RAC-CLINIGENE MEETING
December 2010

Recent advances with Sleeping Beauty and PiggyBac transposons for gene therapy

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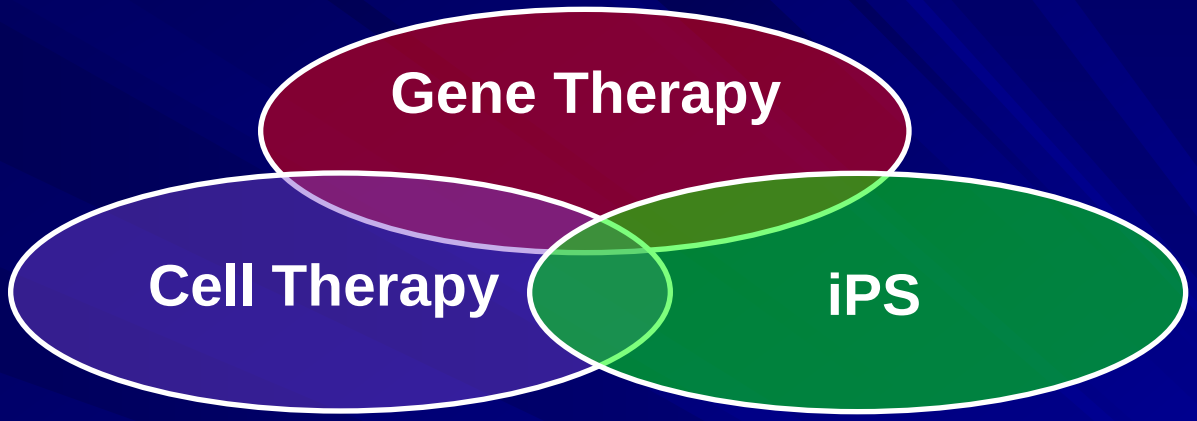
TRANSPOSON APPLICATIONS



ADVANTAGES TRANSPOSONS

- less immunogenic compared to viral vectors
- production at reduced costs
- facilitates implementation of clinical trial
- stable genomic integration: ideal for stem cells & iPS cells
- large transgenes (>10 kb)

TRANSPOSONS: MOBILE GENETIC ELEMENTS

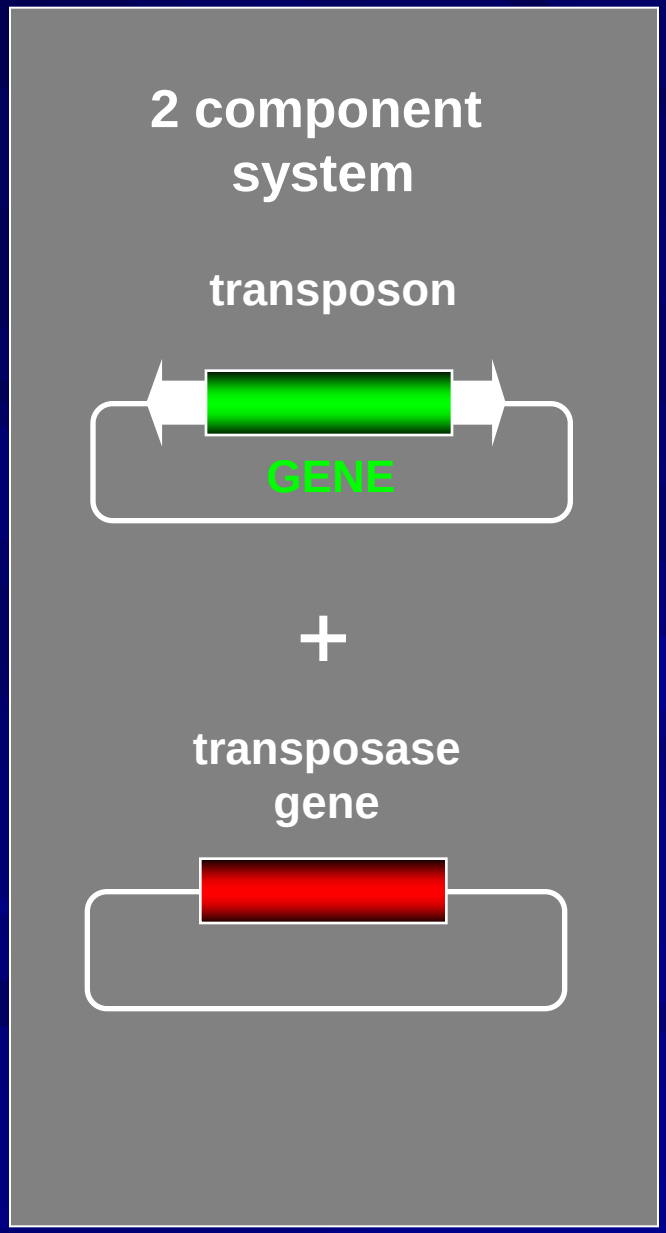
salmon

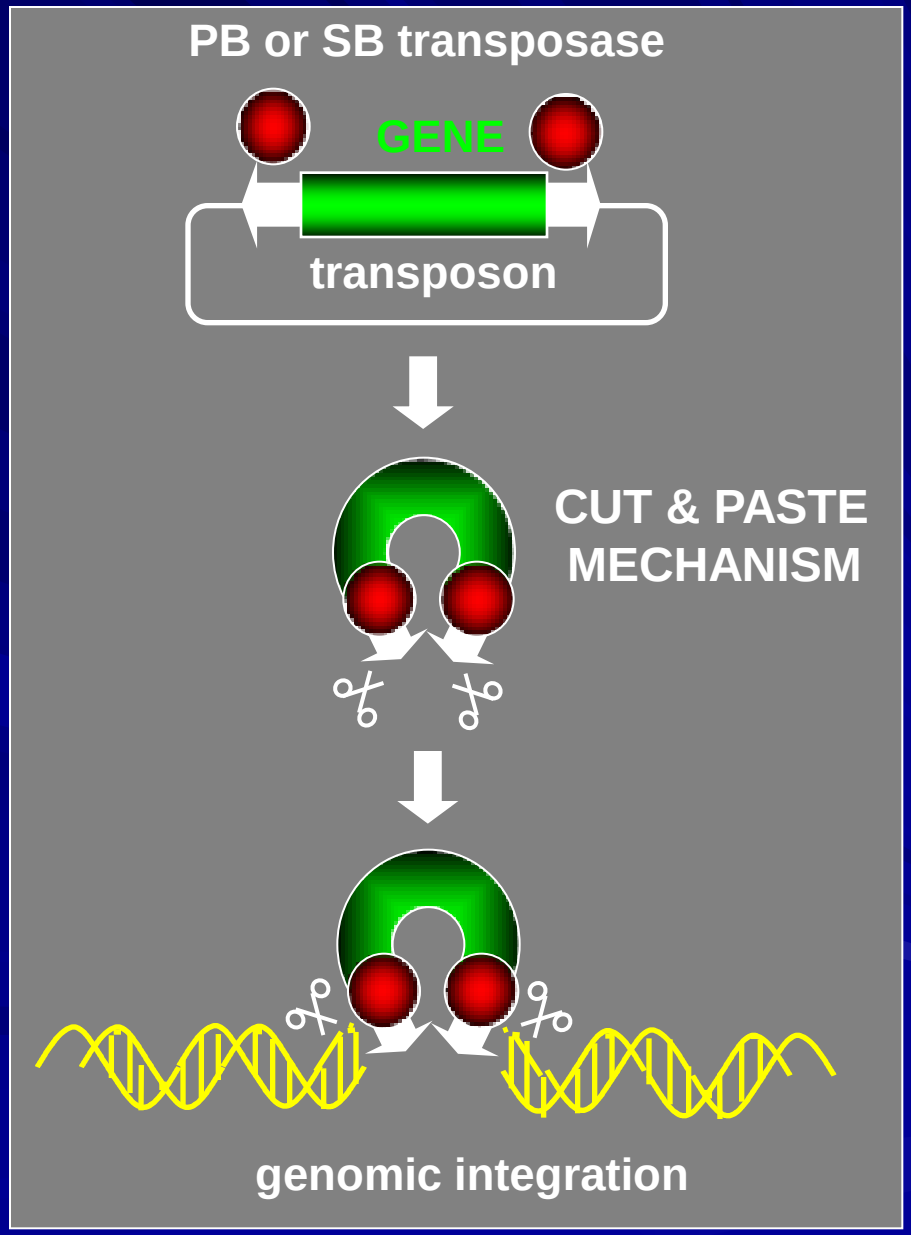
Sleeping Beauty

looper moth

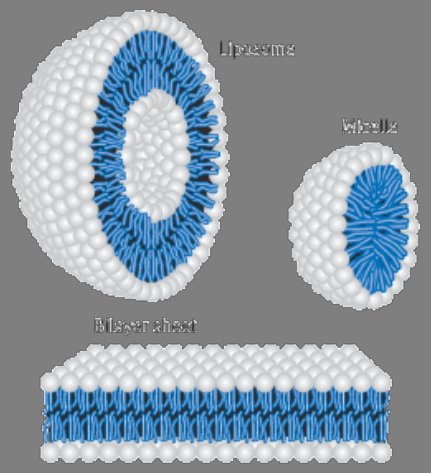
PiggyBac

SLEEPING BEAUTY AND PIGGYBAC TRANSPOSONS FOR GENE DELIVERY



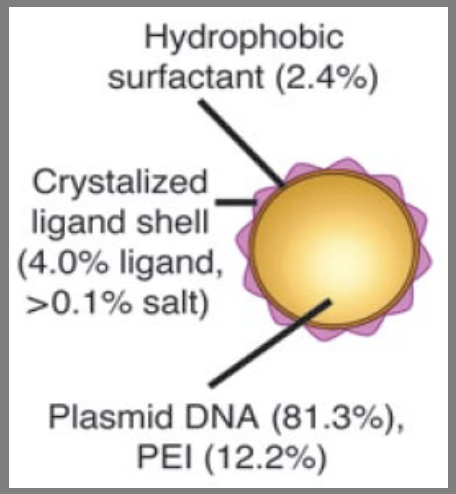


Non-viral methods



PEI
Lipofectamine

Belur et al 2007
Nature Protocols



nanocapsules

Kren et al 2009
J Clin Invest

nucleofection
electroporation

Mates, Chuah et al 2009
Nature Genetics

Limitations of early generation SB transposons:

