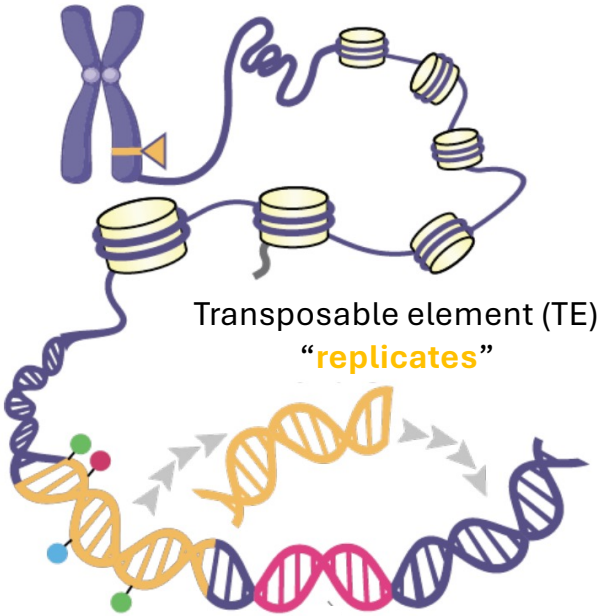
Non-coding RNAs and Transposable Elements



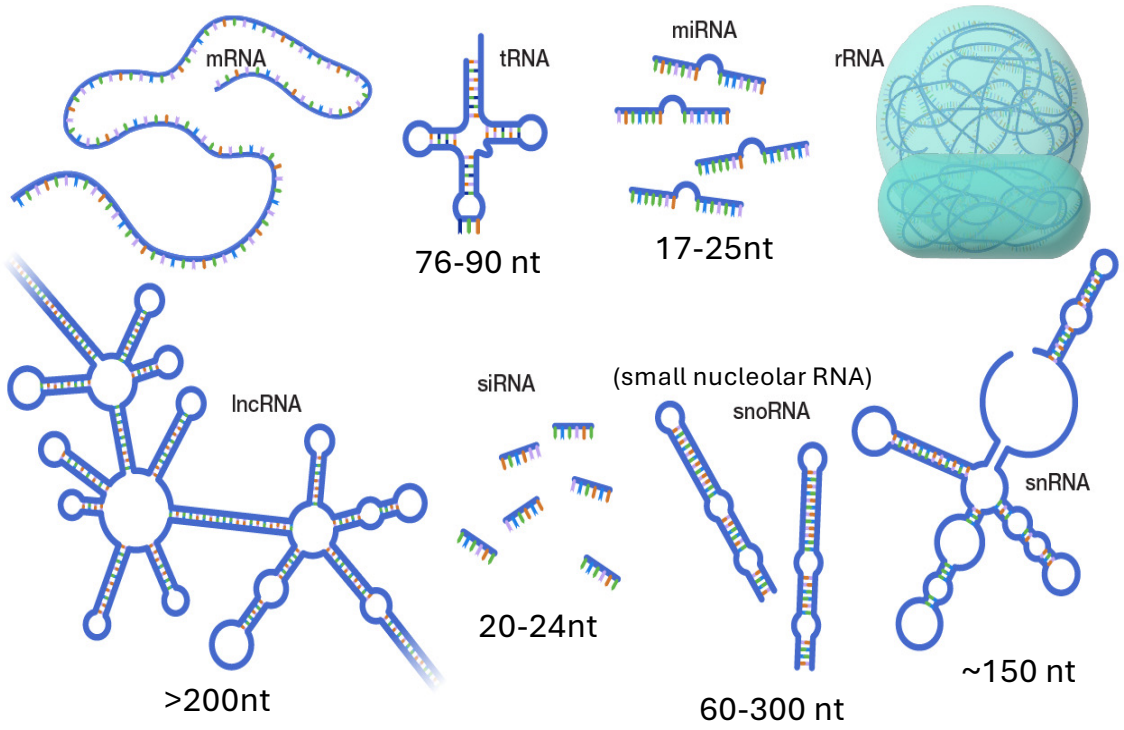
Simon Chu
The Wistar Institute
02/24/2026

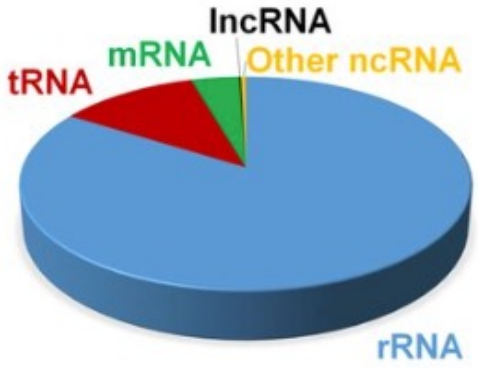
Outline

- **Non-coding RNAs**
  - Types of non coding RNAs
  - Summary of their functions

- **Transposable elements (TEs)**
  - TE annotation
  - TE insertion identification
  - TE RNA expression quantification
  - TE derived neoantigen for mRNA cancer vaccine

Different types of RNA





Mammalian cell

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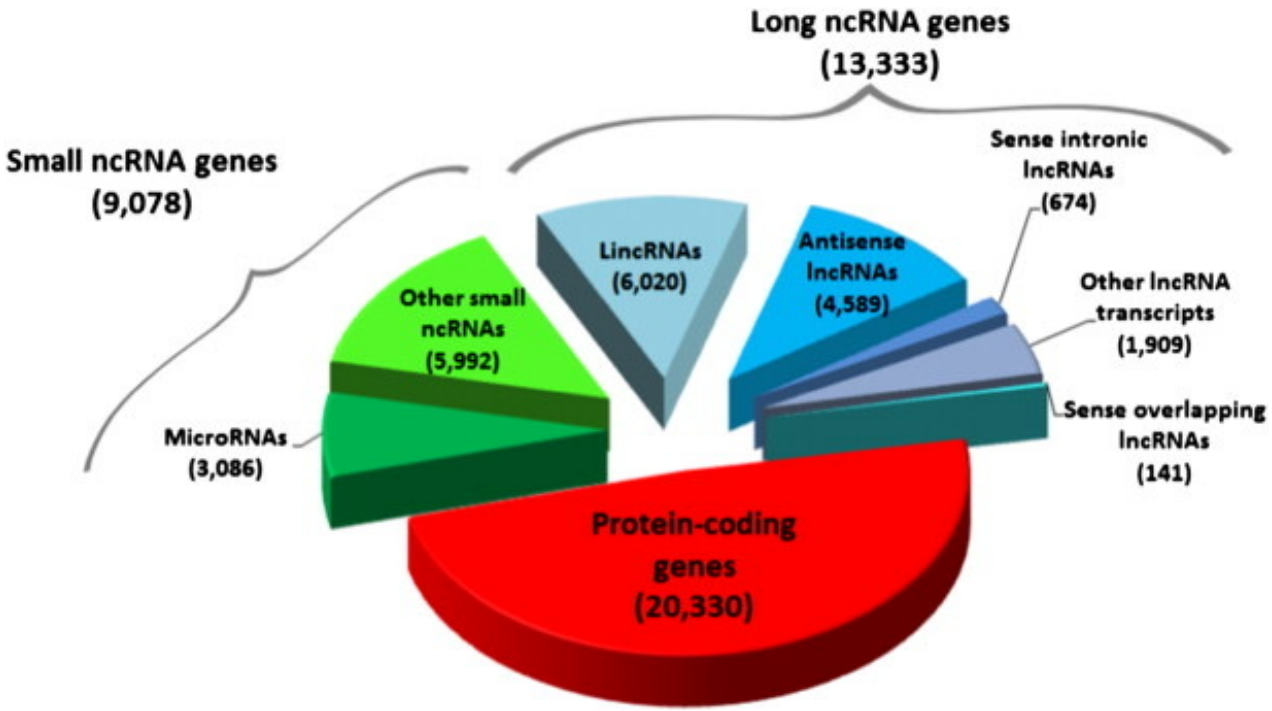
Proportion of non-coding genes

In human genome

- ~2% of coding genes
- ~6% of non-coding genes
  - Due to large copies of ribosomal RNA genes



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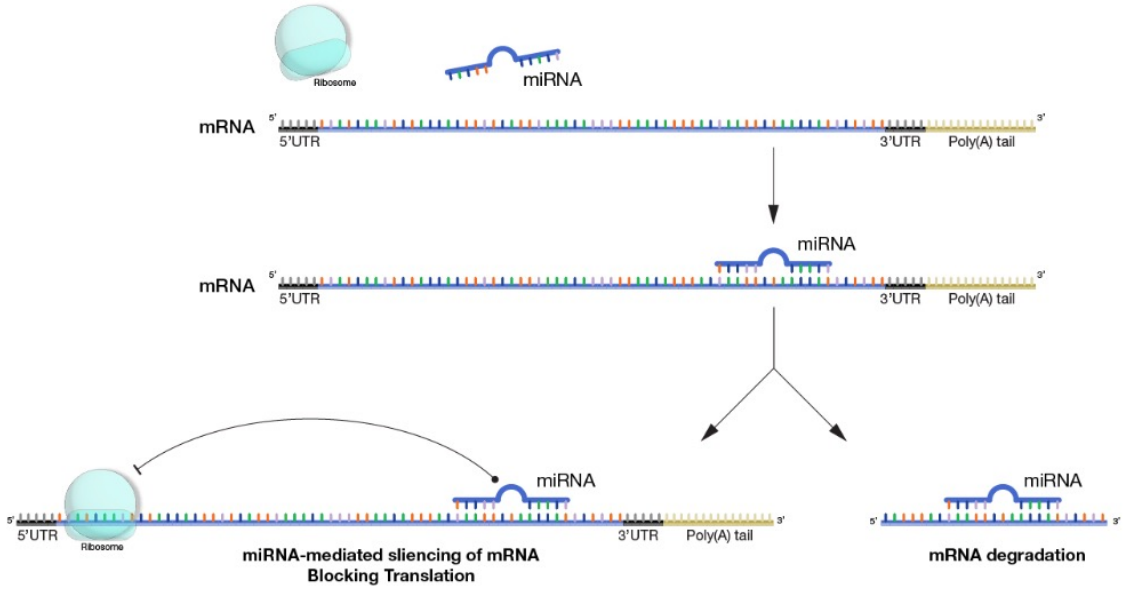
Regulation roles of miRNA and siRNA

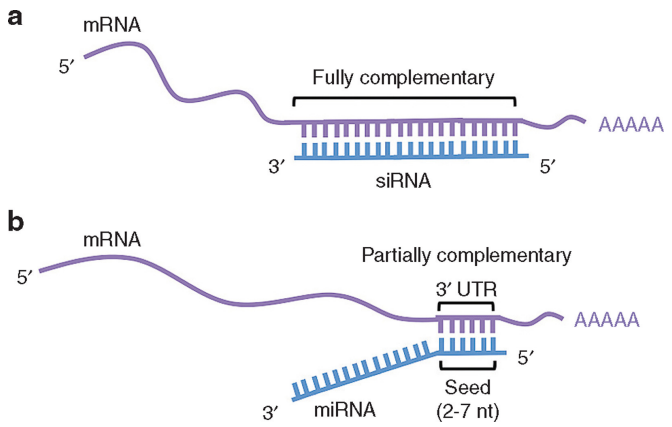
Micro ribonucleic acid (microRNA, miRNA)

- Small, single-stranded, non-coding RNA
- 17-25 nt



Small interfering RNA (siRNA)

