Mechanisms of oncogenesis via insertional mutagenesis: Lessons from cancer screens in mice using the "cut-and-paste" transposon *Sleeping Beauty*

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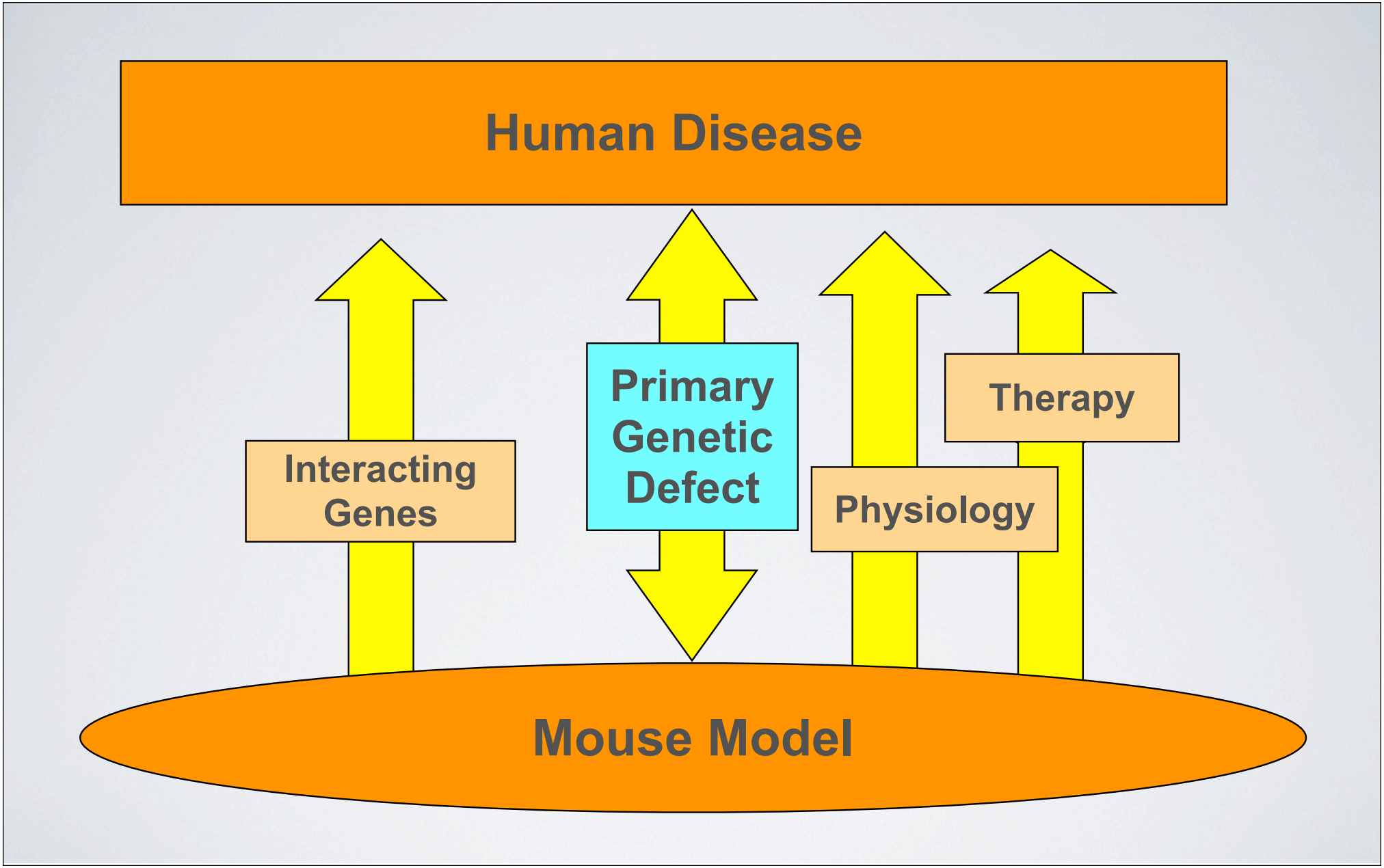
December 9, 2010

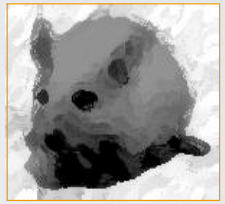

Human Disease
Interacting Genes
Primary Genetic Defect
Physiology
Therapy
Mouse Model

