An introduction to transposable element biology

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16th January 2025, Evomics Workshop on Genomics

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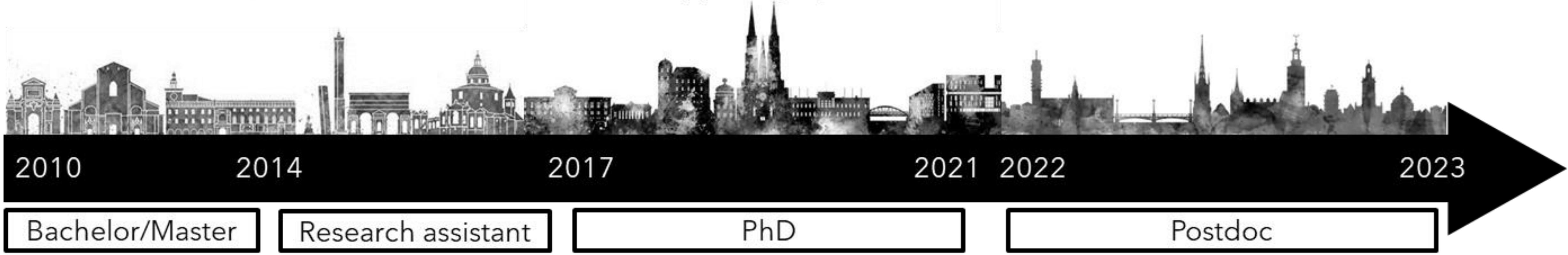


2010 2014 2017 2021 2022 2023

Bachelor/Master

Research assistant

PhD

Postdoc

Ecology/Popgen

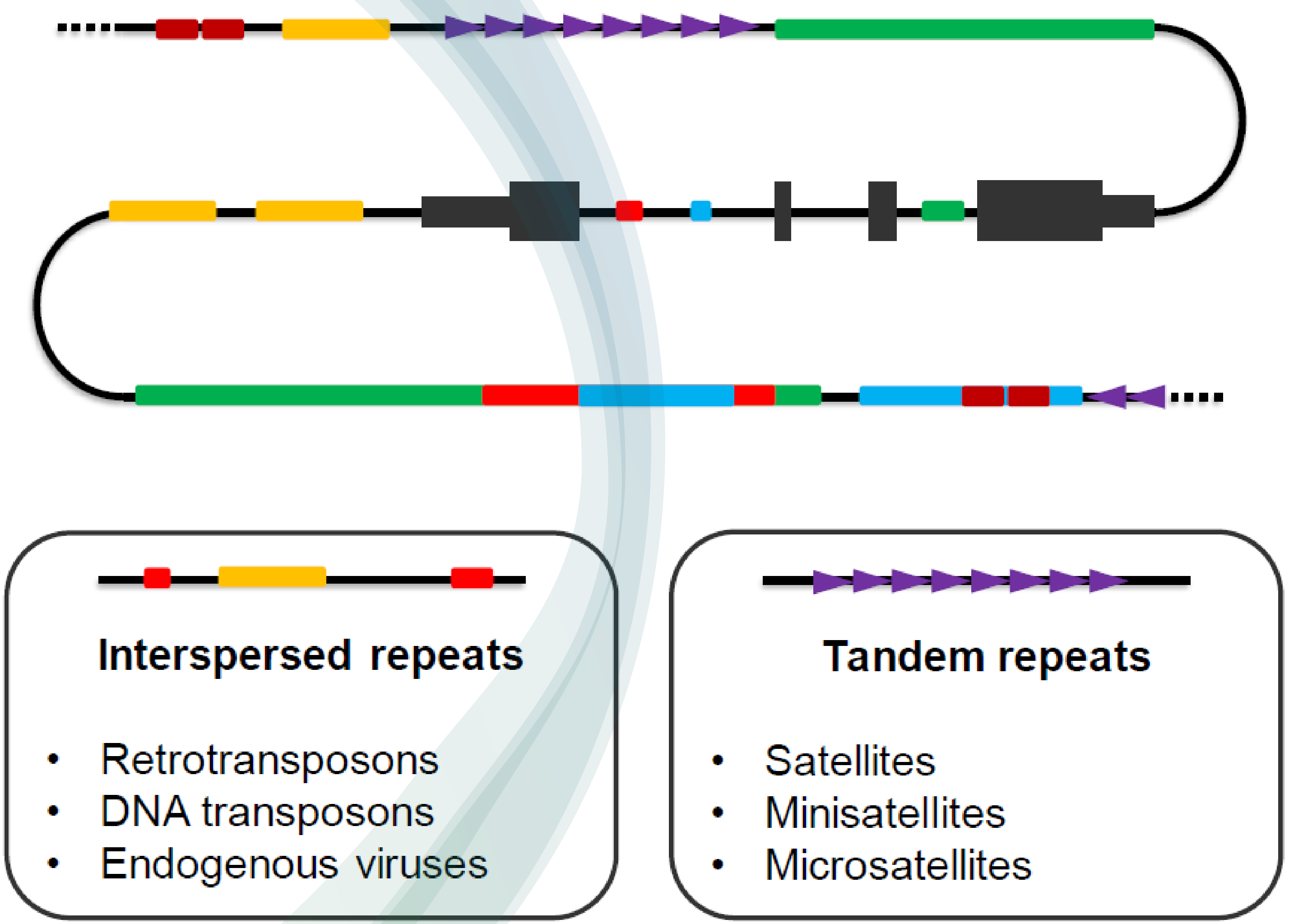
Comparative genomics

Speciation genomics

Overview

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

Genomes: DNA on repeats



Interspersed repeats

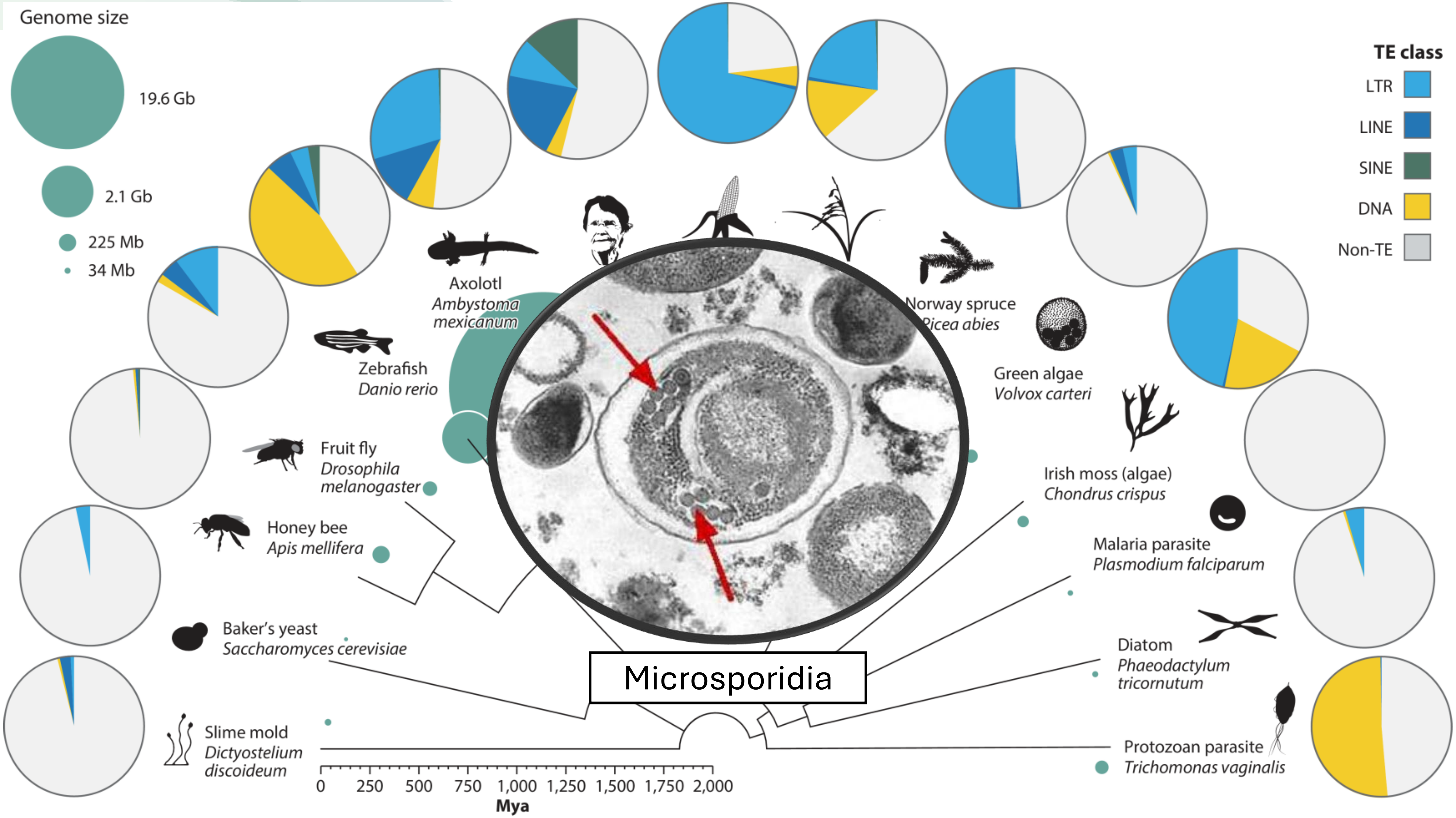
- Retrotransposons
- DNA transposons
- Endogenous viruses

Tandem repeats

- Satellites
- Minisatellites
- Microsatellites

Barbara McClintock

Nobel Prize
1983



Microsporidia

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?

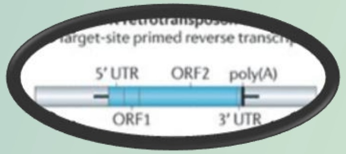
Because

they

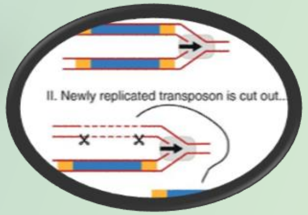
can

Main TE categories

- Sequence structure

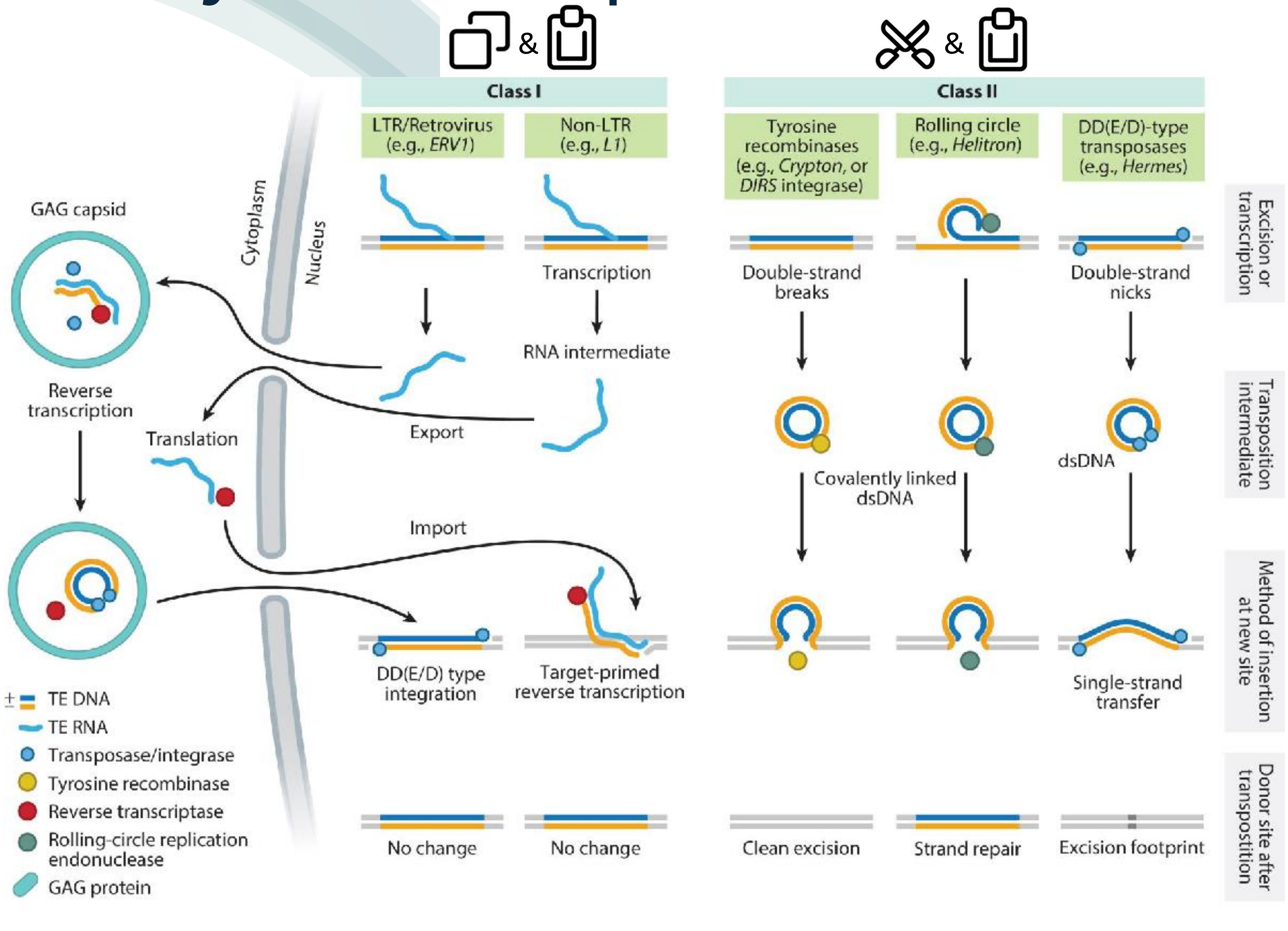


- Transposition mechanisms



- Effects on genome evolution

Eukaryotic transposable elements



Wells JN, Feschotte C. 2020
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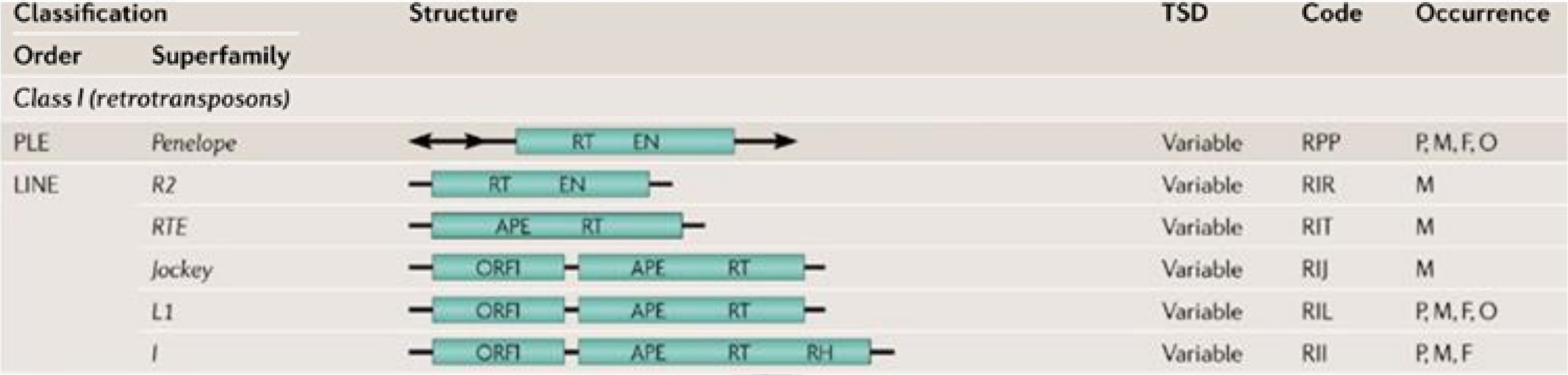
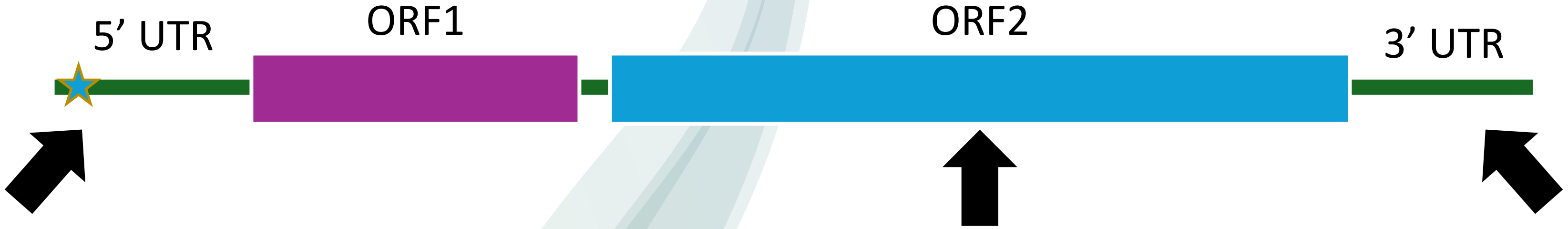
Class I: LINE retrotransposons



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!

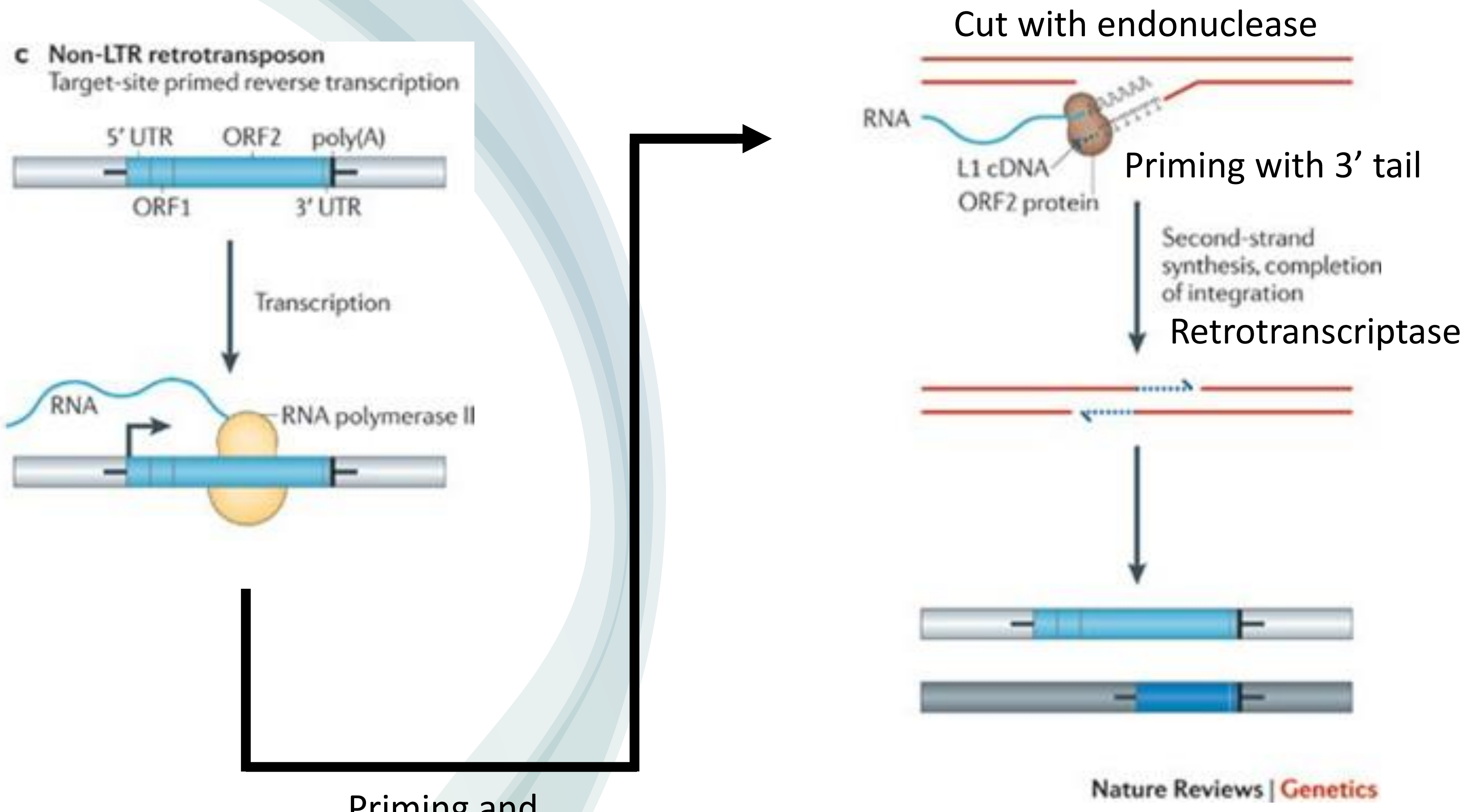
Promoter for RNA polymerase

Open reading frame(s) for transposition proteins

Recognition site for reverse transcriptase

Wicker et al 2007, *Nat Rev Gen*

Target-primed reverse transcription (TPRT)


c Non-LTR retrotransposon
Target-site primed reverse transcription
5′ UTR
ORF2
poly(A)
ORF1
3′ UTR
Transcription
RNA
RNA polymerase II
RNA
L1 cDNA
ORF2 protein
AAAAA
TTTTT
Second-strand synthesis, completion of integration

