Safety Considerations for Gene Editing and Other Gene Therapy Products: An FDA Perspective

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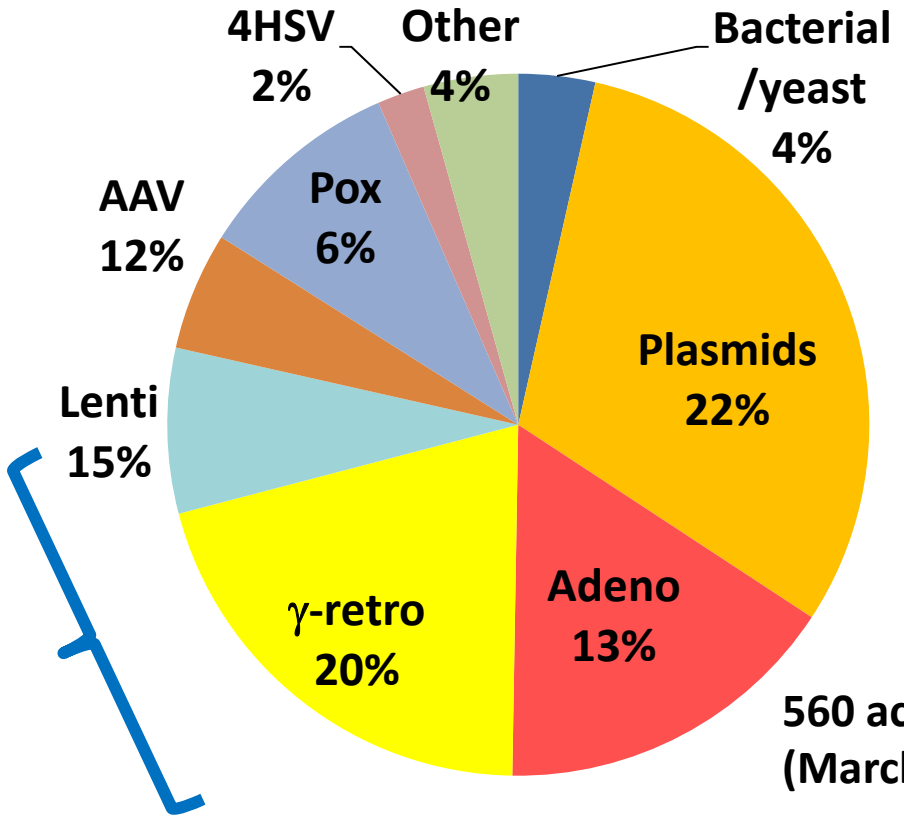
Gene therapy products regulated by OTAT



560 active Gene Therapy INDs
(March 9, 2017)

Clinical applications of lentiviral vectors

Ex vivo applications

- T cells to treat HIV infection
- CAR T cells to treat leukemias and lymphomas
- CD34+ hematopoietic progenitor/stem cells to treat
  - blood disorders
  - metabolic disorders
  - immunodeficiencies

In vivo applications

- Ocular diseases
- CNS disorders
- Immunotherapy applications

Safety issues with HIV-1-based lentiviral vectors used in clinical trials

Potential to form replication-competent lentivirus

Potential for insertional gene activation/inactivation

Potential for off-target transduction *in vivo*

Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector's tissue tropism
- Directing vector integration to genomic "safe harbor sites"
- Modifying the cell substrate for lentiviral vector production

Approaches to improve the safety of lentiviral vectors

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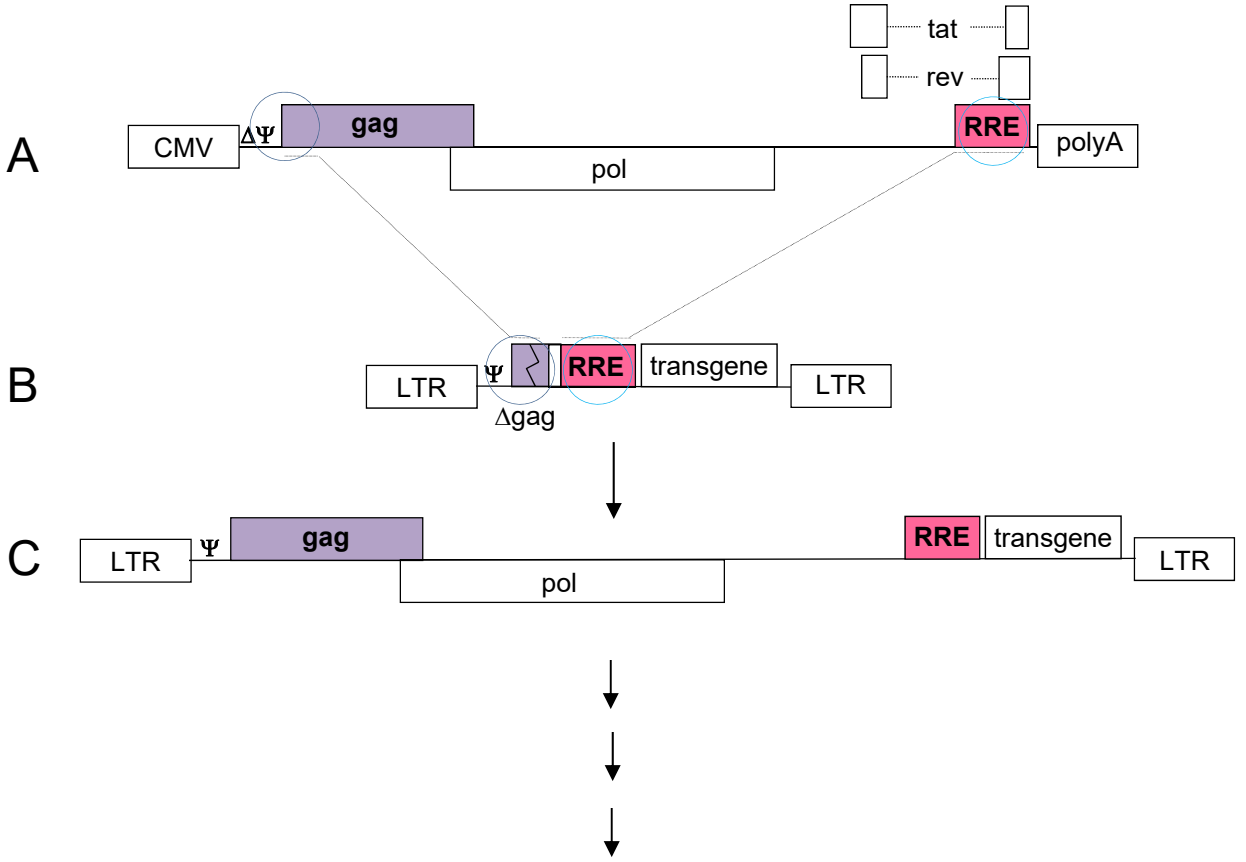
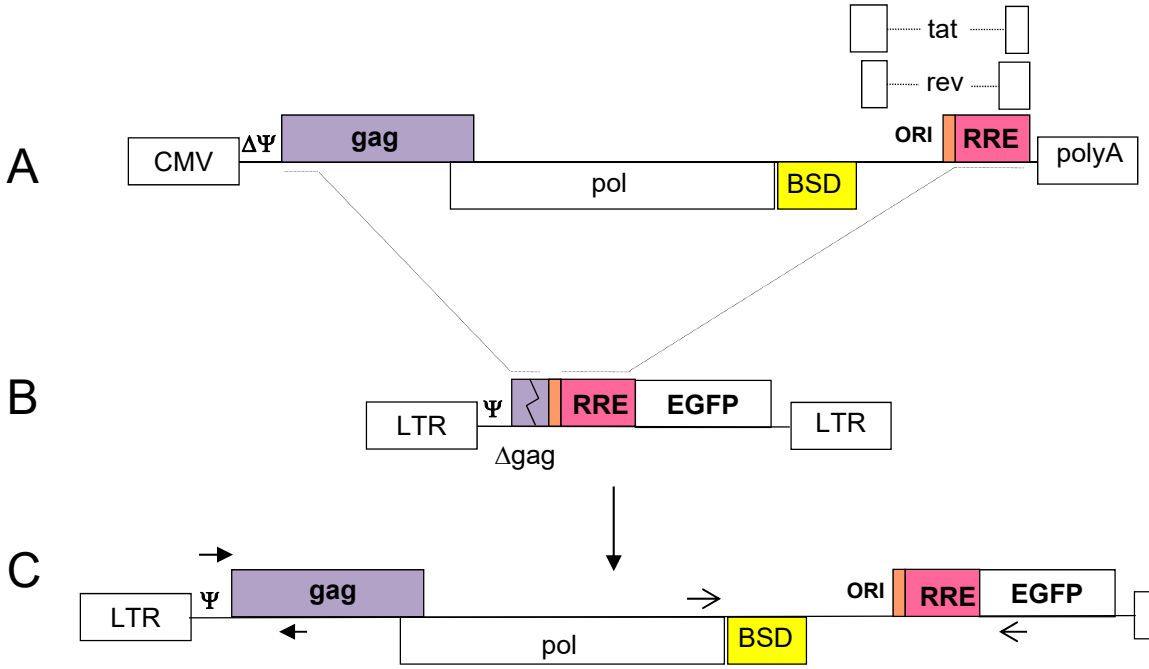
Emergence of partial recombinants



