Exploring the possibility of Alternative Splicing as a path to the regulation of LINE-1 elements in human and mouse

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Overview

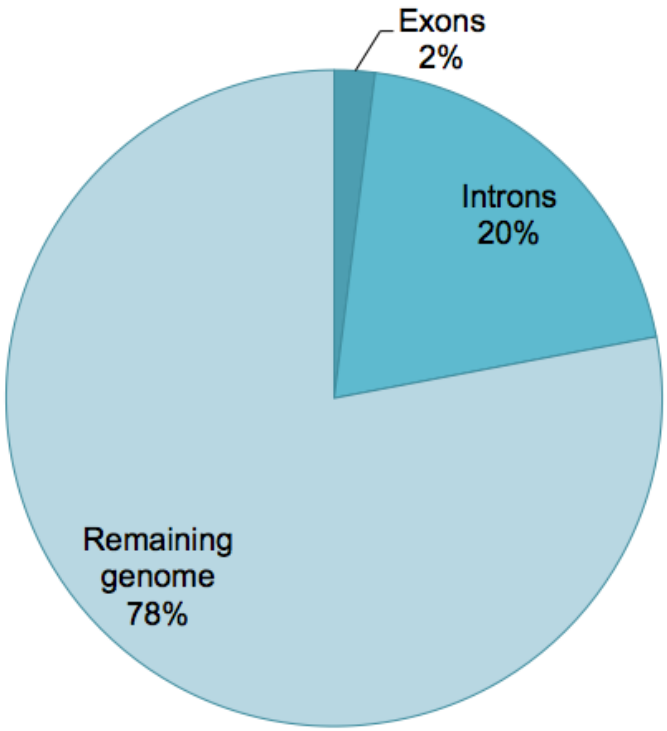
- Background: Transposable Elements (TEs) including L1s

- Regulation of TEs
- Alternative splicing
- Alternative splicing in L1s

Background

The human genome

Repetitive elements are abundant in the human genome



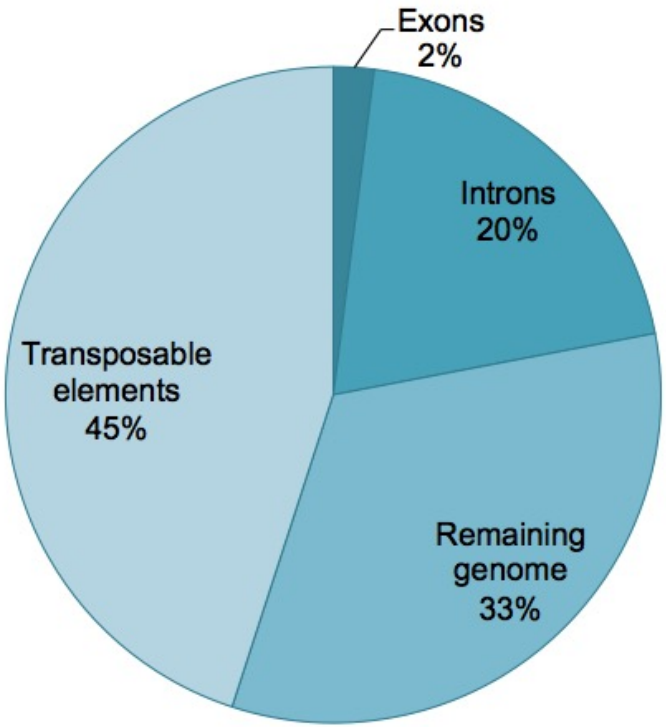
Xu et al. 2010, Singer et al. 2010

Other genome content:

Tandem repeats
Intergenic regions
Duplications
Transposable elements

The human genome

Repetitive elements are abundant in the human genome



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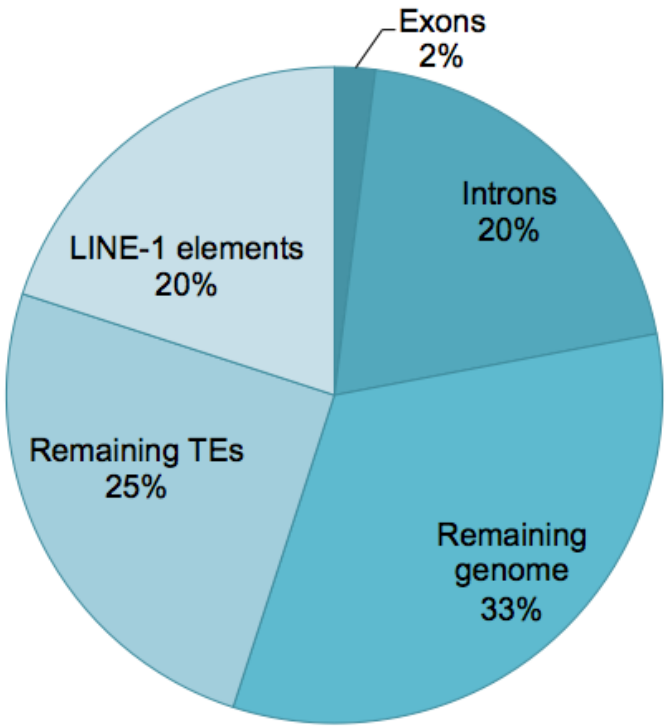
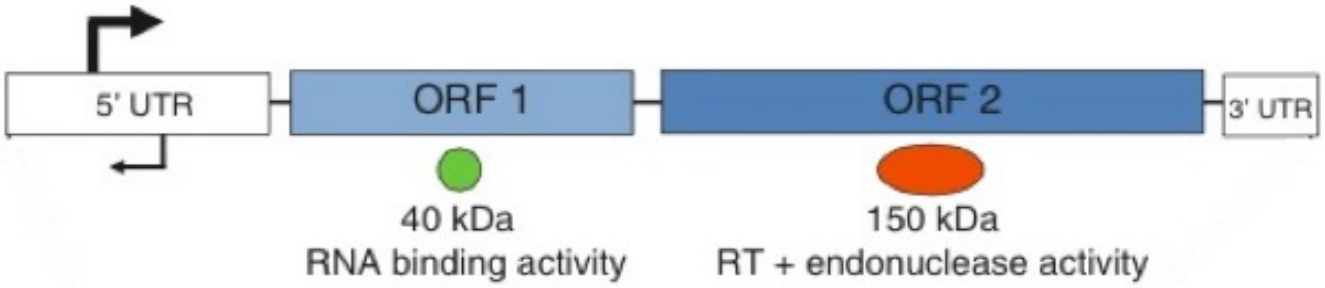


Xu et al. 2010, Singer et al. 2010

LINE-1 (L1) structure

L1s are TEs



L1 structure

- Full length L1s are 6-7kb
- L1s are often 5' truncated, inverted or degraded
- Some variants are 4kb - HAL1s

TE replication cycle

LINE-1s are retrotransposons

- L1s replicate through an RNA intermediate
- They integrate anywhere in the genome - interspersed repeats

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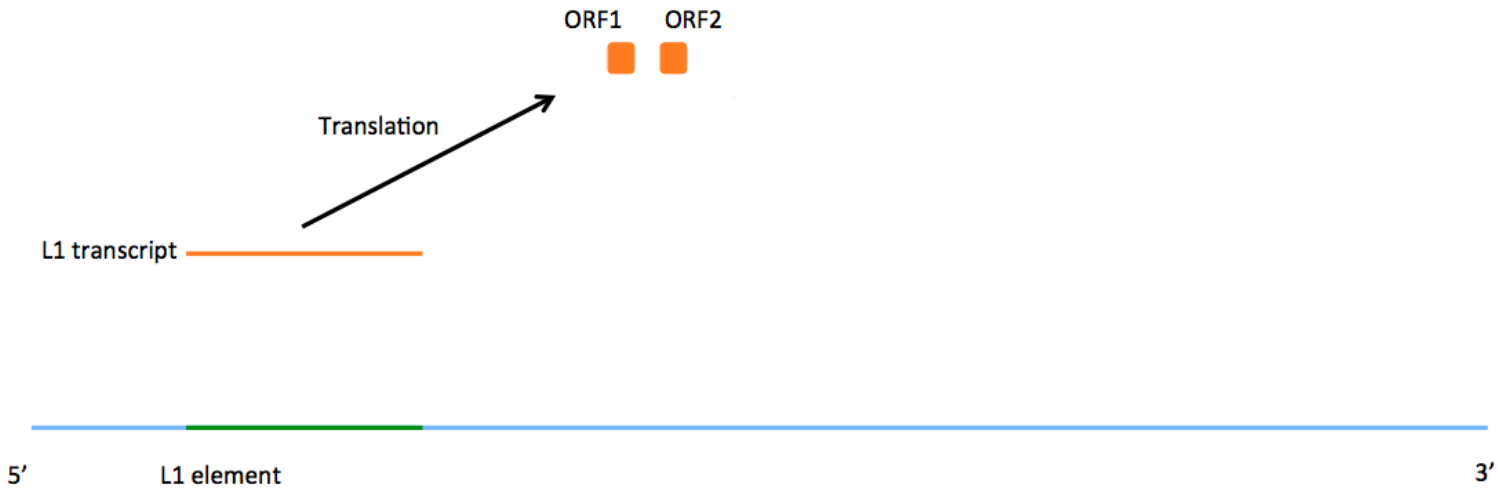