Forward and Reverse Genetics

Reverse Genetics

GENE

Transgenesis



PHENOTYPE

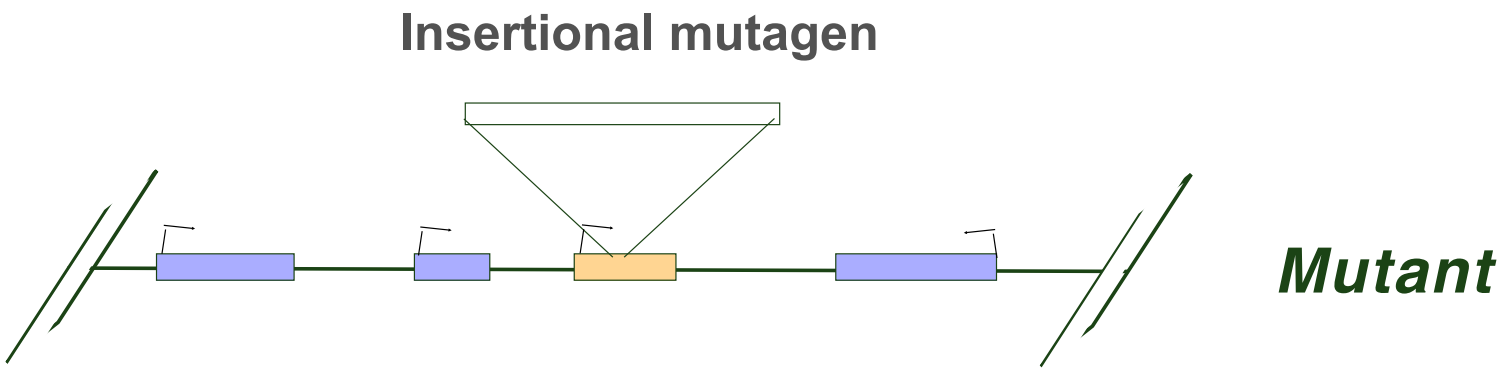
Forward Genetics

Mutagenesis, Natural variation

PHENOTYPE

GENE

Need a Convenient System for Mutagenesis in Mouse



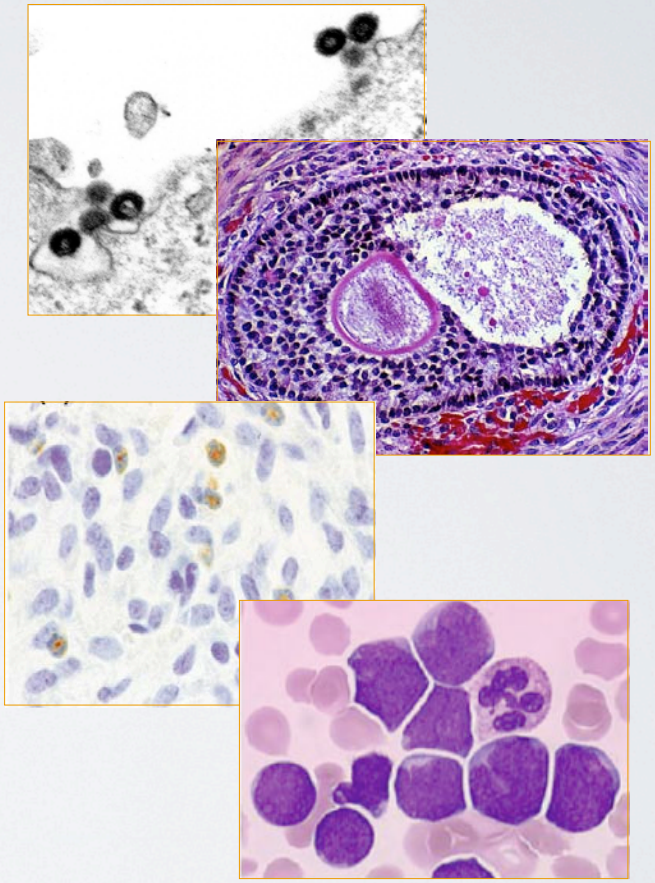
- The generation of mutations via the insertion of DNA
- Simplifies identification of mutant loci

Outline

- *Sleeping Beauty* (SB)
- *SB* based screens for cancer genes
- Mechanisms of cancer gene alteration via SB mutagenesis
- Using SB for gene therapy vector testing

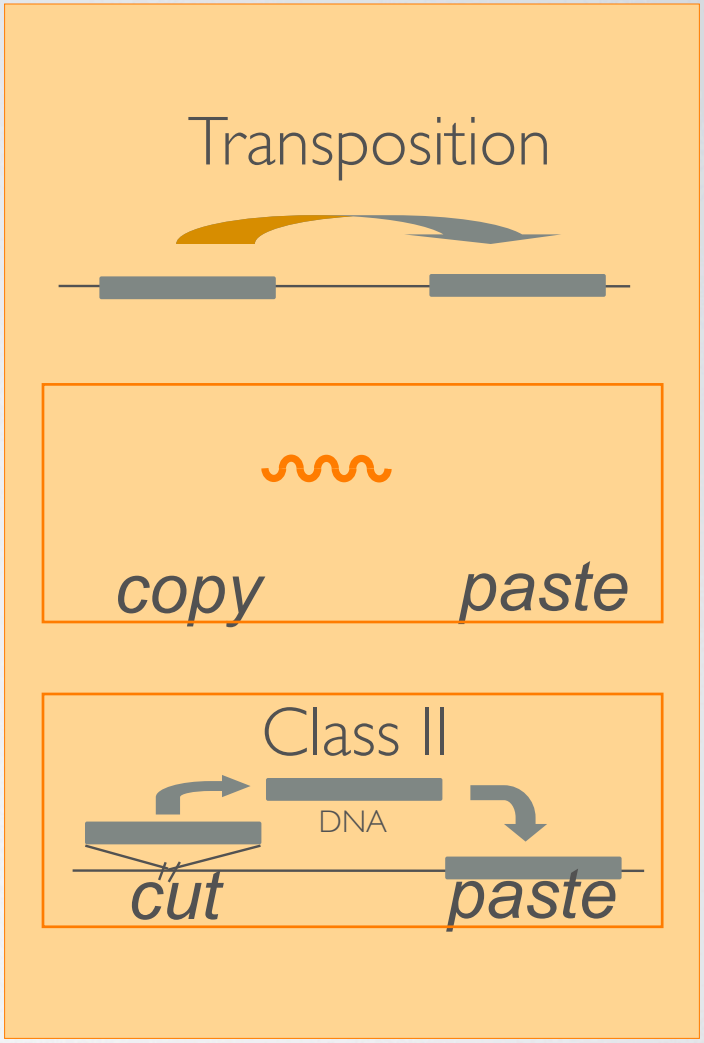
Retroviruses are Powerful Insertional Mutagens in Mice

- Germline acquisition of new proviral insertions in SWR/J – RF/J hybrid mice
- MuLV cause leukemia/lymphoma and MMTV cause mammary tumors in susceptible mouse strains via insertional mutagenesis
- Many host and cell type specific barriers prevent general utility
- Are there other options? Transposons?



Transposable Elements

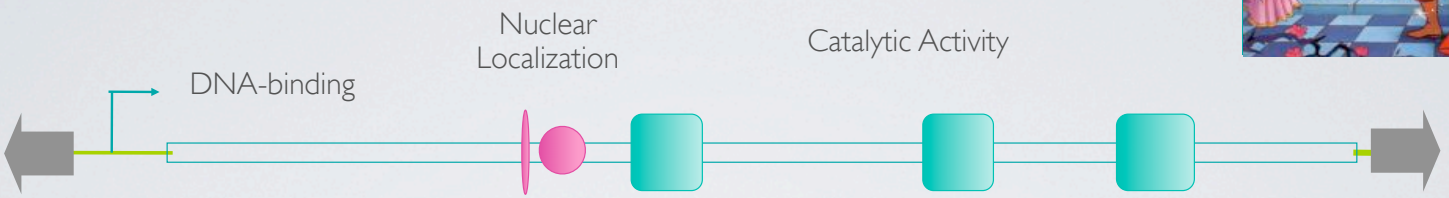
- Sequences of DNA that move around to different positions within genome of a single cell
- "Jumping genes" or "mobile genetic elements"
- Class I or retrotransposons and Class II or DNA transposons
- Encode proteins needed for transposition reaction, e.g. transposases



Sleeping Beauty Based Screens for Cancer Genes

- Sleeping Beauty (SB) is the first vertebrate-active "cut-and-paste" transposable element
- Tc1/mariner family - synthetic element
- Best characterized element, most advanced system