Replication-competent lentivirus?

Assay to assess formation of partial recombinants



Transduction of HEK 293 cells

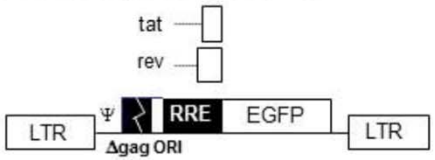
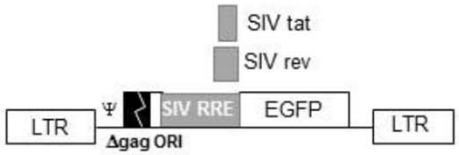
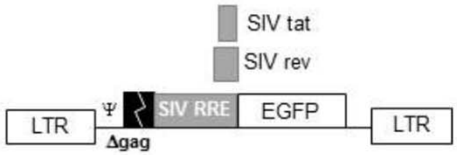
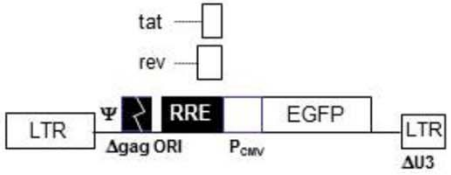
↓

Blasticidin (BSD) selection

↓

Characterization of resistant cell clones by PCR and DNA sequencing

Reducing sequence overlaps

		Sequence overlap (%)	No. of BSD-resistant colonies per 10^6 TU
A	NL-EGFP(MSCV) vector 	13.3	63.4 ± 15.4
B	NL-SRRE-EGFP(MSCV) vector 	9.8	25.6 ± 5.3
C	NL-SRRE/ΔORI-EGFP(MSCV vector 	9.1	9.5 ± 2.4
D	NL(CMV)EGFP/CMV/WPREΔU3 vector 	15.6	3.2 ± 0.5

Kuate et al. *Human Gene Therapy Methods* **25, 126-135 (2014)**

Conclusions

- Partial recombinants are rare
- The frequency of partial recombinants is dependent on vector design

Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector's tissue tropism
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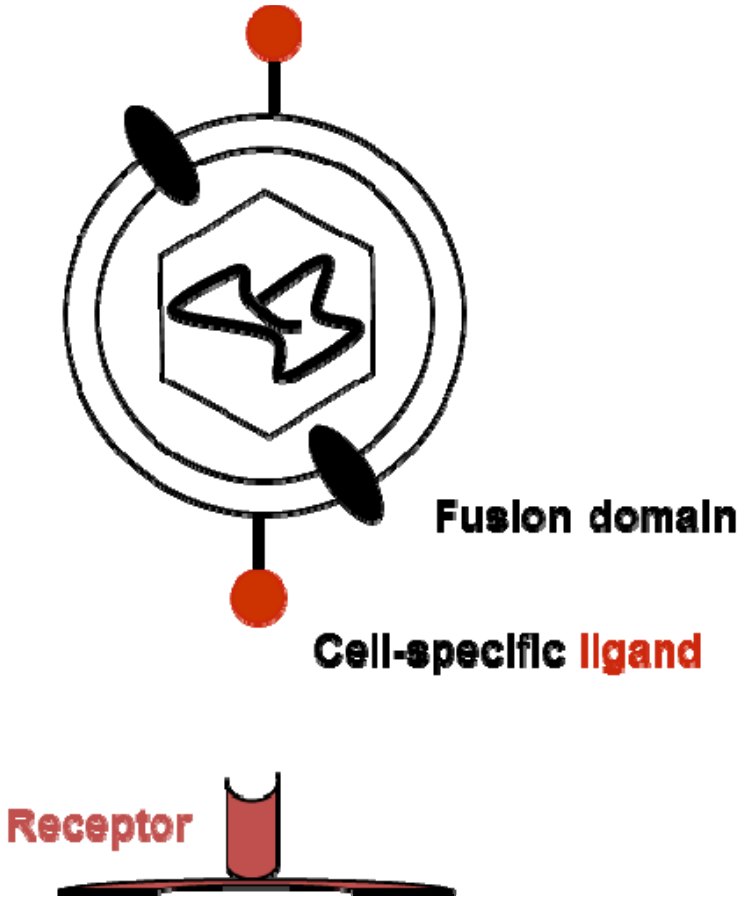
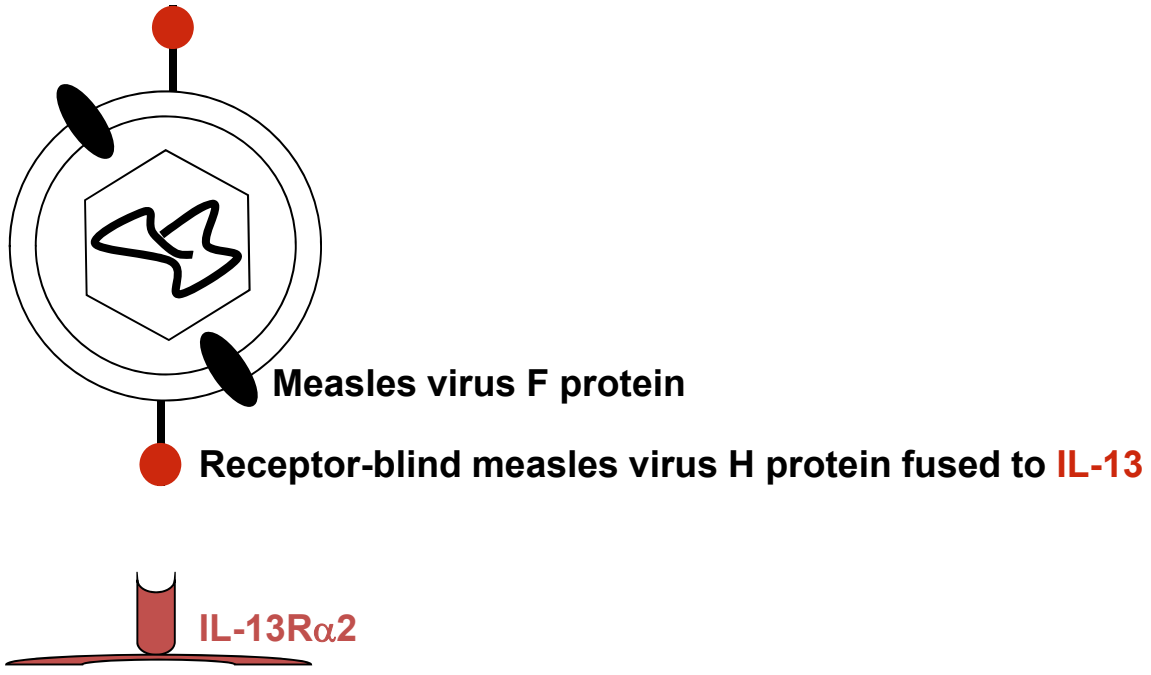
- Modifying the cell substrate for lentiviral vector production

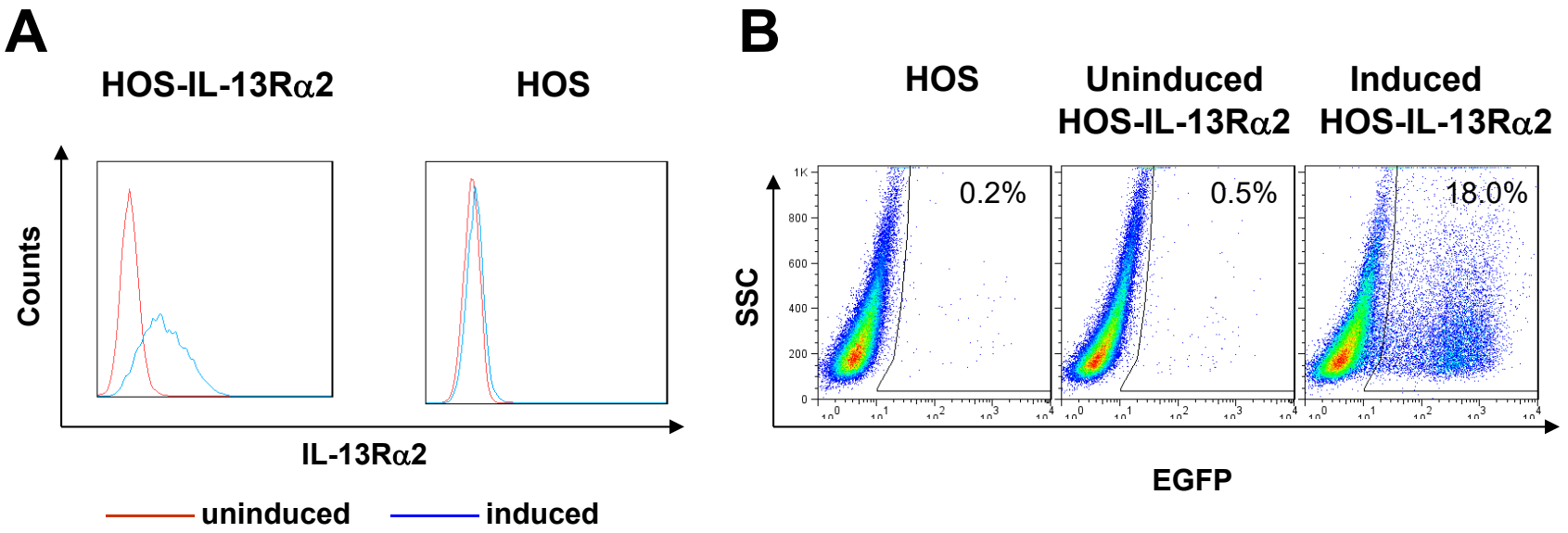
Strategy to target lentiviral vector transduction



Strategy to target IL-13Rα2-positive cells using lentiviral vectors pseudotyped with measles virus-derived hemagglutinin (H) and fusion (F) proteins

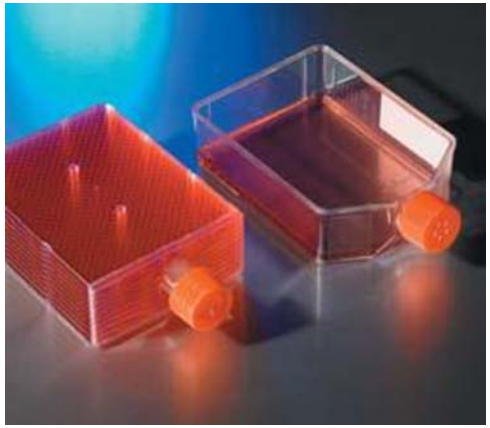


Targeting human osteosarcoma cells that conditionally overexpress IL-13Rα2



Ou et al. *Human Gene Therapy Methods* 23, 137-147 (2012)

