Experience With HIV Based Lentivirus Vectors in Large Animal Models

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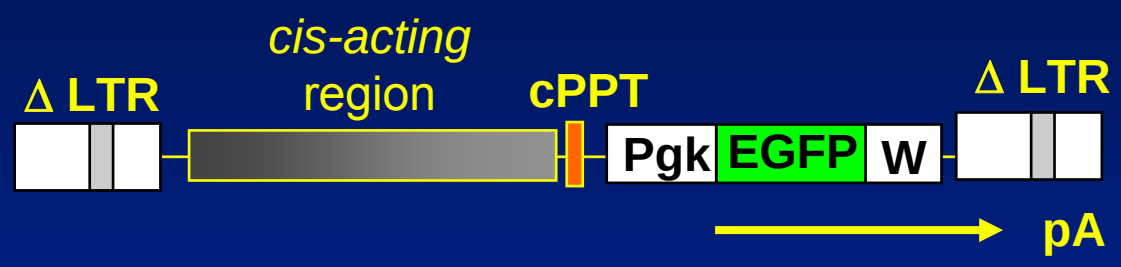
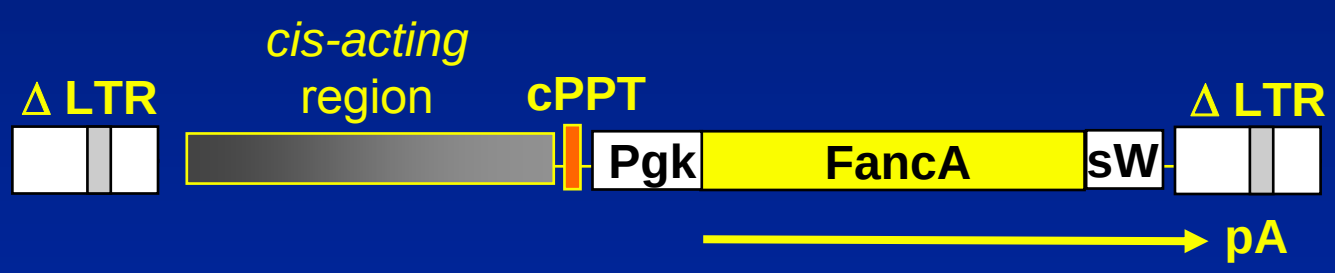
Overview

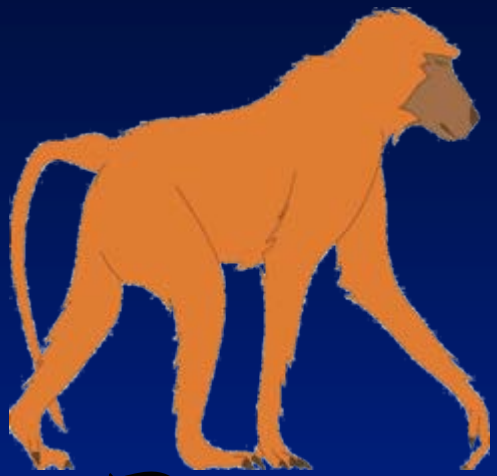
- FANCA vector
- Gene marking levels
- Integration site analysis in vivo
- Copy number analysis in repopulating cells
- Advantages of proposed vector