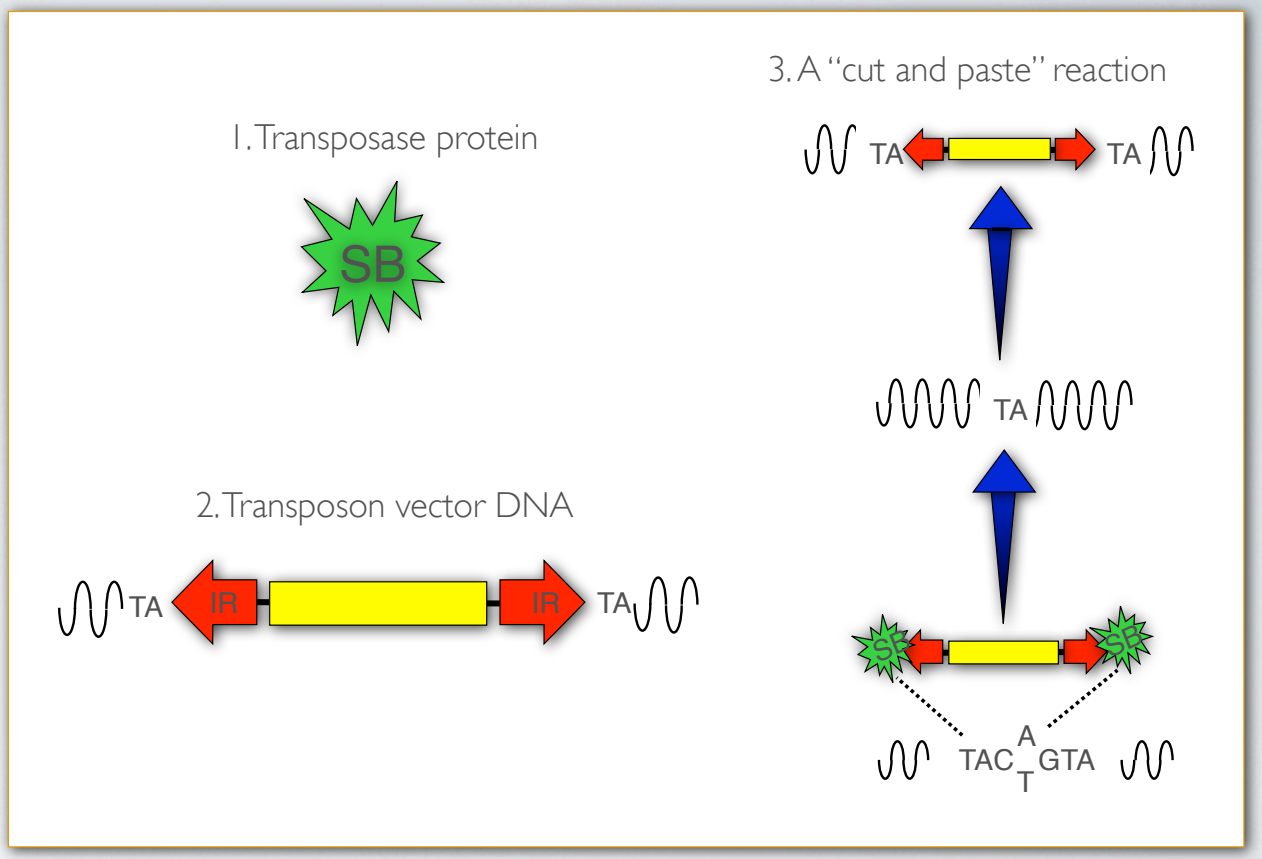
Sleeping Beauty (SB)



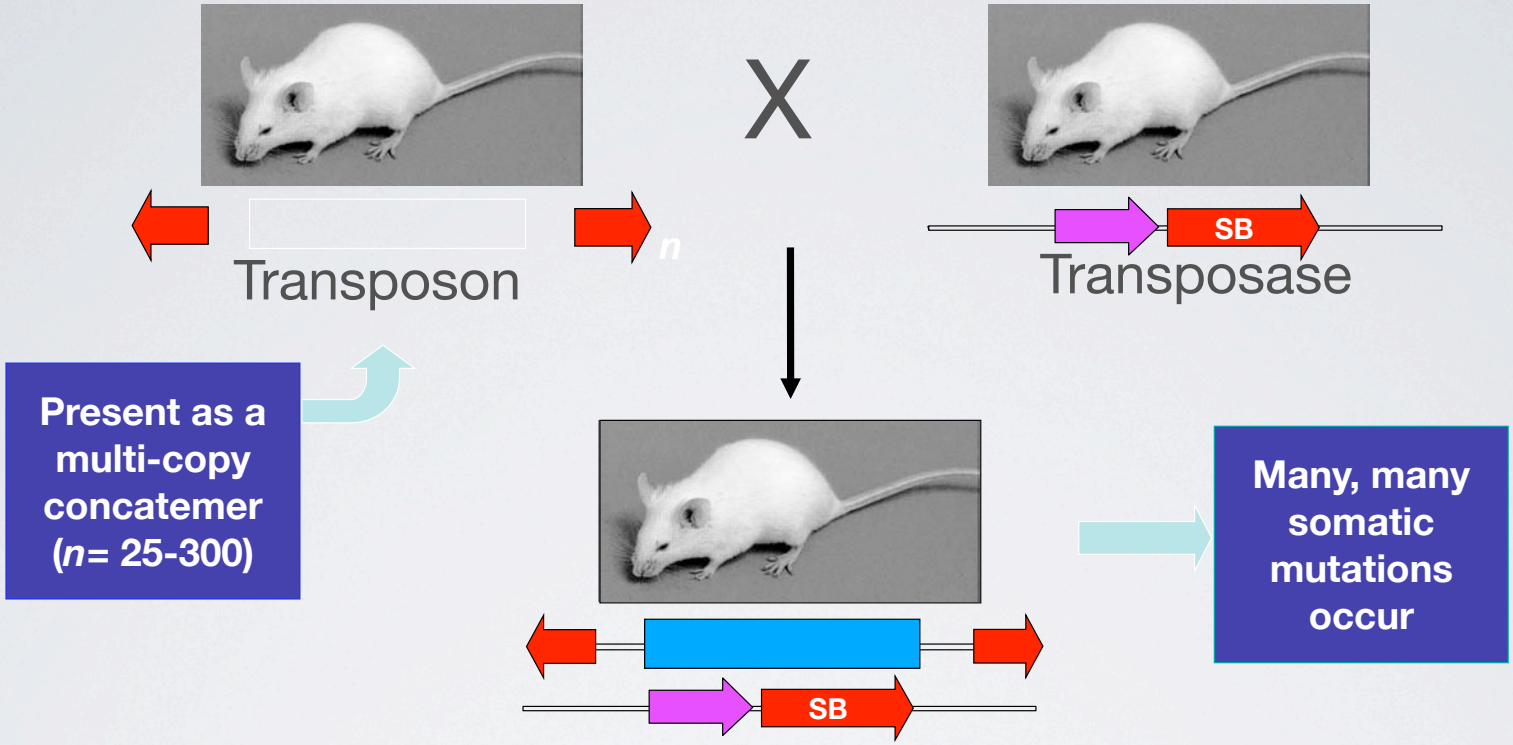
Inverted terminal repeats (IR), internal transposase with paired-like DNA binding and DDE catalytic domains

- **A synthetic "cut-and-paste" DNA transposon**
- **Derived from inactive Tc1/mariner family transposon elements in fish**
- **Created in Dr. Perry Hackett's lab - Dr. Zoltan Ivics and Dr. Zsuzsanna Izsvak - derived a consensus transposase sequence - reverse evolutionary approach (Ivics et al., Cell, 1997).**

