



Mutagenesis in mice and rats using mobile DNA technology

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Disclosures

 I am a consultant, co-founder, and co-owner of NeoClone Biotechnology, Inc., an antibody company. This presentation does not relate to NeoClone's business area.

• I am a consultant, co-founder, and co-owner of Discovery Genomics, Inc. (DGI), a company developing *Sleeping Beauty* (SB) for human gene therapy. This work did not involve DGI personnel or funds and is not directly related to DGI's business area.

The human genome

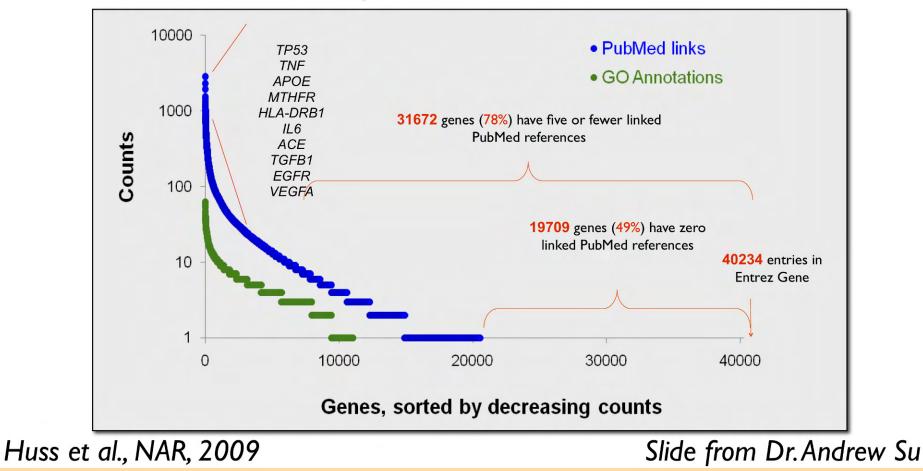


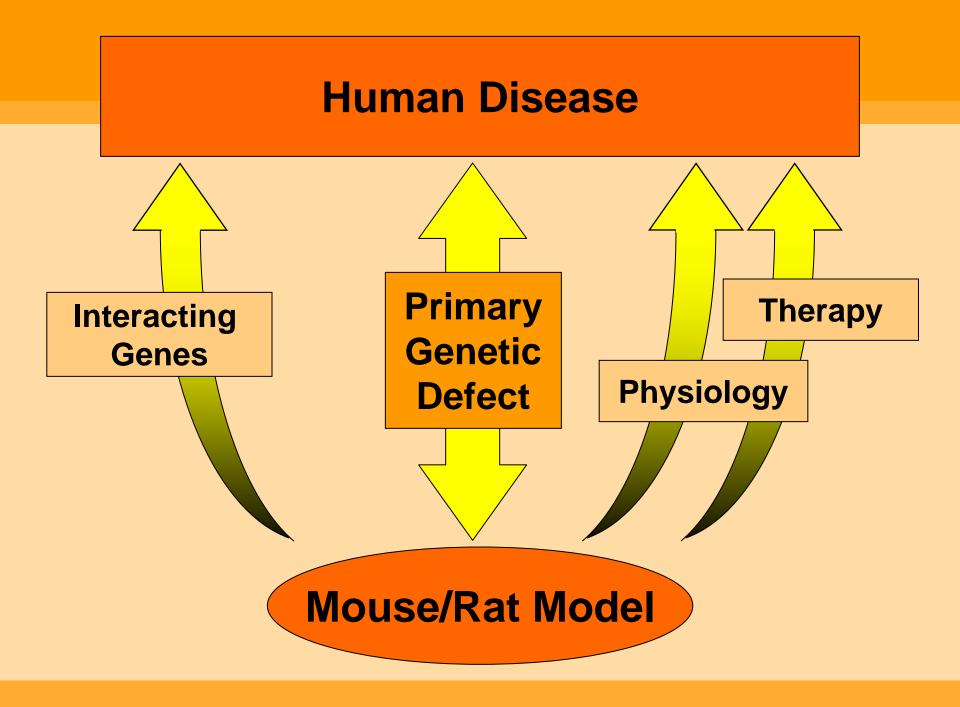
How many genes are there? What do they do? Which are critical in disease? What does the functional landscape of the mammalian genome look like?



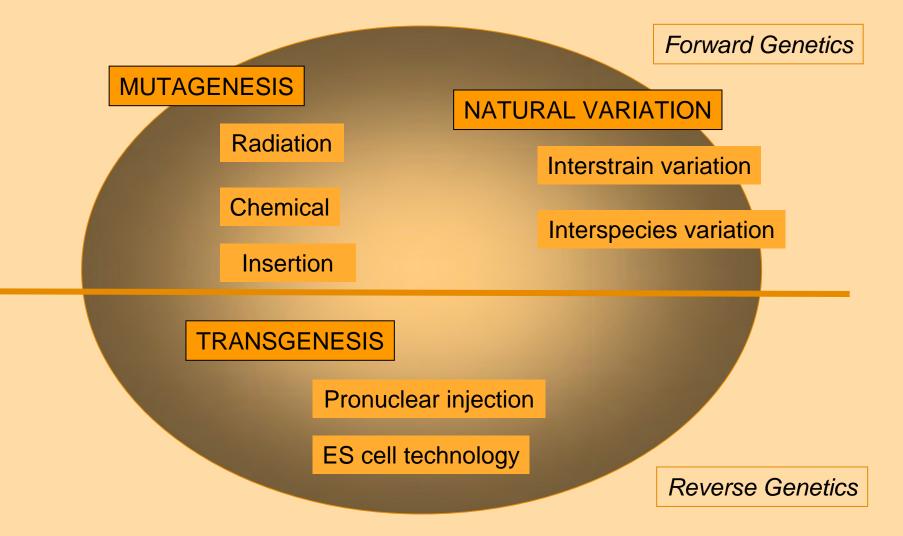
Well Studied Genes Continue to Get all the Attention

•How do we efficiently annotate the function of all the genes in the mammalian genome? •Goal: "Genome-wide functional genomics"