RRL Lenti Self-Inactivating (SIN) FancA Vector and Preclinical Testing Vector





Competitive Repopulation Assay



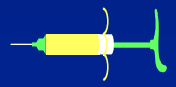
Stem cell harvest
and
CD34 enrichment

split cells in
two aliquots

Transduction

Vector A

Vector B

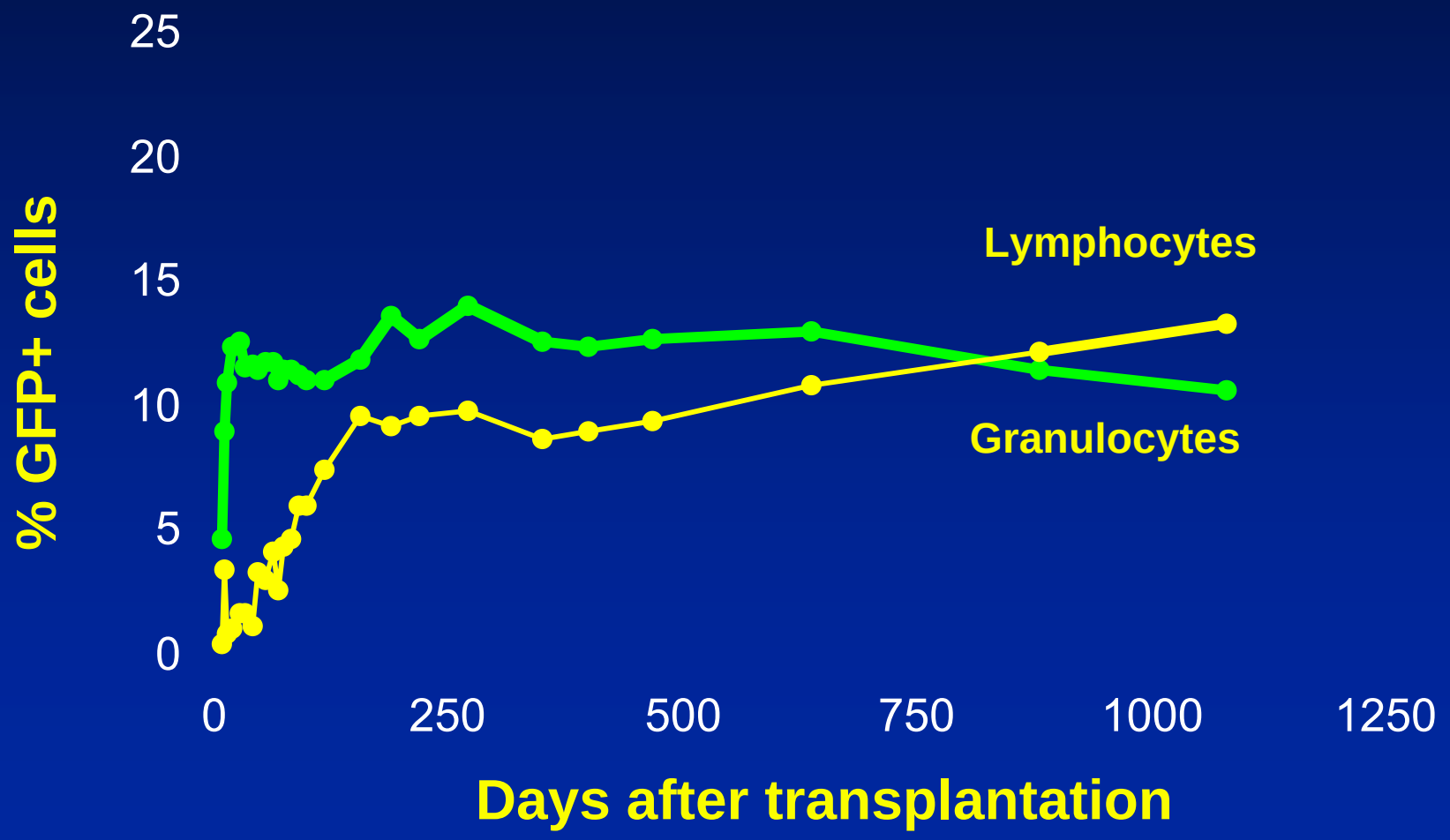


Infusion
of pooled cells

Myeloablative
irradiation

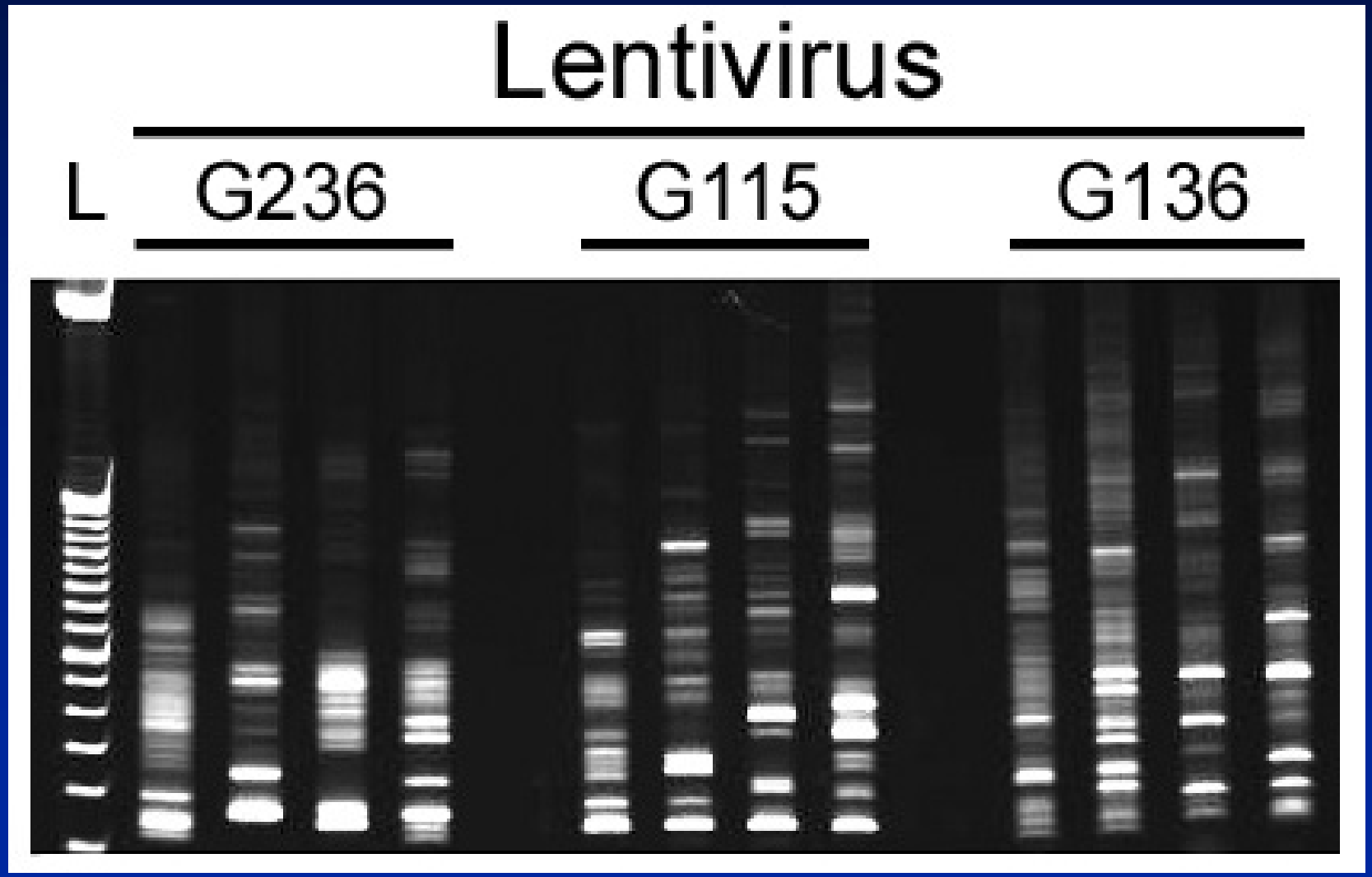
Conditions tested:

Efficient Lentiviral Transduction of Dog Long-Term Repopulating Cells

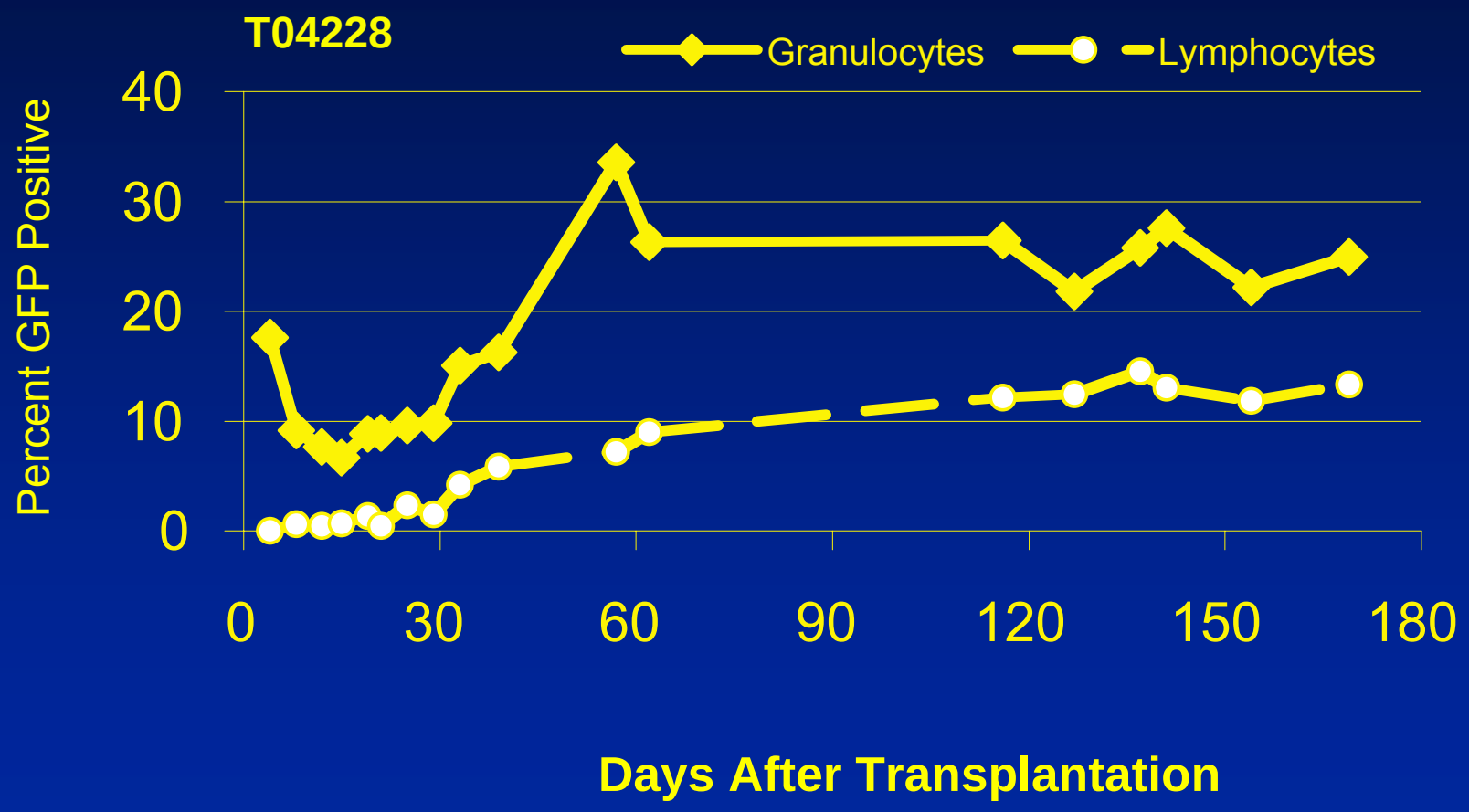


Horn et al. Blood 2004

Polyclonal Hematopoiesis after Lentiviral Transduction of Dog Long-Term Repopulating Cells