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TE replication cycle

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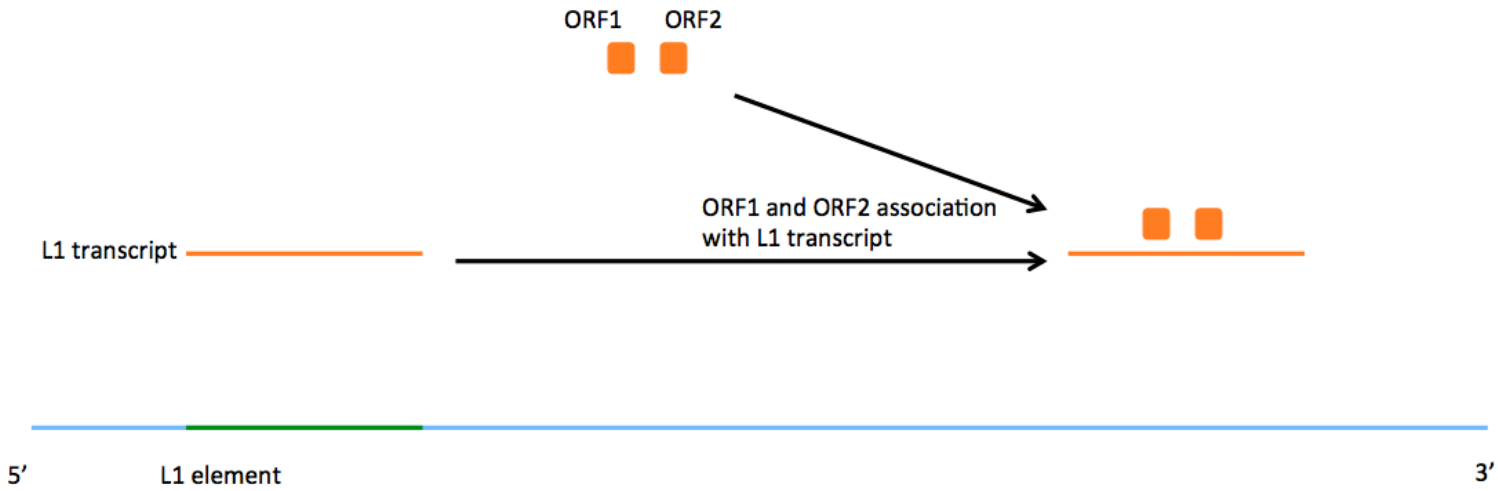

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TE replication cycle

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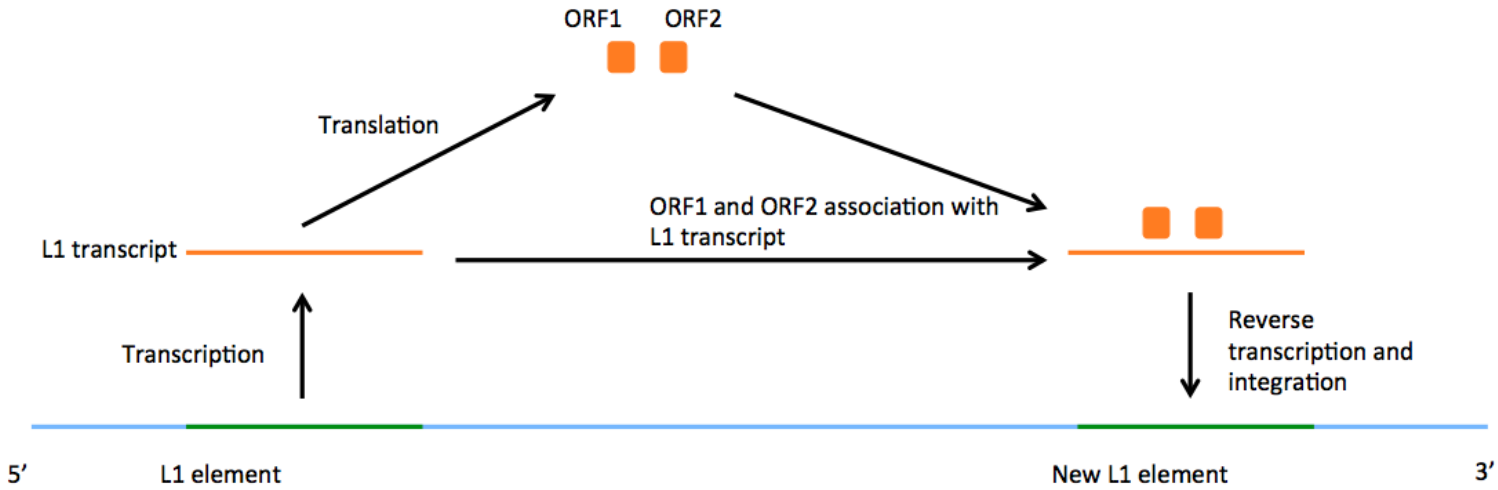