- Small, double-stranded, non-coding RNA
- 20-24 nt



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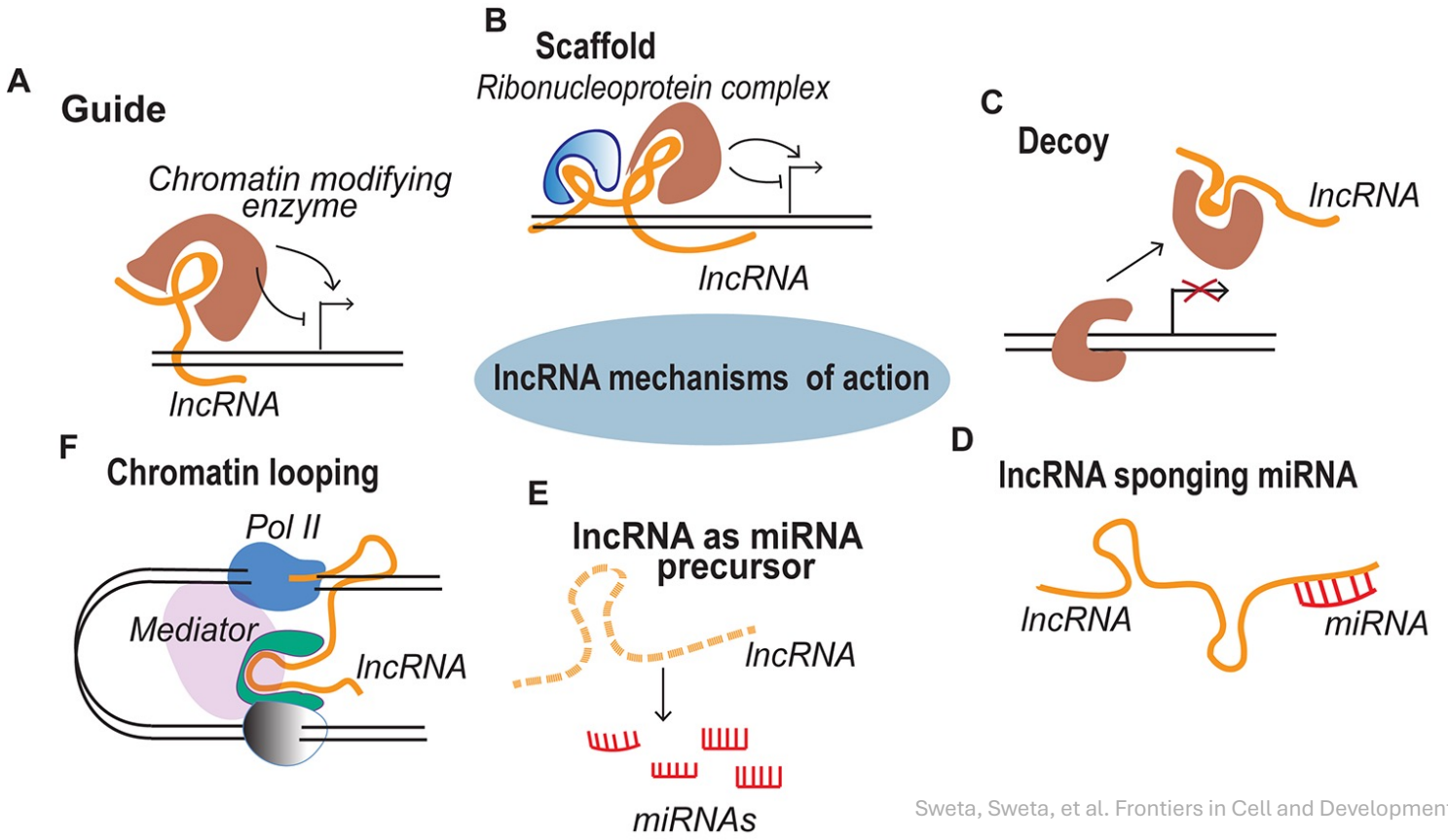
lncRNA mechanisms of action



Sweta, Sweta, et al. Frontiers in Cell and Developmental Biology 2019

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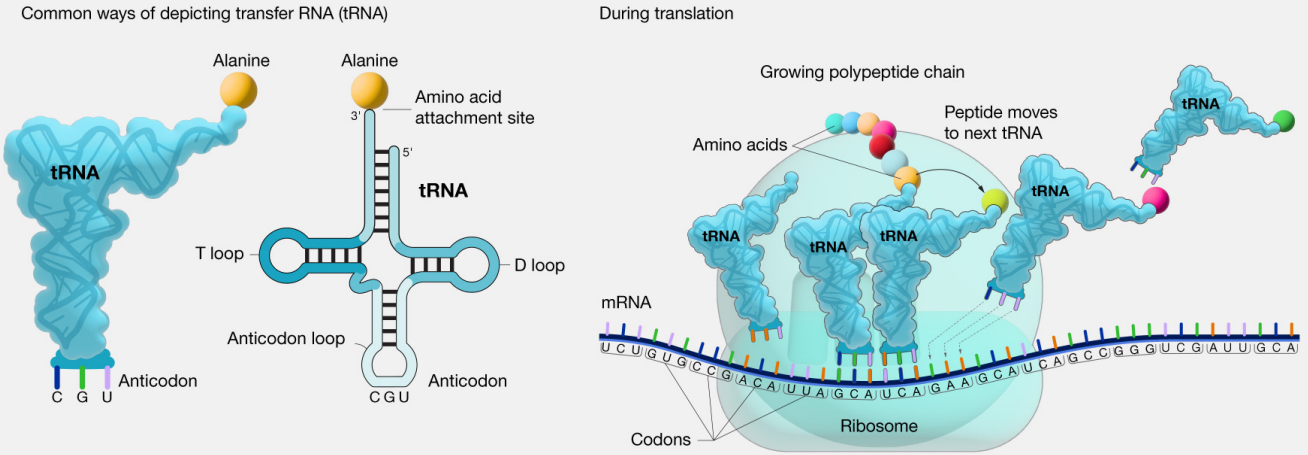
tRNA is essential in protein synthesis

- Transfer ribonucleic acid, typically 76-90 nt
- Provide physical link between mRNA and amino acid



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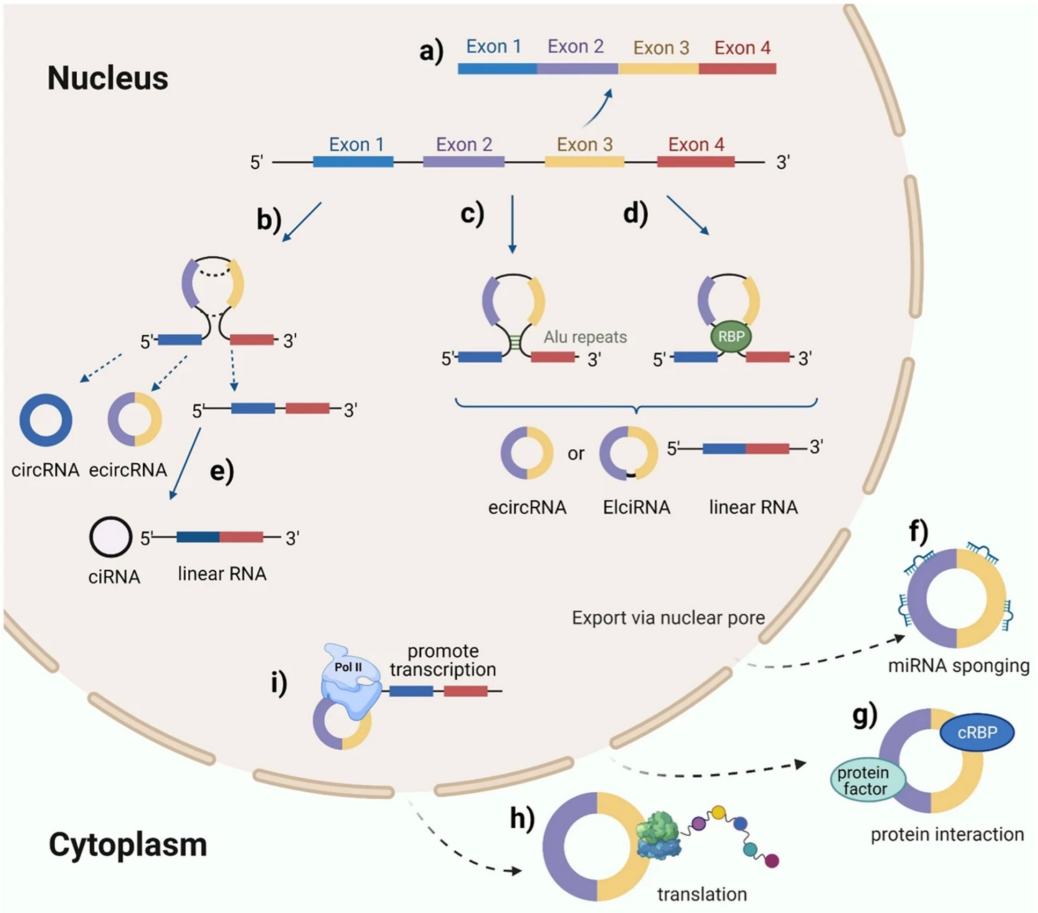
Circular RNAs: Formation and functional impact



Misir, S., Wu, N. and Yang, B.B., 2022. Cell Death & Differentiation

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Outline

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- **Transposable elements (TEs)**
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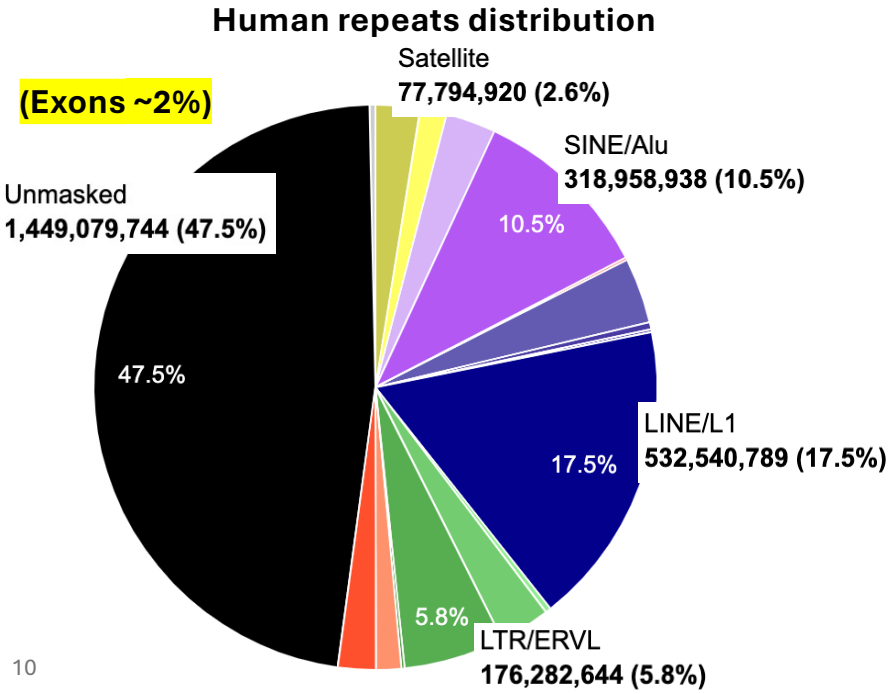
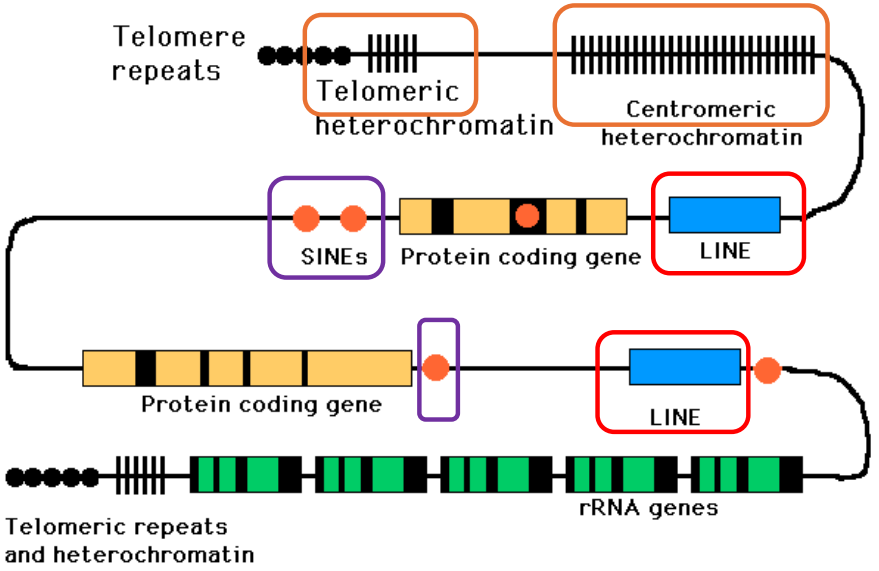
Genomic repeats take half of the human genome

- Transposable elements (TEs):
  - Long interspersed elements (LINE), short interspersed elements (SINE), LTR, DNA transposons
- Tandem repeats and large satellite repeats