Large-scale manufacturing of lentiviral vectors pseudotyped with measles virus H and F proteins



HYPERFlask Cell Culture Vessel

Large-scale manufacturing of lentiviral vectors pseudotyped with measles virus H and F proteins

Mustang Q anion exchange membrane chromatography

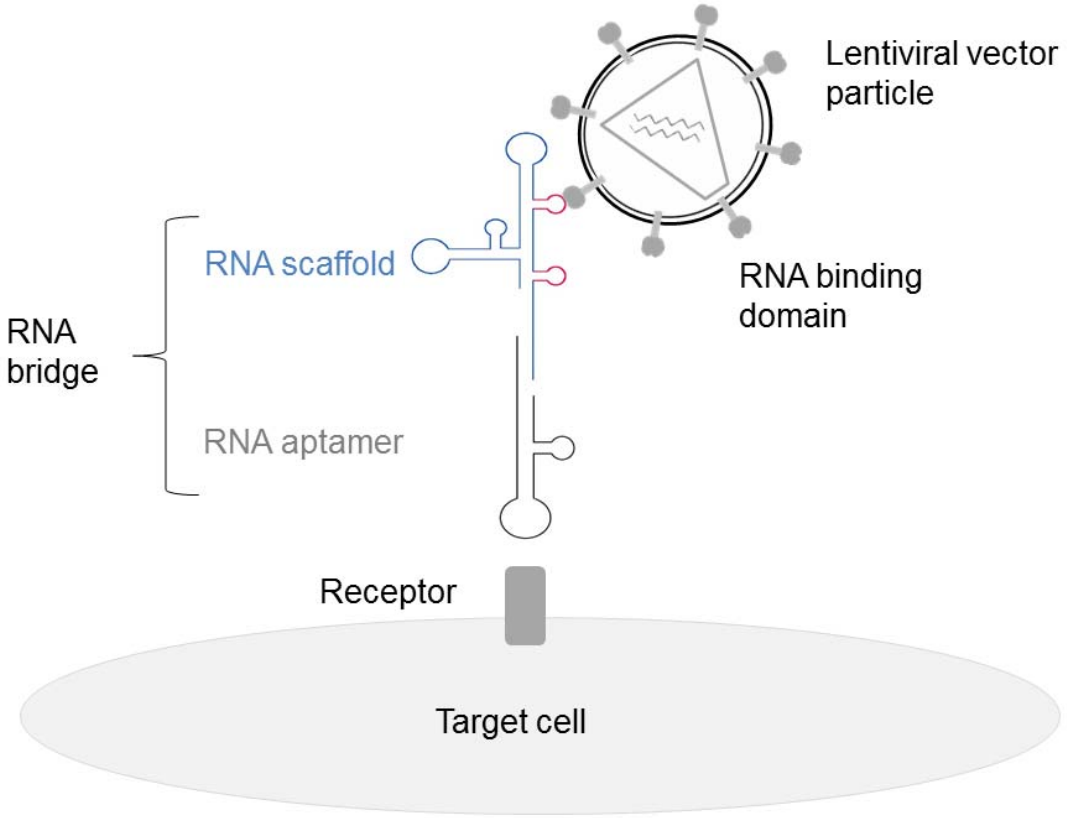
ÄKTA pure chromatography system

Samples	Transducing units, TUs (%)	TUs/mg protein
Input vector samples	100	$2.43 \times 10^4 \pm 1.21 \times 10^4$
Vector eluate	64.36 ± 11.43	$2.87 \times 10^6 \pm 0.15 \times 10^6$
Samples after desalting using Amicon units	60.45 ± 27.64	$2.85 \times 10^6 \pm 0.33 \times 10^6$

100-fold purification

Marino et al. *Gene Therapy* 22, 64-69 (2015)

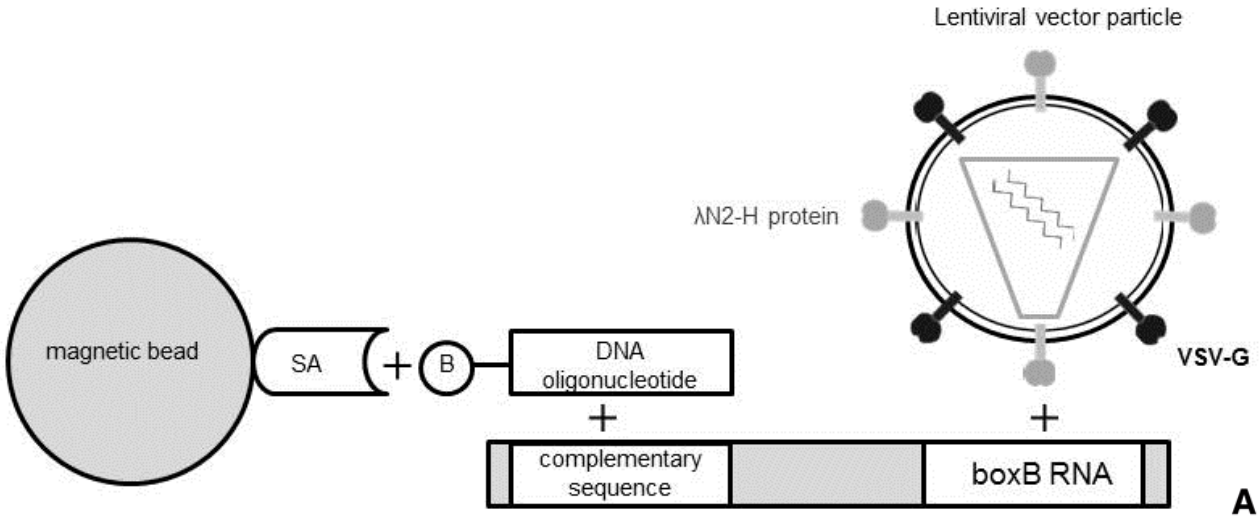
Depiction of a lentiviral vector targeting system involving RNA aptamers

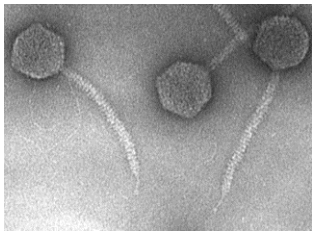


Advantages of aptamer approach:

- RNA sequence space is large
- High affinity aptamer variants are available/can be obtained
- Ease of design/selection/production

