Identification of Transcribed Pseudogenes in Human Genome

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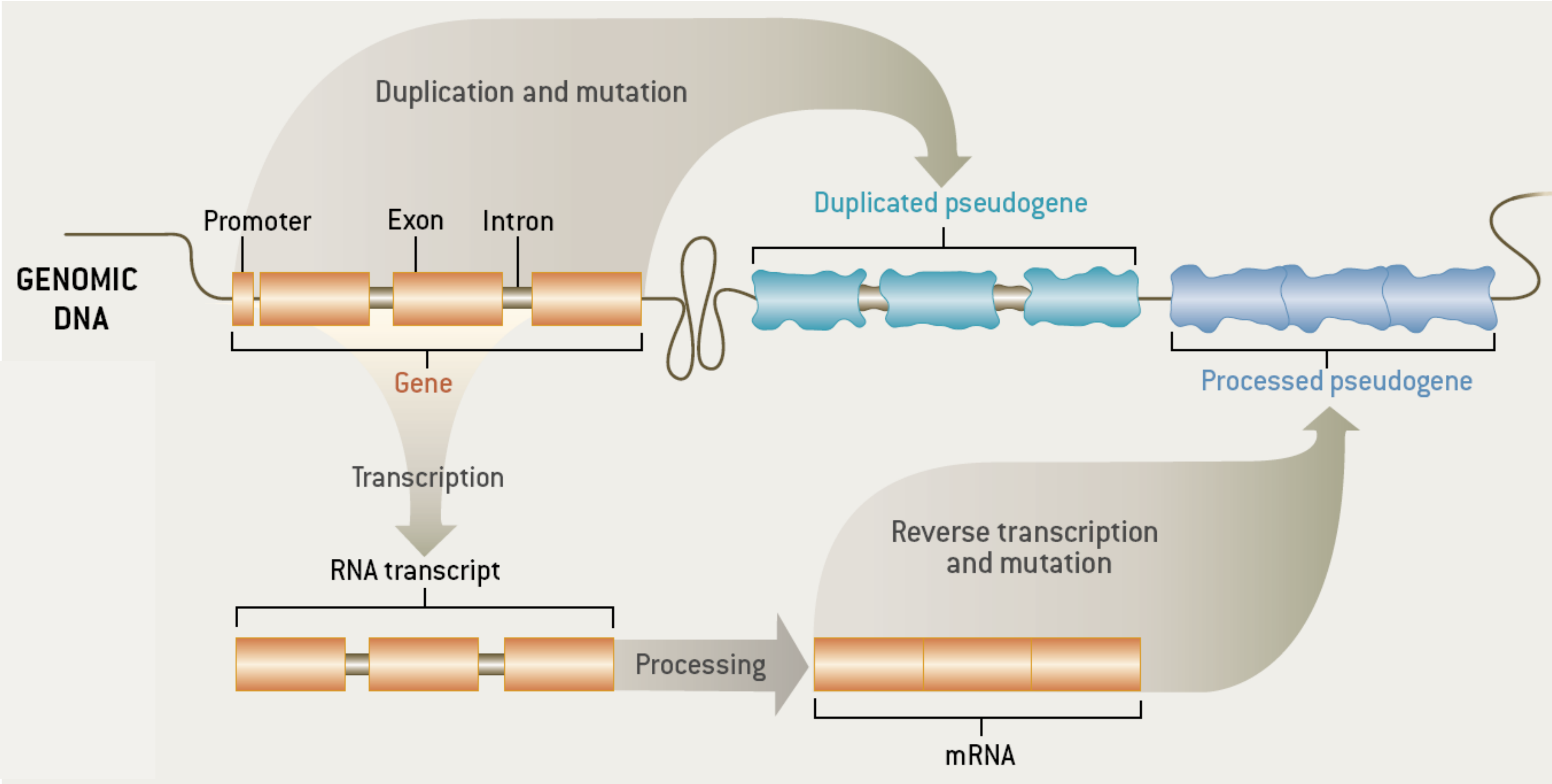
Yale

Pseudogenes are among the most interesting intergenic elements

- Formal Properties of Pseudogenes (ΨG)
  - Inheritable
  - Homologous to a functioning element
  - Non-functional
    - No selection pressure so free to accumulate mutations
      - Frameshifts & stops
      - Small Indels
      - Inserted repeats (LINE/Alu)
    - **What does this mean?** no transcription, no translation?…

[Mighell et al. *FEBS Letts*, 2000]

Two Major Genomic Remodeling Processes Give Rise to Distinct Types of Pseudogenes