Cut with endonuclease

Priming with 3’ tail

Retrotranscriptase

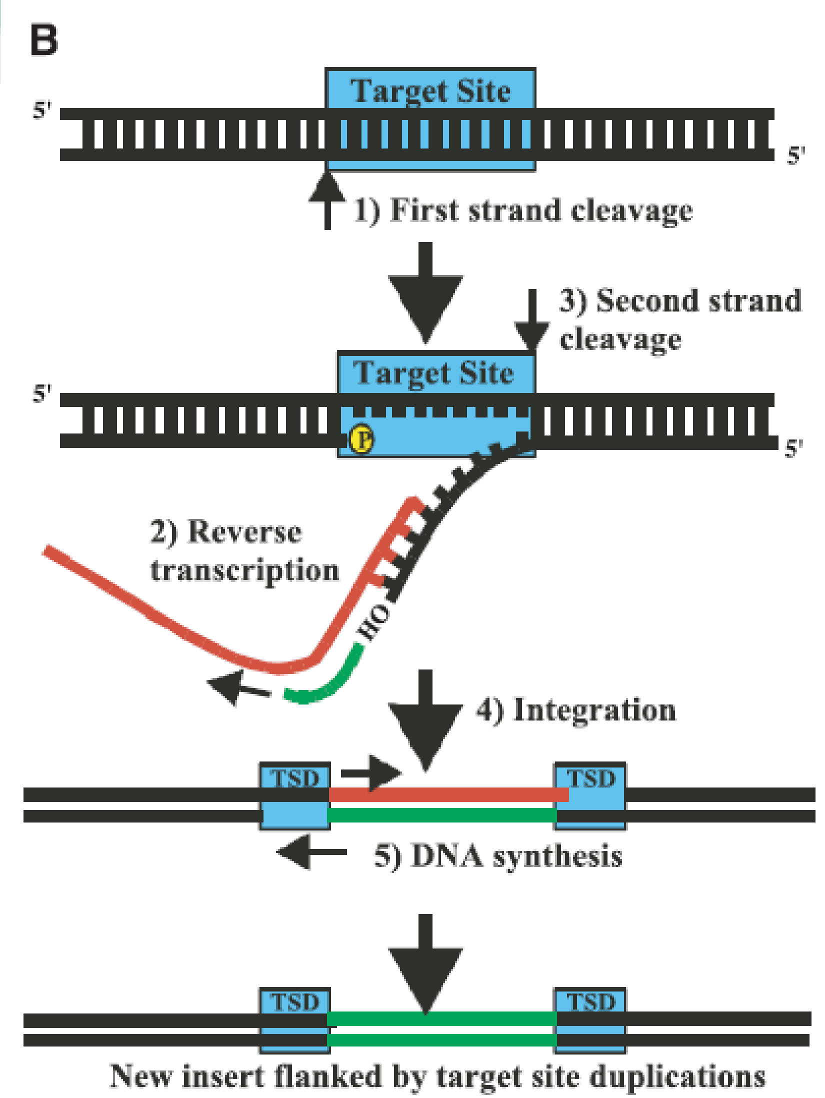
Nature Reviews | Genetics

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

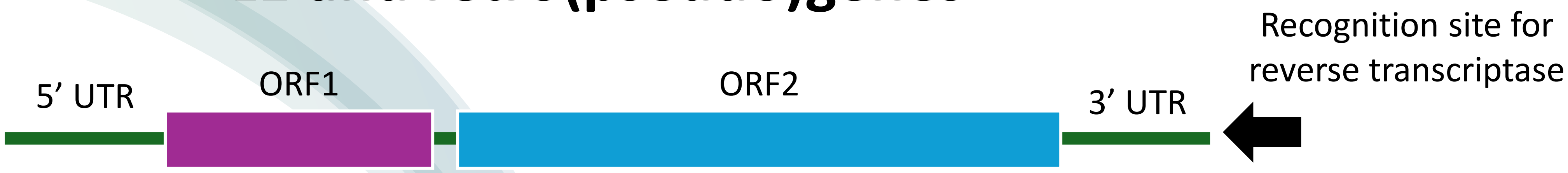


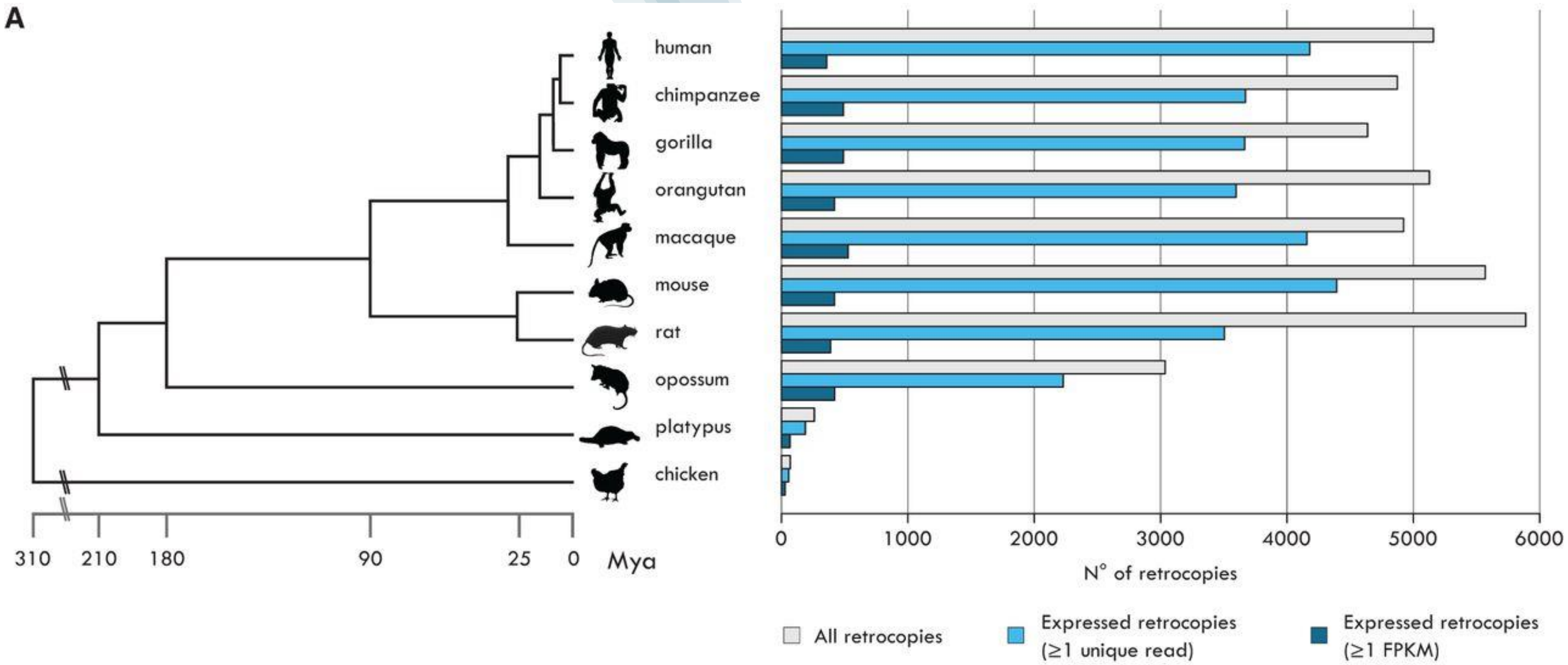
The length of TSDs is important for classification

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

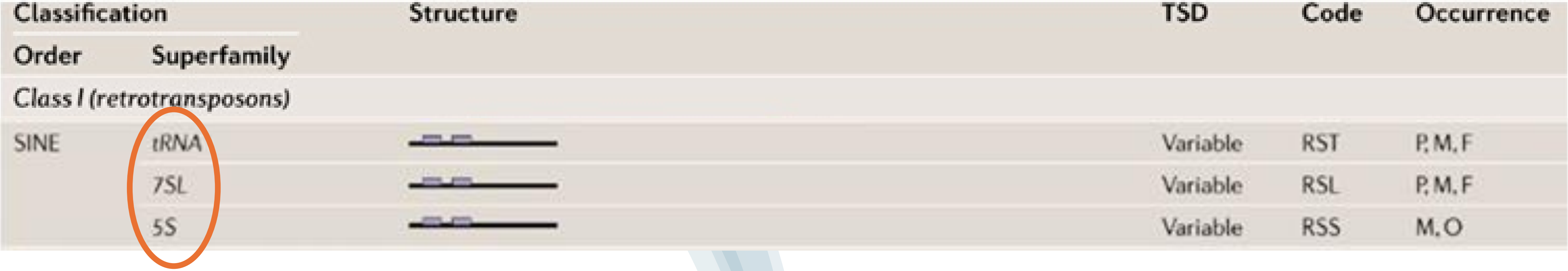




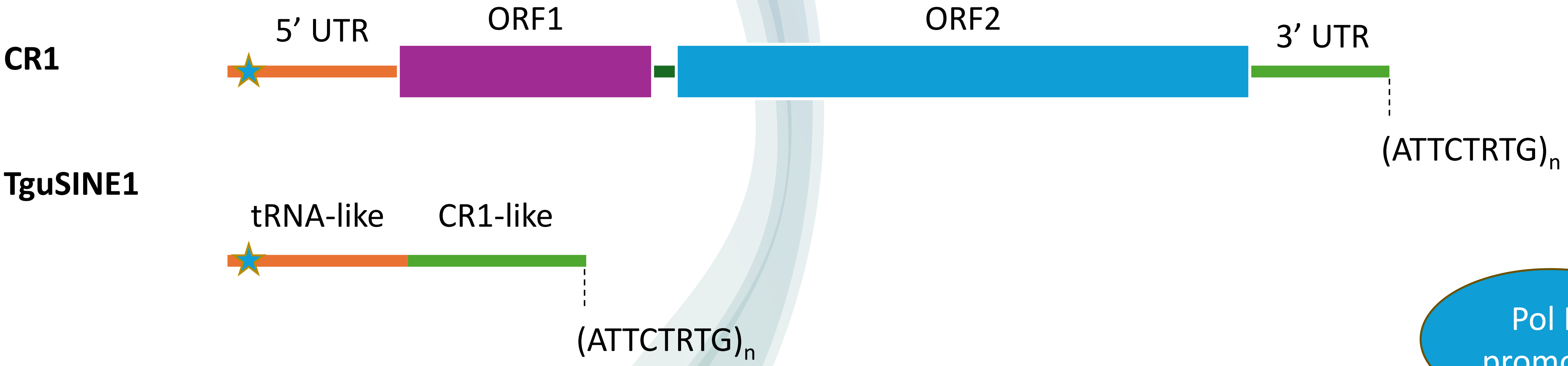
Retrogenes occur when LINE RT recognizes the poly-A tails (L1)

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

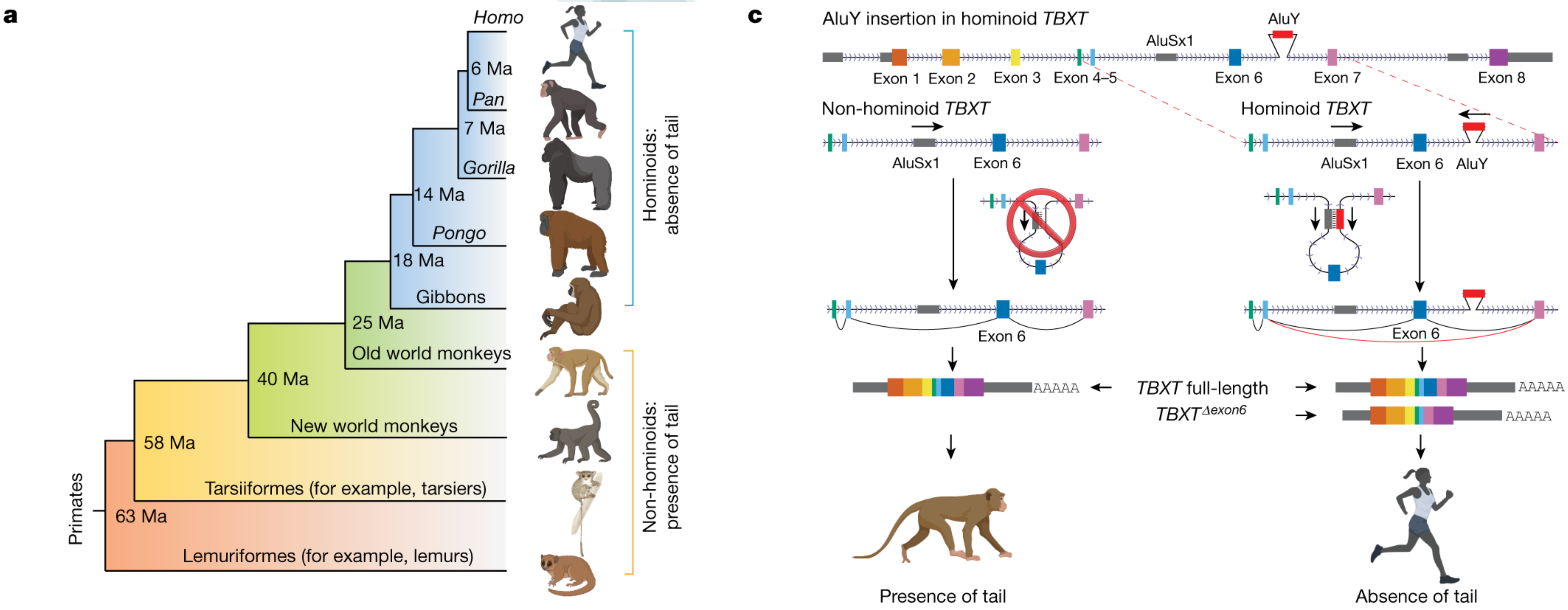


Pol III promoter

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

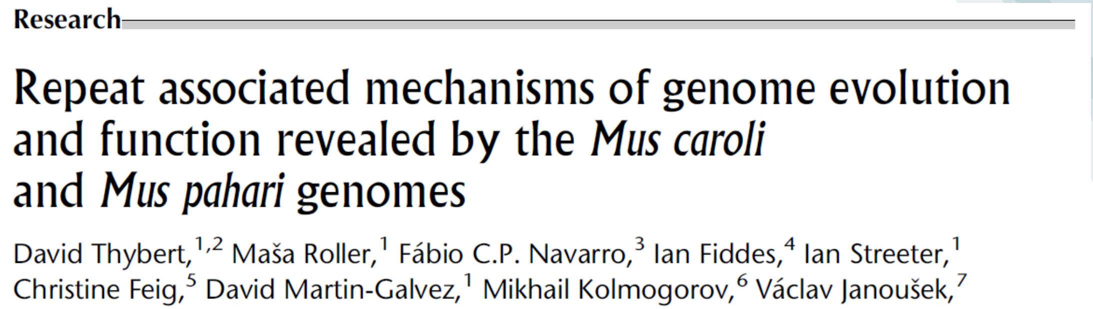
Wicker et al 2007, *Nat Rev Gen*

SINE *Alu* and alternative splicing



Xia et al. 2024, Nature

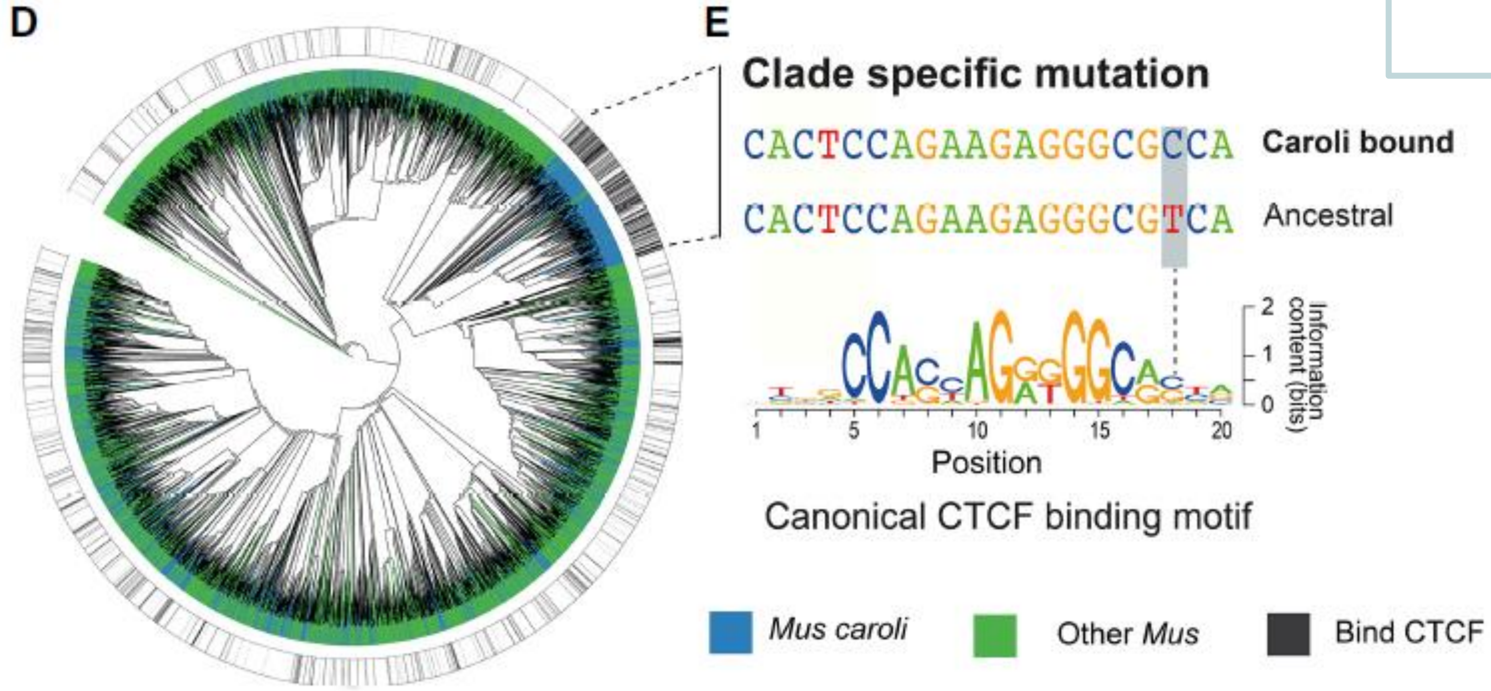
SINEs and 3D genome architecture

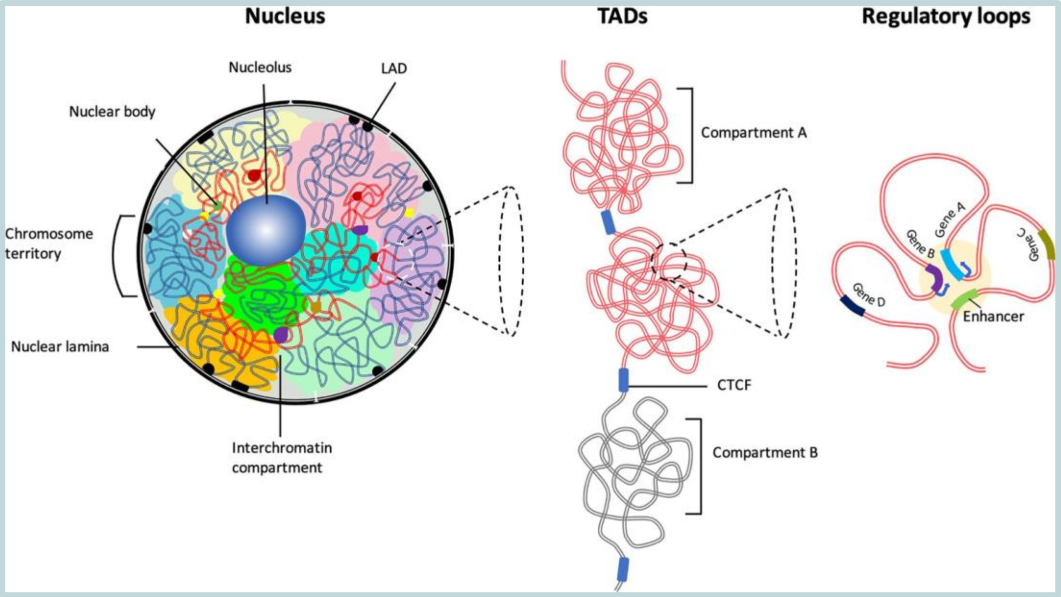


Research

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

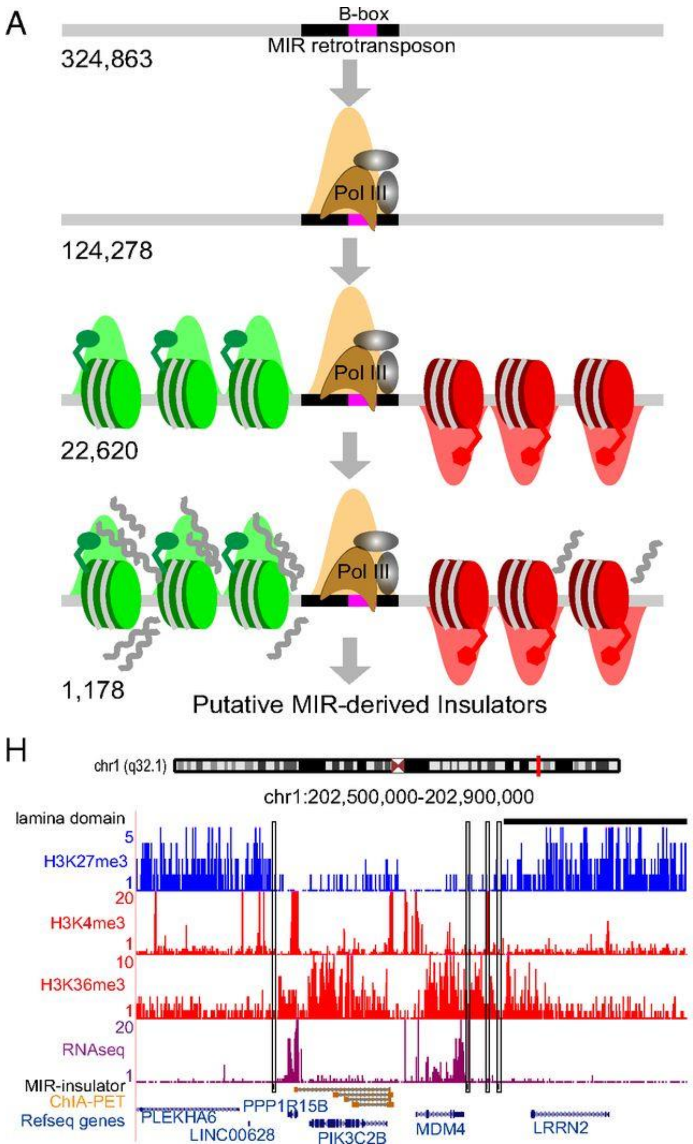
David Thybert,[1,2] Maša Roller,[1] Fábio C.P. Navarro,[3] Ian Fiddes,[4] Ian Streeter,[1]
Christine Feig,[5] David Martin-Galvez,[1] Mikhail Kolmogorov,[6] Václav Janoušek,[7]

