REGOLAZIONE DELLA TRASCRIZIONE DA TRASPOSONI NEL GENOMA

Transcriptional disruption by the L1 retrotransposon implications for mammalian transcriptomes.

Nature. 2004 429:268-74 Han JS

L1 intragenica ed espressione genica

High Low

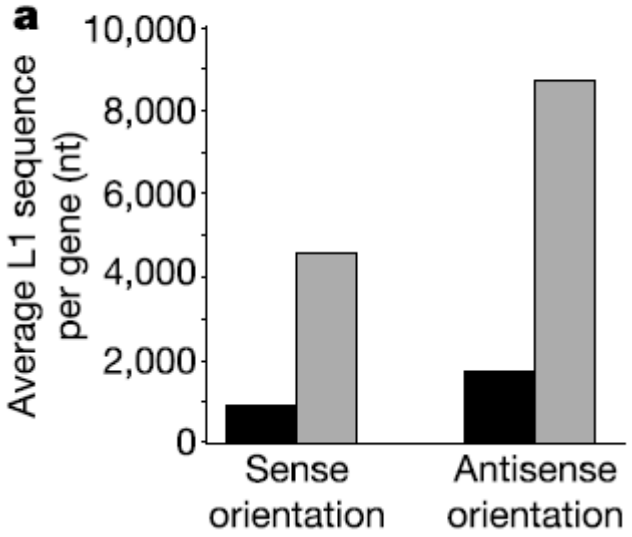
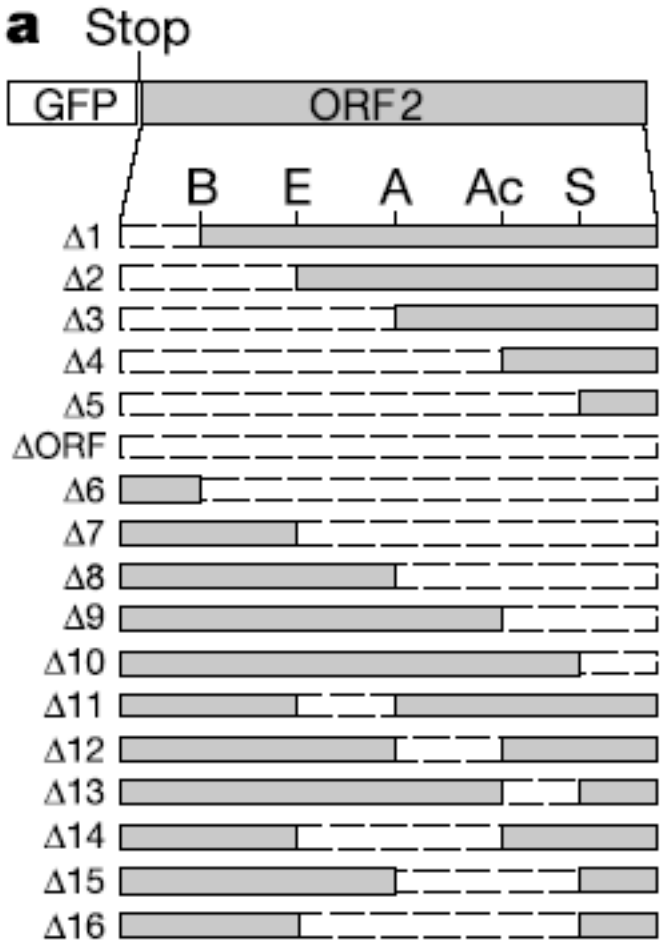


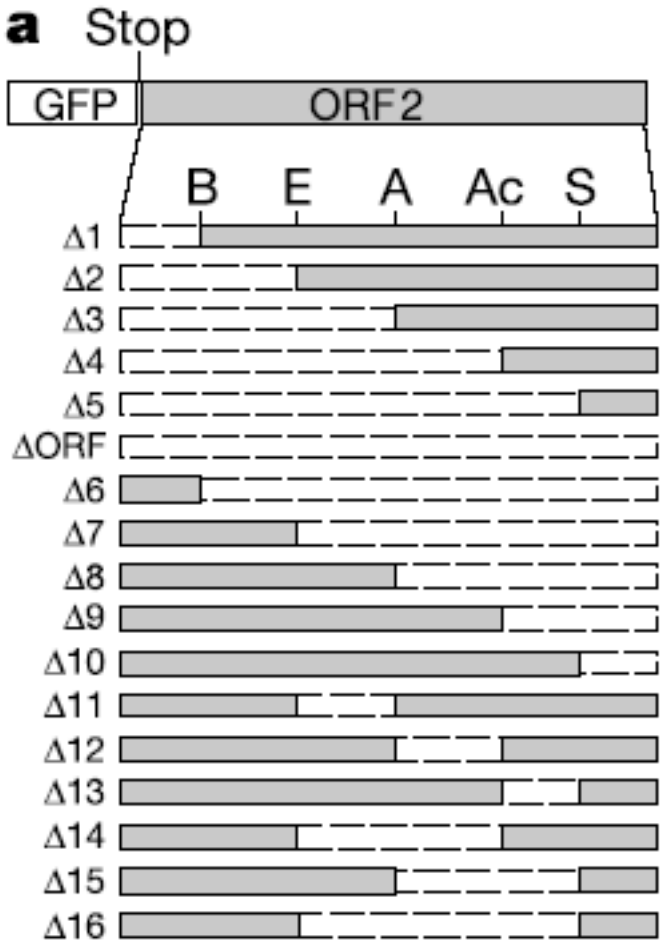
Figure 5 Bioinformatic analysis of L1 content in genes. a, Average L1 content of genomic loci of sets of highly (black bars) and poorly (grey bars) expressed genes (see Methods).