Transposition efficiency relatively low in most primary cells

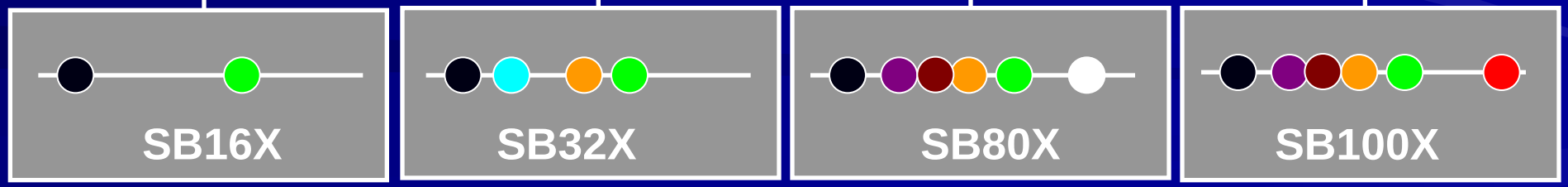
➡ NEED TO DEVELOP EFFICIENT TRANSPOSONS

Sleeping Beauty (SB)

selection evolutionary conserved hyperactive mutants

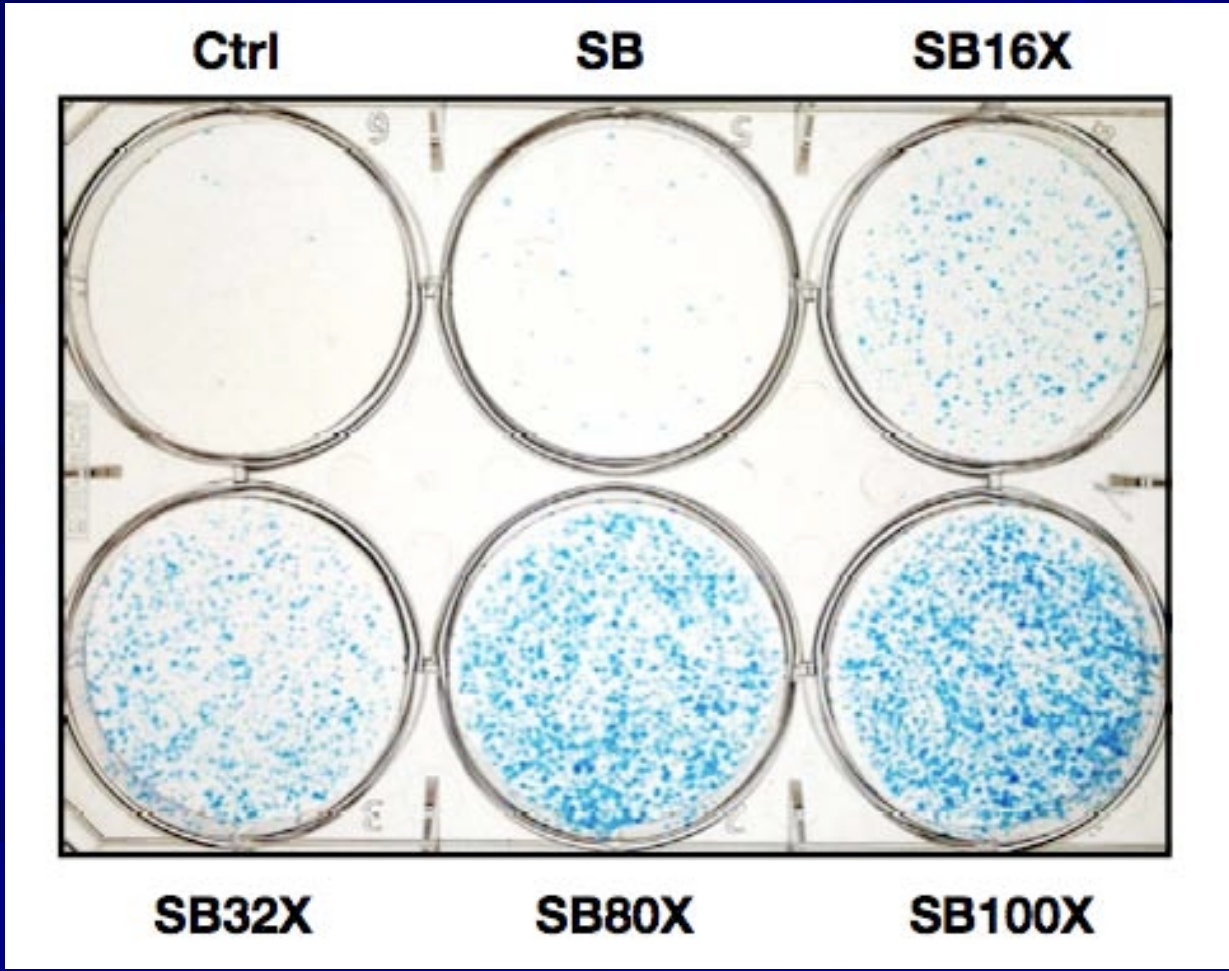
Generation SB library PCR-based DNA shuffling