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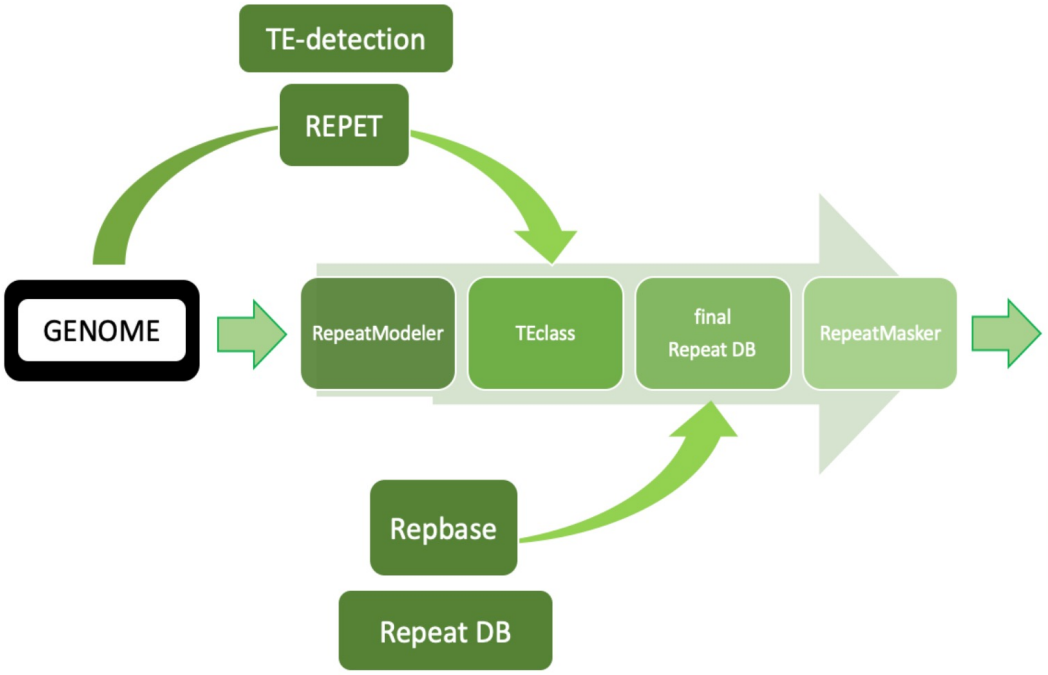
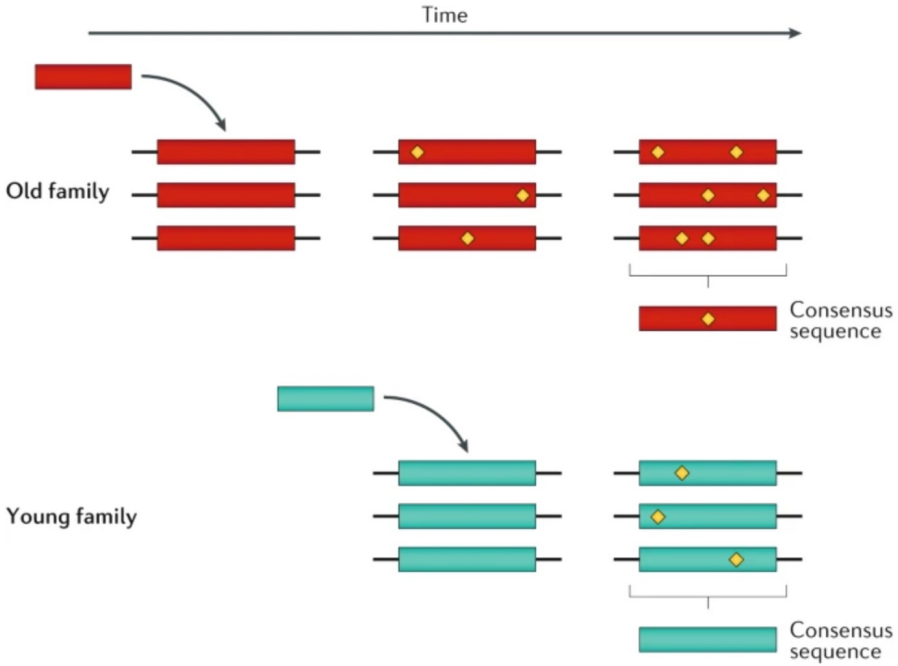
Genomic repeats annotation with RepeatMasker



Repeat Annotation Results	
Total interspersed repeats	**15.89 %**
DNA	8.69 %
LINE	1.42 %
LTR	4.38 %
SINE	0.03 %
Unknown	1.37 %
Low complexity	**0.53 %**
Simple repeats	**3.98 %**
Total	**20.25 %**

Genomic repeats annotation with RepeatMasker




```
 1306 15.6  6.2  0.0 HSU08988  6563  6781  (22462) C  MER7A    DNA/MER2_type    (0)   336   103
12204 10.0  2.4  1.8 HSU08988  6782  7714  (21529) C  TIGGER1  DNA/MER2_type    (0)  2418  1493
  279  3.0  0.0  0.0 HSU08988  7719  7751  (21492) +  (TTTTA)n Simple_repeat      1    33    (0)
 1765 13.4  6.5  1.8 HSU08988  7752  8022  (21221) C  AluSx    SINE/Alu        (23)   289     1
12204 10.0  2.4  1.8 HSU08988  8023  8694  (20549) C  TIGGER1  DNA/MER2_type  (925)  1493   827
 1984 11.1  0.3  0.7 HSU08988  8695  9000  (20243) C  AluSg    SINE/Alu         (5)   305     1
12204 10.0  2.4  1.8 HSU08988  9001  9695  (19548) C  TIGGER1  DNA/MER2_type (1591)   827     2
  711 21.2  1.4  0.0 HSU08988  9696  9816  (19427) C  MER7A    DNA/MER2_type  (224)   122     2
```

```
1306    = Smith-Waterman score of the match, usually complexity adjusted
      The SW scores are not always directly comparable. Sometimes
      the complexity adjustment has been turned off, and a variety of
      scoring-matrices are used.
15.6    = % substitutions in matching region compared to the consensus
6.2     = % of bases opposite a gap in the query sequence (deleted bp)
0.0     = % of bases opposite a gap in the repeat consensus (inserted bp)
HSU08988 = name of query sequence
6563    = starting position of match in query sequence
7714    = ending position of match in query sequence
(22462) = no. of bases in query sequence past the ending position of match
C       = match is with the Complement of the consensus sequence in the database
MER7A   = name of the matching interspersed repeat
DNA/MER2_type = the class of the repeat, in this case a DNA transposon
          fossil of the MER2 group (see below for list and references)
(0)     = no. of bases in (complement of) the repeat consensus sequence
          prior to beginning of the match (so 0 means that the match extended
          all the way to the end of the repeat consensus sequence)
2418    = starting position of match in database sequence (using top-strand numbering)
1465    = ending position of match in database sequence
```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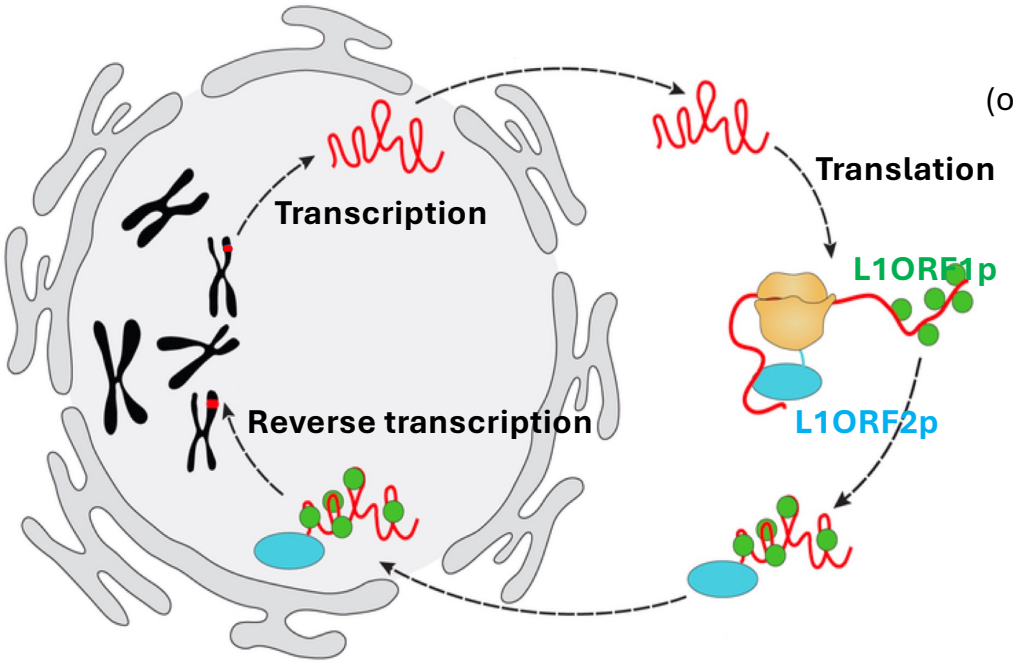
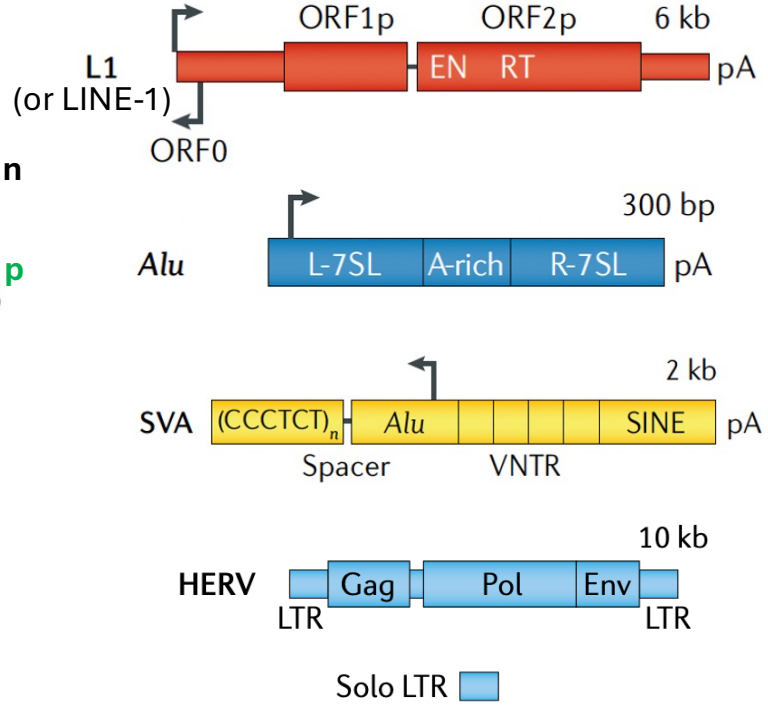
Retrotransposons "mobilize" from one location to another



Transposable elements are ancient viruses



Payer and Burns, 2019, Nature Reviews Genetics

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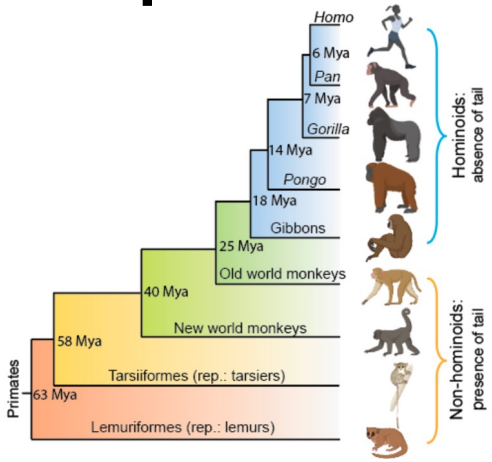
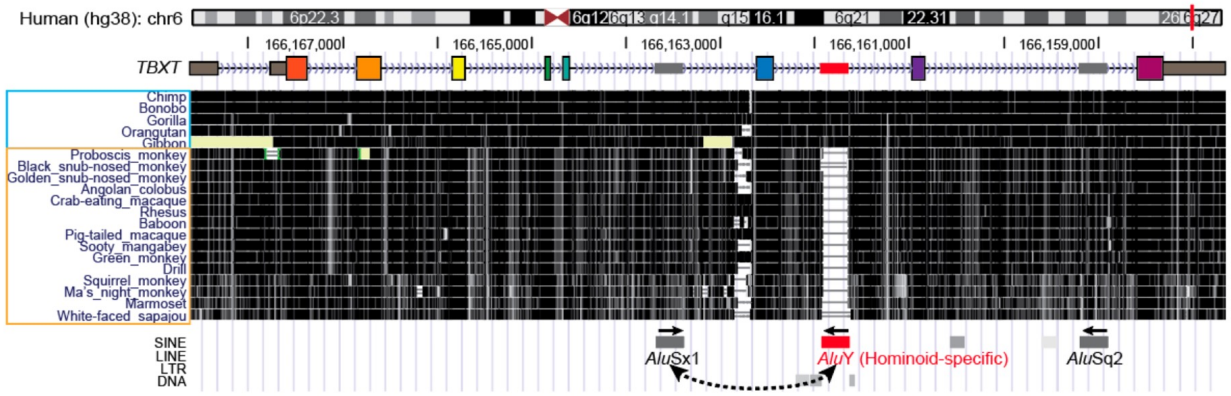
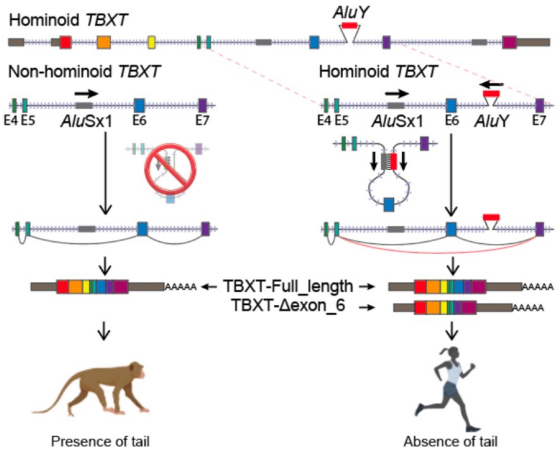
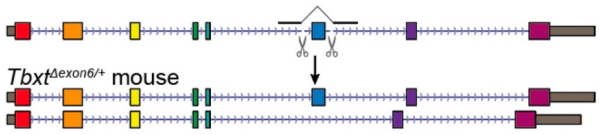
Roles transposable elements are playing (Example: where is our tail?)









