An introduction to transposable element biology

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Bachelor/Master

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Ecology/Popgen

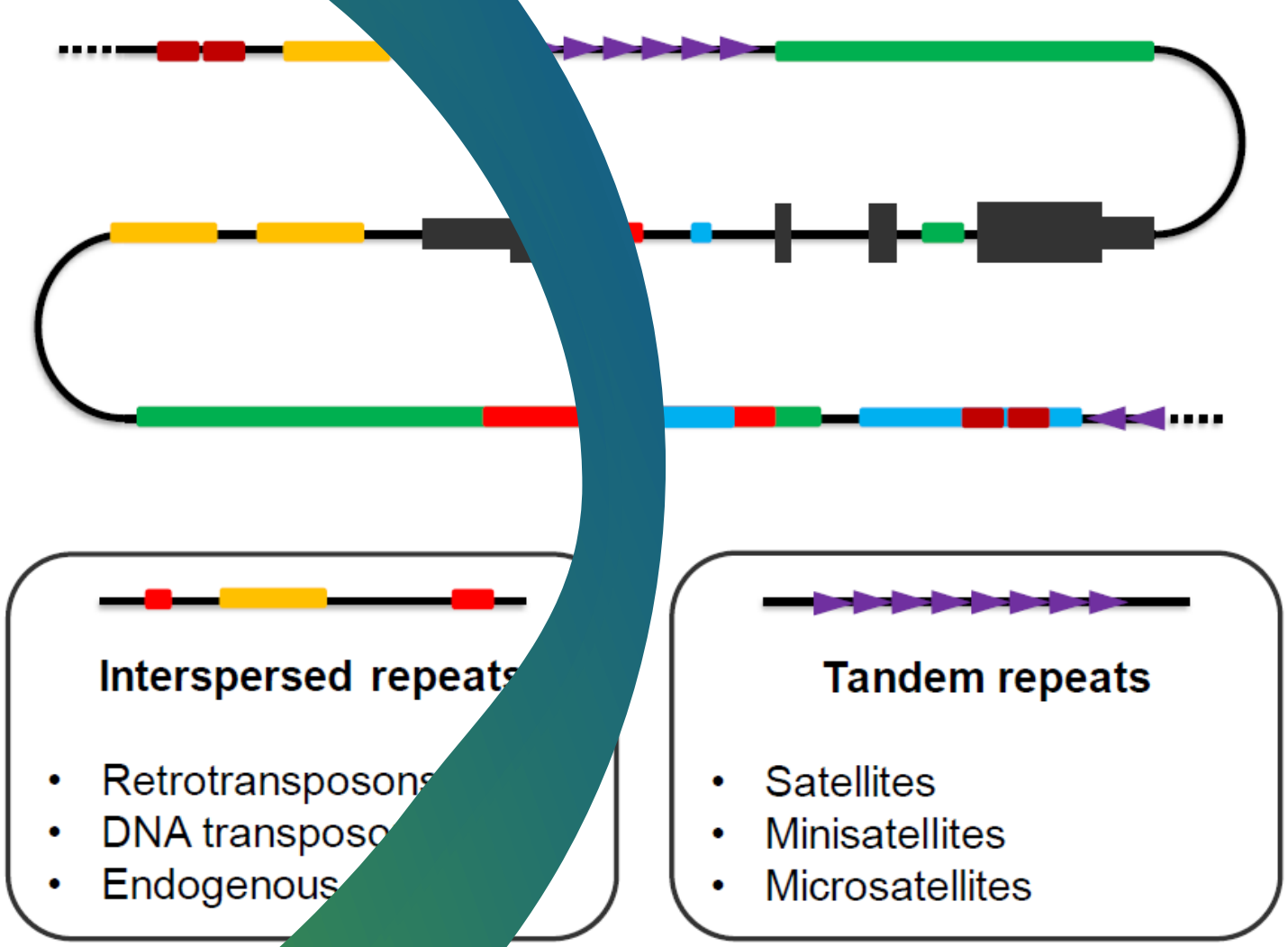
Comparative genomics

Speciation genomics

Overview

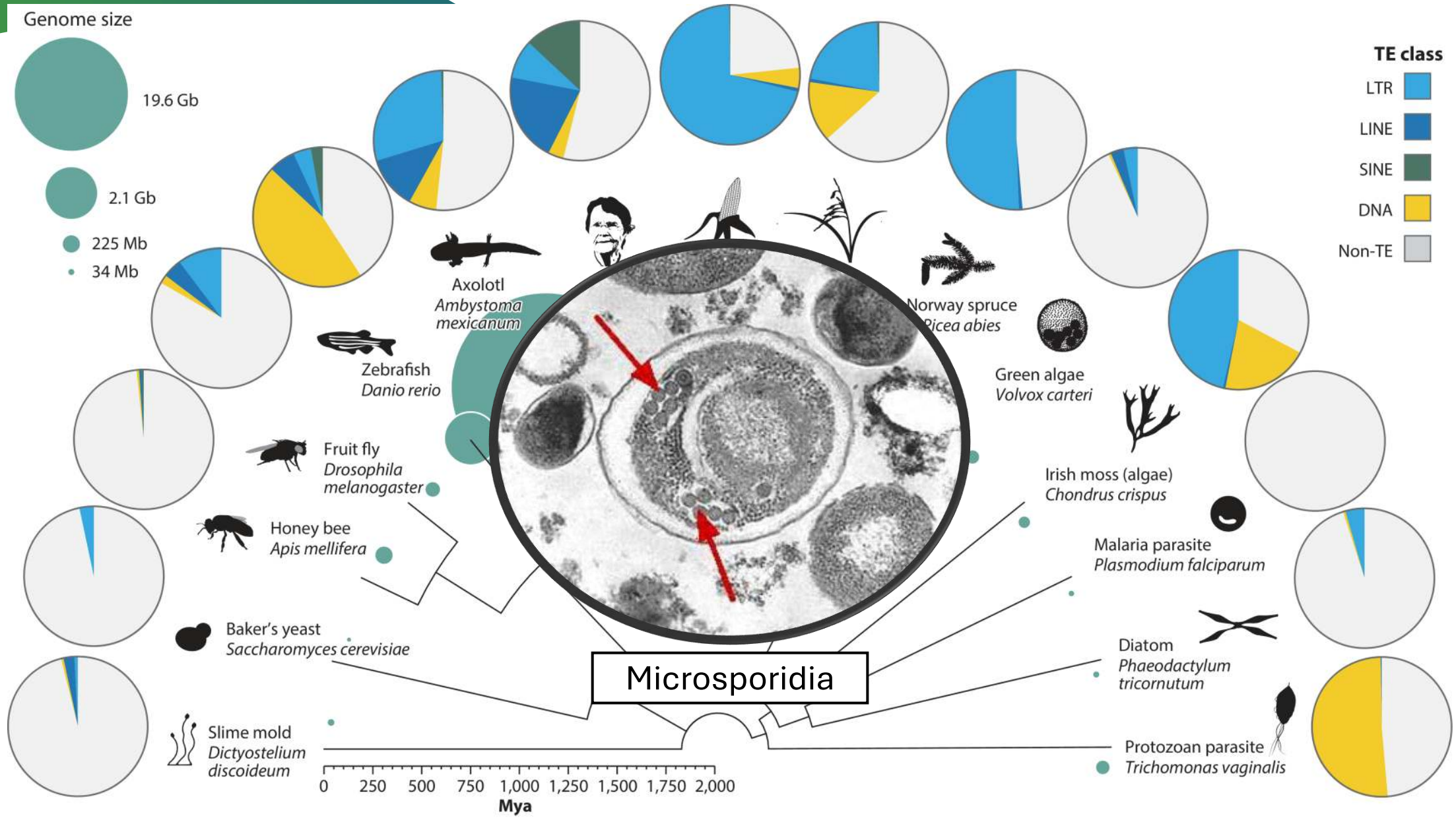
- Part1: intro to TE biology
- Part2: Methods to detect TEs in genomes (+ intro to tutorial)

nes: DNA on repeats



Barbara McClintock

Nobel Prize
1983



Wells JN, Feschotte C. 2020
Annu. Rev. Genet. 54:539–61

TEs are selfish elements

Selfish genetic elements

(anything ranging from single genes or chromosomes to entire genomes)

=

Genetic element with the sole ”purpose” to transmit itself

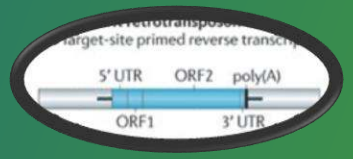
(which often comes with a cost to its host)

Why are they selfish?

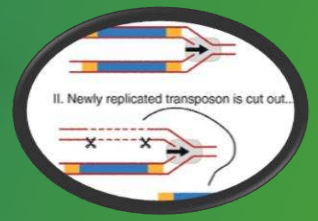
Because they can

Main TE categories

- Sequence structure

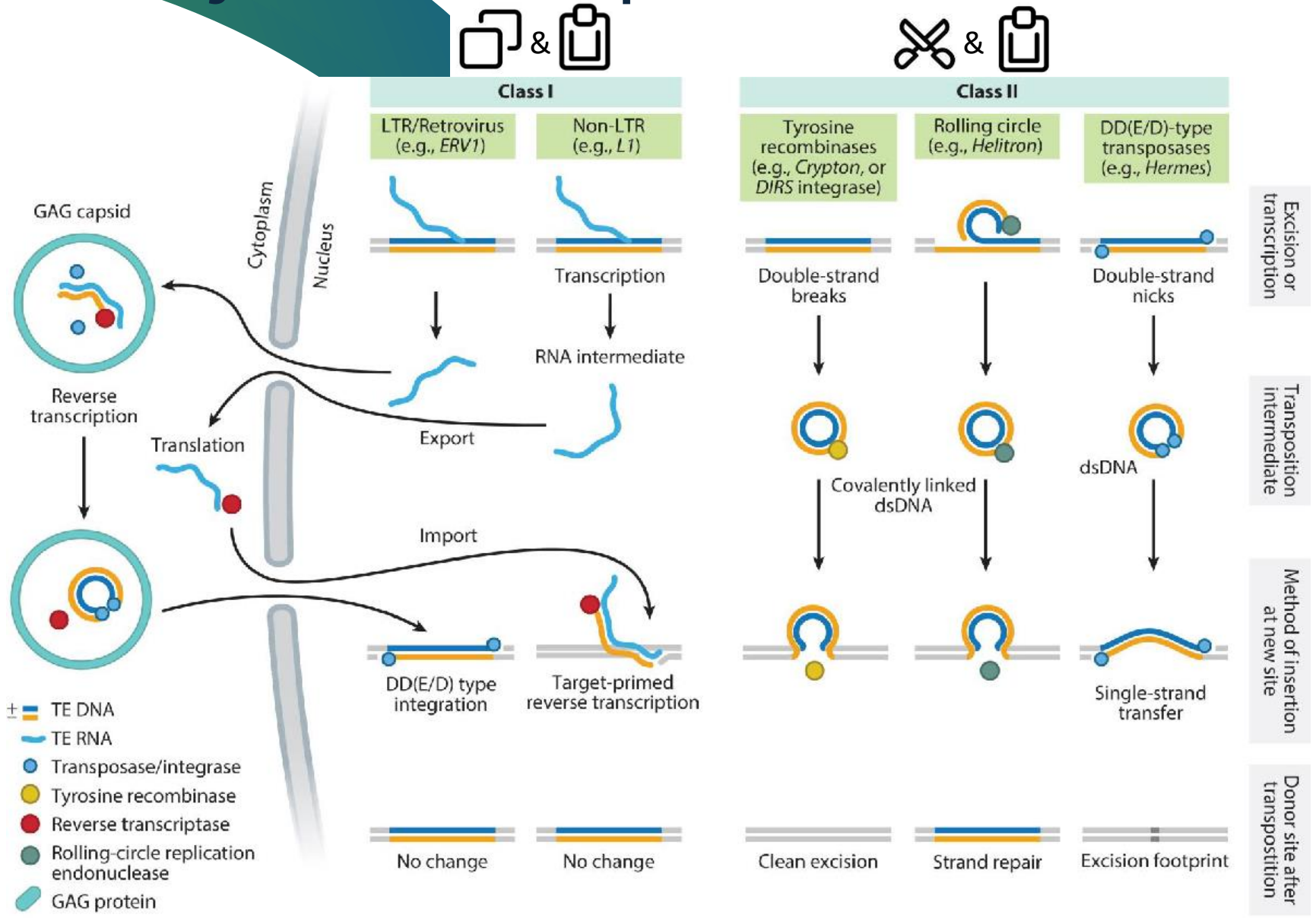


- Transposition mechanisms



- Effects on genome evolution

Eukaryotic transposable elements



Wells JN, Feschotte C. 2020

Class I: LINE retrotransposons

Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
PLE	*Penelope*	RT EN	Variable	RPP	P, M, F, O
LINE	*R2*	RT EN	Variable	RIR	M
	RTE	APE RT	Variable	RIT	M
	Jockey	ORF1 APE RT	Variable	RIJ	M
	L1	ORF1 APE RT	Variable	RIL	P, M, F, O
	I	ORF1 APE RT RH	Variable	RII	P, M, F

RT - retrotranscriptase
EN - endonuclease
RH – RNAse H
APE - DNA (apurinic/apyrimidinic site) endonuclease

Cis-mobilization!

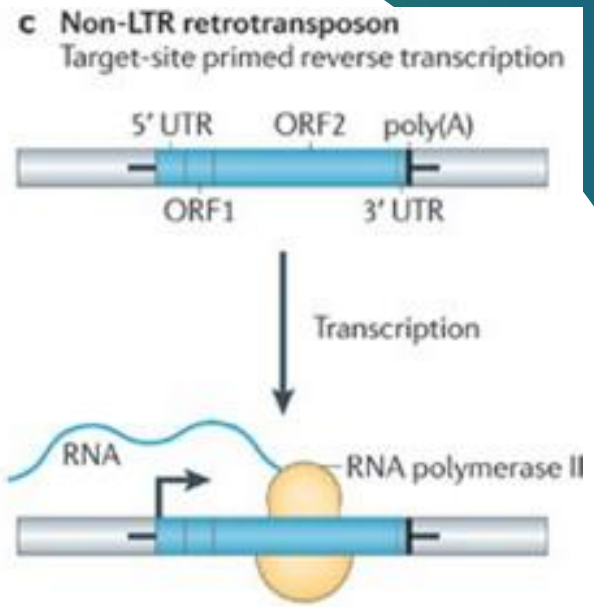
Promoter for RNA polymerase

Open reading frame(s) for transposition proteins

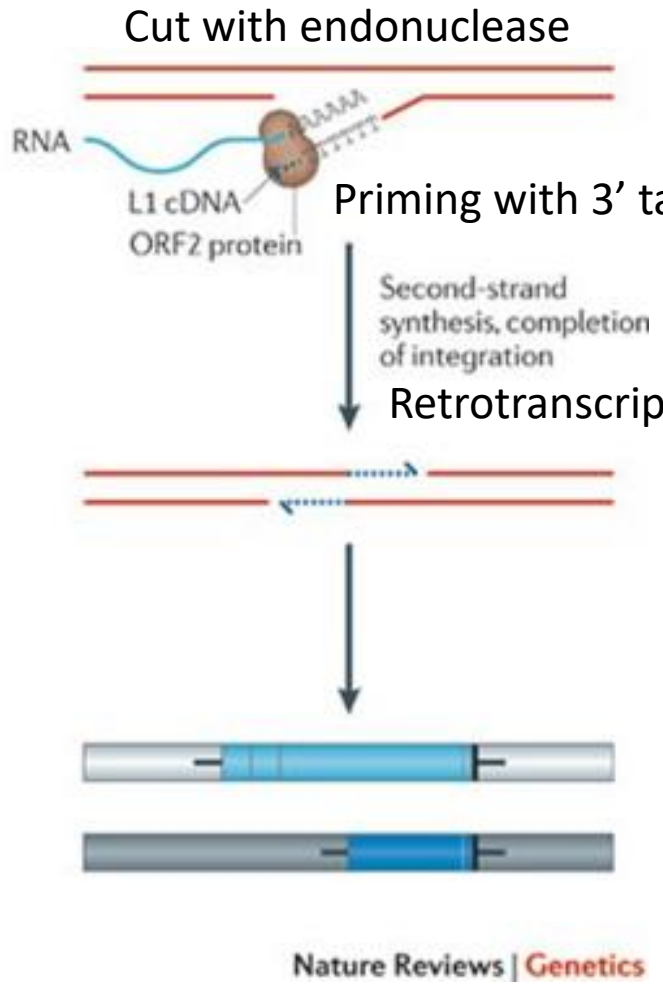
Recognition site for reverse transcriptase

Wicker et al 2007, *Nat Rev Gen*

Target-primed reverse transcription (TPRT)



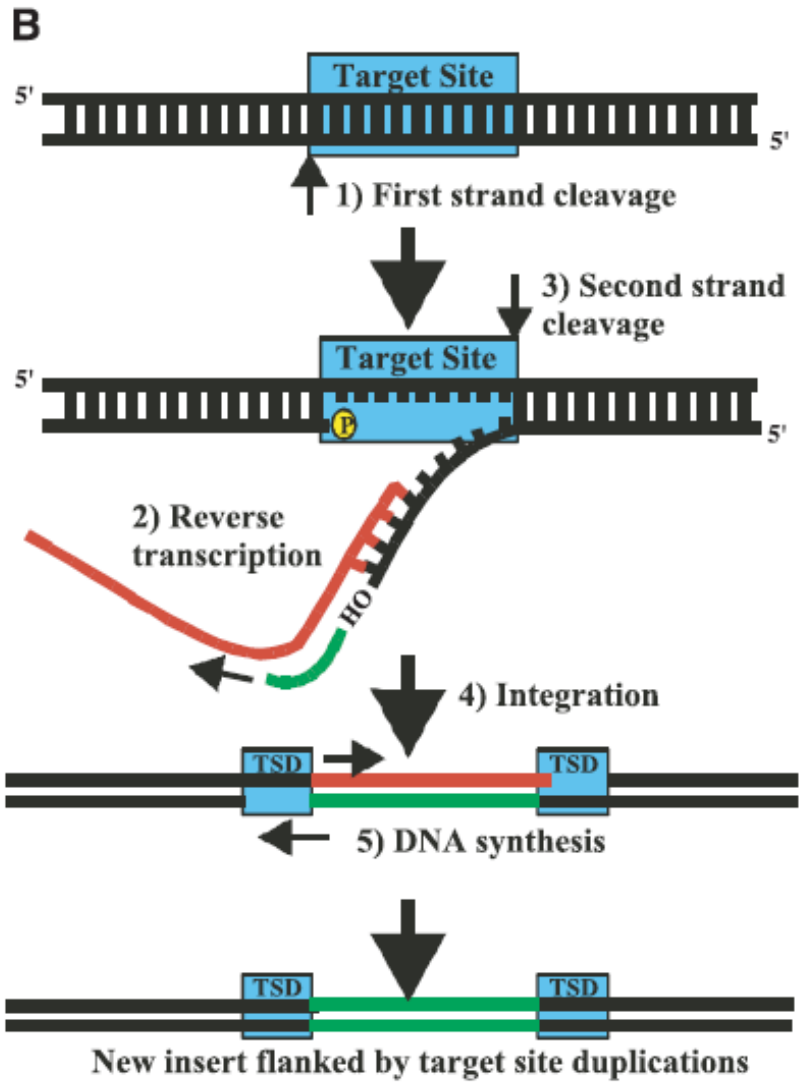
Priming and
reverse transcription



TPRT often undergoes premature 5′ truncation and loss of promoters and/or protein domains