high-throughput (n = 2000) selection



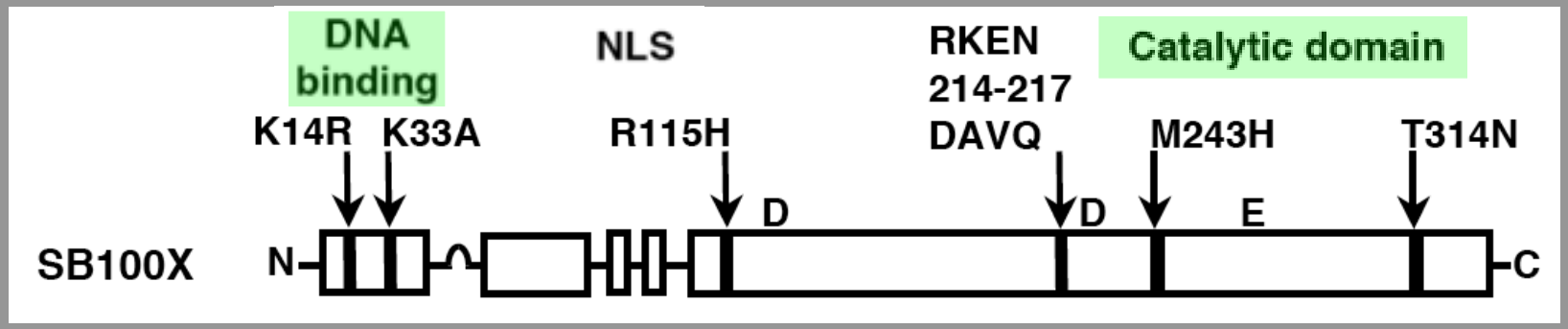
hyperactive transposases

Hyperactive SB selections in HeLa cells



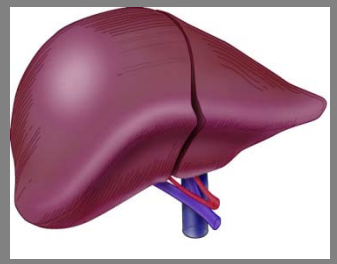
Neo^{R} selection

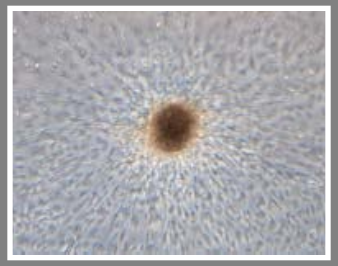
Hyperactivating mutations in SB100X



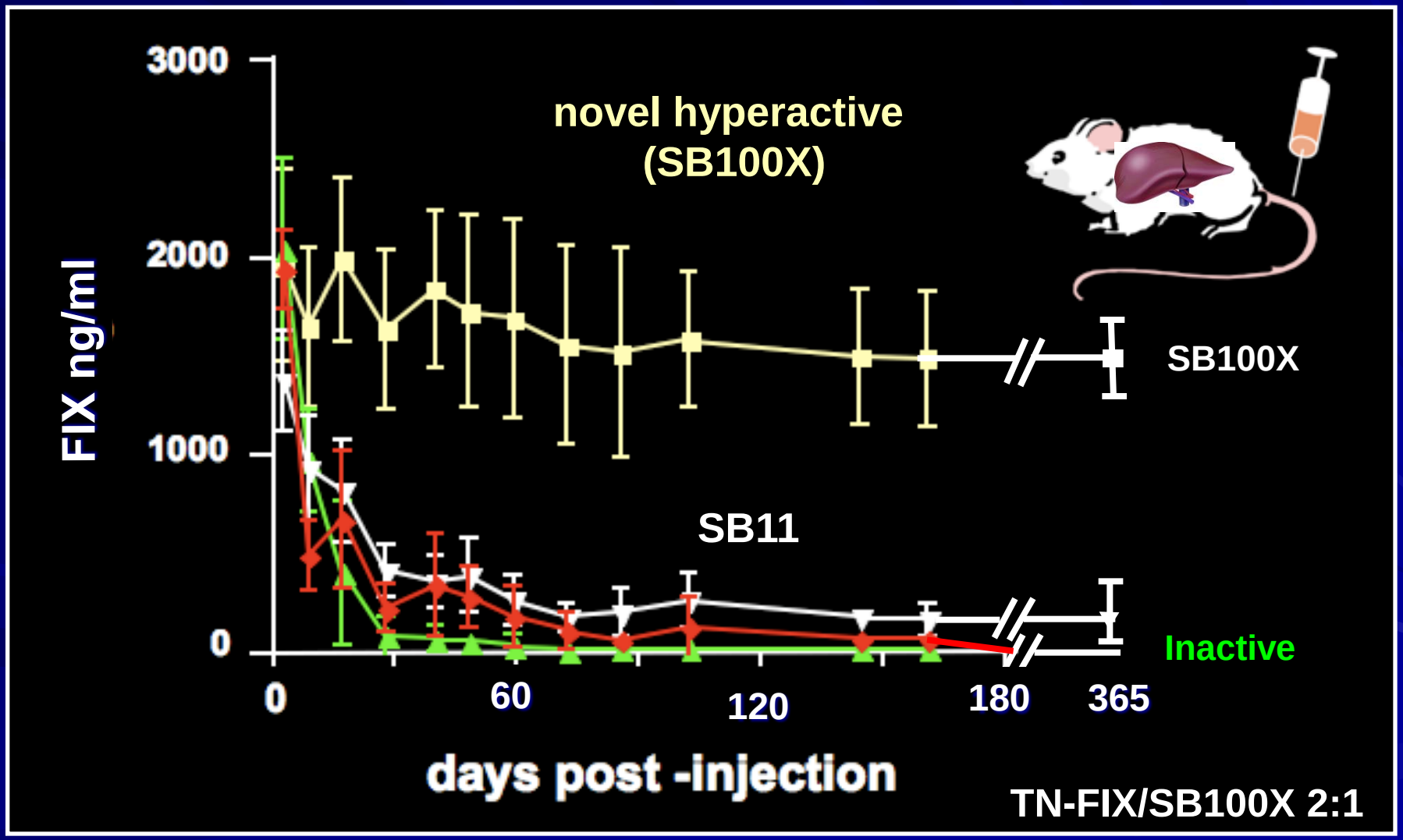
VALIDATION

liver	HSC	MSC	muscle	iPS



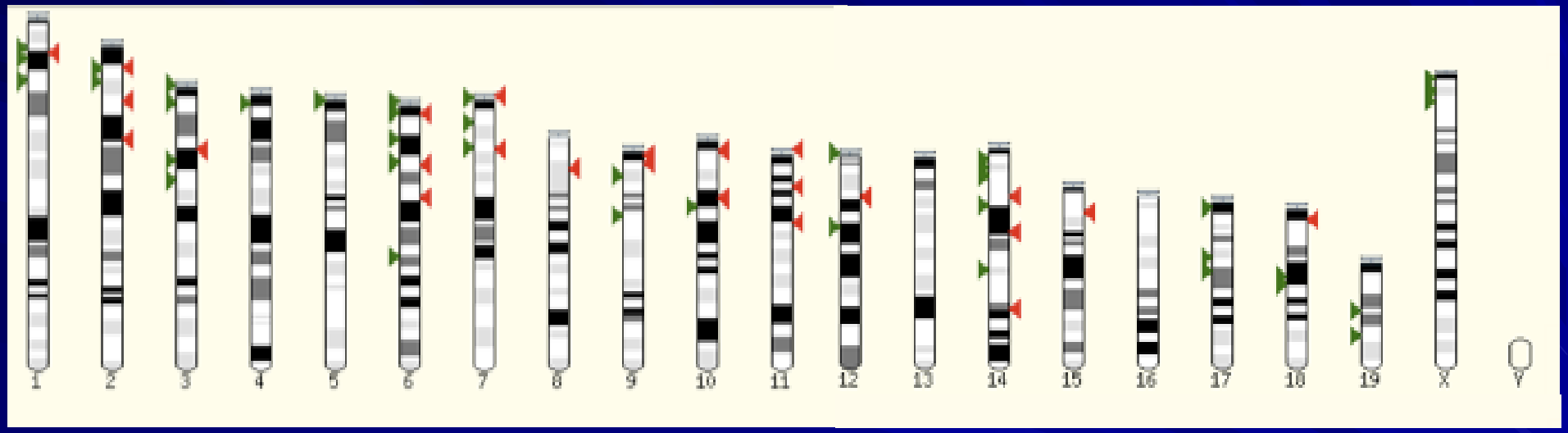


HYPERACTIVE SB TRANSPOSASES RESULT IN ROBUST GENE EXPRESSION IN LIVER



TRANSPOSON INTEGRATION SITE ANALYSIS

Clone	Junction with IR sequence in bold	E-value	Location, Homology
1.	TGTATATATATGTGTGTGTATATA**GACAGTTG**	1e-04	Chr16, not in gene, ref|NT_039625.7|Mm16_39665_37
2.	TGTGTTGTATATAAATAACCCTGGTATTGA**TACAGTTG**	6e-08	Chr9, not in gene, ref|NT_039474.7|Mm9_39514_37
3.	**CAACTGTA**GTATGATGTAACTACCCGGGGAAACATTTT GAAACATCTTAAATCTTTACACAAAGGGTA	2e-25	Chr19, not in gene, ref|NT_039687.7|Mm19_39727_37

