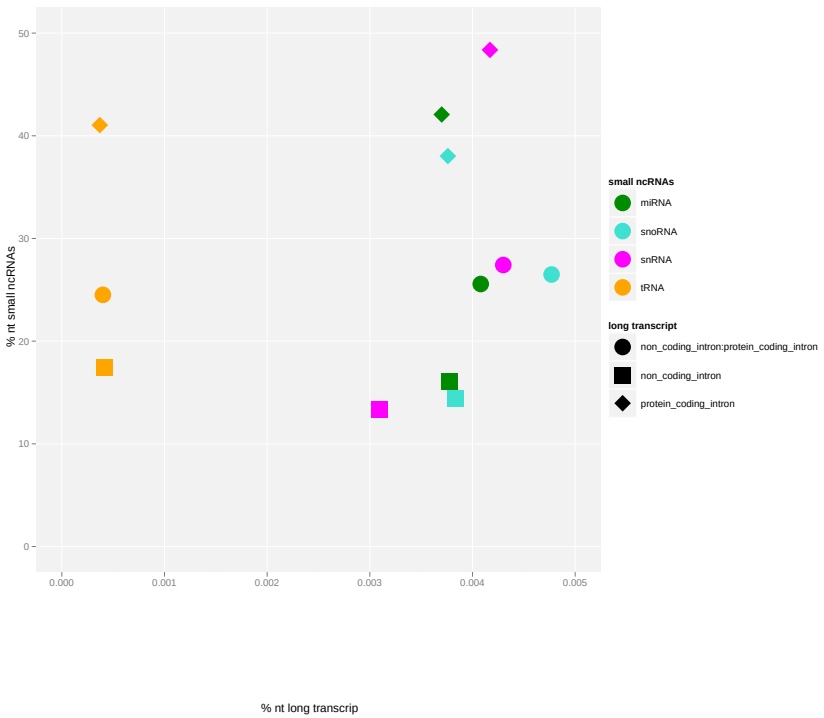




- ca. 60% small ncRNAsintergenic
- miRNAs and snoRNAs more genic
- if genic, then intronic $\rightarrow$ separation of information
- in both coding and noncoding genes