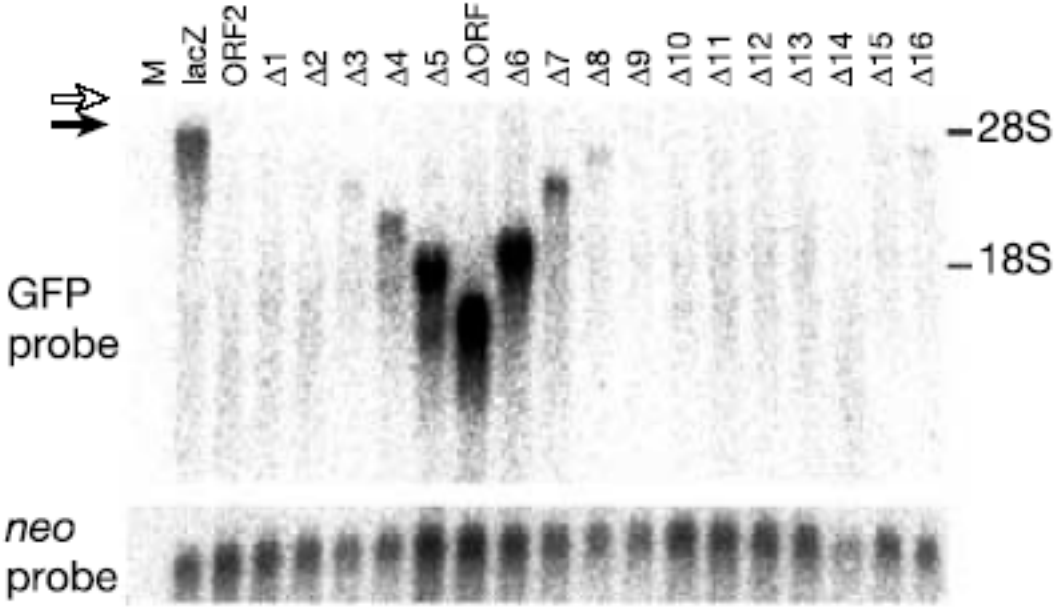
L1 mutants


a
Stop
GFP
ORF2
B
E
A
Ac
S
Δ1
Δ2
Δ3
Δ4
Δ5
ΔORF
Δ6
Δ7
Δ8
Δ9
Δ10
Δ11
Δ12
Δ13
Δ14
Δ15
Δ16

L1 mutants

RNA analysis





Decrease in expression depends on L1 length

Figure 3 Decrease in L1 expression is dependent on length. **a**, The left panel depicts the structures of deletion constructs. Hollow regions represent deleted sequences. B, *Bbv*CI; E, *Eco*RI; A, *Afl*II; Ac, *Acl*I; S, *Spe*I. The right panel shows a total RNA analysis of HeLa transfections. Lanes: M, mock; lacZ, pGFPstoplacZ; ORF2, pGFPstopORF2. Open and black arrows show the expected positions of GFPstopORF2 and GFPstoplacZ, respectively. **b**, The adenosine base composition of the sense strand, in 50-nucleotide windows, was plotted for each position in L1.2 with MacVector 6.5.3 (Oxford Molecular). **c**, The top panel shows the structures of GFPstopORF1, GFPstop4ORF1 and GFPstop5UTR. The 4ORF1 repeat is about 4,500 nucleotides long and the 5′ UTR repeat is about 4,000 nucleotides long. The bottom panel shows a total RNA analysis of HeLa transfections. Open, black and grey arrows show the expected positions of GFPstop4ORF1, GFPstop5UTR and GFPstopORF1, respectively.