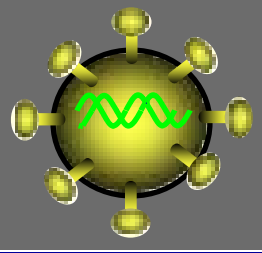


- 70% <u>not</u> in genes
- 30% <u>within</u> genes

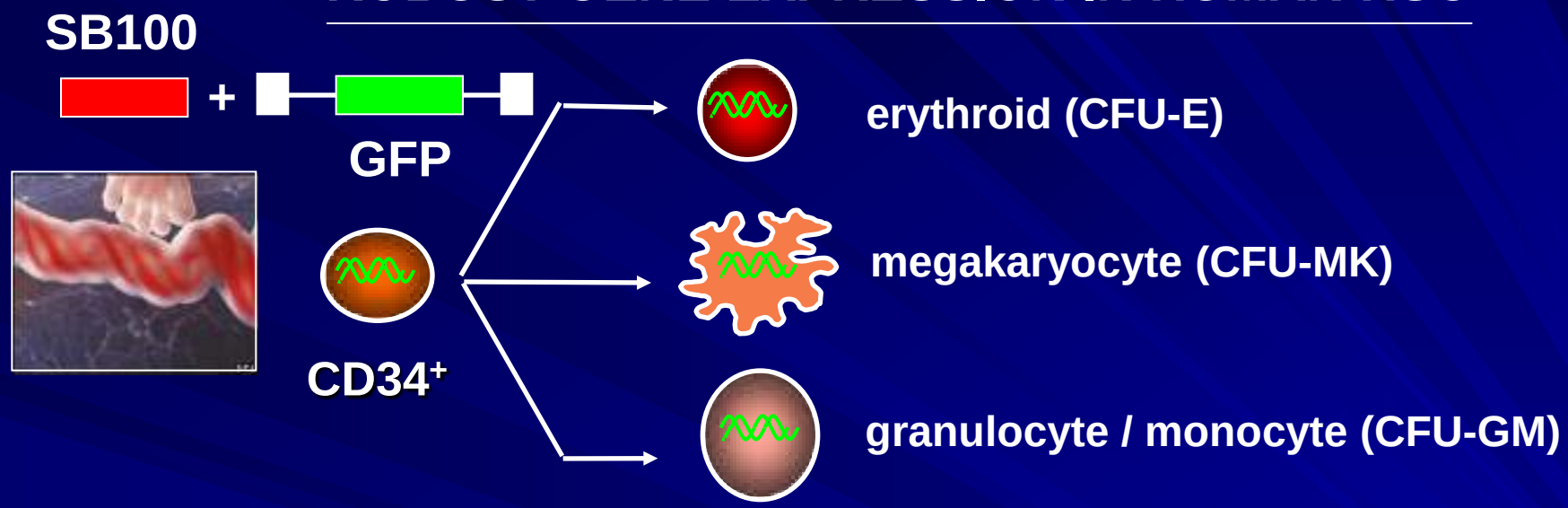
SAFER

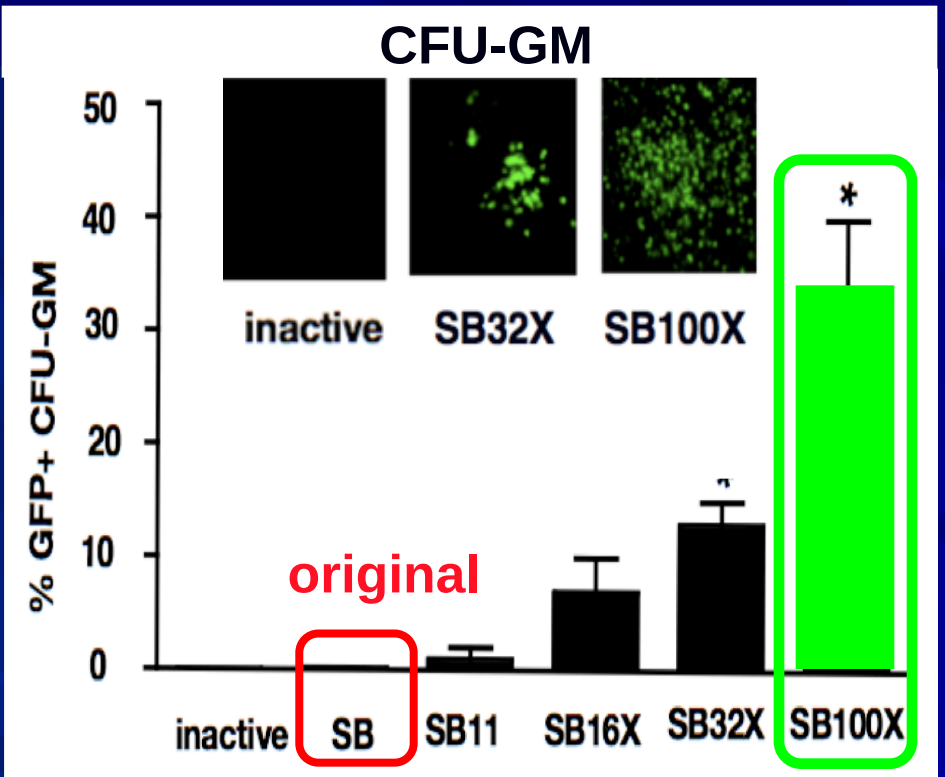
retro / lenti

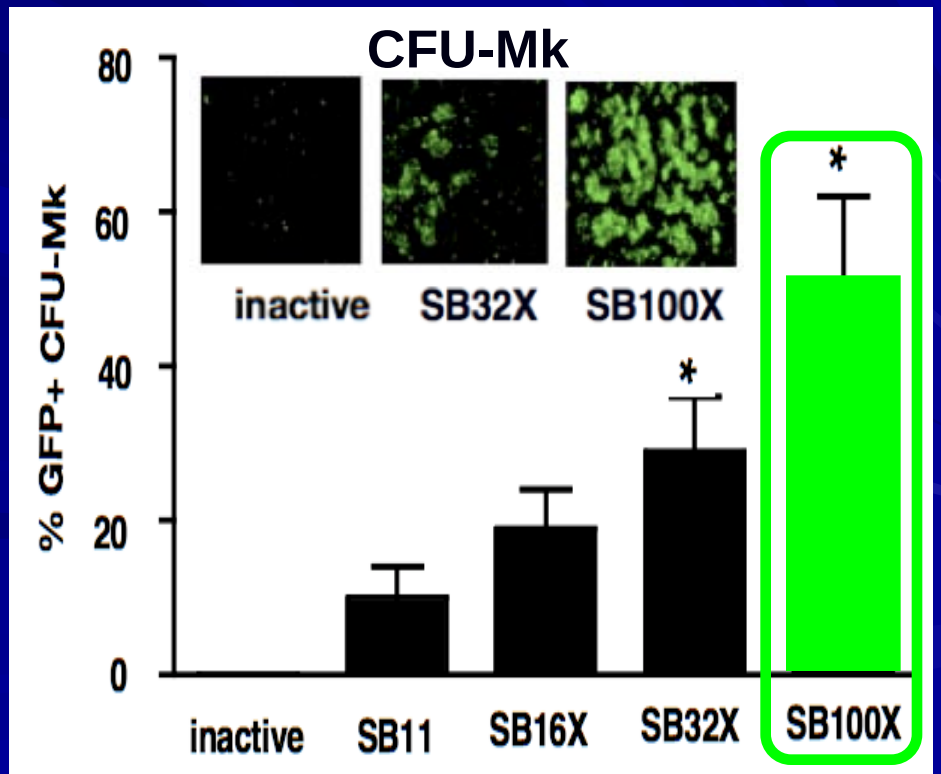
<u>no</u> preference for genes ⟷ preference for genes