Levin and Moran 2011, *Nat Rev Gen*

Target site duplications

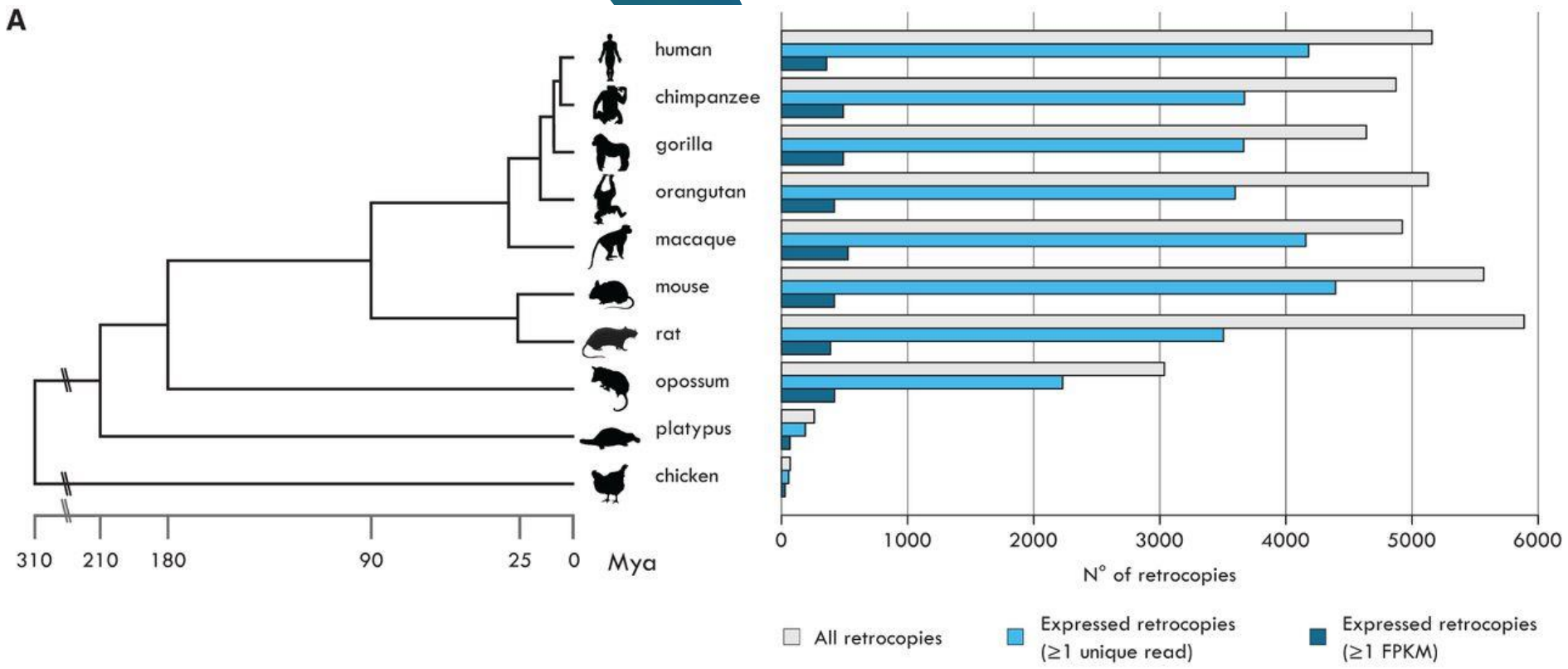


The length of TSDs is important for classification

The length is variable for LINEs but can be of specific lengths for other types of elements

TSDs are the hallmark of most (retro)transposons

L1 and retro(pseudo)genes

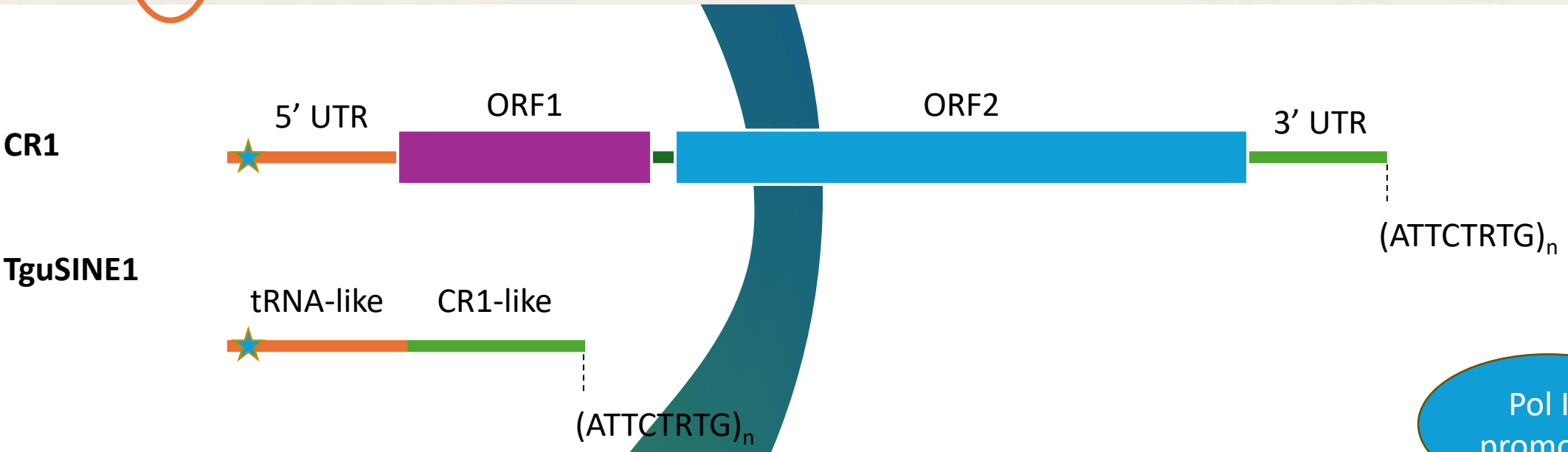


Retrogenes occur when LINE RT recognizes the poly-A tails (L1)

Carelli et al 2016, *Gen Res*

Class I: SINE retrotransposons

Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
SINE	*tRNA*		Variable	RST	P, M, F
	7SL		Variable	RSL	P, M, F
	5S		Variable	RSS	M, O

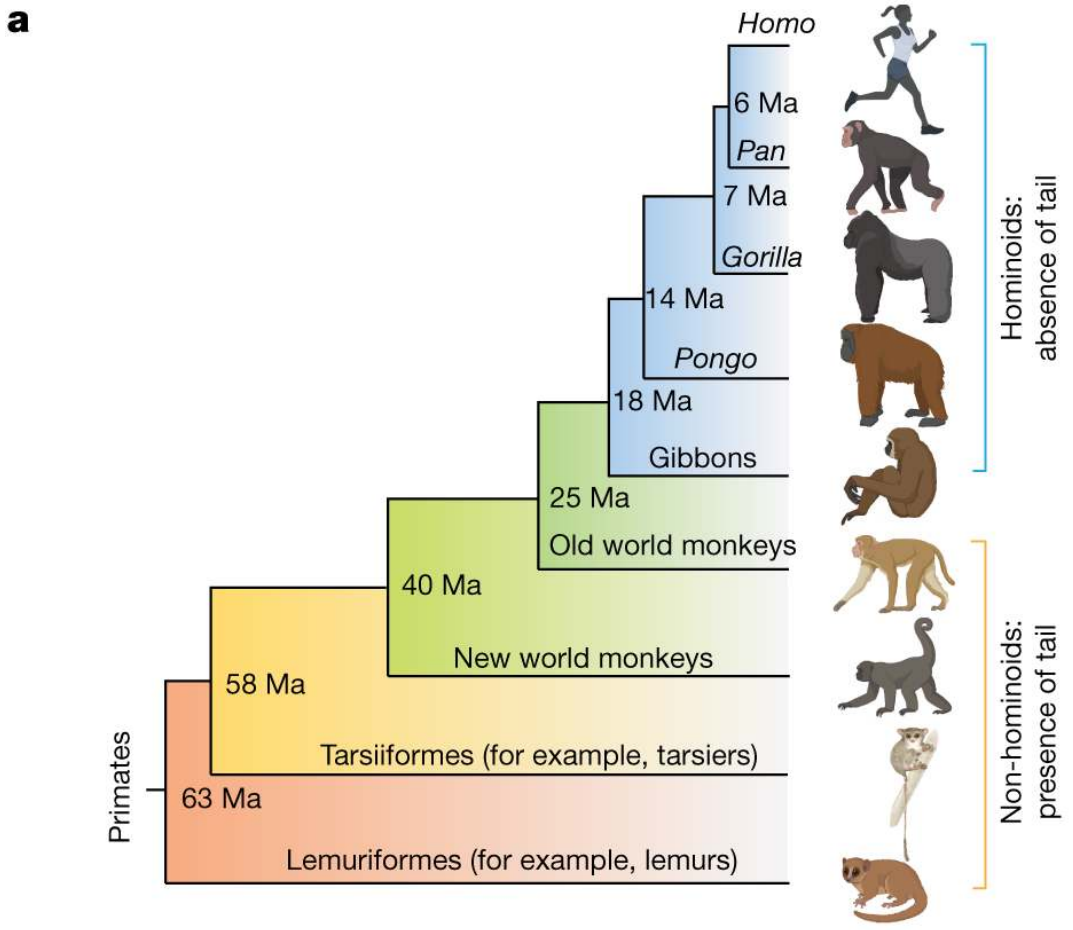


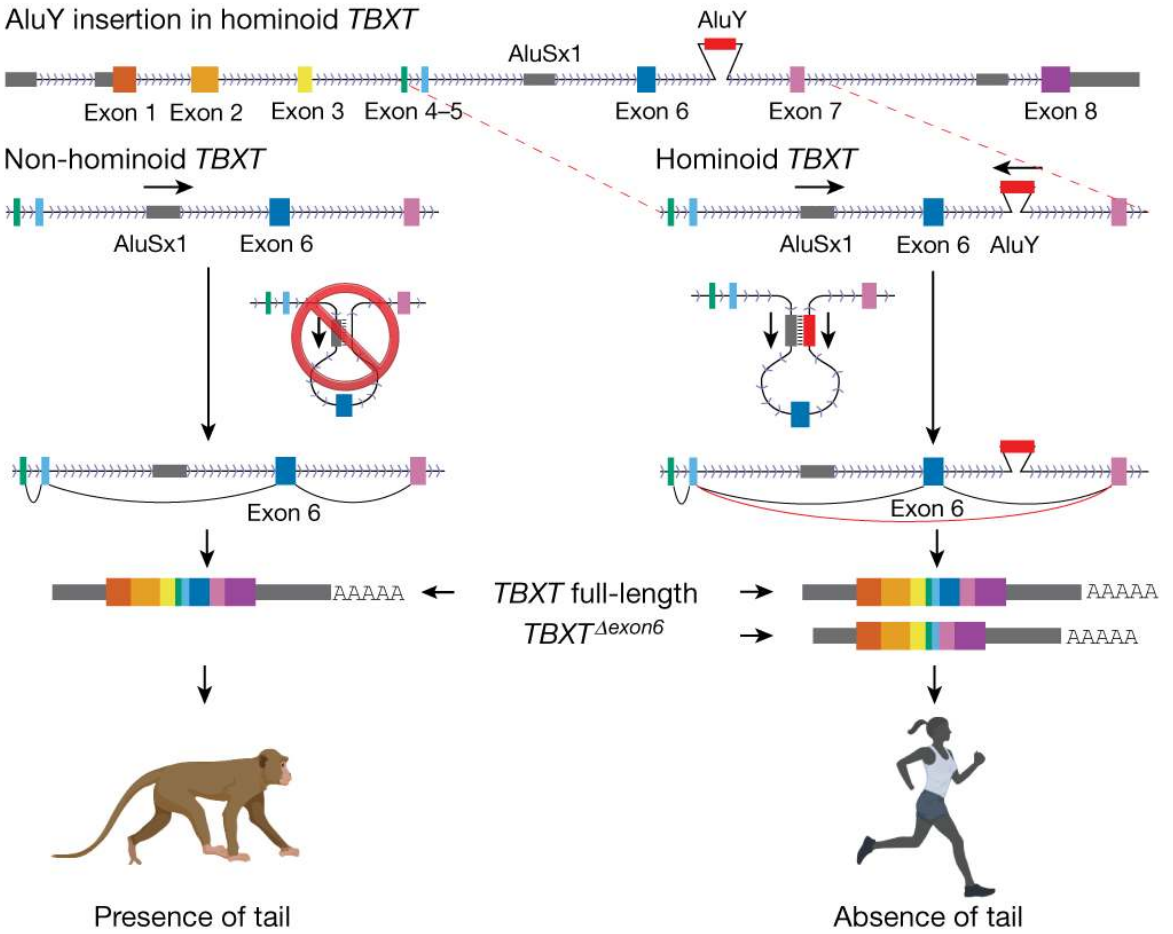
Pol III promoter

SINEs use the LINE protein machinery to move and replicate – *trans*-mobilization!
Non-autonomous elements

Wicker et al 2007, *Nat Rev Gen*

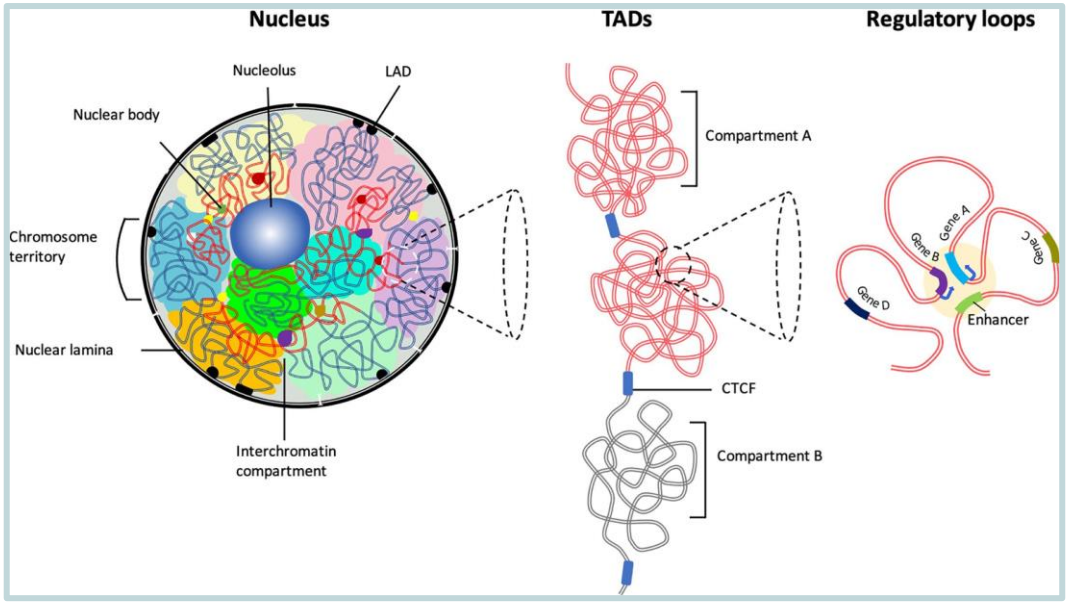
SINE *Alu* and alternative splicing





Xia et al. 2024, Nature

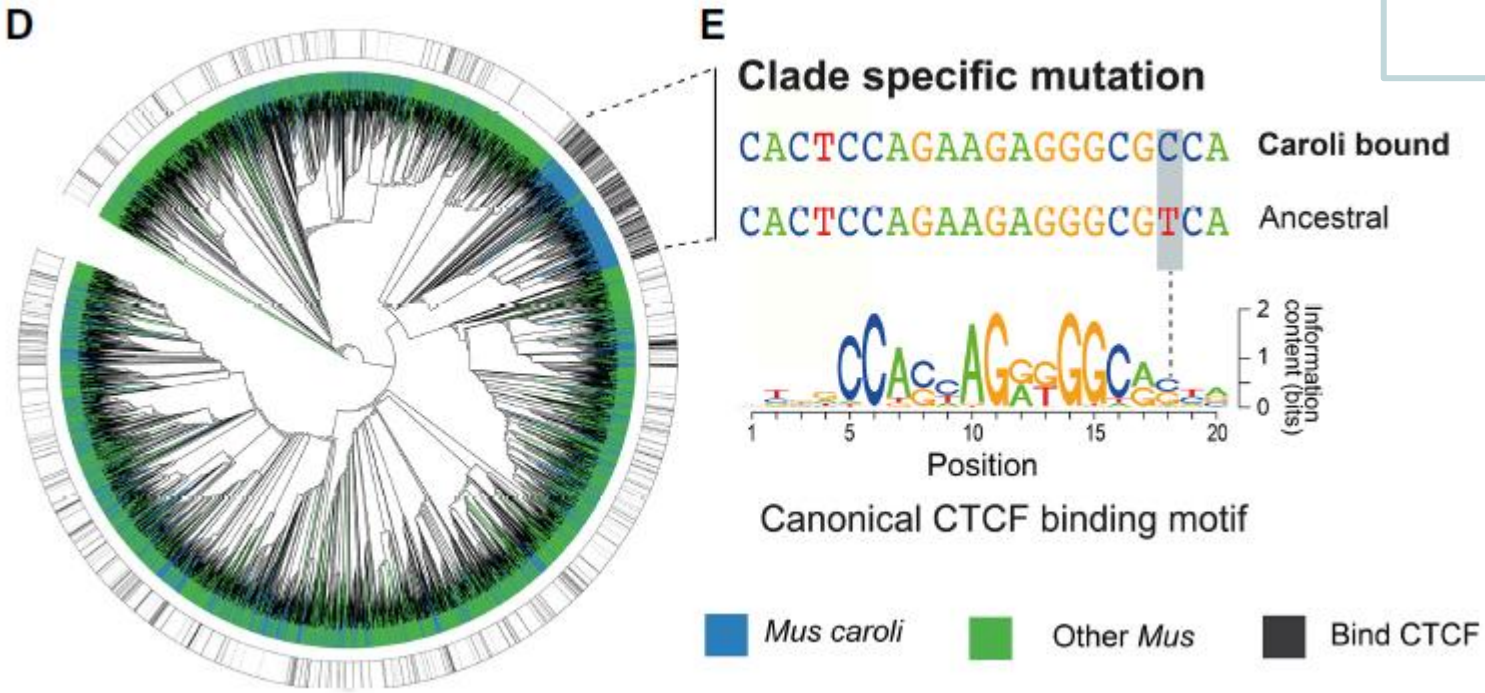
SINEs and 3D genome architecture



Research

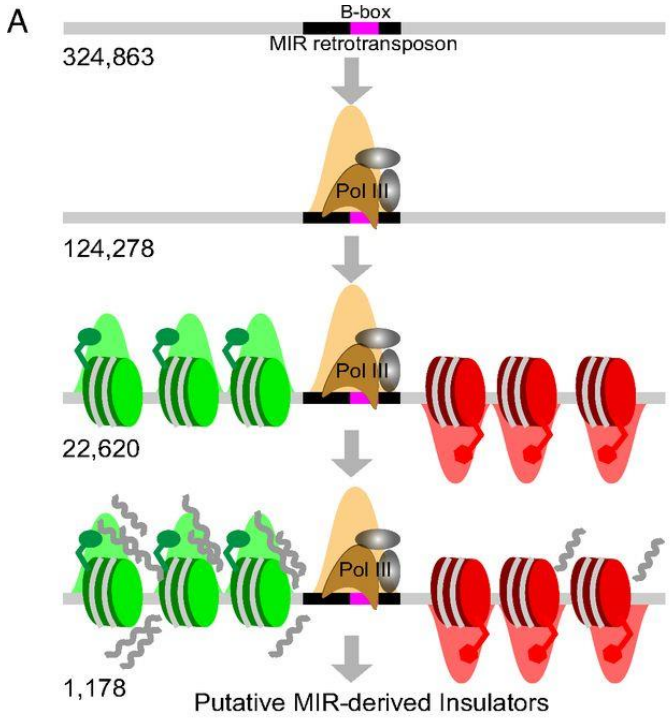
Repeat associated mechanisms of genome evolution and function revealed by the *Mus caroli* and *Mus pahari* genomes

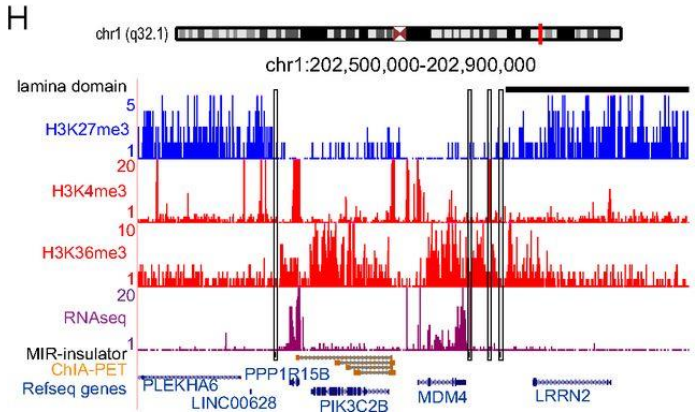
David Thybert,[1,2] Maša Roller,[1] Fábio C.P. Navarro,[3] Ian Fiddes,[4] Ian Streeter,[1] Christine Feig,[5] David Martin-Galvez,[1] Mikhail Kolmogorov,[6] Václav Janoušek,[7]

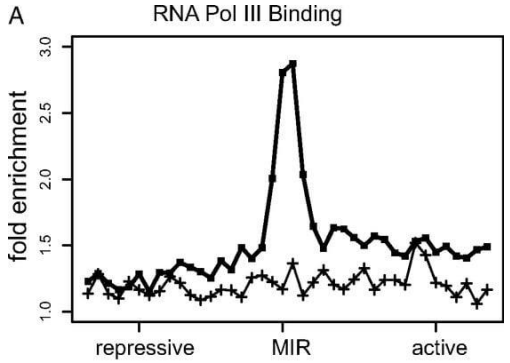


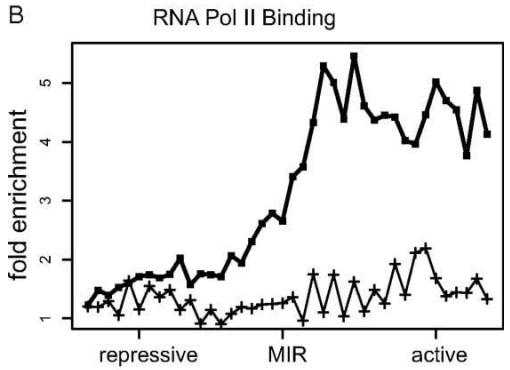
One SNP in the *Mus caroli* lineage turned SINE B2 into CTCF binding sites