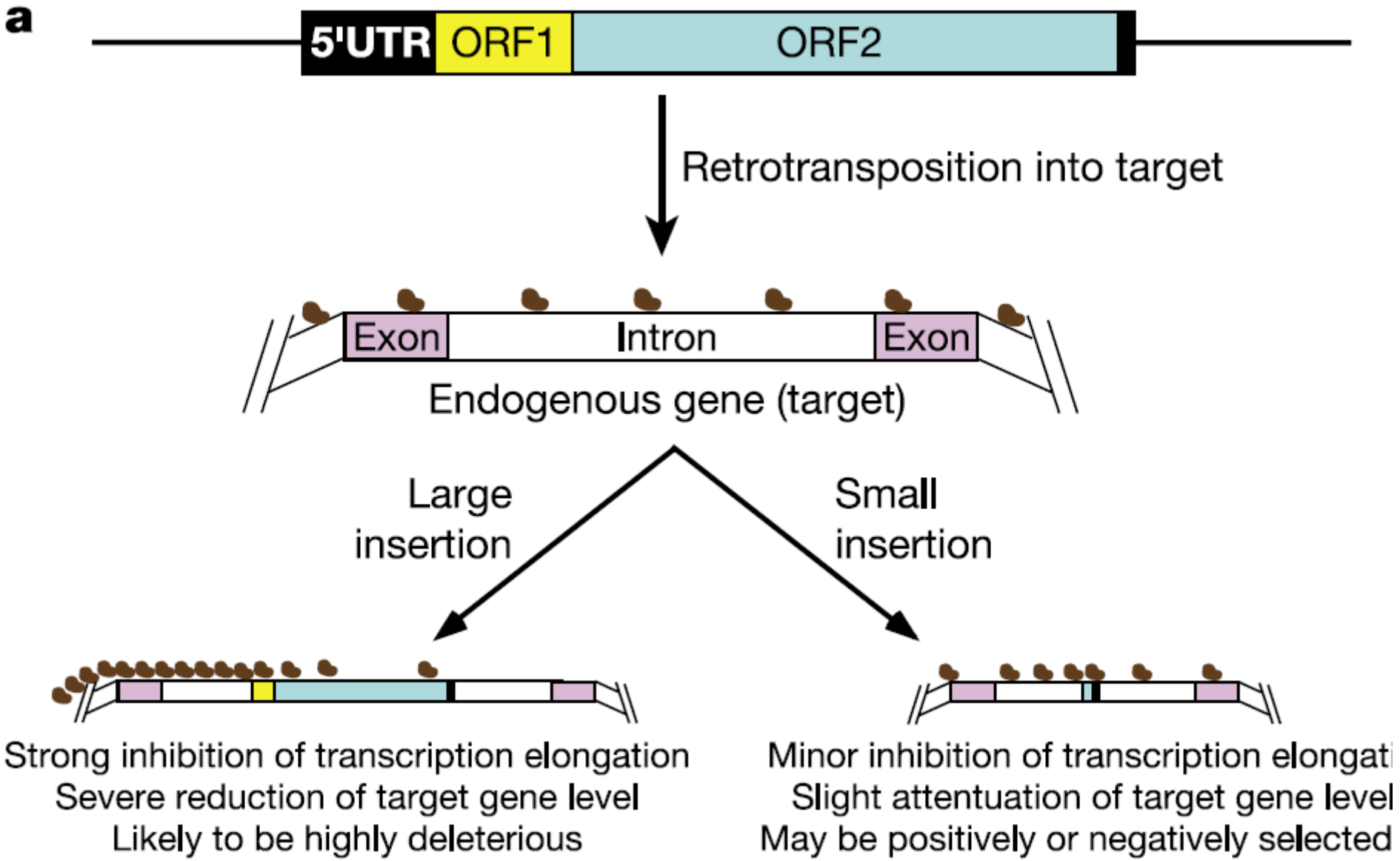

a
5'UTR
ORF1
ORF2
Retrotransposition into target
Exon
Intron
Exon
Endogenous gene (target)
Large insertion
Small insertion
Strong inhibition of transcription elongation
Severe reduction of target gene level
Likely to be highly deleterious
Minor inhibition of transcription elongati
Slight attentuation of target gene level
May be positively or negatively selected

Figure 6 Models for L1-mediated modulation of gene expression/structure. **a**, Effects on transcription. Brown dots represent transcriptional complexes, which could be slowed, paused or dissociated from the templates on encountering significant amounts of L1 sequence. **b**, Effects on mRNA and protein structure. Left, hypothetical gene with three exons. Middle, intronic sense L1 insertions can produce a minor amount of prematurely polyadenylated mRNA, potentially giving rise to a truncated protein with additional, previously untranslated amino acids at the C terminus (white segment). Right, intronic antisense L1 insertions can produce a major amount of prematurely polyadenylated mRNA.

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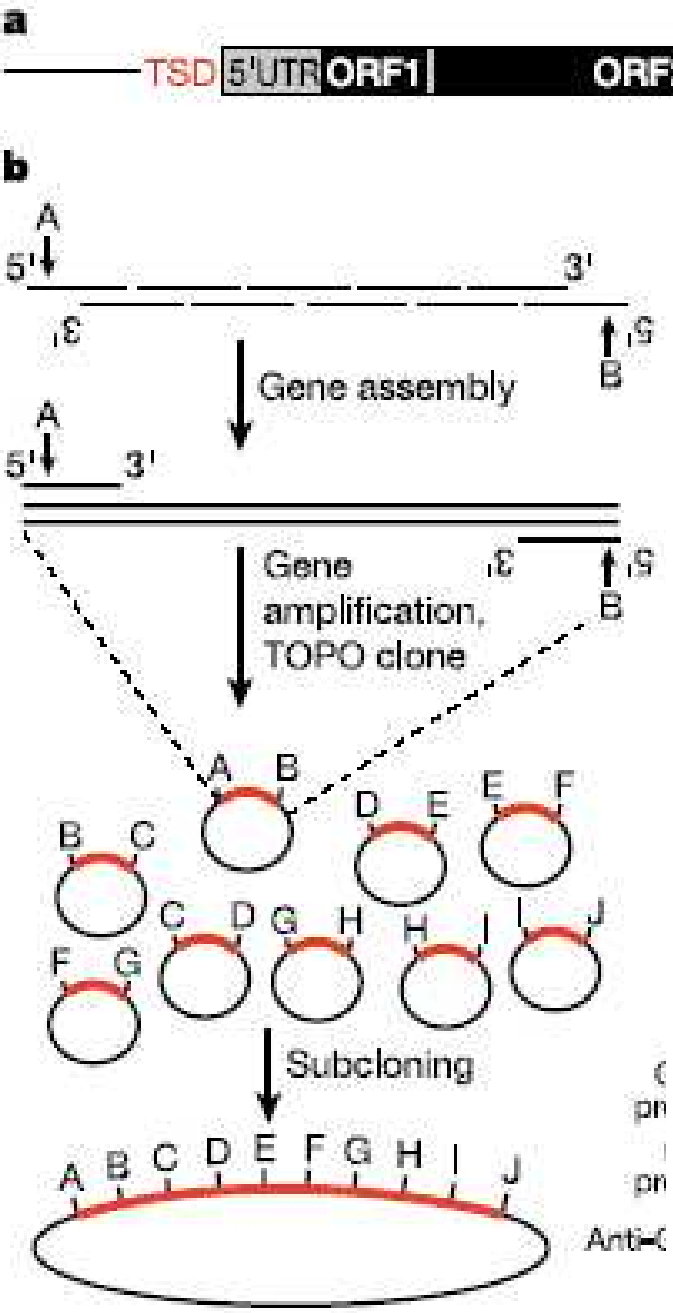
Inserting L1 sequences on a transcript significantly decreases RNA expression and therefore protein expression.

The poor L1 expression is primarily due to inadequate transcriptional elongation.

Because L1 is an abundant and broadly distributed mobile element, the inhibition of transcriptional elongation by L1 might profoundly affect expression of endogenous human genes.