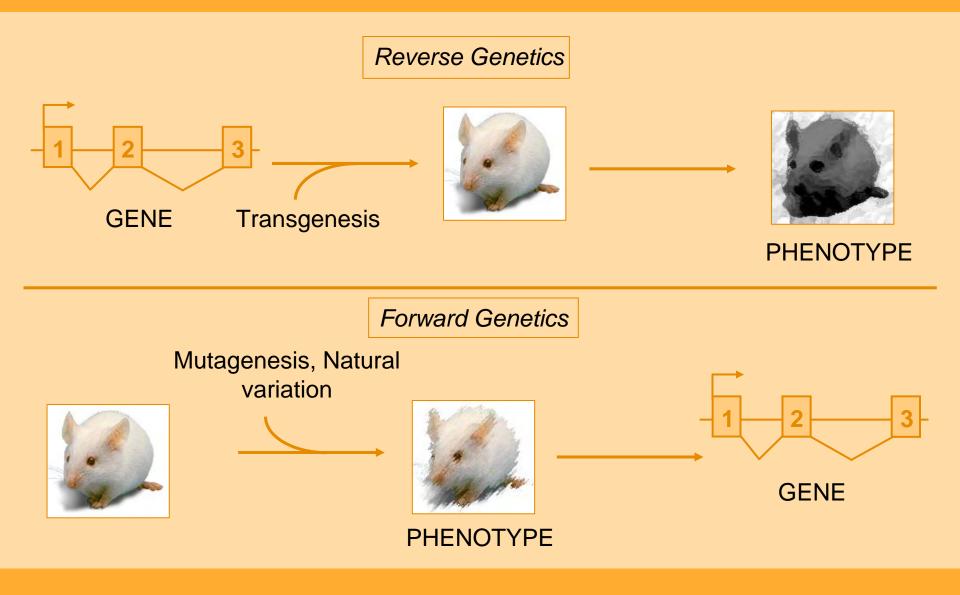




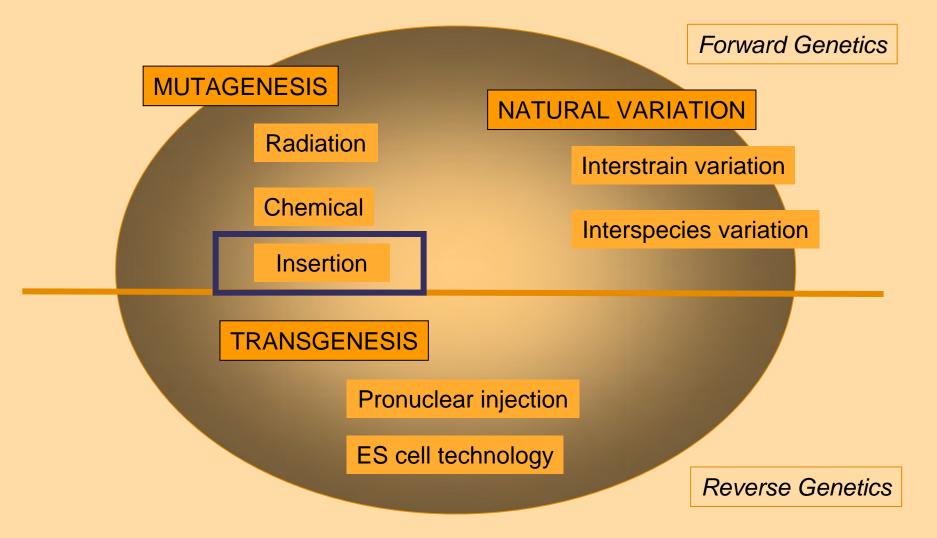
Sources of genetic variation



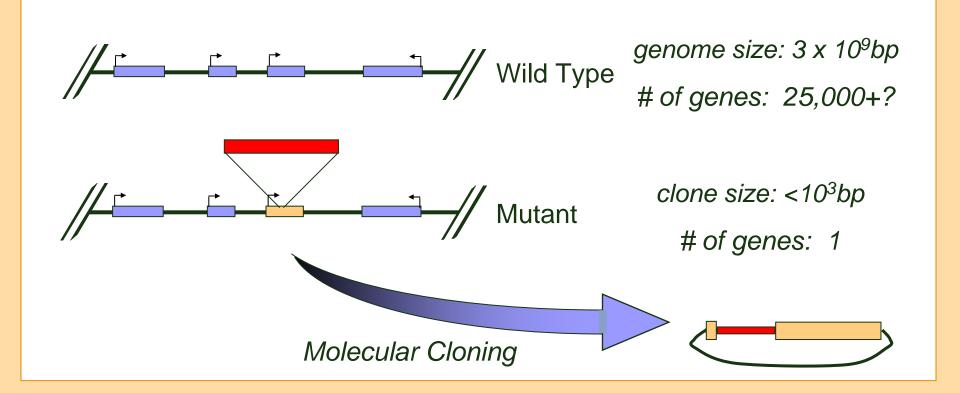
Forward versus reverse genetics



Sources of genetic variation



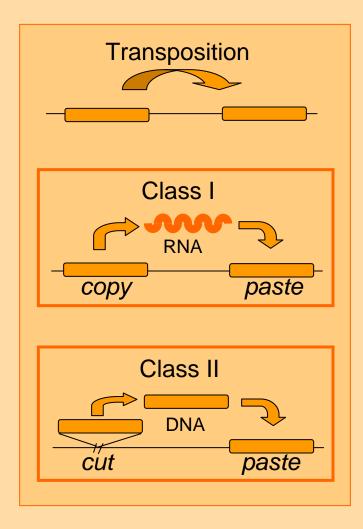
Insertional mutagenesis can simplify gene identification



Naturally occurring systems of mobile DNA are ideal for this purpose

Transposable elements

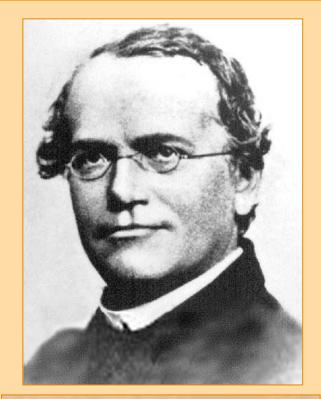
- Sequences of DNA that move around to different positions within genome of a single cell
- "Jumping genes" or "mobile genetic elements"
- Class I or retrotransposons and Class II or DNA transposons
- Encode proteins needed for transposition reaction, e.g. transposases