Sleeping Beauty is a Two Part System: The Transposase and the Transposon Vector

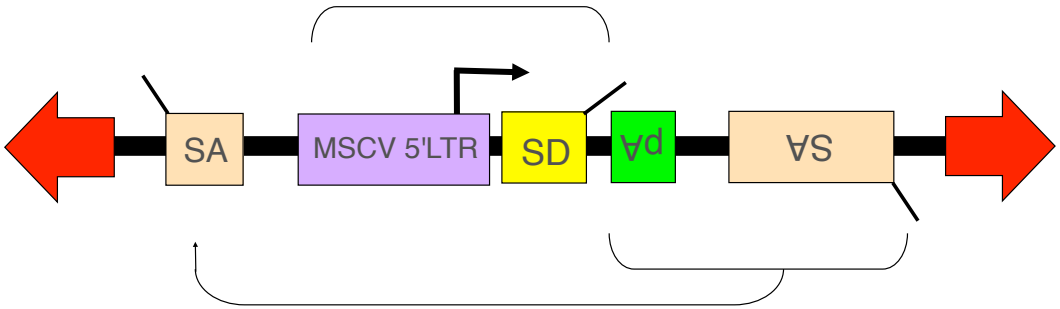


Somatic Mutagenesis with Sleeping Beauty *In Vivo*

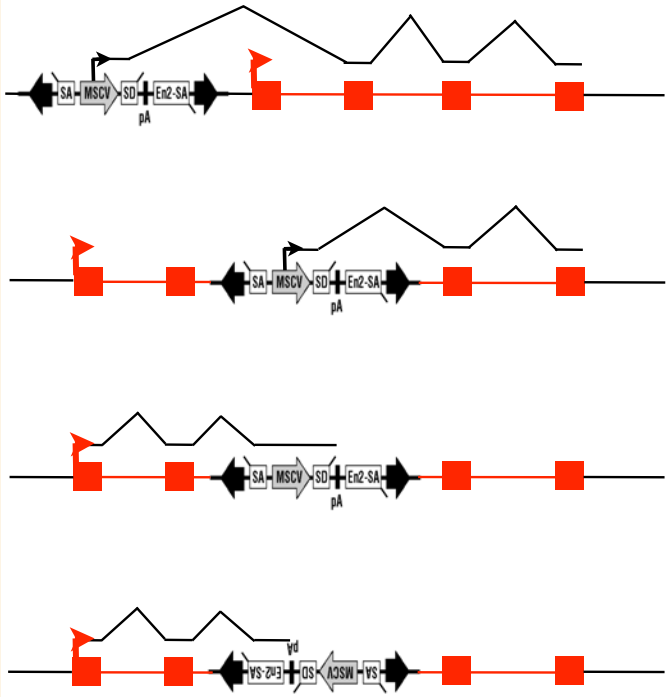


A Sleeping Beauty Transposon Designed to be "Insertionally Oncogenic"

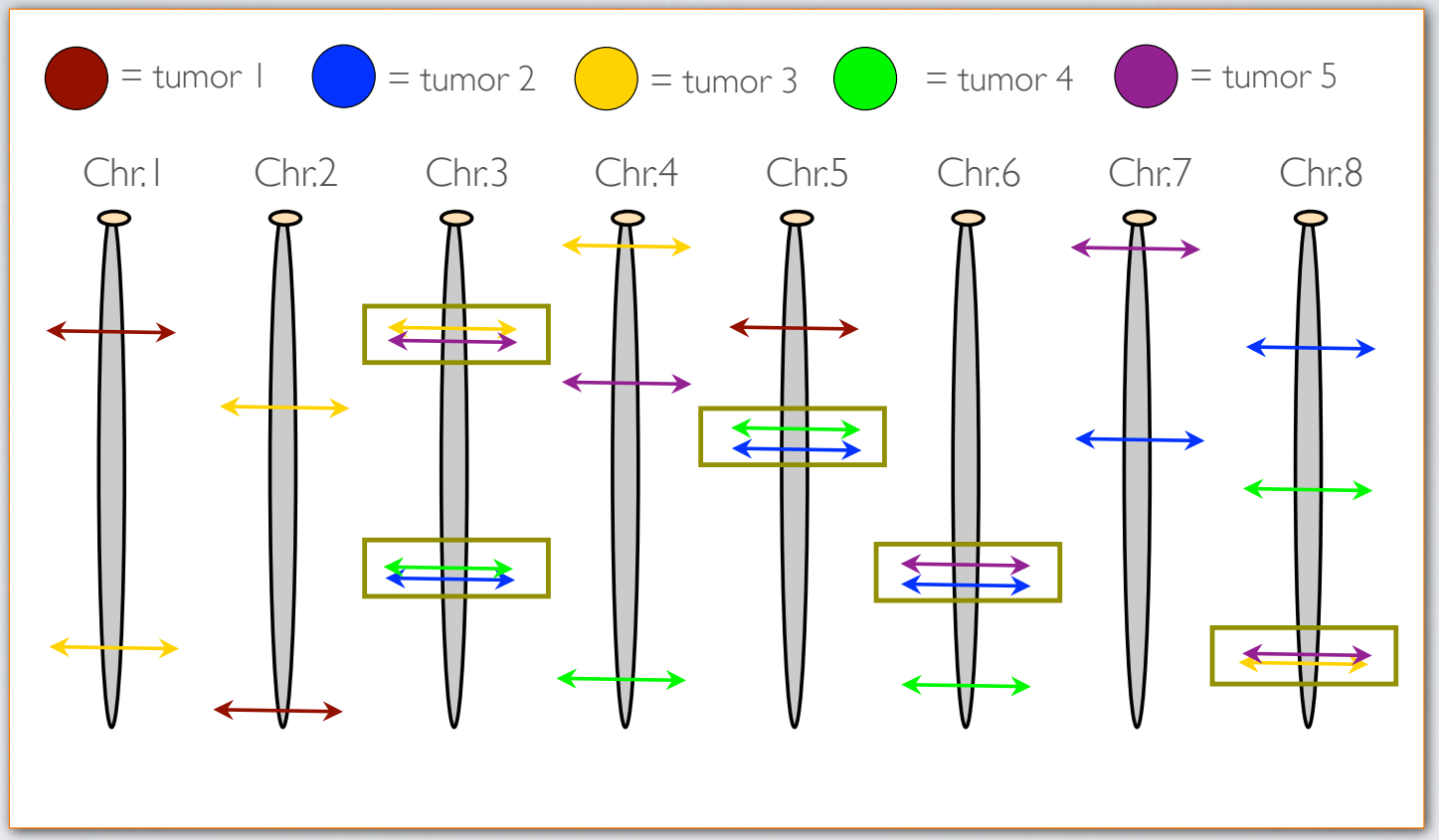
T2/Onc



Gain-of-function or loss-of-function mutations



Identifying Cancer Causing Insertion Mutations



= Common Insertion Site (CIS)

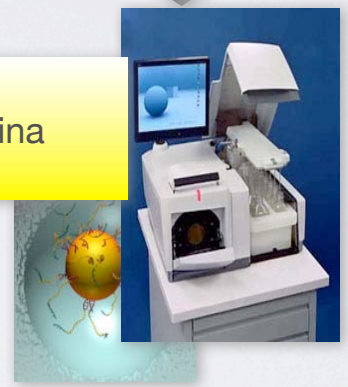
Pipeline for Genetic Analysis of SB-Induced Tumors

Tumors

DNA prepared from *SB*-induced tumors

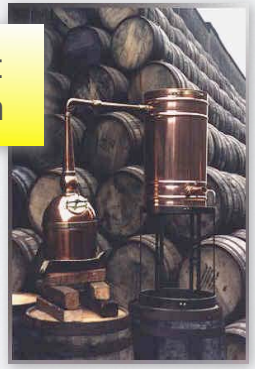
Linker-mediated PCR to amplify T2/Onc-genomic junction fragments - secondary primers barcoded for each tumor