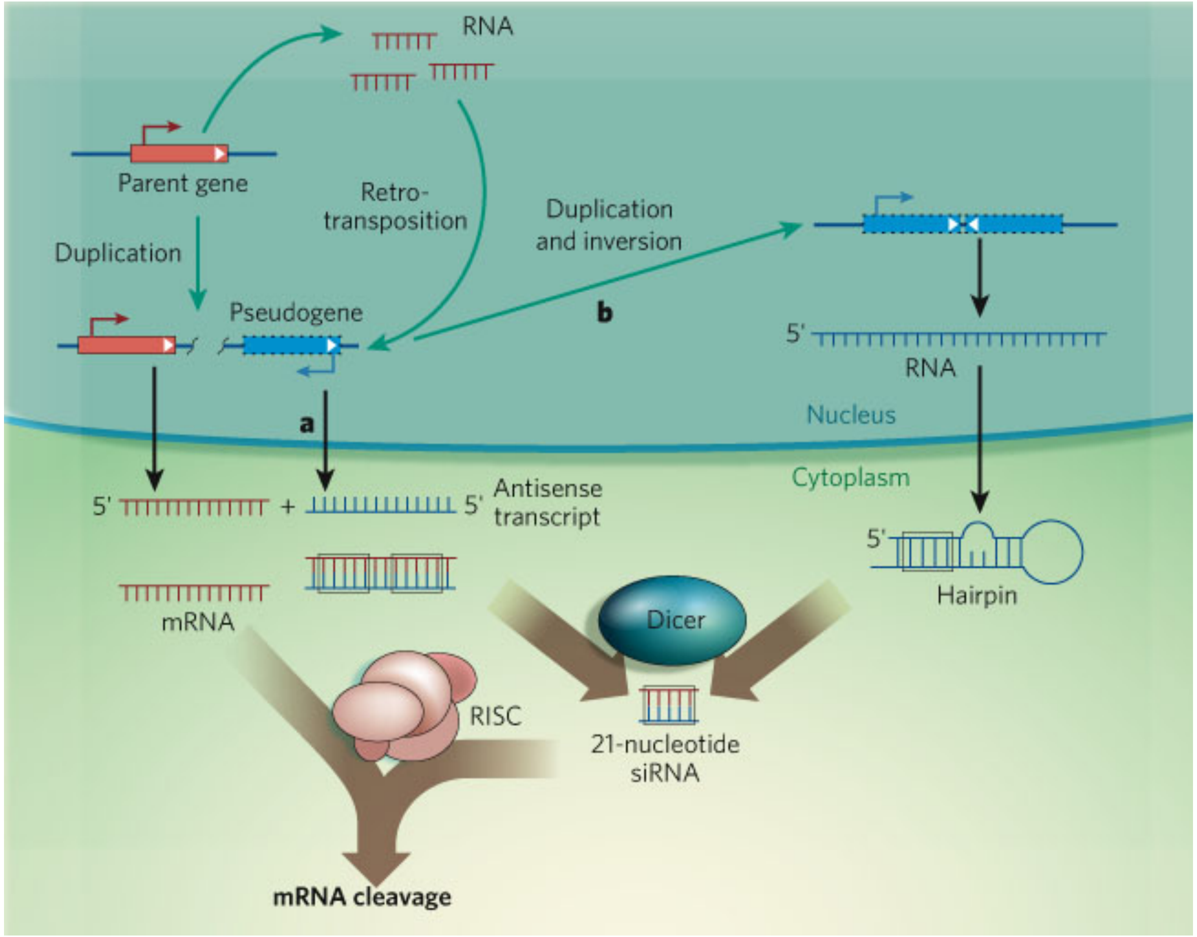


Gerstein & Zheng. Sci Am 295: 48 (2006).

Examples & speculation on the function of pseudogene ncRNAs:

Regulating their parents

- via acting as endo-siRNAs [Recent ex. in fly & mouse, ‘08 refs.]
- via acting as miRNA decoys [PTEN]
- via inhibiting degradation of parent’s mRNA [makorin]



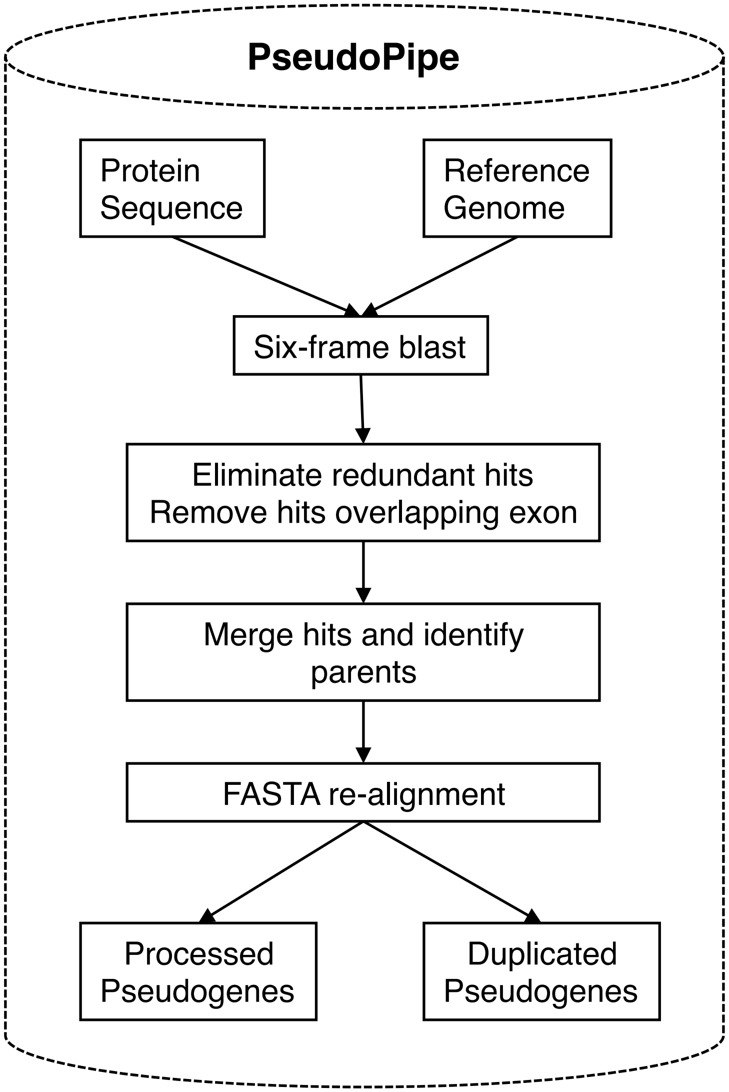
[Sasidharan & Gerstein, Nature ('08)]

Alternatively, just last gasps of a dying gene

Czech *et al. Nature* 453: 798 (‘08).
Ghildiyal *et al. Science* 320: 1077 (‘08).
Kawamur *et al. Nature* 453: 793 (‘08).
Okamura *et al. Nature* 453: 803 (‘08).
Tam *et al. Nature* 453: 534 (‘08).
Watanabe *et al. Nature* 453: 539 (‘08).