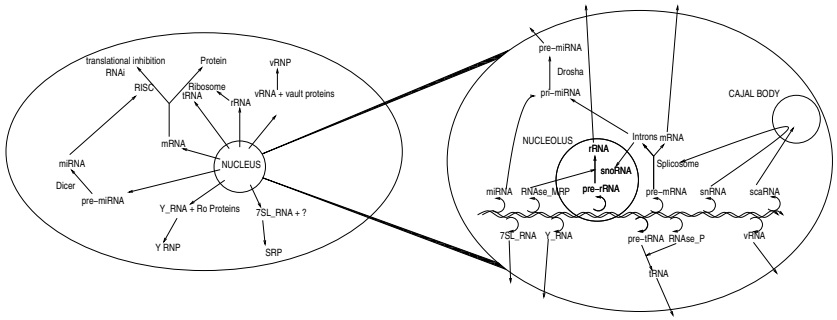
Detailed few of intronic small RNAs

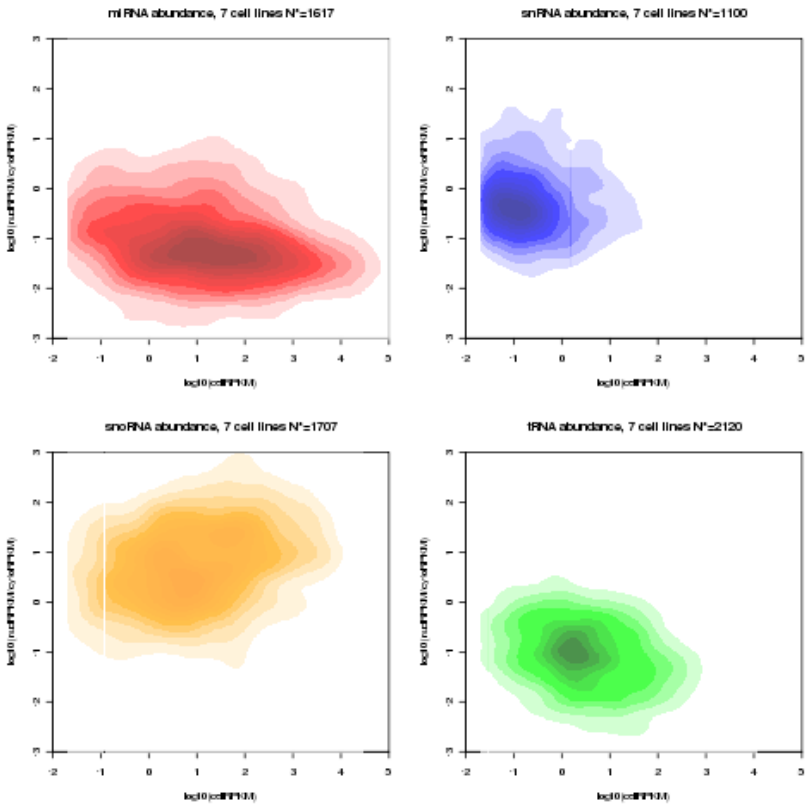


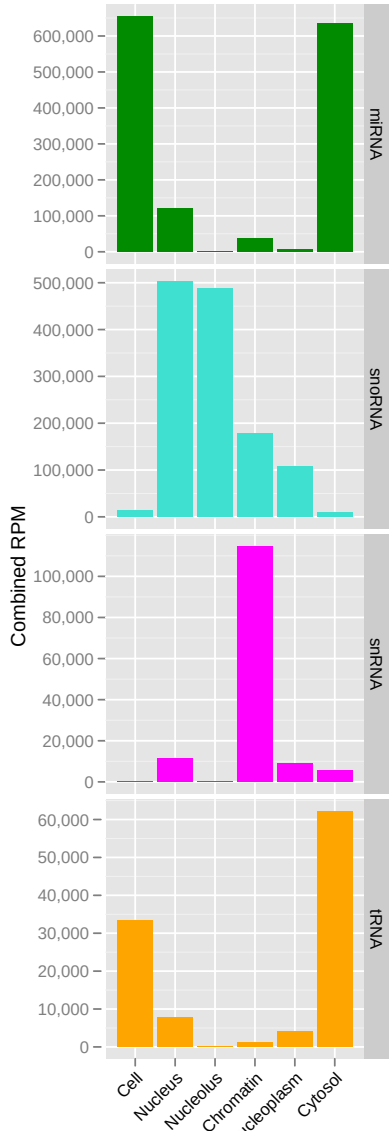


- tRNAs independent loci
- lncRNAs and protein coding gens contain miRNAs, snoRNAs, snRNAs at similar frequency
- lnc-protein overlapping regions: are slightly enriched $\rightarrow$ backup system?

Subcellular compartimentalization of small ncRNAs

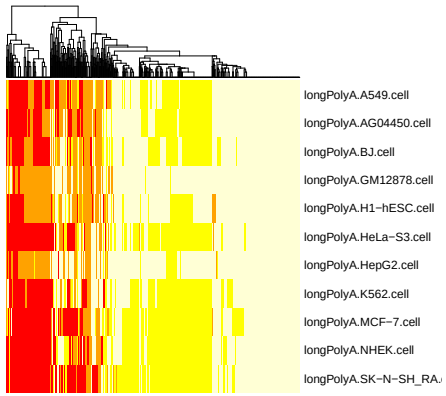




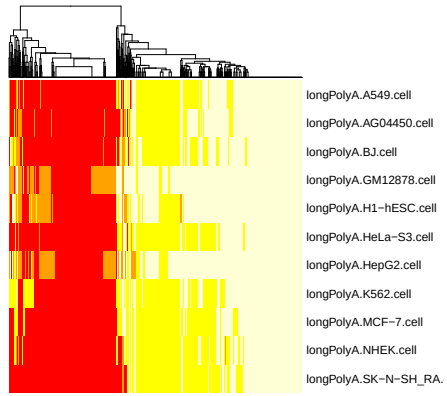


Coexpression of annotated small ncRNA & annotated long transcripts

miRNA



snoRNAs

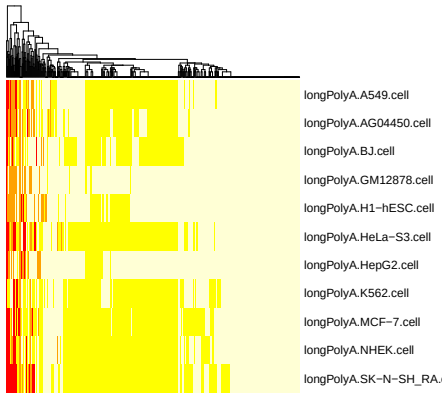


red: both, orange: only small, yellow: only host, white: none

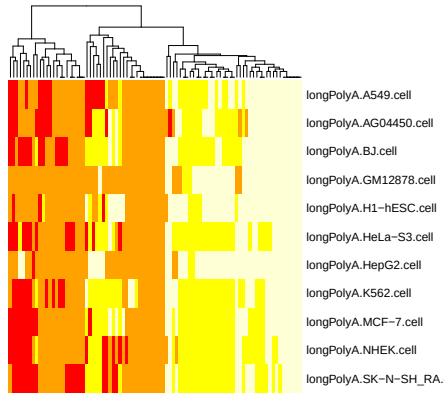
- we see coexpression
- about half of small-long pairs (based on annotation) are expressed
- snoRNAs: uniform expression
- miRNA: some uniformly expressed, but also tissue specific expression
- for both: hosts expressed, but not small $\rightarrow$ annotated long is not the host, or regulation of procesing of small

Coexpression of annotated small ncRNA & annotated long transcripts

snRNA



tRNA



red: both, orange: only small, yellow: only host, white: none

- snRNAs: hardly any genic snRNAs expressed
- tRNAs independent of long

Summary

- overview of ENCODE project
- and GENCODE gene annotation
- small ncRNAs in different types of host genes
- characterized long ncRNAs in gencode annotated regions
- lncRNAs are non protein coding, but share other mRNA features
- small ncRNA reside in introns often shared by coding-noncoding transcripts
- majority of small snoRNAs and miRNAs are intergenic
- 20% of intergenic small ncRNAs in transcribed regions
- coexpression of host-small ncRNA pairs

Acknowledgments

CRG

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CSHL:

Tom Gingeras
Carrie Davies
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...and probably many more

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Barcelona Biomedical Research Park