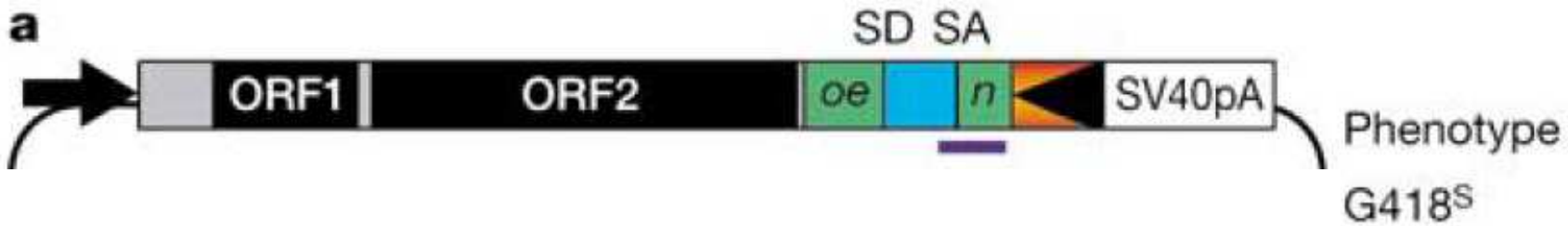
We propose a model in which L1 affects gene expression genome-wide by acting as a 'molecular rheostat' of target genes. Bioinformatic data are consistent with the hypothesis that L1 can serve as an evolutionary fine-tuner of the human transcriptome.

A highly active synthetic mammalian retrotransposon.
Han JS Nature. 429:314

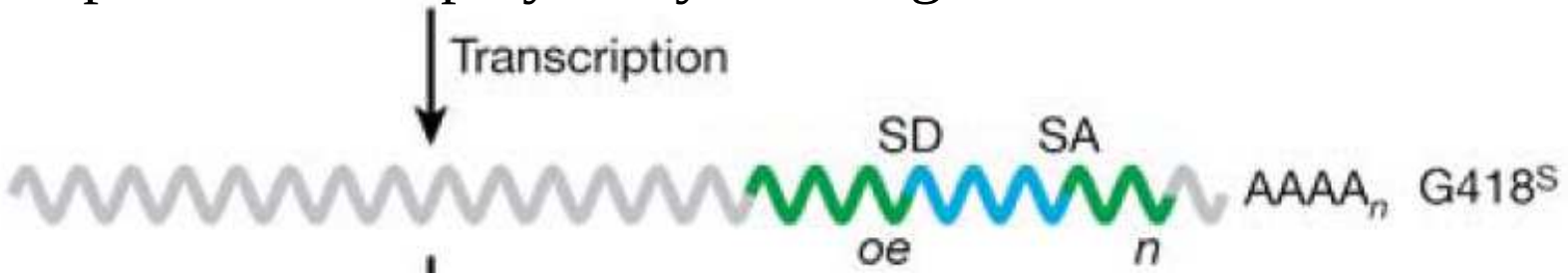


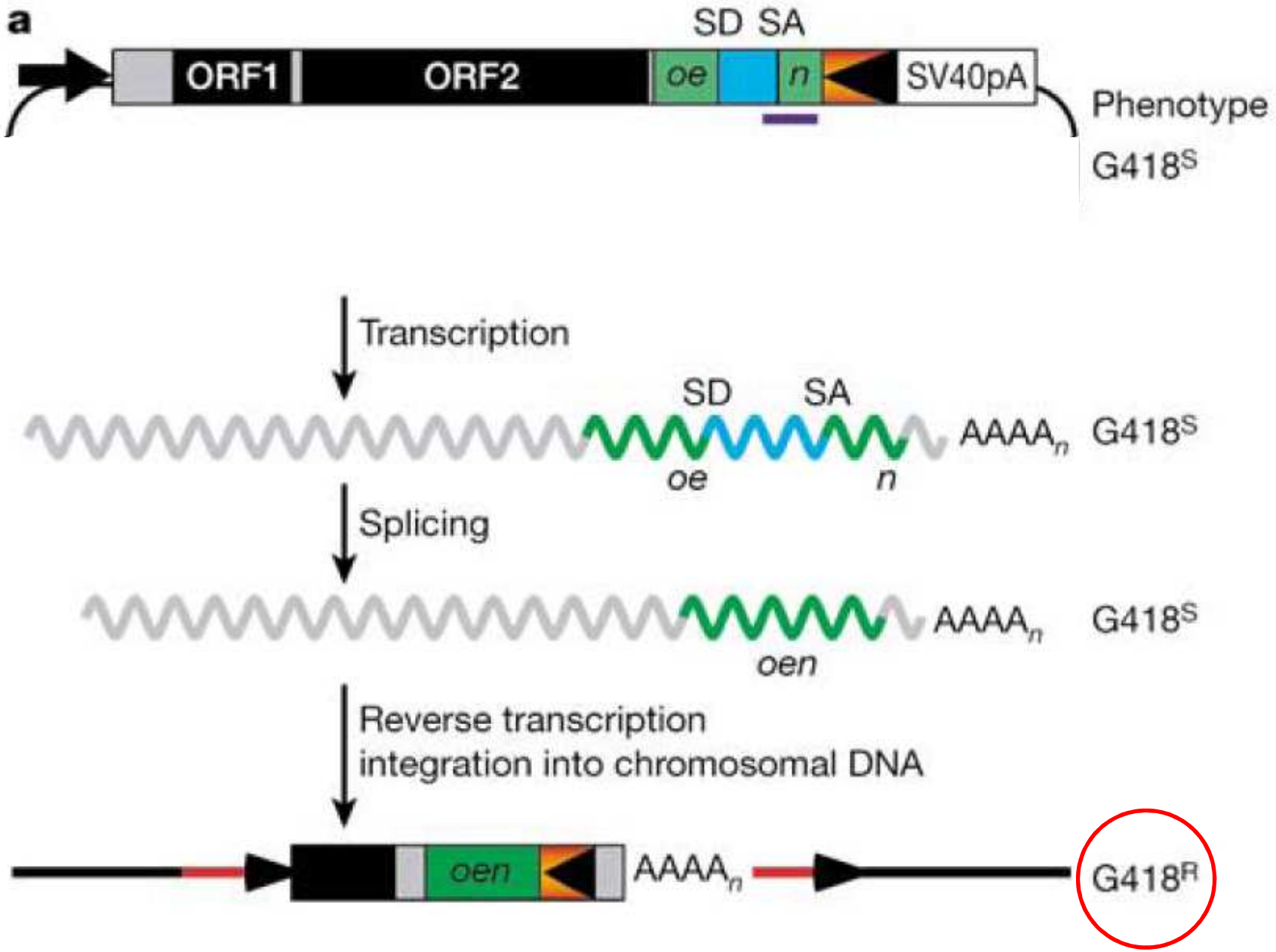
Overview of gene synthesis. Oligonucleotides encoding each fragment were mixed and subsequently used as template amplification.