Xia, Bo et. al. *Nature* 2024

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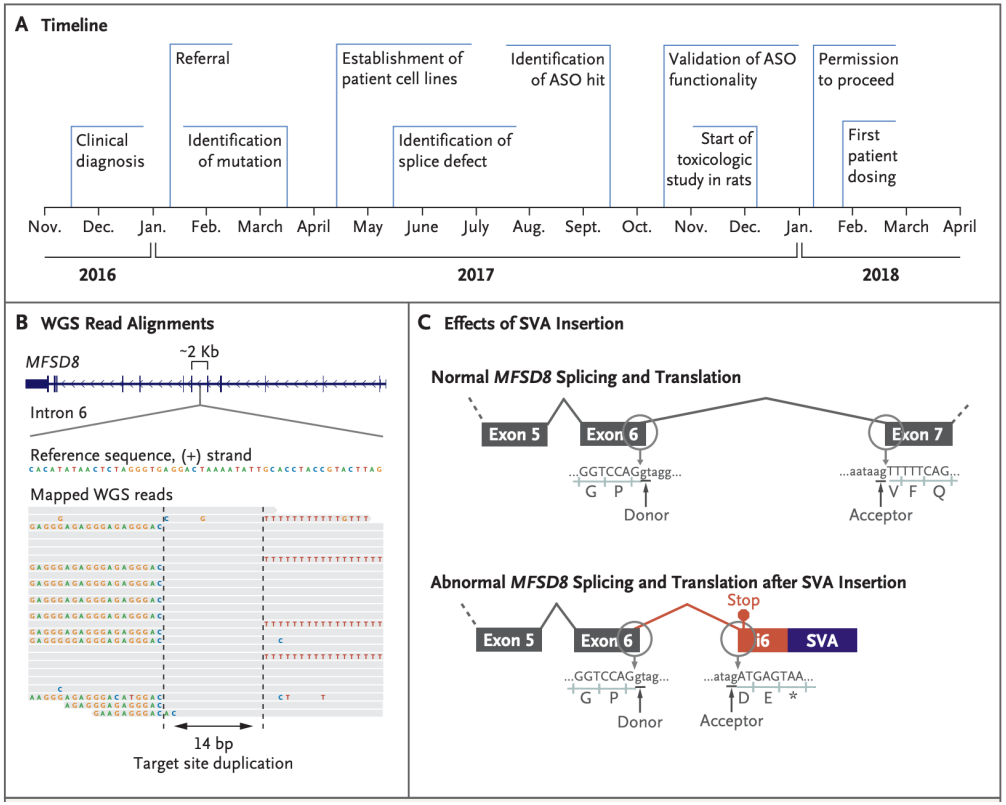
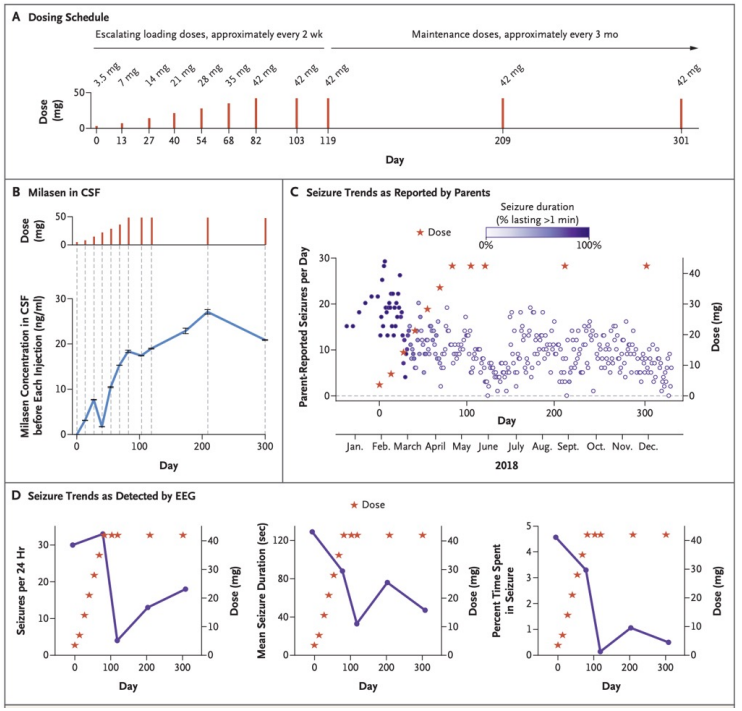
An SVA insertion causes Batten disease (rare disease)





Kim, Jinkuk, et al. *New England Journal of Medicine* (2019).

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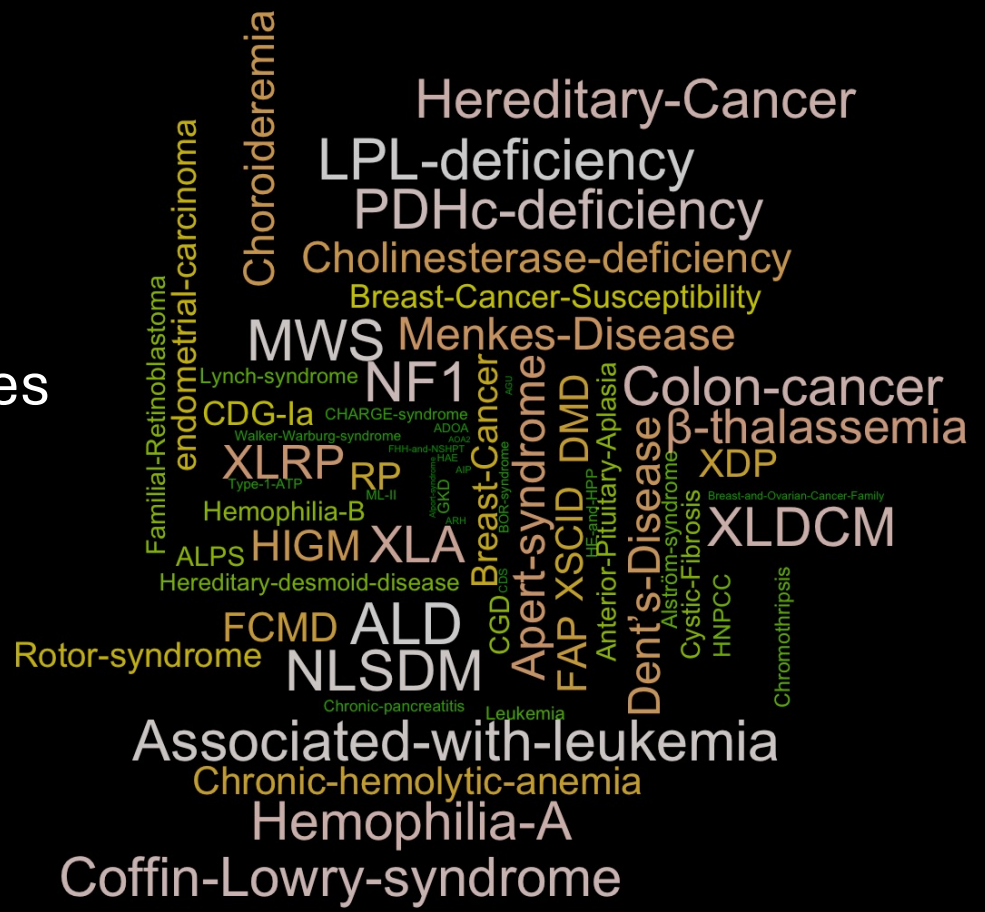
More than **65** human diseases directly caused by retrotransposon insertions



Dustin C. and Kazazian. *Mobile DNA*, 2016

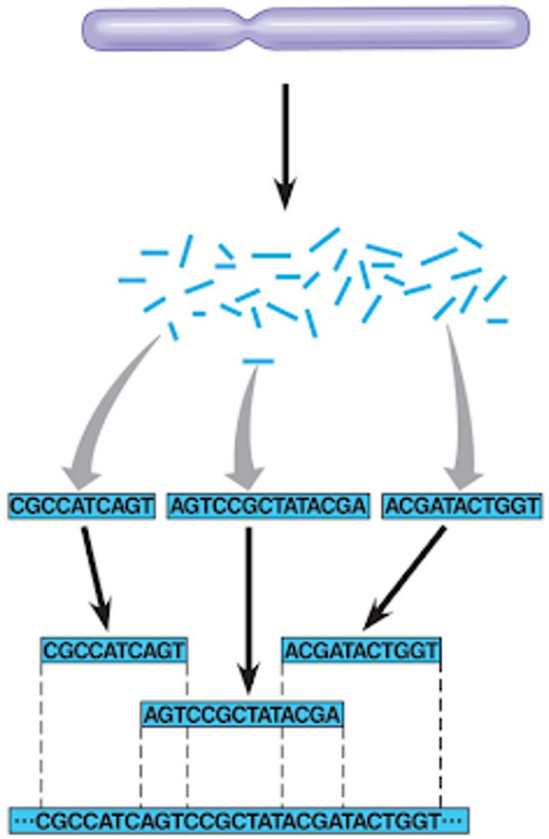
TE insertion identification from sequencing data

Cut the DNA into fragments

Clone the fragments

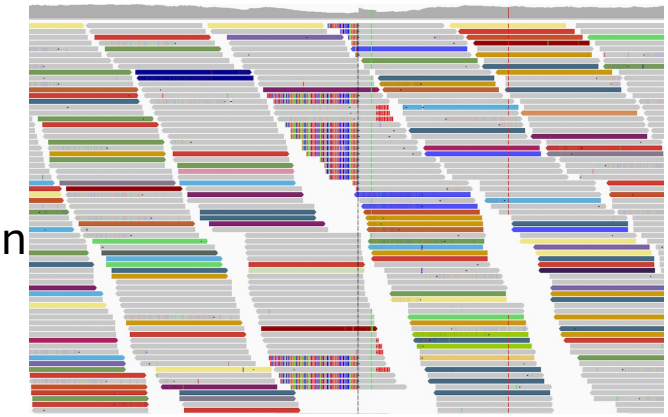
Sequence each fragment

Order/align the sequences

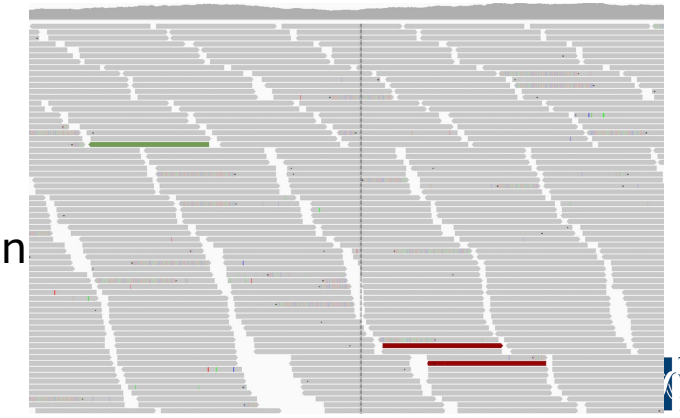


Different patterns

With insertion



No insertion



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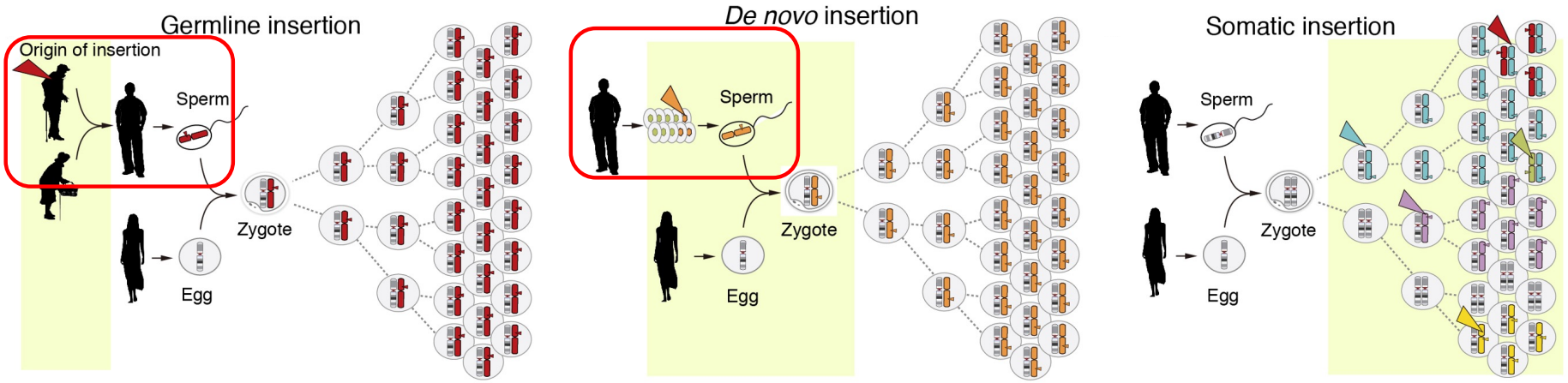
Challenges and motivation (different types of TE insertions)