454/Illumina



Obtain unique T2/Onc insertions

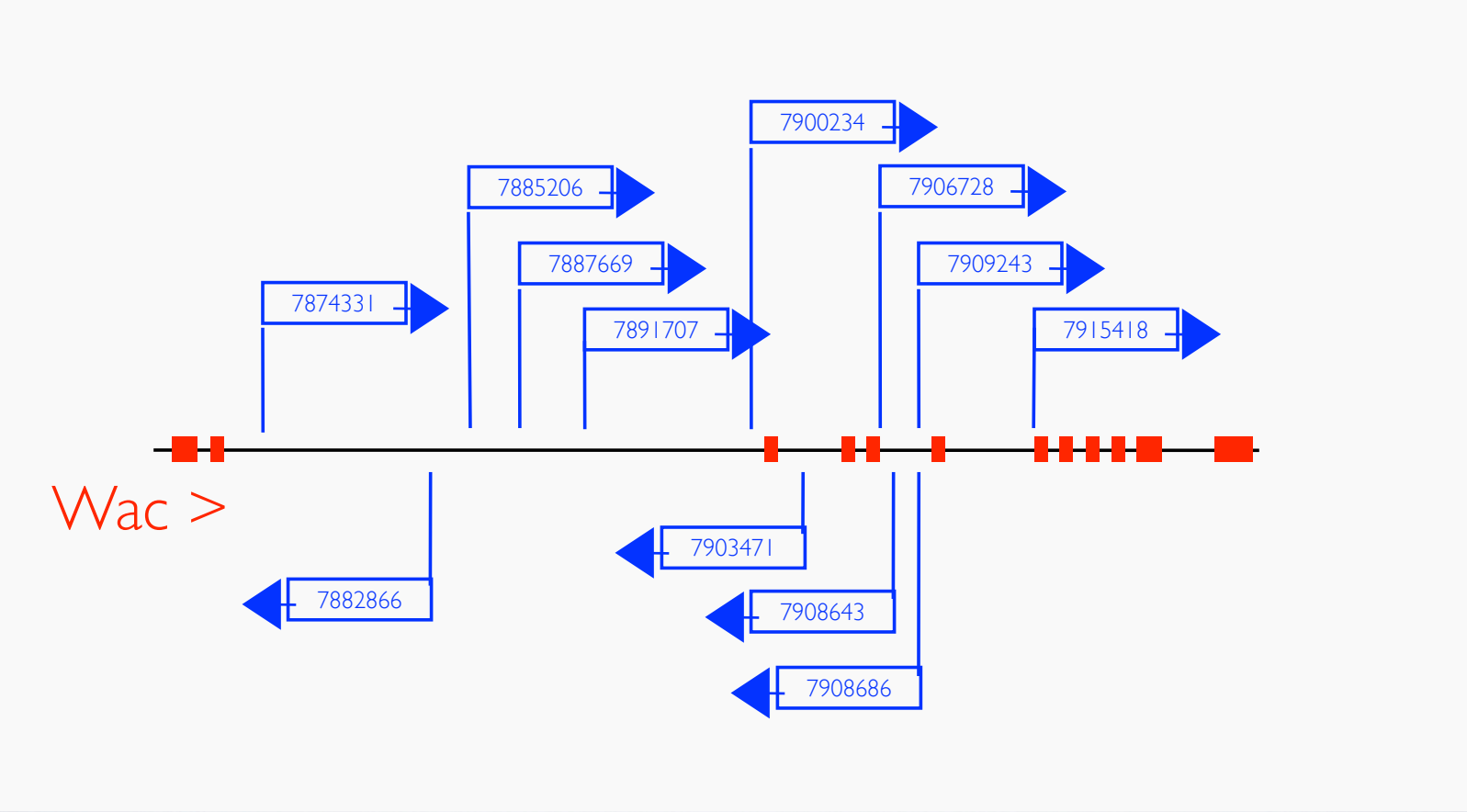
Bioinformat distillation



Common transposon insertion sites

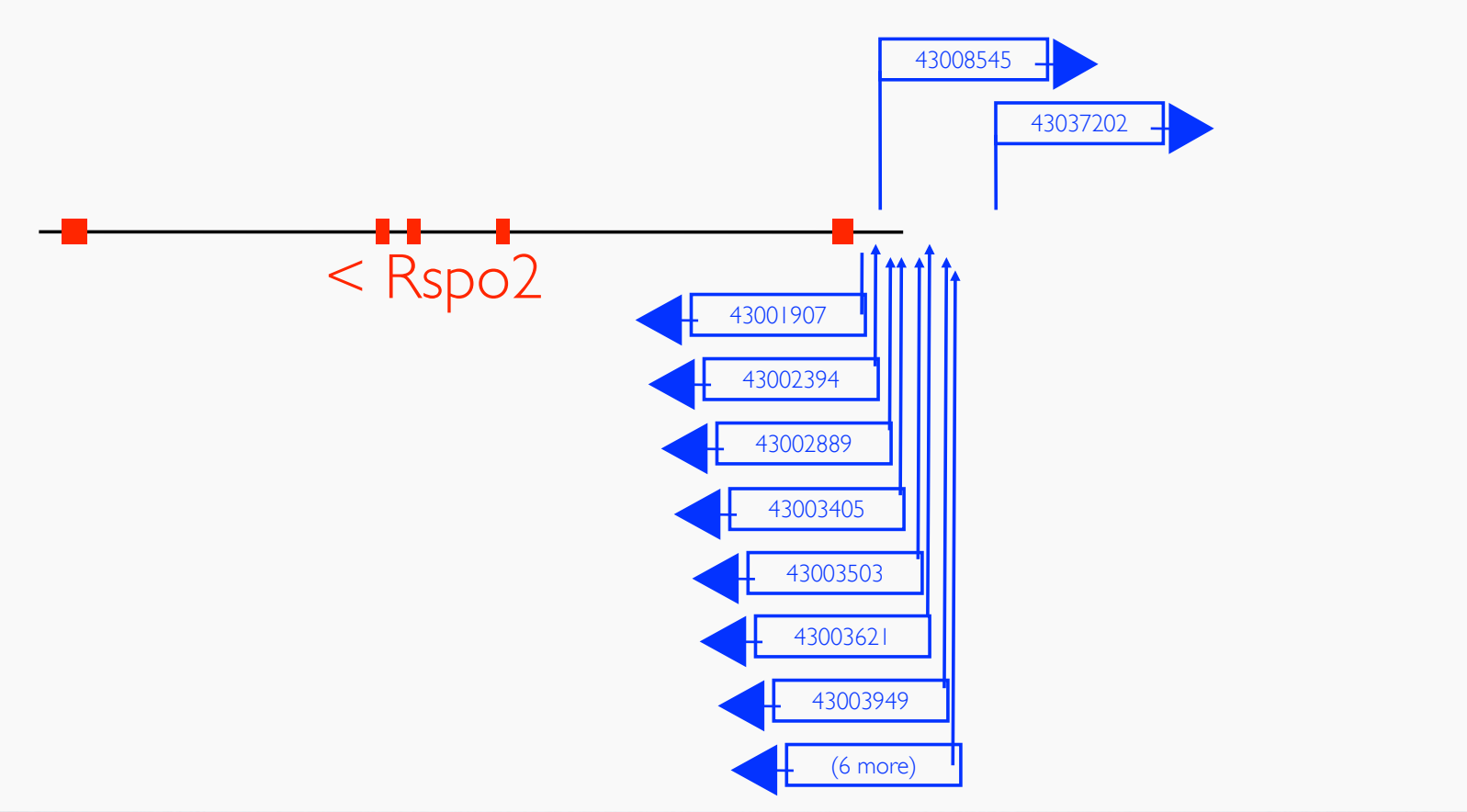
Selection for Loss of Function

GI tract screen: Chr 18: 7,870,000 to 7,930,000