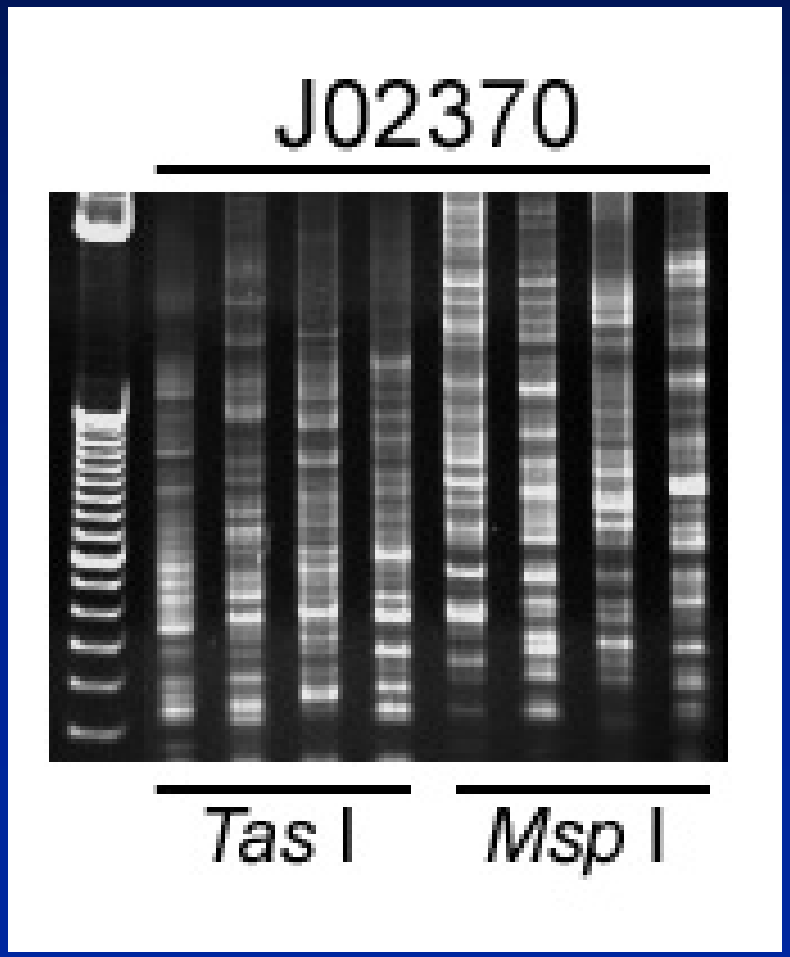


Stable High Level Lentiviral Marking in Macaque Repopulating Cells



Polyclonal Engraftment of HIV Lentivirus Transduced M. nemestrina CD34+ cells

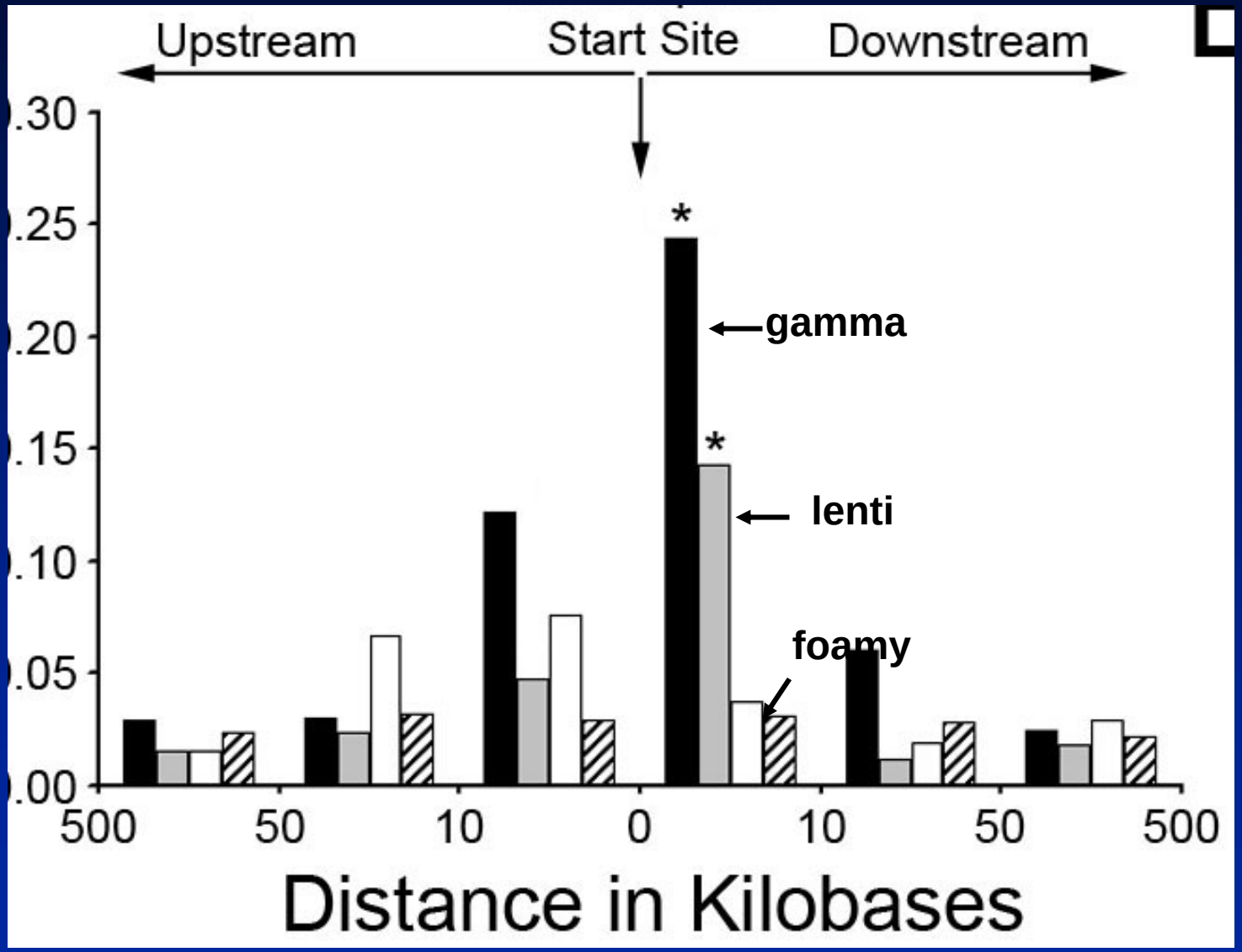
- **Lentivirus modified HSCs**



Animals Transplanted With Proposed Lentivirus Vector Backbone and Short Transduction Protocol

- 5 M. nemestrina with mean marking levels of 15% (1.5 to 35%) and with a mean follow up of 427 days (47 to 834 days)
- 8 dogs with mean marking levels of 10% (1 to 22) and with a mean follow up of 1150 days (360 to 1814).
- All animals have maintained polyclonal hematopoiesis