Binding of boxB RNA to lentiviral vectors displaying an λN2-H fusion protein





Bacteriophage λ

A

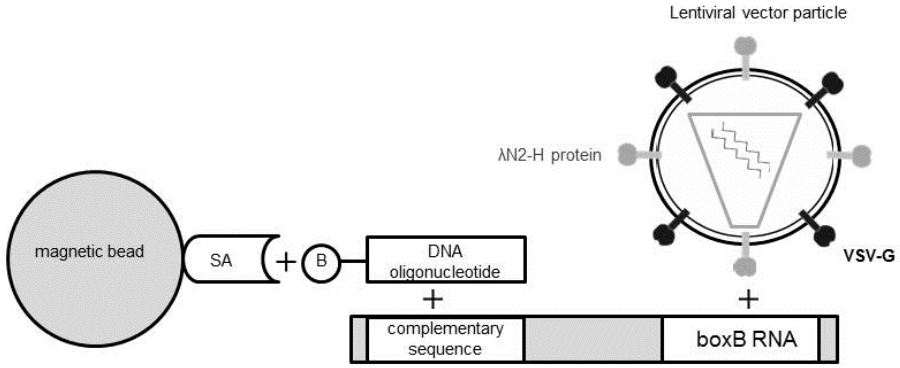
MDAQTRRRERRAEKQAQWKAAN

B

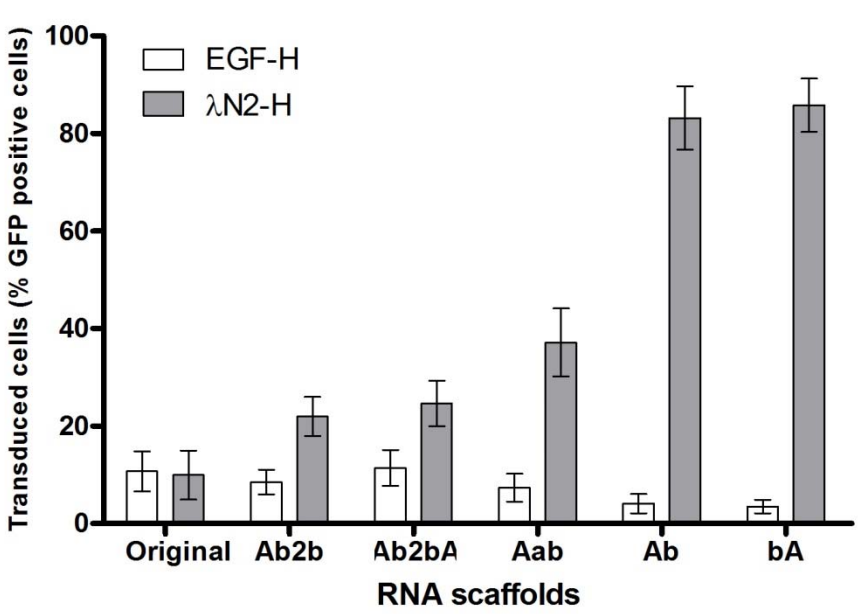
BoxB

Binding of boxB RNA to lentiviral vectors displaying an λN2-H fusion protein

A



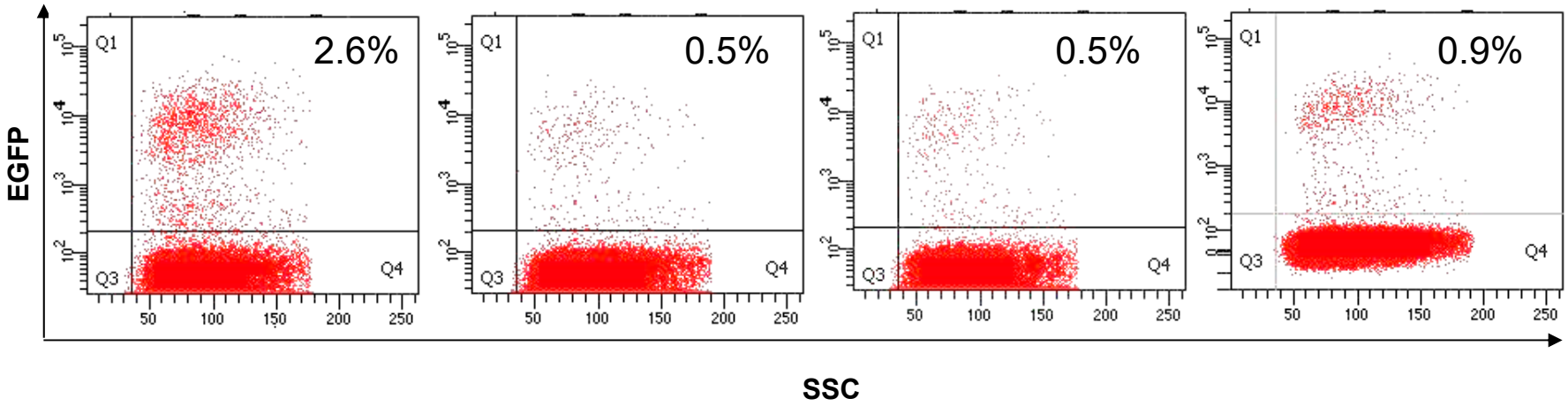
B



A431 cells

Transduction of human epidermoid carcinoma A431 cells using lentiviral vector particles displaying an EGFR-specific RNA aptamer

H-λN	+	+	+	+
Scaffold	+	-	+	+
Aptamer	+	-	+	+
RNase	-	-	+	-
EGF	-	-	-	+



Conclusions

- Strategy involving measles virus H and F proteins allowed selective transduction of IL-13Rα2 positive cells