Poliseno et al. Nature 465:1033 (’10)

Genome-wide Annotation of Pseudogenes



PseudoPipe

Protein Sequence

Reference Genome

Six-frame blast

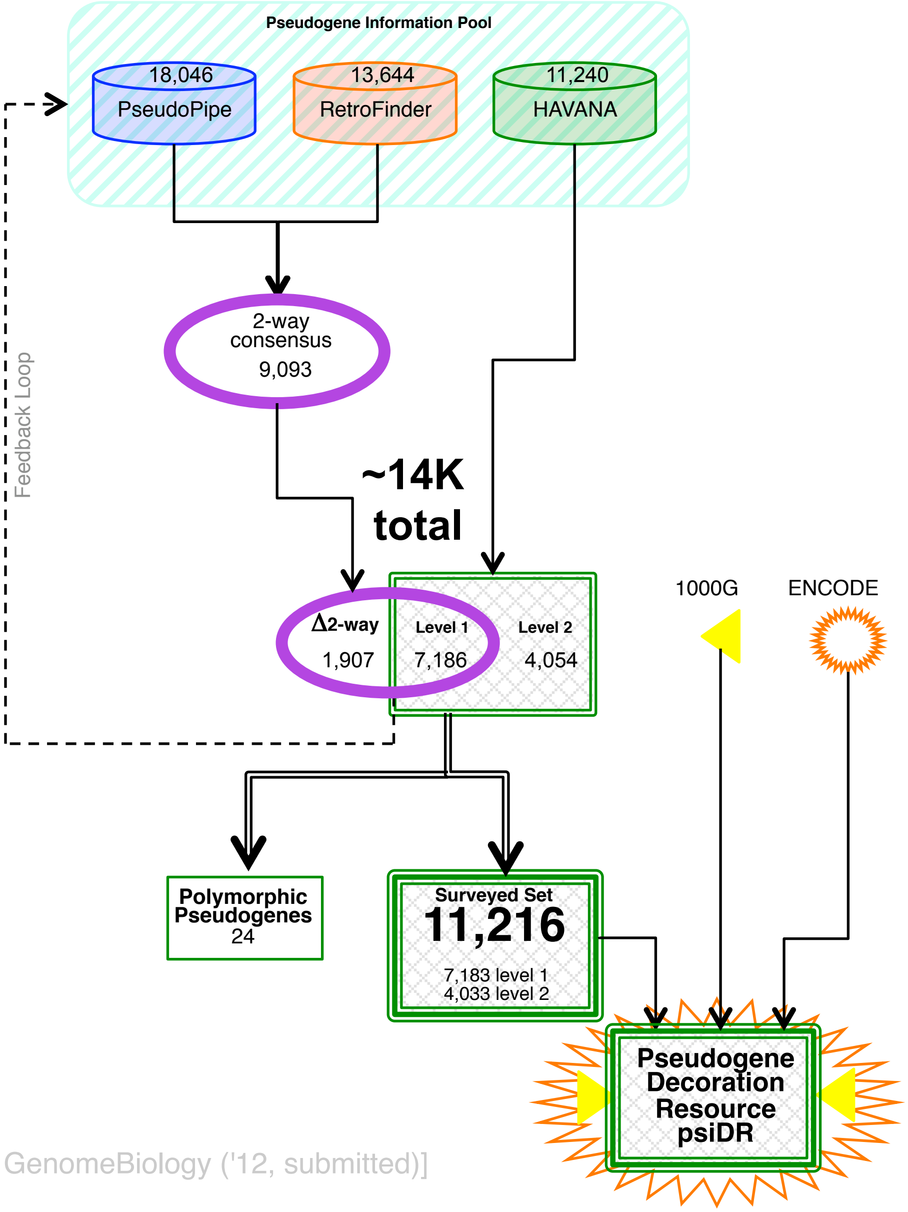
Eliminate redundant hits
Remove hits overlapping exon

Merge hits and identify parents

FASTA re-alignment

Processed Pseudogenes

Duplicated Pseudogenes



Pseudogene Information Pool

18,046 PseudoPipe

13,644 RetroFinder

11,240 HAVANA

2-way consensus 9,093

~14K total

Feedback Loop

Δ2-way 1,907

Level 1 7,186

Level 2 4,054

1000G

ENCODE

Polymorphic Pseudogenes 24

Surveyed Set
11,216
7,183 level 1
4,033 level 2

Pseudogene Decoration Resource psiDR

[Pei et al., GenomeBiology ('12, submitted)]

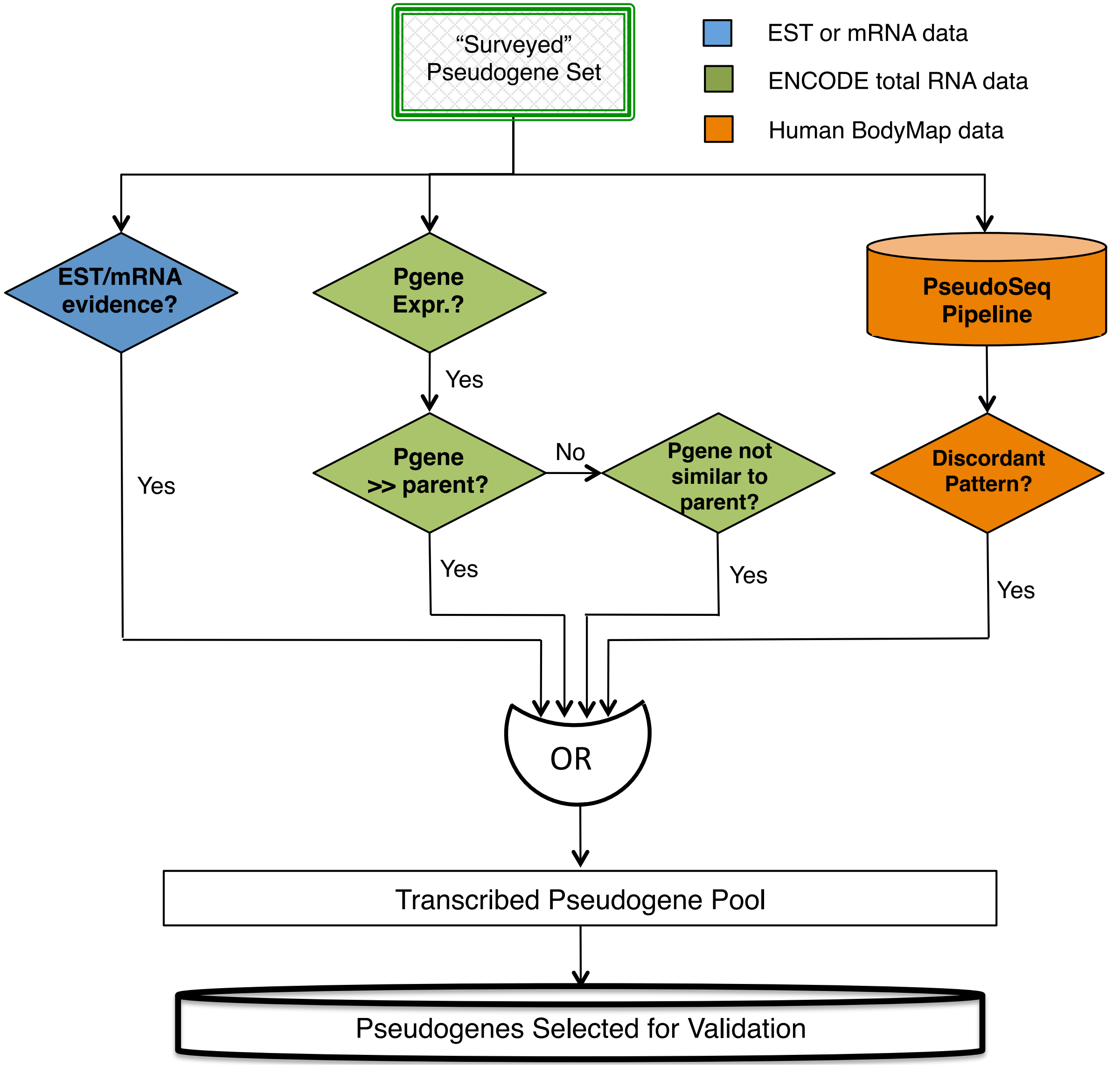
Pipeline to Identify Transcribed Pseudogenes