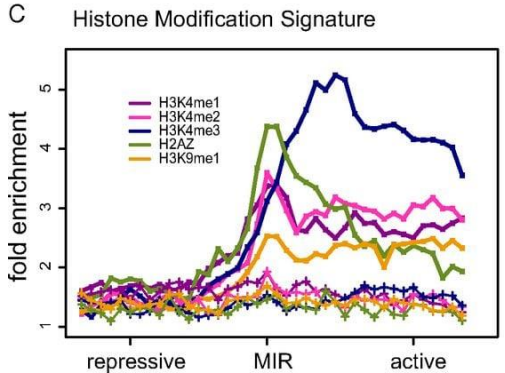
Ancient SINEs became conserved elements and insulators









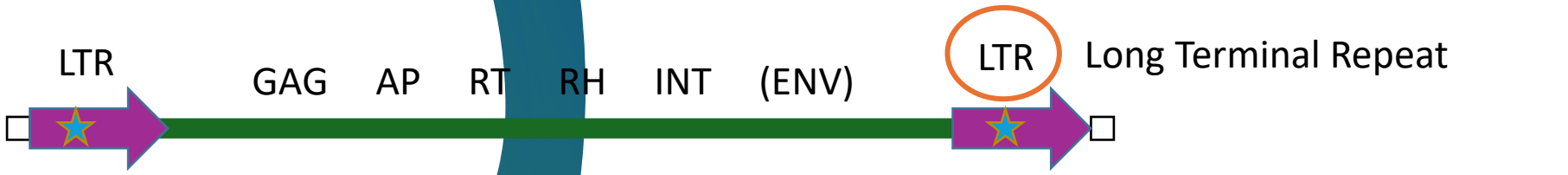


Wang et al. 2015, PNAS

Class I: LTR retrotransposons

Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
LTR	*Copia*	GAG AP INT RT RH	4–6	RLC	P, M, F, O
	Gypsy	GAG AP RT RH INT	4–6	RLG	P, M, F, O
	Bel–Pao	GAG AP RT RH INT	4–6	RLB	M
	Retrovirus	GAG AP RT RH INT ENV	4–6	RLR	M
	ERV	GAG AP RT RH INT ENV	4–6	RLE	M

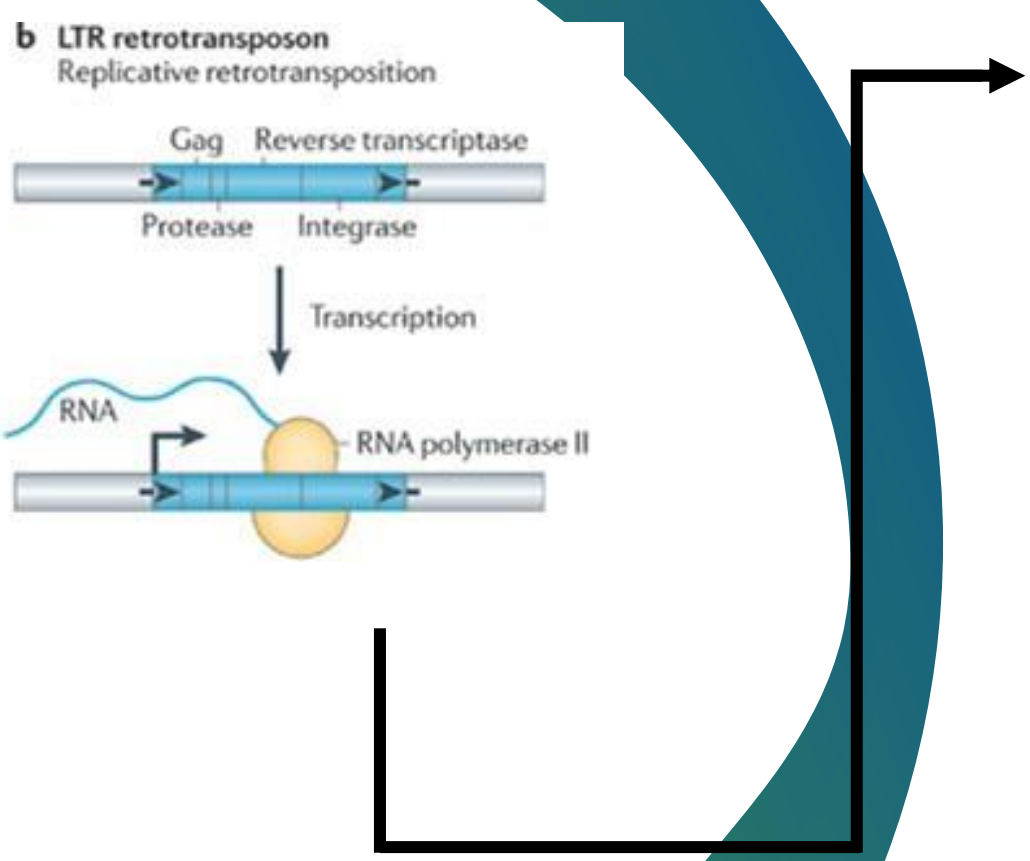
GAG – capsid protein
AP – aspartic proteinase
RT - retrotranscriptase
RH – RNAse H
INT - integrase
ENV – envelope protein



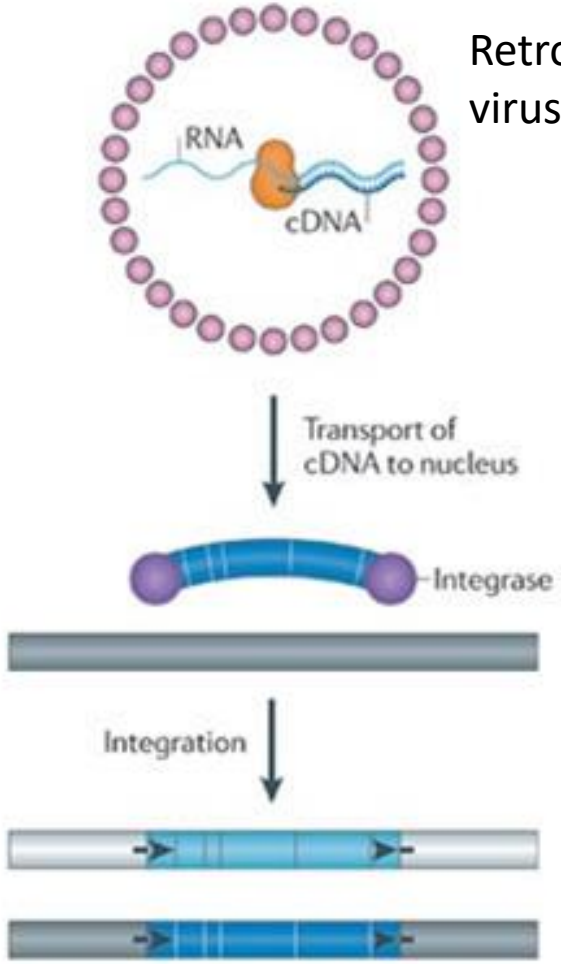
Non-allelic homologous recombination (NAHR)

Wicker et al 2007, *Nat Rev Gen*

Replicative retrotranposition



Reverse transcription

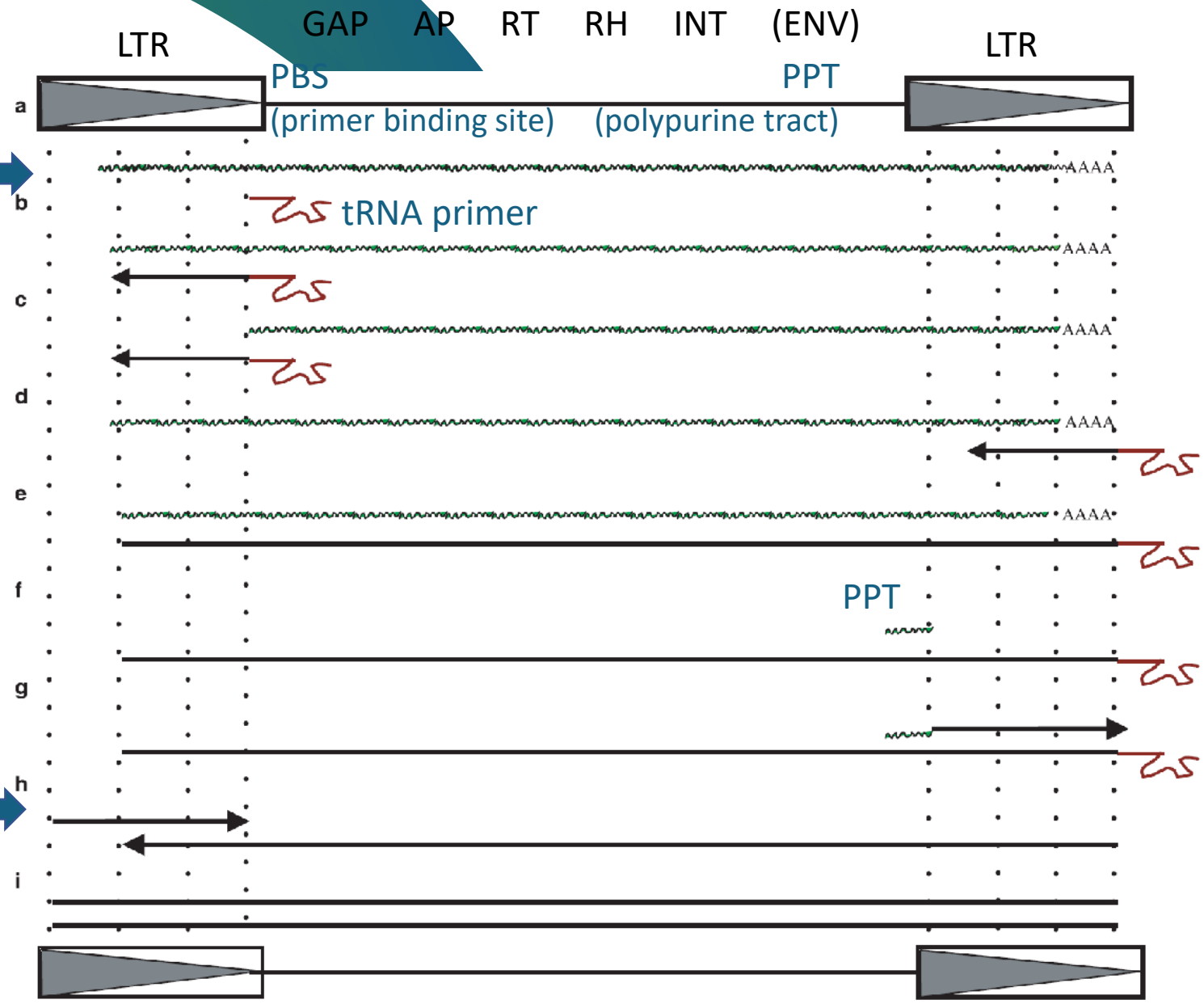


Retrotranscription inside a virus-like particle

Levin and Moran 2011, *Nat Rev Gen*

LTRs are essential for retrotransposition

Transcription starts from an internal promoter!



The second LTR ensure the restoring of the entire element

Insertion in the genome

Transcribed LTR retrotransposon

The synthesis of ds-DNA copy happens within the viral-like particle

Complete new copy ready to be integrated in the genome

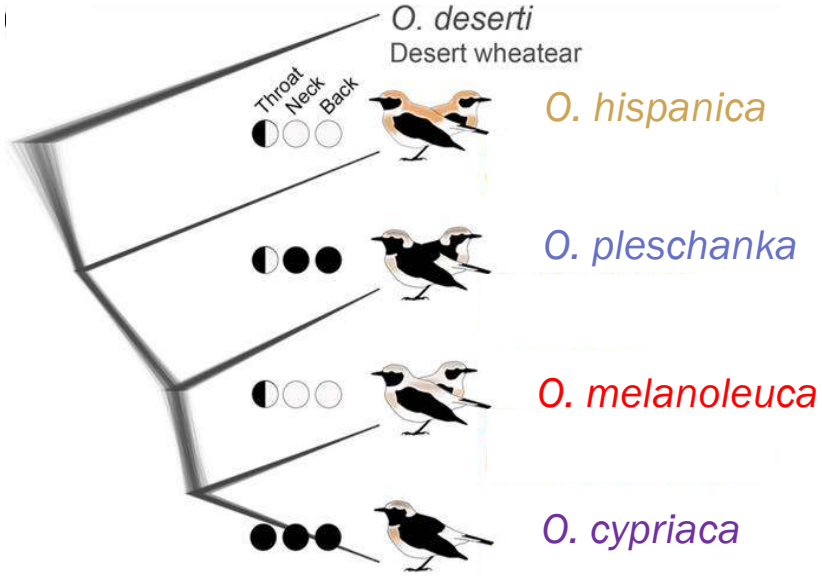
Kazazian 2004, *Science*

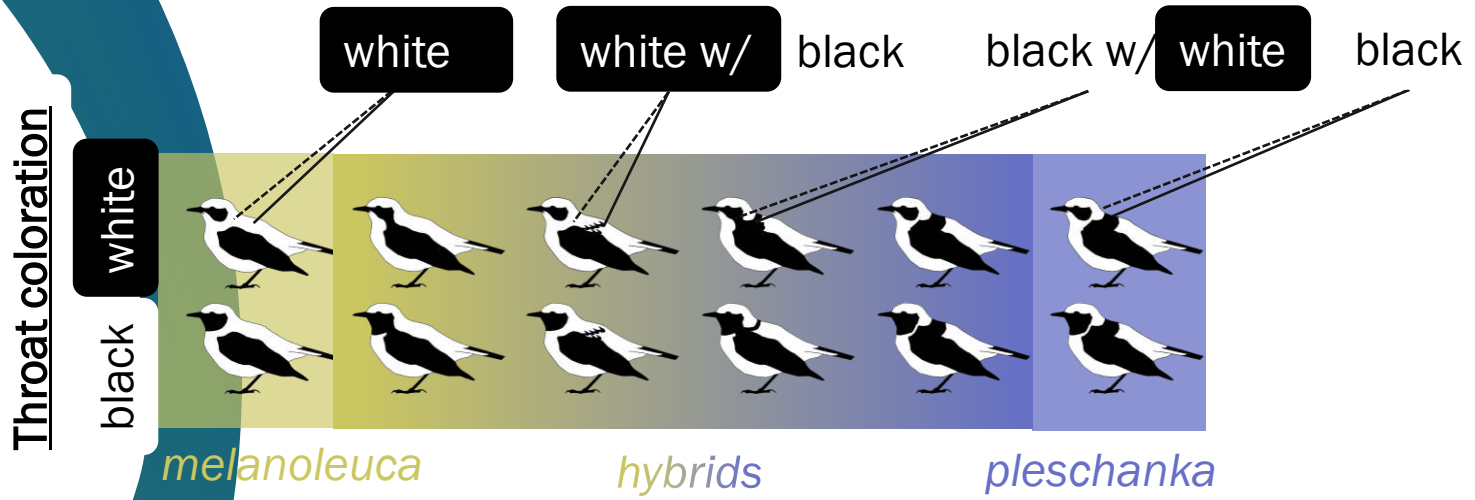
LTRs and coloration

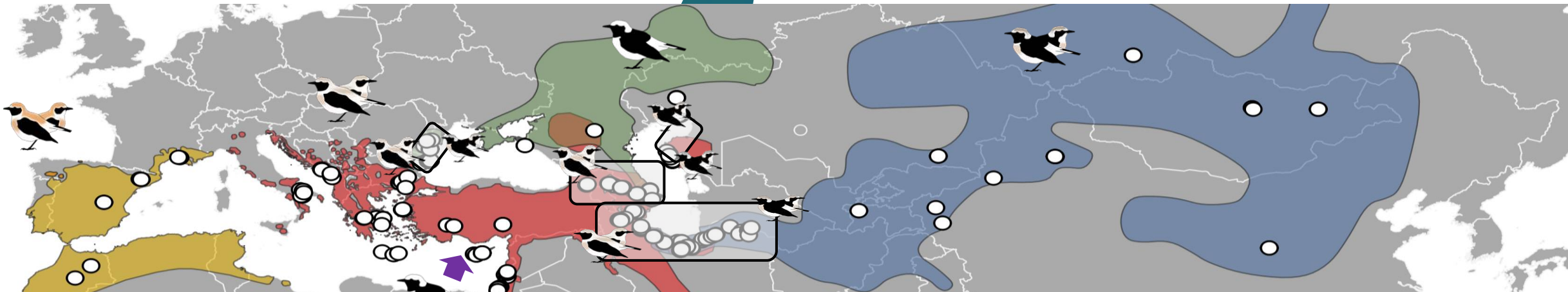

Dave Lutgen


Madeline Chase

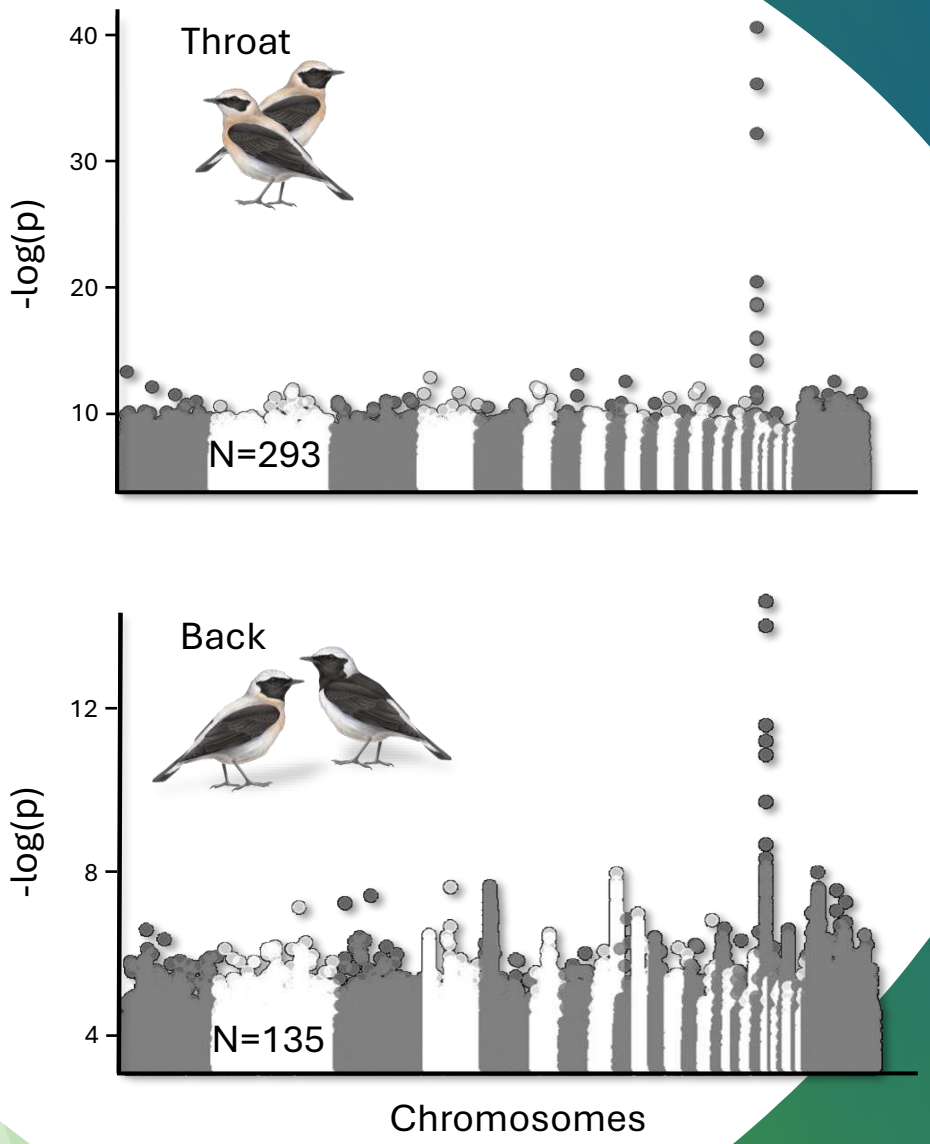

Fritjof Lammers







LTRs and coloration



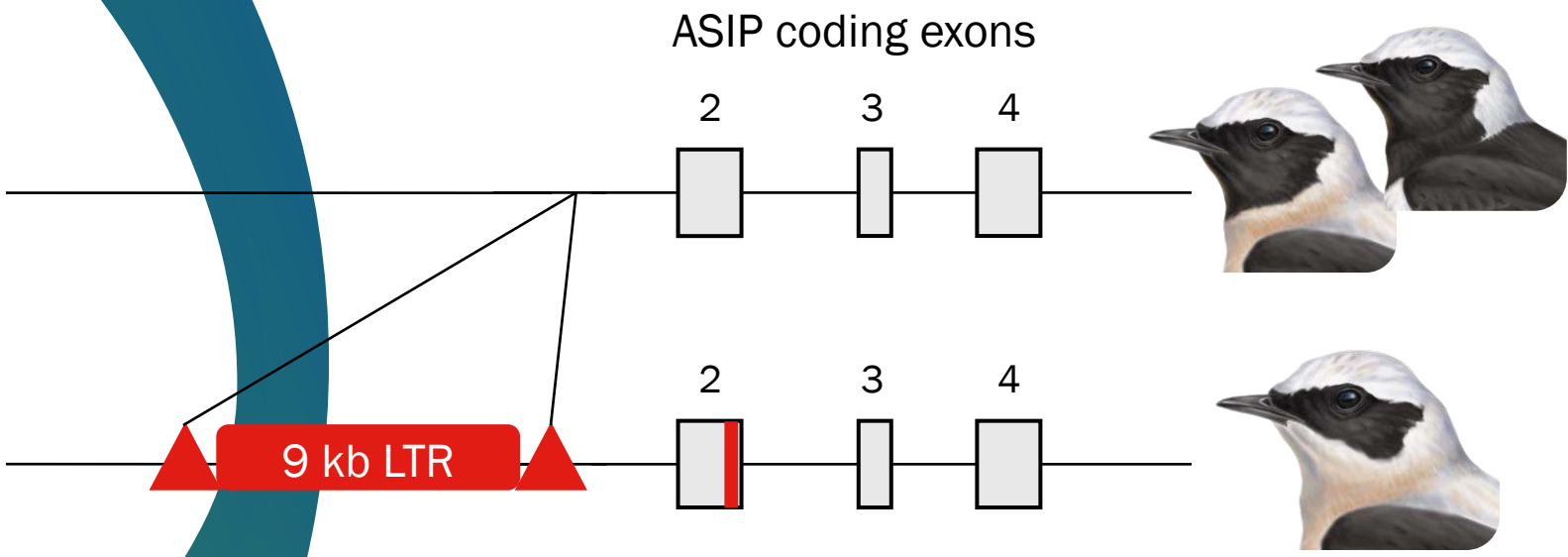
Agouti signalling protein (ASIP): 2 non-synonymous coding SNPs