Transposons at the beginning of modern genetics

- Mendel's wrinkled pea mutation
- Transposon insertion into a gene encoding a starch branching enzyme

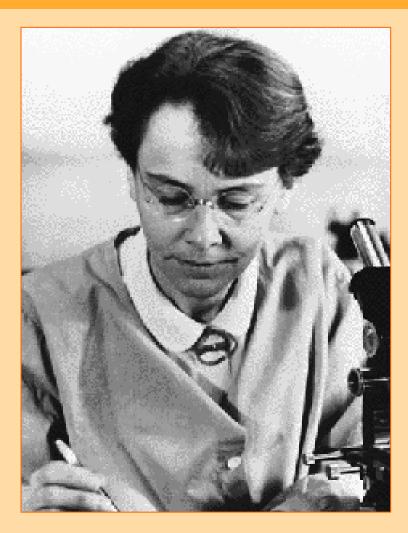




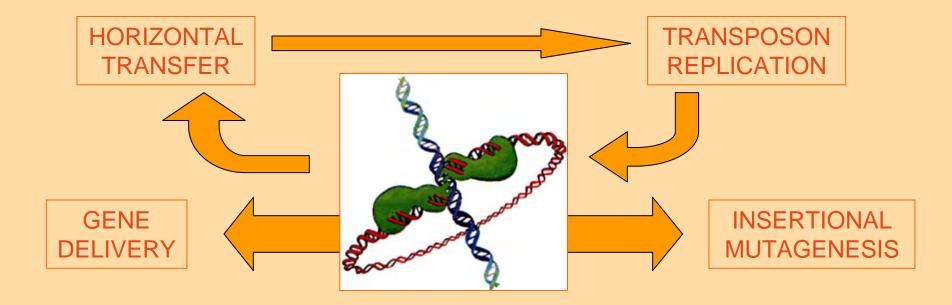


Barbara McClintock discovered transposable elements

- Definitive proof in 1952
- Showed linkage arrangement of genes could change in Maize
- Nobel Prize in 1983



The properties of transposable elements make them useful for genetics



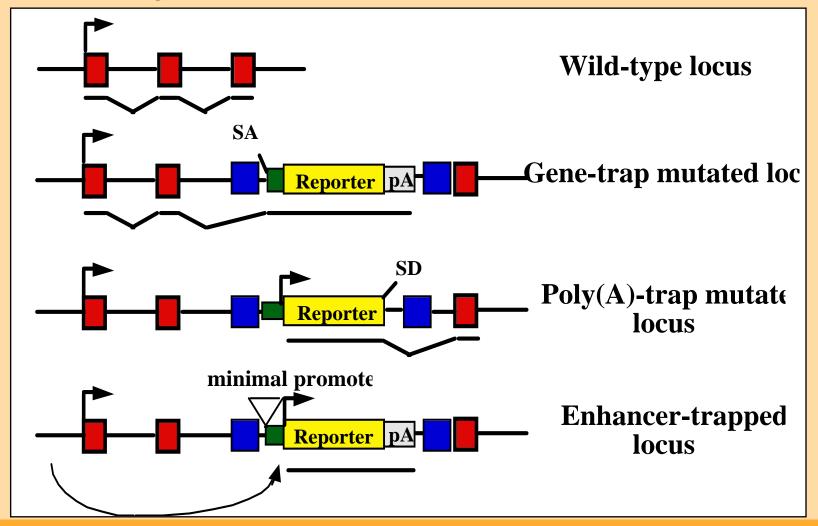
Transposable elements become useful tools for geneticists

- 1982: The P element used to genetically transform *Drosophila*
- 1989: P element "enhancer traps" reveal patterned gene expression in Drosophila



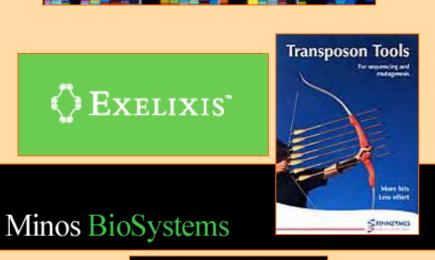
Transposable elements are probes for the functional parts of a gene

Reporter gene vectors sensitive to insertion site context



Transposable elements have entered mainstream basic and applied science

- Now that thousands of elements have been identified the possibilities for their use have expanded
- Biotechnology companies founded on transposable elements





Transposons for genetics/genomics

- Comprehensive insertion mutation libraries established in many species: *Drosophila*, plants, bacteria, fungi
- Complement chemical mutagenesis approaches
- Can be used to create useful "tagged" libraries (e.g. GFP-tagged ORFs)
- Fundamental platform for functional genomics
- Vertebrate genetics/genomics?

Sleeping Beauty (SB)

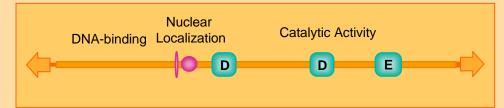
•A synthetic "cut-and-paste" DNA transposon system

•Derived from inactive Tc1/mariner family transposon elements in fish

-Tc1/mariner elements widespread animals, plants, fungi, ciliates and bacteria but all vertebrate elements dead