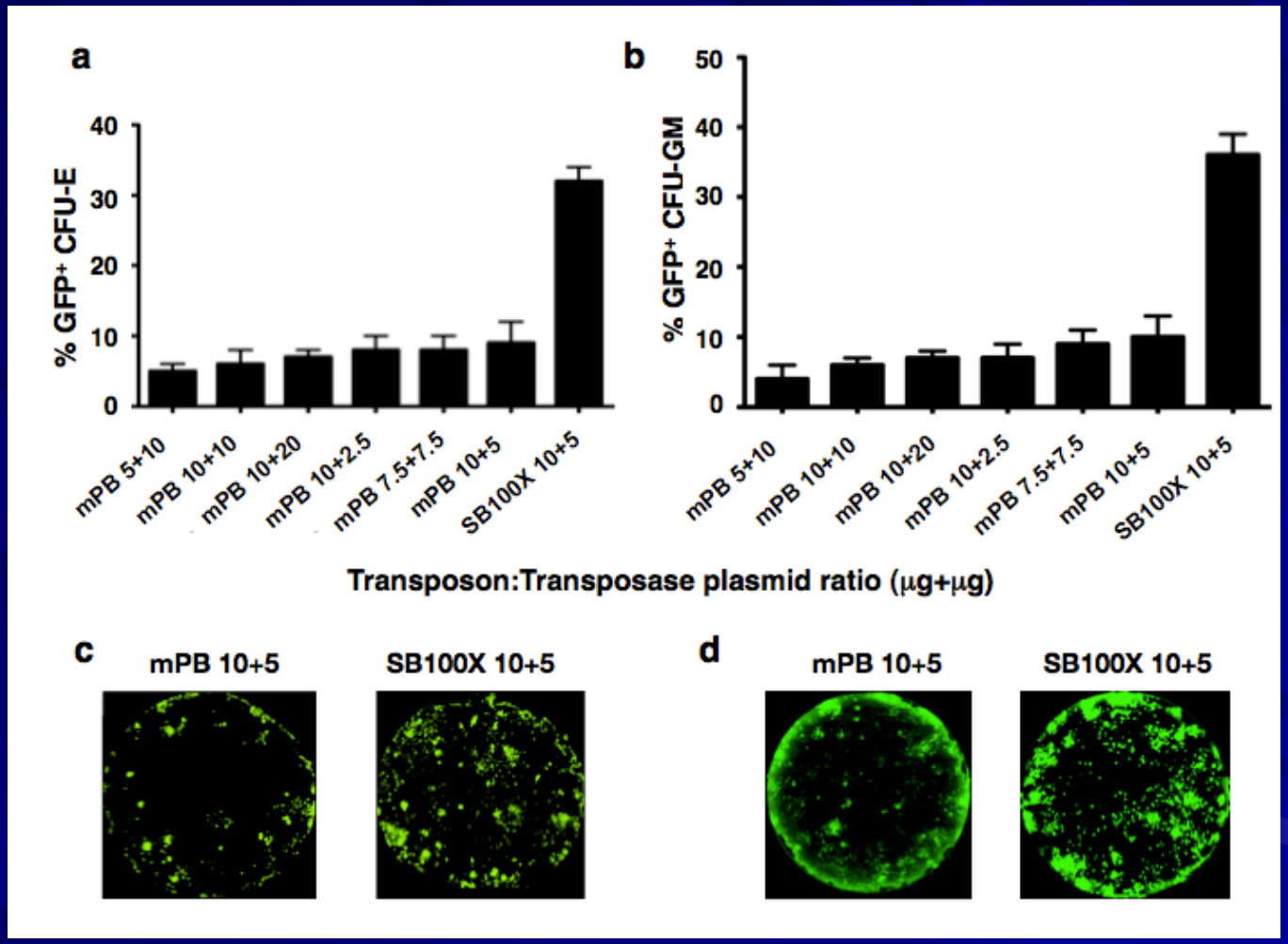
ROBUST GENE EXPRESSION IN HUMAN HSC



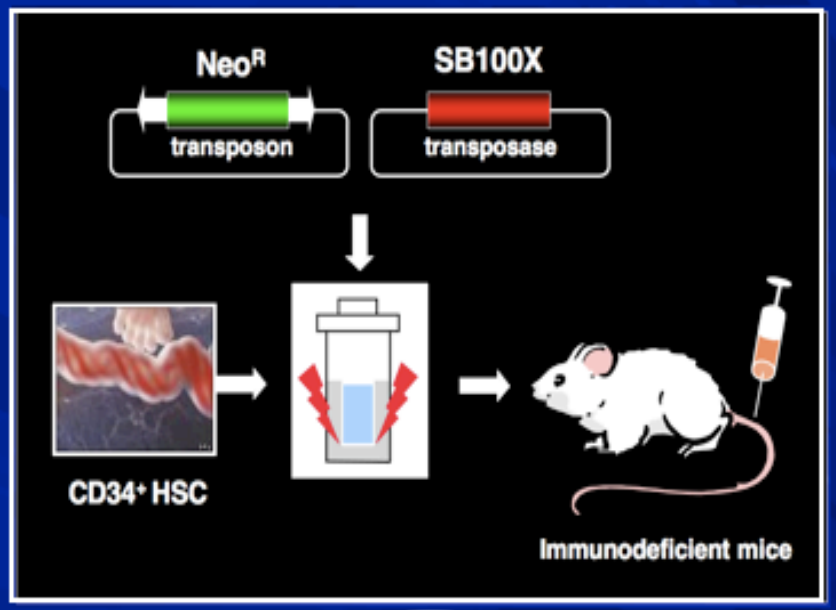


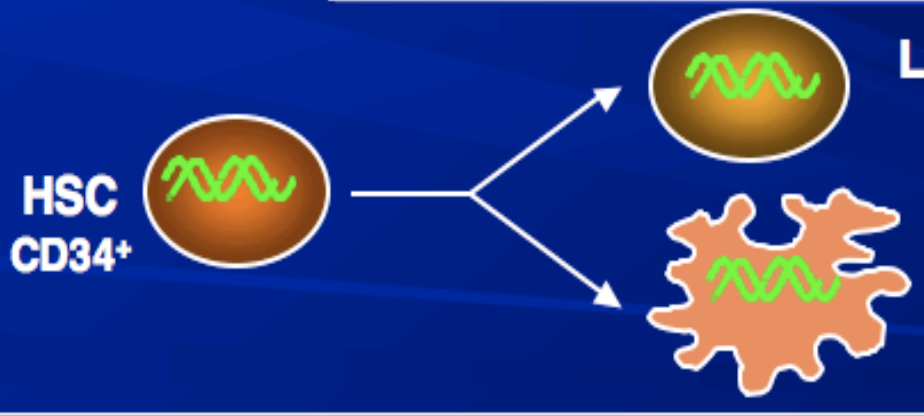


SB100 MORE EFFICIENT THAN PB IN HSC



LONG-TERM GENE MARKING AND HEMATOPOIETIC RECONSTITUTION





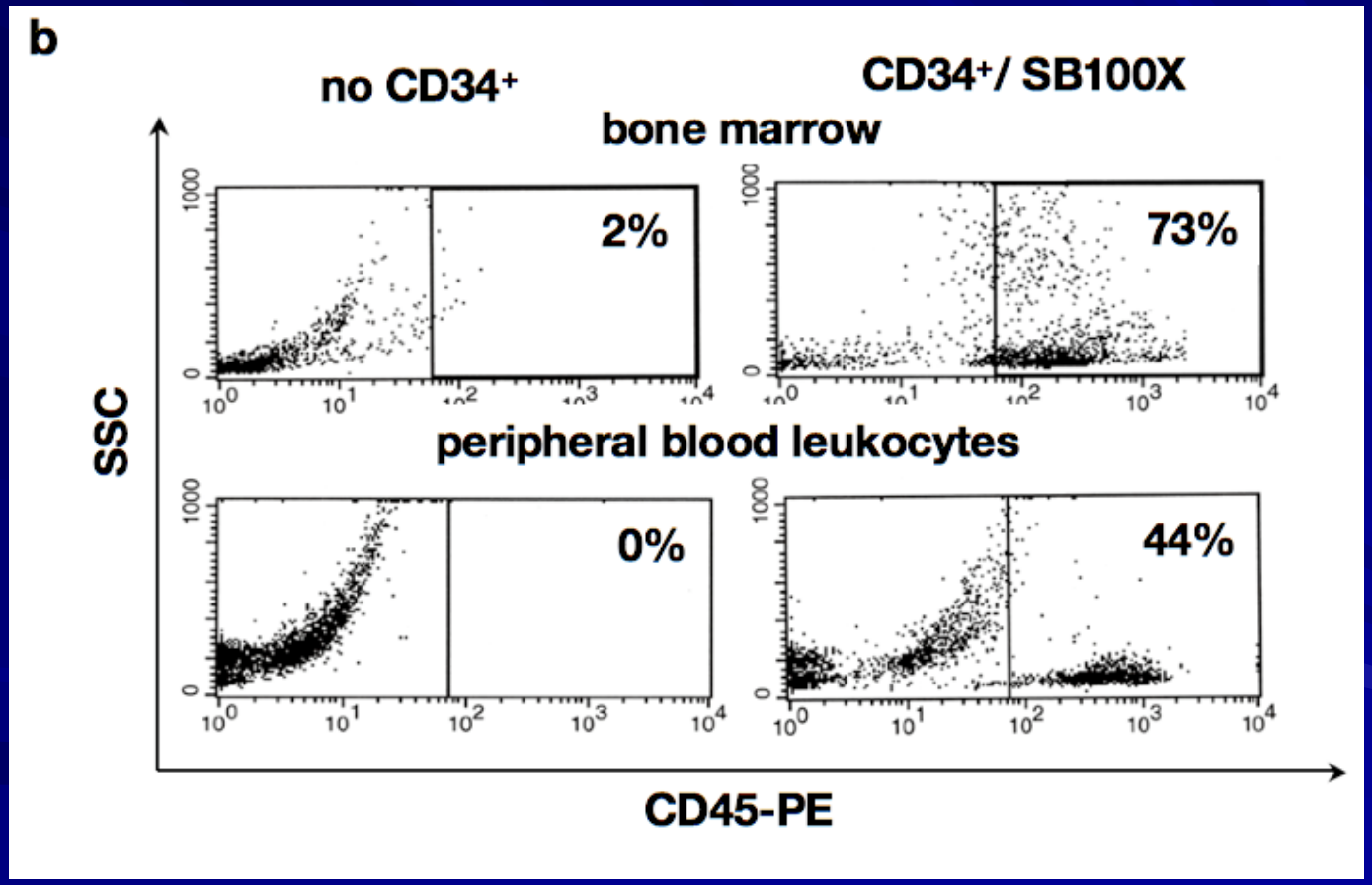
long-term NeoR marking

+++

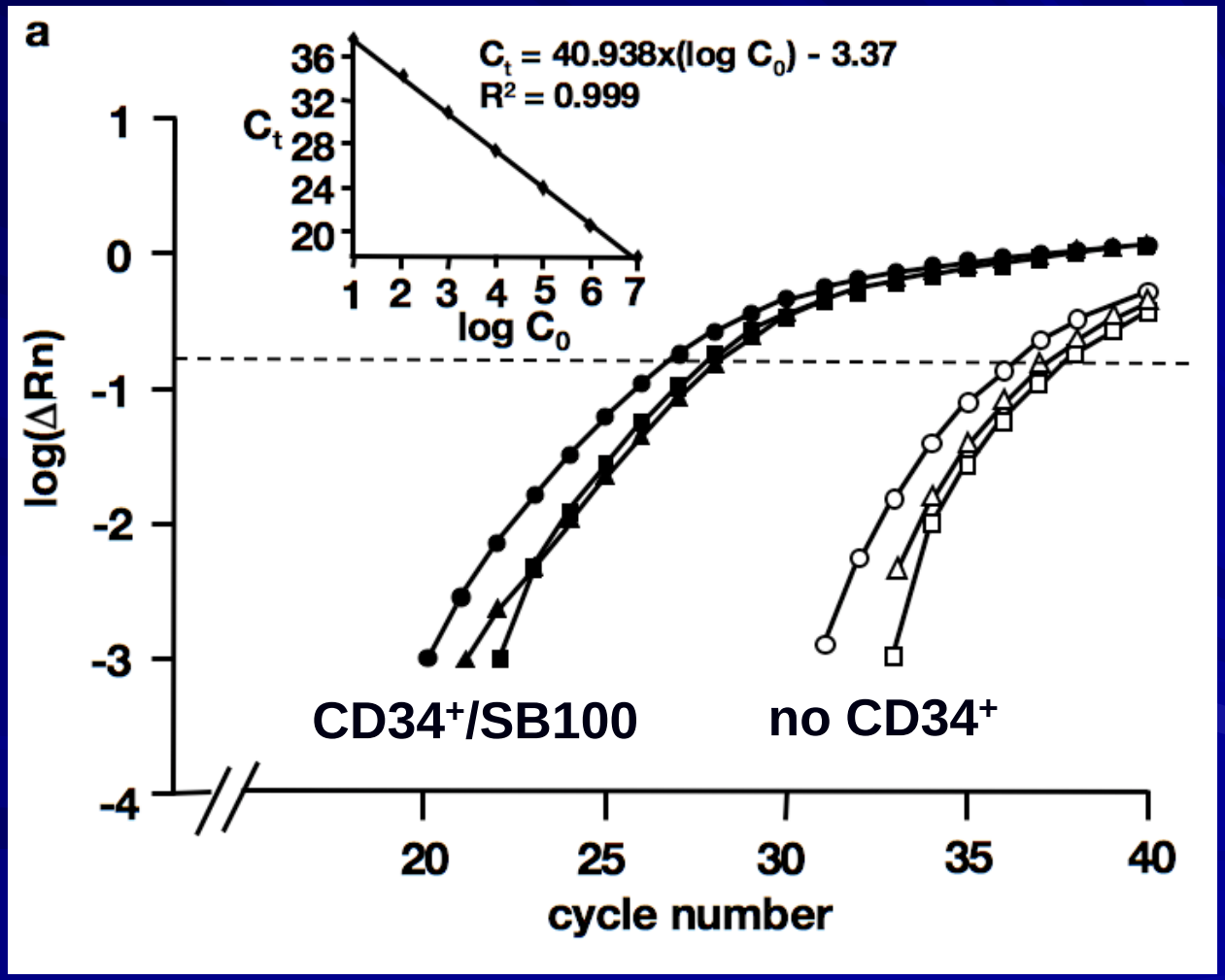
+++

1st successful HSC gene transfer using non-viral vectors

LONG-TERM ENGRAFTMENT AND HEMATOPOIETIC RECONSTITUTION WITH GENE-MARKED CD34$^+$ HSC TRANSFECTED WITH HYPERACTIVE SB



LONG-TERM ENGRAFTMENT AND HEMATOPOIETIC RECONSTITUTION WITH GENE-MARKED CD34$^+$ HSC TRANSFECTED WITH HYPERACTIVE SB



0.4 Neo^R copies/human cell

COMMON SB INTEGRATION SITES IN MYELOID & LYMPHOID LINEAGE

Supplementary Table 3: Integration sites in the myeloid and lymphoid lineage

Clone	CD33+ samples Junction with IR sequence inbold	E-value	Location, Homology	CD20+ samples Junction with IR sequence inbold	E-value	Location, Homology
1*.	CTAGTTTAACTACCCGGTTTGAAGAAACA ATTCATGAAACAGGCTCAGCAAATAGAAA AACTGTCACAGCCACG**TACAGTTG**	1e-29	Chr12, not in gene, ref|NT_009714.16|Hs12_9871	CTAGTTTAACTACCCGGTTTGAAGAAAC AATTCATGAAACAGGCTCAGCAAATAGA AAA**TACAGTTG**	9e-31	Chr12, not in gene, ref|NT_009714.16|Hs12_9871
2*.	**CAACTGTA**TATGGTGATAGGAAGTGGTCT AG	3e-3	Chr6, galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase, ref|NT_007299.12|Hs6_7456	ACTAGACCACTTCCTATCACCATA**TACA GTTG**	1e-3	Chr6, galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase, ref|NT_007299.12|Hs6_7456
3*.	**CAACTGTA**CCTAGTAAGCTTTAATTCAAA CTCAAAACTTCTAATTGAAAGTGGTTGCT GTTGTTGTGATTCCATTGCTTTTTCTAGG TACTATACCTTCATCCTACCTAG	1e-44	Chr3, not in gene, ref|NT_005612.15|Hs3_5769	**CAACTGTA**CCTAGTAAGCTTTAATTCAA ACTCAAAACTTCTAATTGAAAGTGGTTG CTGTTGTTGTGATTCCATTGCTTTTTCT AGGTACTATACCTTCATCCTACCTAGAA TTGCTTTTGAAGTTTTTCTGCCTTGCCT TTGATTGAACAGAATTTACAATAAAAGA TTTTCTACATCATCAAGGCTTCCACAGA GTTCAAATGAAAACACTTTTTAGGGGTA GCTGTTAACTGCCTACTTTTATAGTCTC TTGATTGAAAGCTACTGCTCTAAATTTA	2e-136	Chr3, not in gene, ref|NT_005612.15|Hs3_5769

	long-term Neo^R marking
Lymphoid CD20+	+++
Myeloid CD33+	+++

➡ confirmed gene transfer in common bona fide hematopoietic stem/progenitor

1st successful HSC gene transfer and repopulation in vivo using non-viral vectors

nature genetics

Mates, Chuah, et al., Nature Genetics, 41(6): 753-761, 2009

Molecular evolution of a novel hyperactive *Sleeping Beauty* transposase enables robust stable gene transfer in vertebrates

blood

VandenDriessche et al., Blood, 114(8):1461-8, 2009

Emerging potential of transposons for gene therapy and generation of induced pluripotent stem cells