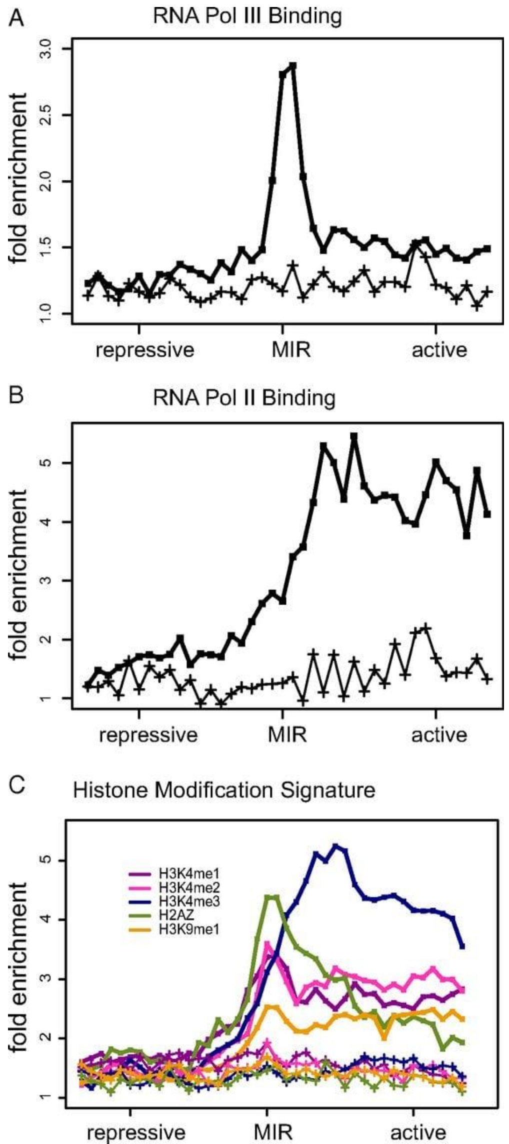




One SNP in the *Mus caroli* lineage turned SINE B2 into CTCF binding sites

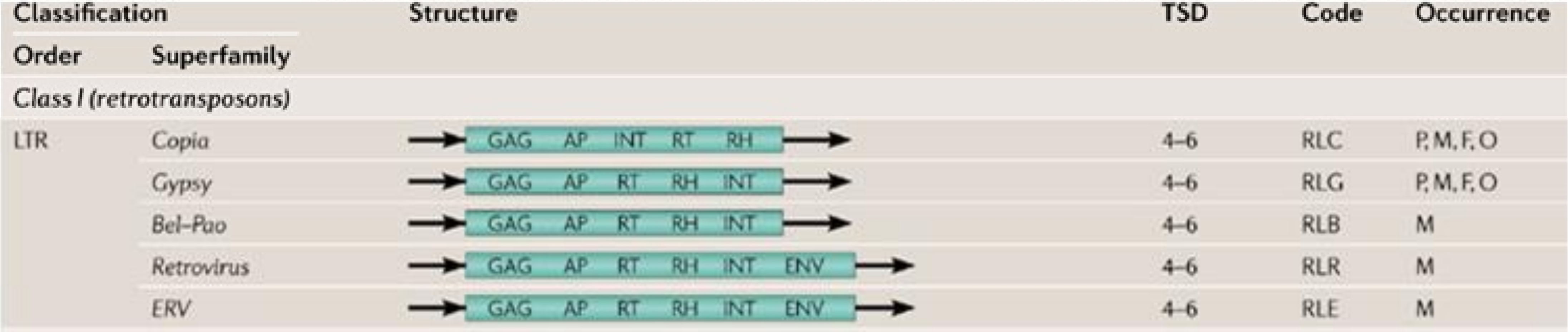
Ancient SINEs became conserved elements and insulators





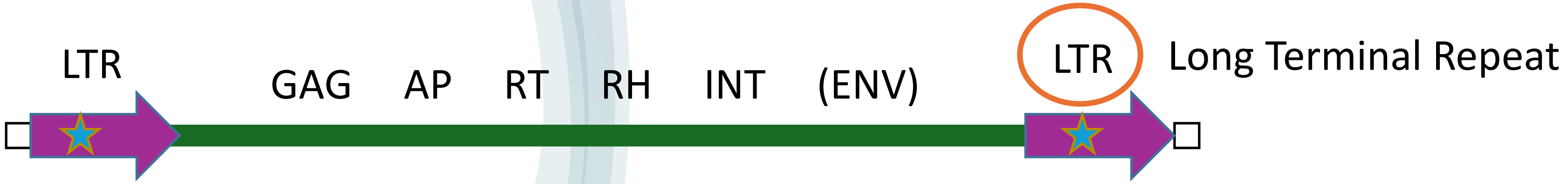
Wang et al. 2015, PNAS

Class I: LTR retrotransposons

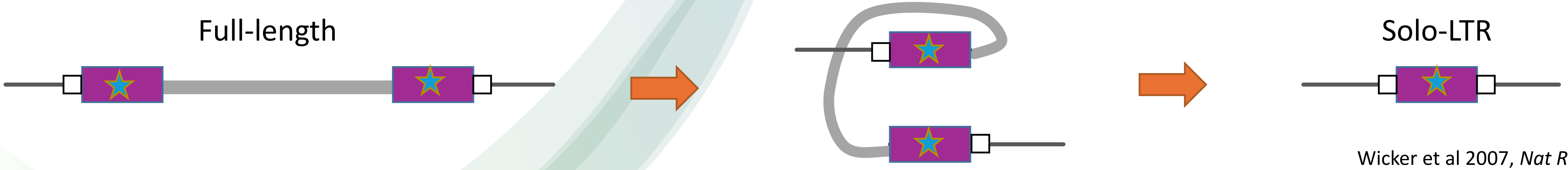


Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
LTR	*Copia*	→ GAG AP INT RT RH →	4–6	RLC	P, M, F, O
	Gypsy	→ GAG AP RT RH INT →	4–6	RLG	P, M, F, O
	Bel–Pao	→ GAG AP RT RH INT →	4–6	RLB	M
	Retrovirus	→ GAG AP RT RH INT ENV →	4–6	RLR	M
	ERV	→ GAG AP RT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

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

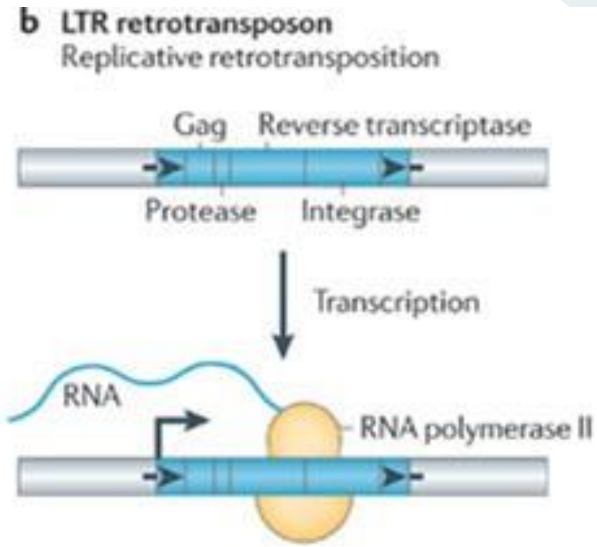


Non-allelic homologous recombination (NAHR)

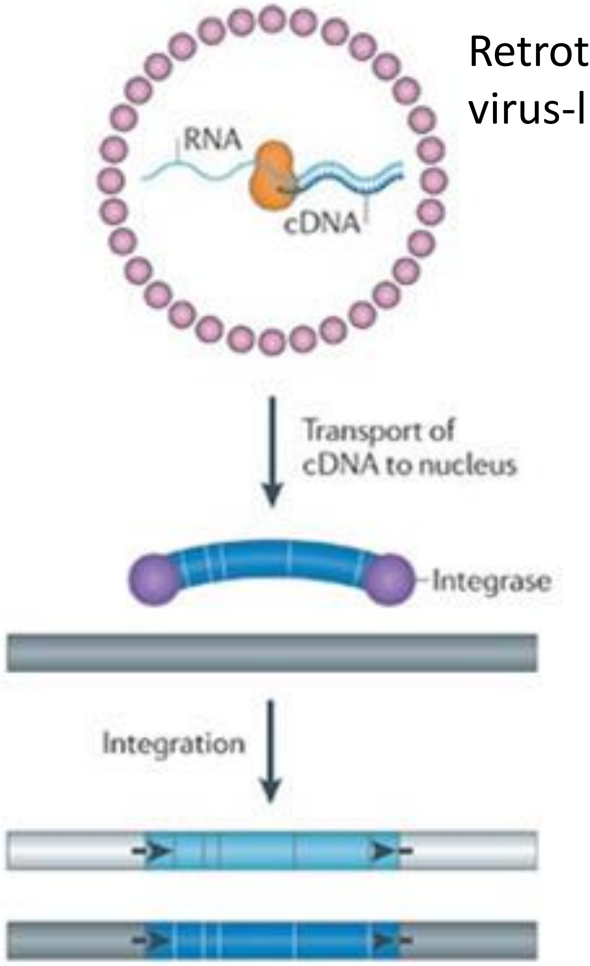


Wicker et al 2007, *Nat Rev Gen*

Replicative retrotranposition



Reverse transcription



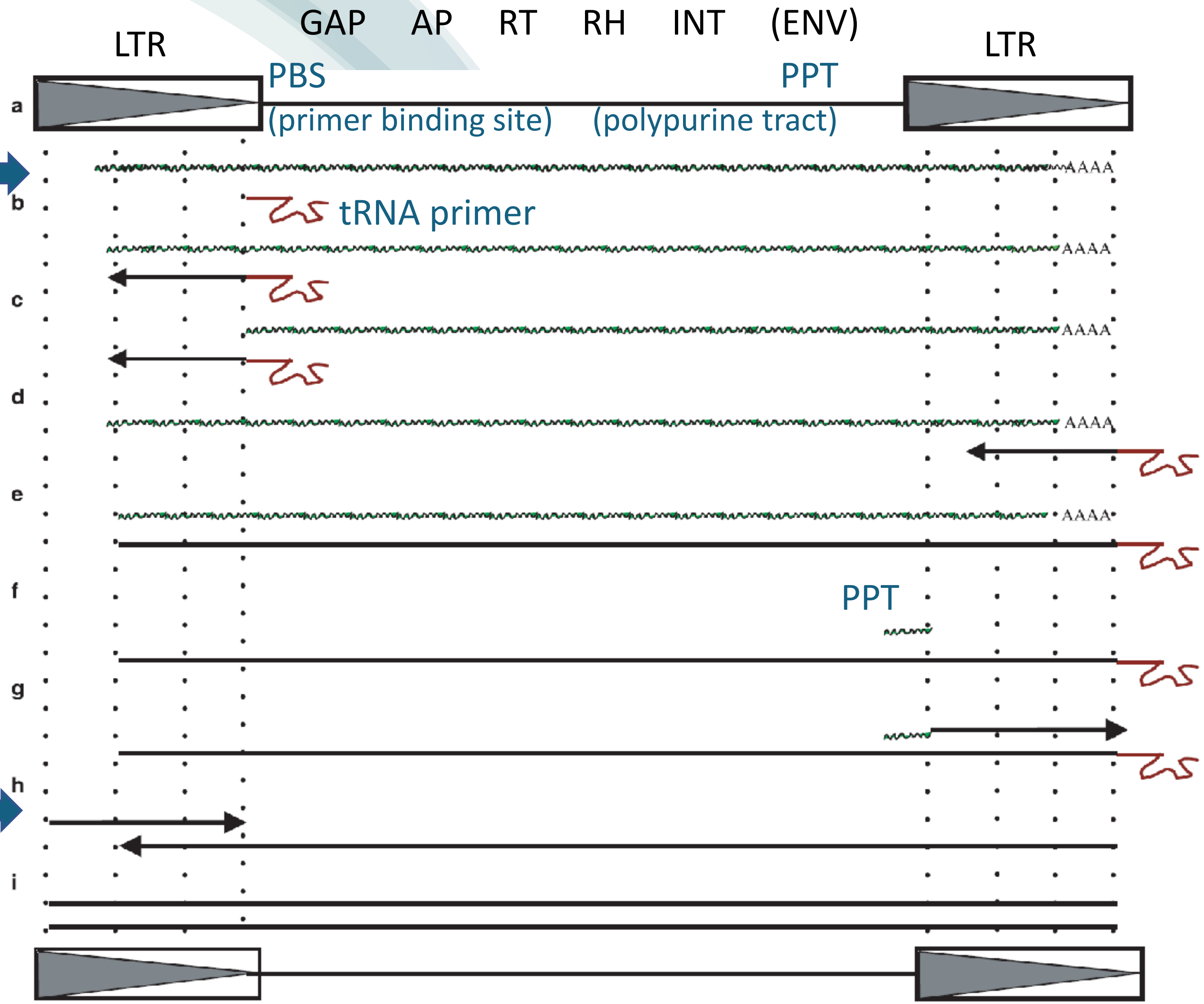
Retrotranscription inside a virus-like particle

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

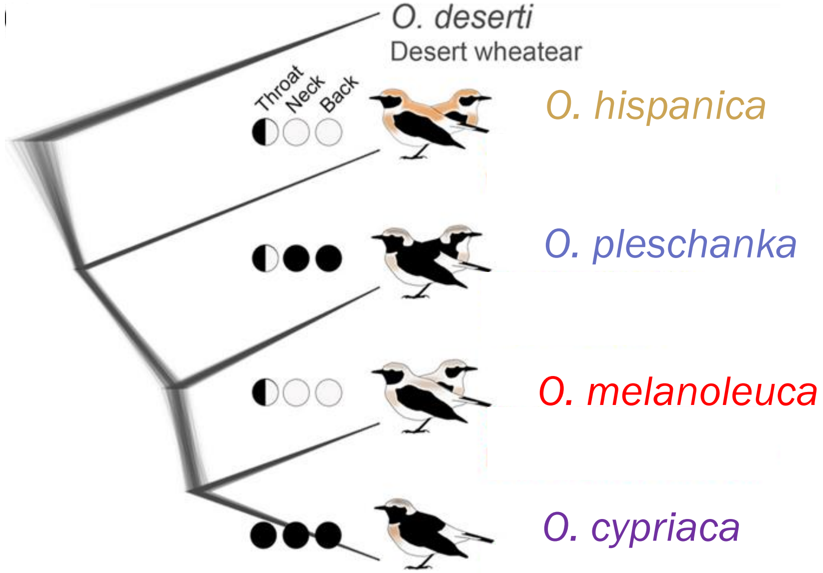
Kazazian 2004, *Science*

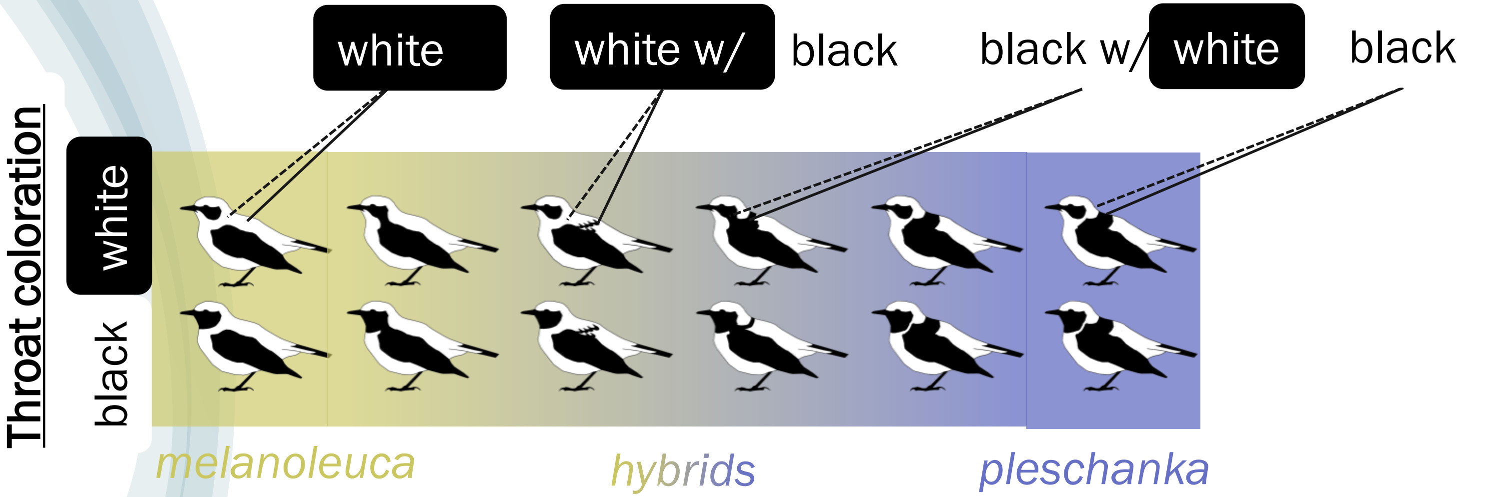
LTRs and coloration

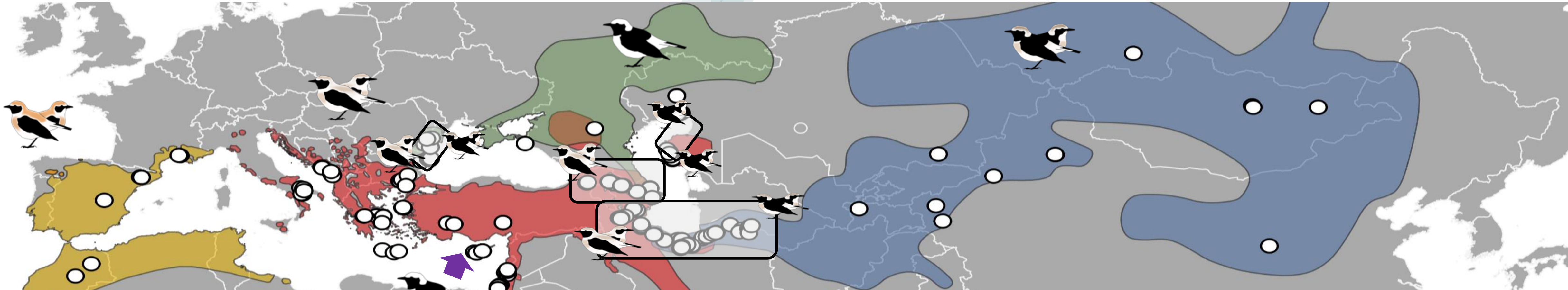
Dave Lutgen

Madeline Chase

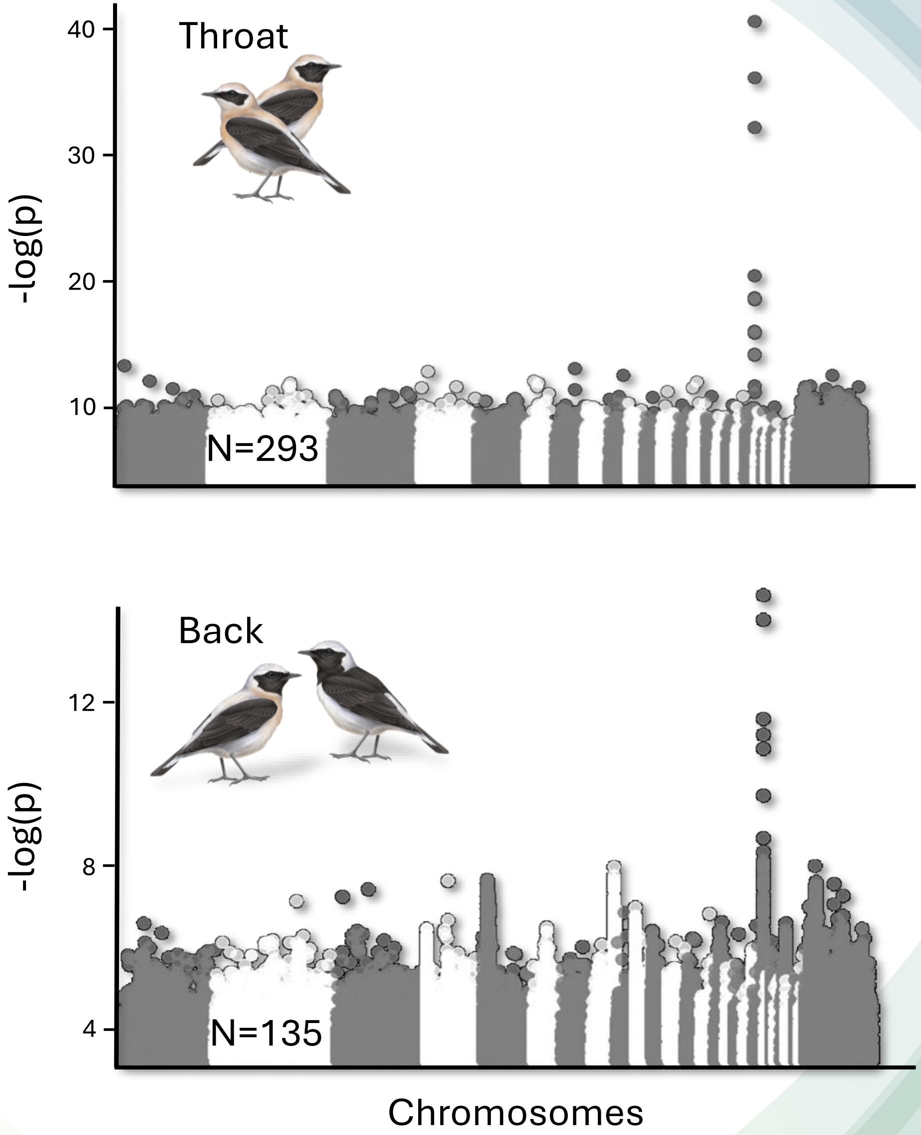
Fritjof Lammers







LTRs and coloration



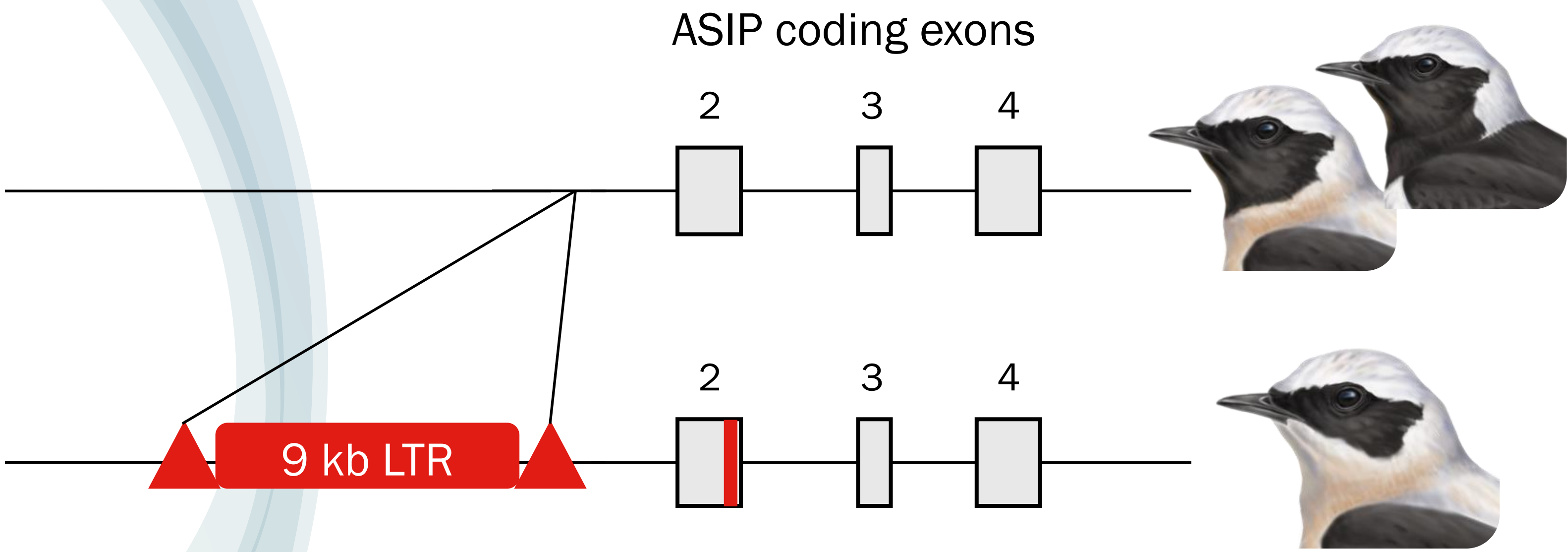
Agouti signalling protein (ASIP): 2 non-synonymous coding SNPs