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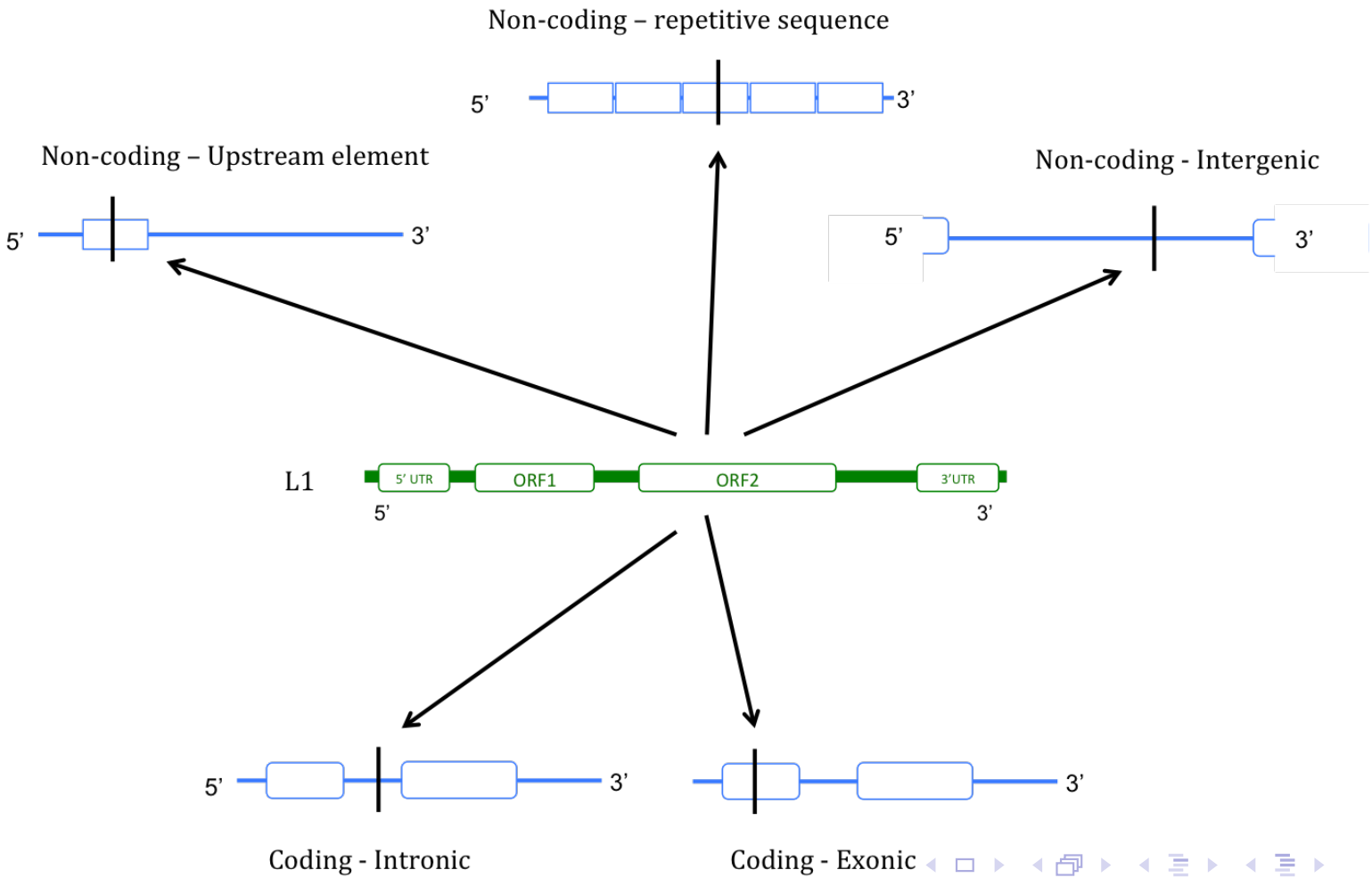
Regulation of TEs