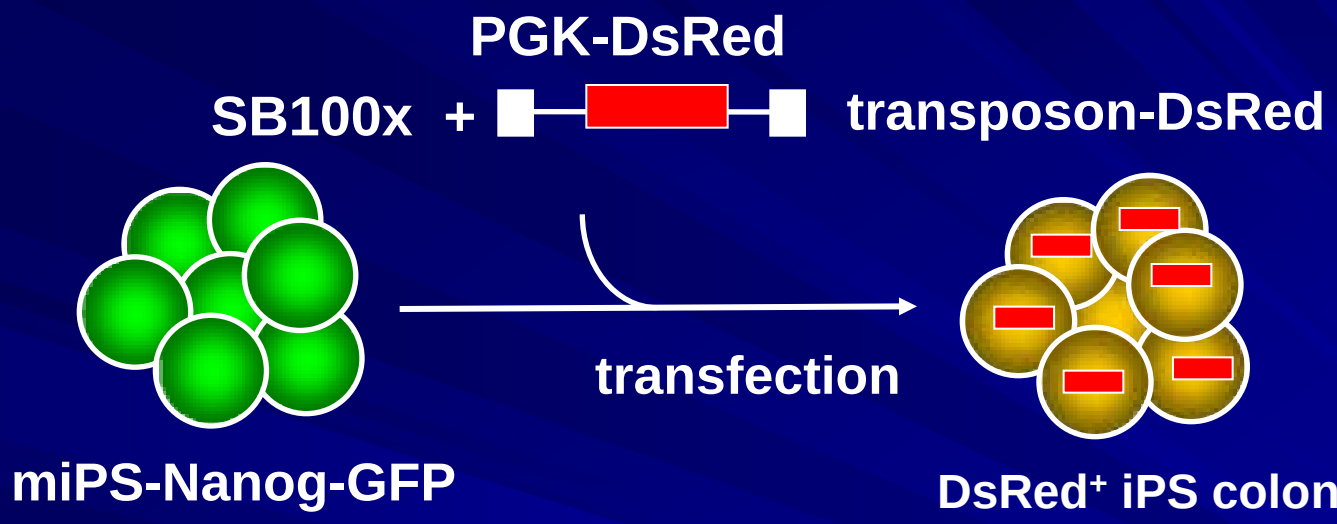
Molecular Therapy

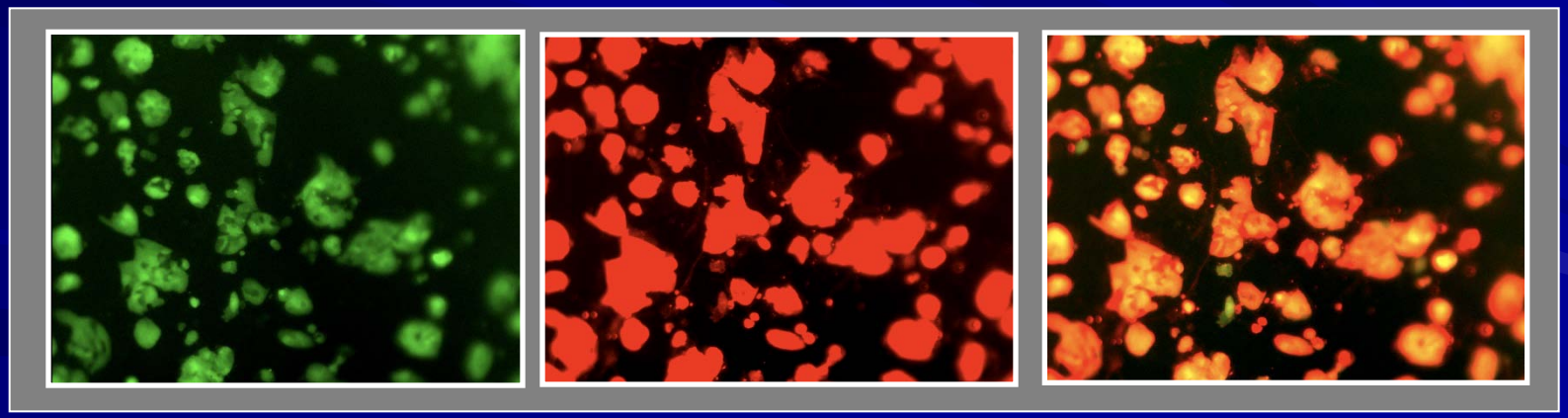
© The American Society of Gene & Cell Therapy

Grabundzija et al., Mol. Ther. 18(6): 1200-1209, 2010

Comparative Analysis of Transposable Element Vector Systems in Human Cells

Further validation of transposon mediated gene transfer into iPS





Transposed RFP+ iPS colonies

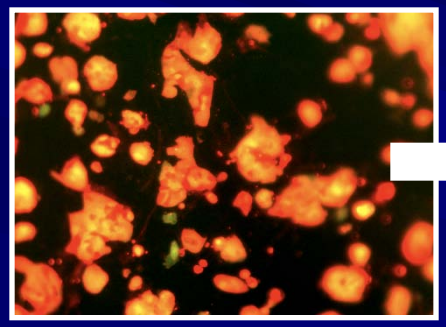
Transposon integration sites in iPS

TATGTGGGAGCACTCAGTTTGTTAACTCAGATTCTCT CCTCAGAGCTCTATGAACCTGCCTCCTGACAAGGCCC GGCTGCTGCGACAGTATGACAACGAGAAGAAGTGGGA TCTGATCTGTGACCAGGTATGAGCTTGGCA**TACAGTT** **G**	7e-66	Chr15, formin-like 3 protein ref|NT_039621.7|Mm15_39661_37
CATGAATCTACTTGTCTCTGCCTTTCCTACACTAGAG TTATGAGTACATACCACCCTGCCTAAATA**TACAGTTG**	1e-25	Chr12, not in gene, ref|NT_166318.1|Mm12_163492_37
CATGTCTGGGGGAAGATGTAGATATAGCTCTCAAATT TGAAAAGGAAATAAATTTGTTTAGAAAGAATGGGTCA GTACCTACCAAGAAACAAGCTTTTCCTATATGGACCT TGAGTAATACTTAAGGTCTGTATATTTACTATATA**TA** **CAGTTG**	1e-68	Chr5, not in gene, ref|NT_039299.7|Mm5_39339_37
CATGAGTCATACATAGTCCCCACCATCTATCTCCCTG TGTCCCCCCACTGAATCCATTTTCCACTTGGAGTGGA CACCTAATCCCATTTGCTTCAAGCTGATTCTTCTTGT ACTTTAGGACTGTGATTCCAAGTTA**TACAGTTG**	3e-63	Chr5, not in gene, ref|NT_039305.7|Mm5_39345_37

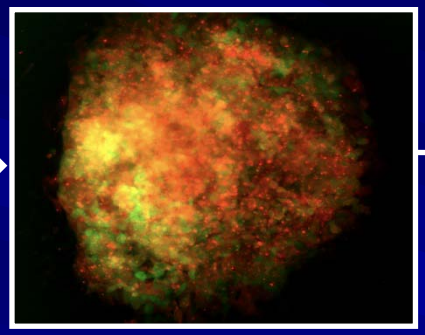
Transposon inverted repeat

Are transposed iPS pluripotent ?

Transposed iPS
DsRed^{+} GFP^{+}



Embryoid body differentiation



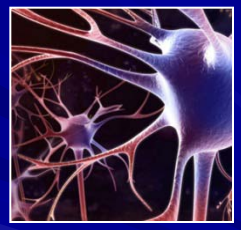
Differentiation

cardiomyogenic

mesoderm

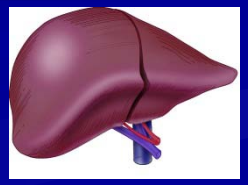
neuronal

ectoderm

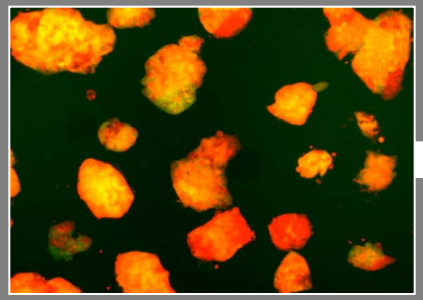


hepatic

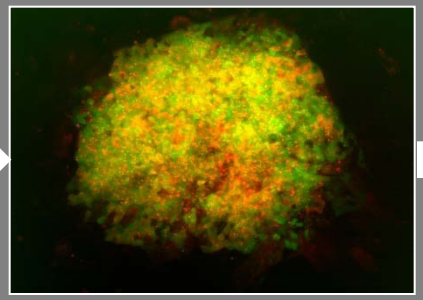
endoderm



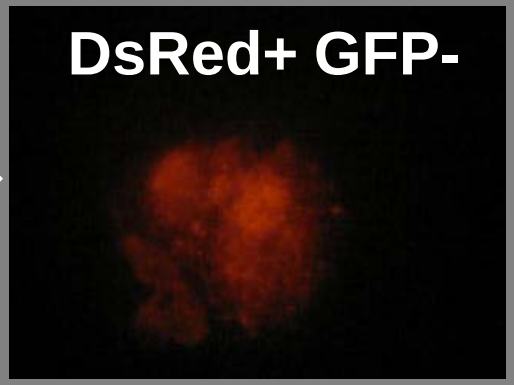
Transposed iPS capable of cardiomyogenic differentiation