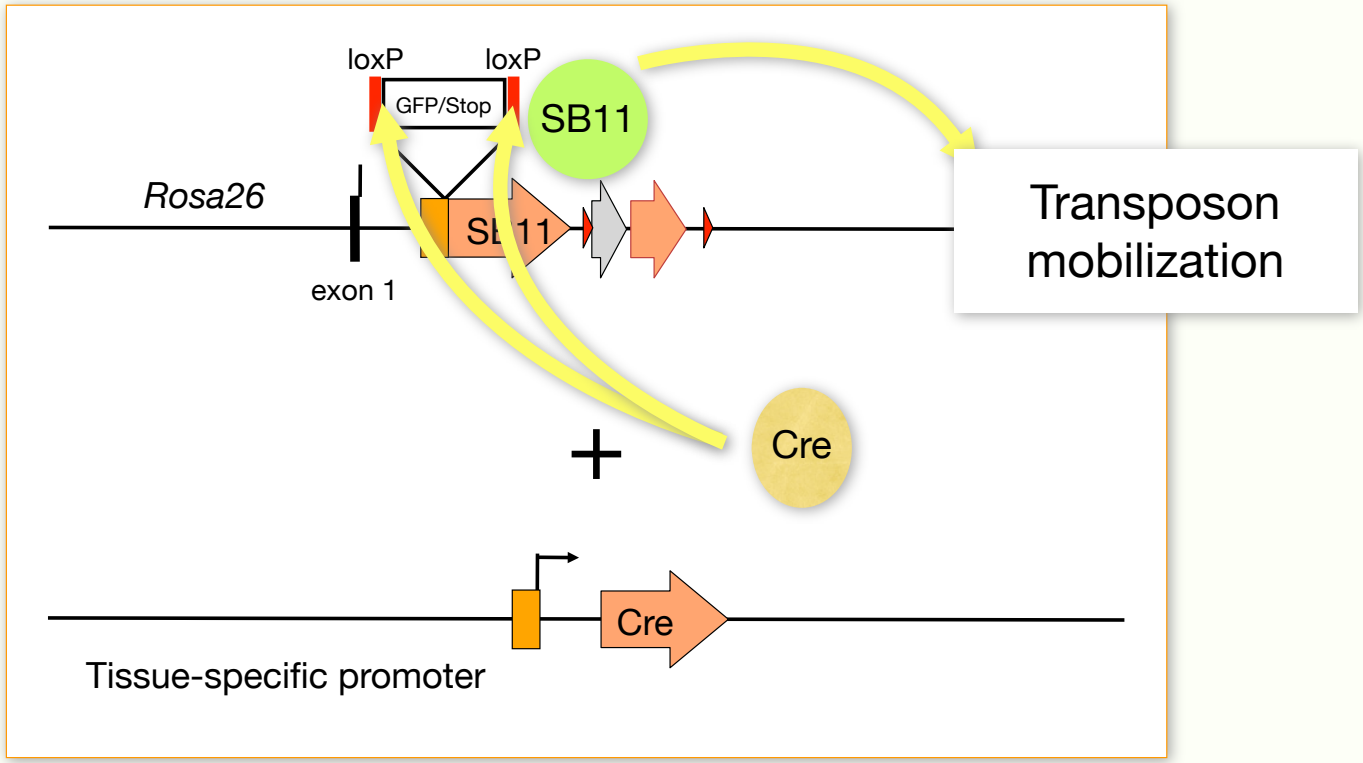


Selection for Gain of Function

GI tract screen: Chr 15: 42,850,000 to 43,050,000

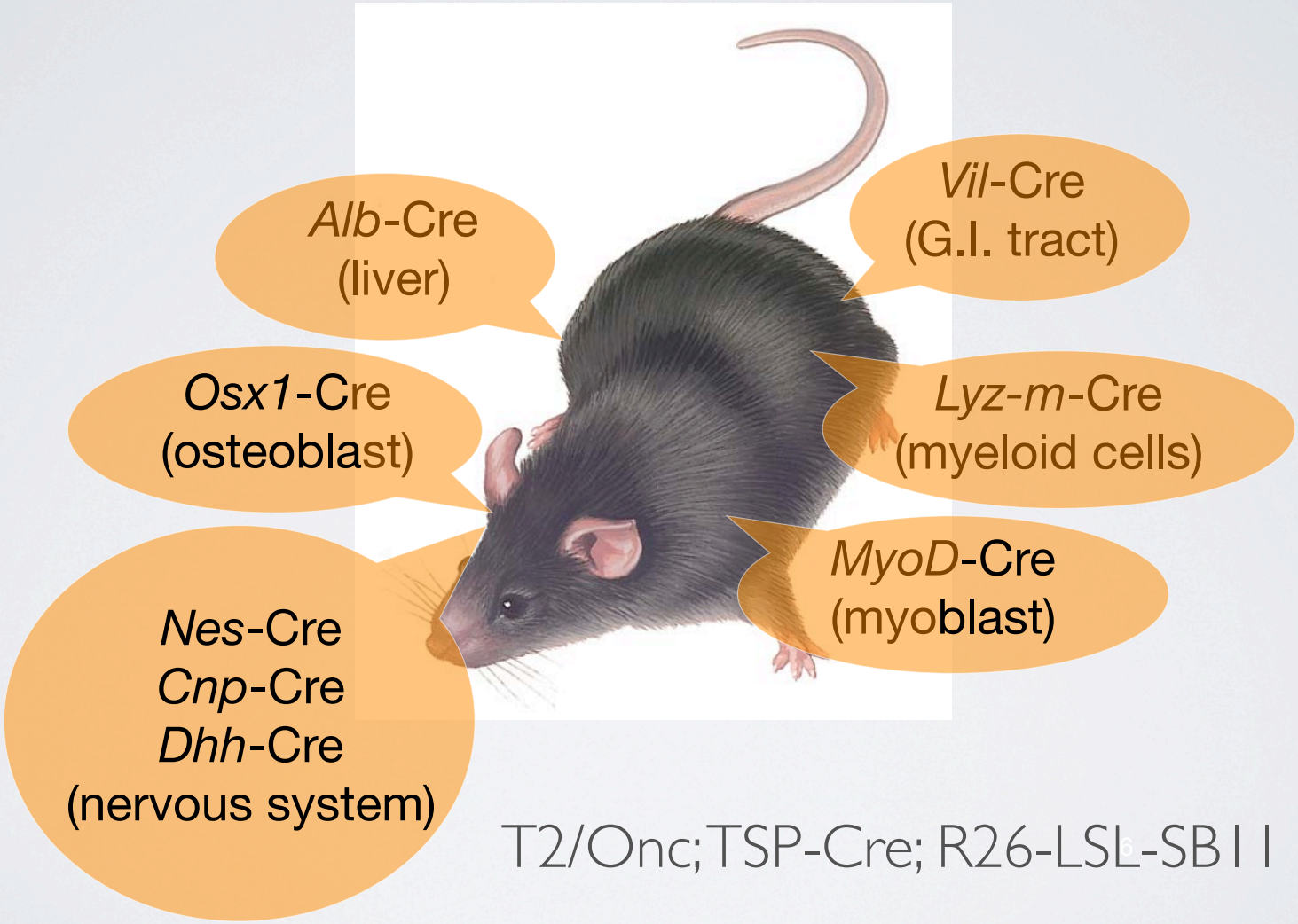


Targeting Transposition to Tissues of Interest using a Conditional SB Transposase Allele