Regulation of TEs

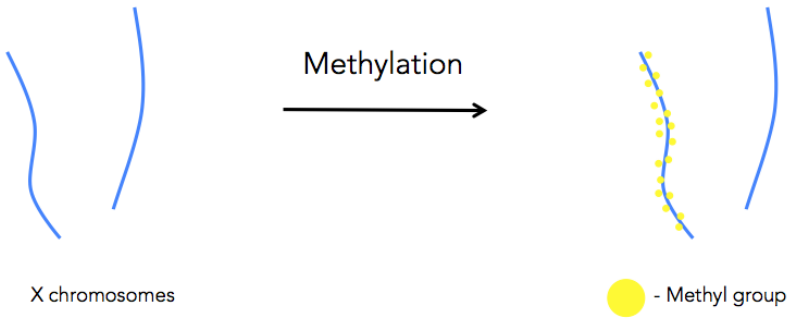
Why is regulation required?



DNA methylation

Methylation of DNA is widespread throughout the genome

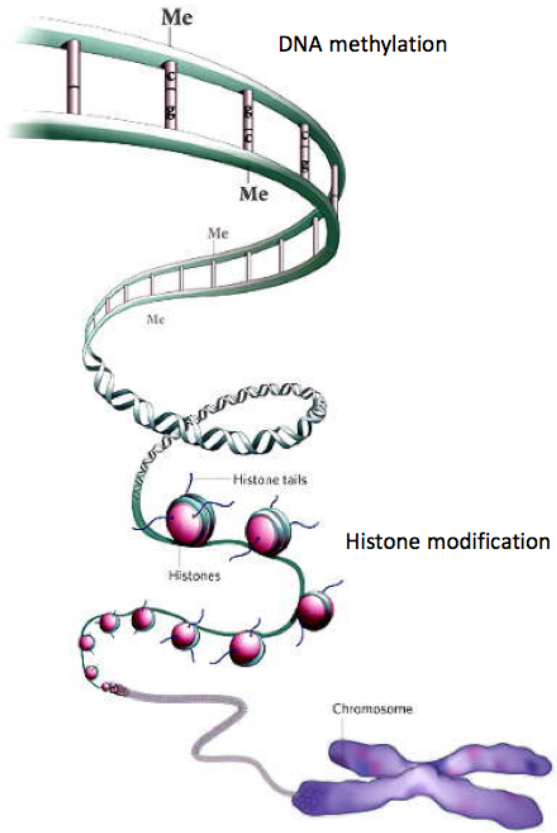
- X chromosome inactivation, TE silencing
- Absence of methylation occurs with TE accumulation
- Levels fluctuate in development
- L1s in the female mature gamete aren't fully methylated



Other regulation

Many other mechanisms have been shown to suppress TEs

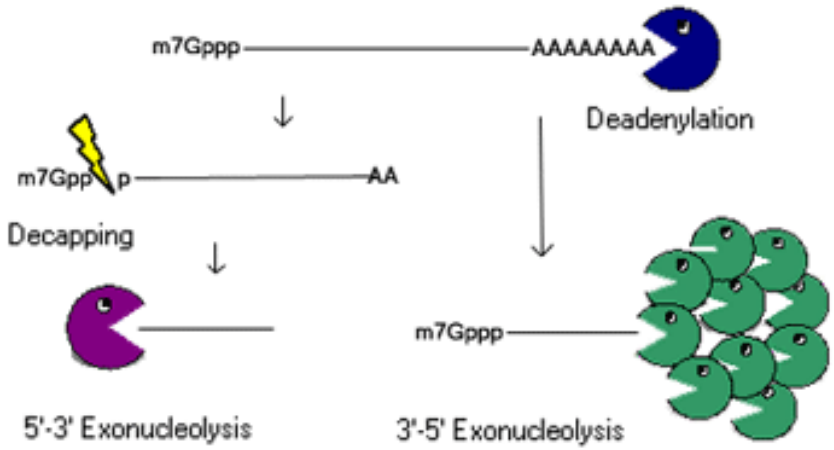
- Histone modifications
  - Methylation - SETB1
  - Ubiquitination
  - Acetylation
- RNA interference
  - miRNAs
  - siRNAs
  - piRNAs
- RNA editases
  - APOBEC