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



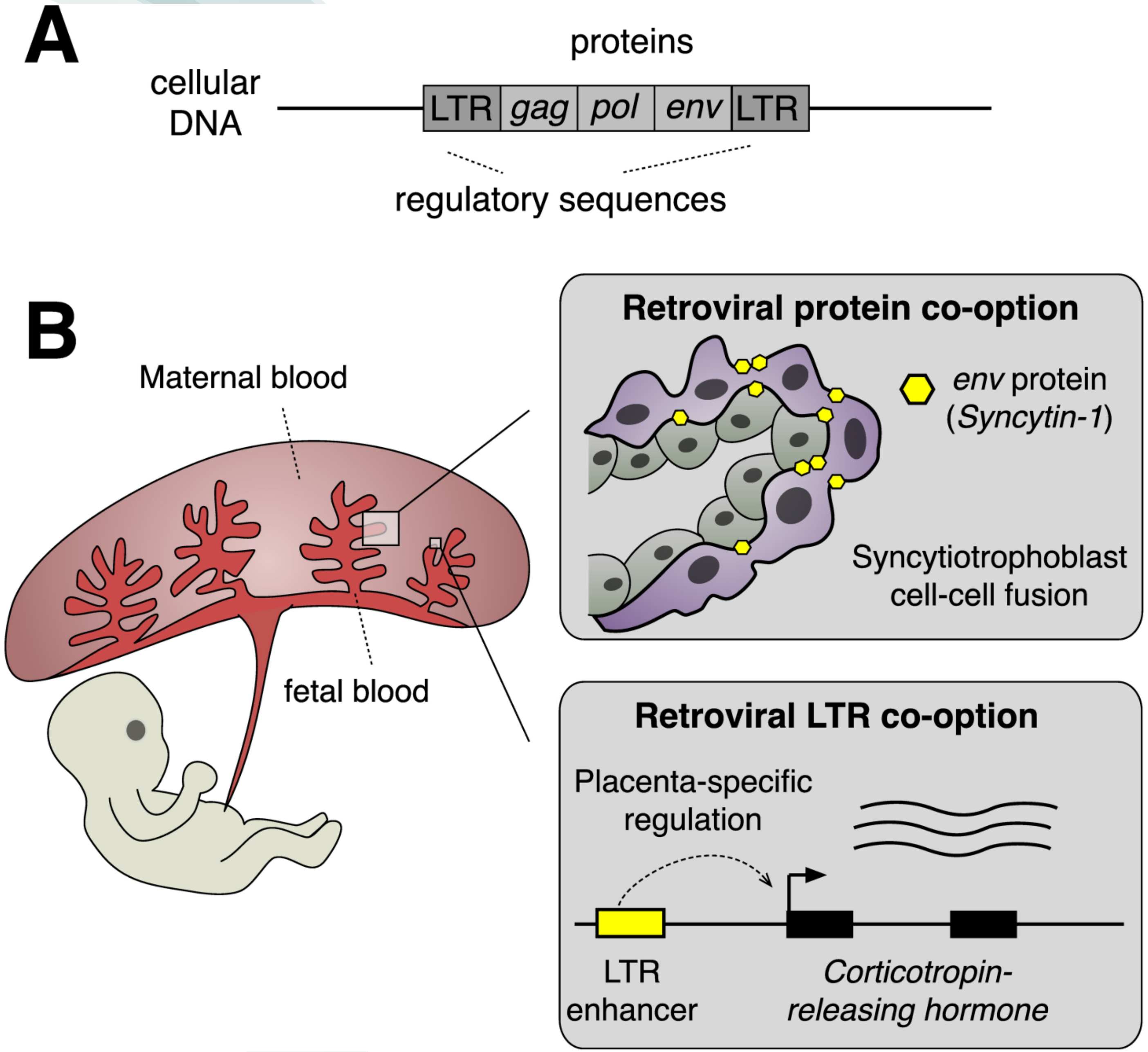
ASIP coding exons

2 3 4

9 kb LTR

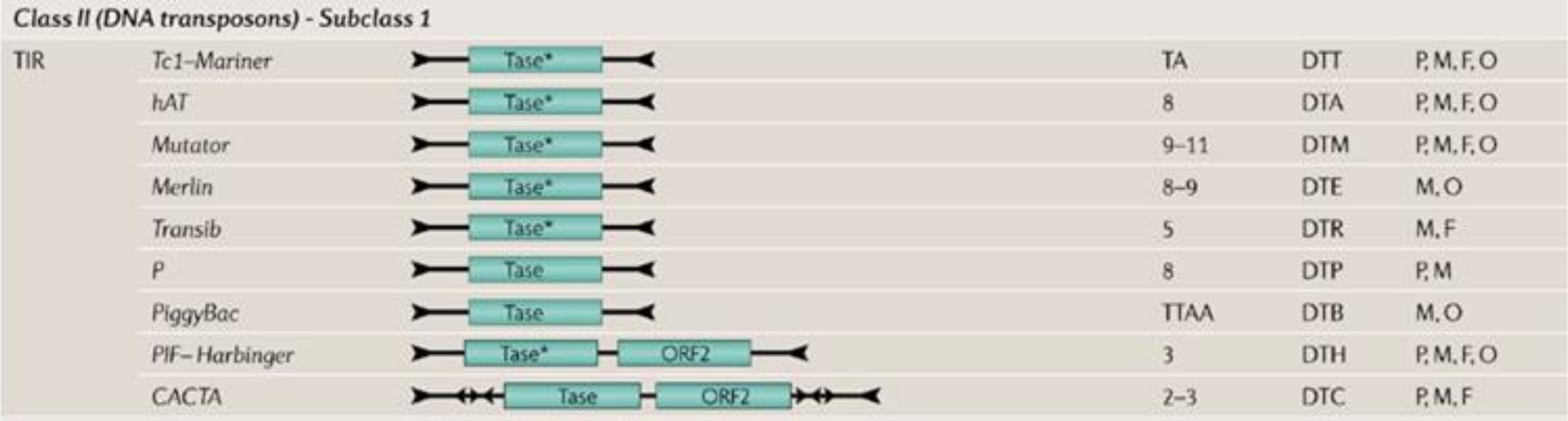
2 3 4

LTRs and placenta

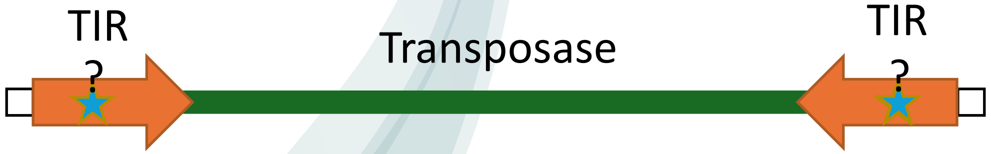


Choung 2018, PLOS Biology

Class II: DNA transposons

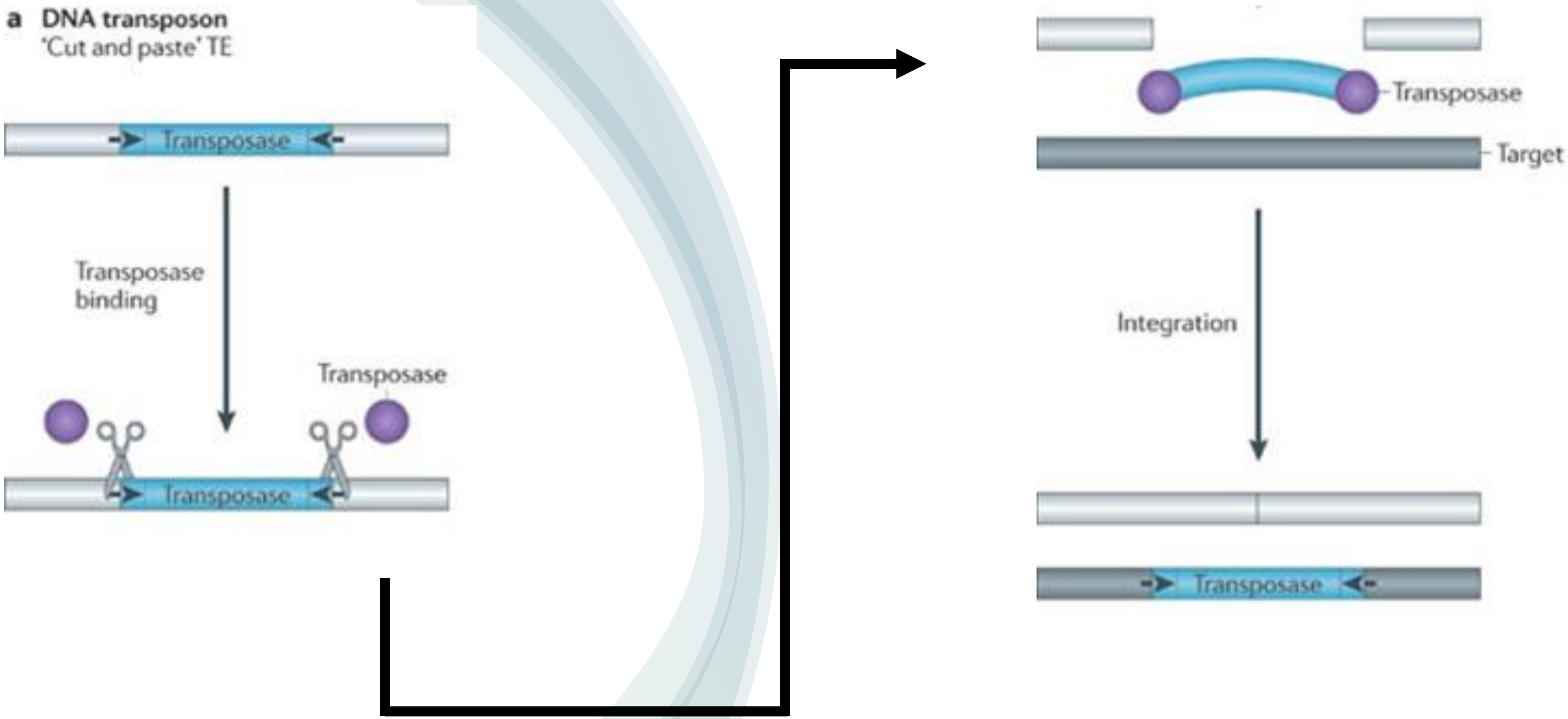


Class II (DNA transposons) - Subclass 1					
TIR	*Tc1–Mariner*	Tase*	TA	DTT	P, M, F, O
	hAT	Tase*	8	DTA	P, M, F, O
	Mutator	Tase*	9–11	DTM	P, M, F, O
	Merlin	Tase*	8–9	DTE	M, O
	Transib	Tase*	5	DTR	M, F
	P	Tase	8	DTP	P, M
	PiggyBac	Tase	TTAA	DTB	M, O
	PIF–Harbinger	Tase* ORF2	3	DTH	P, M, F, O
	CACTA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



Wicker et al 2007, *Nat Rev Gen*

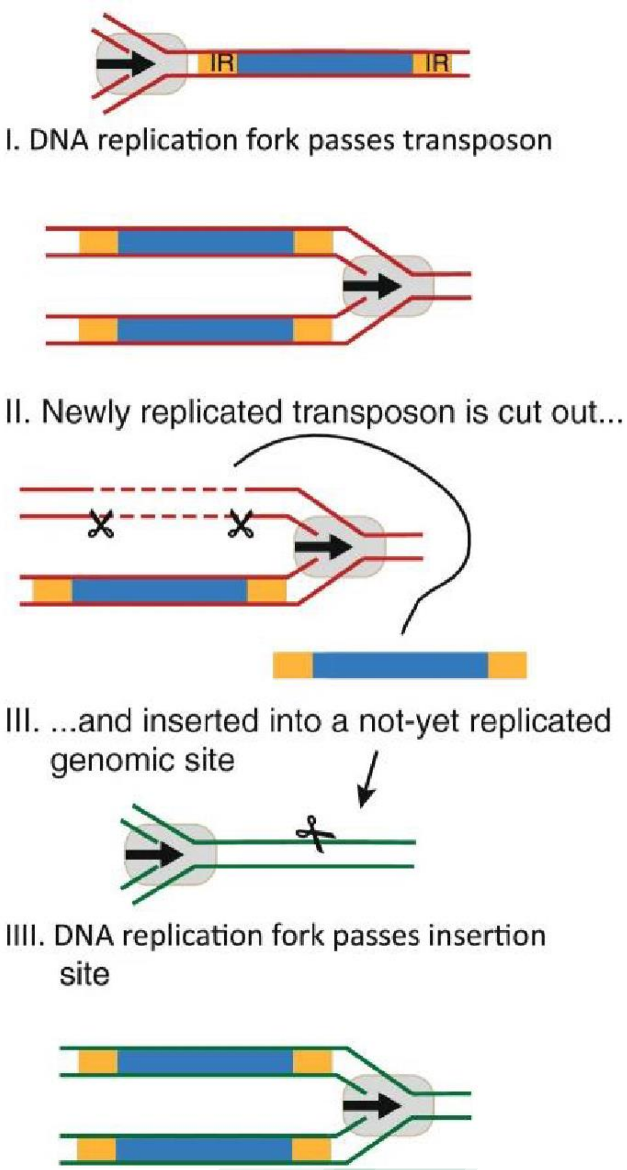
Cut and paste transposition (TIRs)



Excision

Levin and Moran 2011, *Nat Rev Gen*

How to increase in copy number?

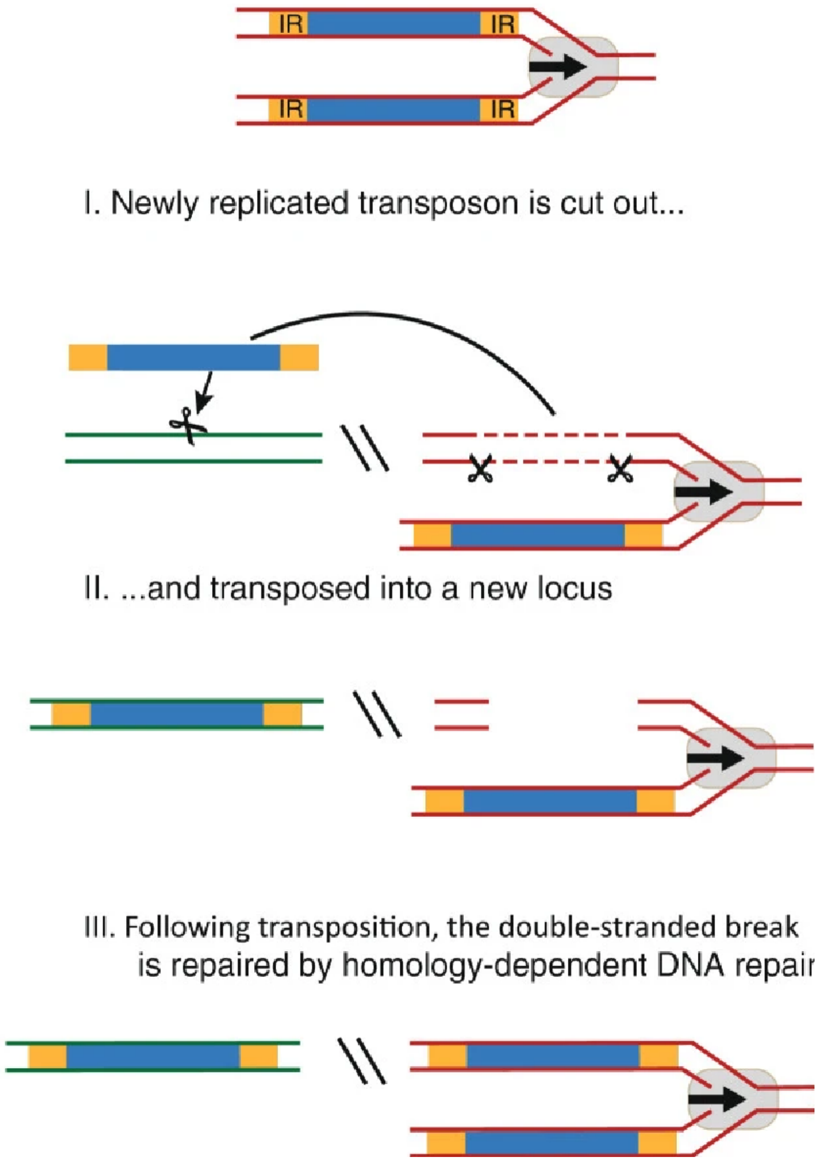


I. DNA replication fork passes transposon

II. Newly replicated transposon is cut out...

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

IIII. DNA replication fork passes insertion site



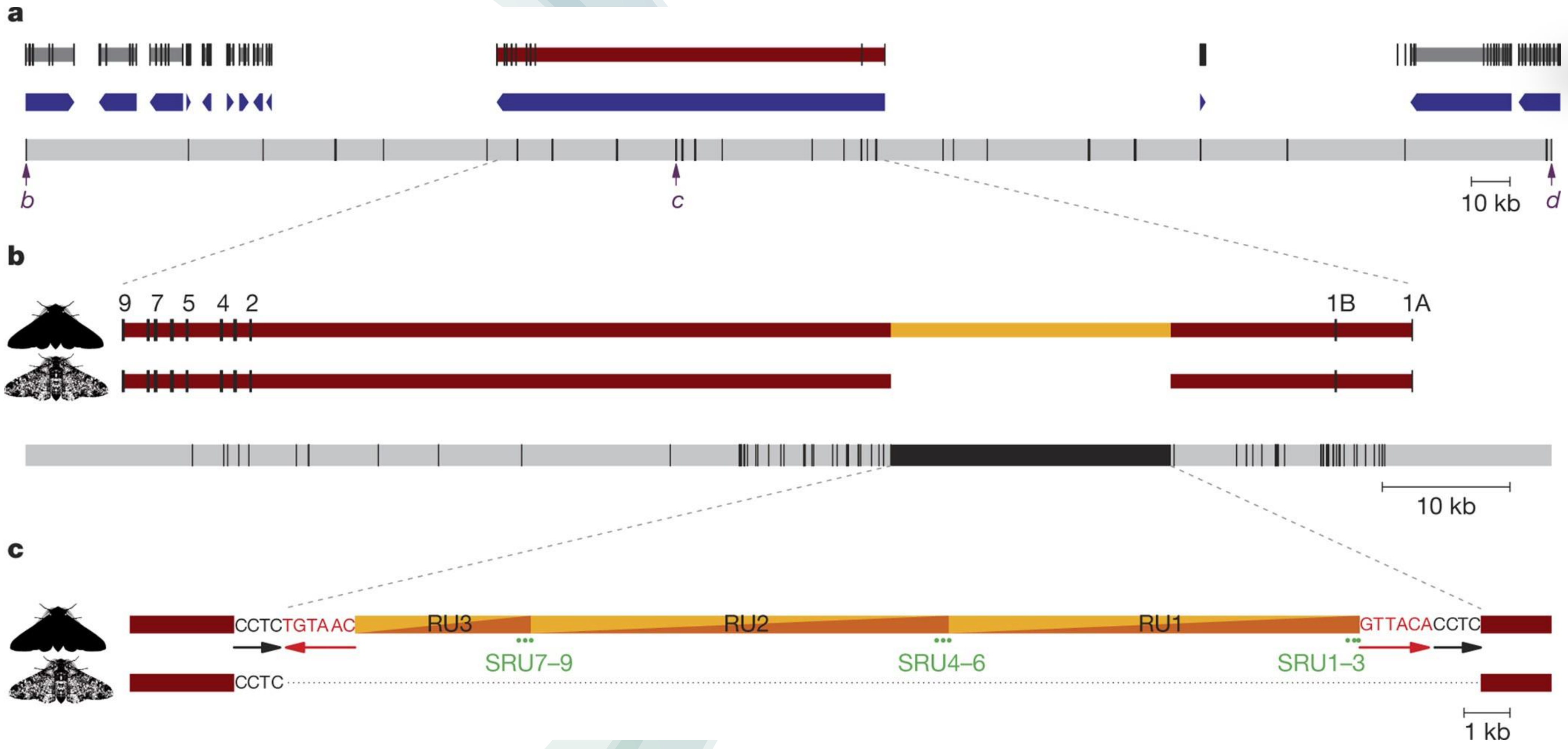
I. Newly replicated transposon is cut out...