- Aptamer strategy looks promising but needs optimization

Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector's tissue tropism
- Directing vector integration to genomic "safe harbor sites"
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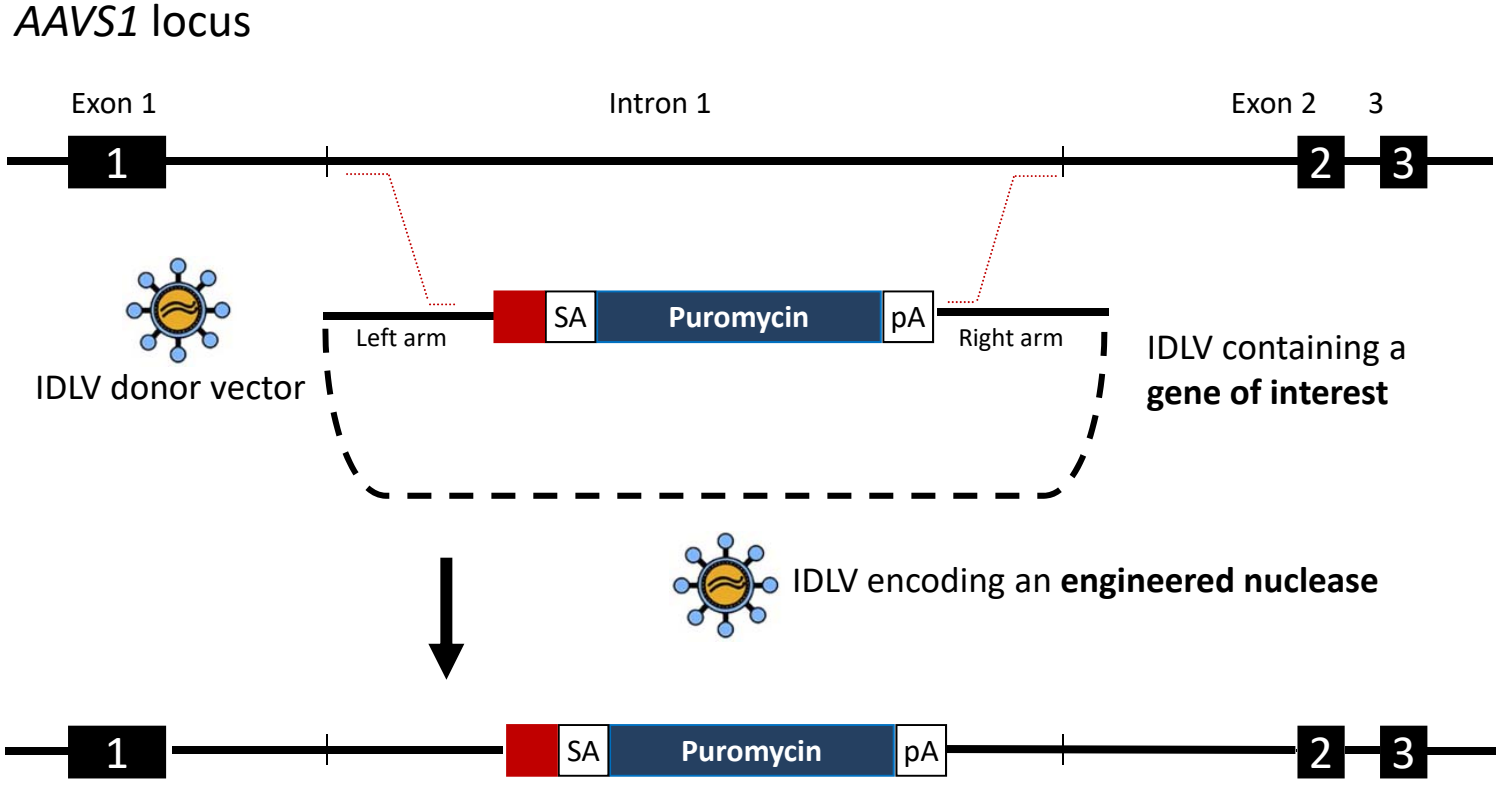
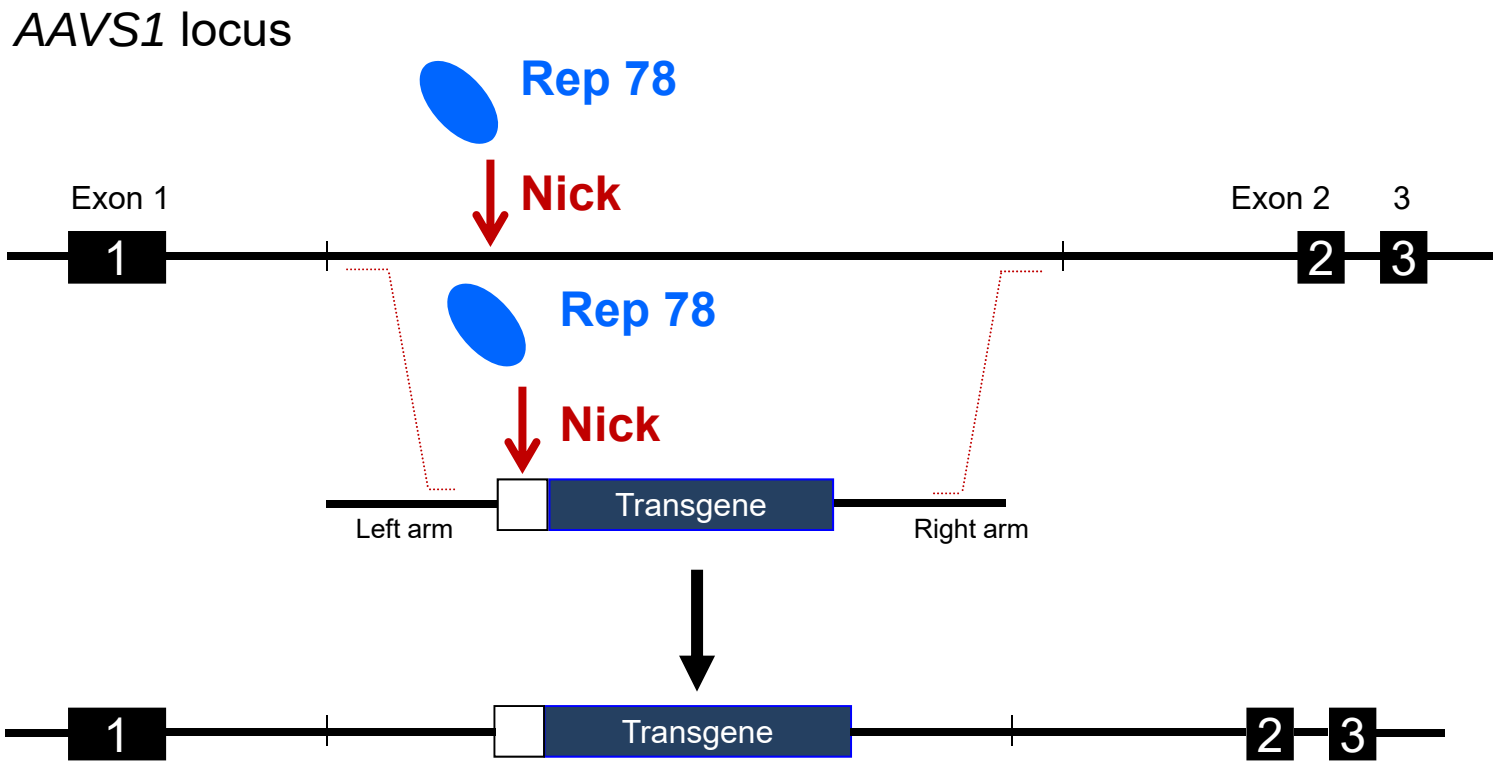
Nuclease-mediated integration of integrase-defective lentiviral vectors (IDLVs) at the *AAVS1* "safe harbor" site on chromosome 19



