

Session II

Review of Strategies to Promote Persistence of T cells

GENE DELIVERY CONSIDERATIONS

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MD Anderson Cancer Center

September 10, 2013

Session begins 2:30 PM

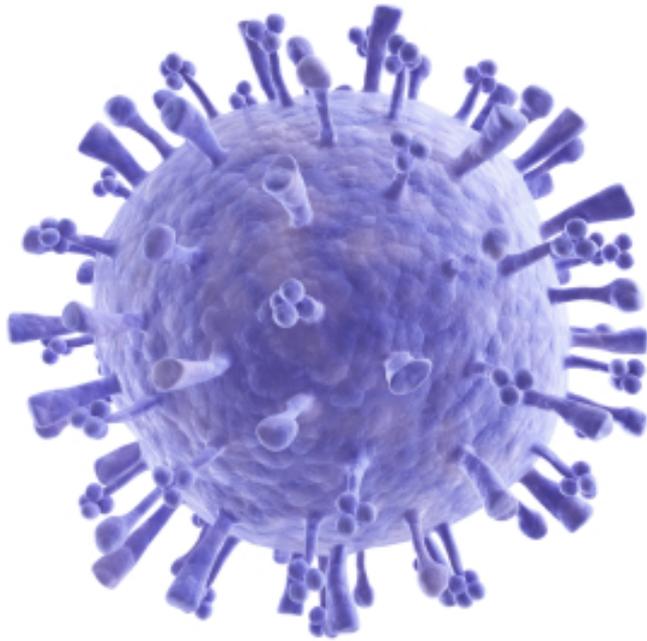


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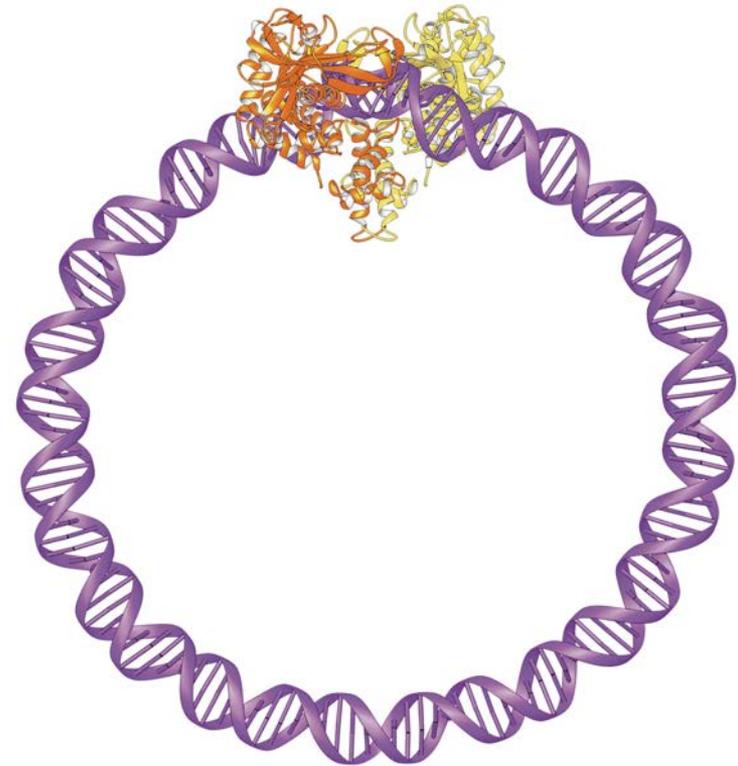


Gene Delivery Systems for CAR-Based T-cell Immunotherapy: An overview of what's being deployed in the clinic