transposed iPS
DsRed^{+} GFP^{+}

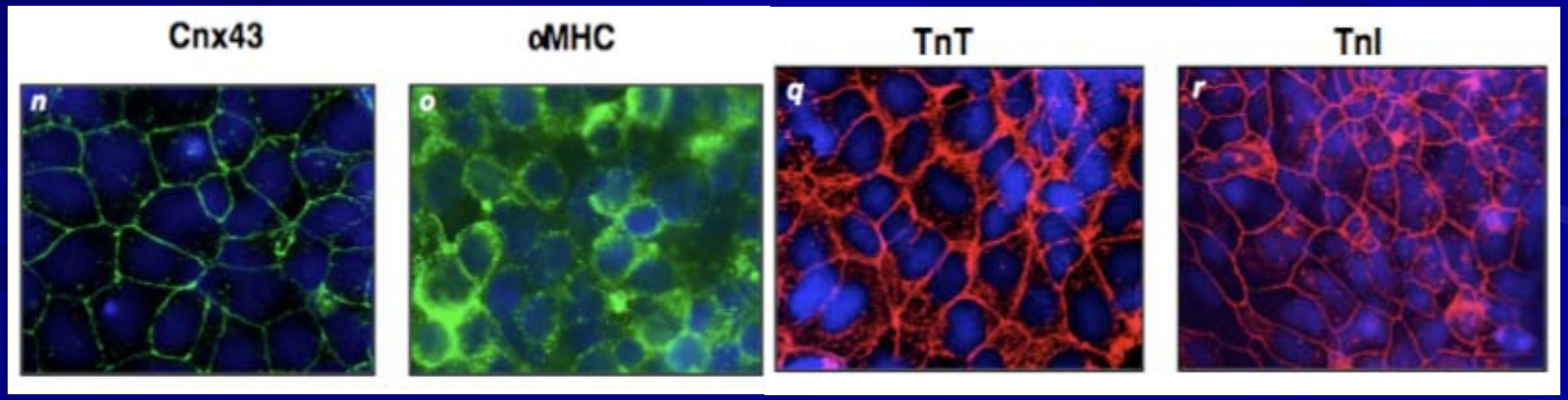


embryoid
body



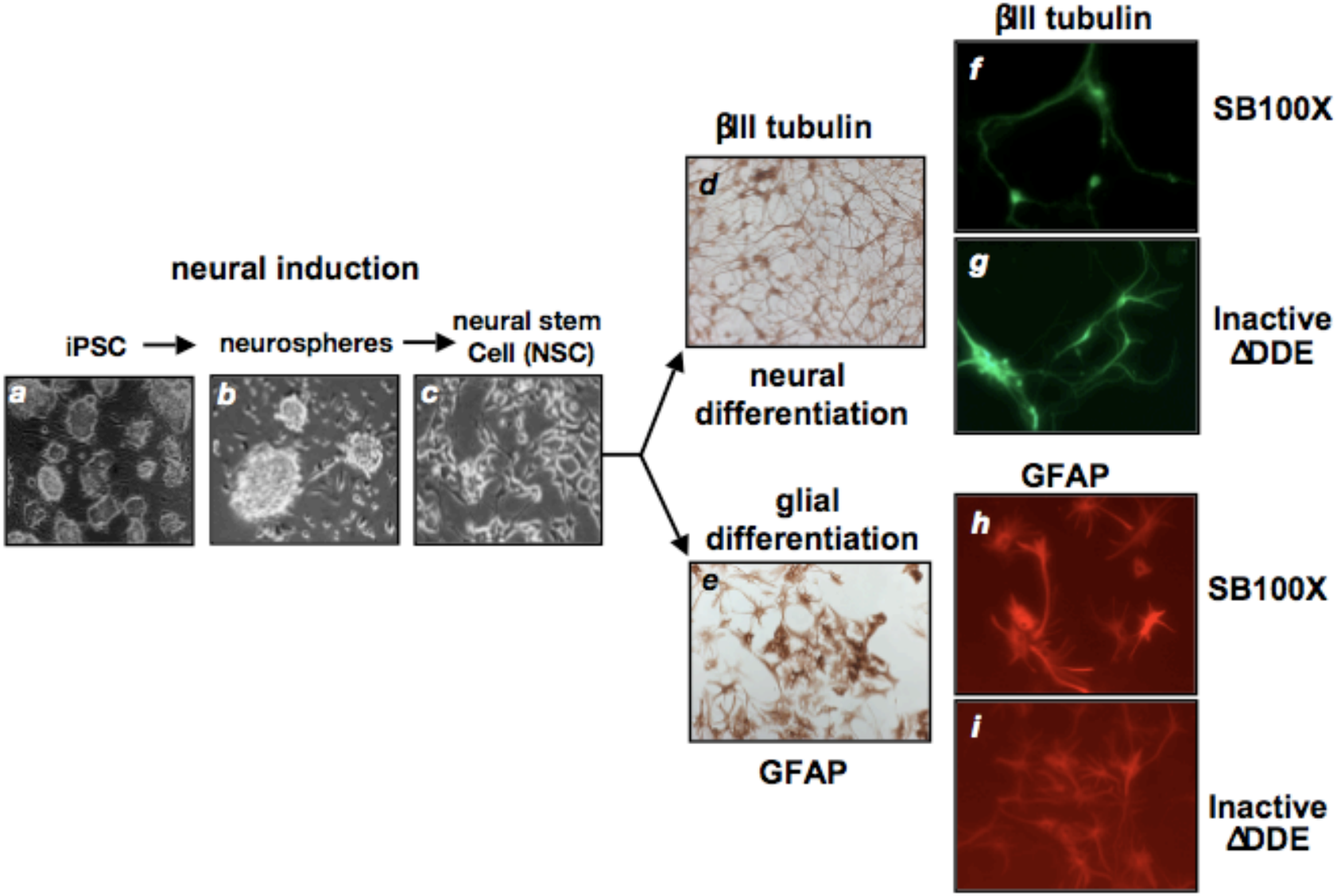
contracting
cardiomyocyte-like cells

CARDIAC DIFFERENTIATION MARKERS



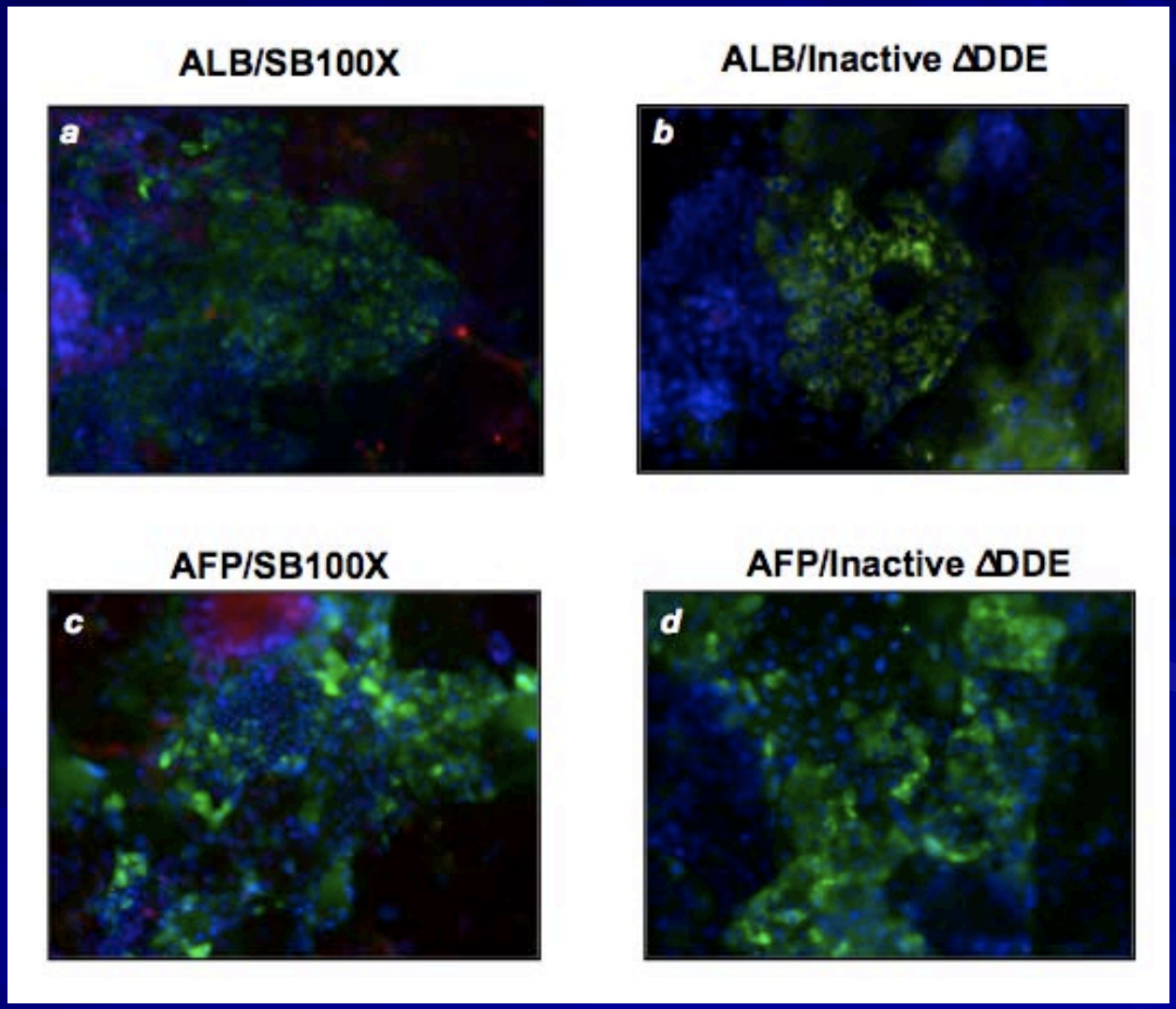
SB100 Transposed iPS capable of neuronal and glial differentiation