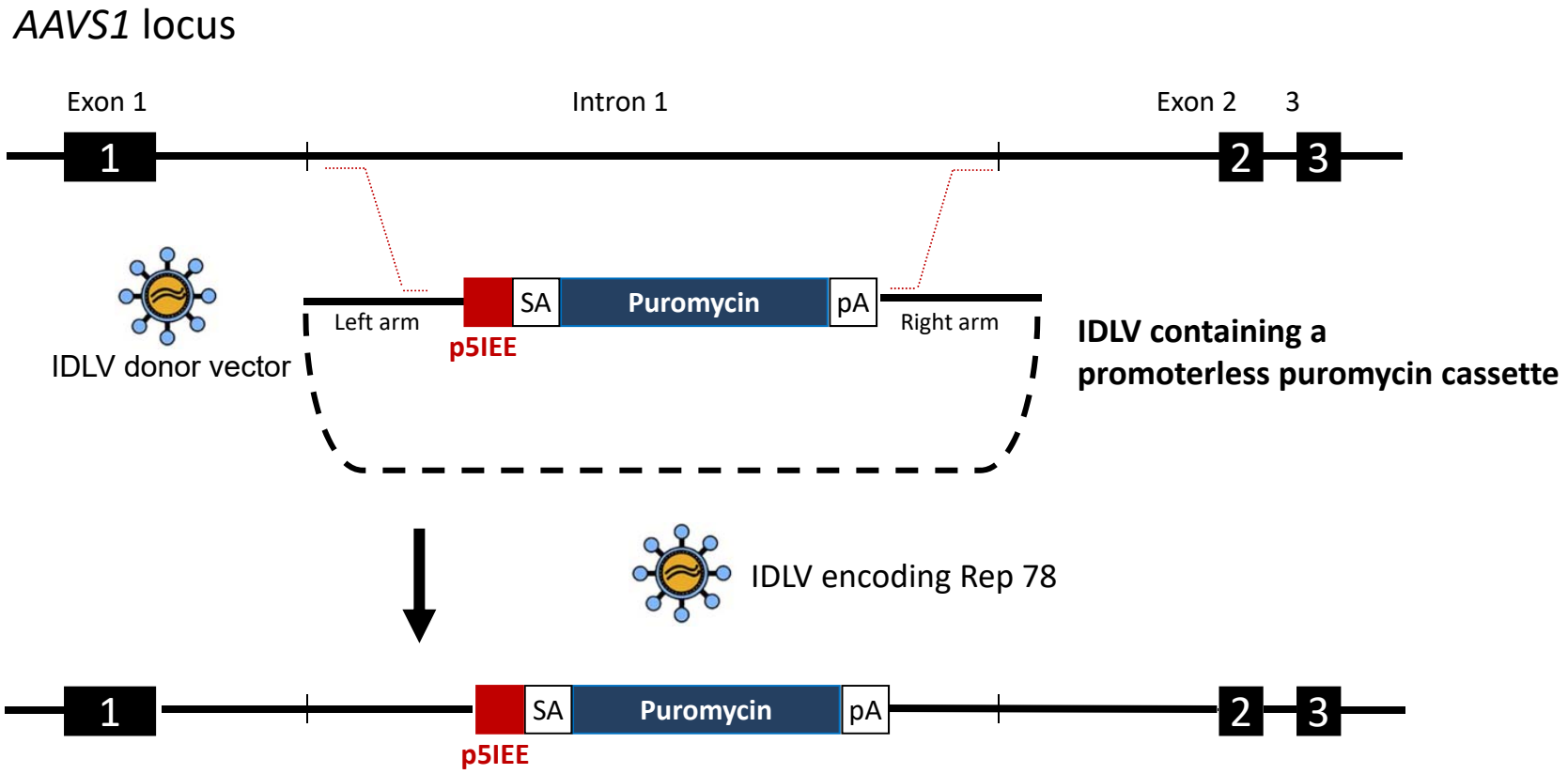
Puromycin cassette integrated at *AAVS1* site

Double nick strategy involving the AAV2 Rep protein to promote "homologous recombination" at the AAVS1 locus

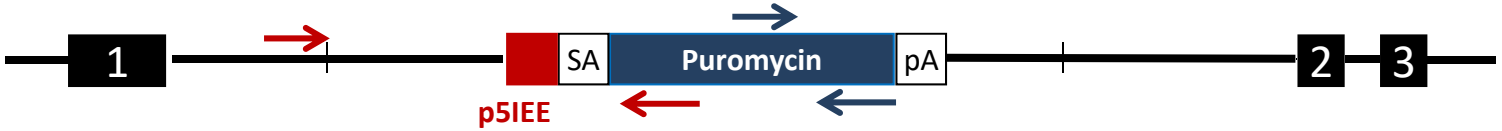


Gonçalves MA et al. *Nucleic Acids Res.* 40, 3443-3455, 2012

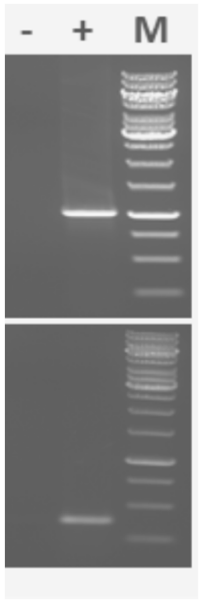
Rep-mediated integration of IDLVs at the *AAVS1* "safe harbor" site



Rep and ZFN-mediated insertion of transgene sequences at the *AAVS1* site mediated by IDLVs



Samples	Number of puromycin-resistant clones*	PCR positive clones
Donor	66 ± 12	0% (0/10)
Donor + Rep	275 ± 14	84% (21/25)
Donor + ZFN	200 ± 10	100% (19/19)



Primers used:

AAVS1-Puro

Puro-Puro

*10^5 cells used for transduction

Conclusions

- The overall efficiencies of the nuclease/nickase approaches need to be improved