Agouti signalling protein (ASIP): 17 SNPs up to 45 kb upstream ASIP

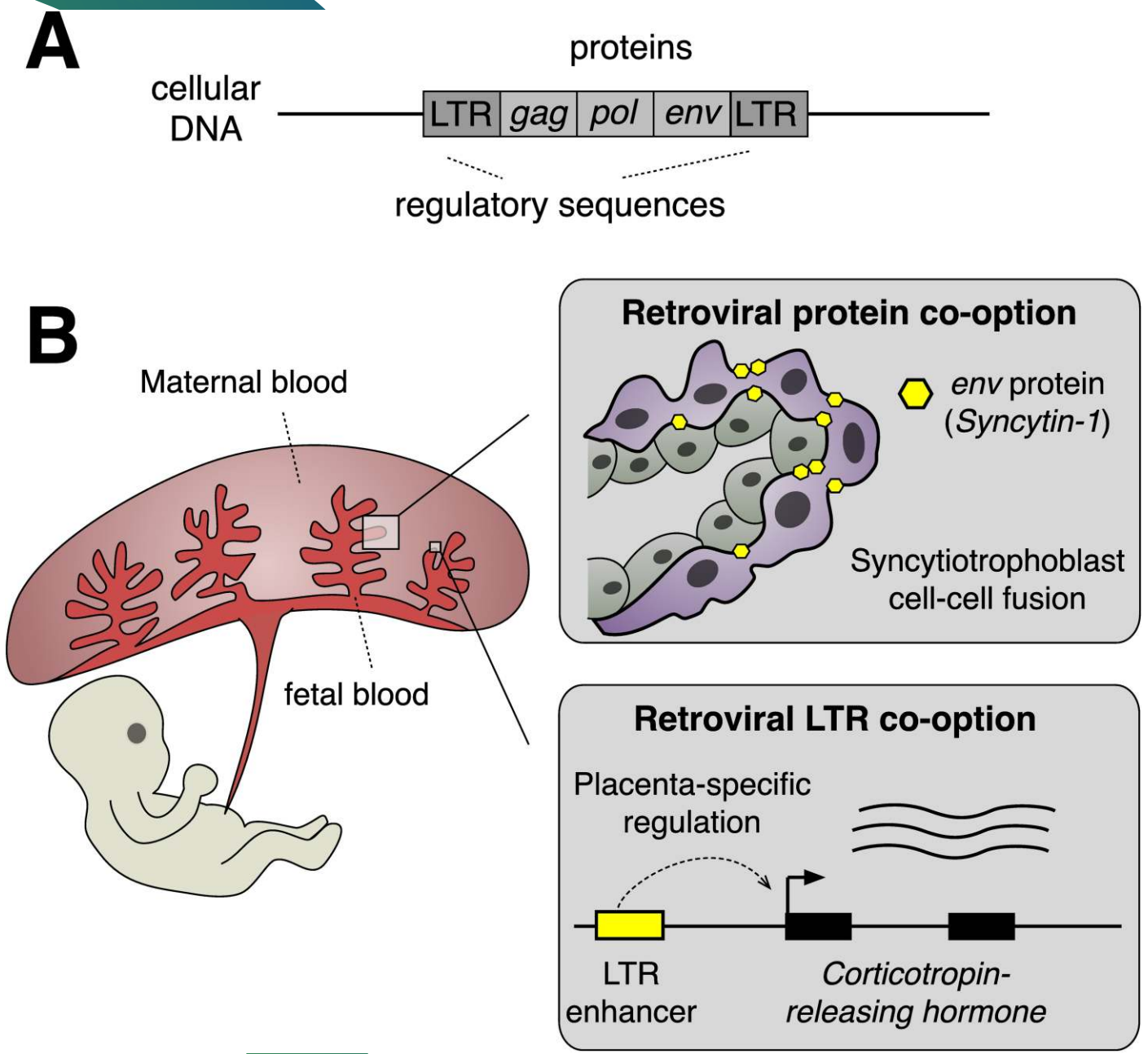
LTRs and coloration

hispanica and *melanoleuca*

		SNP		
		W / W	W / b	b / b
LTR	+ / +	9	2	0
	+ / -	0	40	2
	- / -	0	2	273



LTRs and placenta



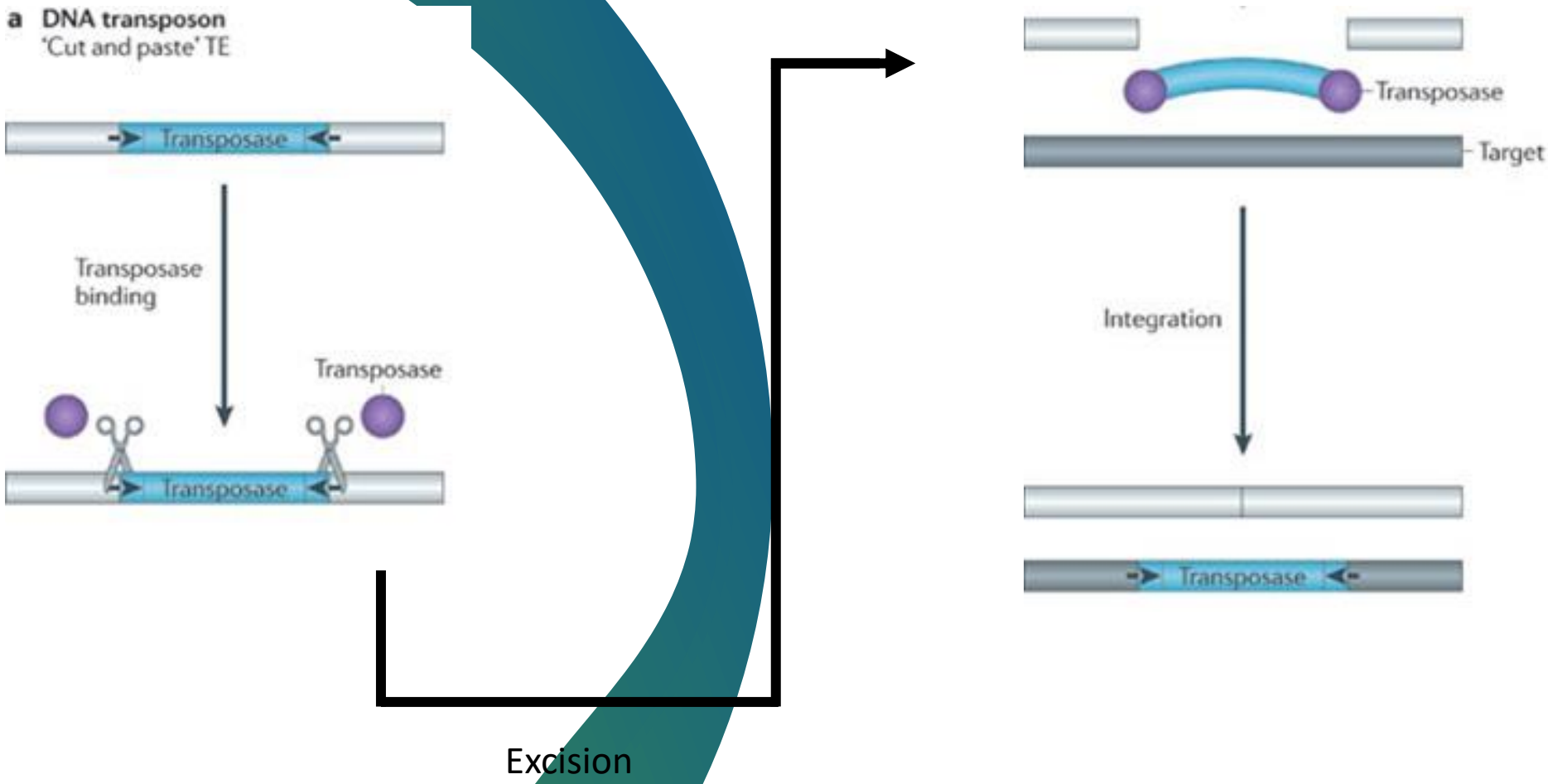
Choung 2018, PLOS Biology

Class II: DNA transposons

Class II (DNA transposons) - Subclass 1					
TIR	*Tc1–Mariner*	Tase*	TA	DTT	P, M, F, O
	hAT	Tase*	8	DTA	P, M, F, O
	Mutator	Tase*	9–11	DTM	P, M, F, O
	Merlin	Tase*	8–9	DTE	M, O
	Transib	Tase*	5	DTR	M, F
	P	Tase	8	DTP	P, M
	PiggyBac	Tase	TTAA	DTB	M, O
	PIF–Harbinger	Tase* ORF2	3	DTH	P, M, F, O
	CACTA	Tase ORF2	2–3	DTC	P, M, F

Wicker et al 2007, *Nat Rev Gen*

Cut and paste transposition (TIRs)

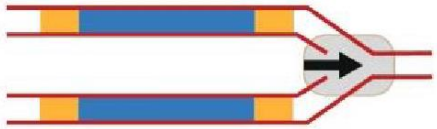


Mobile DNA

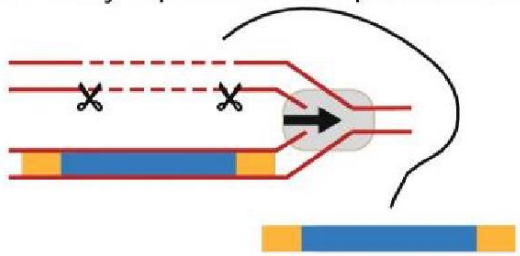
Levin and Moran 2011, *Nat Rev Gen*

How to increase in copy number?

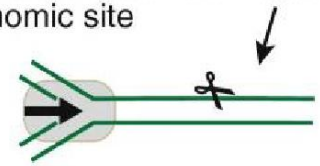
I. DNA replication fork passes transposon



II. Newly replicated transposon is cut out...

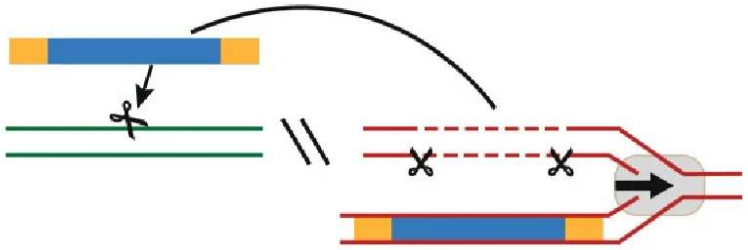


III. ...and inserted into a not-yet replicated genomic site

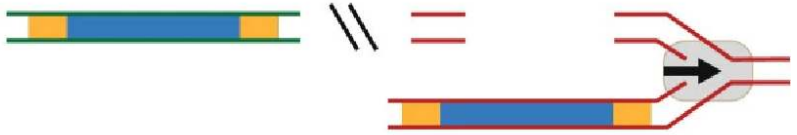


IIII. DNA replication fork passes insertion site

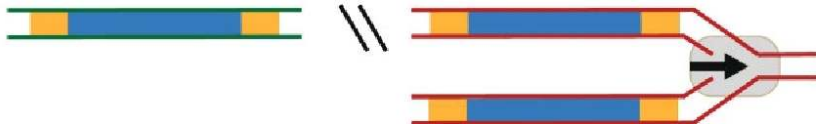
I. Newly replicated transposon is cut out...