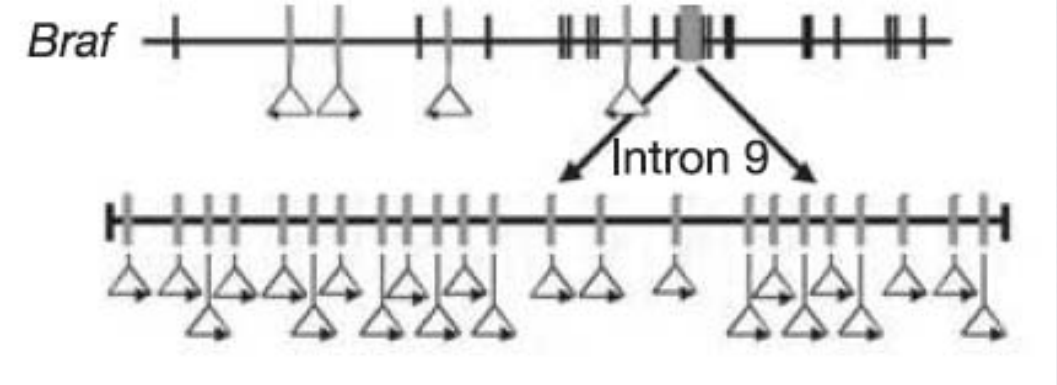
Adam Dupuy, Nancy Jenkins & Neal Copeland

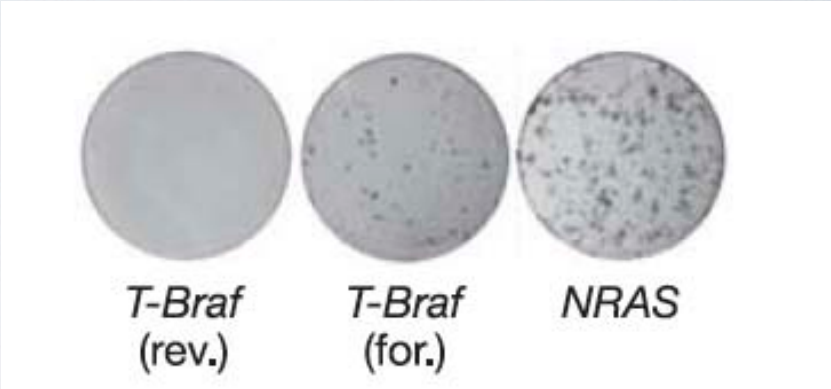
Tissue-specific SB Transposon Mutagenesis

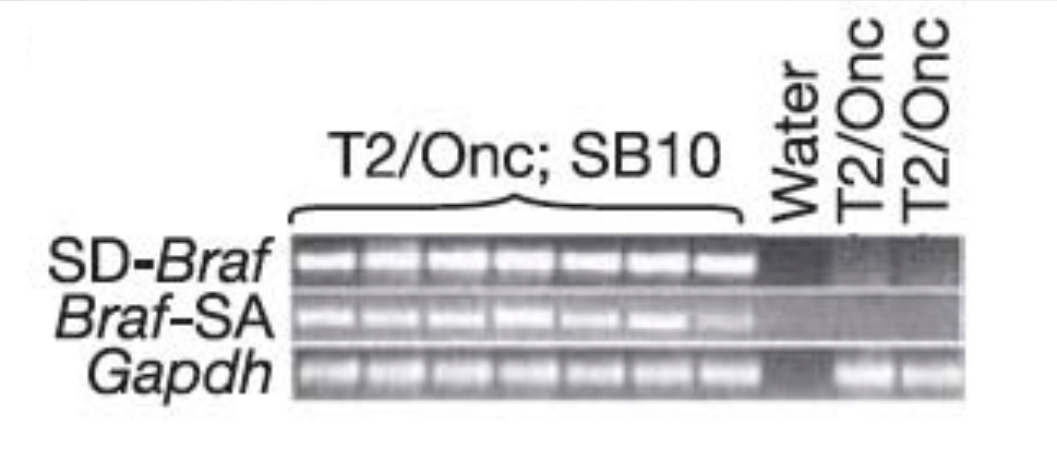


T2/Onc;TSP-Cre; R26-LSL-SB11

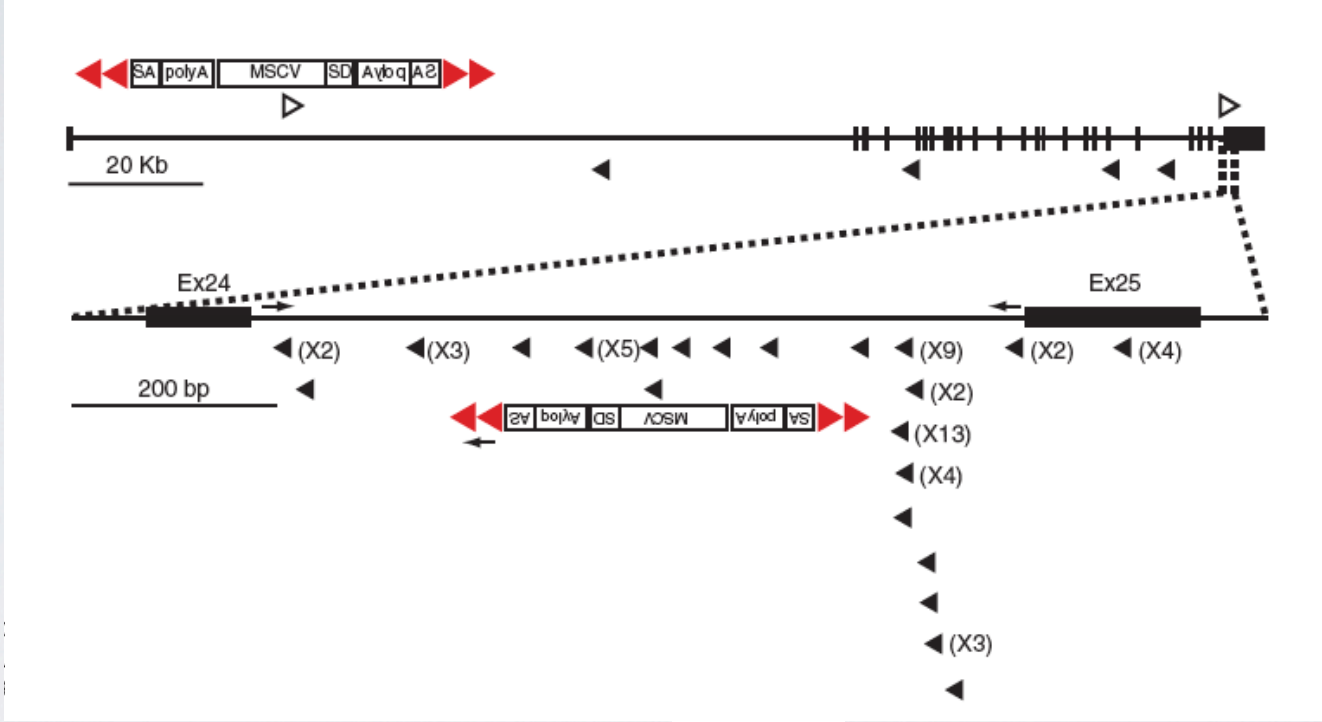
The Transposon can Drive Expression of Oncogenic N-Terminally Truncated Proteins: *Braf*