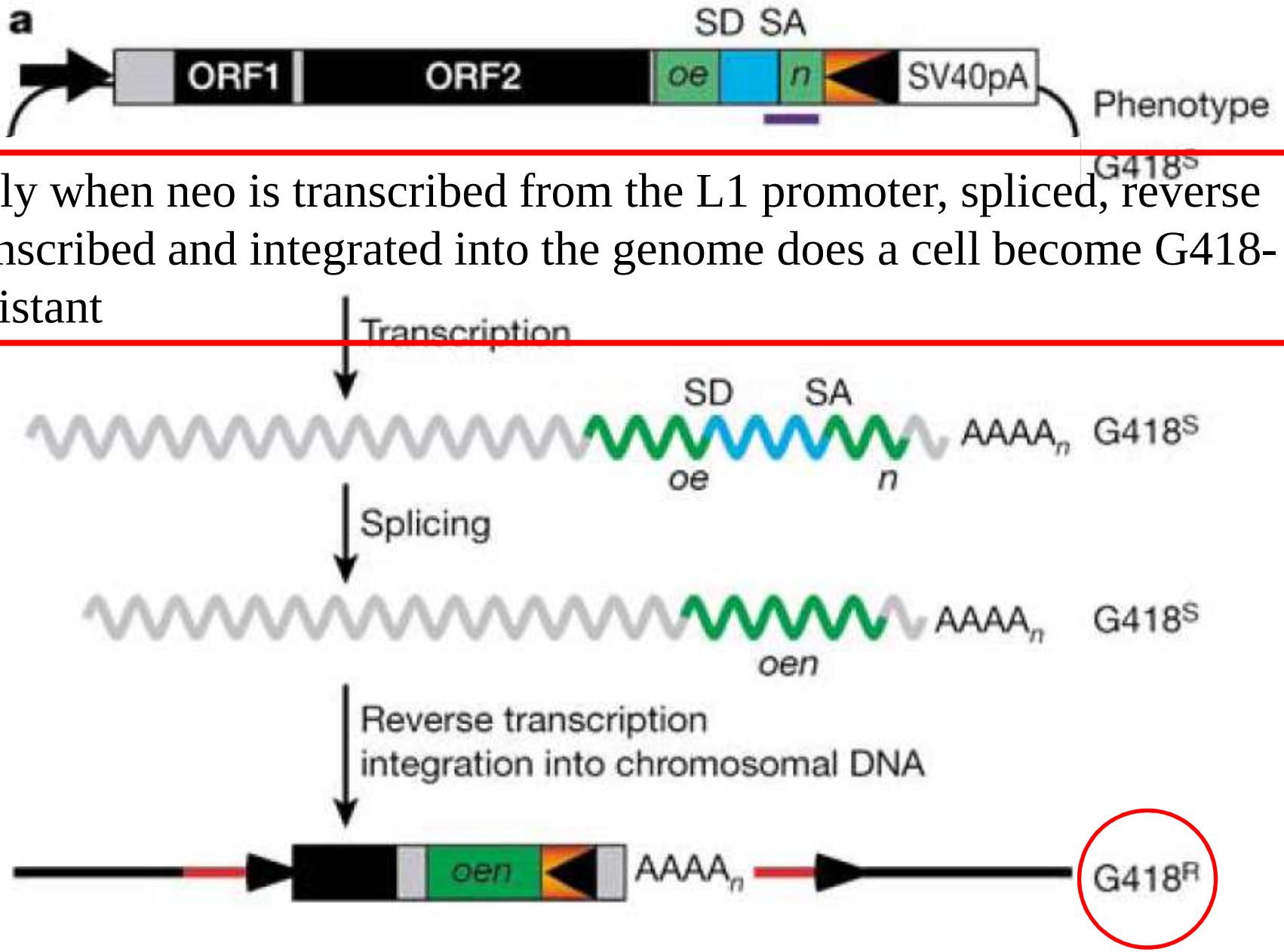
products were cloned and ligated together with unique restriction sites (labelled A to J)