mRNA decay

Targeting the L1 RNA intermediate

- Targets aberrant transcripts
- Nonsense mediated decay

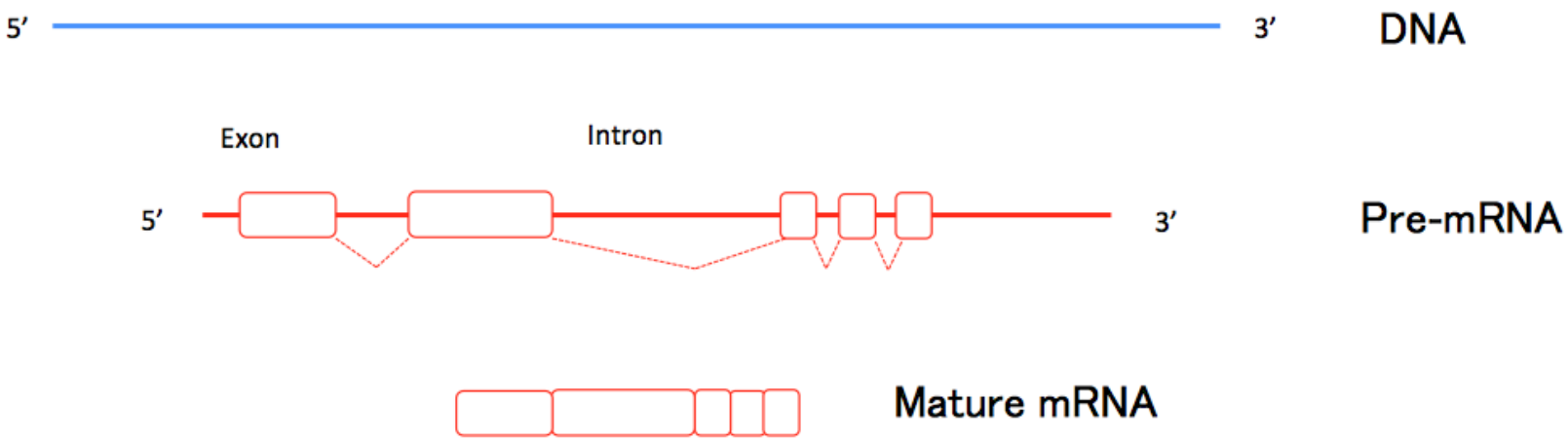


- Alternative splice event → Premature Termination Codon → Target for NMD
- Low coding potential → Target for NMD