II. ...and transposed into a new locus

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

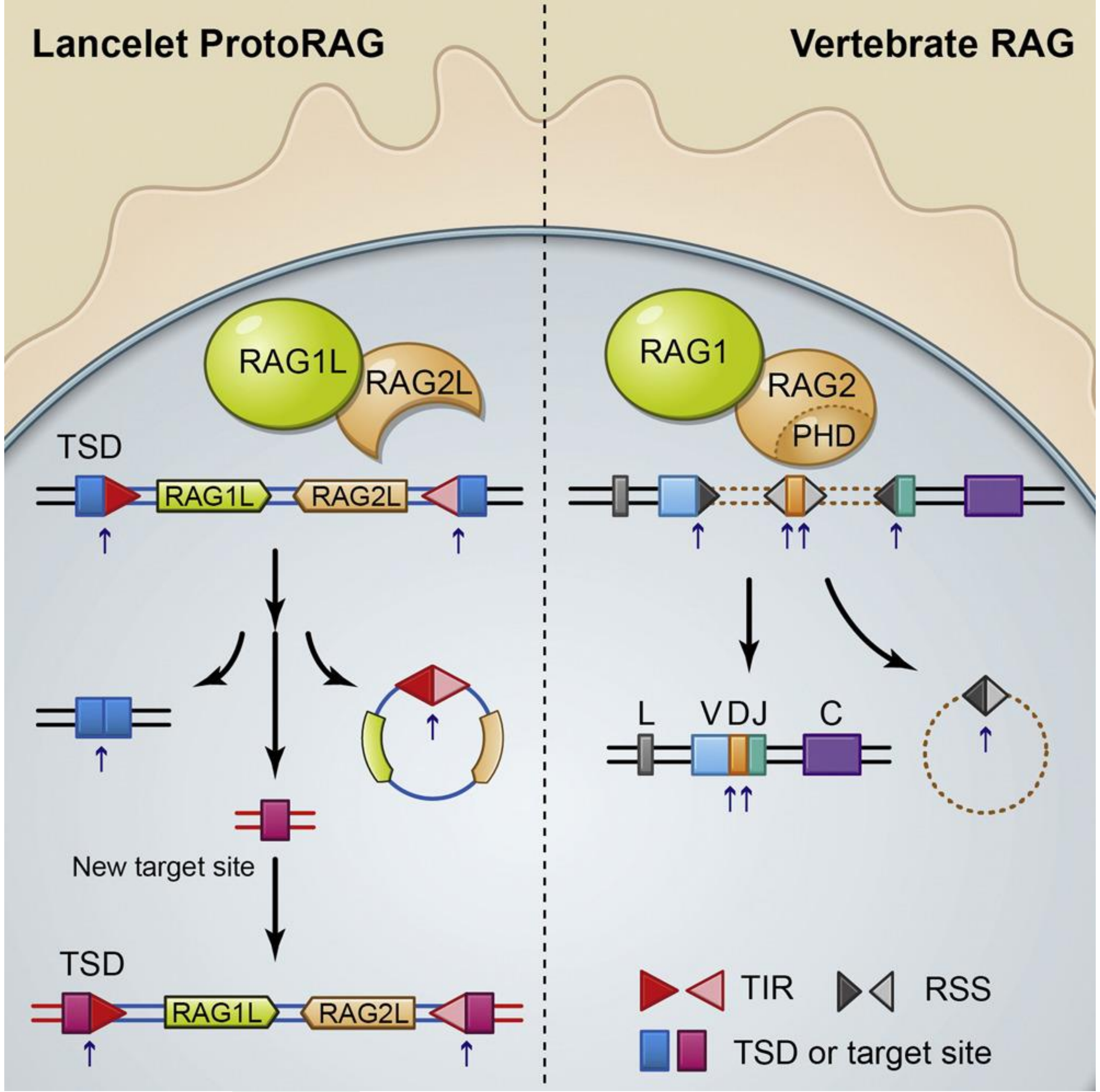
Skipper et al. 2013, *J. Biomed. Sci.*

DNA transposons and coloration



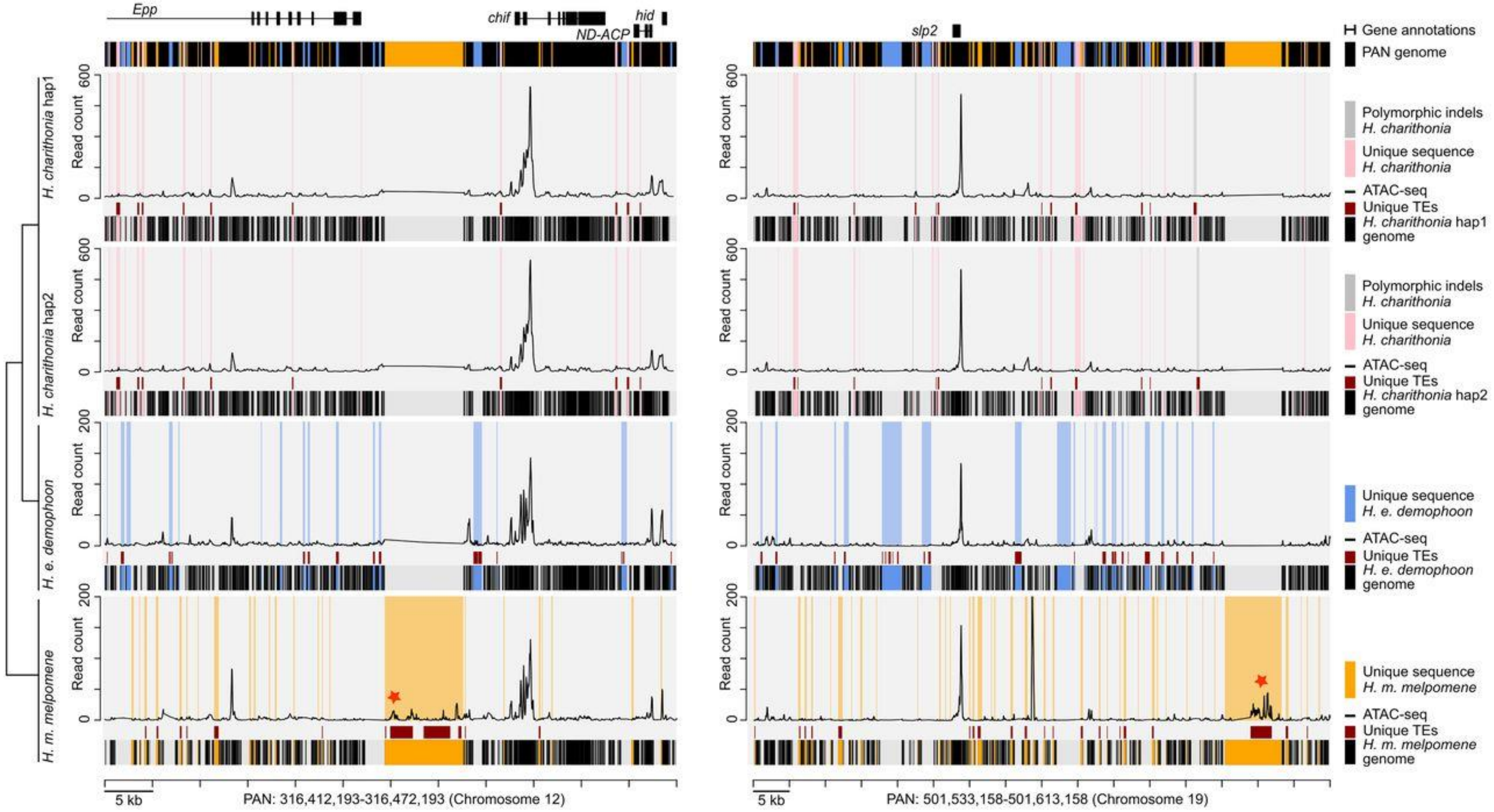
Choung 2018, PLOS Biology

DNA transposons and immune system



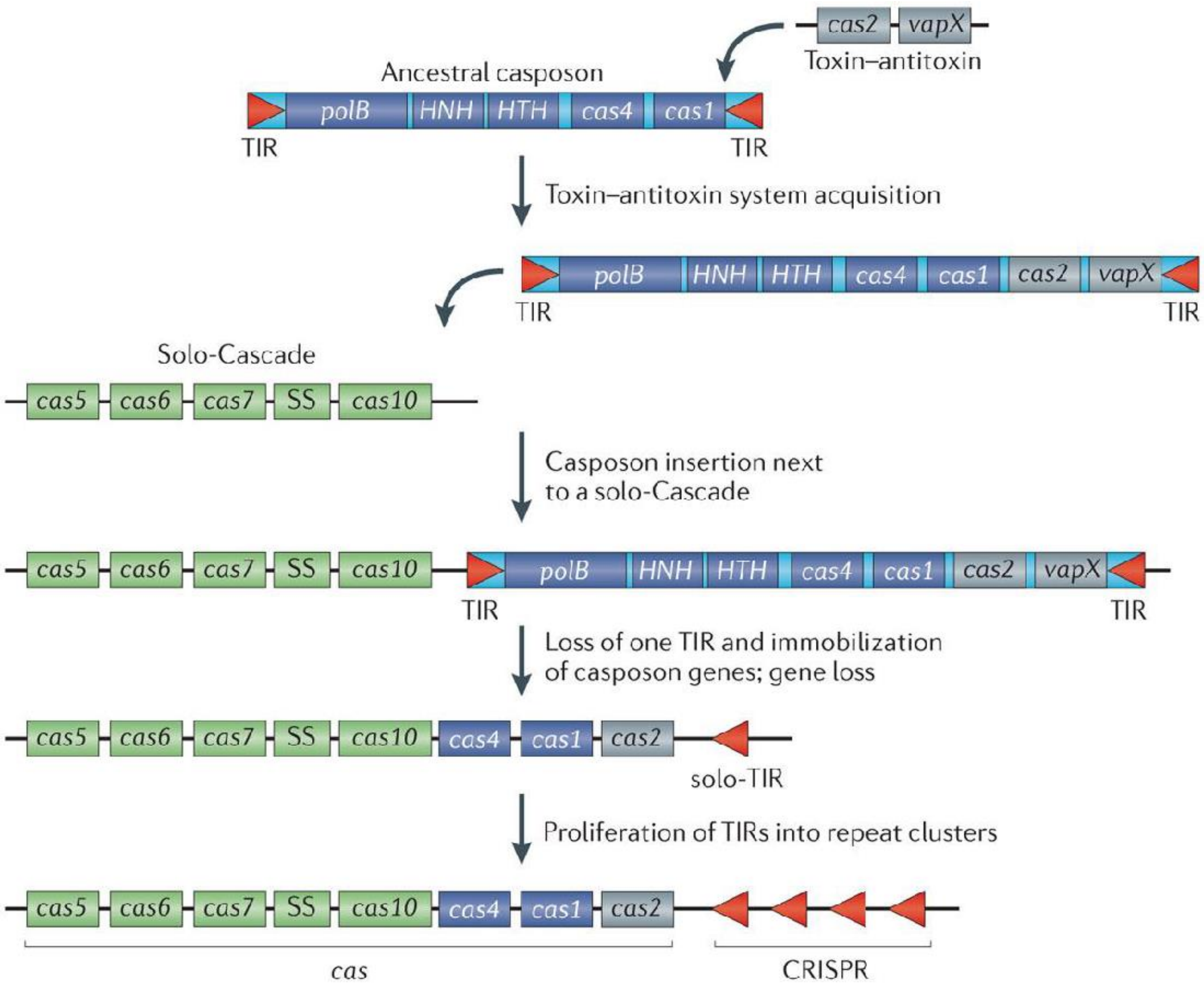
Huang et al. 2016, Cell

ATAC (Assay for Transposase-Accessible Chromatin)



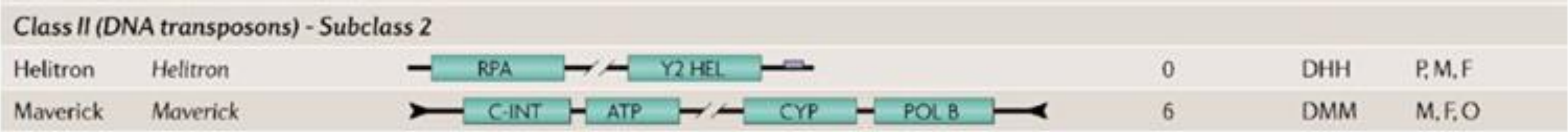
https://github.com/francicco/ComparativeGenomicsLab/blob/main/PartIII/IndentificationConservedElements.md#part-iiib-atac-peak-enrichment

CRISPR-Cas and transposons

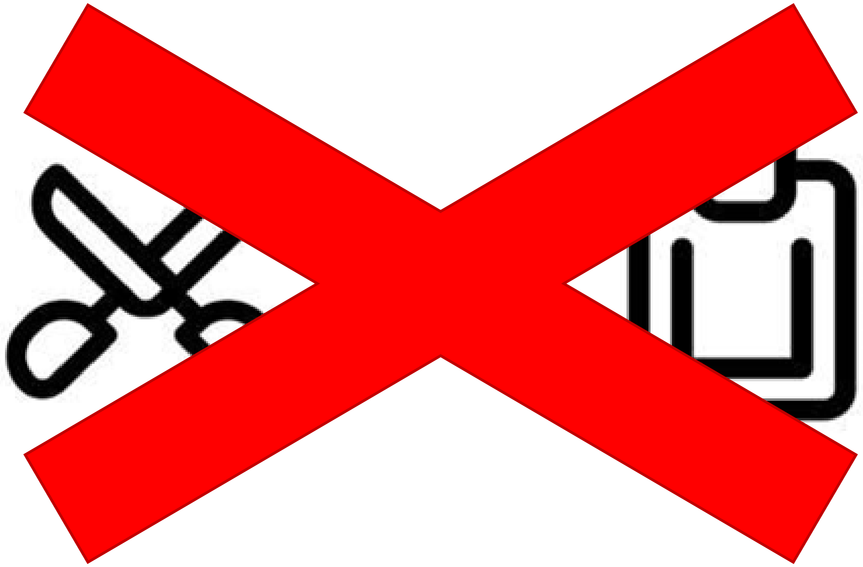


Koonin and Krupovic 2015, *Nat Rev Genet*

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

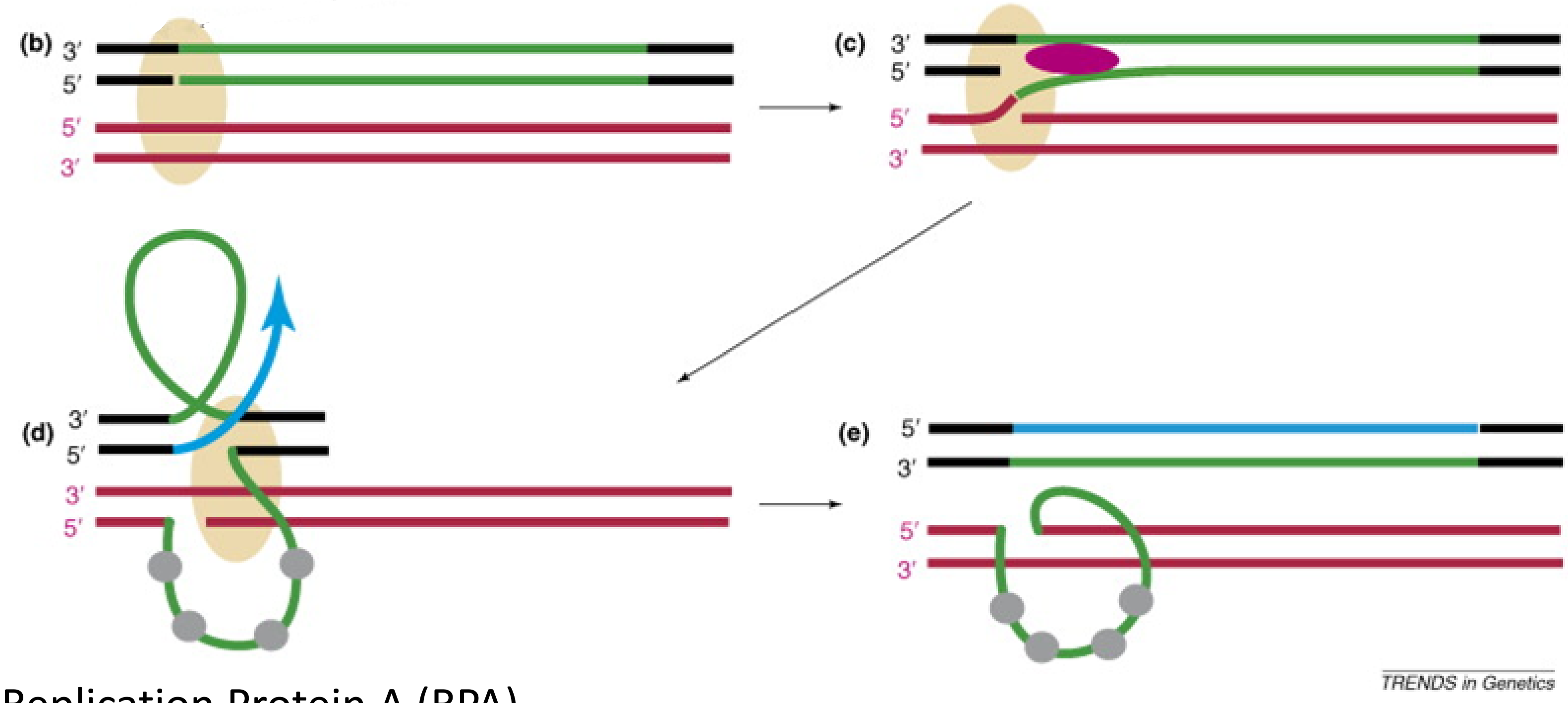


Wicker et al 2007, *Nat Rev Gen*

Rolling circle transposition: Helitrons

Replicase

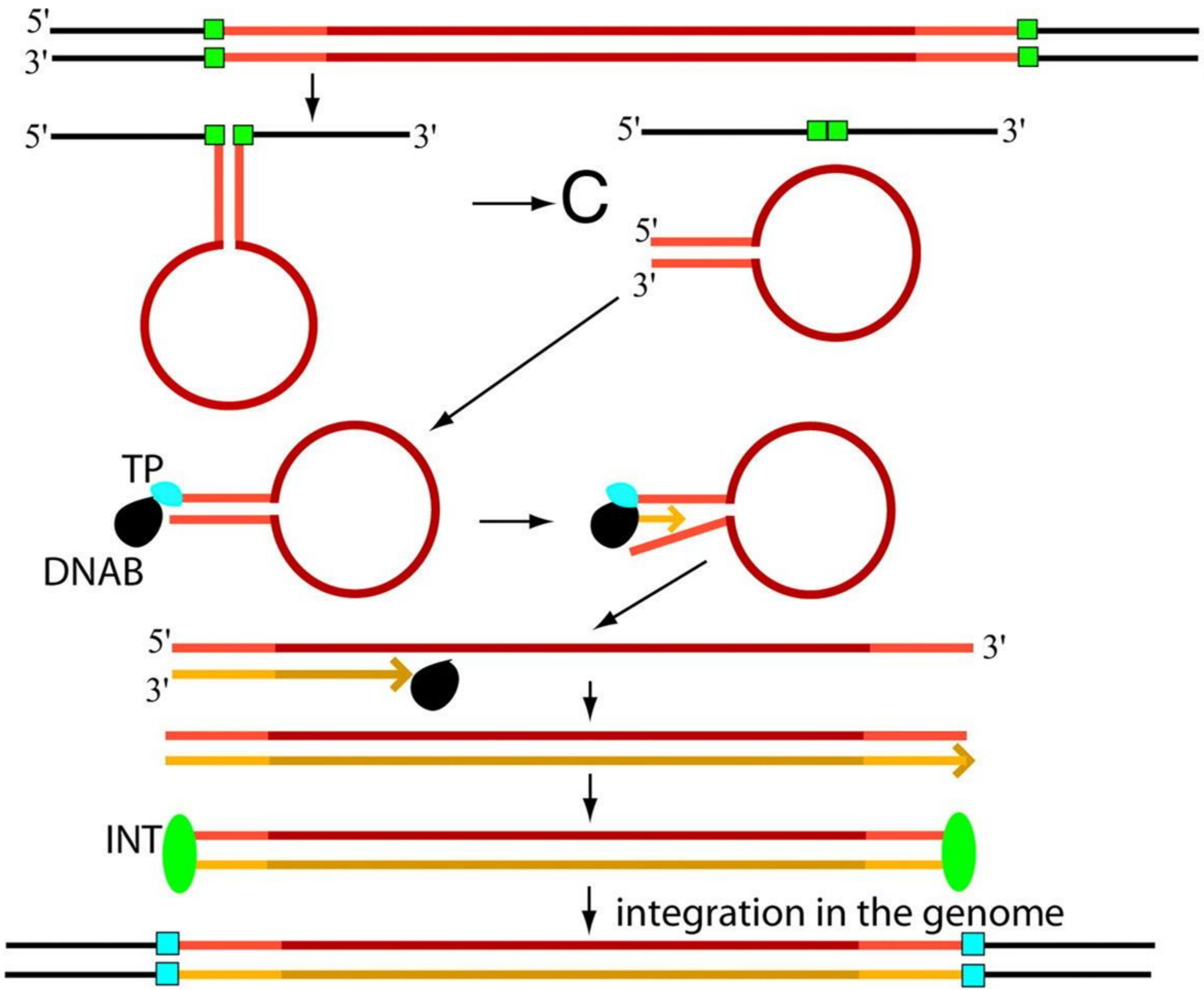
Helicase



Replication Protein A (RPA)