- ~2,000 TE insertions each person
- Specificity is important in building a database

- *De novo* insertion is rare
- Sensitivity is more important in detection

- Low allele-frequency if TE insertions happened late

Chu*, Zhao*, et al. *Current Protocols in Human Genetics* 2020

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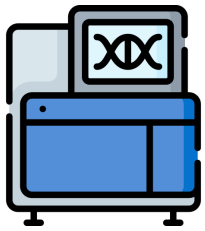
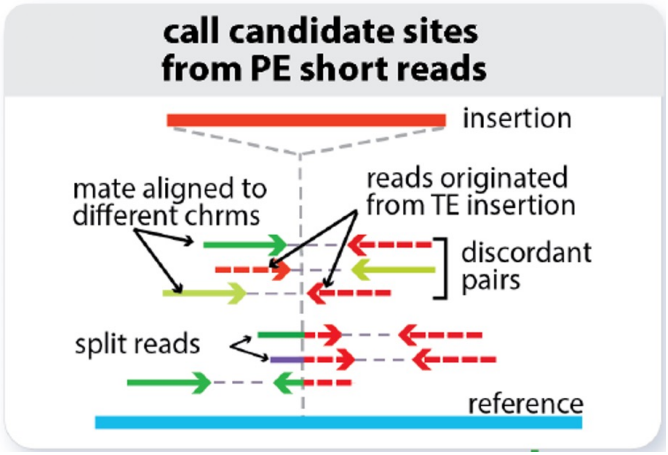
xTea: TE insertion identification from short read sequencing data

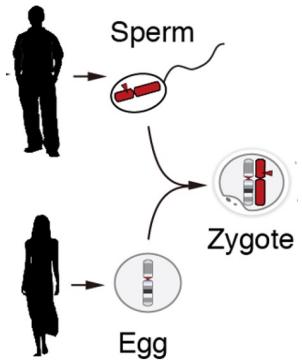
TE insertion **location** identification

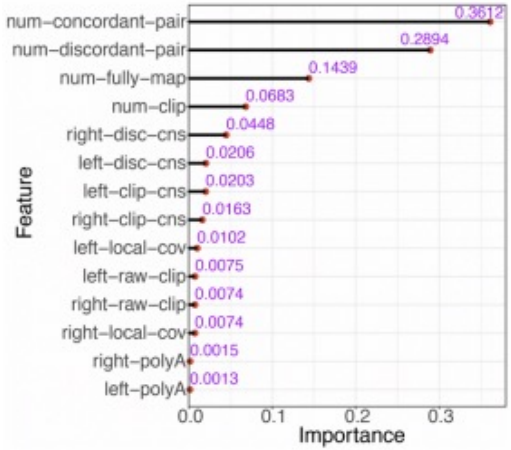


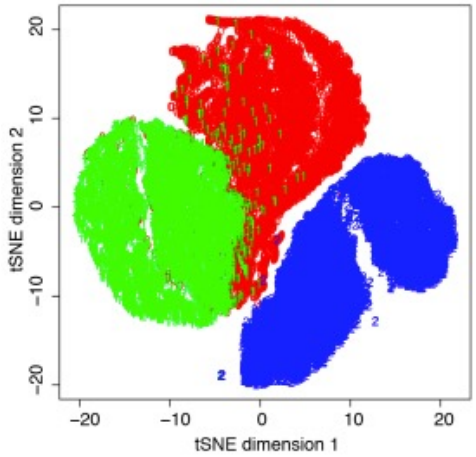
Personal sequencing data



Germline TE insertion **genotype** identification







Chu et. al. *Nature Communications* 2021

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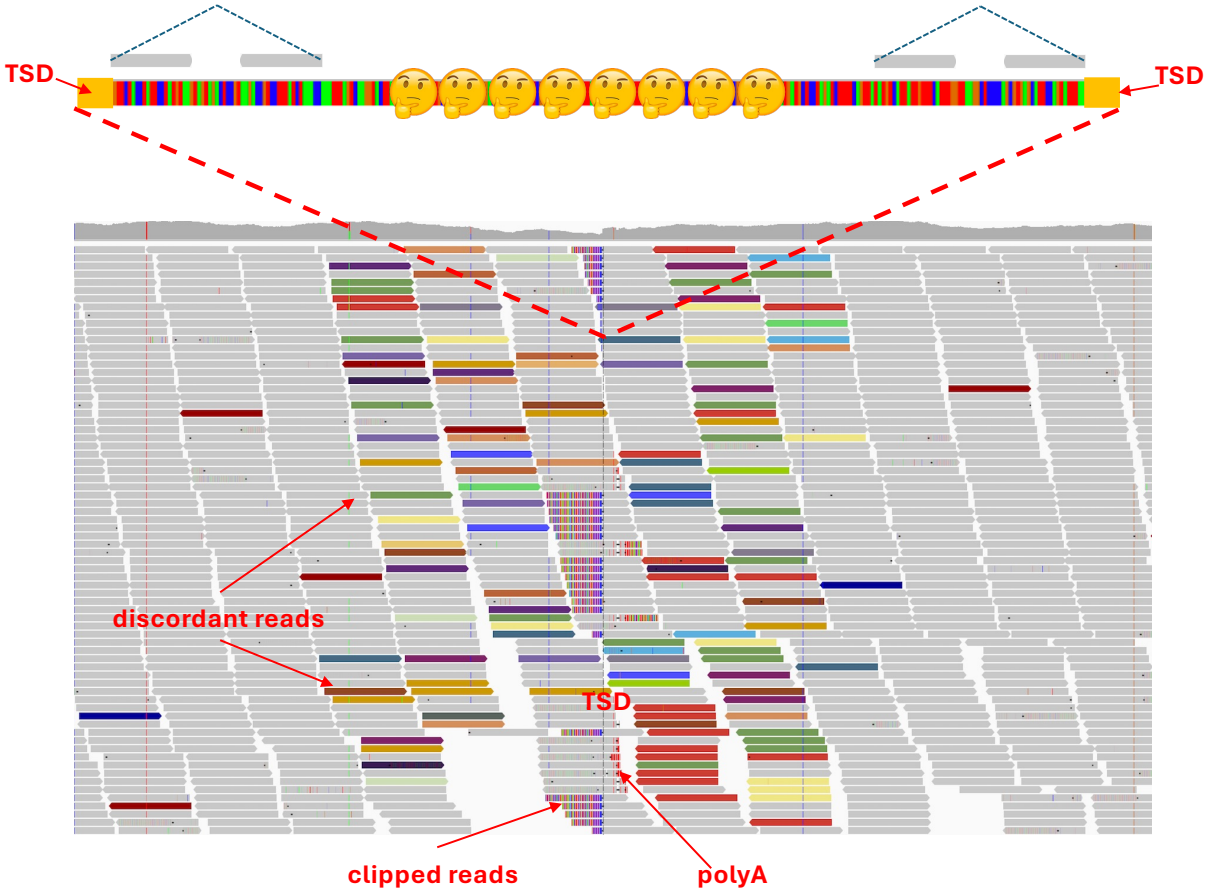
We cannot construct the full insertion from short reads



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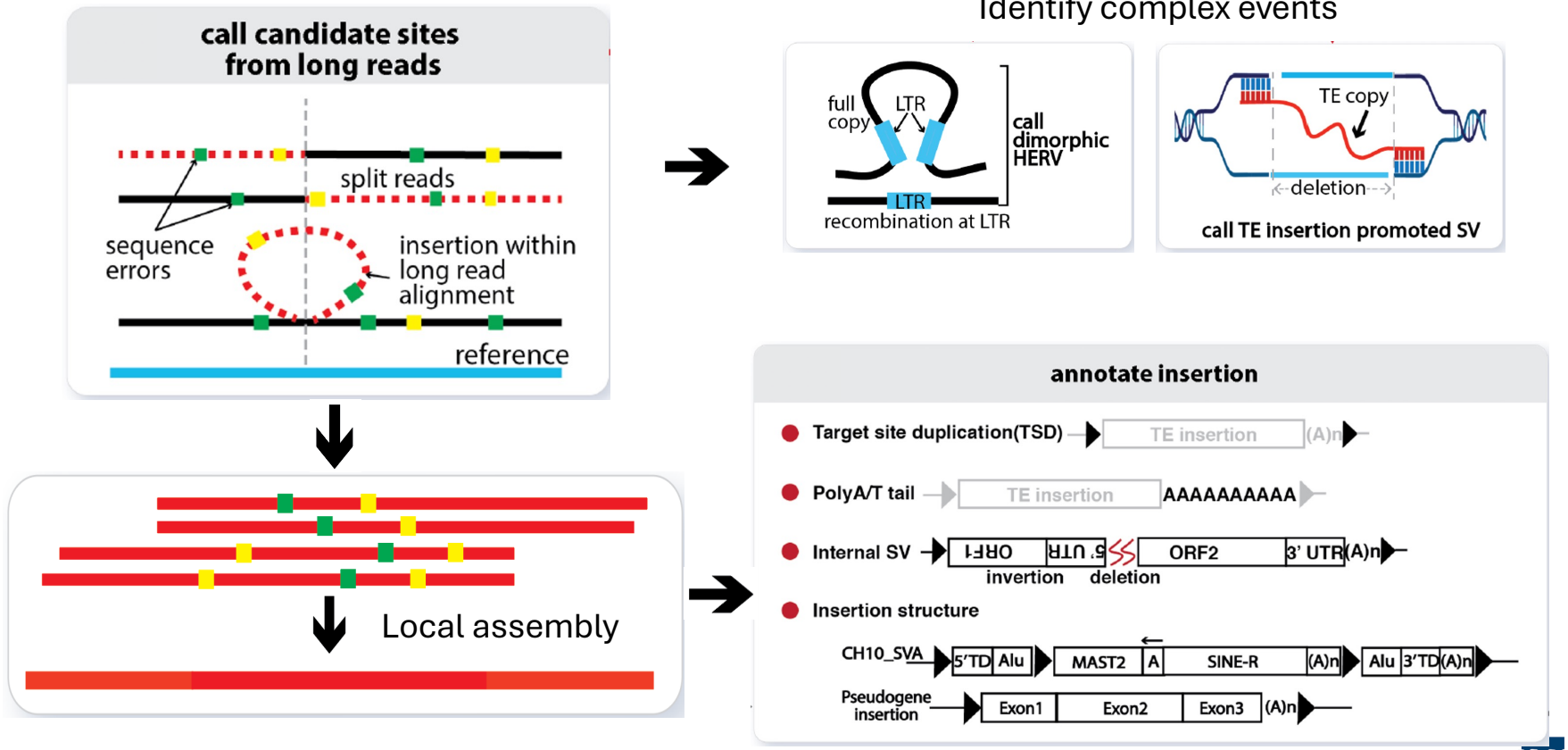
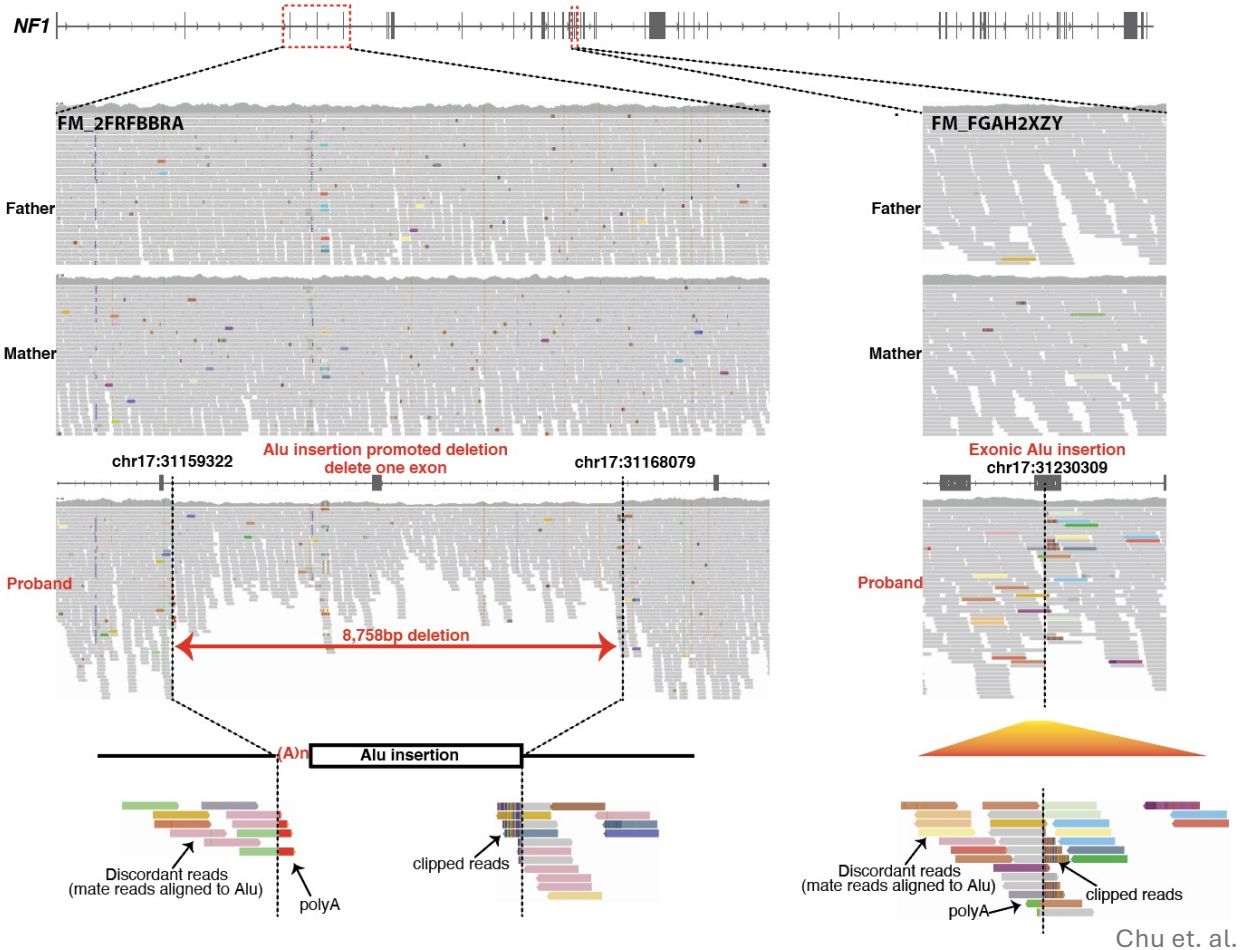
xTea: TE insertion identification from long read sequencing data