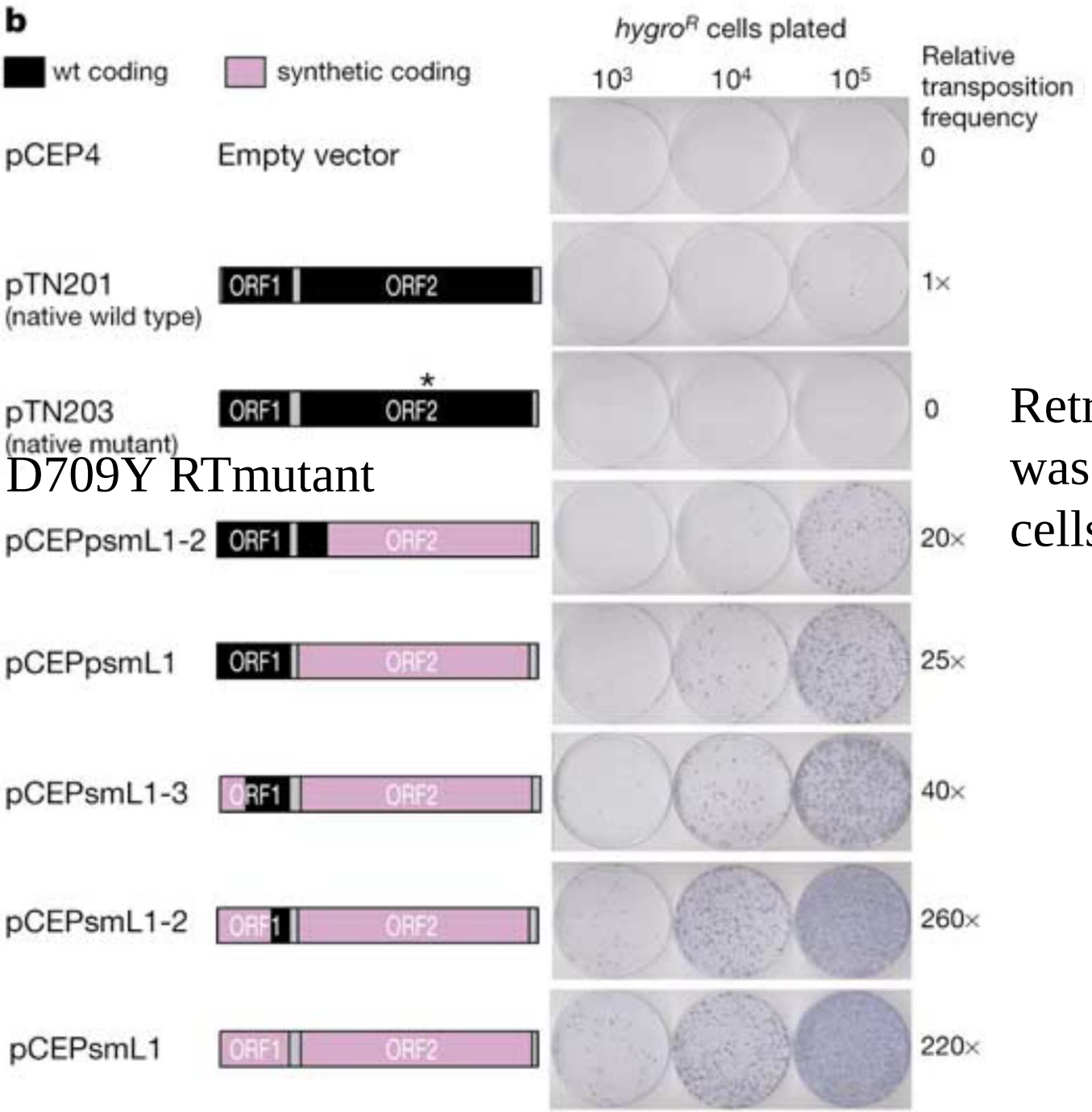
an intron-interrupted neo reporter in the 3’ untranslated region with its own promoter and polyadenylation signal




a
SD SA
ORF1
ORF2
oe
n
SV40pA
Phenotype
G418S
Transcription
SD
SA
AAAAn
G418S
oe
n
Splicing
AAAAn
G418S
oen
Reverse transcription
integration into chromosomal DNA
oen
AAAAn
G418R



Only when neo is transcribed from the L1 promoter, spliced, reverse transcribed and integrated into the genome does a cell become G418-resistant



D709Y RTmutant

Retrotransposition was assayed in HeLa cells

Figure 2 Retrotransposition of synthetic mL1. **a**, The retrotransposition assay. The L1 element contains an intron-interrupted *neo* reporter in the 3′ untranslated region with its own promoter and polyadenylation signal. Only when *neo* is transcribed from the L1 promoter, spliced, reverse transcribed and integrated into the genome does a cell become G418-resistant[4]. Blue lines represent probes for RNA analysis (Fig. 4). SD, splice donor; SA, splice acceptor. **b**, Retrotransposition was assayed in HeLa cells ($N = 3$). pTN201 contains only wild-type native mouse L1 sequence, and pTN203 contains wild-type native mouse L1 sequence with a D709Y reverse transcriptase point mutation[22]. The average absolute number of colonies for pTN201 was 440 events per 10^6 transfected cells.

A highly active synthetic mammalian retrotransposon.

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Transcription through L1 open reading frames is inefficient owing to an elongation defect

This elongation defect probably controls L1 transposition frequency in mammalian cells.

We report bypassing this transcriptional defect by synthesizing the open reading frames of L1 from synthetic oligonucleotides, altering 24% of the nucleic acid sequence without changing the amino acid sequence.

When the synthetic open reading frames were substituted for the wild-type open reading frames in an established retrotransposition assay, transposition levels increased more than 200-fold.

These synthetic retrotransposons are also the most highly active L1 elements known so far and have potential as practical tools for manipulating mammalian genomes