Expression of neuronal and glial differentiation markers

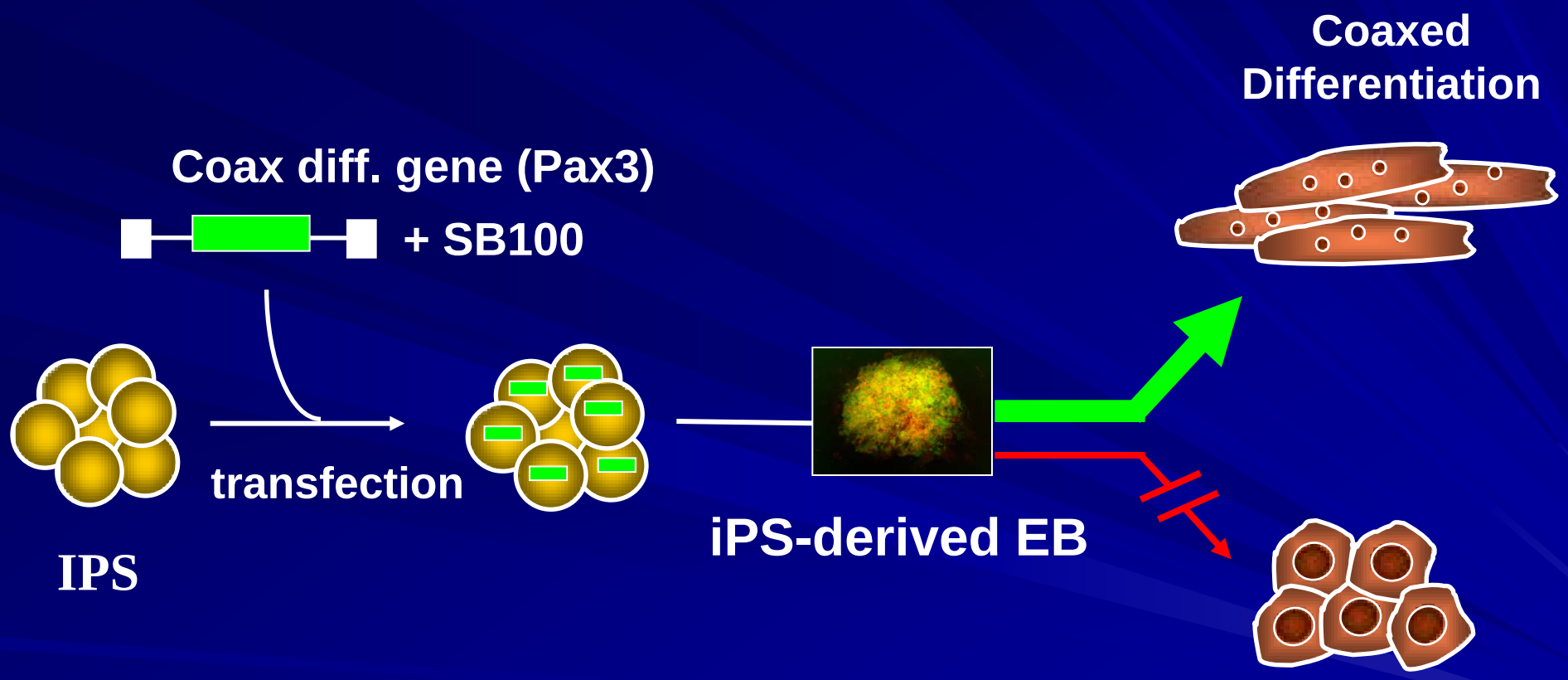


Transposed iPS capable of hepatic differentiation

Expression of hepatic markers in hepatic cells derived from transposed iPS

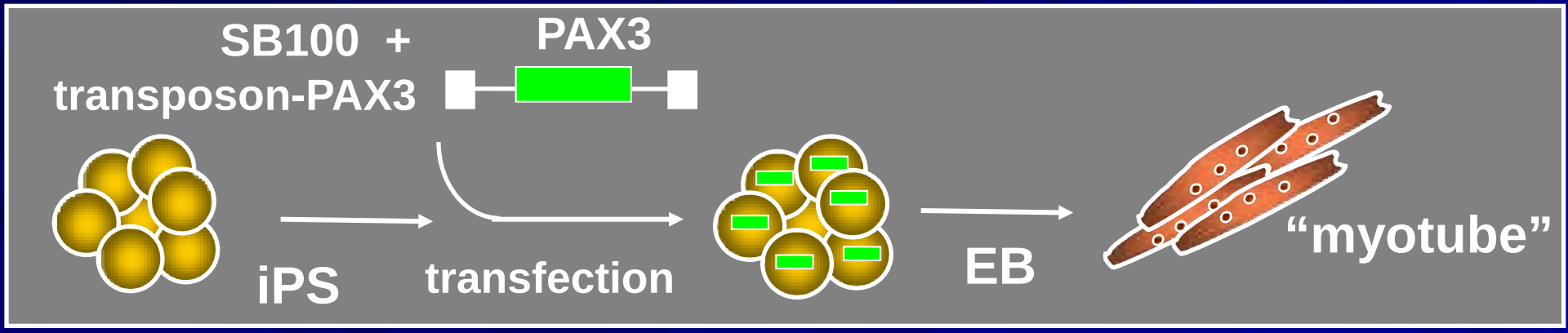


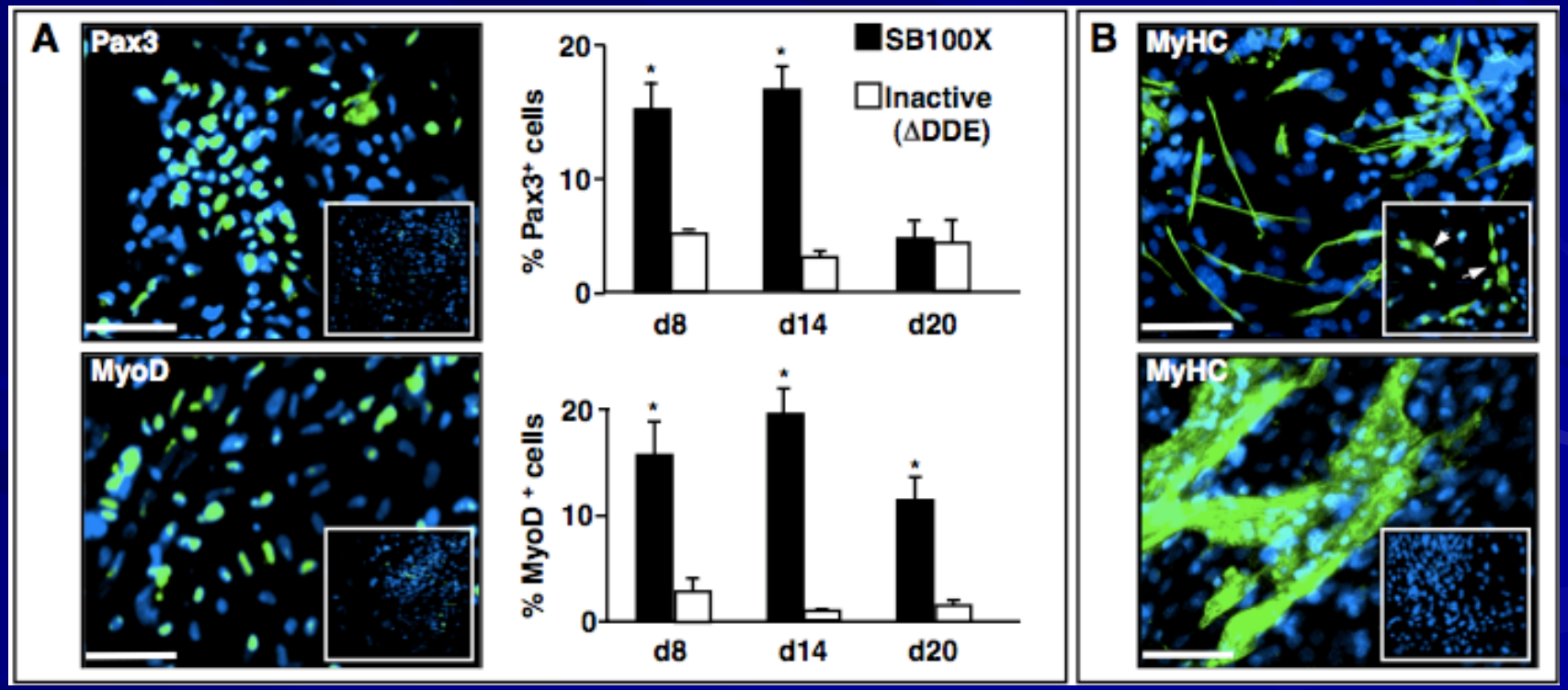
Can we use Transposition into iPS: a technology platform for "coaxed" differentiation



HYPOTHESIS: Can we "coax" differentiation of iPS into myogenic precursor cells and myotubes by expressing the myogenic transcription factor PAX3?

Transposon-mediated PAX3 gene transfer in iPS: A novel non-viral paradigm for coaxed myogenic differentiation





STEM CELLS®

EMBRYONIC STEM CELLS/INDUCED PLURIPOTENT STEM CELLS

Novel Hyperactive Transposons for Genetic Modification of Induced Pluripotent and Adult Stem Cells: A Non-Viral Paradigm for Coaxed Differentiation

Eyayu Belay[1,2,*], Janka Mátrai[1,2,*], Abel Acosta-Sanchez[1,2], Ling Ma[1], Mattia Quattrocelli[4], Lajos Mátés[3], Pau Sancho-Bru[4], Martine Geraerts[4], Bing Yang[1], Vermeesch Joris[5], Melvin Yesid Rincón[1,2], Ermira Samara-Kuko[1,2], Zoltán Ivics[3,6], Catherine M. Verfaillie[4], Maurilio Sampaolesi[4], Zsuzsanna Izsvák[3,6,#], Thierry VandenDriessche[1,2,#], Marinee K. L. Chuah [1,2,#,]

CONCLUSIONS

➡ **successful and robust stable in vivo gene marking in bona fide HSC by non-viral gene delivery**
(implications for gene therapy of hematopoietic disease)

➡ **Long-term expression in liver**

➡ **State of the art SB and PiggyBac transposons represent an attractive platform technology for :**

- **gene therapy**
- **functional genomics**
- **stem cell engineering**
- **iPS generation / coaxed differentiation**
- **regenerative medicine**

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EU FP6 – CLINIGENE
EU FP7 – PERSIST
FWO, VIB