 Chu et. al. *Nature Communications* 2021

Example TE insertions cause brain tumors



Chu et. al. medrxiv, 2024

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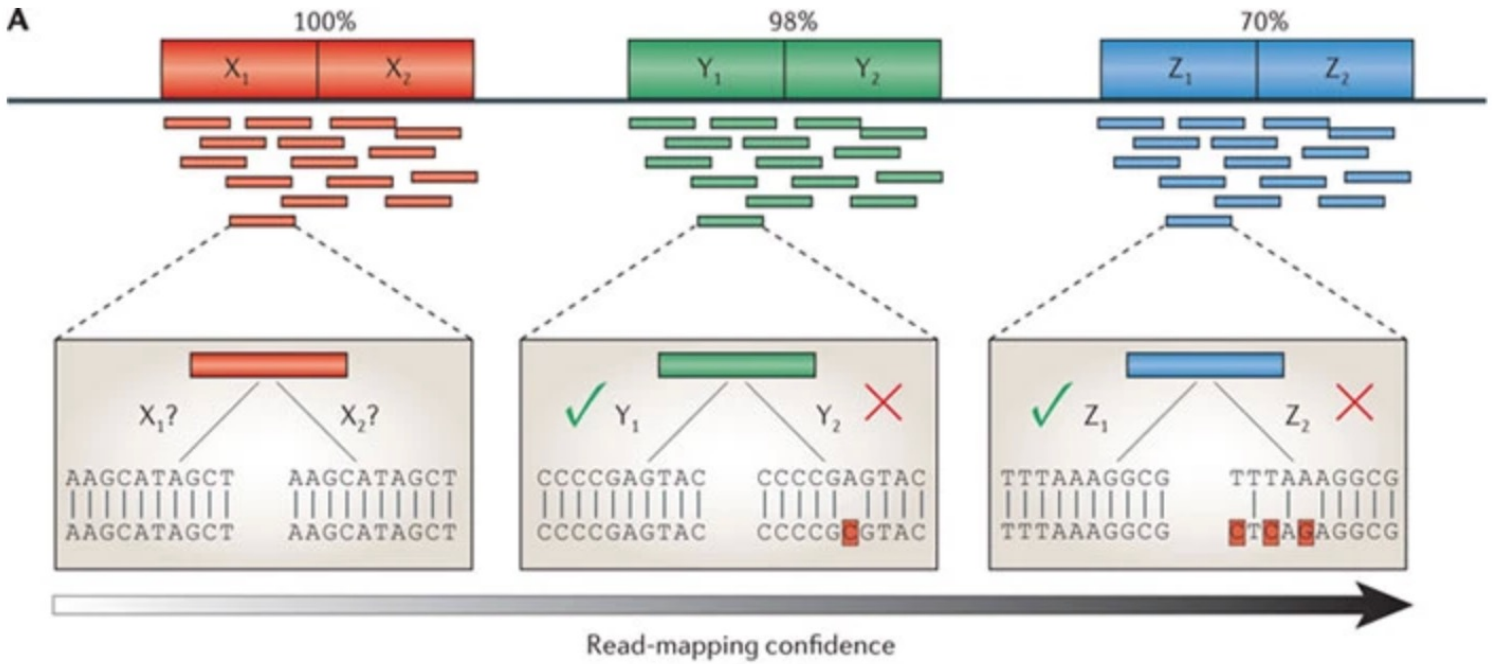
TE RNA expression quantification is challenging



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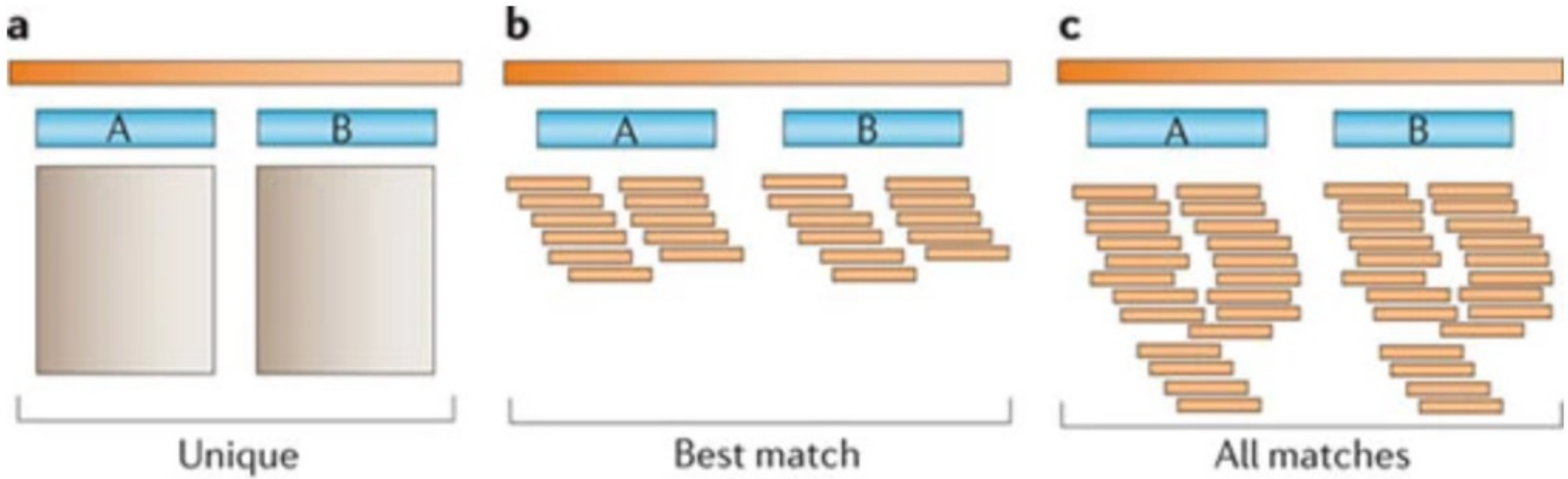
Different strategies in dealing with multi-mapped reads



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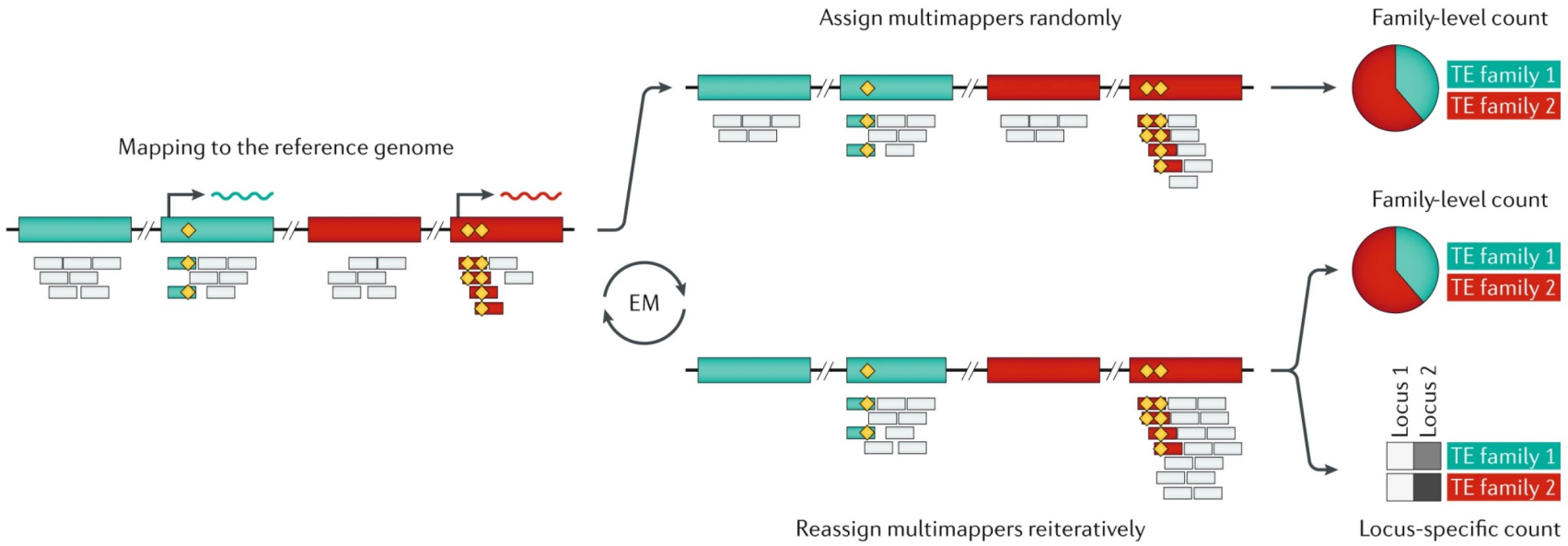
TE RNA expression quantification approaches



e.g. TEtranscript, SQuIRE

Lanciano and Gael, Nature Reviews Genetics, 2020

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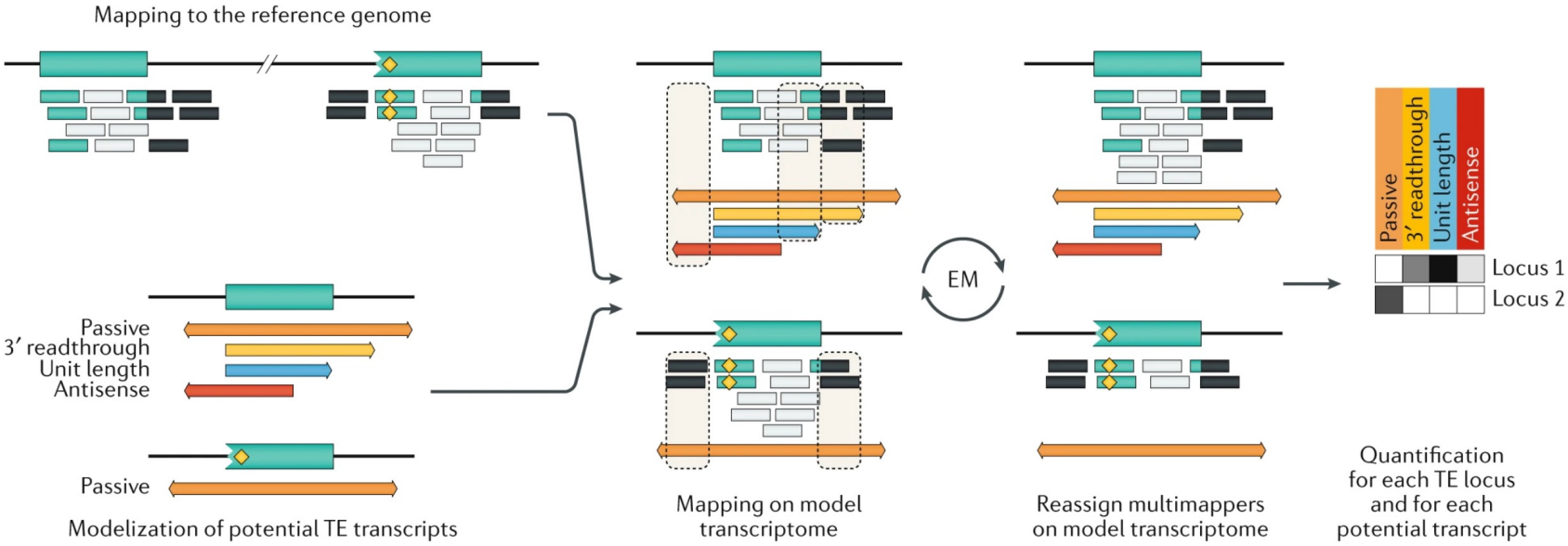
TE RNA expression quantification approaches