EVOLUZIONE RECENTE DEI TRASPOSONI

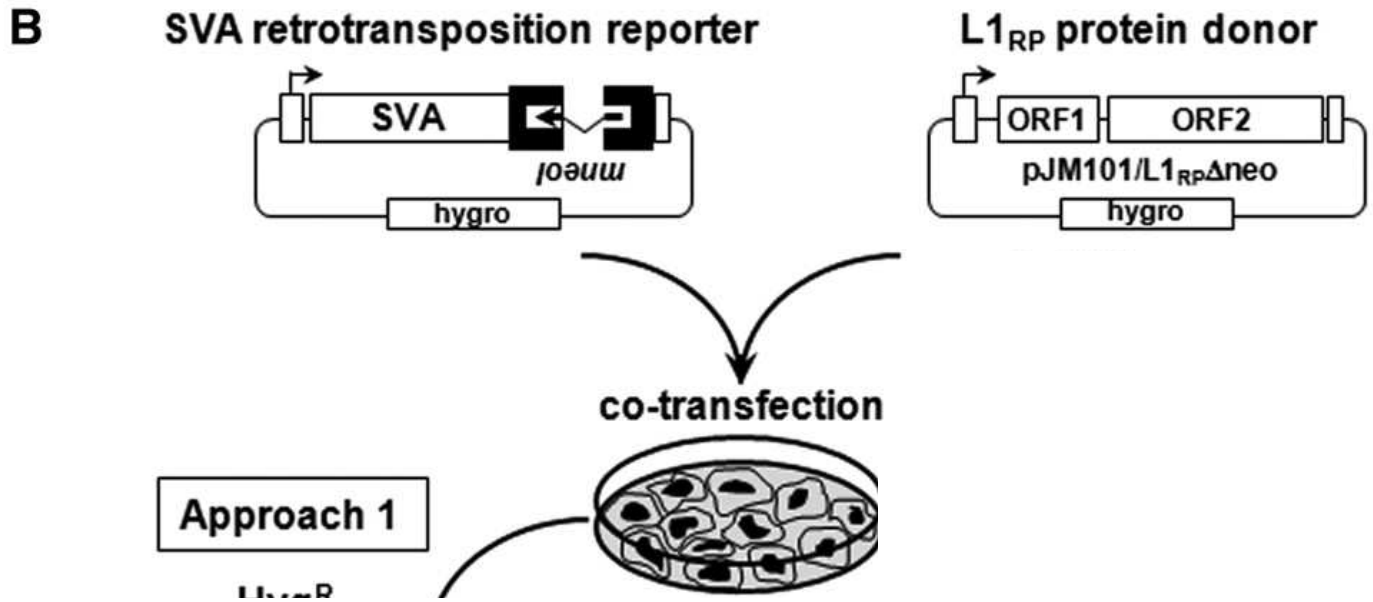
SVA

SINE-VNTR-Alu (SVA) elements are nonautonomous, hominid-specific non-LTR retrotransposons

composite mobile elements.

They represent the evolutionarily youngest, currently active family of human non-LTR retrotransposons

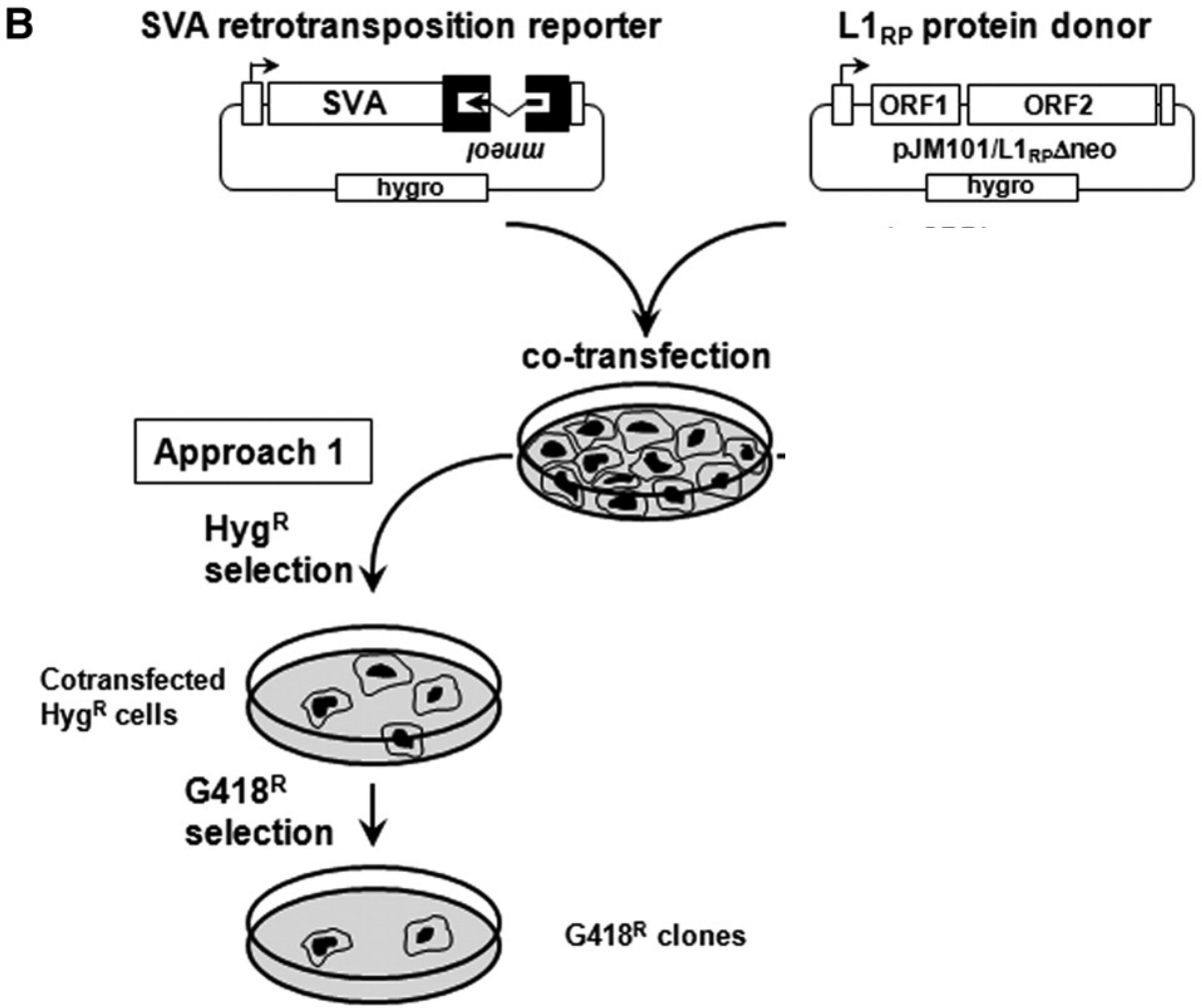
Rationale of the SVA trans-mobilization assay.



trans-mobilization of mneoI-tagged SVA elements by the L1 protein machinery?