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

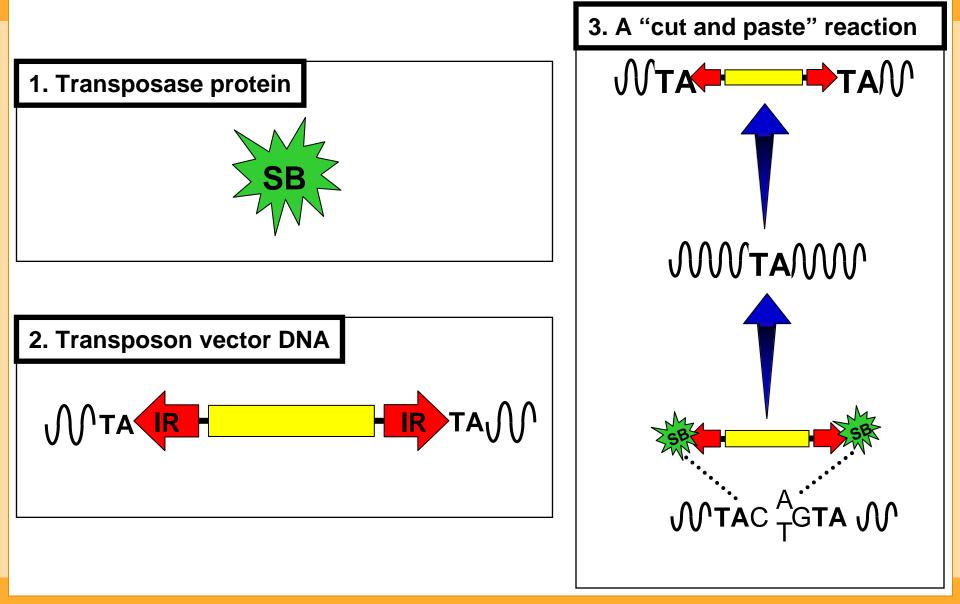




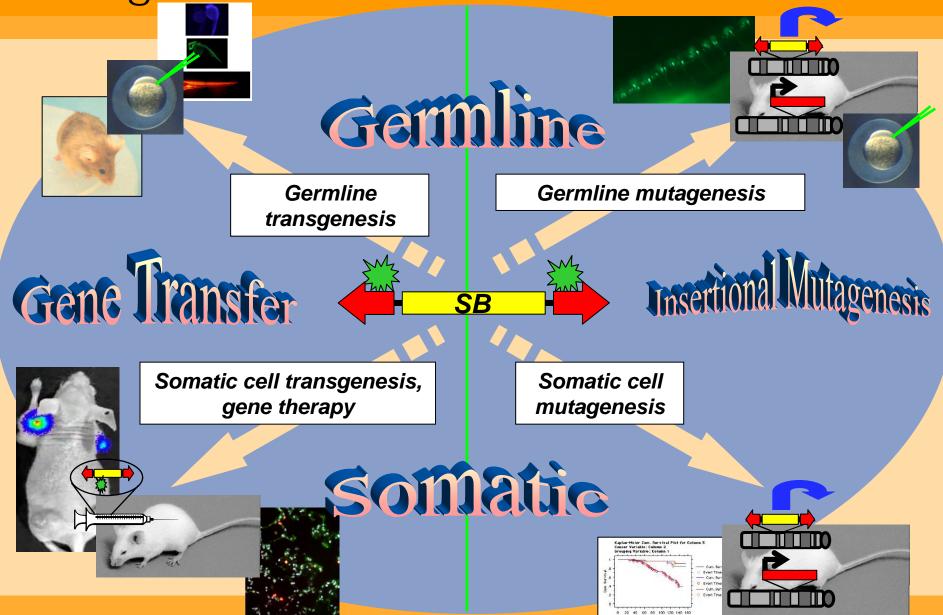
•Created in Dr. Perry Hackett's lab by Dr. Zoltan lvics and Dr. Zsuzsanna Izsvak - creating a consensus transposase sequence reverse evolutionary approach

lvics et al., Cell, 1997

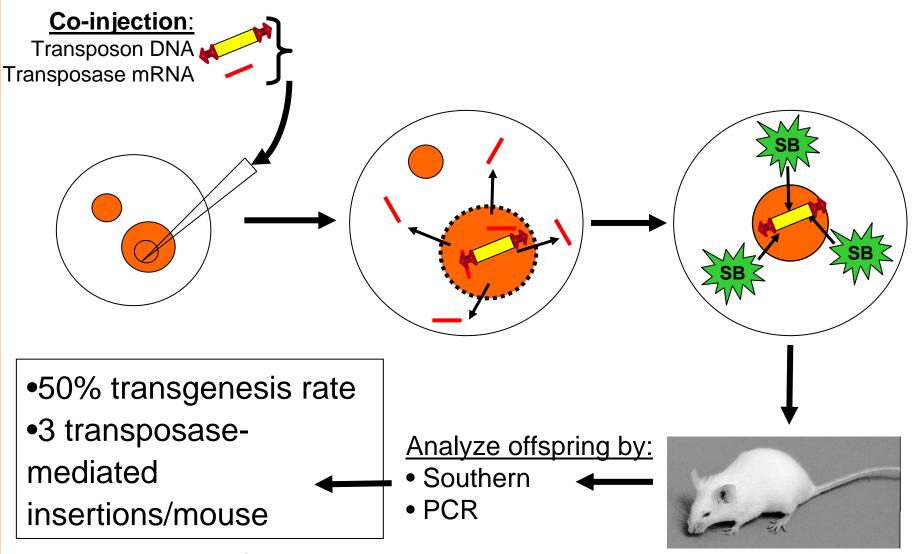
Sleeping Beauty is a two part system: The transposon and the transposase



SB: A versatile tool for applied and basic biological research



Germline transgenesis by transposition

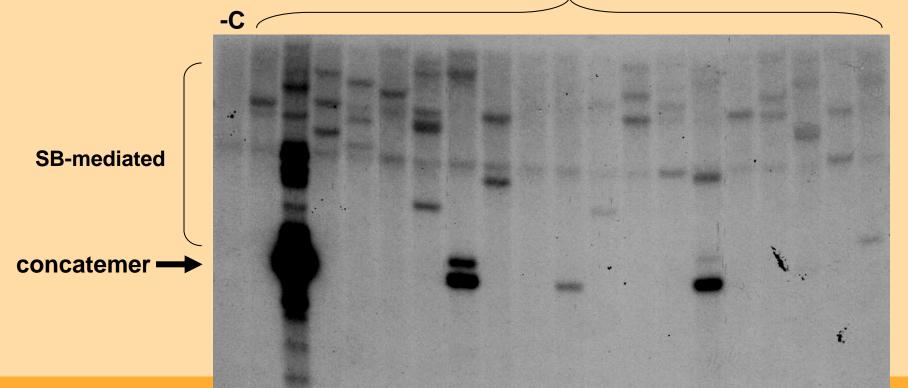


Dupuy et al., PNAS, 2002.