II. ...and transposed into a new locus

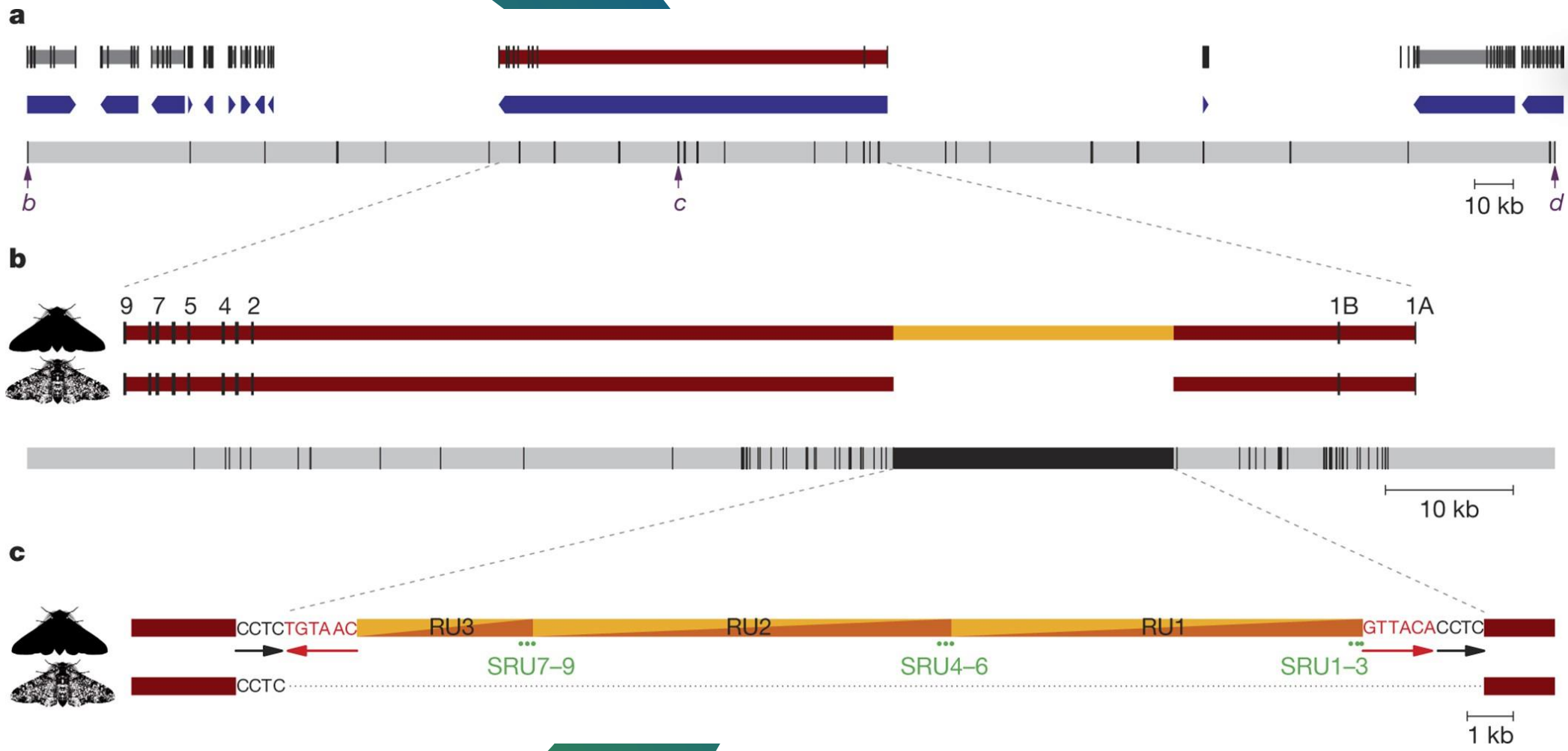


III. Following transposition, the double-stranded break is repaired by homology-dependent DNA repair



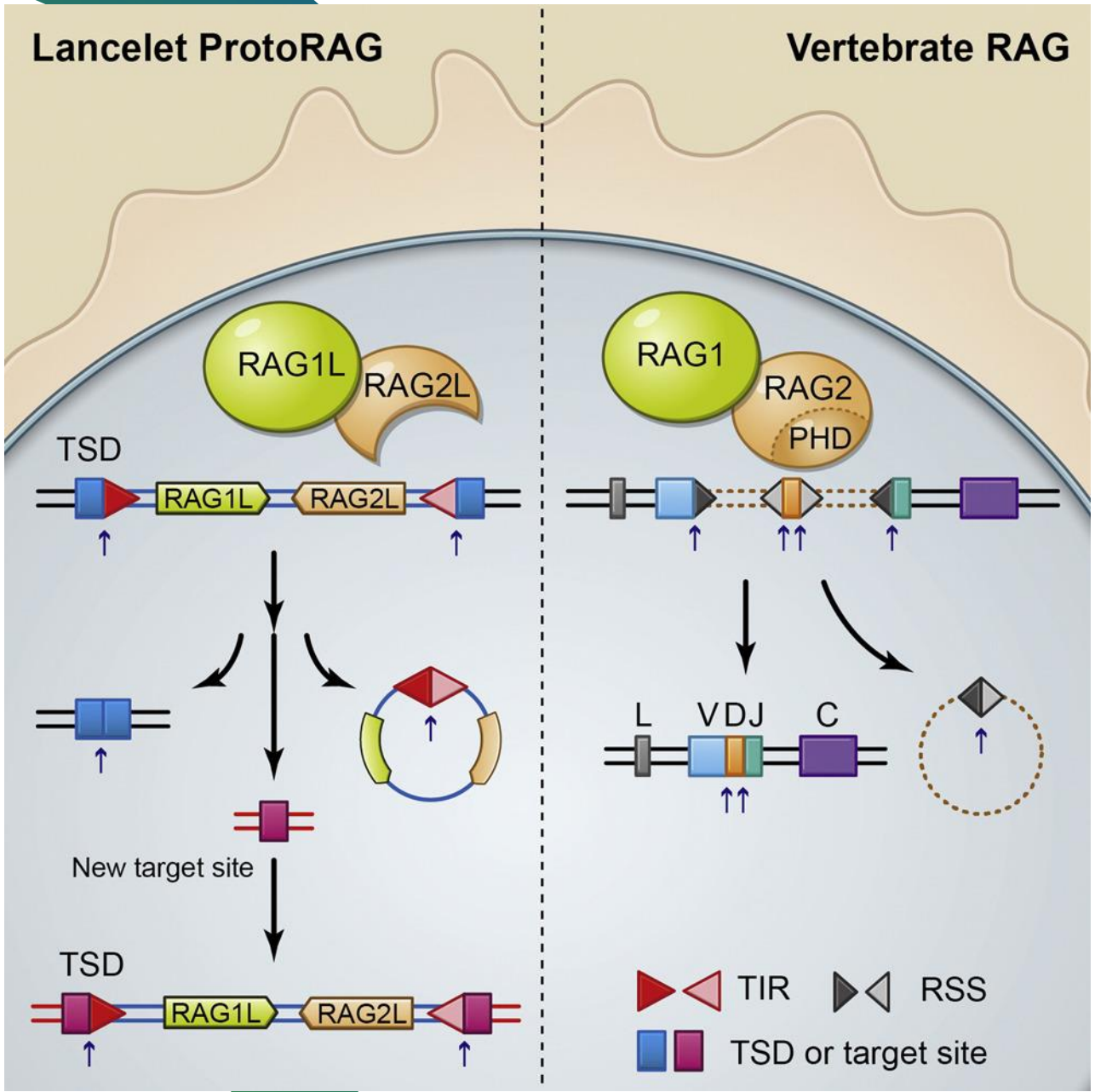
Skipper et al. 2013, *J. Biomed. Sci.*

DNA transposons and coloration



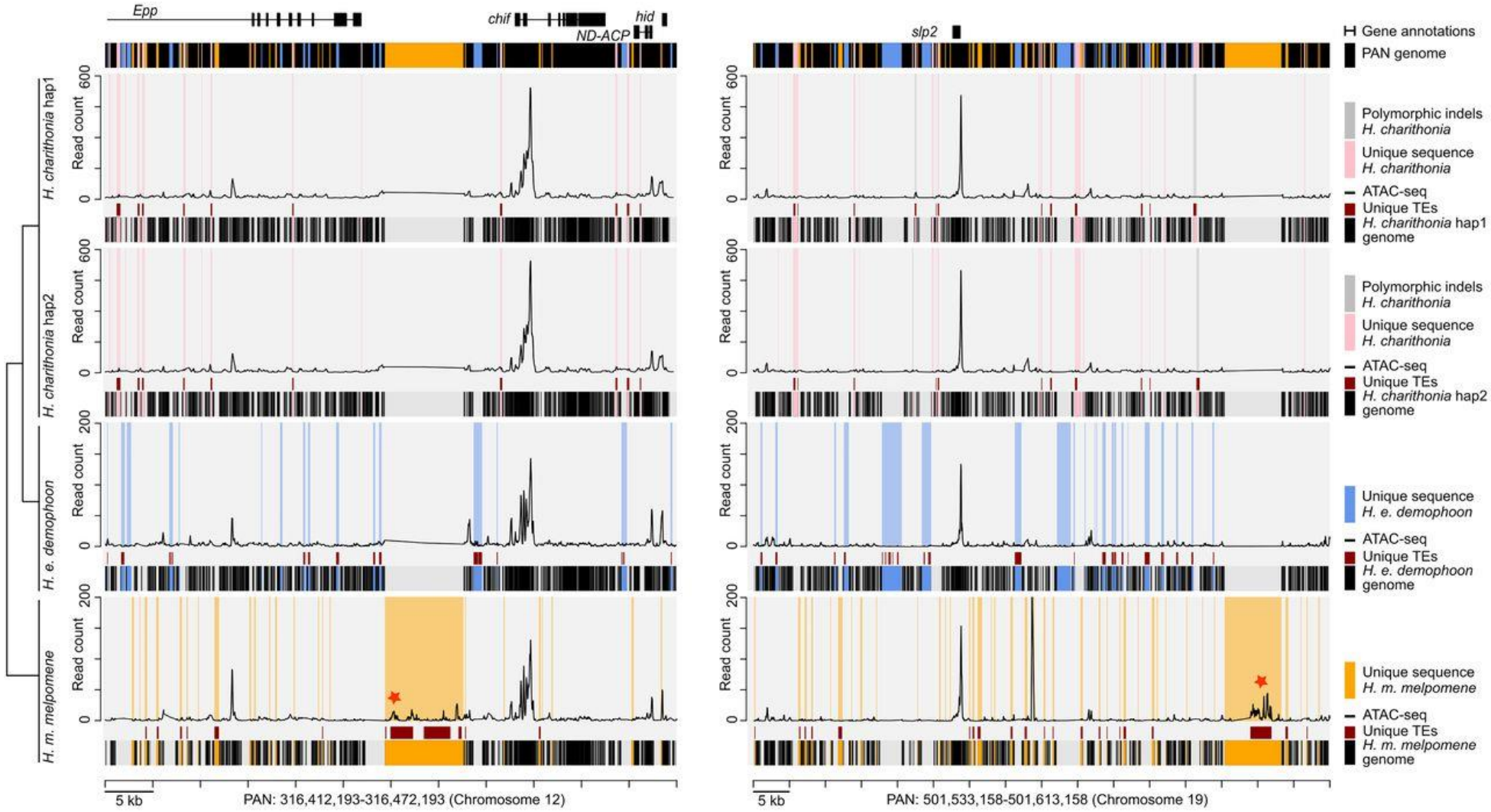
Choung 2018, PLOS Biology

DNA transposons and immune system



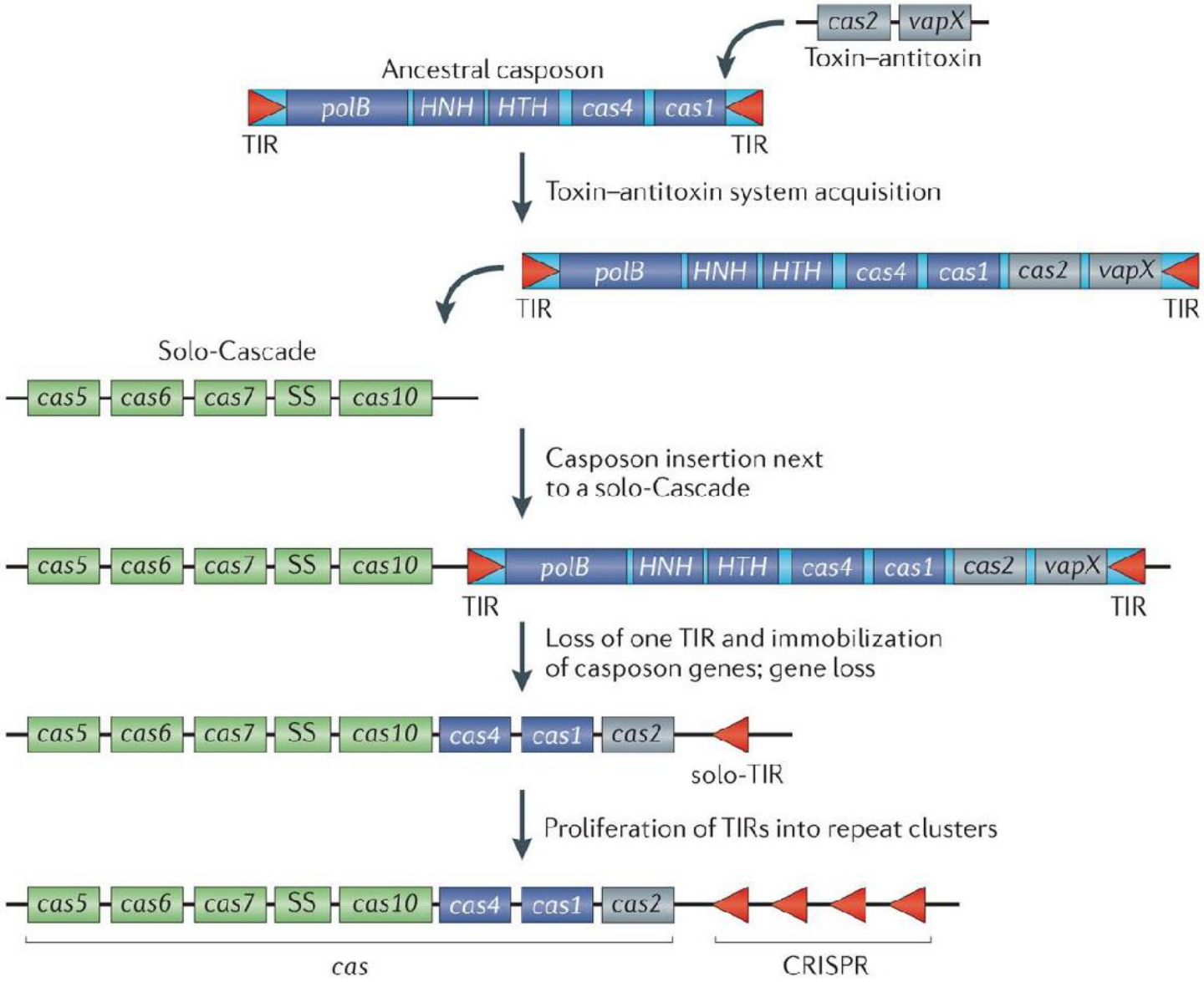
Huang et al. 2016, Cell

ATAC (Assay for Transposase-Accessible Chromatin)



https://github.com/francicco/ComparativeGenomicsLab/blob/main/PartIII/IndentificationConservedElements.md#part-iiib-atac-peak-enrichment

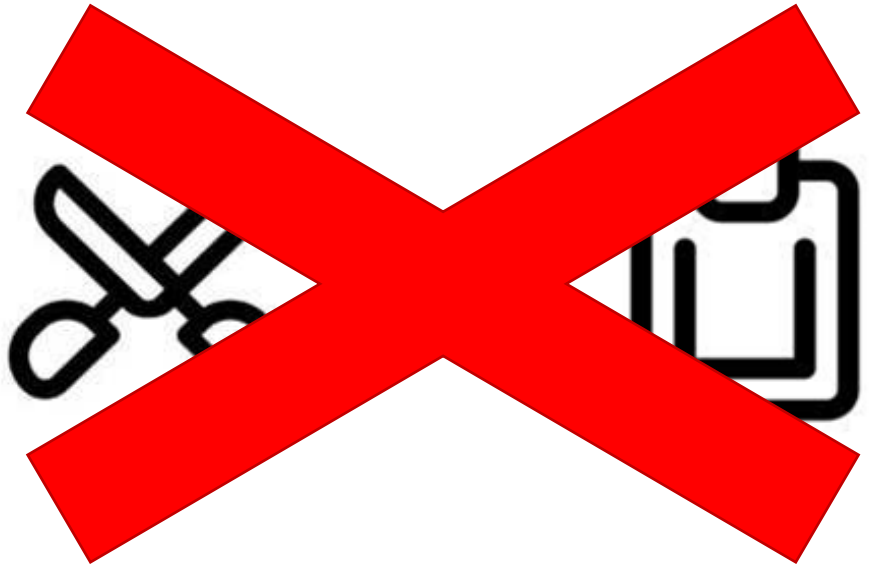
CRISPR-Cas and transposons



Koonin and Krupovic 2015, *Nat Rev Genet*

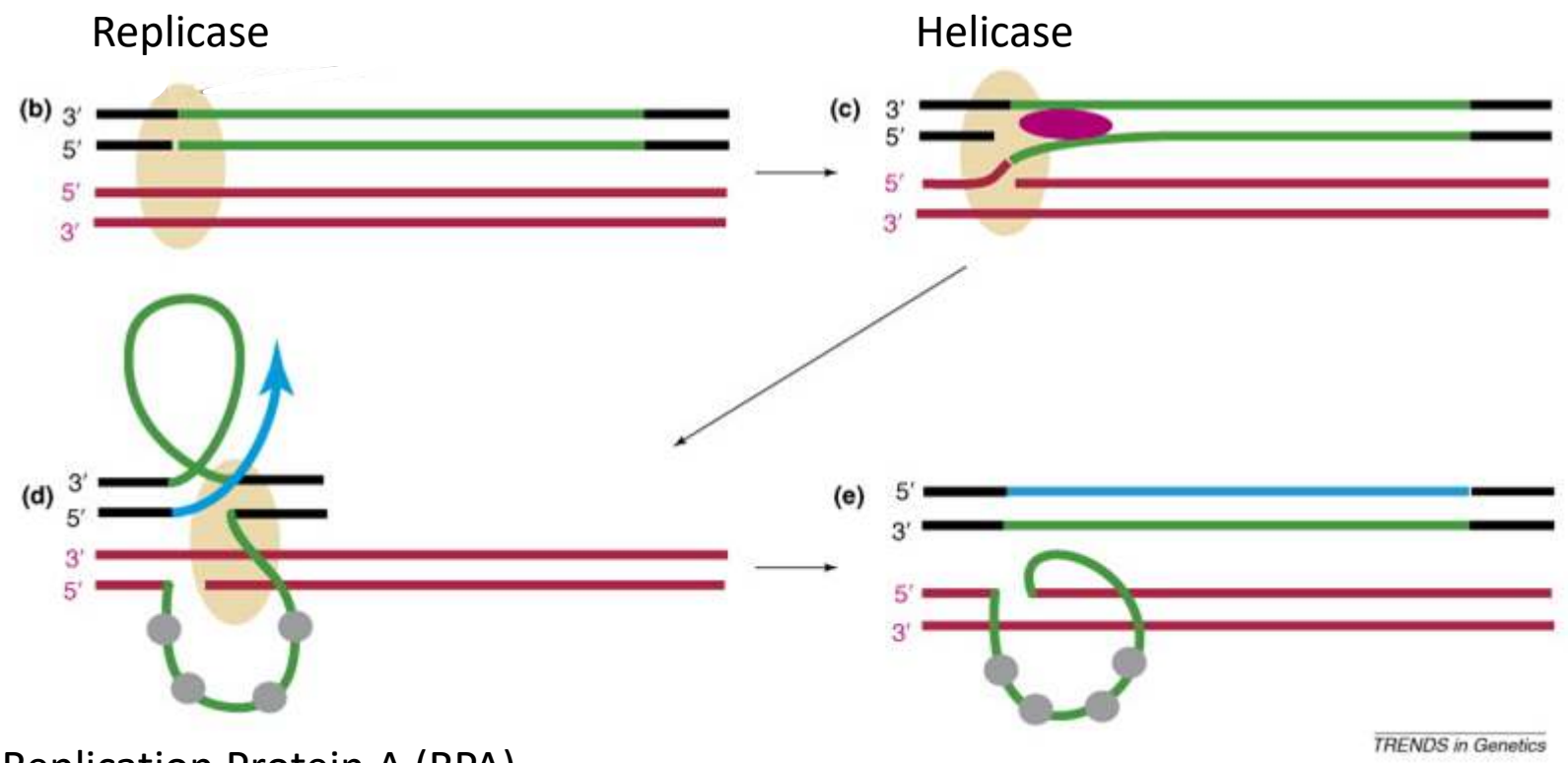
Class II: DNA transposons (subclass 2)

Class II (DNA transposons) - Subclass 2					
Helitron	*Helitron*	RPA — Y2 HEL	0	DHH	P, M, F
Maverick	*Maverick*	C-INT — ATP — CYP — POL B	6	DMM	M, F, O



Wicker et al 2007, *Nat Rev Gen*

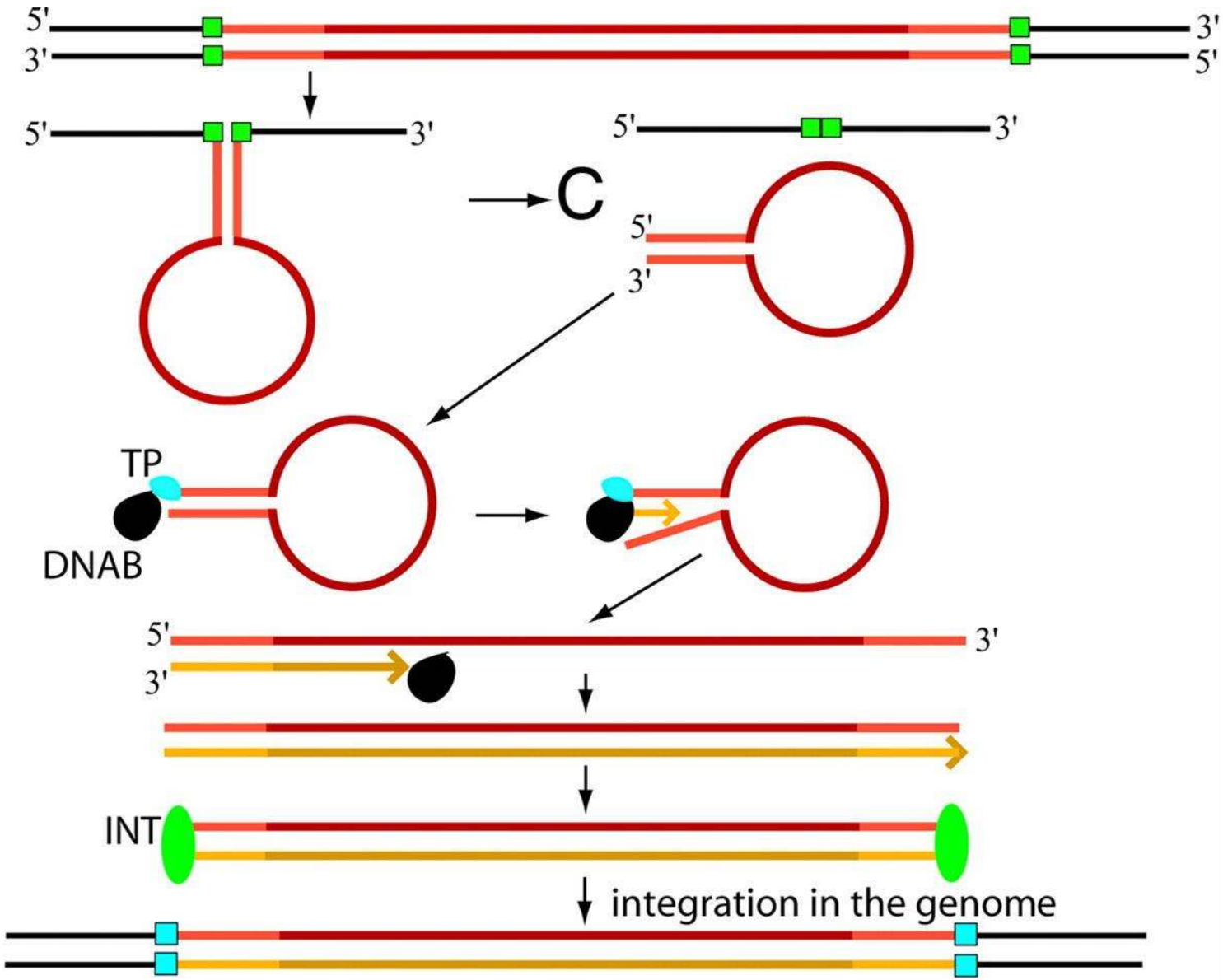
Rolling circle transposition: Helitrons



Replication Protein A (RPA)

Kapitonov and Jurka 2007, *Trends Genet*

Self-synthesizing transposition: Mavericks/Polintons



Kapitonov and Jurka 2006, *PNAS*

TEs with tyrosine recombinase

Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
DIRS	*DIRS*		0	RYD	P, M, F, O
Class II (DNA transposons) - Subclass 2					
Crypton	*Crypton*		0	DYC	F

TE integration mechanism occurs via:

- **Endonuclease**: LINE, SINE, PLE
- **DDE-Transposase**: TIR
- **Integrase**: LTR, Maverick/Polinton
- **Rep protein**: Helitron
- **Tyrosine recombinase**: DIRS, Crypton

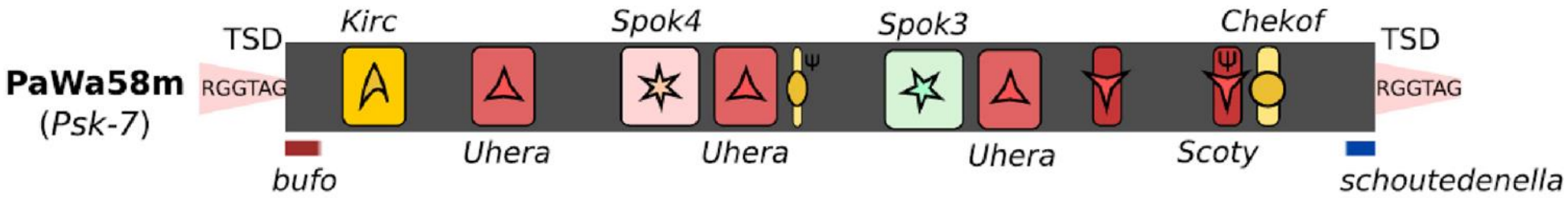
Class I: retrotransposons
Class II: DNA transposons

Wicker et al 2007, *Nat Rev Gen*

A hyper-selfish Crypton

Transposable elements carrying meiotic drive genes

YR DNA transposons: *Enterprise*(247 kb, fungus *Podospora anserina*)