Retroviral/Lentiviral



Transposase/Transposon



CAR CD19-specific Trials

Institute	Clinical trial.gov identifier	Vector for T-cell modification	Env	CAR Design	References
BCM	NCT00586391	Gammaretrovirus (MoMLV)	GALV (PG13)	FMC63 CD19scFv-IgG1 Fc- CD28-CD3z	Savoldo et al., JCI 2011
	NCT00840853				
	NCT00709033				
FHCRC	NCT01475058	Lentivirus (SIN-hEf1a)	VSV.G	FMC63 CD19scFv-IgG4 Fc- CD28-CD3z -T2A-huEGFRt	Wang et al., Blood, 2011
MDACC	NCT00968760	Plasmid (SB hEF1a Transposon/Transposase)	N/A	FMC63-CD19scFv-IgG4 Fc-CD28-CD3z	Singh et al., PLOS One, 2013
	NCT01497184				
	NCT01362452				
MSKCC	NCT01430390	Gammaretrovirus (MoMLV)	GALV (PG13)	SJ25C1 CD19scFv-CD8a-CD28-CD3z	Brentjens et al., CCR, 2007
	NCT00466531				
	NCT01416974				
NCI	NCT01044069	Gammaretrovirus (MSCV)	RD114 (293-GP)	FMC63 CD19scFv-CD28(114-153)-CD28-CD3z	Kochenderfer et al., 2009 J of Immunotherapy
	NCT00924326				
	NCT01087294				
UPENN	NCT01593696	Lentivirus (SIN-hEf1a)	VSV.G?	FMC63 CD19 scFV-CD8a-CD8aTM-4-1BB-CD3z	Porter et al., 2011, NEJM
	NCT01029366				
	NCT01626495				

MoMLV - Maloney murine leukemia virus
 MSCV - Mouse stem cell virus
 huEGFRt- human EGFR truncated

GALV - Gibbon Ape Leukemia Virus
 VSV.G = Vesicular Stomatitis Virus glycoprotein
 RD11.4 - Endogenous Feline Virus glycoprotein

Retroviral and lentiviral constructs are flanked by Long Terminal Repeats (LTRs). Transposon constructs are flanked by IR/DR-type inverted repeats

General Comparison of viral versus transposon based gene delivery systems

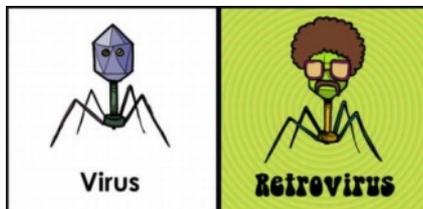
Retroviral/Lentiviral

Advantages

- Efficient gene delivery
- High expression of transgene
- Well characterized systems
- Flexibility of pseudotyping env genes of choice to maximize transduction efficiency

Disadvantages

- Potentially hazardous – need to demonstrate lack of replication competent virions in each prep
- Requirement for packaging cell line (labor intensive)
- Expense of production/release of clinical-grade material
- Concern over integration at sites of active transcription
- May activate oncogenes
- Requires T-cell activation for transduction (including lentivirus)



Sleeping Beauty

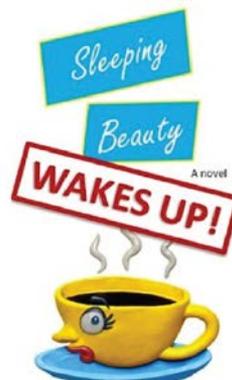
Transposase/Transposon

Advantages

- More efficient than “naked” DNA
- High level of expression
- Plasmid DNA is not cost prohibitive and not hazardous to produce (allows for testing of multiple constructs)
- Does not appear to integrate at sites of active transcription (not shown to activate oncogenes)
- Can electroporate whole resting PBMC and then numerically expand T cells