Alternative Splicing

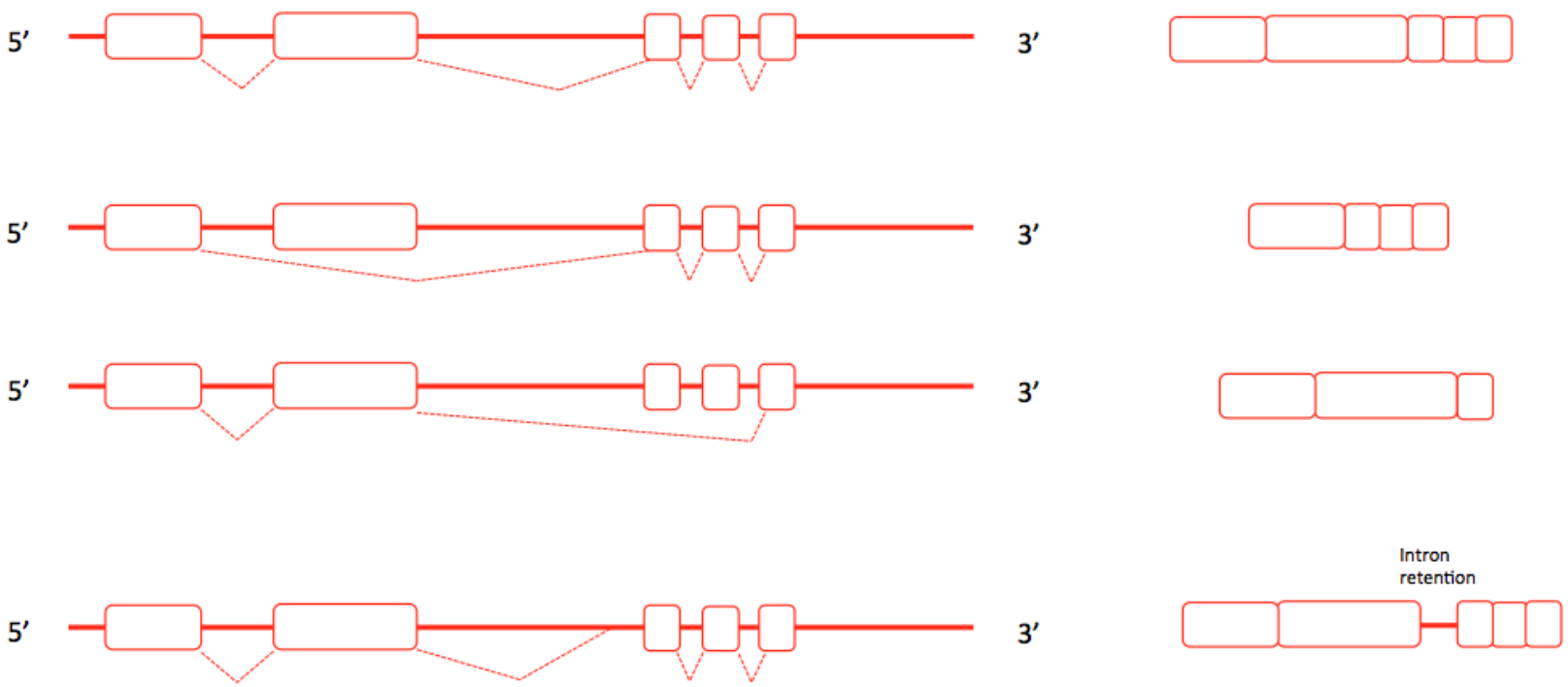
mRNA processing

DNA is transcribed to RNA, which is processed to form mature mRNA



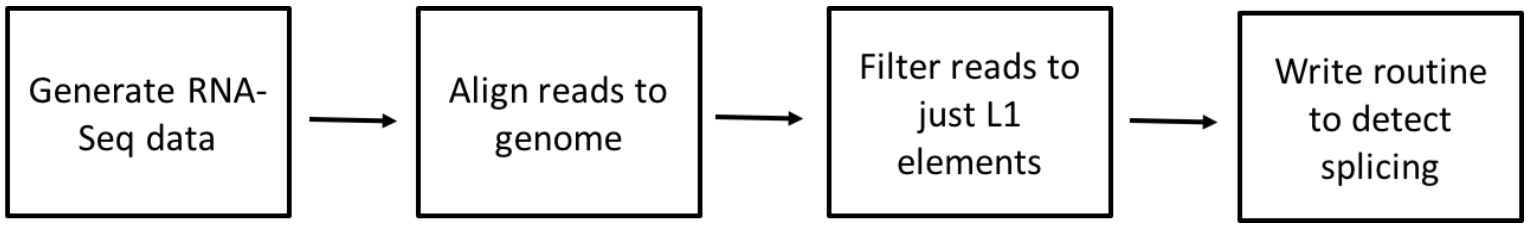
mRNA processing

Alternative splicing can form multiple splice variants



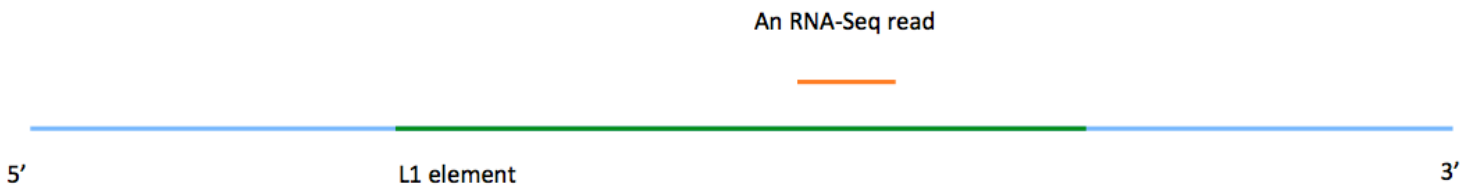
Project Aims

Detecting Alternative Splicing in L1 elements



Detecting Alternative Splicing in L1 elements

RNA-Seq reads can be aligned to the genome



- The alignment file will give information about each read
- Genome coordinates, read quality