Nucleic Acids Research

Rationale of the SVA trans-mobilization assay.



Raiz J et al. Nucl. Acids Res. 2011;nar.gkr863

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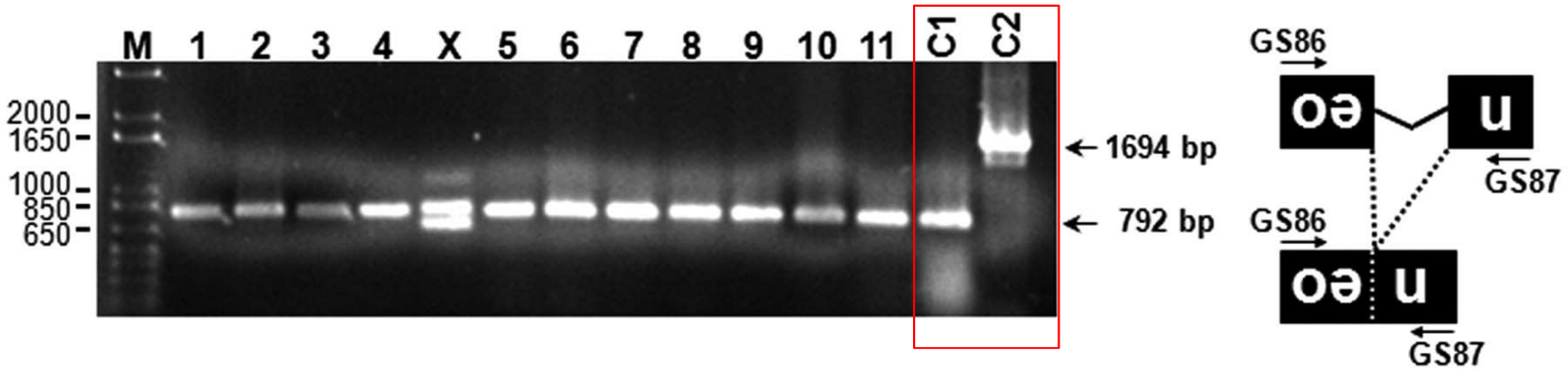
Diagnostic PCR to test for correct splicing of the intron from the mneoI indicator cassette.

PCR to test for correct splicing of the intron from the mneoI cassette



integration into the genome via authentic “retro”transposition

Raiz J et al. Nucl. Acids Res. 2011;nar.gkr863

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L1 ORF1p is required for trans-mobilization of SVA reporter elements.

	Retrotransposition Frequency [x 10^{-6}]	L1 *Cis* activity [%]
		100
n=6	1.3 ± 0.67	0.005
	197 ± 13	0.68
	3.2 ± 2.3	0.01

pCEP4 empty vector
intact (L1RP) and
mutant (ΔORF1) L1 protein donor plasmid

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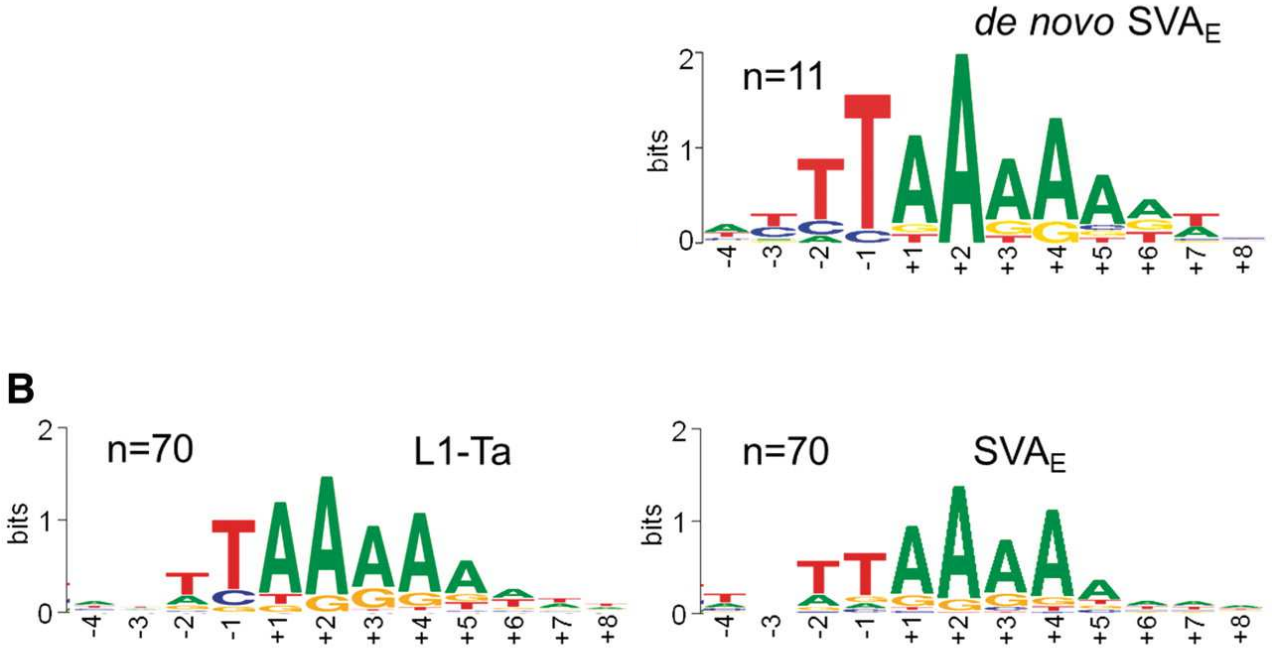
The nucleotide profile of SVAE de novo insertion sites resembles the consensus target sequence of pre-existing human-non-LTR retrotransposons.



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