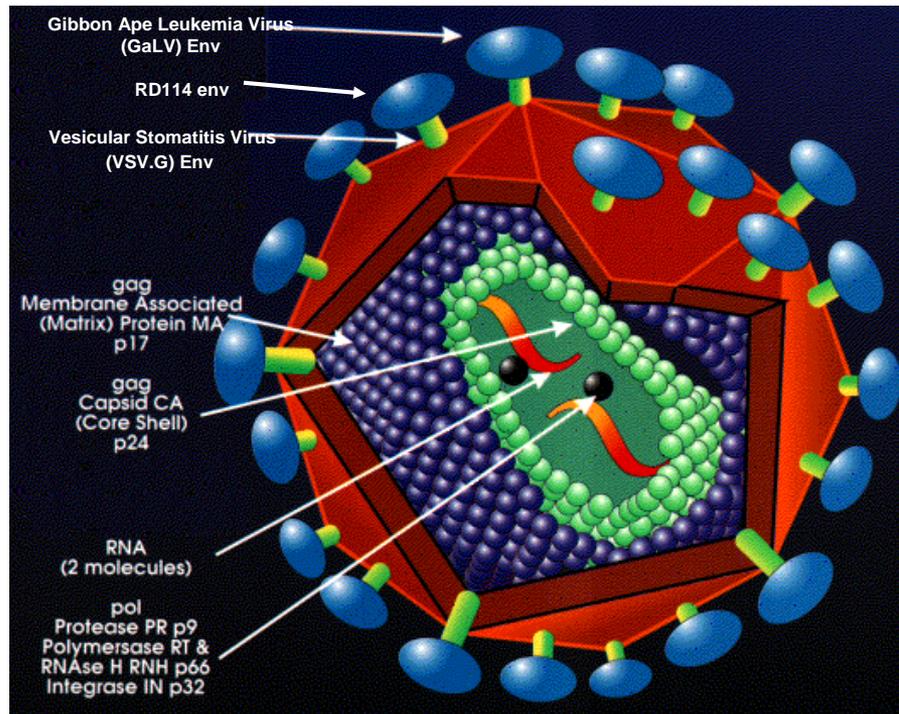
Disadvantages

- Low to mid/variable transfection efficiency
- Ensure that the transposase does not integrate (separate non-integrating expression plasmid is used or *in vitro*-transcribed mRNA) to prevent introduced transposon from “jumping”



Retroviral and Lentiviral Gene Delivery

Viruses offer the flexibility of pseudotyping with multiple env proteins but are encoded by infectious genomes.



Lentivirus

It is required to separate the gag/pol, env and LTR flanked transgene into separate plasmids for transfection to limit the chance of the generation of replication competent virions. Must test for p35 expression as from HIV in cell after transduction. (Larger cargo load).

Retrovirus

Engineering of permanent viral producing cells (e.g. PG13 co-expressing gag/pol/GALV cells) is acceptable.