876 transcribed pseudogenes:

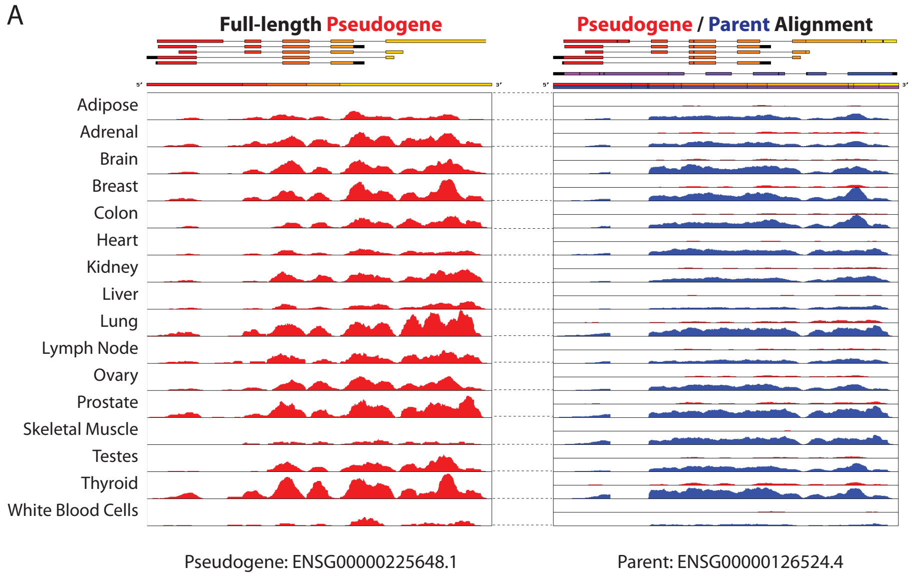
- 422 from EST evidence;

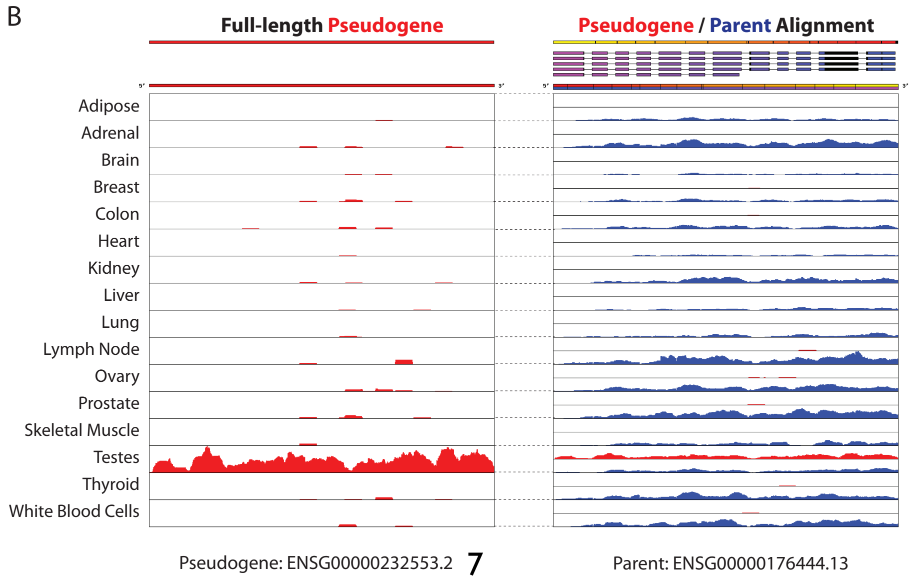
- 344 from pseudoSeq pipeline on BodyMap data;

- 110 from total RNA data of GM12878 and K562.