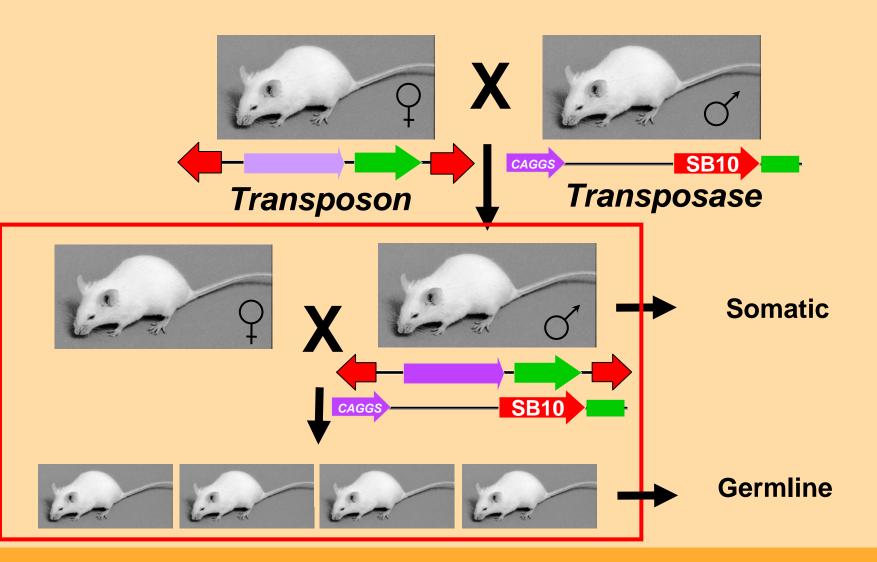
Improved germline transgenesis using SB

- Optimized SB11 transposase mRNA created
- Methylated transposon vector DNA
- Experiment repeated: ~90% of offspring transgenic

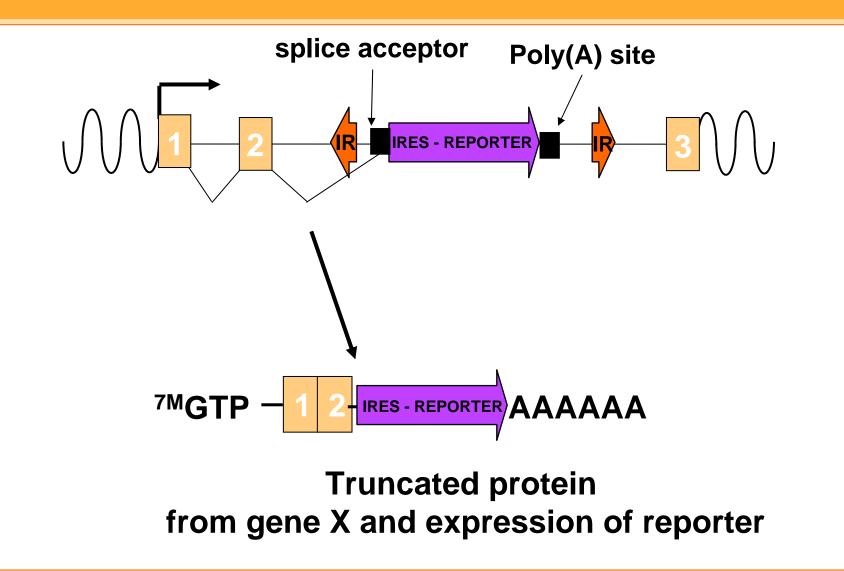
Founder tail biopsy DNA



Mutagenesis with *Sleeping Beauty:* Inducing transposition of chromosomally-resident transposon vectors



Gene trap transposons are designed to disrupt genes



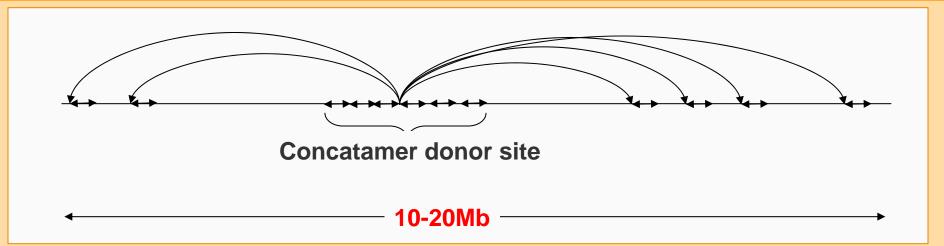
SB can efficiently induce germline mutations

- •Up to three insertions per gamete
- •Roughly1/4 transposition events land in known or predicted transcription unit
- No strong tendency toward or away from genes
- No strong tendency to land in promoters of genes
- •Transposons can hop into and mutate genes (some are nulls, some hypomorphs, some silent)
- Mutations can be reverted by remobilization
- •Transposons land near donor locus 50% of the time i.e "local hopping"

Dupuy et al., Genesis, 2001. Carlson et al., Genetics, 2003.

| Chr | position | mouse gene hit | NRAA definition or Panther best hit | | | | | | |
|-----|-----------------------------------------------------|----------------|------------------------------------------------------------|--|--|--|--|--|--|
| 8 | 5745814 | EST | No known function | | | | | | |
| 7 | unknown | mCG67976 (-) | Krueppel-related C2H2-type zinc-finger | | | | | | |
| 9 | 14154737 | mCG1034501 (+) | No known function | | | | | | |
| 14 | 32352513 | mCG52624 (-) | No known function | | | | | | |
| 9 | 4309928 | EST | No known function | | | | | | |
| 9 | 34541781 | mCG127192 (-) | No known function | | | | | | |
| 3 | unknown | mCG9496 (-) | Guanylate cyclase alpha 2 subunit | | | | | | |
| 7 | 88114871 mCG1028279 (-) Reverse Transcripta related | | | | | | | | |
| 3 | unknown | mCG1044682 (+) | No known function | | | | | | |
| 3 | unknown | mCG59825 (-) | No known function | | | | | | |
| 12 | unknown | mCG1039718 (+) | No known function | | | | | | |
| 17 | 80326798 | mCG12054 (+) | No known function | | | | | | |
| 6 | 93423488 | mCG127714 (+) | Mitochondrial carrier protein- related | | | | | | |
| 8 | 1931386 | mCG1814 (-) | Shc SH2-binding protein 1 | | | | | | |
| 9 | 95742434 | mCG1032876 (+) | No known function | | | | | | |
| 9 | 60,950,446 | mCG7690 (+) | Similar to Carbonic anhydrase XII [<i>Hs</i>] | | | | | | |
| 1 | 116,266,773 | mCG132548 (+) | Similar to Caspr 5 protein isoform 1 [<i>Hs</i>] | | | | | | |
| 1 | 32,392,298 | | | | | | | | |
| | | mCG121449 (+) | Family not named | | | | | | |
| 1 | 41,557,972 | mCG121450 (-) | Similar to Eukaryotic initiation factor 4B [Hs] | | | | | | |
| 1 | 41,987,901 | | | | | | | | |
| 1 | 43,571,384 | mCG116075 (-) | Similar to axonemal dynein heavy chain 7 [Hs] | | | | | | |
| 1 | 62,781,818 | mCG123134 (-) | Parathyroid hormone receptor (Panther) | | | | | | |
| 13 | 61,342,598 | mCG121043 (-) | Kruppel-related C2H2-type zinc-finger protein (Panther) | | | | | | |
| 10 | 121,530,761 | mCG49173 (-) | Glyceraldehyde-3-phosphate dehydrogenase | | | | | | |
| 9 | 59,566,450 | mCG1034698 (-) | unnamed protein product (pseudogene) | | | | | | |
| 9 | 60,485,914 | mCG7689 (-) | Death-associated kinase 2 | | | | | | |
| | unknown | | | | | | | | |
| 10 | 88,689,166 | mCG1039017 (+) | no description | | | | | | |
| 1 | 138,297,839 | | | | | | | | |
| 16 | 7,487,380 | mCG1038218 (-) | no description | | | | | | |