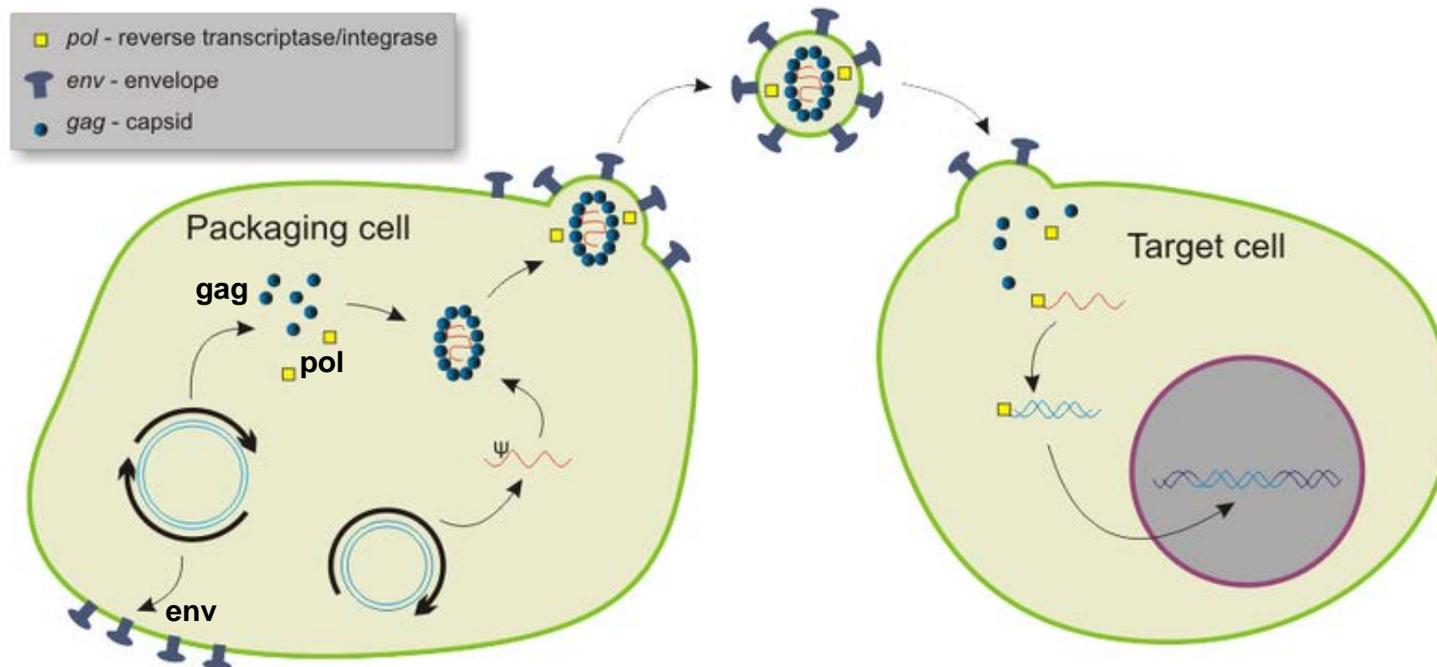
Note

To express GALV or RD114 on lentivirus, a chimeric env protein consisting of the TM/cyt domains of the Amphotropic Murine Leukemia Virus env and the ectodomain of GALV or RD114 must be used (GALV/TR , RD114/TR)

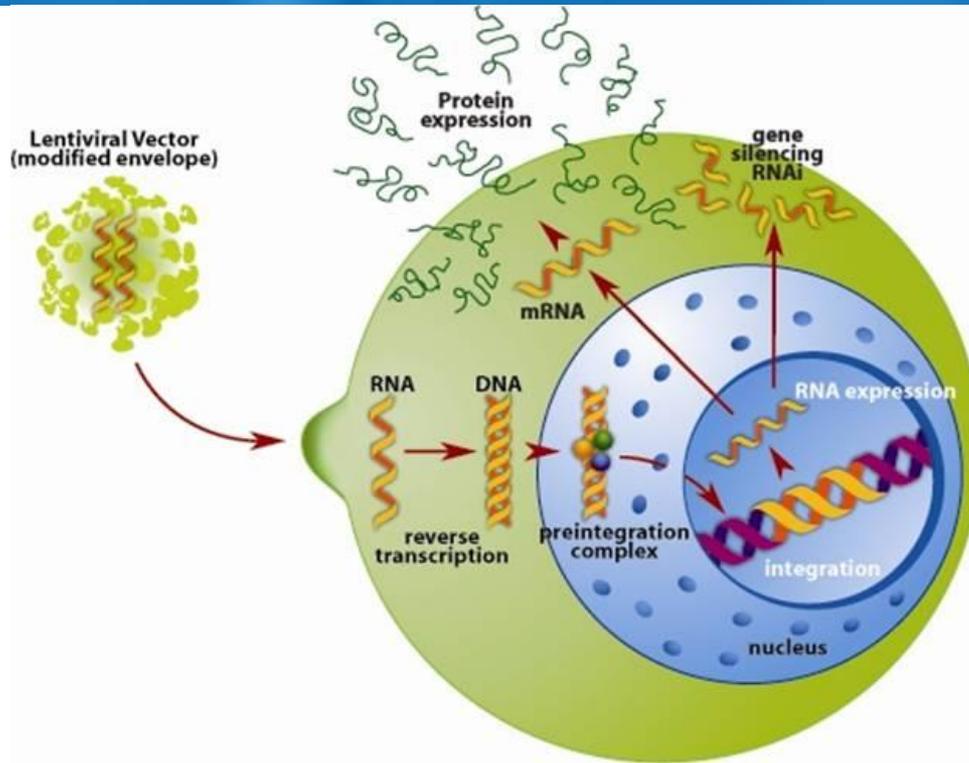
Envelope Protein	Receptor	Cell type transduced	Toxicity
GALV	sodium-dependent phosphate transporter protein (Pit-1)	Lymphocytes and hematopoietic cells	Low
RD114	neutral amino acid transporter SLC1A5	Stems cells and hematopoietic cells	Low
VSV.G	LDL receptor and its family members	Pantropic	High

Retroviral and Lentiviral Gene Delivery

- Requires the production of genomic-integrating replication-deficient virus
- Employs helper plasmids and packaging cells
- Production is complex as it needs manufacturing components for safety.
 - Lentiviral production is dependent on transient transfection of packaging cells.
 - Retroviral production can be based on “stable” packaging cell lines.