Detecting Alternative Splicing in L1 elements

RNA-Seq reads can be aligned to the genome

- Reads will overlap, indicating where the L1s are

Detecting Alternative Splicing in L1 elements

RNA-Seq reads can be aligned to the genome

- If there is a gap, that will suggest that there has been some splicing,

Detecting Alternative Splicing in L1 elements

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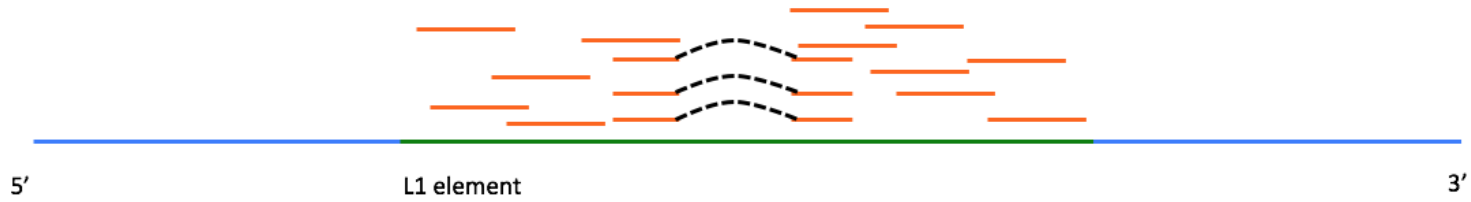
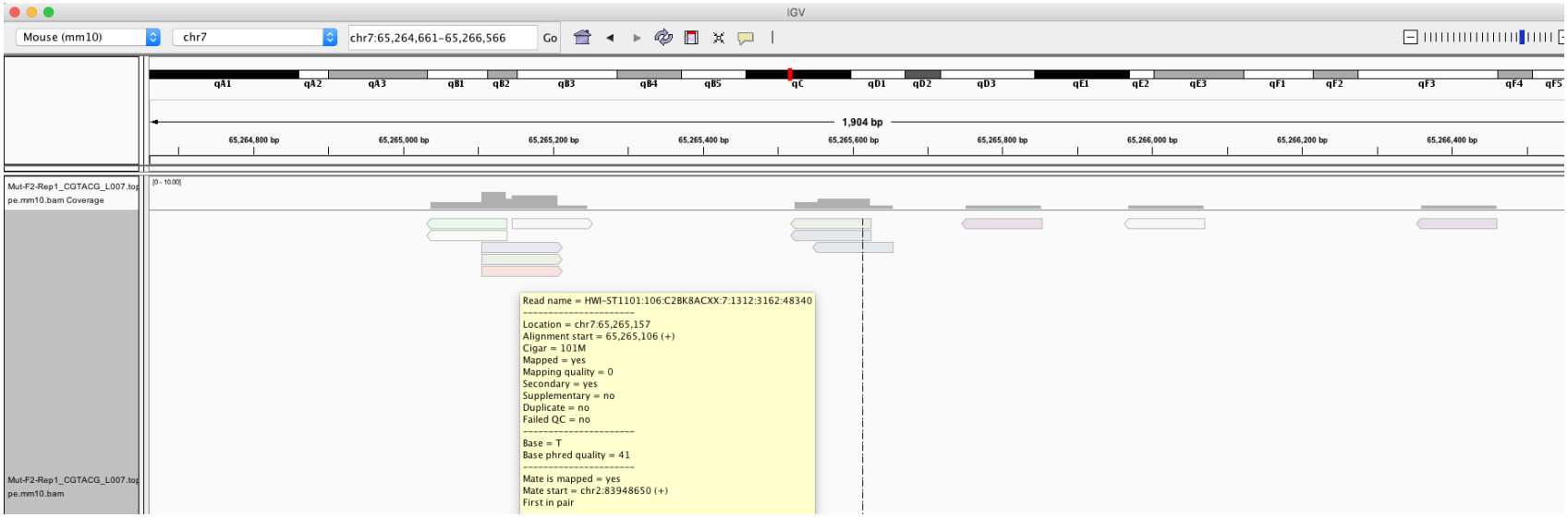


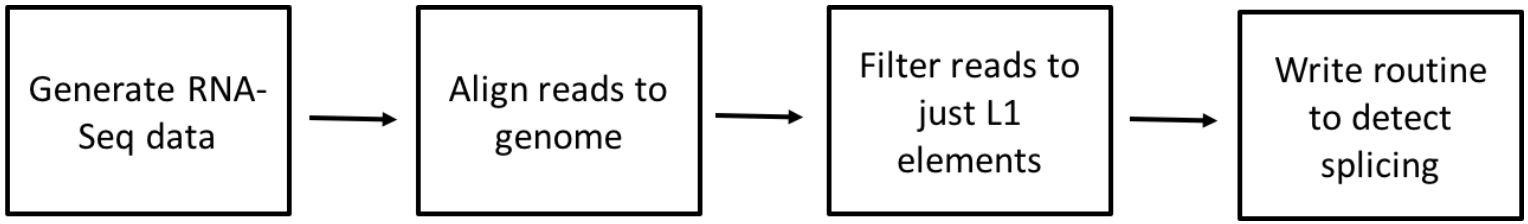
- Reads that are split over two locations on the genome with a gap indicate splicing

Read visualisation with IGV



Summary

- L1s are the most abundant TEs in the human genome, and we are using them as a candidate for TE regulation
- We know they are regulated by a range of mechanisms
- To start analysis we are looking for evidence of alternative splicing



Further analysis

- Investigate if the genome itself has alternatively spliced, retrotransposed L1s
- Comparative analysis; compare the mouse data with human

Further analysis

If there is no evidence for alternative splicing in the genome

- Continue with investigation of the genome,
  - Alternative splicing may still be found in the genome, not the transcriptome
- Continue with investigation in mice
  - Alternative splicing may still occur in L1 transcripts in other organisms