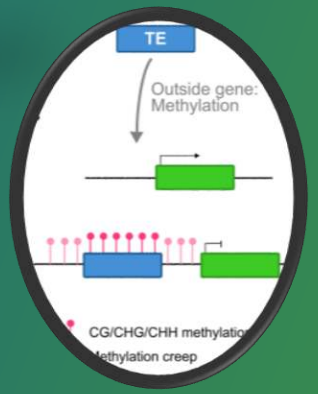


Vogan et al. 2021, *Genome Res.*

TEs as dynamic mutations

- NAHR

- Methylation



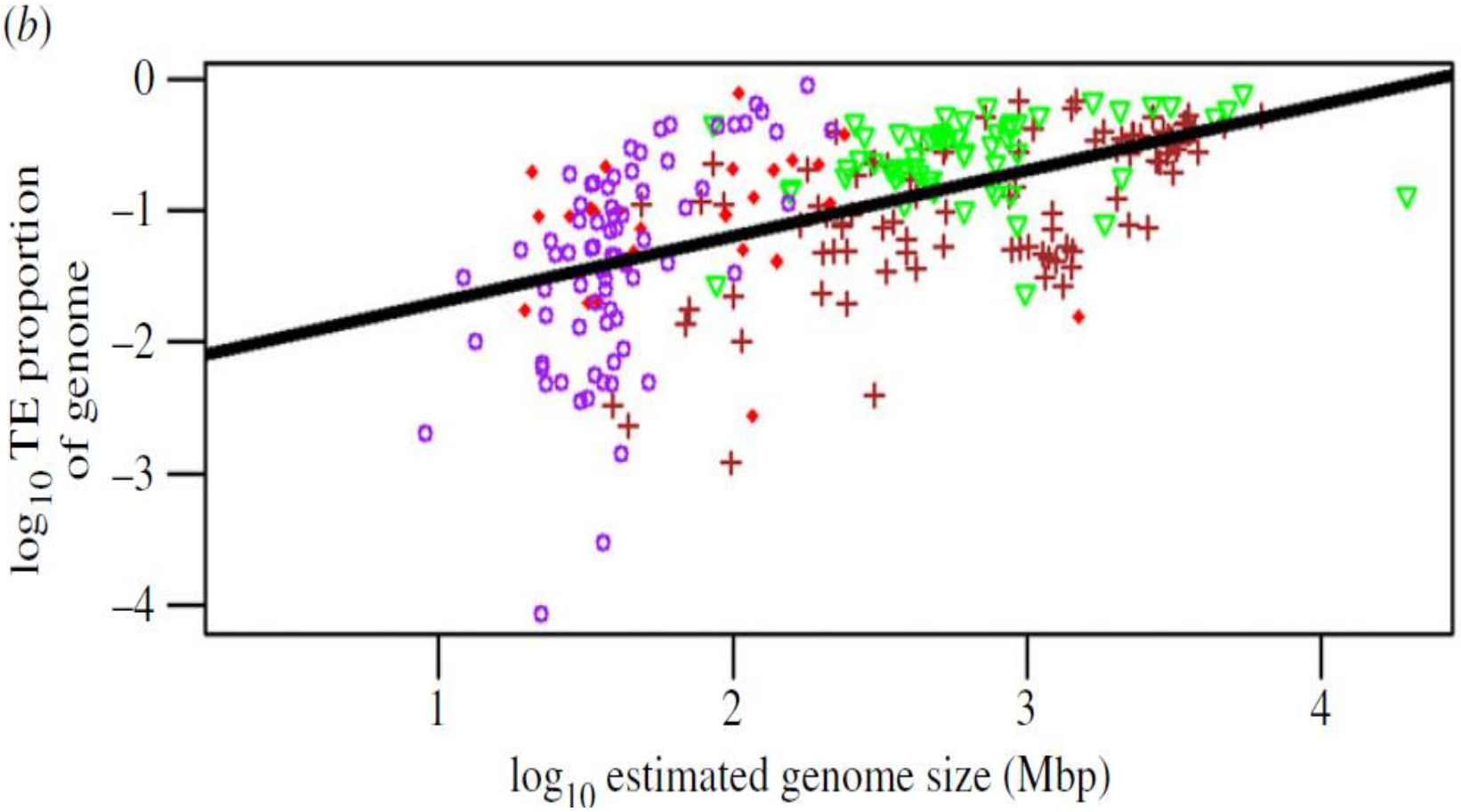
Non-allelic homologous recombination NAHR

Full-length LTR → solo LTR

Larger scale deletions and copy number variation

Inversions

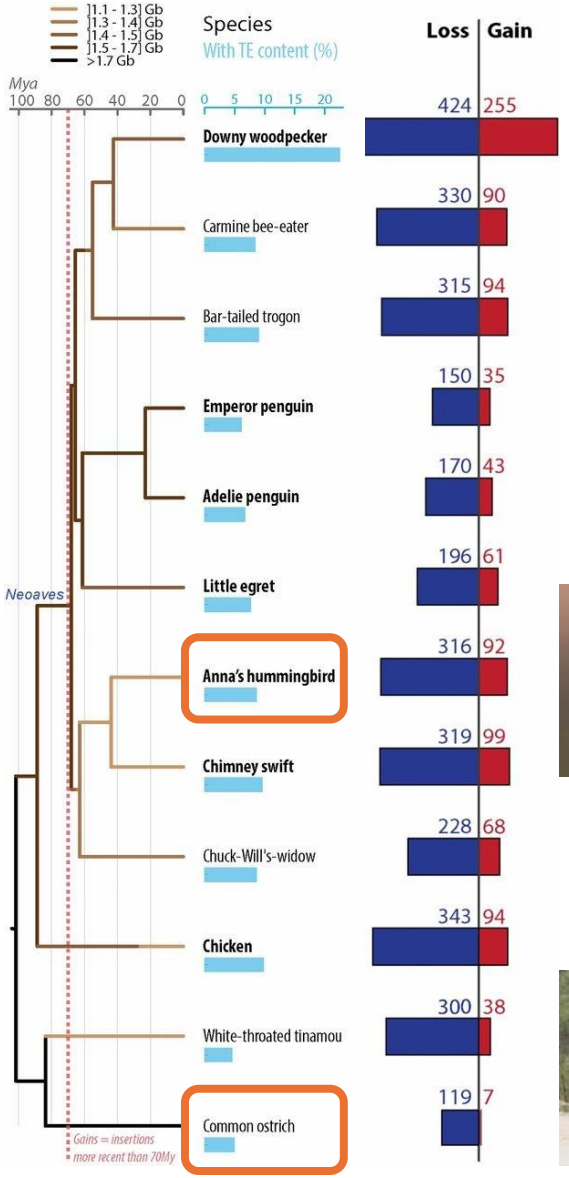
Genome size evolution



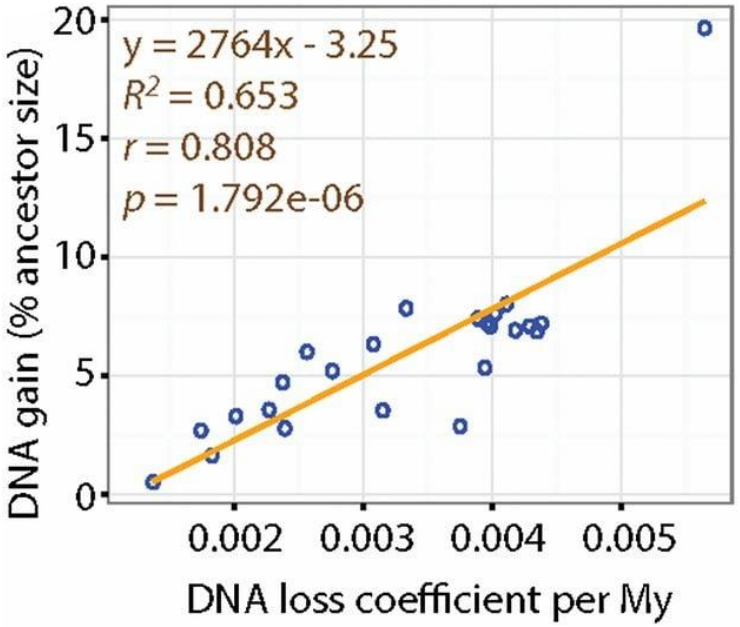
Elliot and Gregory, 2015 *Phil Trans B*

Genome size evolution

Accordion model



No constraint

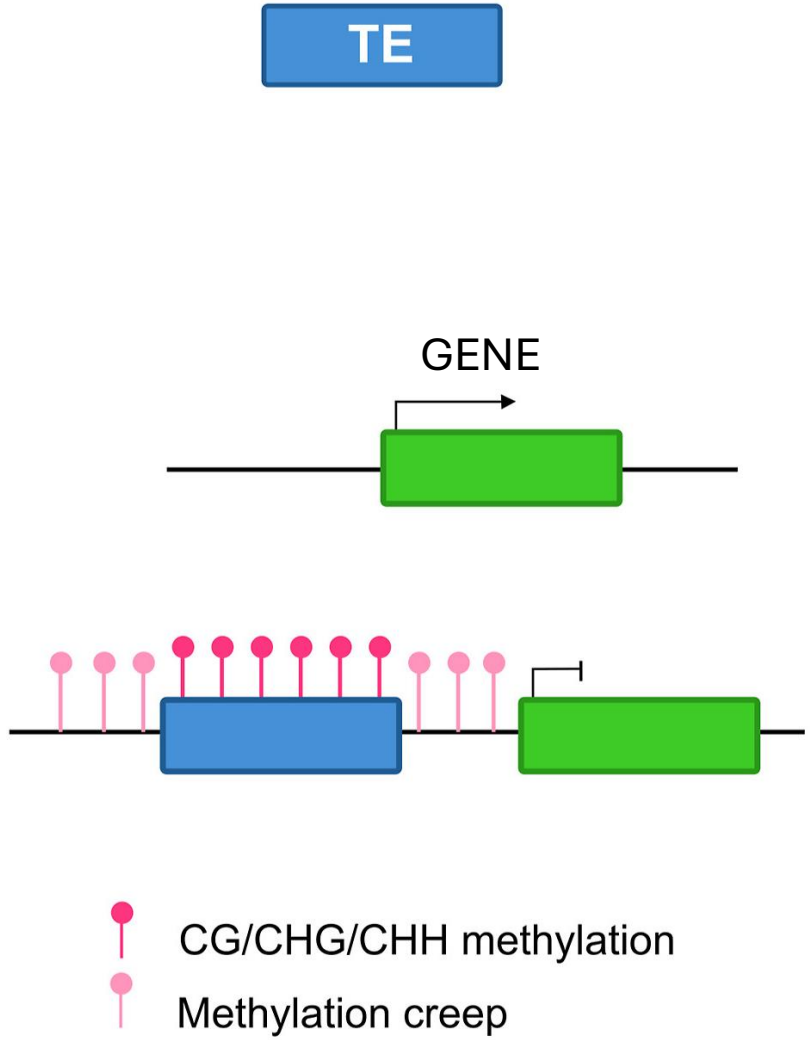


Consider not only host popgen, but also TE popgen!

Need to clean up their genomes
VS
genome structure reflects popgen of the host and TEs

Kapusta et al 2017, *PNAS*

Methylation spillover

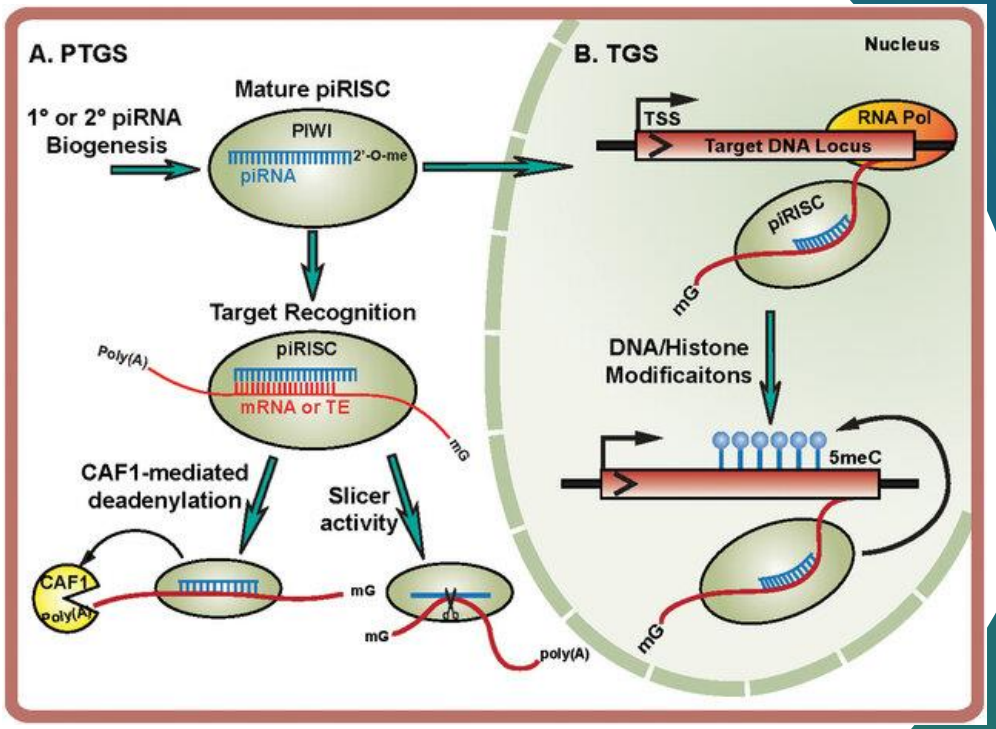


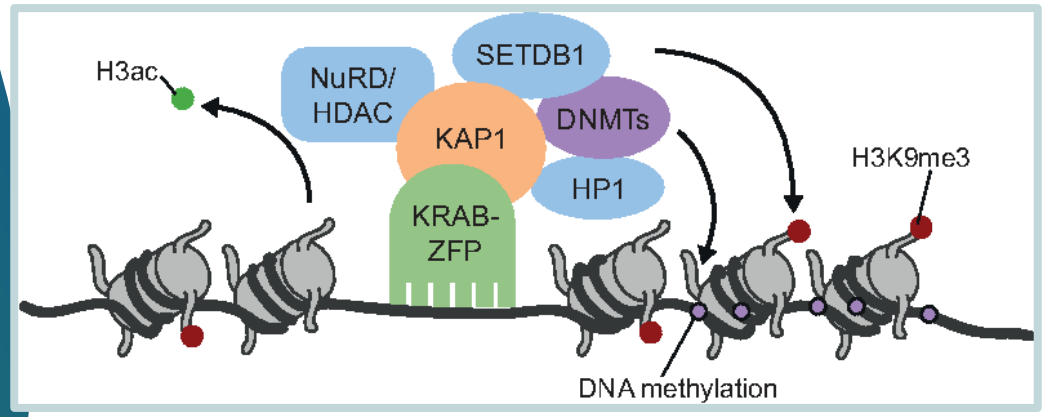
Anderson et al. 2022, Current Opinion in Plant Biology

Silencing mechanisms

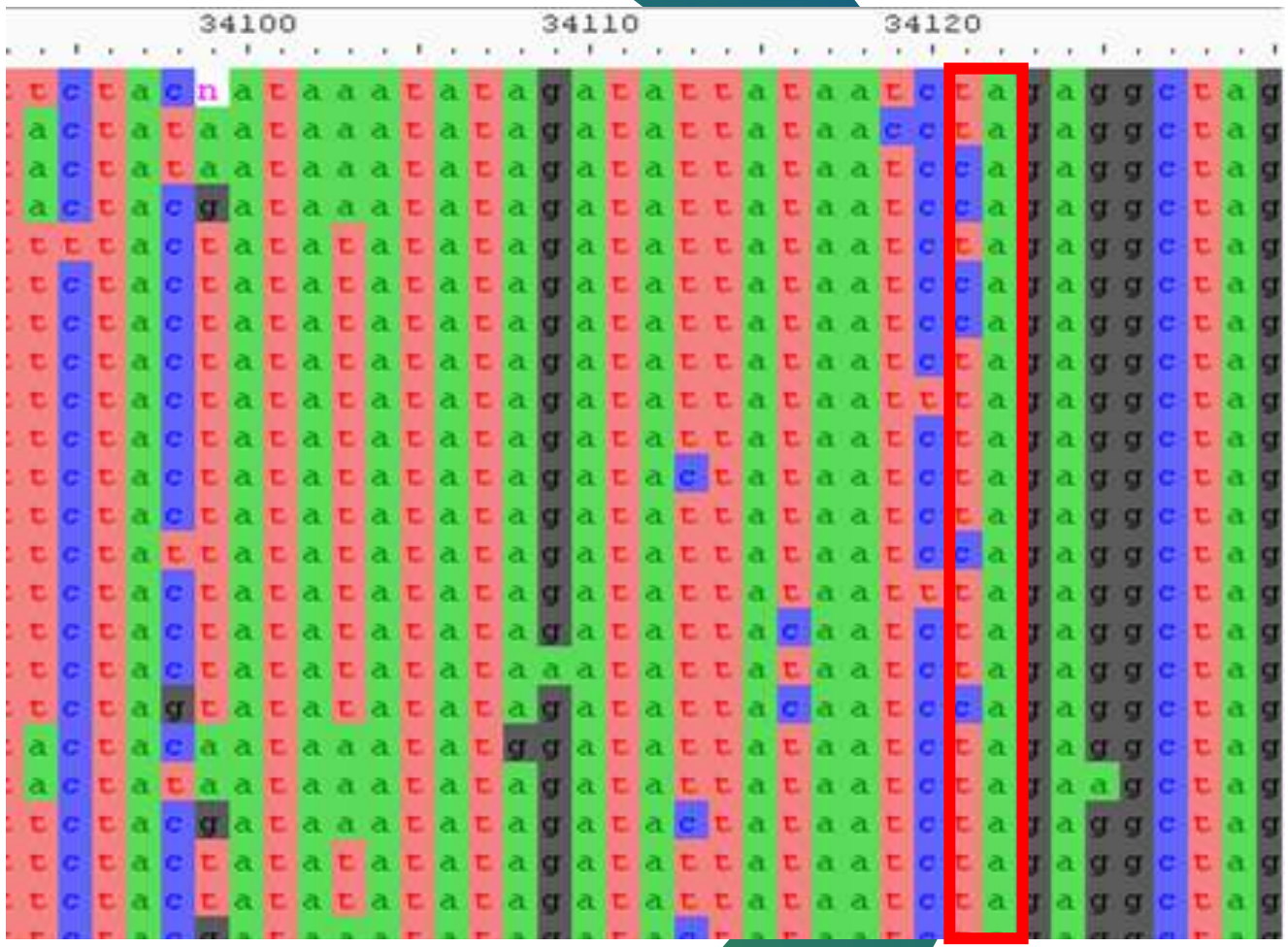
- DNA level
- Post-transcriptional level

DNA methylation, KRAB and PIWI





Repeat induced point mutation (RIP)



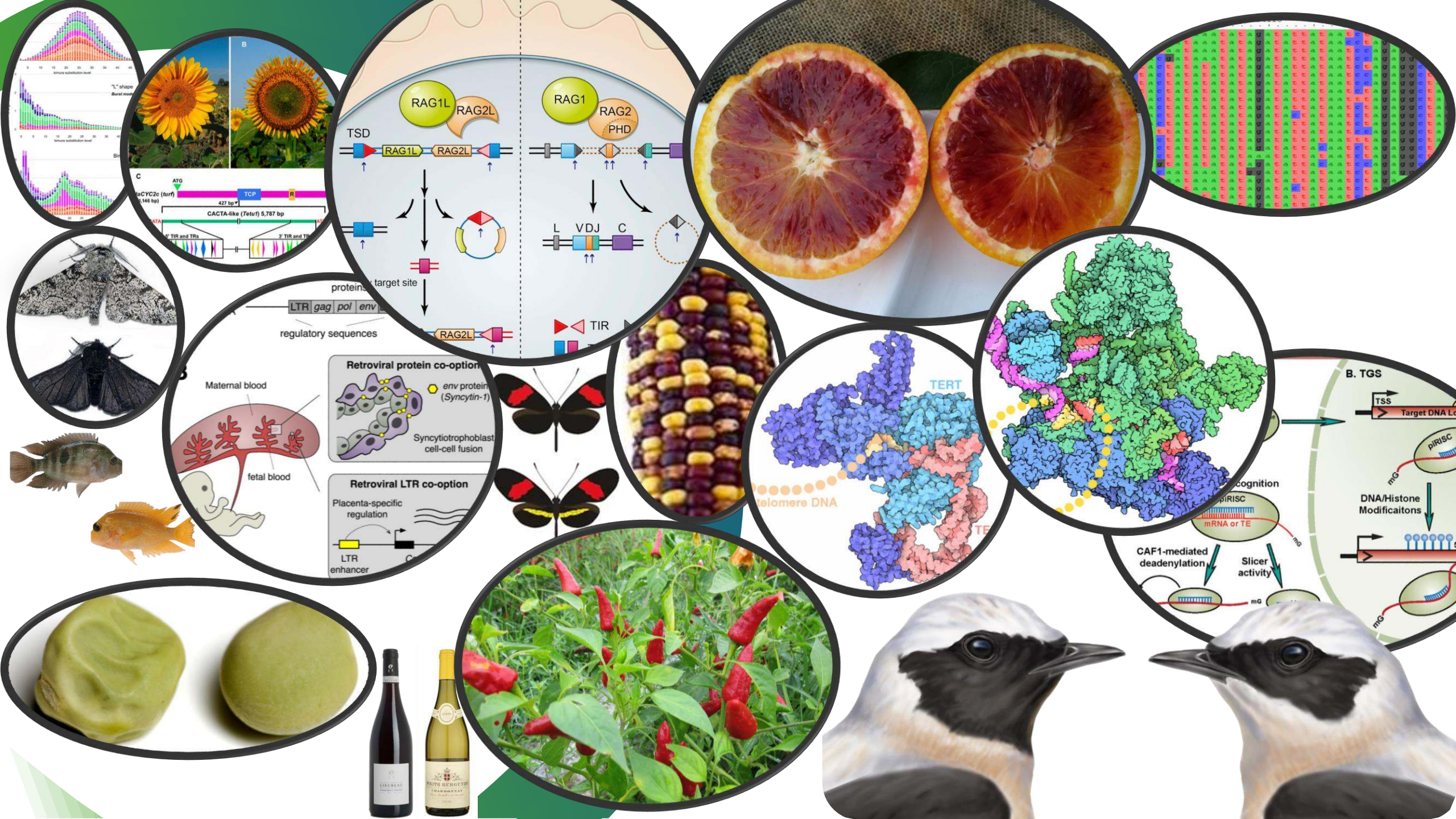
C -> T mutations
In *Neurospora*, mutations that are induced by RIP occur preferentially in CpA dinucleotides