Integrants in Repopulating Cells Near Proto-oncogenes in Dogs



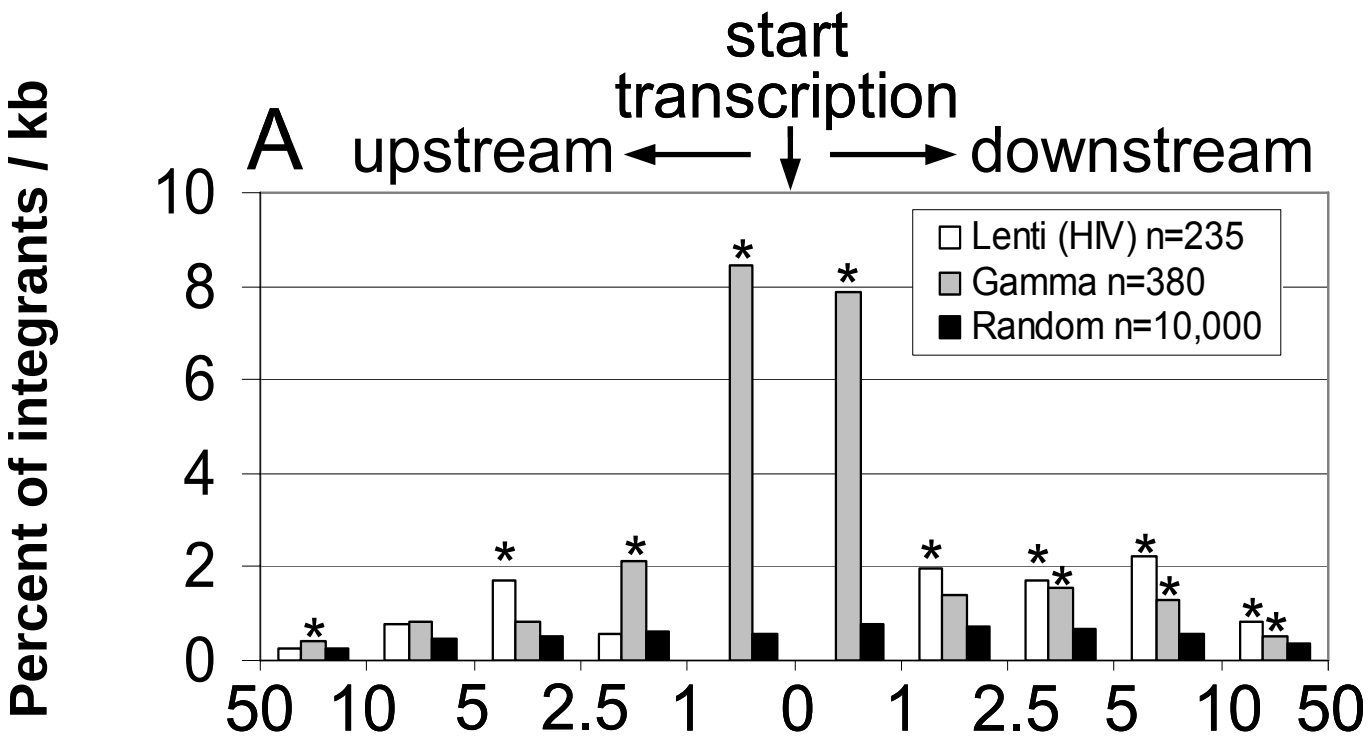
Beard et al HGT 2007

Retrovirus Integration Sites Relative to RefSeq Transcripts

Retrovirus	*Unique Sites*[†]	*% In Genes*	*% Within 10 kb/50 kb of Transcription Start Sites*	*% In Proto-Oncogenes*	*% Within 10 kb/50 kb of Proto-Oncogene Transcription Start Sites*
Gammaretrovirus	**82**	**48.8**	**34.2** / 73.2**	**7.3**	**3.66** / 7.32*
Lentivirus	**210**	**58.6**	**15.2 / 44.3**	**4.3**	**1.90* / 3.33**
Random	**10000**	**37.3**	**14.2 / 44.7**	**2.4**	**0.6 / 3.02**

[†]Retrovirus integration sites containing retrovirus LTR and linker cassette flanking canine genomic sequence that aligned with ≥35 bp to the publicly accessible (May 2005) dog genome with a minimum BLAT score of 70, percent **match** of ≥95 %, and the second rank alignment score ≤ 98.% of the first rank score.*p* values = *$p<0.01$, and **$p<0.001$