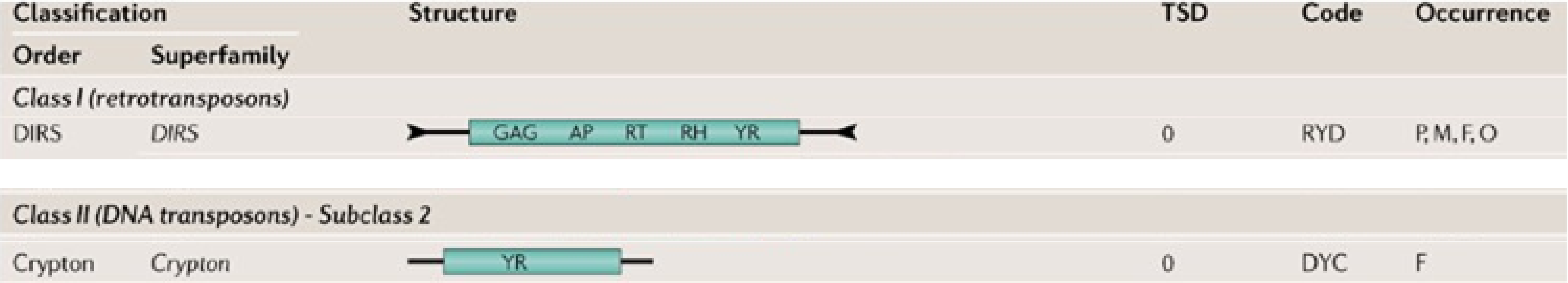
Kapitonov and Jurka 2007, *Trends Genet*

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	GAG AP RT RH YR	0	RYD	P, M, F, O
Class II (DNA transposons) - Subclass 2					
Crypton	Crypton	YR	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

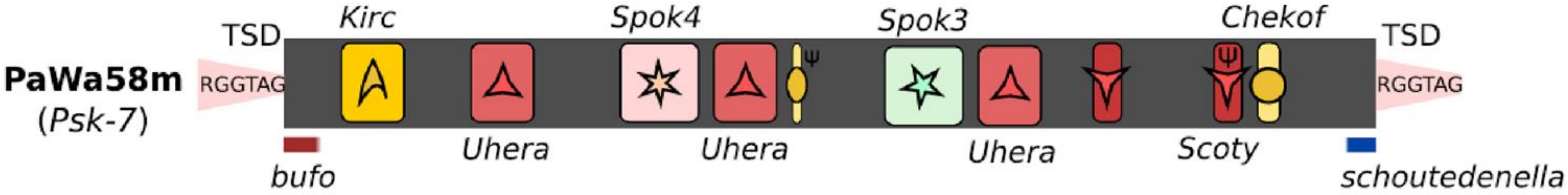
Class I: retrotransposons
Class II: DNA transposons

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*)

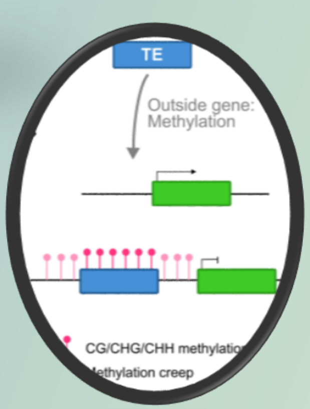


Vogan et al. 2021, *Genome Res.*

TEs as dynamic mutations

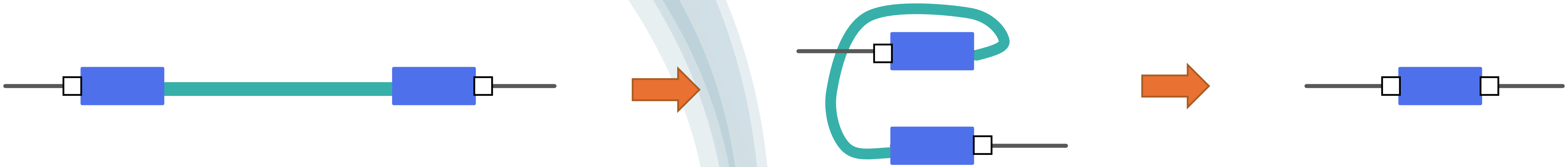
- NAHR

- Methylation



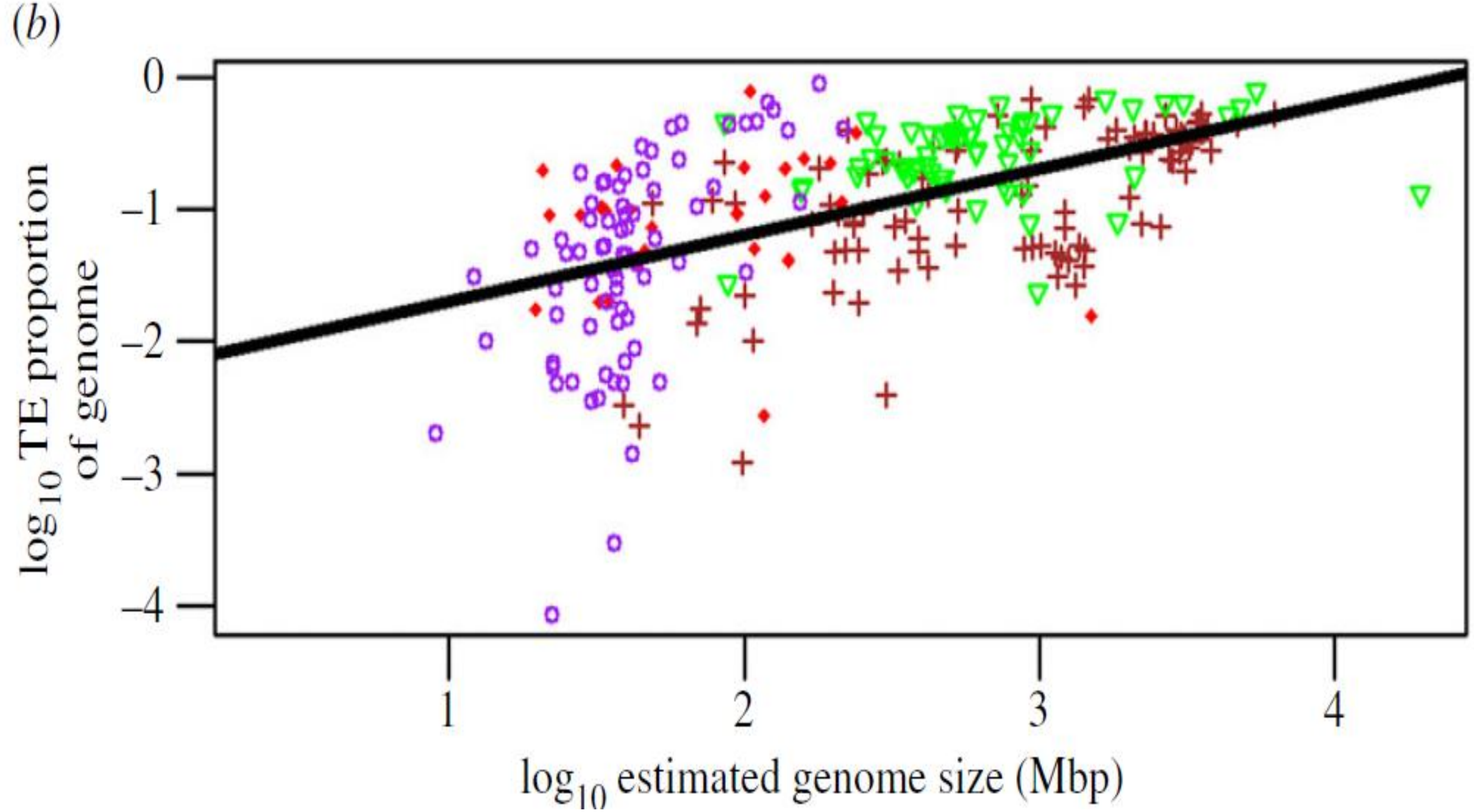
Non-allelic homologous recombination NAHR

Full-length LTR → solo LTR



- Larger scale deletions and copy number variation
- Inversions

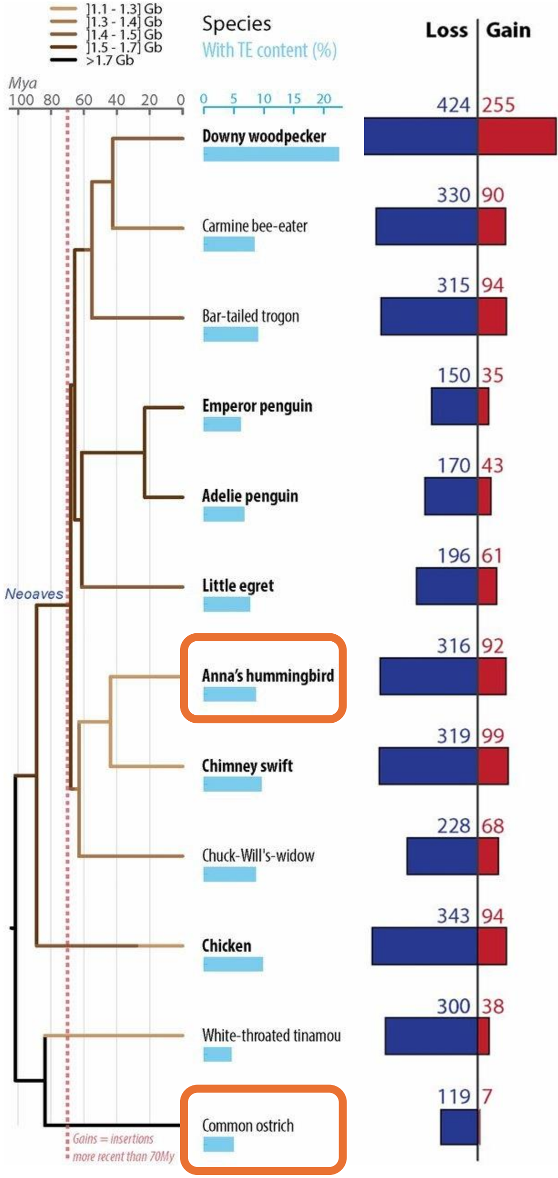
Genome size evolution



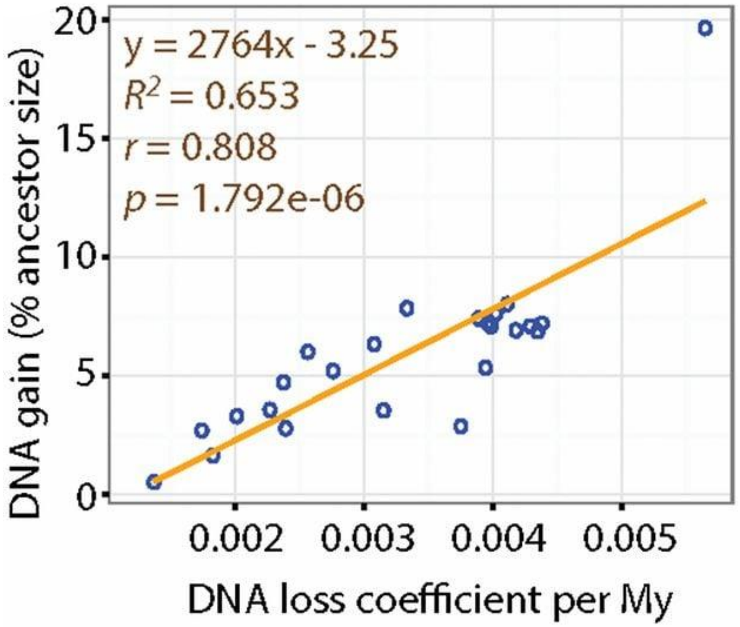
Elliot and Gregory, 2015 *Phil Trans B*

Genome size evolution

Accordion model



No constraint



Consider not only host popgen, but also TE popgen!

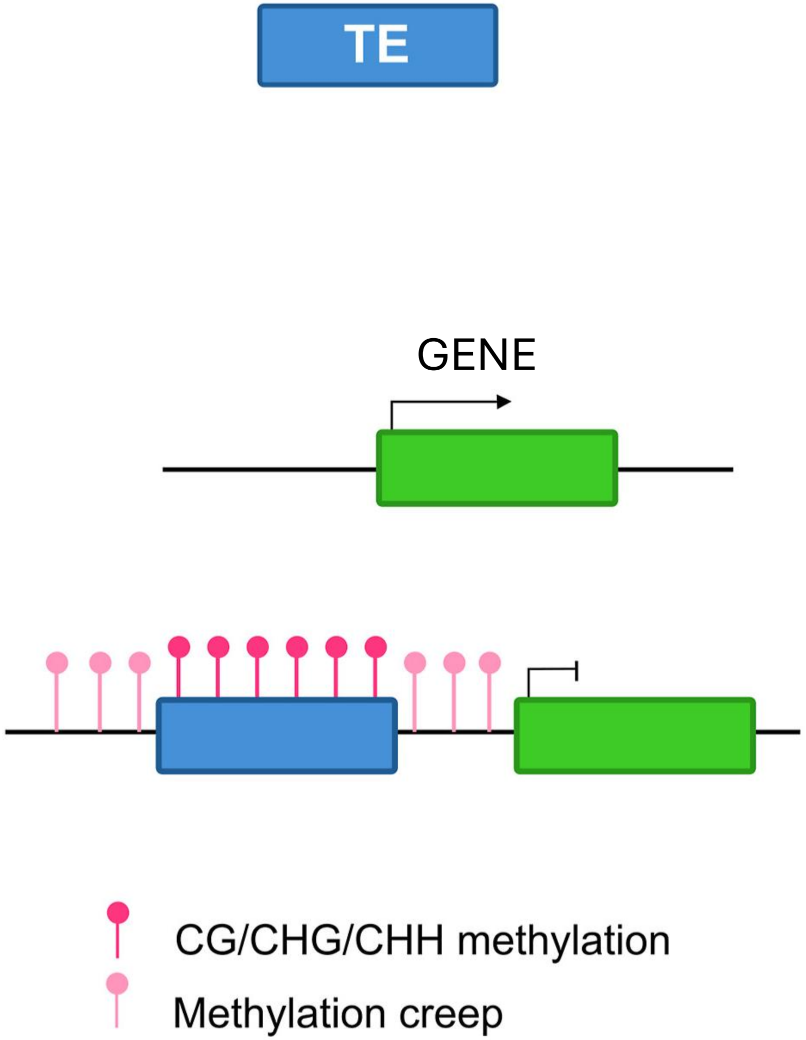
Need to clean up their genomes

VS

genome structure reflects popgen of the host and TEs

Kapusta et al 2017, *PNAS*

Methylation spillover

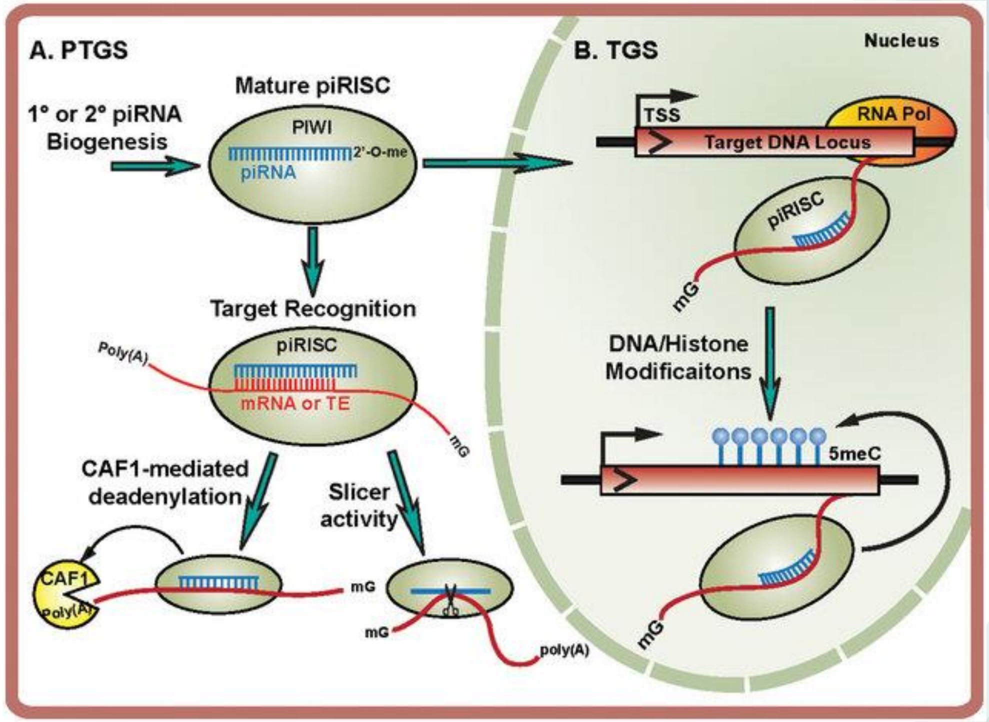


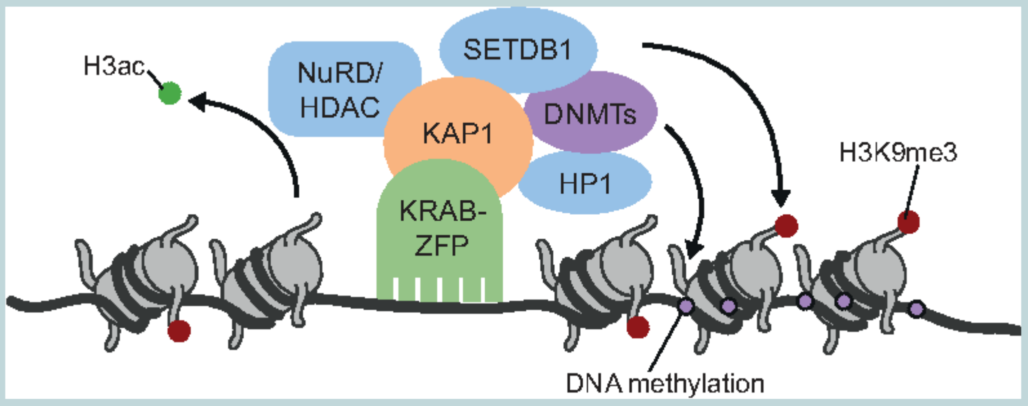
Anderson et al. 2022, Current Opinion in Plant Biology

Silencing mechanisms

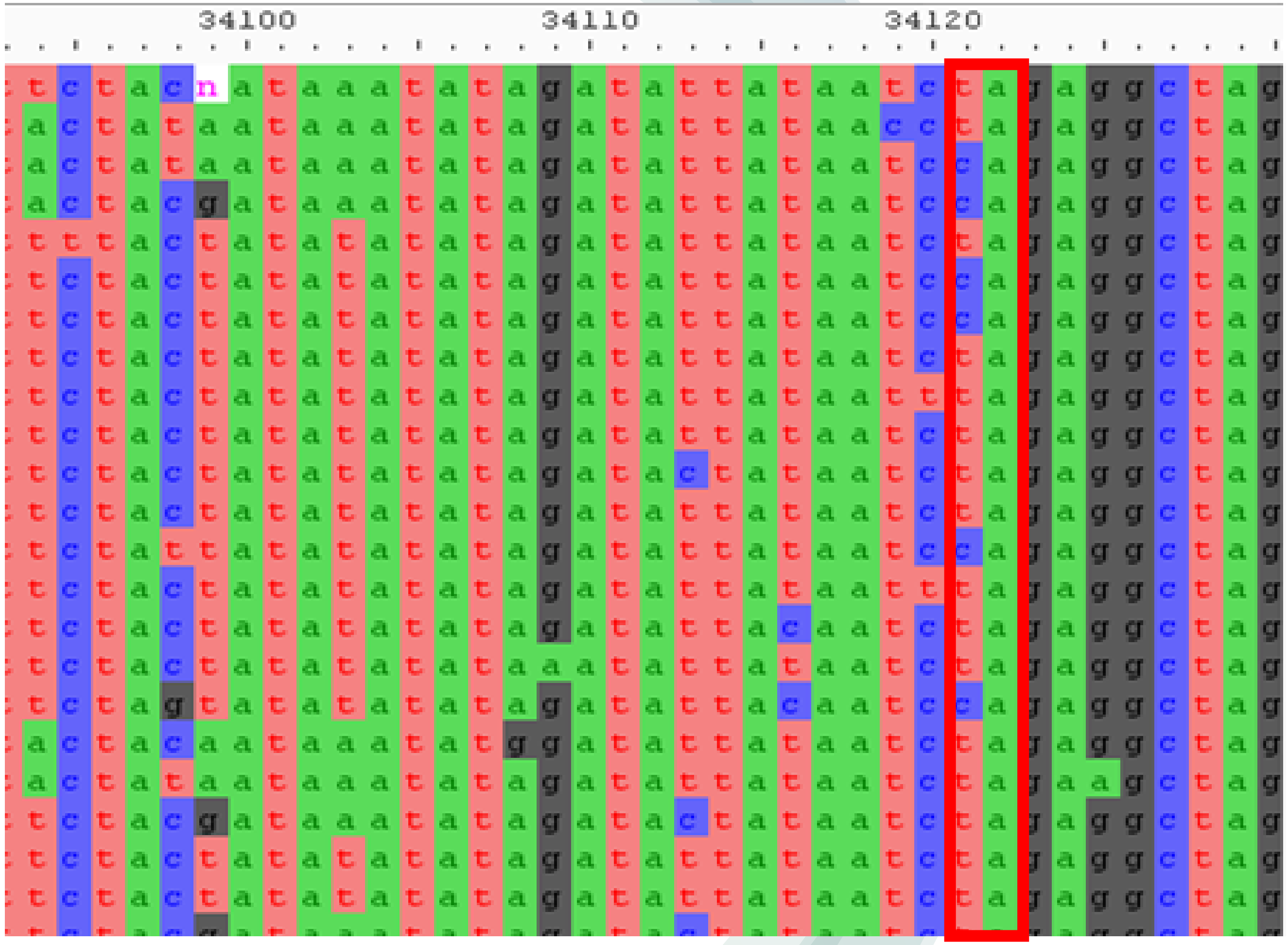
- DNA level
- Post-transcriptional level