e.g. L1EM

Lanciano and Gael, Nature Reviews Genetics, 2020

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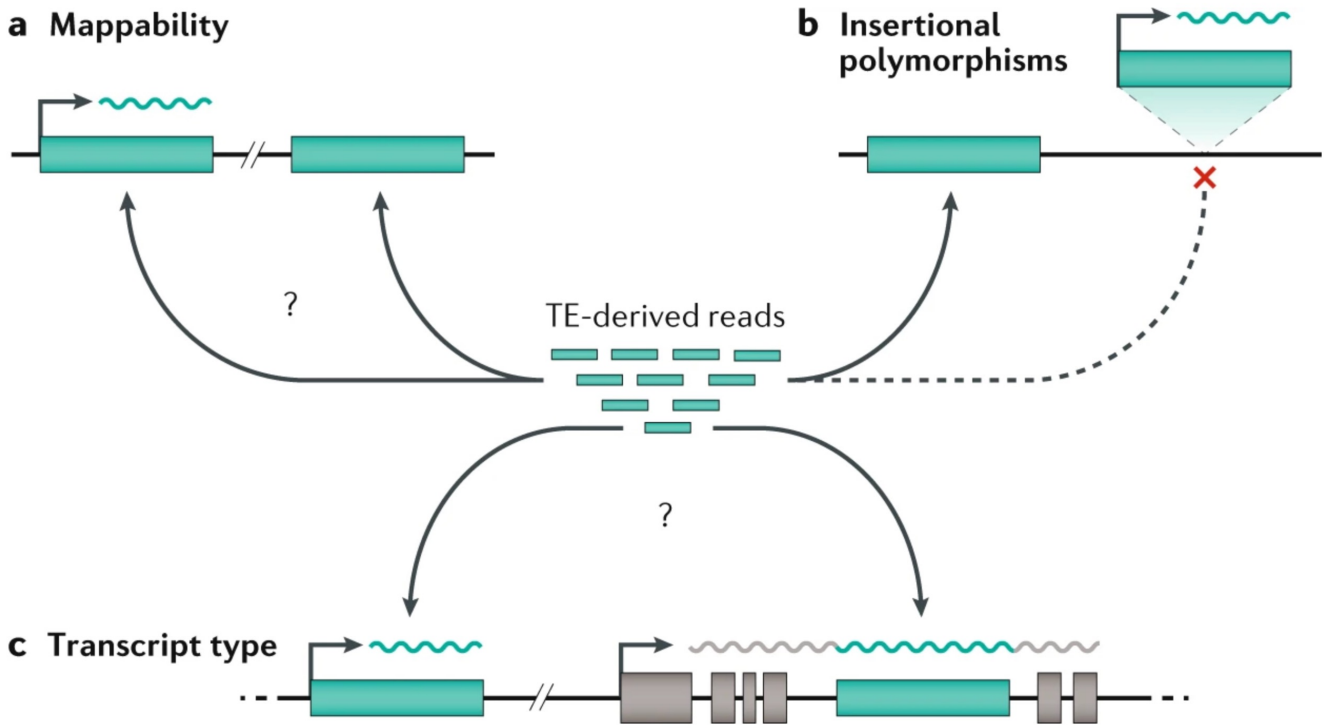
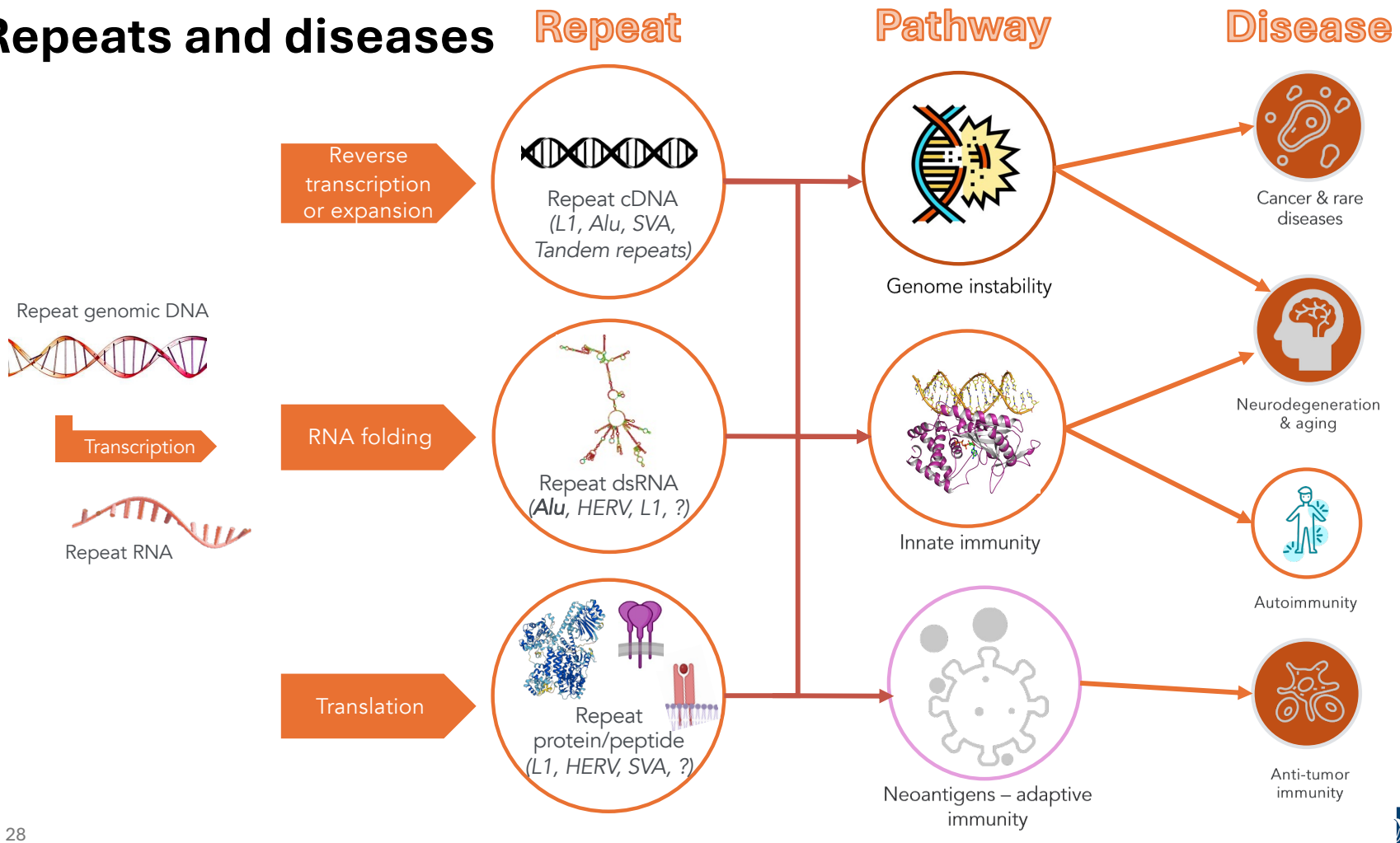
TE RNA expression quantification remains challenging



Lanciano and Gael, Nature Reviews Genetics, 2020

Repeats and diseases



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Dark genome matters

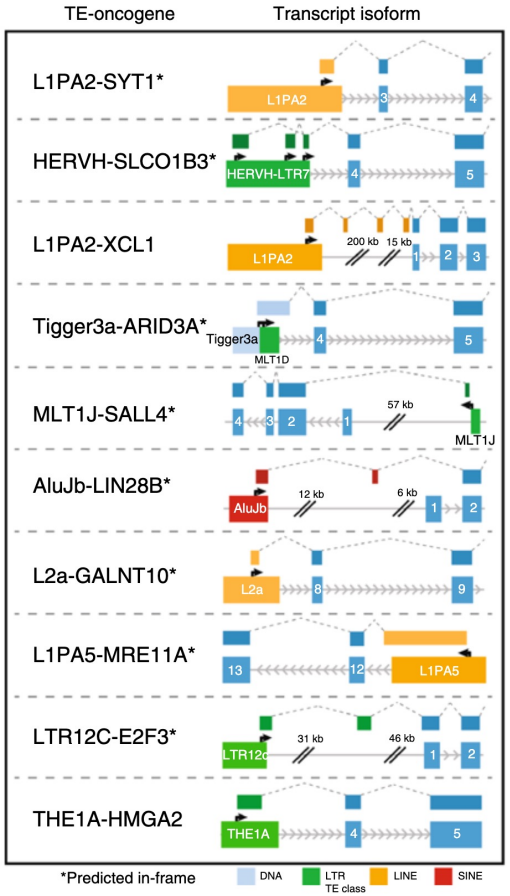
Transcribed smORFs

ORF class	RNA type	Median size (codons)	Translation[15]	Conservation	Coding features	Function
Intergenic ORFs	None	22	None	None[6,8]	Non-canonical AA	None
uORFs	5′ UTRs of mRNAs	22	Low	None[8,30]	• Nonrandom AA • No domains	• Non-coding • Translation regulation
lncORFs	lncRNAs	24	Low	None[8,10]	• Nonrandom AA • No domains	Non-coding or coding
Short CDSs	Short mRNAs	79	High	Class	• Positively charged AA • Transmembrane α-helices	• Coding • Regulators of canonical proteins
Short isoforms	Spliced mRNAs	79	High	Kingdom	• Canonical AA • Protein domain loss	• Coding • Small interfering peptides
Canonical ORFs	mRNAs	491	High	Kingdom	• Canonical AA • Multiple protein domains	• Coding[42] • Structural, enzymatic, regulatory

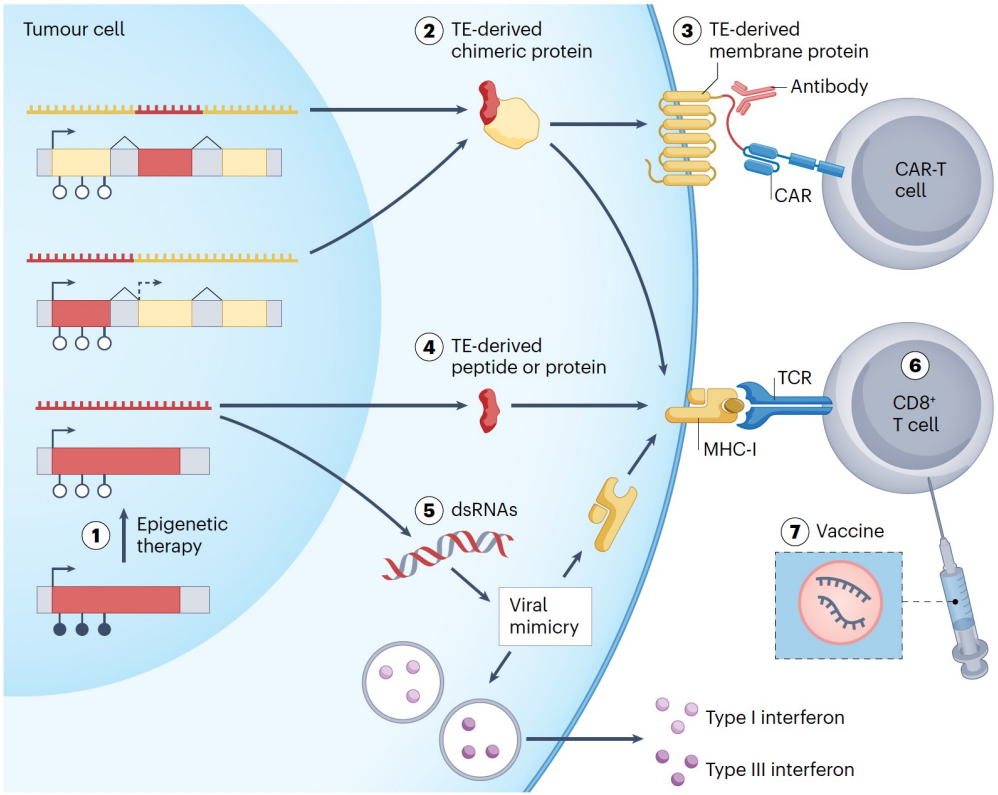
Untranslated region · ORFs · DNA · Other coding sequences · RNA splicing · Ribosome profiling signal