Local hopping could allow regional saturation mutagenesis



•Generate concatemer donor sites in gene-rich regions of high interest

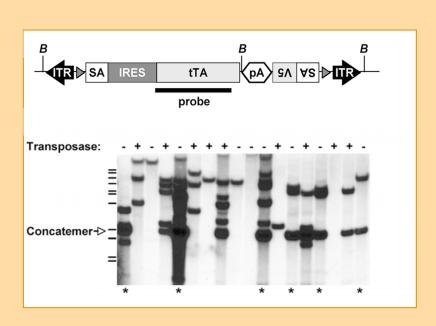
•Need high hopping rate, concatemer donor homozygous viable, with no phenotype

•Selected a transposon transgenic line on chr. 11 in the *Trp53-Wnt3* interval for this project

•Kile et al. (2003 Nature) had performed ENU based screen in this interval

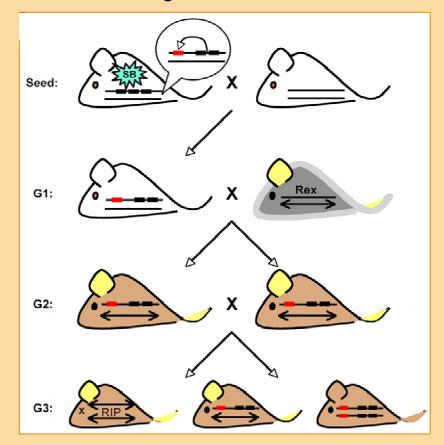
•Keng et al. (2005 *Nature Methods*) suggested SB can be used for regional mutagenesis

Three-generation forward genetic screen for recessive lethal, visible, and behavioral mutants



•The GT3A donor on Chr. 11 hops at a high rate

•The breeding scheme



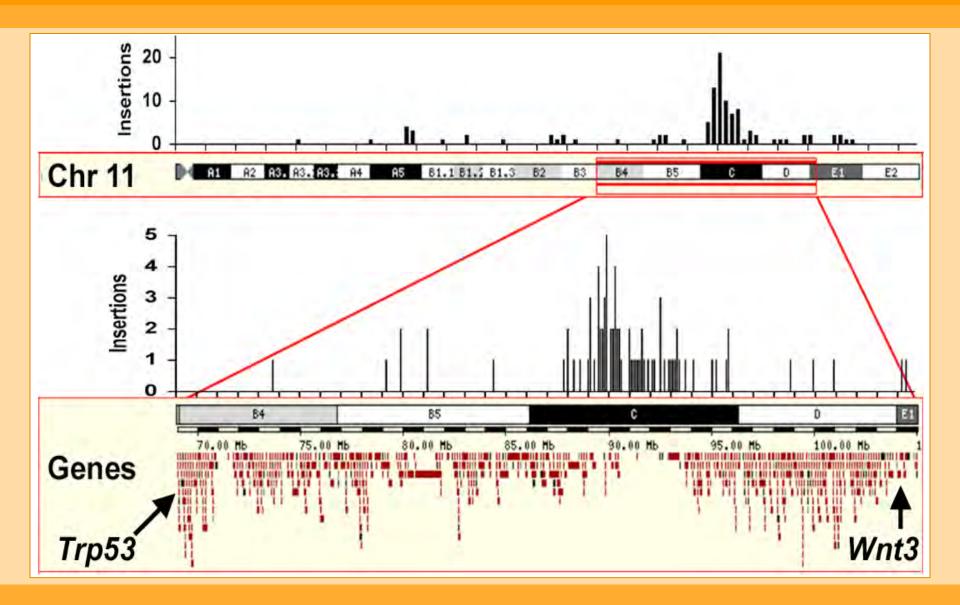
•Also cryopreserved sperm, cloned transposon insertions

Geurts et al., PLoS Genetics, 2006.

Results of 50+ pedigrees

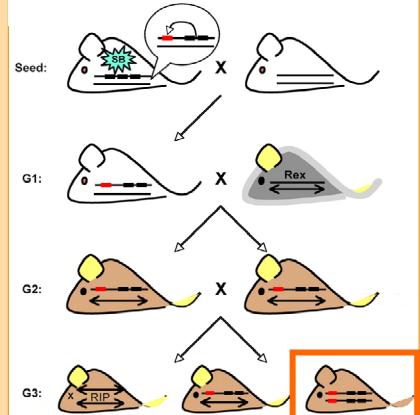
- Cloned hundreds of germline transposon insertions from G2 or G3 animals
- 50% inserted into chromosome 11 sequences
- Of those, about 70% landed in a known or predicted transcription unit

The distribution of gene-trap transposon insertions on chromosome 11 show localized mutagenesis in pedigrees