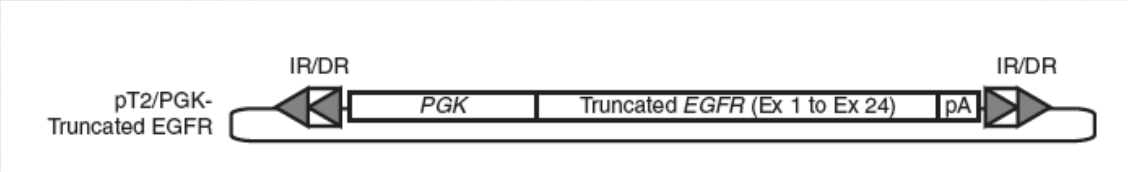






The Transposon can Cause Oncogenic C-Terminal Truncation: *Egfr*





The transposon can cause loss of TSG expression via insertion and LOH: *Pten* and *Nf1*

A Transposon with a Different Promoter Induces a Different Spectrum of Cancer

Molecular Biology, Pathobiology, and Genetics

A Modified *Sleeping Beauty* Transposon System That Can Be Used to Model a Wide Variety of Human Cancers in Mice

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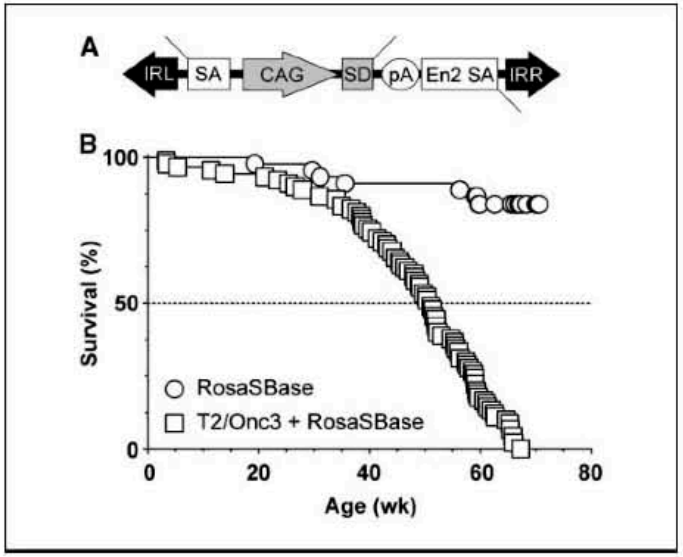


Figure 2. Structure and function of the T2/Onc3 transposon. *A*, the T2/Onc3 transposon is similar to the earlier T2/Onc2 element except that a CMV_{ie} enhancer/CAG promoter replaces the MSCV 5′-LTR. *B*, survival of T2/Onc3;RosaSBase double-transgenic mice compared with RosaSBase littermates.

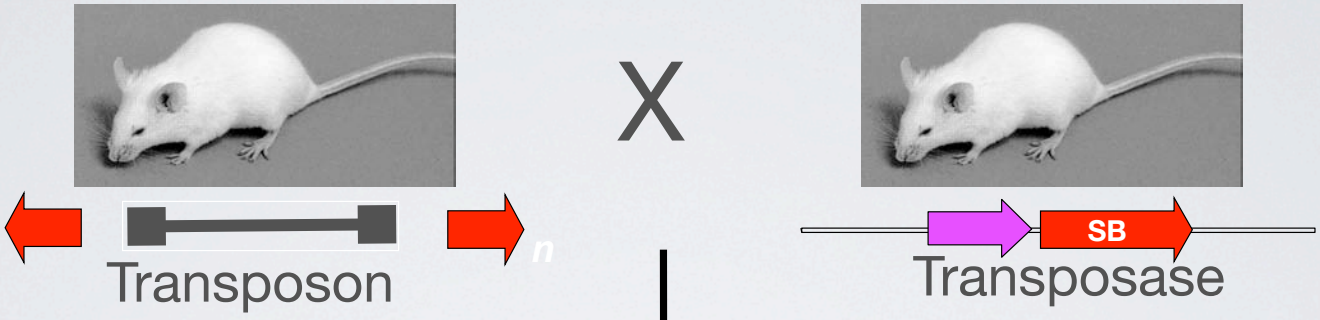
Table 1. Pathology of T2/Onc3-induced tumors

Site	Type	No.*
Skin	Squamous cell carcinoma	27
	Melanoma	1 (1)
	Basal cell carcinoma	1
Liver	Hepatocellular carcinoma	23 (1)
	Hepatoblastoma	1
	Adenoma	58
Colon	Carcinoma	1
	Adenoma	1
Lung	Carcinoma	3
	Adenoma	21
Brain	Astrocytoma	1
Mammary gland	Carcinoma	5
Ovary	Adenoncarcinoma	1
Clitoral gland	Squamous cell carcinoma	1
Salivary gland	Carcinoma	1 (1)
	Squamous cell carcinoma	1
Preputial gland	Carcinoma	2
Parathyroid	Carcinoma	1
Nasal cavity	Carcinoma	2
Adrenal gland	Pheochromocytoma	1
Lymphoma	T cell	2
	B cell	5
Multiple	Sarcoma	2
	Hemangiosarcoma	4 (1)
	Hemangioma	7
	Adenoma (other)	23

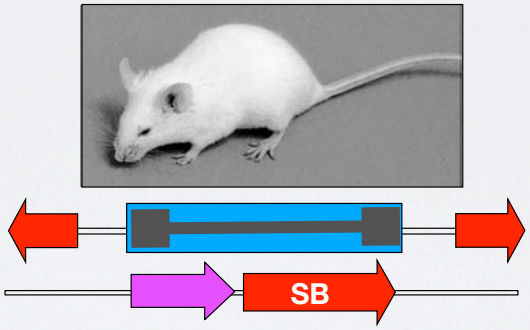
*Number of tumors in 62 T2/Onc3;RosaSBase mice. The number of metastatic tumors of each type is shown in parentheses.

A transposon without a promoter is weakly oncogenic

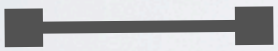
SB transgenic mice could be used to determine the relative oncogenic genotoxicity of various vectors



Create transgenic lines of mice with concatemer of SB IR-flanked vector



Relative oncogenicity, tissues at risk, genetic loci favored during tumor development



New Vector

Masonic Cancer Center, University of Minnesota

A Comprehensive Cancer Center designed by the National Cancer Institute

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John Ohlfest (UMN)
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Nancy Ratner (Cincinnati)

Paul Marker (UW-Madison)

Lara Collier (UW-Madison)

Michael Taylor (Toronto)

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Fausto Rodriquez
Aaron Bender

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