DNA methylation, KRAB and PIWI





Repeat induced point mutation (RIP)



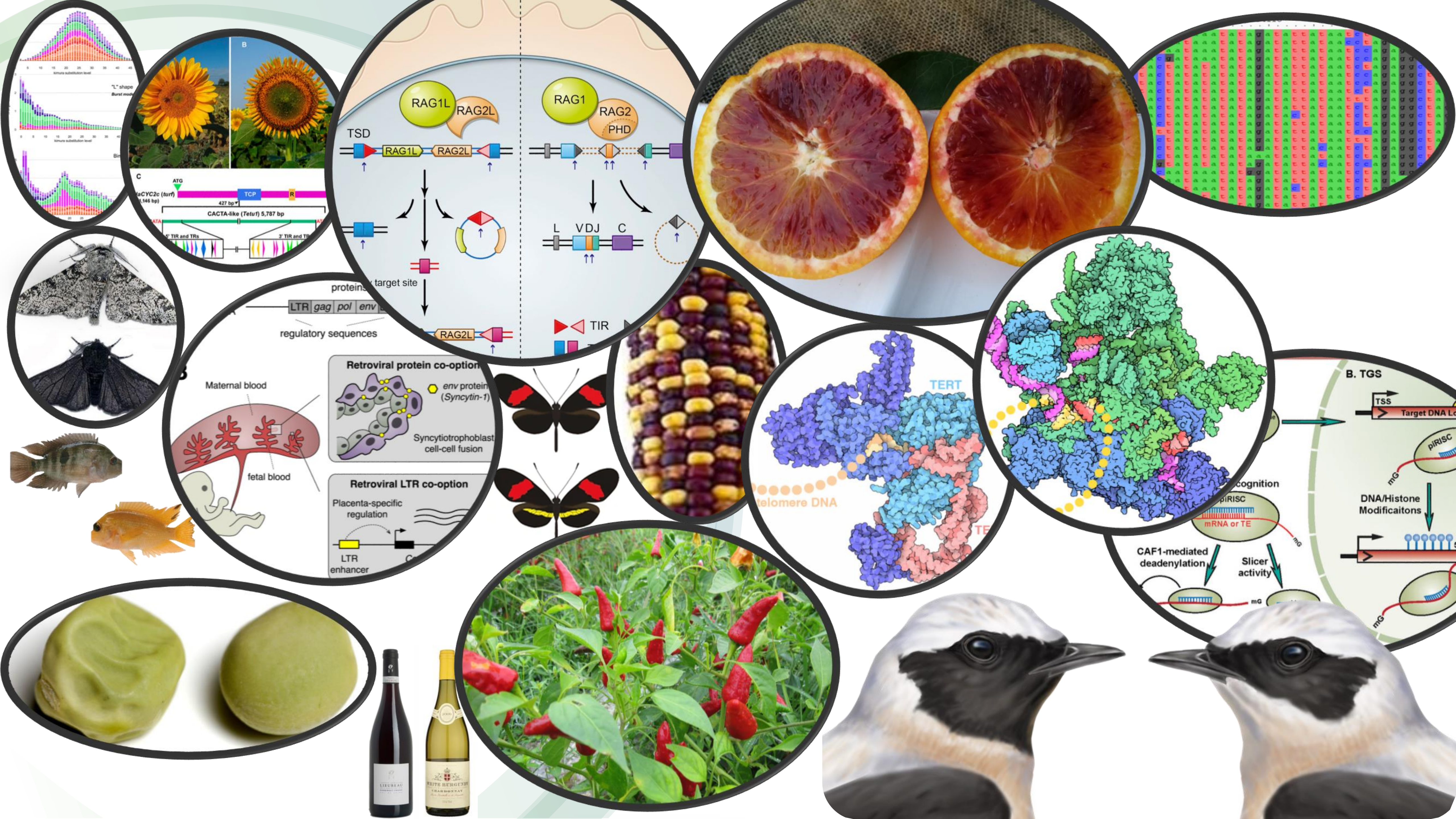
C -> T mutations
In *Neurospora*, mutations that are induced by RIP occur preferentially in CpA dinucleotides

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

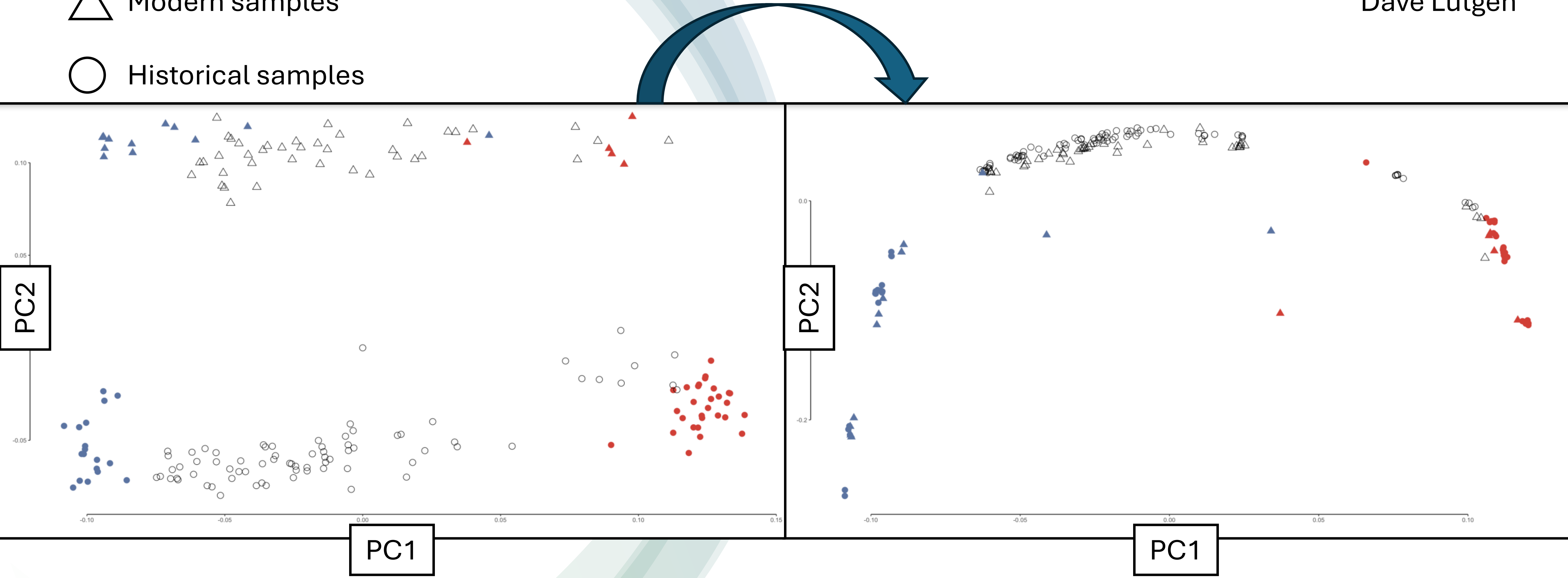

"L" shape
Burst mode
kimura substitution level
B
C
ATG
HaCYC2c (turf)
TCP
R
427 bp
CACTA-like (Tetuf) 5,787 bp
5' TIR and TRs
3' TIR and TRs
RAG1L
RAG2L
TSD
RAG1L
RAG2L
target site
RAG2L
RAG1
RAG2
PHD
L
VDJ
C
TIR
proteins
LTR
gag
pol
env
regulatory sequences
Maternal blood
fetal blood
Retroviral protein co-option
env protein
(Syncytin-1)
Syncytiotrophoblast
cell-cell fusion
Retroviral LTR co-option
Placenta-specific
regulation
LTR
enhancer
TERT
telomere DNA
B. TGS
TSS
Target DNA Lo
piRISC
mG
DNA/Histone
Modificaitons
cognition
piRISC
mRNA or TE
CAF1-mediated
deadenylation
Slicer
activity
mG
LIEUBEAU
WHITE BURGUNDY
CHARDONNAY

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

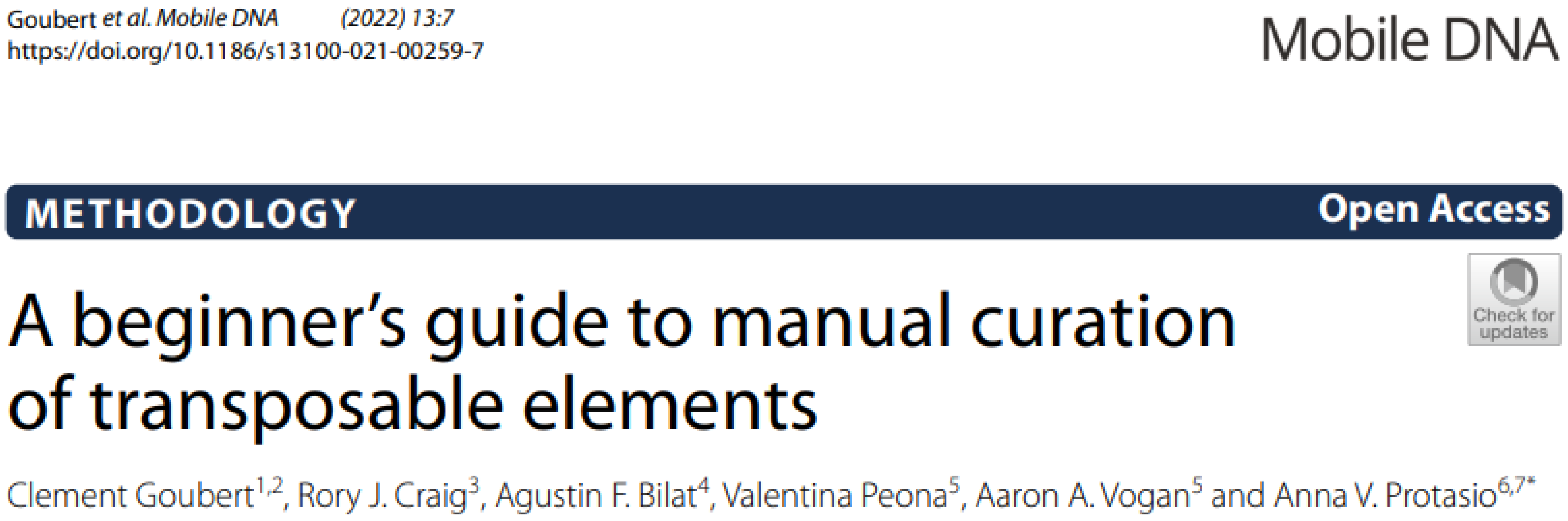
Transposable elements

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**

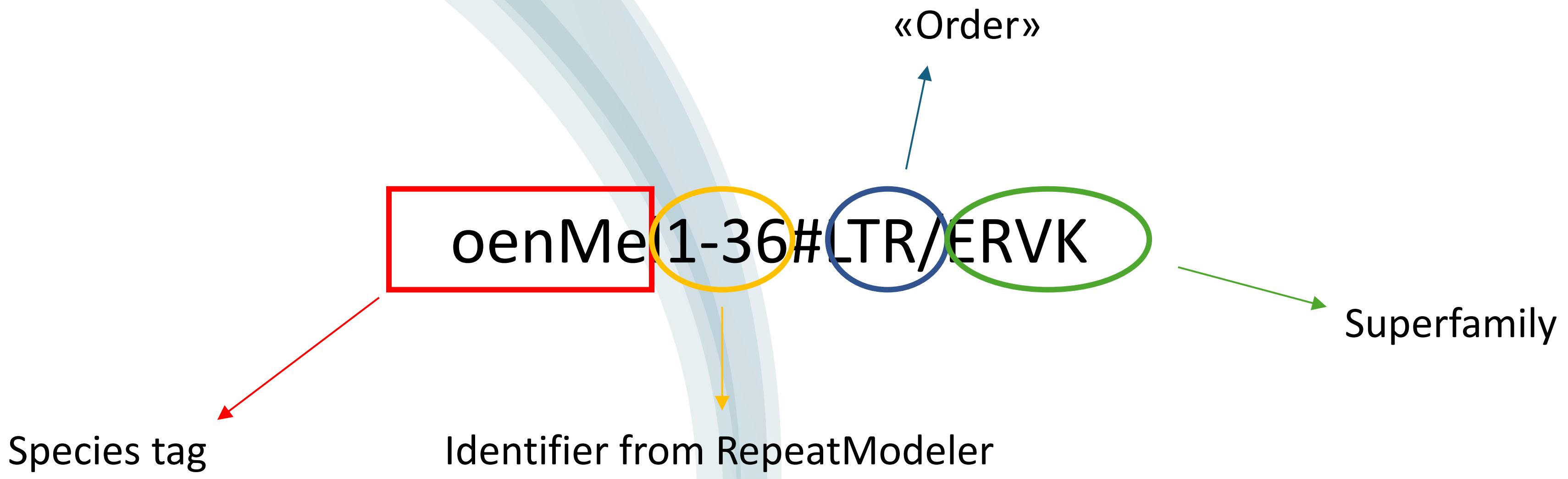
A beginner's guide to manual curation of transposable elements

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



«Order»

oenMel1-36#LTR/ERVK

Superfamily

Species tag

Identifier from RepeatModeler

Other examples

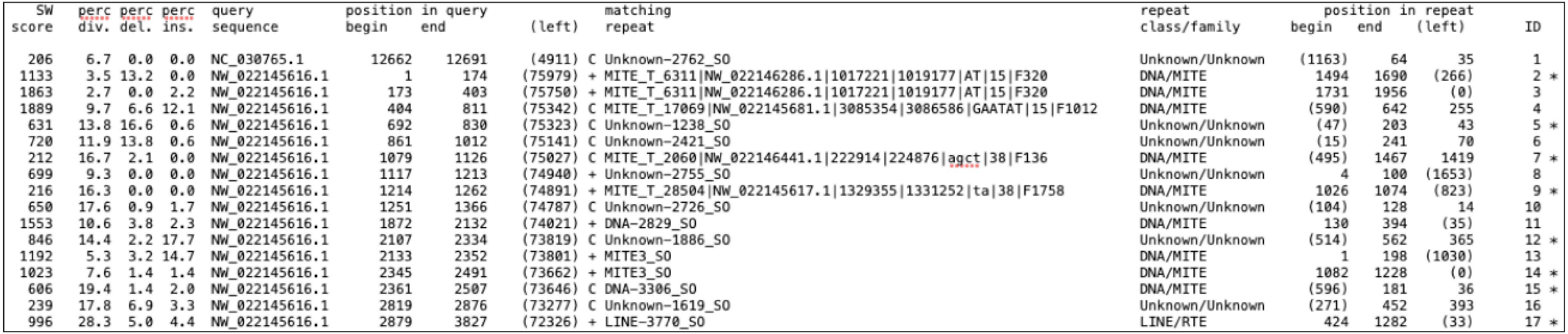
oenMel1-36**_LTR**#LTR/ERVK

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

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

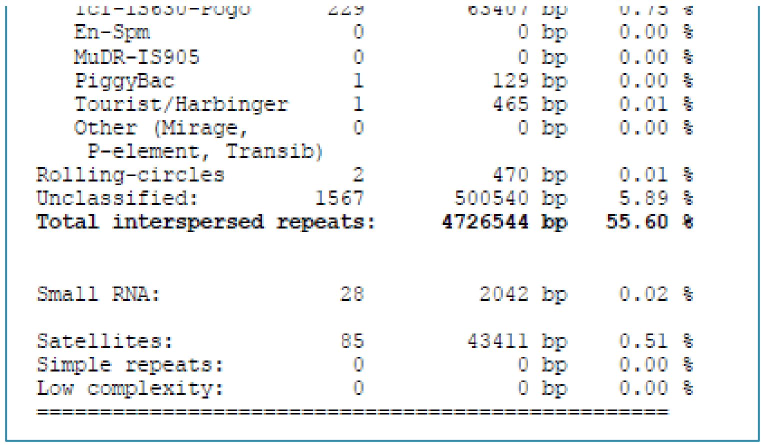
TE annotation

Genome.fasta.out (main output)



```
   SW   perc perc perc  query             position in query            matching                                                      repeat              position in repeat
score   div. del. ins.  sequence          begin     end        (left)    repeat                                                        class/family        begin   end    (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 *
```

Genome.fasta.tbl



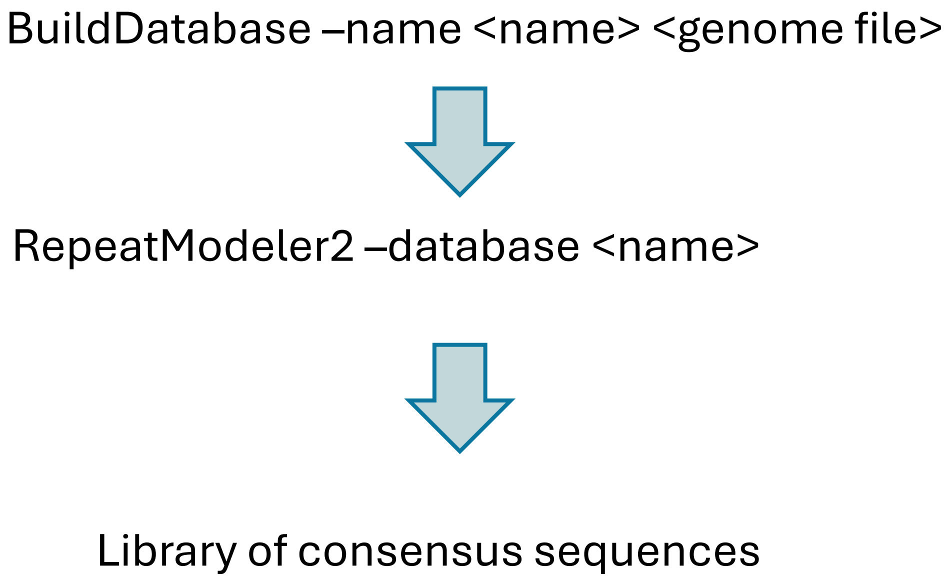
```
==================================================
    Tc1-IS630-Pogo        229       63407 bp    0.75 %
    En-Spm                  0           0 bp    0.00 %
    MuDR-IS905              0           0 bp    0.00 %
    PiggyBac                1         129 bp    0.00 %
    Tourist/Harbinger       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
```

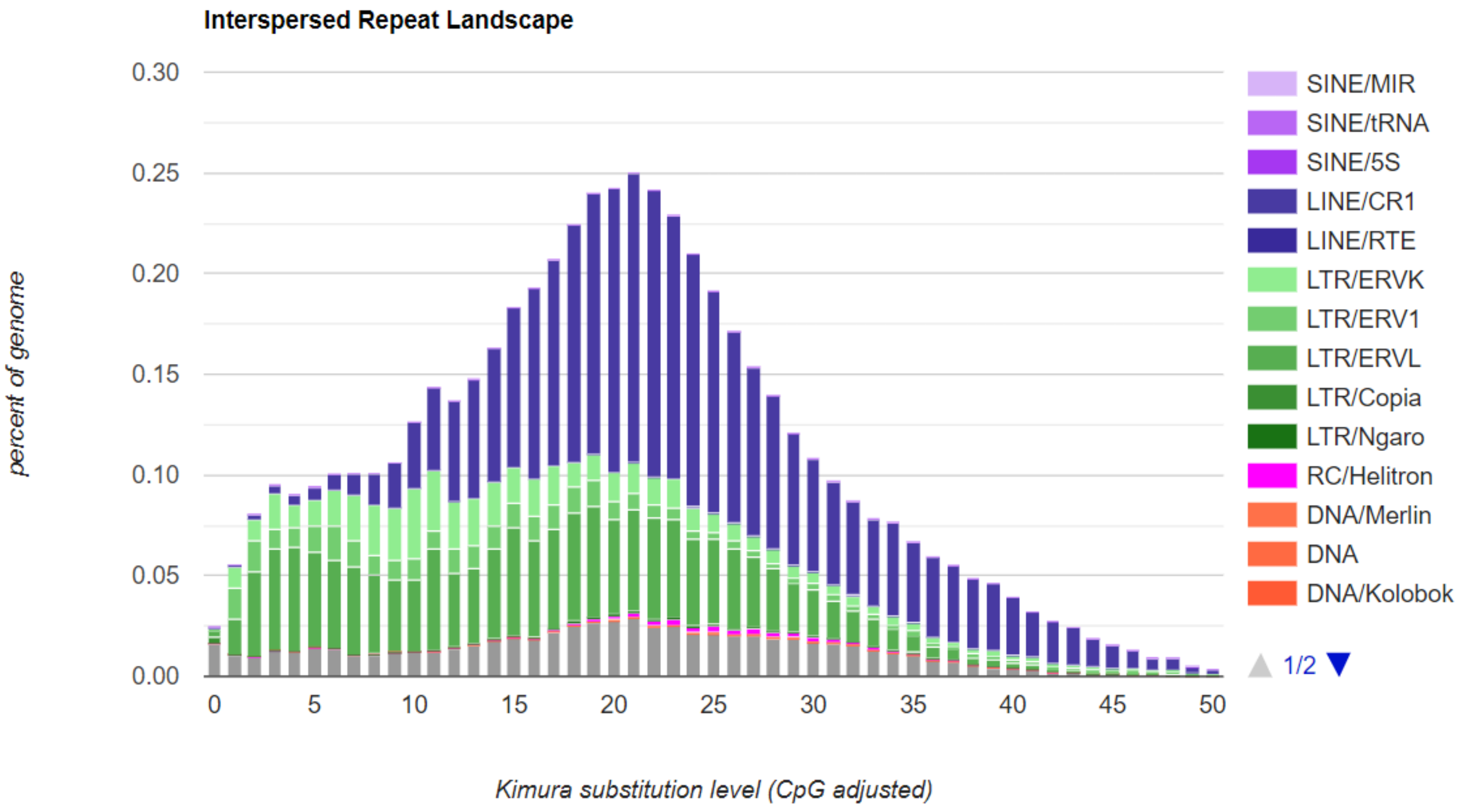
RepeatMasker –lib <library> <genome file>