Results of 50+ pedigrees

- Phenotypes sought in G3 mice
- Roughly 50% of pedigrees carry a pre-natal lethal mutation
- Some viable mutants also obtained



Insertion mutations present in pedigrees

Table 1. Pedigree Phenotype and Cumulative Insertion Data

| GT3A; CAGGS-SB10 | Number | Pedigree Designation | Chromosome-11 Insertions ^a | In Genes ^b |
|---------------------------|--------|-----------------------------------------------------------------------|---------------------------------------|-----------------------|
| | | | | |
| Recessive prenatal lethal | 19 | M, Q, V, W, Z, AG, AL, AO, AP, AS, AU, AX, AY, BA, BB, BC, BH, BL, BM | 50 | 32 |
| Behavioral | 1 | BG | 4 | 3 |
| Skeletal | 1 | BMc | 2 | 1 |
| No phenotype | 9 | T, AD, AM, AQ, AT, AV, AZ, BI, BK | 19 | 10 |
| GT3A; RosaSB11 | | | | |
| Recessive prenatal lethal | 2 | CD, CQ | 4 | 3 |
| No phenotype | 6 | CE, CF, CG, CH, CJ, CL | 1 | 1 |
| | | | | |

^aNumber of independent insertions identified.

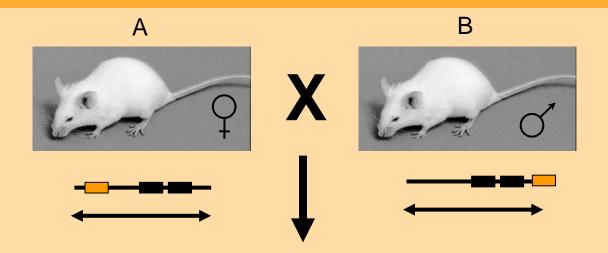
^bIncludes insertions in known or predicted genes and mapped expressed sequence tags.

CThis pedigree also displays recessive early embryonic lethality.

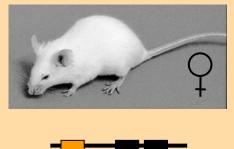
DOI: 10.1371/journal.pgen.0020156.t001

Complementation testing performed on many of the lethal mutants

Complementation testing



•Can we obtain mice carrying a copy of each mutagenized chromosome 11 one from pedigree A plus one from pedigree B?





We found six complementation groups in 19 pedigrees

•Two major complementation groups (I and II)

 Indicating common mutations occurring repeatedly

| GROUP I | | | | | | | | | | | | | GF | roui | P II | | | | | |
|---------|---|----|----|----|----|----|----|----|----|----|----|---|----|------|------|----|----|----|----|--|
| M | V | AL | AO | AU | BB | CQ | BM | AS | AG | AX | вс | Ζ | BL | W | AP | AY | BH | CD | | |
| - | - | | - | - | - | | | | + | + | | - | - | + | | + | + | + | М | |
| | - | | | - | - | - | - | - | | + | + | - | | + | + | + | + | + | v | |
| | | - | | - | | | - | | + | + | + | | | + | + | + | + | | AL | |
| | | | - | - | - | | | - | + | + | + | | | ÷ | + | + | + | | AO | |
| | | | | - | - | - | | | + | | + | | | ÷ | + | + | + | | AU | |
| | | | | | - | - | | - | + | + | + | - | - | + | + | + | + | + | BB | |
| | | | | | | - | - | | | | | | | ÷ | + | + | + | | CQ | |
| | | | | | | | - | | | | | | | | | | + | | BM | |
| | | | | | | | | - | + | - | + | | - | + | + | + | + | | AS | |
| | | | | | | | | | - | + | + | | + | + | + | + | | | AG | |
| | | | | | | | | | | - | | + | | + | | | + | | AX | |
| | | | | | | | | | | | - | + | | + | + | | + | | вс | |
| | | | | | | | | | | | | - | | - | - | - | - | | z | |
| | | | | | | | | | | | | | - | - | | | - | | BL | |
| | | | | | | | | | | | | | | - | | - | - | - | w | |
| | | | | | | | | | | | | | | | - | - | - | | AP | |
| | | | | | | | | | | | | | | | | - | - | | AY | |
| | | | | | | | | | | | | | | | | | - | | BH | |
| | | | | | | | | | | | | | | | | | | - | CD | |

Most lethal pedigrees carry deletions/rearrangements

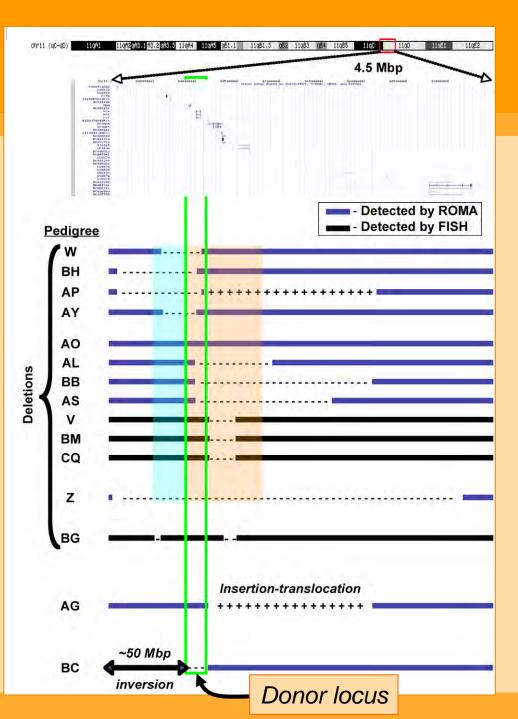
•FISH or CGH used to define sequences lost in pedigrees carrying lethal mutations

•Complementation group I pedigrees deletions extending telomeric from the donor site

•Complementation group II pedigrees deletions extending centromeric from the donor site

•Deletions are 100's of kb in size, up to 4.3 Mbp (pedigree Z)

•In pedigree AG, donor site plus several hundred kb has jumped to chromosome 5 and in pedigree BC, a 50 Mbp inversion



Viable phenotypes are recovered in forward transposon-based screen

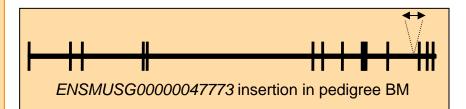
•Dominant polydactyly (extra digits) or polysyndactyly (extra, fused digits)