Only a few repeated genes are known to survive RIP

Characterisation and annotation of transposable elements

Valentina Peona

16th January 2025, Evomics Workshop on Genomics


RAG1L
RAG2L
TSD
RAG1L
RAG2L
RAG1
RAG2
PHD
L
VDJ
C
target site
RAG2L
TIR
regulatory sequences
LTR
gag
pol
env
Retroviral protein co-option
env protein
(Syncytin-1)
Maternal blood
Syncytiotrophoblast
cell-cell fusion
fetal blood
Retroviral LTR co-option
Placenta-specific
regulation
LTR
enhancer
TERT
B. TGS
TSS
piRISC
mRNA or TE
CAF1-mediated
deadenylation
Slicer
activity
DNA/Histone
Modificaitons

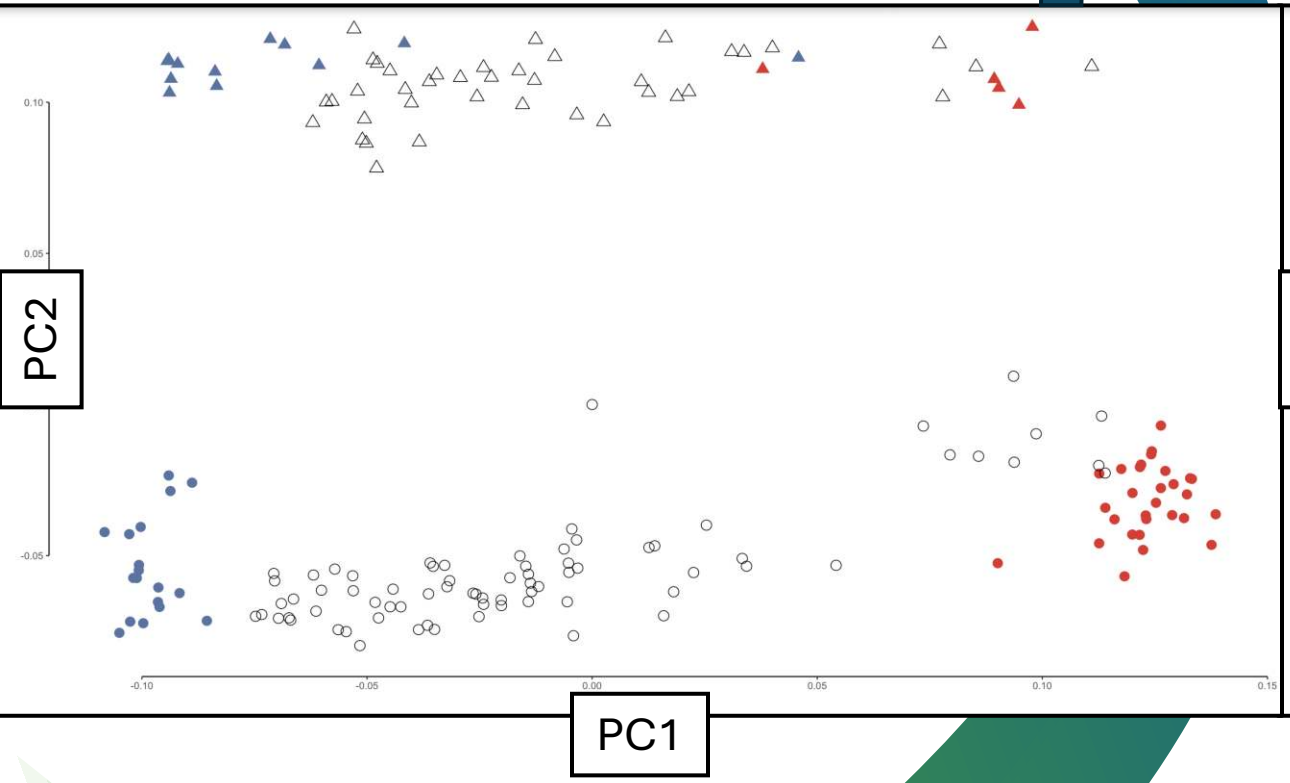
Effects on PCA

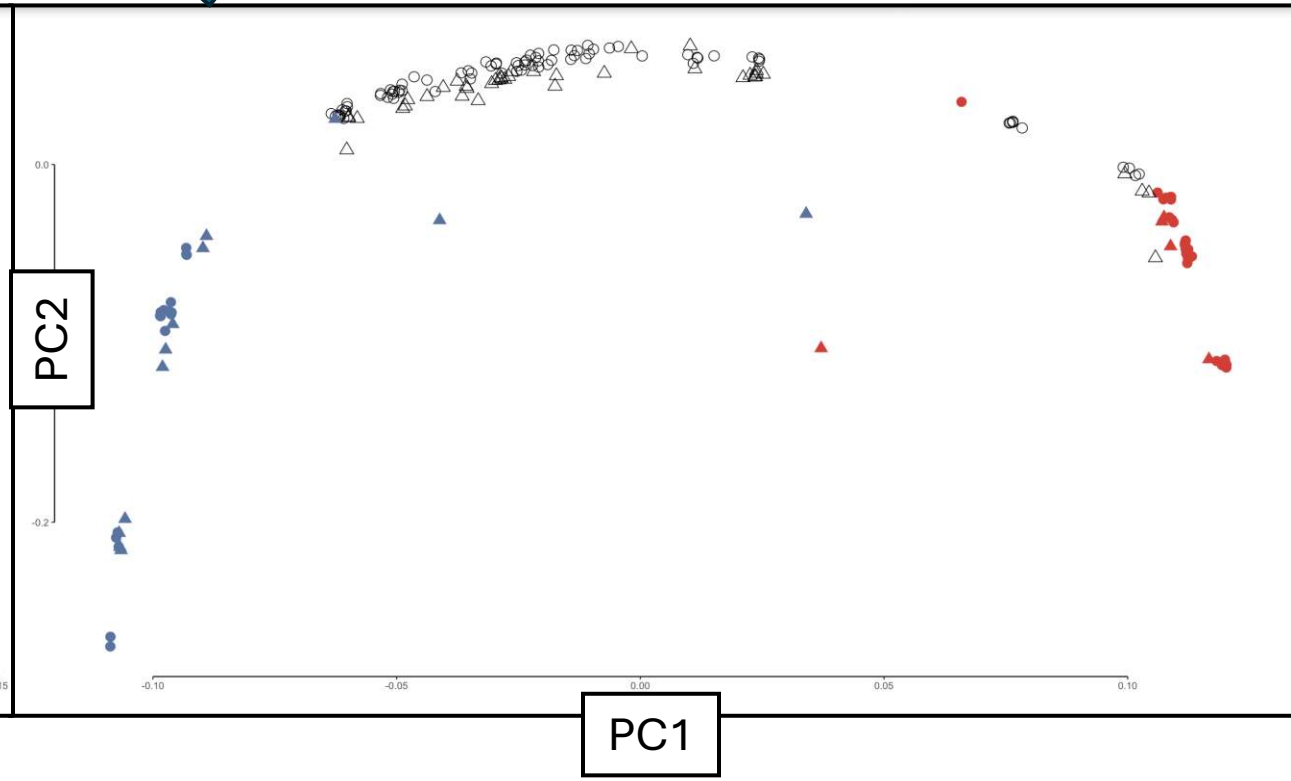
Dave Lutgen

△ Modern samples

○ Historical samples

Removal of SNVs in repeats
(and INDELS)





Lutgen et al. *in preparation*

TE annotation and lab tutorial

- Characterise the diversity of TEs: RepeatModeler2
- Annotate TEs: RepeatMasker

Characterise the diversity of TEs

We need to know what types of TEs are present to then know where they are

We then need a library, a set of reference/representative sequenc (**consensus sequences**) to use as

We can use consensus sequences already available in various databases OR create a **de novo library** of consensus sequences

Different approaches for different types of input sequences

A consensus sequence can be a fasta or an HMM model

Genome assembly

- All vs all alignment of the genome
  Cluster similar sequences
  Consensus sequences from multi-sequence alignments
  RepeatModeler2, REPET, CARP

Raw reads

- All vs all alignment of the reads (downsampled at 0.1X)
  Cluster similar sequences
  Assembly of the clusters
  Tools like RepeatExplorer2, DNAPipeTE

Classification system

Cellular organisms
Phylum
Class
Order
Family
Genus
Species
Individual

Transposable elements
Class
Subclass
Order
Superfamily
Family
Subfamily
Copy

Consensus sequence

Proprieties of a high-quality TE library

- is **complete** - the entire diversity of repeats is represented
- contains **nonredundant** consensus sequences - each element is represented only once
- contains **full-length consensus sequences** - each elements is not fragmented/truncated

Goubert *et al. Mobile DNA* (2022) 13:7
https://doi.org/10.1186/s13100-021-00259-7

Mobile DNA

METHODOLOGY Open Access

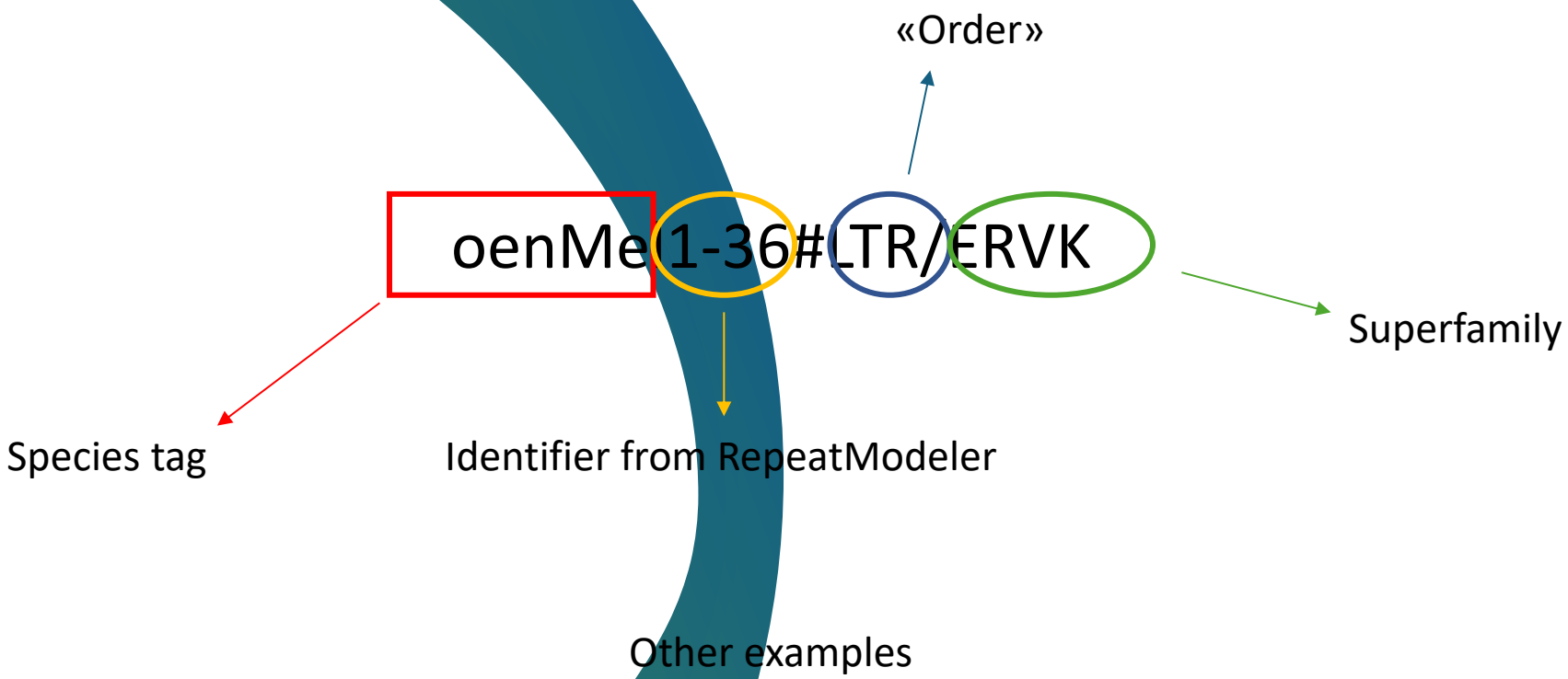
A beginner's guide to manual curation of transposable elements

Clement Goubert[1,2], Rory J. Craig[3], Agustin F. Bilat[4], Valentina Peona[5], Aaron A. Vogan[5] and Anna V. Protasio[6,7*]

https://mobilednajournal.biomedcentral.com/articles/10.1186/s13100-021-00259-7

Extensive section of supplementary materials with (among the others) video tutorials of how to curate the consensus sequences!

Structure of the consensus sequence name



Other examples

oenMel1-36_**LTR**#LTR/ERVK

oenMel1-36_**int**#LTR/ERVK

oenMel1-90.**inc**#LINE/CR1

TE annotation

Genome.fasta.tbl

Genome.fasta.out (main output)

SW score	perc div.	perc del.	perc ins.	query sequence	position in query begin	position in query end	(left)		matching repeat	repeat class/family	position in repeat begin	position in repeat end	position in repeat (left)	ID	
206	6.7	0.0	0.0	NC_030765.1	12662	12691	(4911)	C	Unknown-2762_SO	Unknown/Unknown	(1163)	64	35	1	
1133	3.5	13.2	0.0	NW_022145616.1	1	174	(75979)	+	MITE_T_6311|NW_022146286.1|1017221|1019177|AT|15|F320	DNA/MITE	1494	1690	(266)	2	*
1863	2.7	0.0	2.2	NW_022145616.1	173	403	(75750)	+	MITE_T_6311|NW_022146286.1|1017221|1019177|AT|15|F320	DNA/MITE	1731	1956	(0)	3	
1889	9.7	6.6	12.1	NW_022145616.1	404	811	(75342)	C	MITE_T_17069|NW_022145681.1|3085354|3086586|GAATAT|15|F1012	DNA/MITE	(590)	642	255	4	
631	13.8	16.6	0.6	NW_022145616.1	692	830	(75323)	C	Unknown-1238_SO	Unknown/Unknown	(47)	203	43	5	*
720	11.9	13.8	0.6	NW_022145616.1	861	1012	(75141)	C	Unknown-2421_SO	Unknown/Unknown	(15)	241	70	6	
212	16.7	2.1	0.0	NW_022145616.1	1079	1126	(75027)	C	MITE_T_2060|NW_022146441.1|222914|224876|agct|38|F136	DNA/MITE	(495)	1467	1419	7	*
699	9.3	0.0	0.0	NW_022145616.1	1117	1213	(74940)	+	Unknown-2755_SO	Unknown/Unknown	4	100	(1653)	8	
216	16.3	0.0	0.0	NW_022145616.1	1214	1262	(74891)	+	MITE_T_28504|NW_022145617.1|1329355|1331252|ta|38|F1758	DNA/MITE	1026	1074	(823)	9	*
650	17.6	0.9	1.7	NW_022145616.1	1251	1366	(74787)	C	Unknown-2726_SO	Unknown/Unknown	(104)	128	14	10	
1553	10.6	3.8	2.3	NW_022145616.1	1872	2132	(74021)	+	DNA-2829_SO	DNA/MITE	130	394	(35)	11	
846	14.4	2.2	17.7	NW_022145616.1	2107	2334	(73819)	C	Unknown-1886_SO	Unknown/Unknown	(514)	562	365	12	*
1192	5.3	3.2	14.7	NW_022145616.1	2133	2352	(73801)	+	MITE3_SO	DNA/MITE	1	198	(1030)	13	
1023	7.6	1.4	1.4	NW_022145616.1	2345	2491	(73662)	+	MITE3_SO	DNA/MITE	1082	1228	(0)	14	*
606	19.4	1.4	2.0	NW_022145616.1	2361	2507	(73646)	C	DNA-3306_SO	DNA/MITE	(596)	181	36	15	*
239	17.8	6.9	3.3	NW_022145616.1	2819	2876	(73277)	C	Unknown-1619_SO	Unknown/Unknown	(271)	452	393	16	
996	28.3	5.0	4.4	NW_022145616.1	2879	3827	(72326)	+	LINE-3770_SO	LINE/RTE	424	1282	(33)	17	*

```
    Tc1-Mariner-Pogo          229          63407 bp    0.75 %
    En-Spm                      0              0 bp    0.00 %
    MuDR-IS905                  0              0 bp    0.00 %
    PiggyBac                    1            129 bp    0.00 %
    Tourist/Harbinger           1            465 bp    0.01 %
    Other (Mirage,              0              0 bp    0.00 %
     P-element, Transib)
Rolling-circles              2          470 bp    0.01 %
Unclassified:             1567       500540 bp    5.89 %
Total interspersed repeats:       4726544 bp   55.60 %


Small RNA:                  28         2042 bp    0.02 %

Satellites:                 85        43411 bp    0.51 %
Simple repeats:             0             0 bp    0.00 %
Low complexity:             0             0 bp    0.00 %
==================================================
```

We can find the coordinates of repeats in the genome by aligning our library to the genome

RepeatModeler2

GENOME FASTA FILE

```
>oenMel
ATGAGCGCGAGAGGG
CGAATCCCTAGGCTA
ACATCGTCCCGCGAT
GCTTGCTTAGAACCT
TGCCTAGACCTGAGC
TCTAGCTTACTGCTA
GCTTCCGATTTACAC
GATCACCCTACATAT
CTTCACATCCATCTC
```

BuildDatabase –name <name> <genome file>

RepeatModeler2 –database <name>

Library of consensus sequences

RepeatMasker

GENOME FASTA FILE

```
>oenMel
ATGAGCGCGAGAGGG
CGAATCCCTAGGCTA
ACATCGTCCCGCGAT
GCTTGCTTAGAACCT
TGCCTAGACCTGAGC
TCTAGCTTACTGCTA
GCTTCCGATTTACAC
GATCACCCTACATAT
CTTCACATCCATCTC
```

+

```
>consensus1
ATTGCGCGTTAGGAT
ATCCCGATCGCCC
>consensus2
TGTAGGGAGTCTTGA
CA
>consensus3
ATTTCGGGCTAGGCT
TGAGGC
```

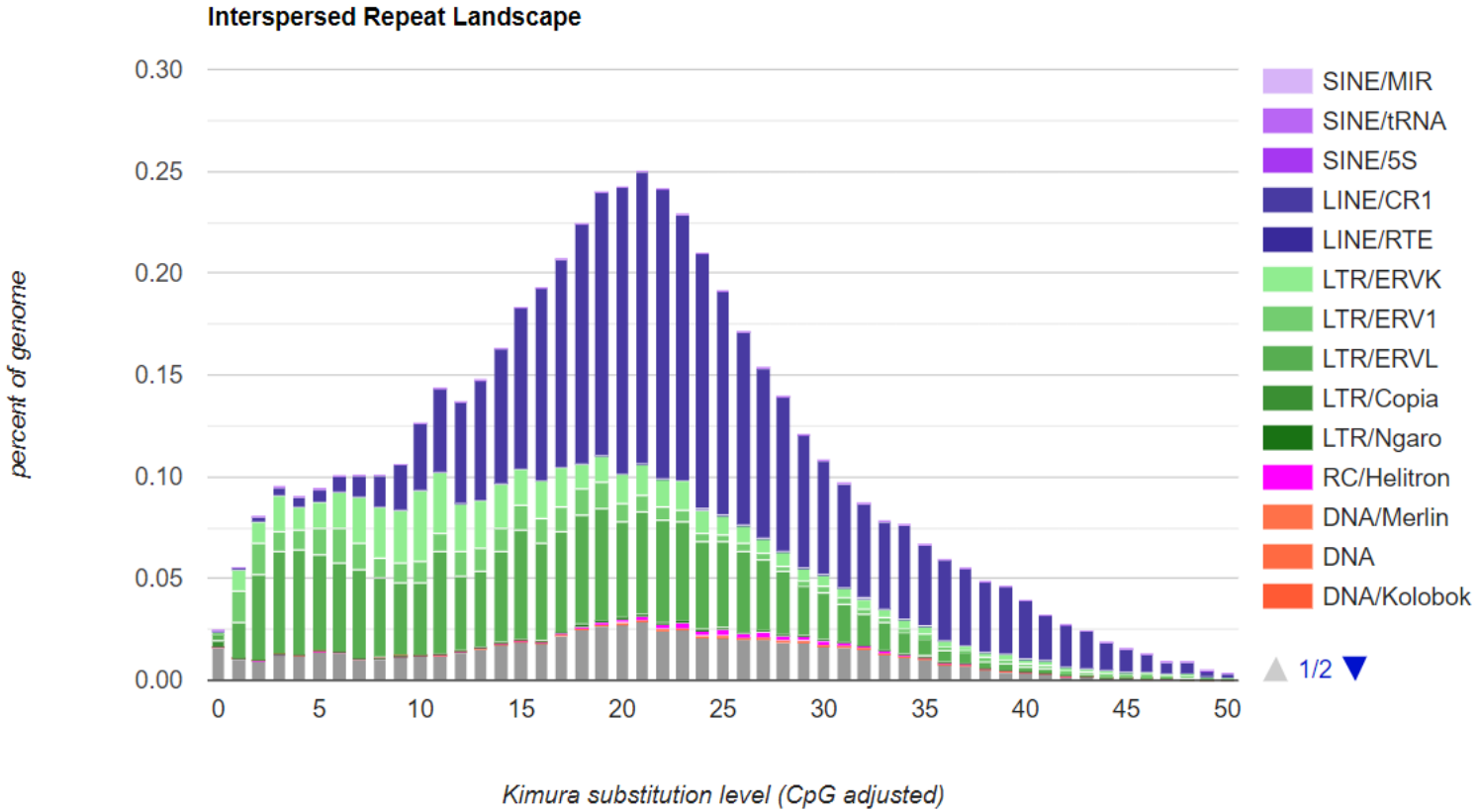
RepeatMasker –lib <library> <genome file>