- ZFNs and Rep can be toxic if expressed at high levels

Approaches to improve the safety of lentiviral vectors

- Reducing the likelihood to form replication-competent lentivirus
- Narrowing the vector's tissue tropism
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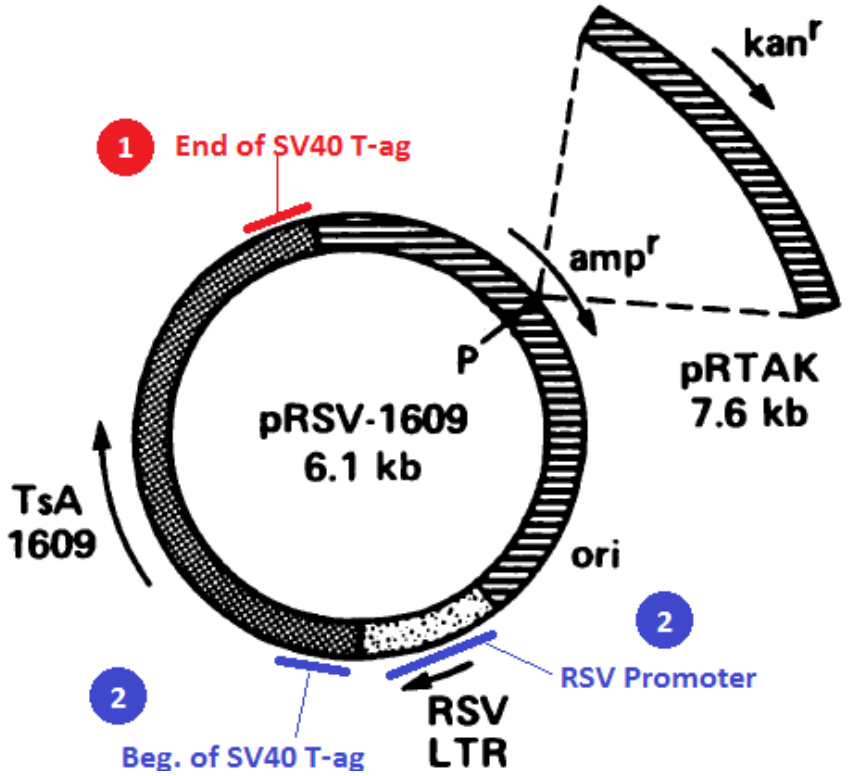
Design of a HEK 293T cell line bearing a genomic deletion of the SV40 T antigen coding region

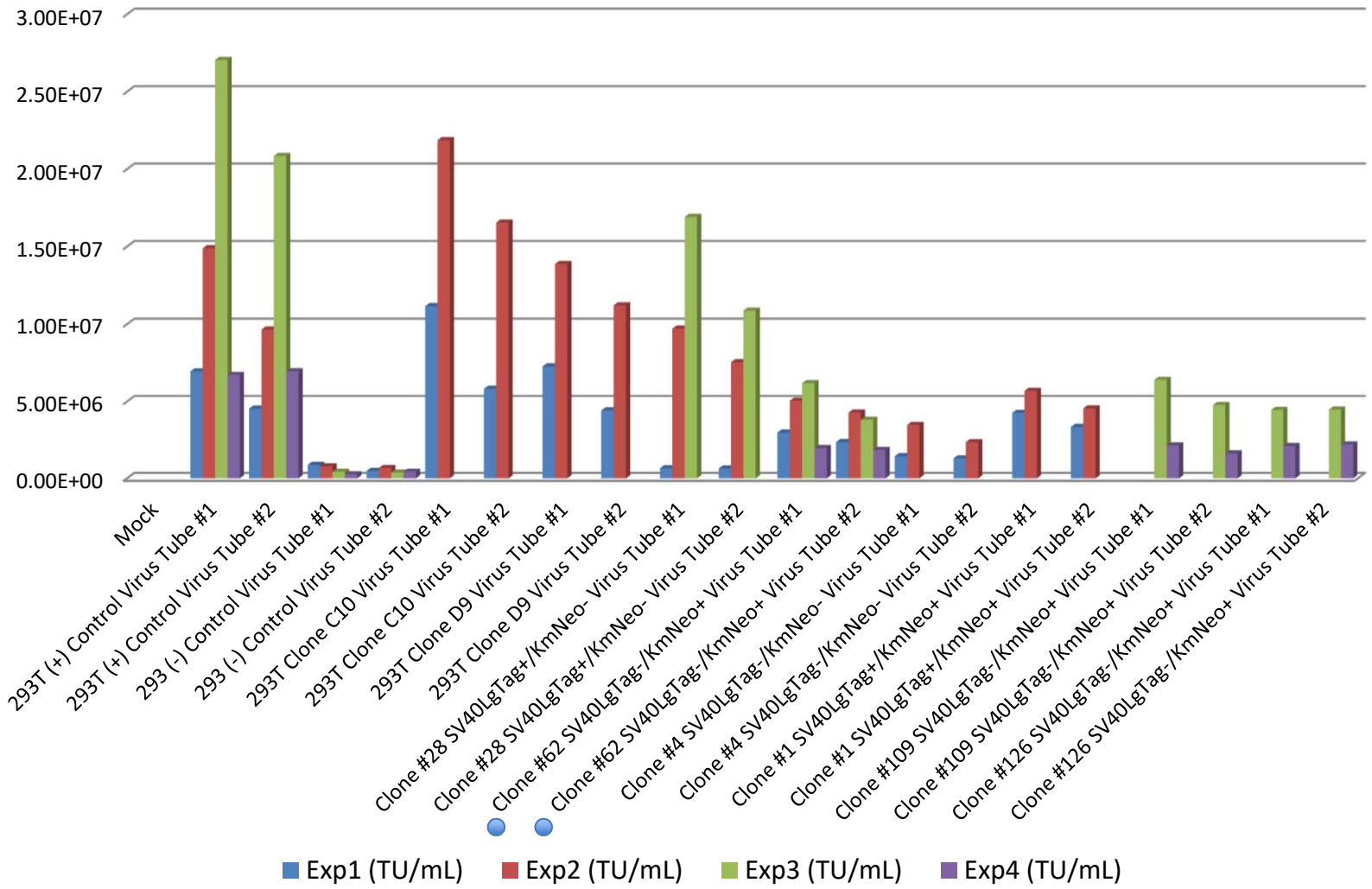


Heinzel et al. *J. Virol.* 62, 3738-3746 (1988)

PCR screens

Total # of clones screened by PCR	176
• # of clones negative both for SV40 T-ag and Km/Neo resistance gene sequences	55
• # of clones negative for Km/Neo sequence	37
• # of clones negative PCR result for SV40 T-ag sequence	3 **Clone #62,** **Clone #109,** **Clone #126**
• # of wild type clones	81

Lentiviral vector production using modified HEK 293T cell clones



Conclusions

- Three out of the 176 cell clones screened revealed deletions of the T antigen sequence

- Vector titers for the three T antigen knock out clones were lower than those obtained using HEK 293T cells but higher than those obtained using HEK 293 cells

Acknowledgments

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Questions?