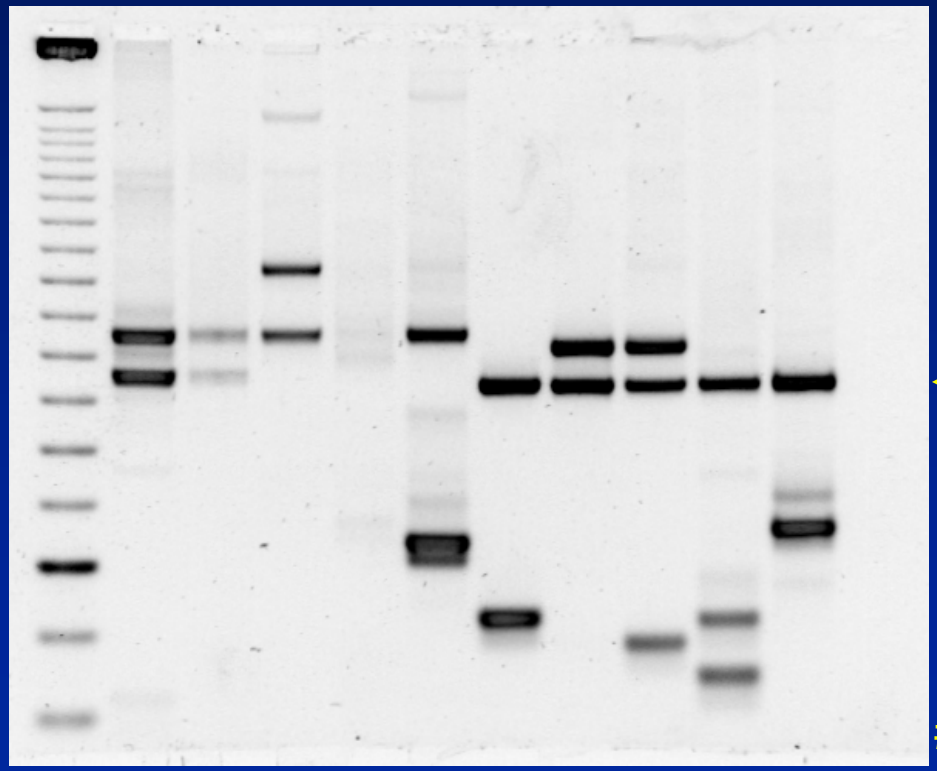
Distribution of Integration Sites Relative to Genes in Macaque Repopulating Cells



Over-represented Gene Classes Near Integrants in Repopulating Cells

Vector	Gene Class	# Integrants	% Integrants	p-value
Gammaretroviral	phosphorylation	57	18.6%	1.90E-11
	intracellular signaling cascade	42	13.7%	1.50E-08
	chromosomal translocation	12	3.9%	5.20E-06
	proto-oncogene	13	4.2%	5.40E-06
	nucleotide-binding	35	11.4%	2.30E-05
HIV-lentiviral	metal-binding	45	22.5%	3.40E-08
	nuclear protein	54	27.0%	6.80E-08
	zinc-finger	33	16.5%	3.60E-07
	splice variant	67	33.5%	5.00E-06
	phosphorylation	34	17.0%	2.40E-05

Low Number of Integration Sites in Repopulating Cells Despite High MOI Transduction (MOI of 100)



Colony Integration Sites

Summary of Colony analysis after Tx

Backbone	MOI	# of Colonies	integrations / colony (average)
Gamma	<1	118	1.76
Lenti	100	65	1.45

Before and after Transplantation

Animal	# of Colonies	integrations / colony (average)
M99267 (Pre-tx)	18	2.94
M99267 (Post-tx)	17	2.35

Safety and Potential Advantages of Proposed Lentivirus Vector

- Stable polyclonal hematopoiesis in 13 large animals with proposed vector backbone.
- FANCA vector has SIN configuration and a relatively weak internal PGK promoter
- Better, likely safer integration profile in long-term repopulating cells than gammaretrovirus vectors
- Ability to efficiently transduce long-term hematopoietic repopulation cells with a short transduction protocol

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- Canine Facility

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Keith Humphries

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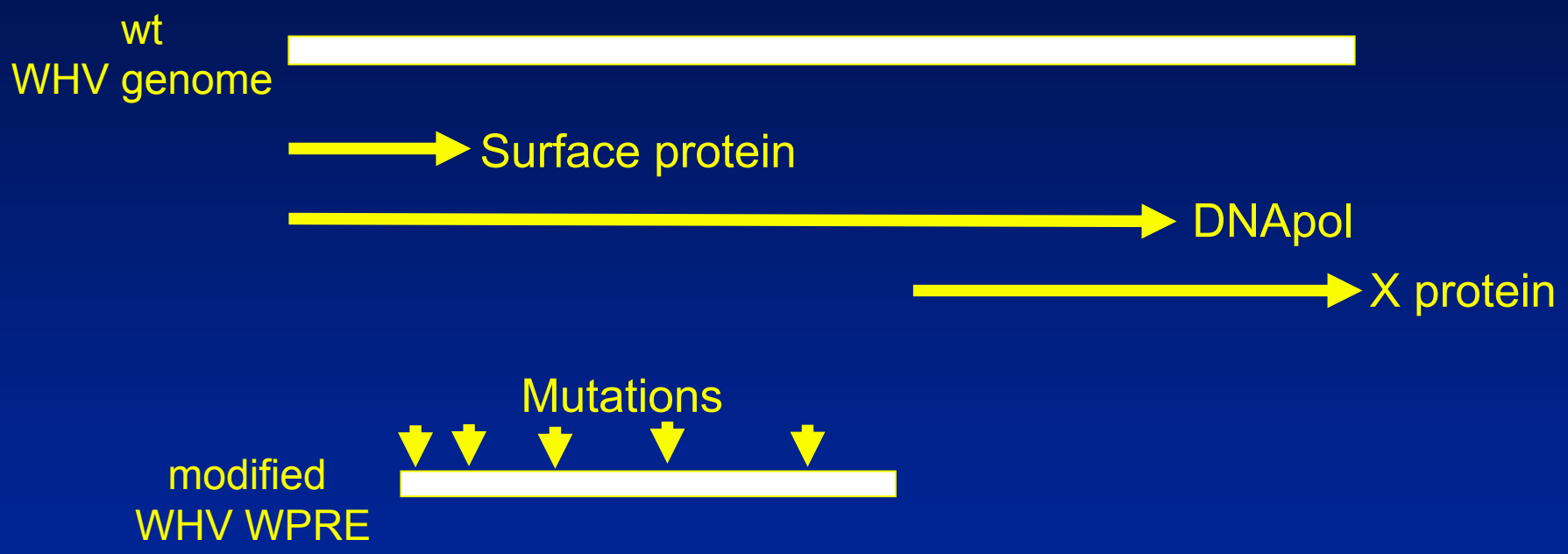
- John Zaia