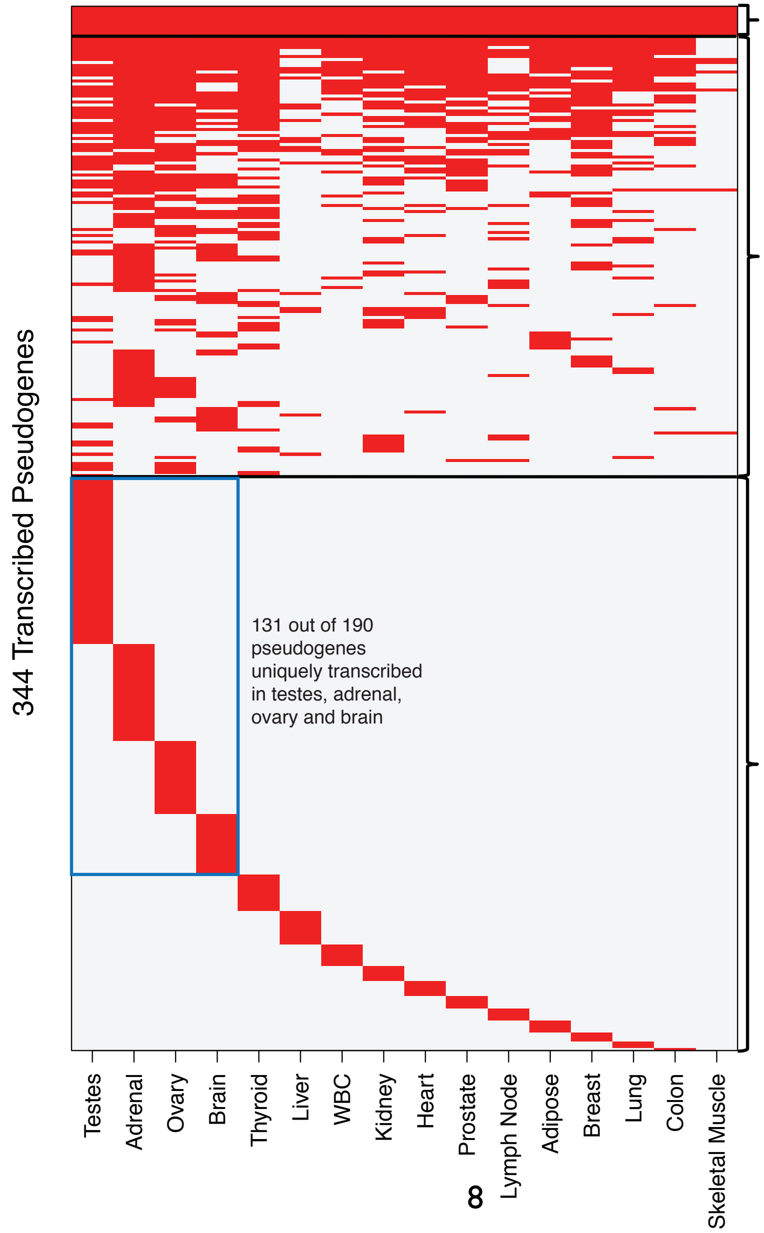


Transcribed Pseudogenes by PseudoSeq





Transcribed Pseudogenes by PseudoSeq



131 out of 190 pseudogenes uniquely transcribed in testes, adrenal, ovary and brain

10 pseudogenes transcribed in all tissues

144 pseudogenes transcribed in multiple tissues

190 pseudogenes transcribed in one tissue

Validation of Transcribed Pseudogene

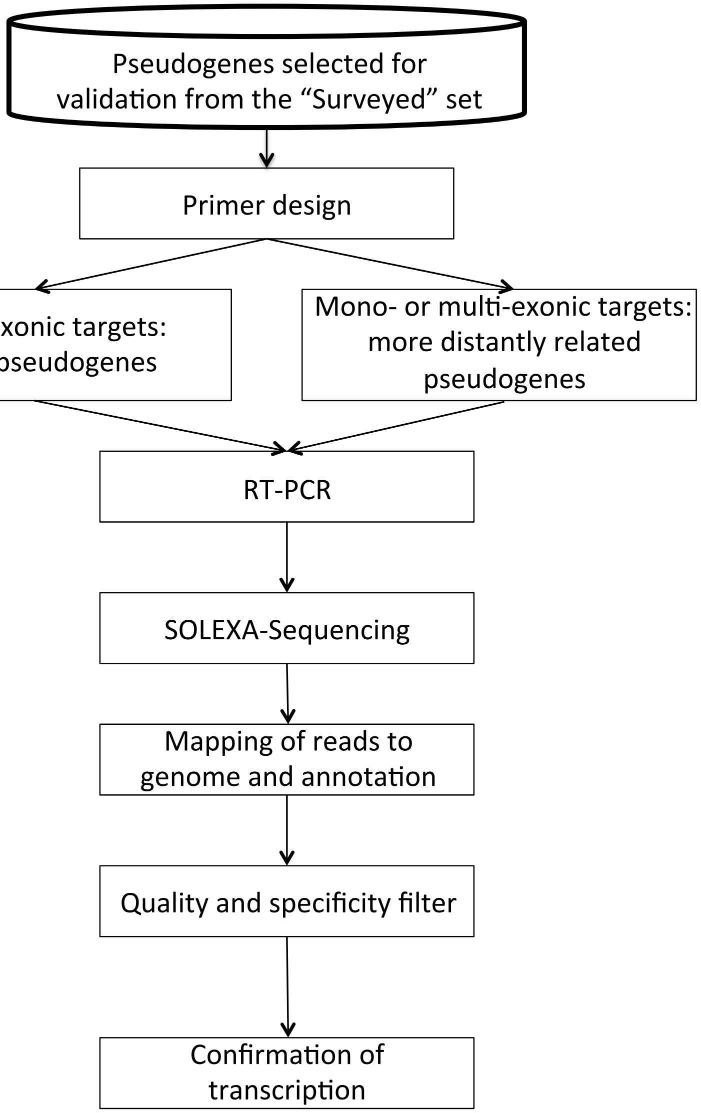
Mono-exonic RT-PCR:

Target to pseudogene exons. One target for each pseudogene;

Multi-exonic RT-PCR:

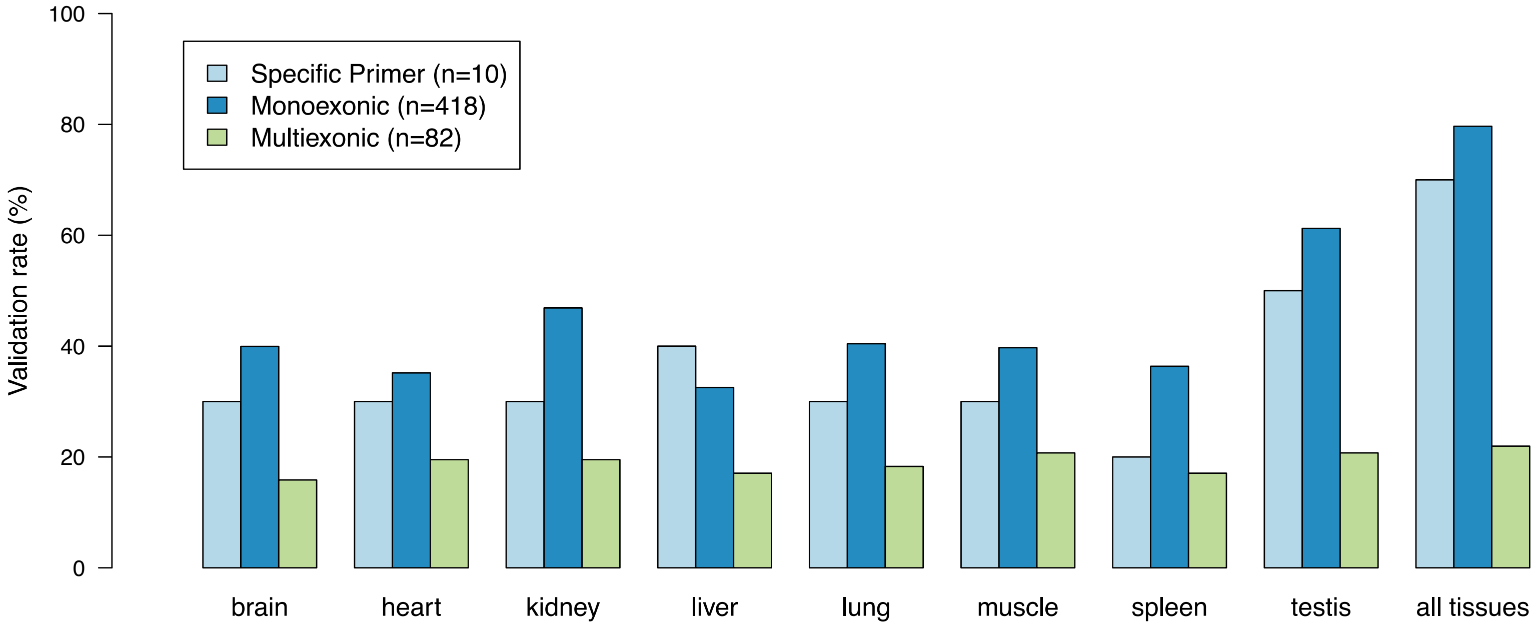
Target to exon-exon junctions. Multiple targets for each pseudogene;

Statistical model to make sure reads mapped to pseudogene annotation are indeed from pseudogene transcription, but not from parents.



Pseudogenes selected for validation from the “Surveyed” set
Primer design
Mono-exonic targets: young pseudogenes
Mono- or multi-exonic targets: more distantly related pseudogenes
RT-PCR
SOLEXA-Sequencing
Mapping of reads to genome and annotation
Quality and specificity filter
Confirmation of transcription

Validation of Transcribed Pseudogene



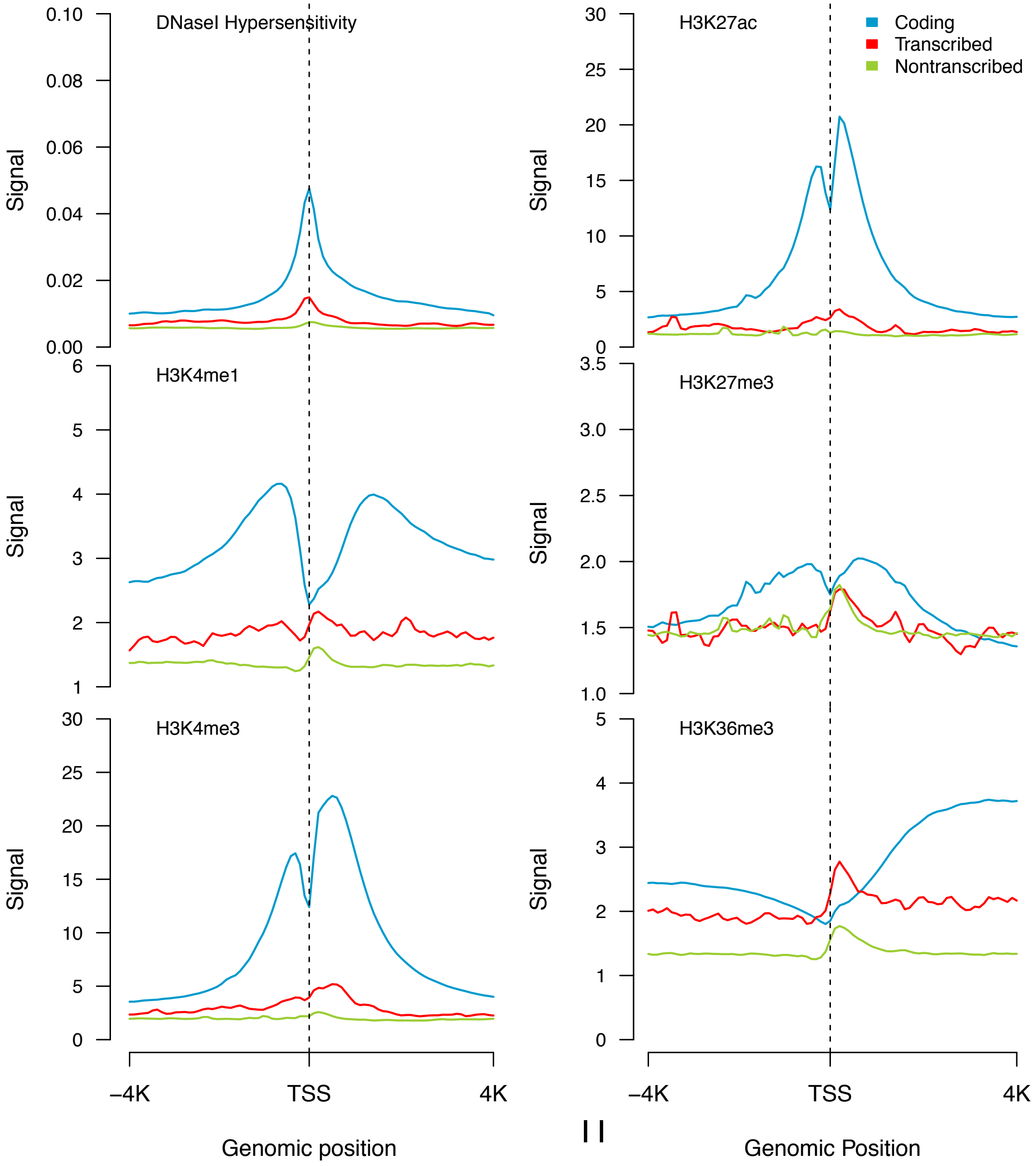
Total number of transcribed pseudogenes being validated: 469