Retroviral and Lentiviral Transduction



Advantages

- High transduction efficiency and level of transgene expression

Considerations

- Gene silencing via LTR methylation: more prominent in MoMLV- than MSCV- (from MoMLV, but less prone to LTR methylation) based retroviruses
- Use of self inactivating (SIN) viruses with mutated 3' LTRs and internal promoters offer flexibility of internal promoter choice (cellular compatibility and decreased promoter methylation)
- All lentiviruses used for gene therapy are SIN

Institutions using Lentivirus

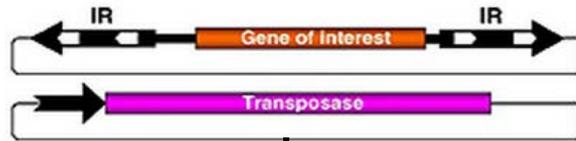
- University of Pennsylvania (VSV.G)
- Fred Hutchinson Cancer Research Center (VSV.G)

Institutions using Retrovirus

- National Cancer Institute (MSCV; RD114)
- Memorial Sloan-Kettering Cancer Center (MoMLV; GALV)
- Baylor College of Medicine (MoMLV;GALV)

The *Sleeping Beauty* Transposon/Transposase System

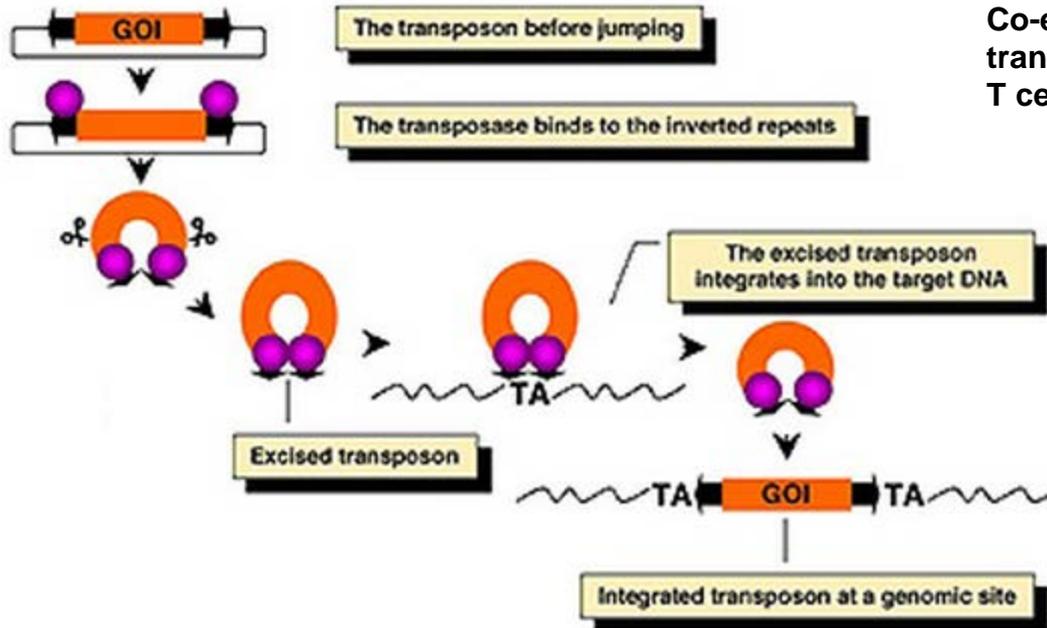
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Encode CAR between IR/DR-type inverted repeats