NIH

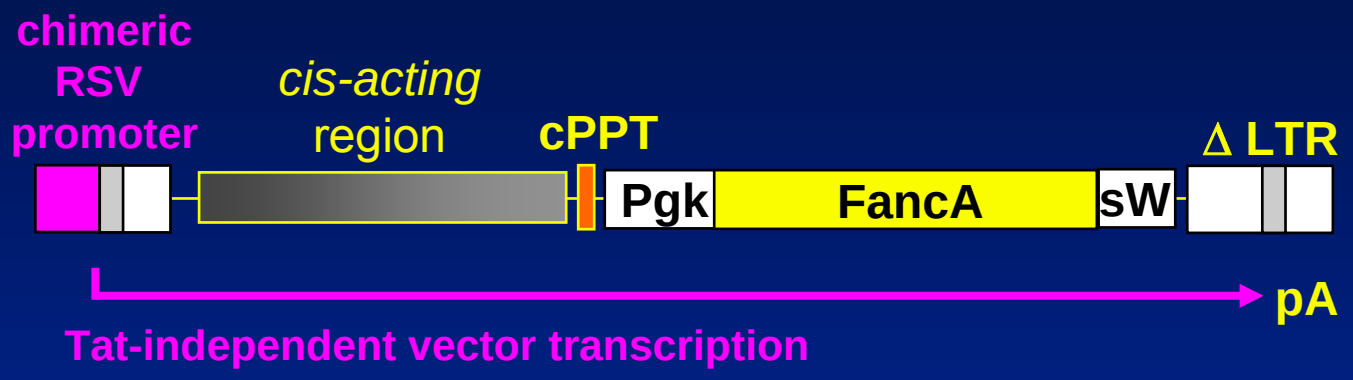
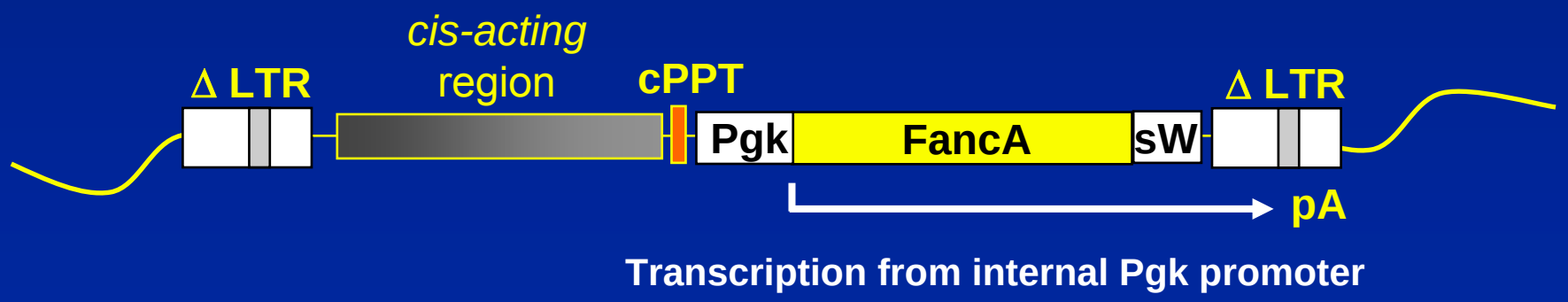
Safety-Modified WPRE (woodchuck post-transcriptional regulatory element)



- No X protein ORF
- interrupted partial DNAPol ORF
- interrupted partial surface protein ORF

Lenti RRL Tat-independent Self-Inactivating (SIN) Vector

Vector plasmid



Integrated vector provirus