- 94 from EST pipeline;
- 97 from totalRNA pipeline;
- 271 from BodyMap data pipeline;
- 7 are manually chosen due to their discordant expression patterns of pseudogenes and parents

Overall validation rate: 75.5% (354 out of 469)

- Specific primer: 70% (7 out of 10)
- Monoexonic: 79.7% (333 out of 418)
- Multiexonic: 22.0% (18 ouf of 82)

Chromatin Signatures of Pseudogenes



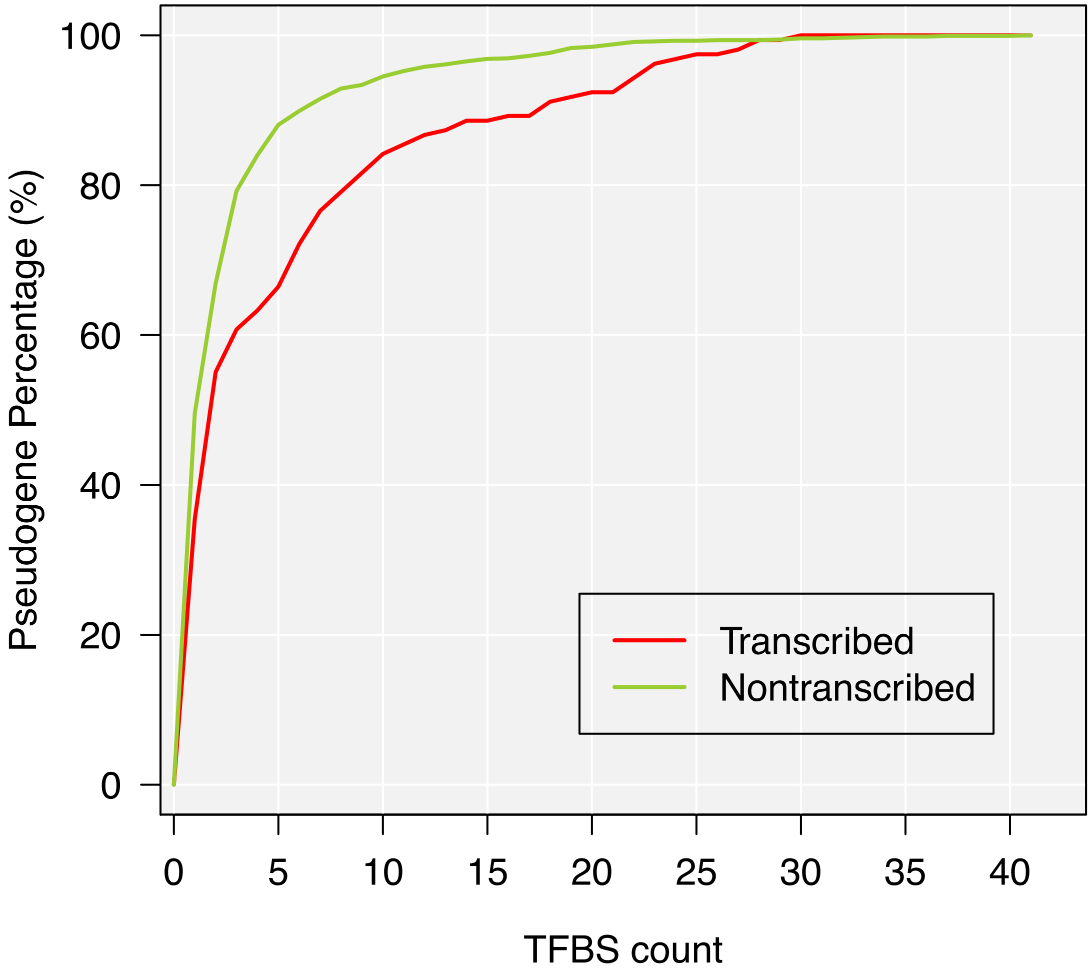
Transcription Factor Binding Sites of Pseudogenes

TF binding sites in upstream regions of pseudogenes in K562:

- Most pseudogenes have 0 or very few TFBS in their upstream regions

- Transcribed pseudogenes have more TFBS than non-transcribed pseudogenes (p-value = 3.8e-3)