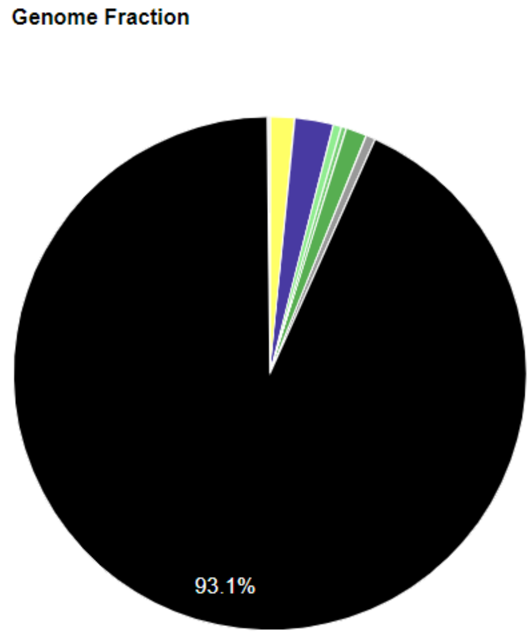
.out + .gff files with coordinates of repeats

Repeat landscape

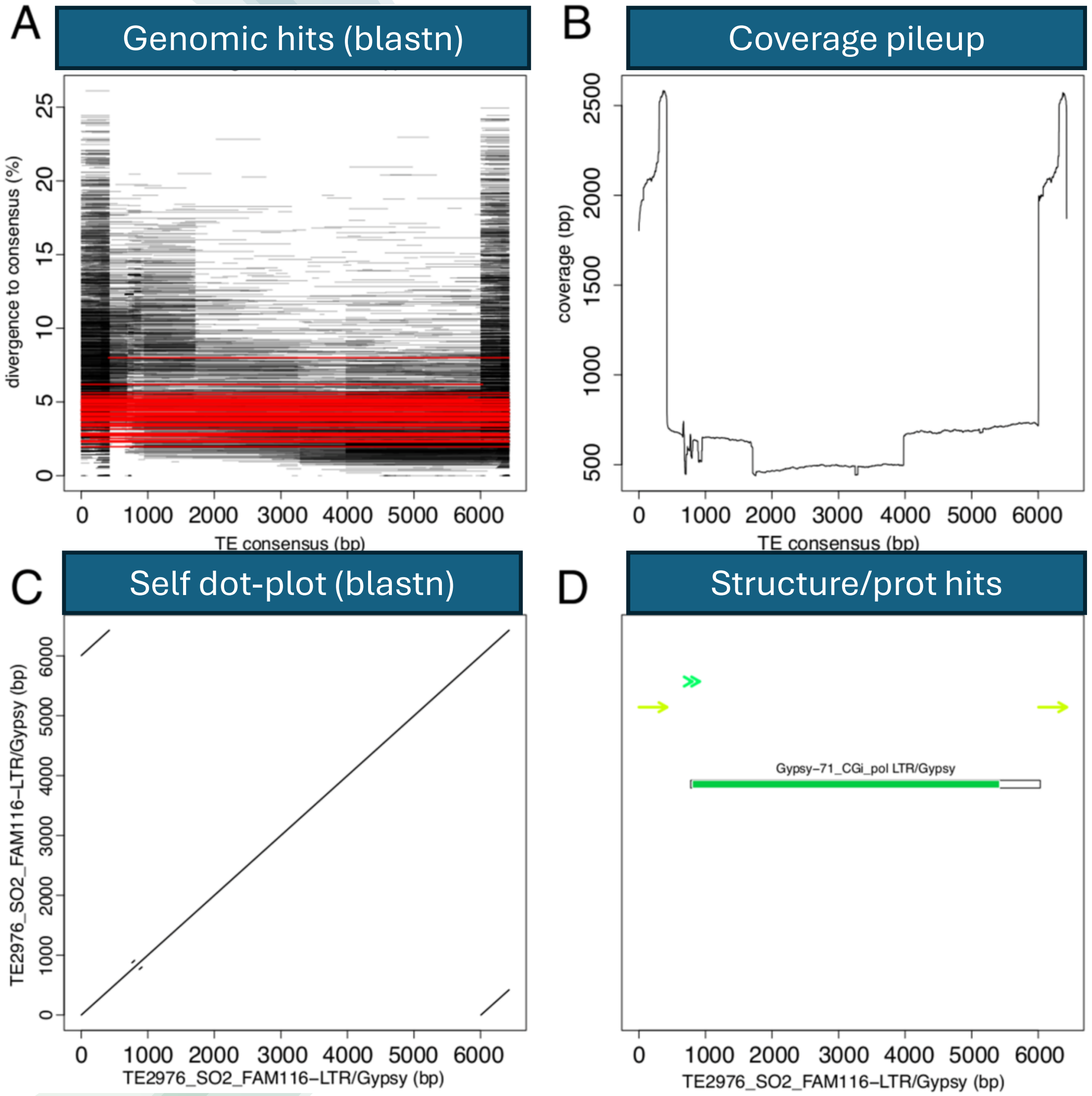
Use outputs from RepeaMasker to visualise the repetitive content of the genome

- calcDivergenceFromAlig.pl
- createRepeatLandscape.pl



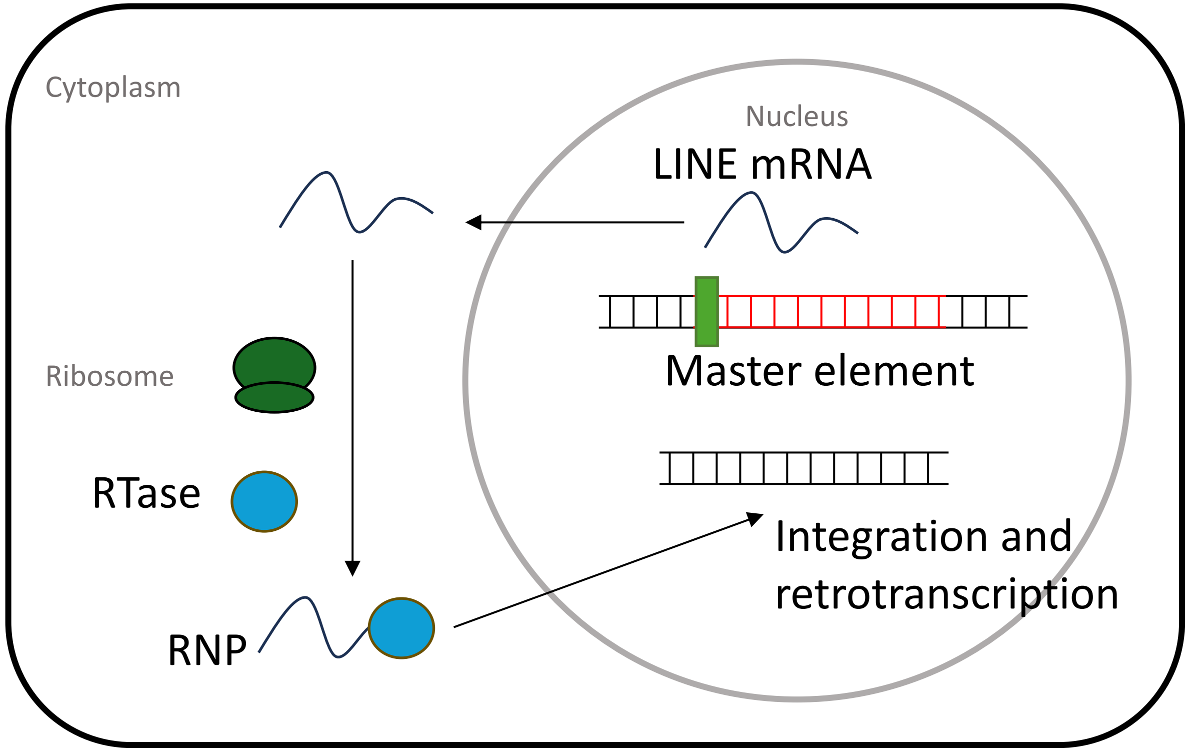


Analyse sequence characteristics of TEs



LINE retrotransposons

Where/when/how in the cell



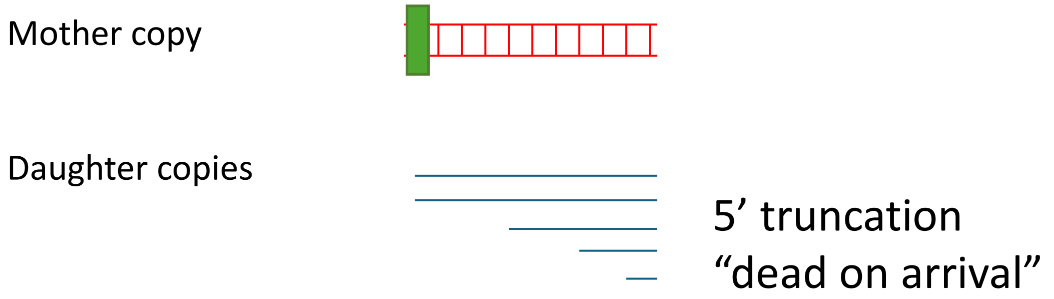
Requirements for mobility

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

Target site preference and TSD

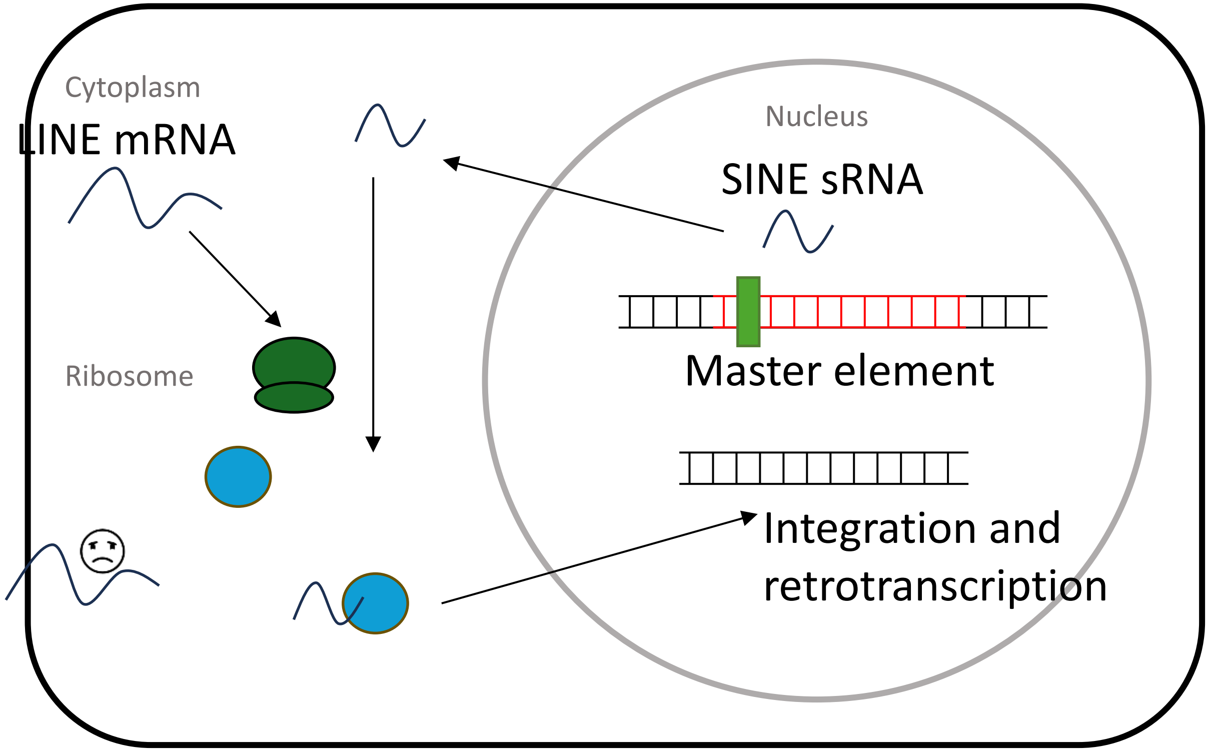
Target site preferentiality for sequences similar to the 3’ UTR
Target site duplications are of variable length

Content of new copies



SINE retrotransposons

Where/when/how in the cell



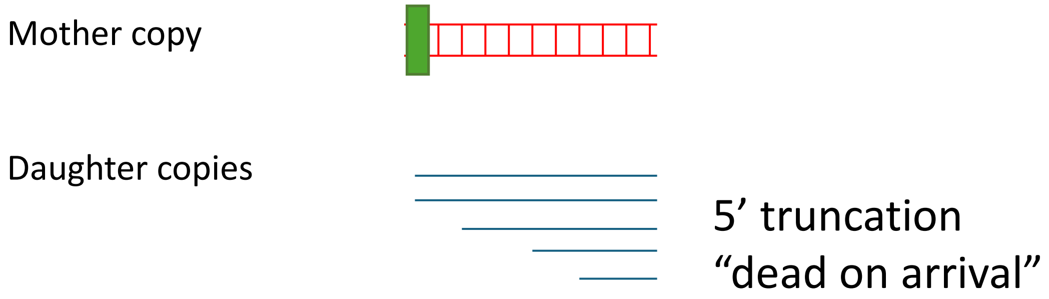
Requirements for mobility

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

Target site preference and TSD

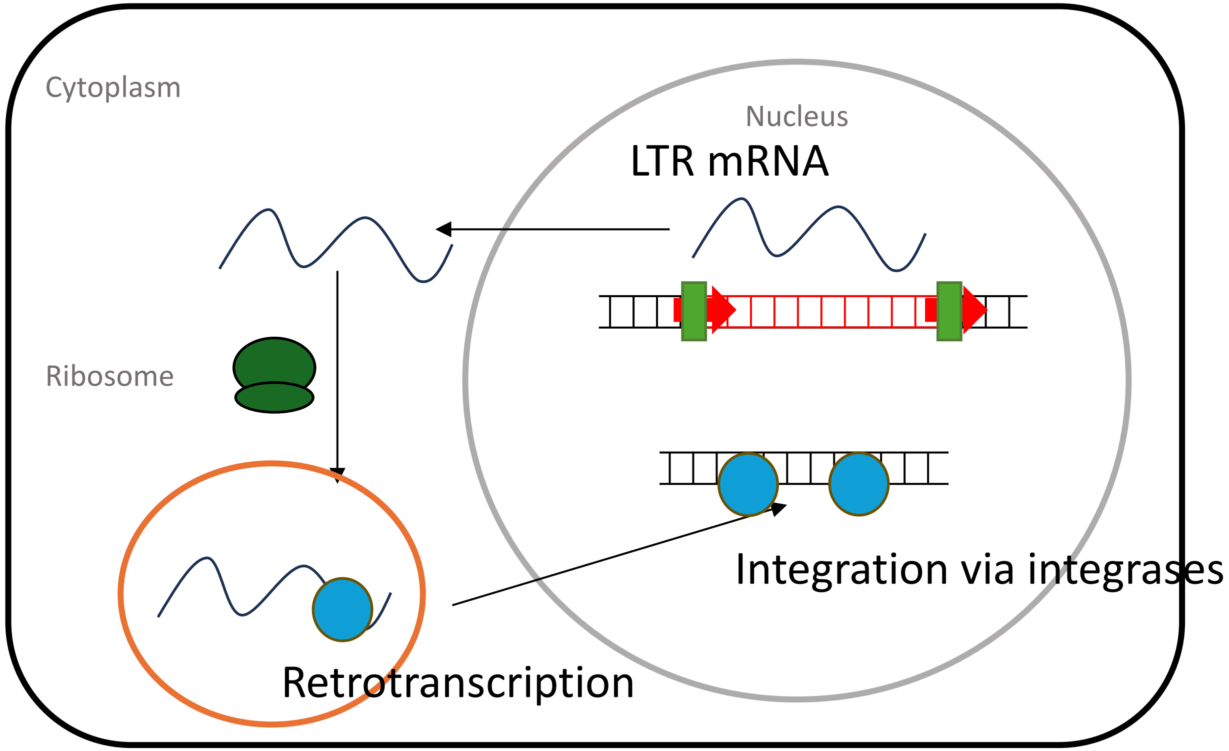
Target site preferentiality for sequences similar to the 3’ UTR
Target site duplications are of variable length

Content of new copies



LTR retrotransposons

Where/when/how in the cell



Cytoplasm

Nucleus

LTR mRNA

Ribosome

Integration via integrases

Retrotranscription

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

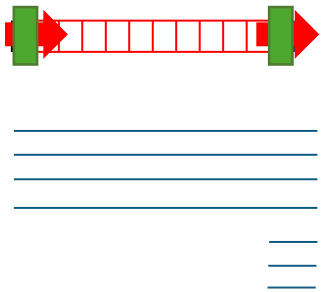
No preferentiality for target site

Specific length of target site duplications (4 bp, 5 or 6 bp)

Content of new copies

Mother copy

Daughter

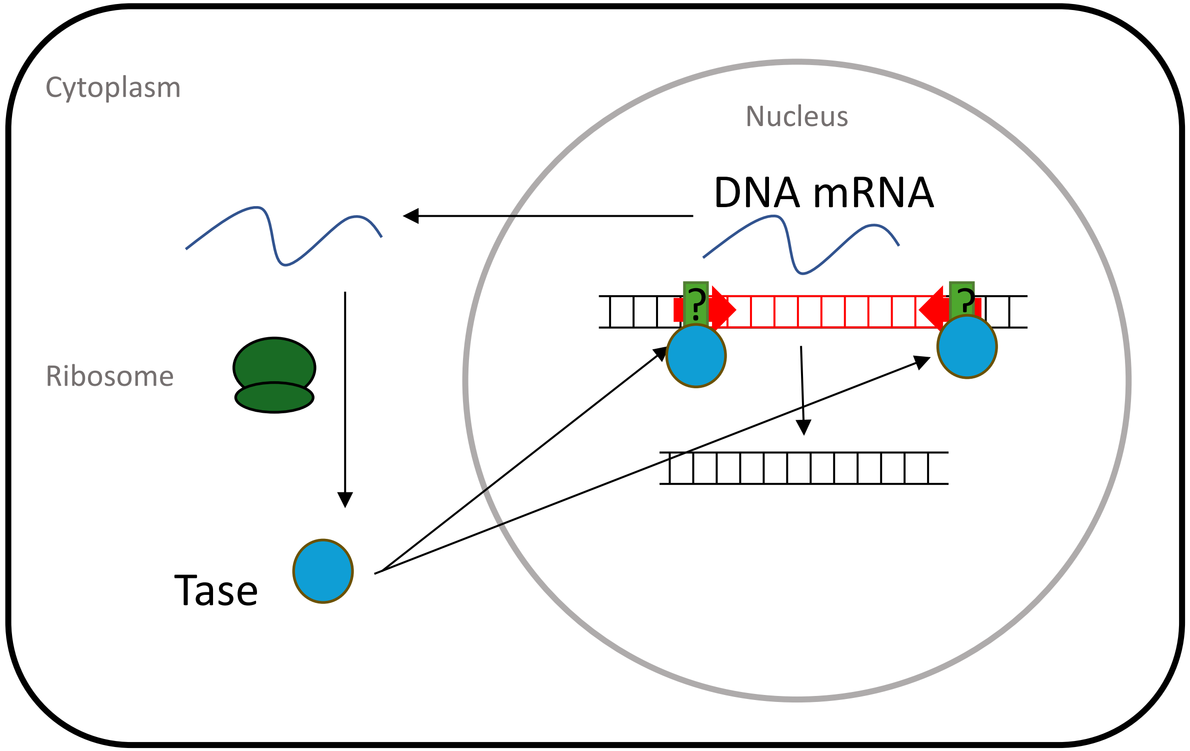


NAHR

Solo-LTR

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

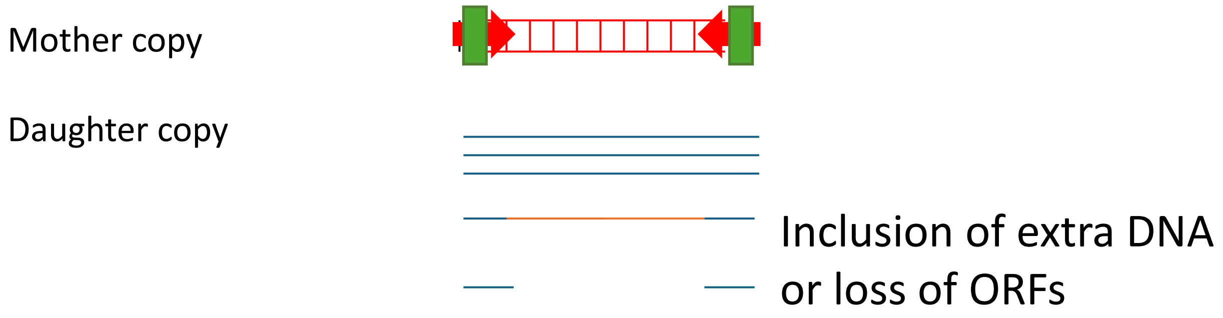
Recognition site in TIRs allows for both *cis*- and trans-mobilisation (with different probabilities)

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



Inclusion of extra DNA or loss of ORFs