Encode Transposase in standard expression vector

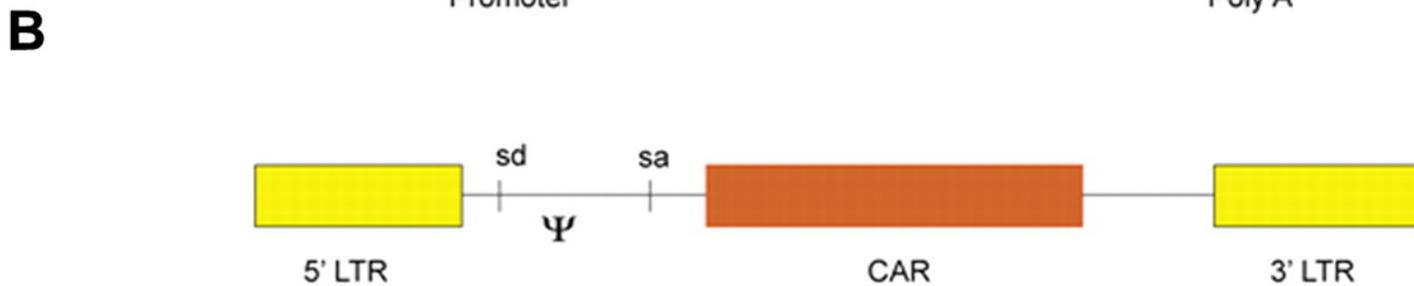
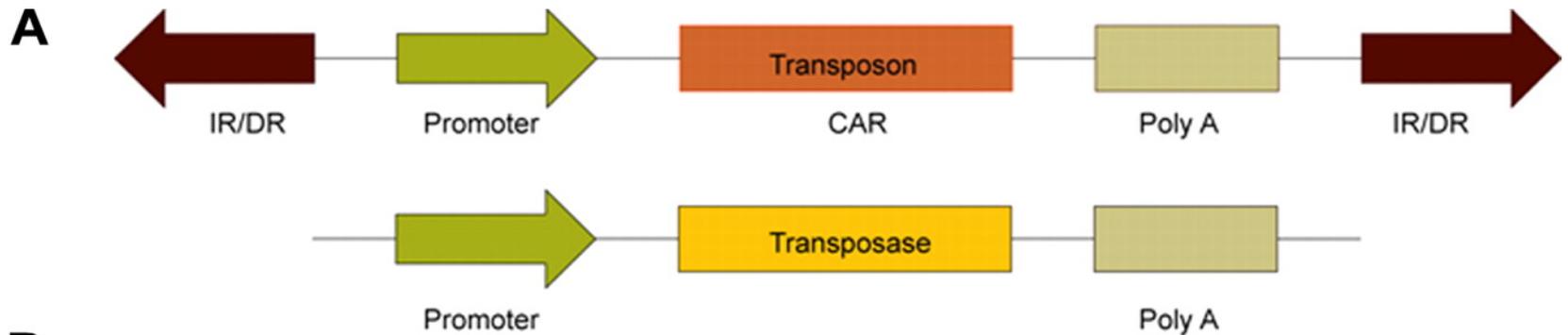
Each T cell prep is checked for integration of transposase via RT-PCR. None has been observed. Nevertheless, we are moving towards using mRNA



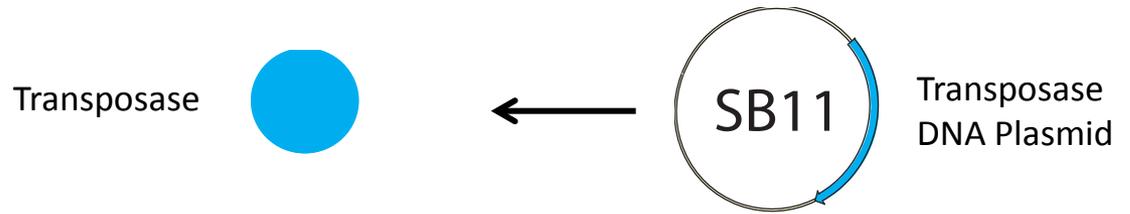
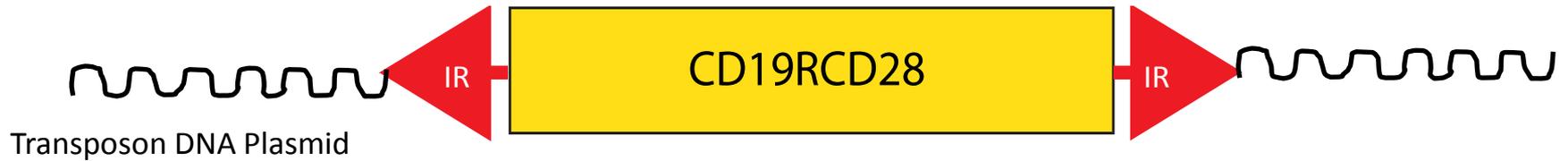
Co-electroporate the transposon and transposase plasmids into PBMC or T cells

Transposon integrates at TA rich sites in the genome. T cells expressing CAR can be selected on artificial APC expressing CD19