- Similar results in GM12878, HeLa-S3, h1-Hesc and HepG2 cell lines

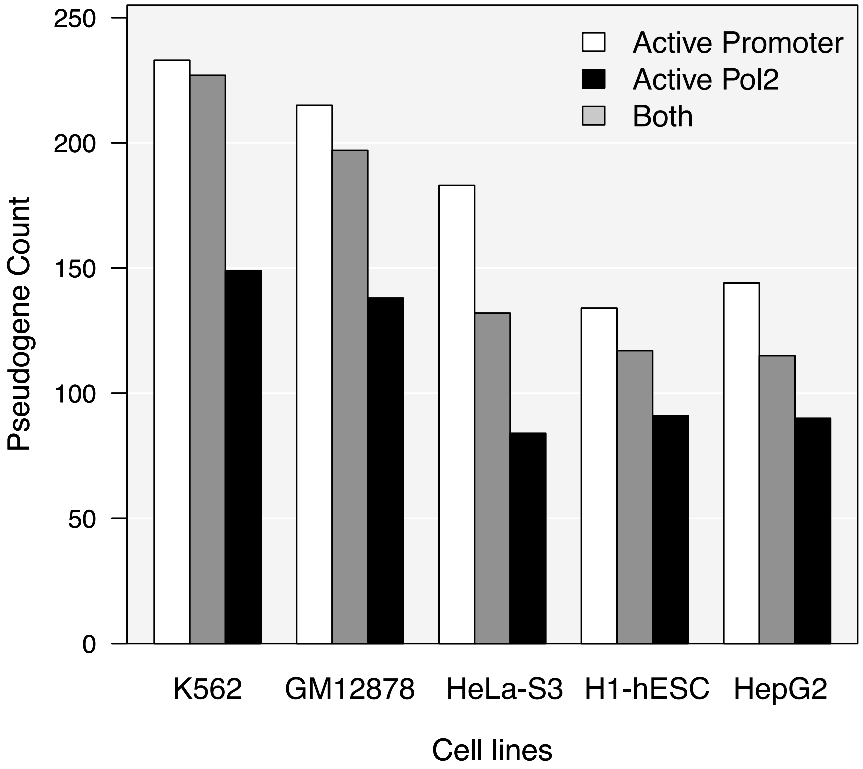


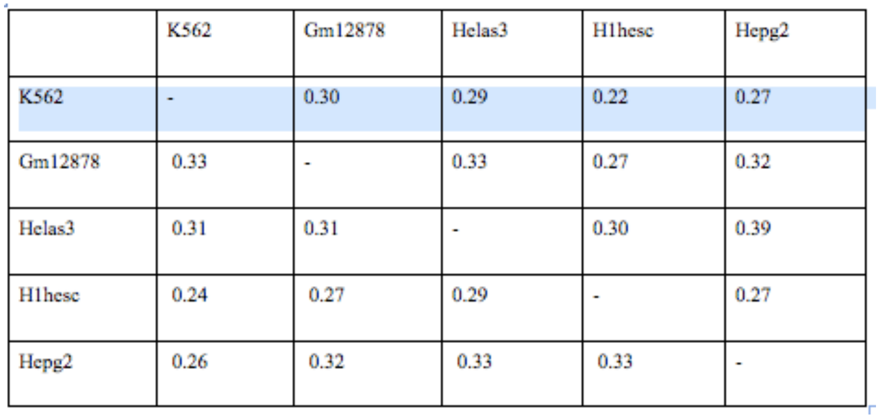
Pseudogenes with Active Upstream Sequences

Active promoters predicted by Kevin Yep's random forest model, using open chromatin, histone modification and TFBS data;

Active Pol2 bindings are from upper 5% of Pol2 binding peaks, in terms of peak widths and heights, plus binding of Pol2 co-factors;

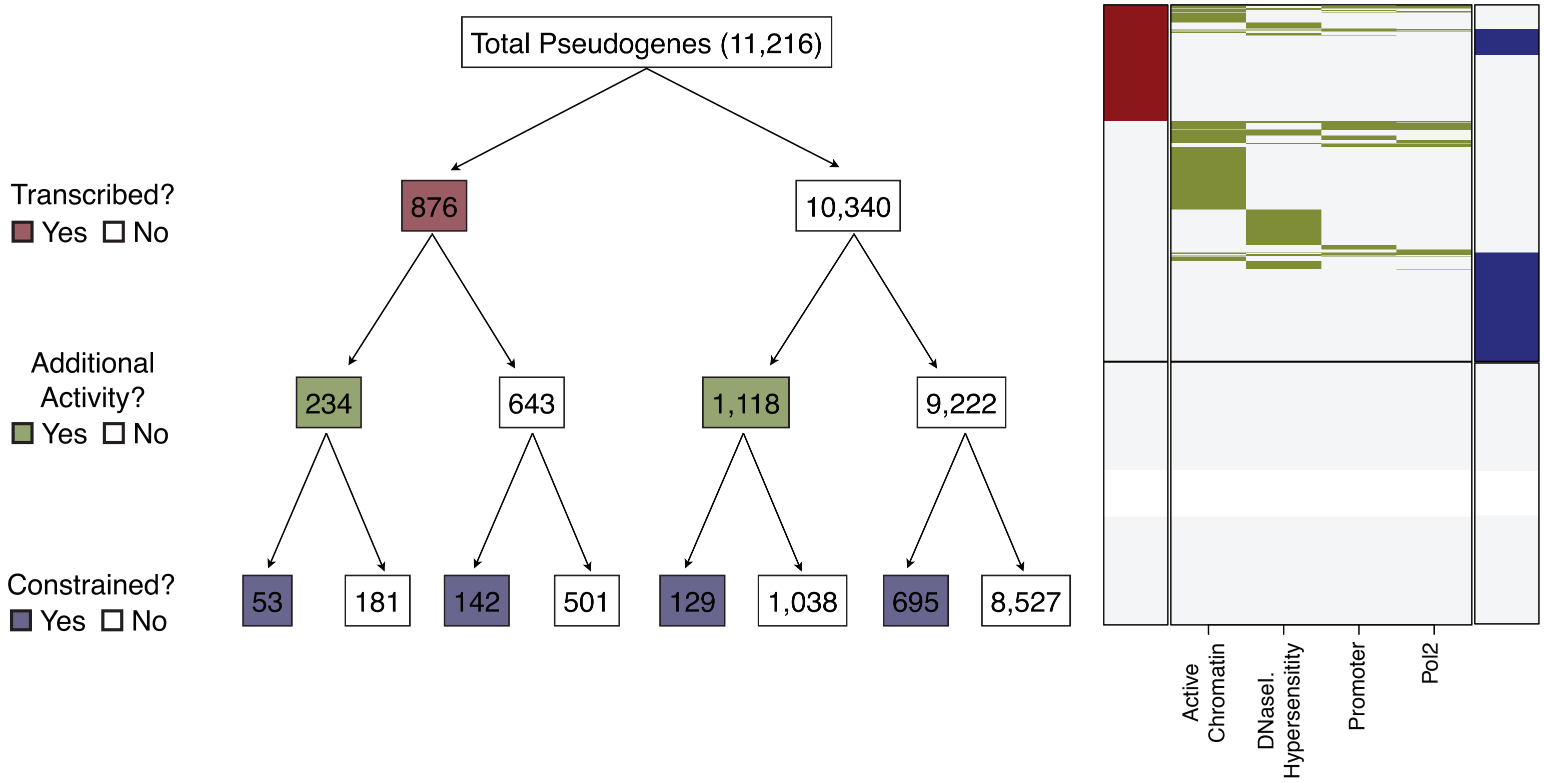
Both active promoters and Pol2 binding sites are more abundant in upstream of transcribed pseudogenes than that of non-transcribed pseudogenes





	K562	Gm12878	Helas3	H1hesc	Hepg2
K562	-	0.30	0.29	0.22	0.27
Gm12878	0.33	-	0.33	0.27	0.32
Helas3	0.31	0.31	-	0.30	0.39
H1hesc	0.24	0.27	0.29	-	0.27
Hepg2	0.26	0.32	0.33	0.33	-

Partial Activity of Pseudogenes



Total Pseudogenes (11,216)

Transcribed?
🟥 Yes □ No

876

10,340

Additional Activity?
🟩 Yes □ No

234

643

1,118

9,222

Constrained?
🟦 Yes □ No

53

181

142

501

129

1,038

695

8,527

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