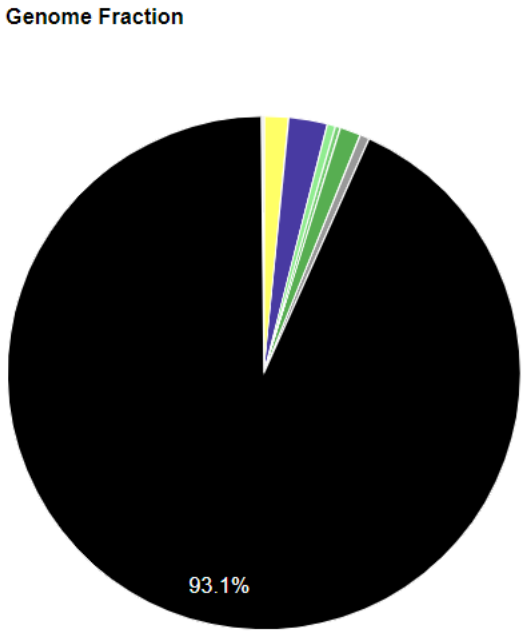
.out + .gff files with coordinates of repeats

Repeat landscape

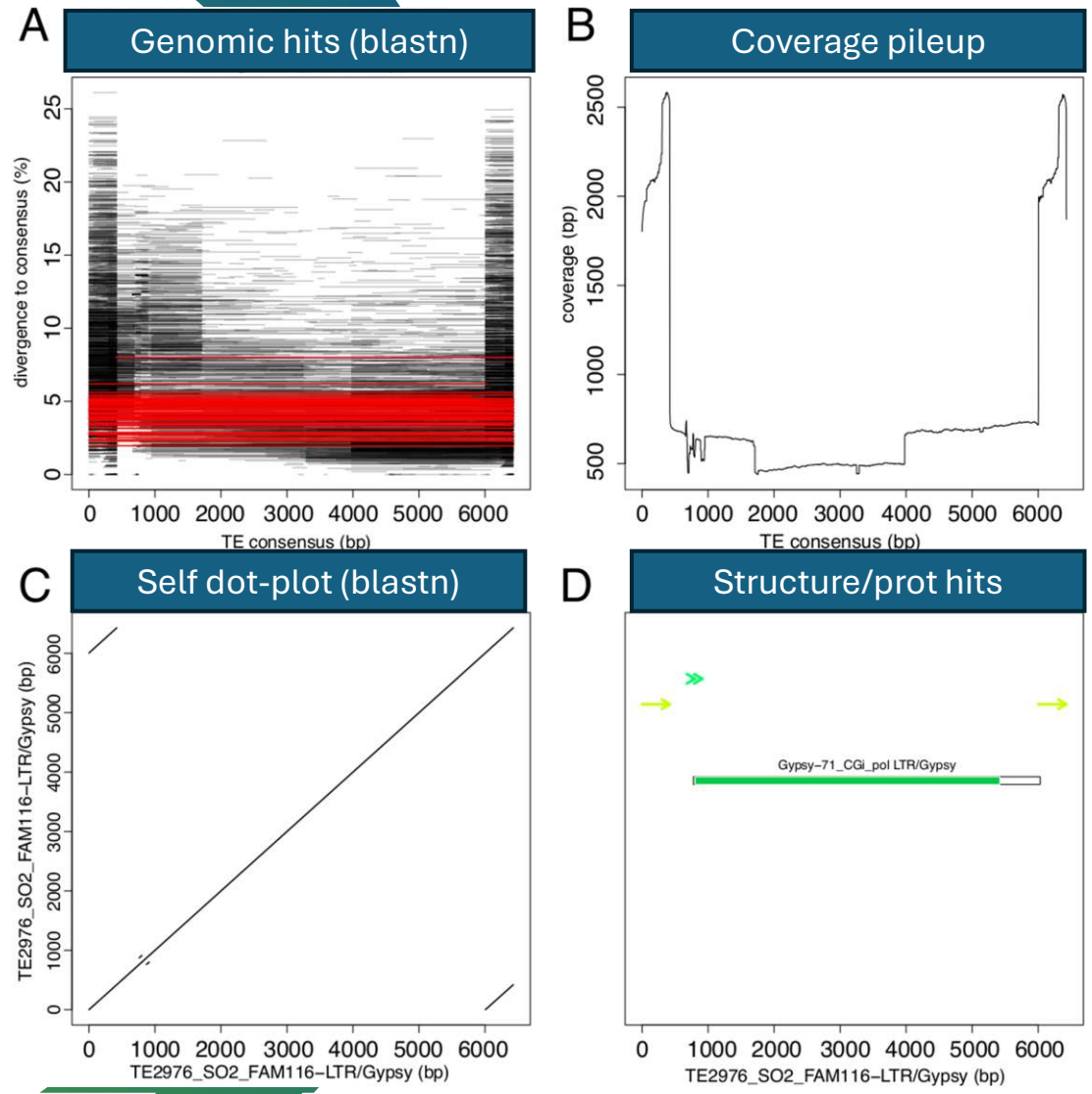
Use outputs from RepeaMasker to visualise the repetitive content of the genome

- calcDivergenceFromAlig.pl
- createRepeatLandscape.pl



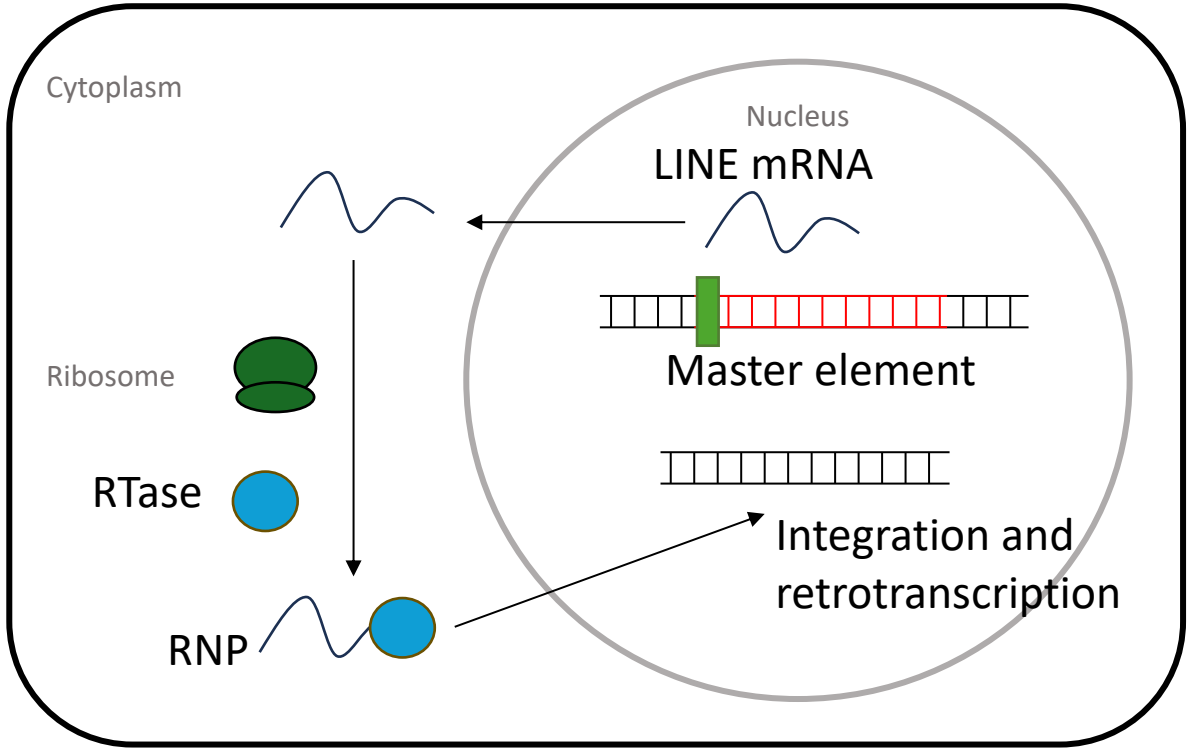


Analyse sequence characteristics of TEs



LINE retrotransposons

Where/when/how in the cell



Requirements for mobility

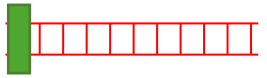
Transcription: promoter for pol II -> mRNA + polyA
Replication: RTase
Recognition site for *cis*-mobilisation
Integration: endonuclease
No introns

Target site preference and TSD

Target site preferentiality for sequences similar to the 3' UTR
Target site duplications are of variable length

Content of new copies

Mother copy

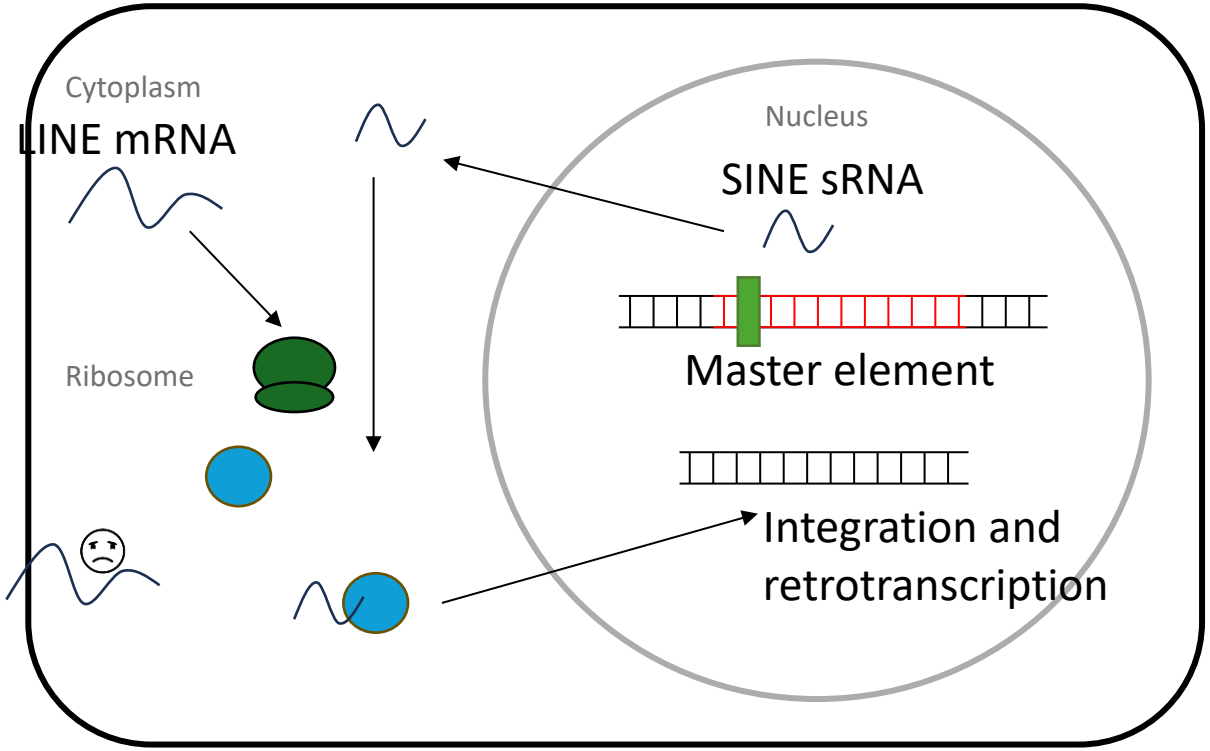


Daughter copies

5' truncation
"dead on arrival"

SINE retrotransposons

Where/when/how in the cell



Requirements for mobility

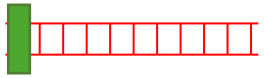
Transcription: promoter for **pol III** -> sRNA
Replication + integration: using LINE derived proteins
Recognition site for *trans*-mobilisation
No introns

Target site preference and TSD

Target site preferentiality for sequences similar to the 3' UTR
Target site duplications are of variable length

Content of new copies

Mother copy

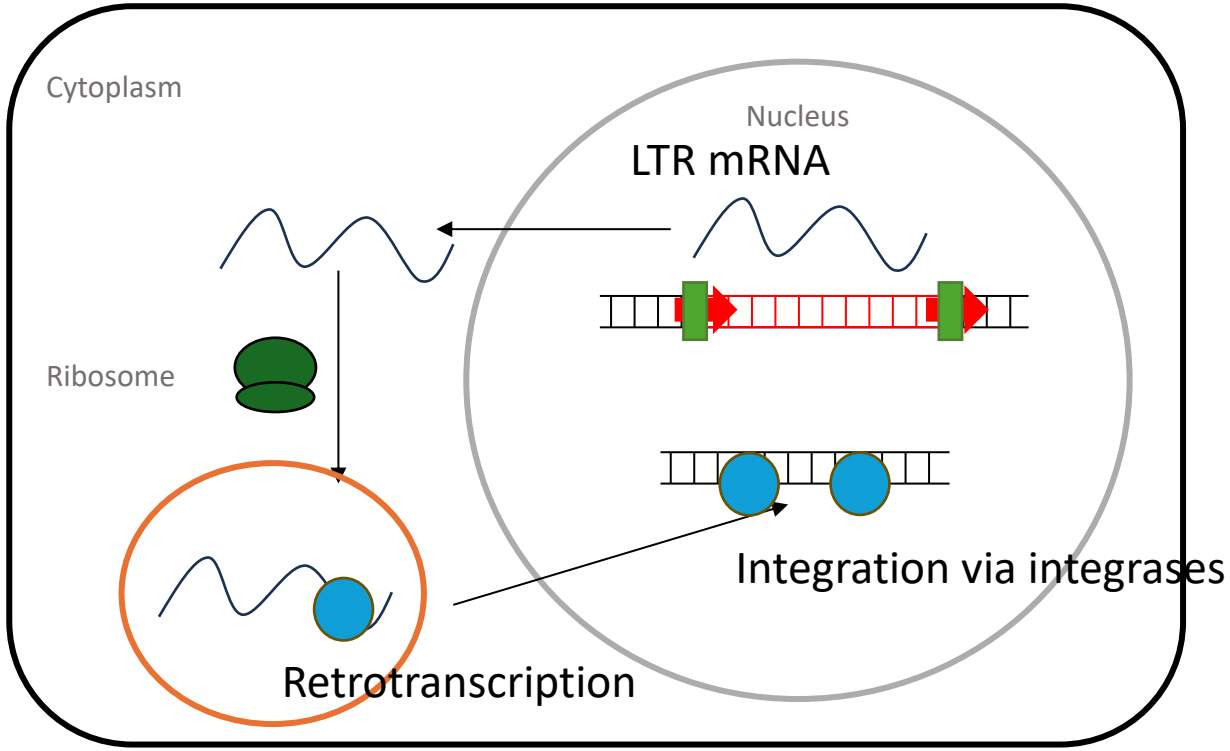


Daughter copies

5' truncation
"dead on arrival"

LTR retrotransposons

Where/when/how in the cell



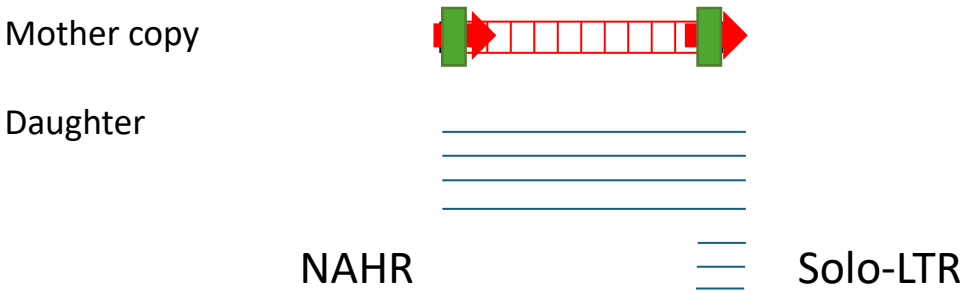
Requirements for mobility

Transcription: promoter for pol II -> mRNA + polyA
Replication: within viral-like particle, gag (capsid), protease, RTase, RNAse H
Integration: integrase
Recognition site for *cis*-mobilisation (LTR)
No introns

Target site preference and TSD

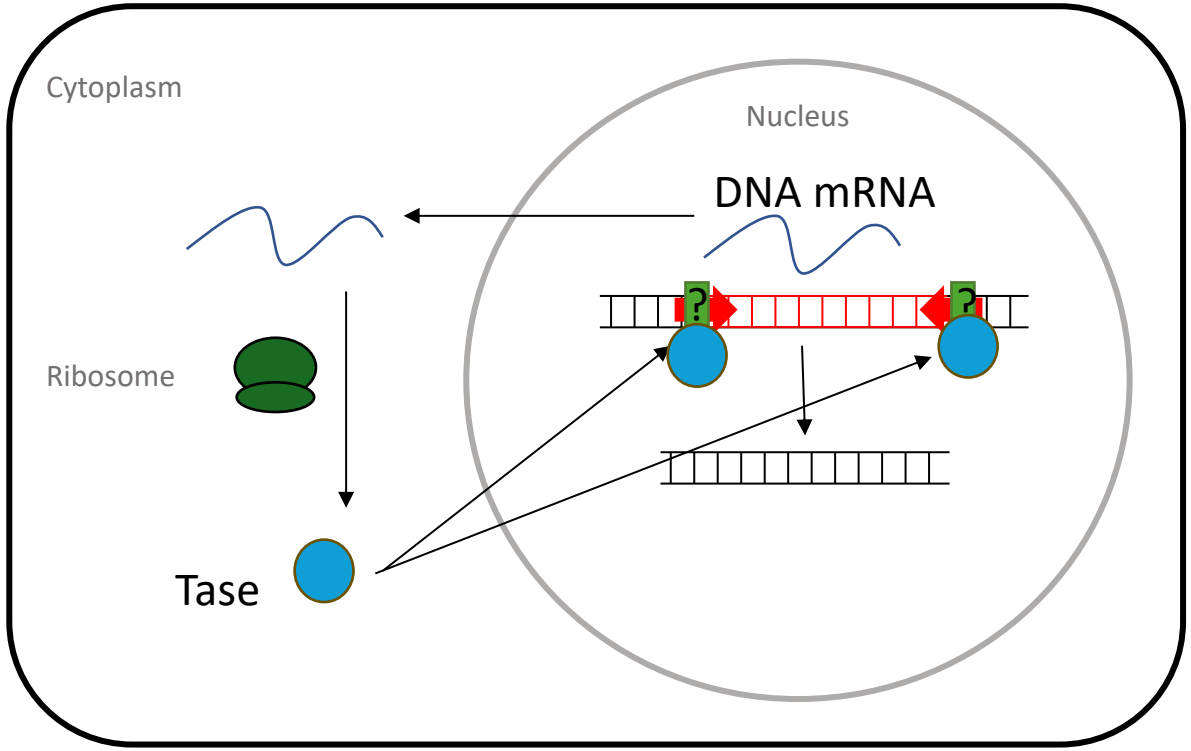
No preferentiality for target site
Specific length of target site duplications (4 bp, 5 or 6 bp)

Content of new copies



DNA transposons (TIR)

Where/when/how in the cell



Requirements for mobility

Transcription: promoter for pol II -> mRNA + polyA
Mobilisation and integration: transposase (Tase)
Replication: dependent on host DNA replication
Recognition site in TIRs allows for both *cis*- and trans-mobilisation (with different probabilities)
Might have introns

Target site preference and TSD

There can be specificity for target site (e.g., TA, TTAA) and there can be specific target site duplication length (e.g., 8 bp)

Content of new copies

Mother copy

Daughter copy

Inclusion of extra DNA or loss of ORFs