Vector systems to express CAR transgenes used in clinical trials

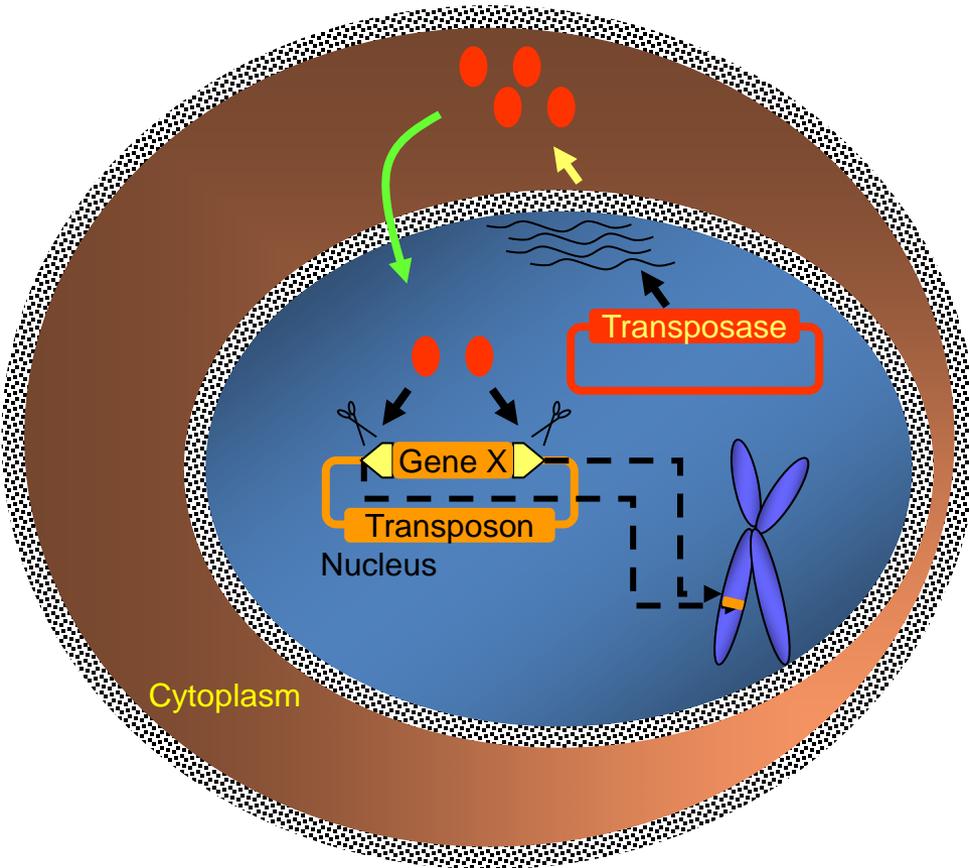


Sleeping Beauty transposition



T-cell genome

Potential Risk in using plasmid DNA as a source for SB11

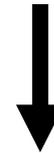


SB11 from plasmid DNA

Potential risk of integration
into host genome

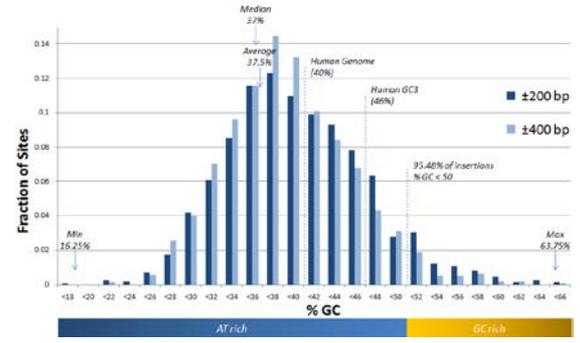
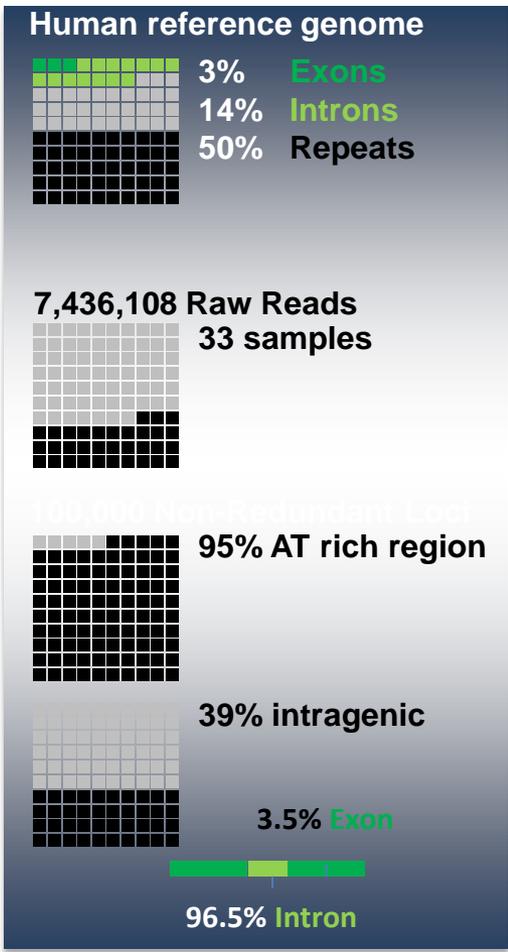
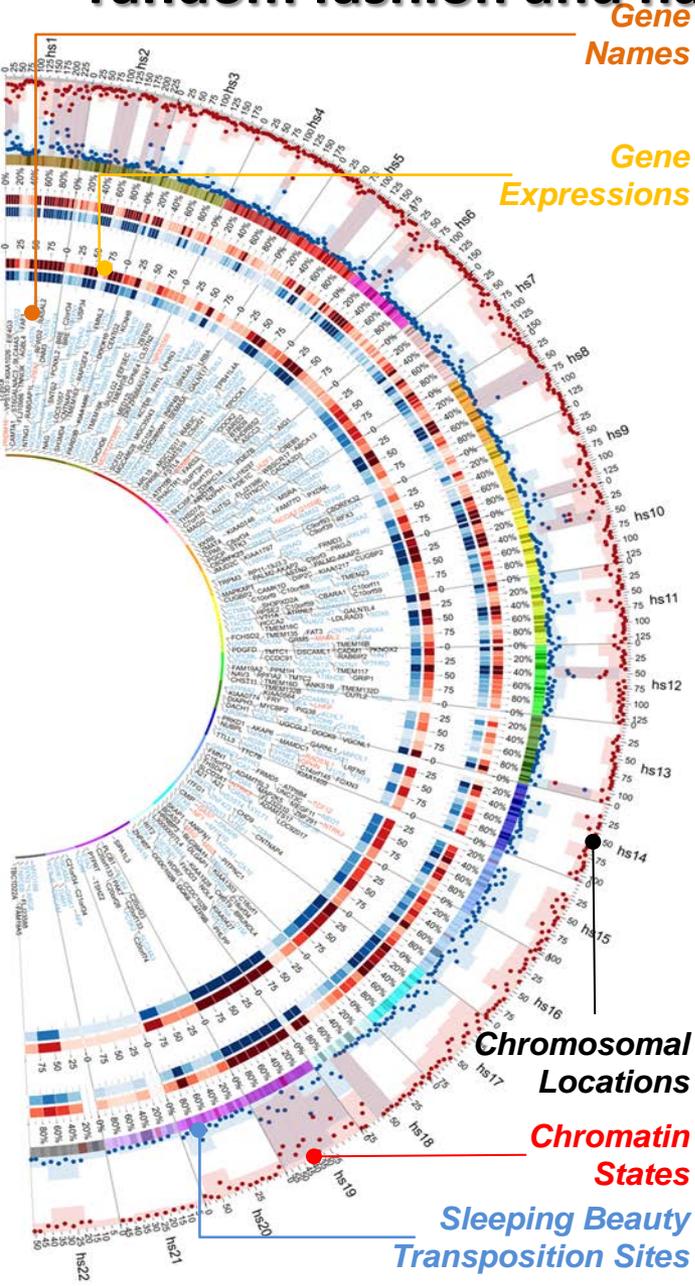


Re-excision of integrated transgene,
paste into another site
(transgene "hopping")



mRNA for Transposase

The Sleeping Beauty Transposon integrates into the T cell genome in a random fashion and has not been observed to activate oncogenes



- No obvious hotspots
- No insertion bias towards potentially dangerous loci (e.g. oncogenes [LMO2], tumor suppressors, miRNAs etc.).
- No insertions in constitutively heterochromatic centromeric regions of chr 1, 9, 16
- No insertions in constitutively heterochromatic regions of acrocentric chr 13, 14, 15, 21, 22
- TSS associated with quiescent T cells favored
- Conformationally open sites favored

Acknowledgements

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Thank You

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