Couso, J.P. and Patraquim, P., 2017. Nature reviews Molecular cell biology

TE exonization as an enriched reservoir of novel targets



Jang, et. al., *Nature Genetics*, 2019



Liang, Yonghao, et al. *Nature Reviews Cancer*, 2024

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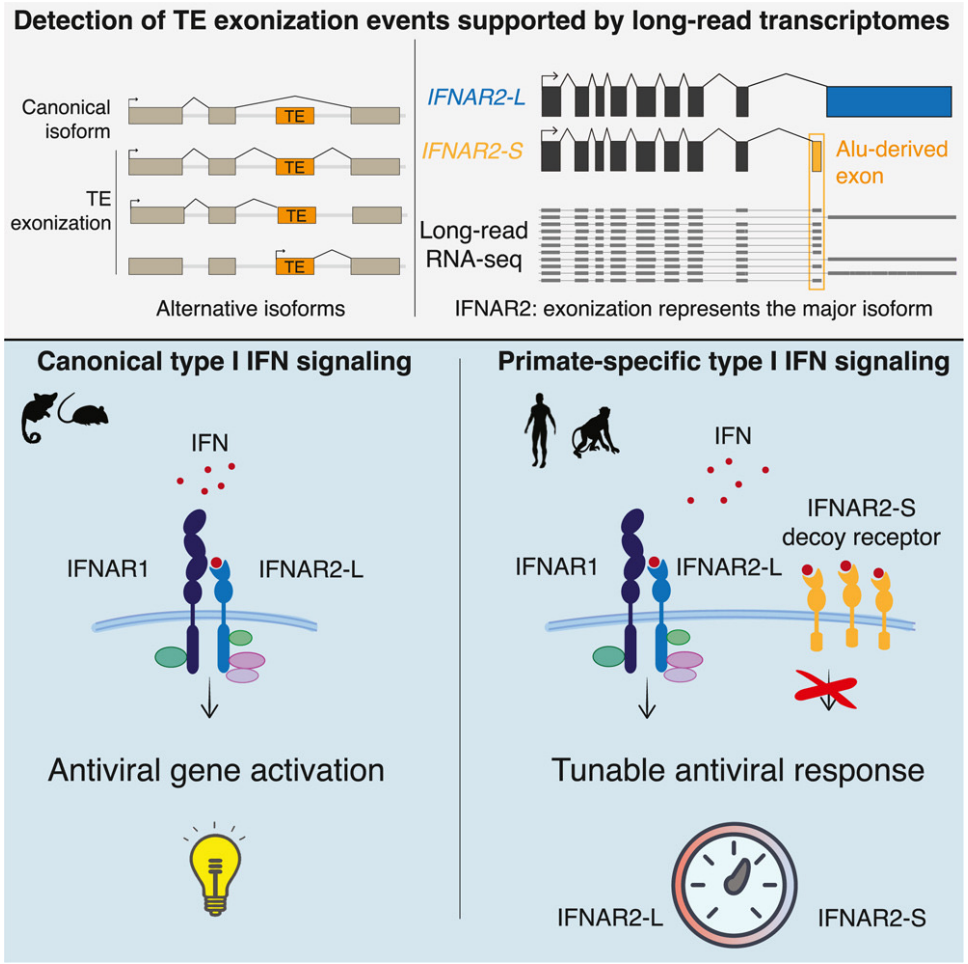
Functional TE exon-trapping



Pasquesi, Giulia Irene Maria, et al. *Cell,* 2024

31

TrapHunter: TE derived exon-trapping identification

Different types of exon-trapping events:

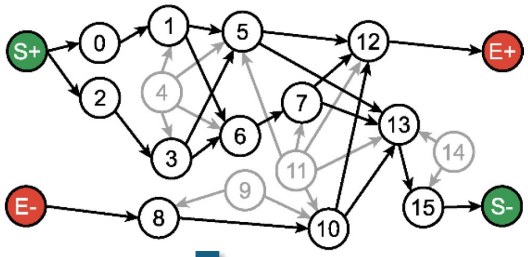
Initiated from repeats:

Spliced to repeats:

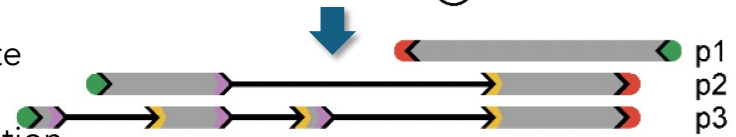
Terminated at repeats:

TrapHunter

Short-reads assembly

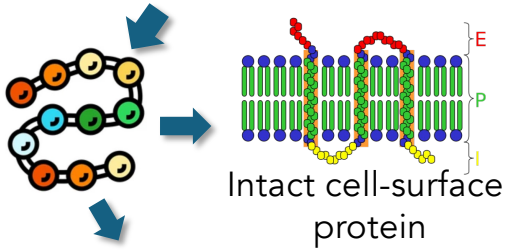


Candidate fusion identification



Second-stage contig assembly

Translate to aa sequence



Chu et. al. in prep.

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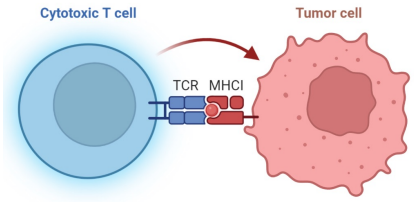
Three major criteria in neoantigen discovery

Tumor specific

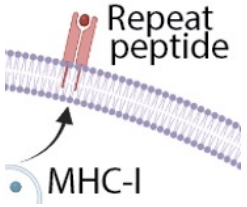


- Identified in tumor RNA but not normal
  - Differentially expressed

Prevalent in patients

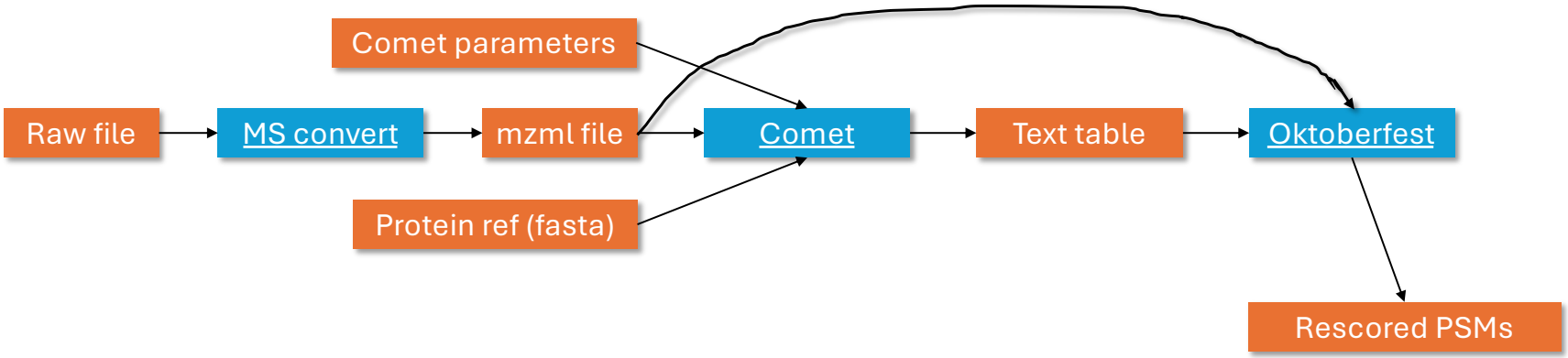
- Prevalent in tumors
  - Prevalent in TCGA tumors

On cell surface

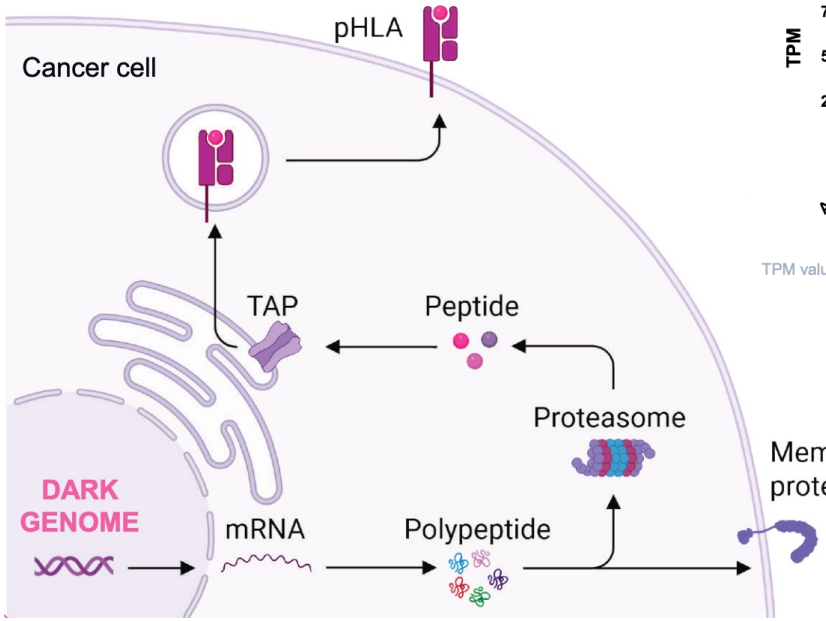


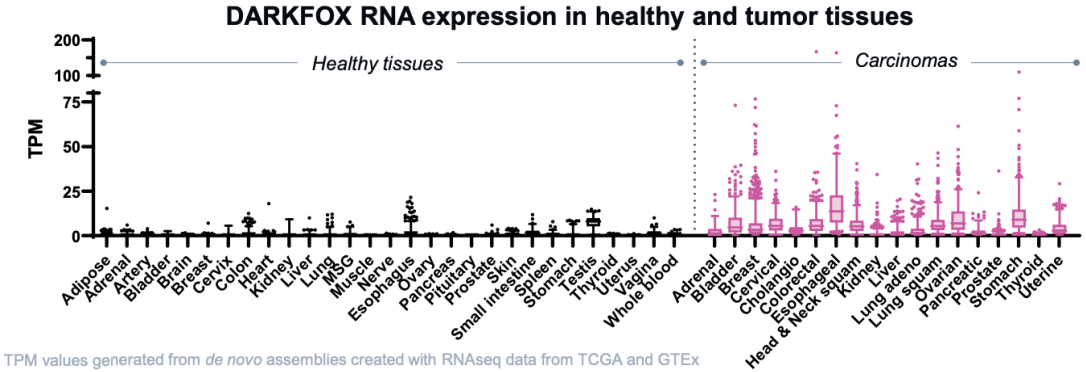
- Translated and on cell surface
  - Immunopeptidomics data

Neoantigen discovery pipeline with immunopeptidomics data

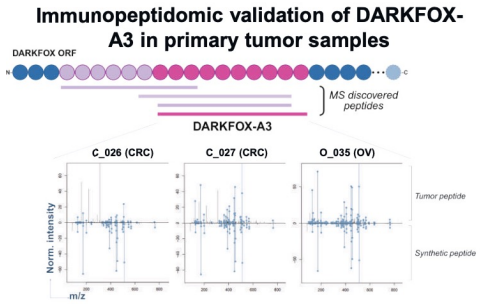


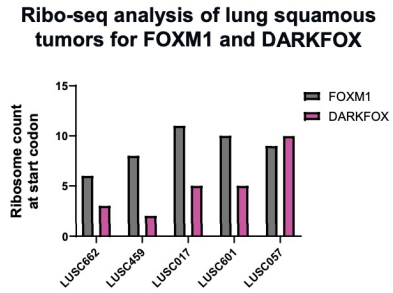
Implication example: Dark genome derived neoantigens for mRNA cancer vaccines





TPM values generated from *de novo* assemblies created with RNAseq data from TCGA and GTEx





Figures from Enara Biosciences

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Summary

- **Non-coding RNAs**
  - Different types of non coding RNAs
  - Summary of their functions

- **Transposable elements (TEs)**
  - TEs play important regulation roles
  - Sequence annotation with RepeatMasker
  - TE insertion identification with xTea
  - Current approaches in TE RNA expression quantification
  - TE derived neoantigen for mRNA cancer vaccine