Bal/wt Bal/BM



Bal/wt Bal/BM Bal/BM

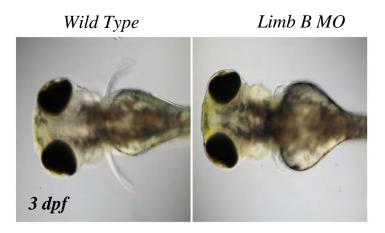




•Pioneer gene, ankyrin-repeats and NLS, well-conserved

•Two zebrafish versions of this gene (LimbA and limbB)

•Morpholino (MO) knockdown causes absence of pectoral fins other hedgehog LOF phenotypes



•BM mutation in mice may be a GOF mutation

Germline mutagenesis conclusions

- Local mutagenesis with SB very high yield of mutants
- Genes required for viability, limb development, and behavior identified
- But, complementation testing and FISH/CGH analyses show SB can induce chromosomal deletions and these are responsible for most of the lethal mutants. (Roughly 40% of pedigrees)
- Local saturation mutagenesis of the germline requires measures to eliminate recurrent chromosomal deletions from screen
 - Alternatively, do genome-wide project after improving transposition rate
- It may be more practical to generate germline insertion mutations by transposase mRNA + transposon DNA pronuclear injections.

Other transposon systems function in mammalian cells **TECHNICAL REPORT** genetics

- Tol2
- PiggyBac
- Minos
- **Frog Prince**

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piggyBac (PB) Insertional mutations and their characterizations in mice

Ling Y. Dun¹⁶⁴, Ke Jin⁹, Yiming Lin⁹, Wansai Yang¹⁶, Xing Xia⁹, Lin Ye⁹, Li Wang¹⁶, Lin Zin⁹, Sheng Ding¹⁸, Yi Su⁶, Je Zhen⁹, Min Han¹⁶⁶, Yuan Zheong¹⁶⁸, Tian Xu^{16,7}, Naolini Wa¹⁶, Hing Gu⁴s and Yang Zhang¹⁶⁴

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Hyperactive • transposases being developed (e.g. SB100)

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

Lajos Mártis^{1,4}, Marines K L Chush^{1,6}, Ryoyu Beley², Boris Jevenow², Namitha Manoj¹, Abel Acento-Sancher², David P Gerole¹, Andrea Schmitt¹, Korja Recker¹, Janisa Marinl², Ling Me², Krmire Samaro-Kalar², Como Gynemant², Diana Prypatindrek¹, Casha Milakoy¹, Brailsy Fletcher¹, Thierry VandenDeisench², Zalita Nick³ & Zumanna Itarik^{1,1}

The Weyley Benty (RD) incorporate is a precising registration of the formation is contained where detections are a contained by formation in the formation of t

Resource

- cell types. A large-scale genetic screen in manufalan cells yielded a hyperactive transposase (581693) with ~ 109-fold enhancement in efficiency when compared to
- tow-tan emanement in microsci, with compared to investigation manyawanes. SII 00X supported 17:-30%-gene transfer in human CD34" colls evelched in hemation stam or progenities cells. Transplantation of game-marked CD34" colls in immunicationed asks resulted in loss-fer

Col. Vol. 183, 473-483, August 12, 9936, Cauright (1909) or Enador Inc. DOI 10.1016/scil.000407.013

Efficient Transposition of the piggyBac (PB) Transposon in Mammalian Cells and Mice

Shang Ding,^{1,4} Xiaohui Wu,^{1,5,4} Gang Li,¹ Idin Han,^{1,6} Yean Zhuang,^{1,6} and Tian Xu^{4,4,4} ¹Institut of Developmental Biology and Molecular Medicine School of Life Sciences Sohool of Life Scences Hudin University 359 Handian Rosal Bhanghai 200453 China Phoward Hughes Mechael Institute Dagetimetri of NICOB University of Colonatio at Braider Boulder, Colonatio B0009 Phoneshead or Instancian Bourney, Coolinain Buder [®]Department of Immunology Duite University Neckloal Center Duitem, North Canolina 27710 [®]Howard Hughes Medical Institute [®]Howard Hughes Medical Institute Department of Ganatics Yele University School of Medicine Royer Center for Melicine 295 Congrists Antinue Nere Haven, Connectious 08338

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Efficient Transposition of Tol2 in the Mouse Germline

Vincent W. Keng, ** Barbara J. Rym, ** Kirk J. Wangensteen, 'Darius Balciusses, Christian Schmott, Stephen C. Ekker^a and David A. Largaospada* '

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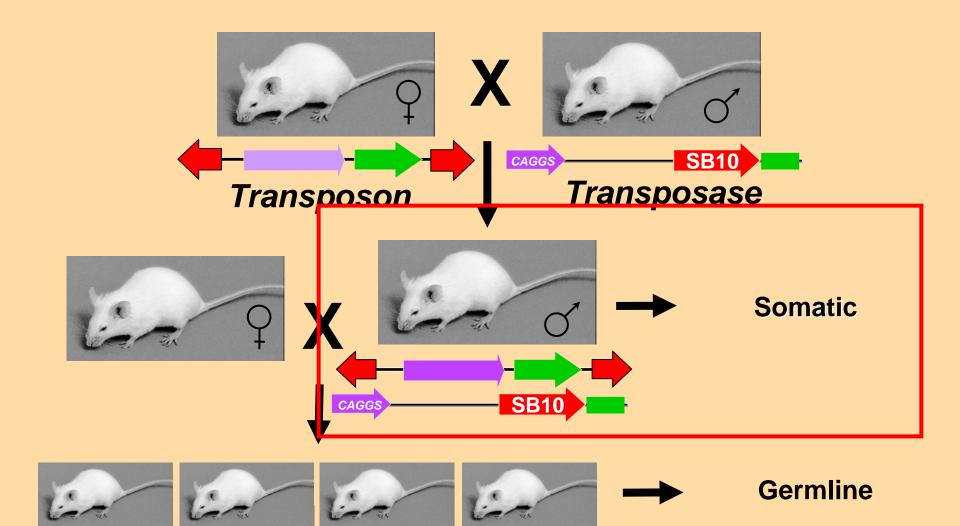
Servard genetic mutagenesis screen (Table 1). Using an 5 strain general principles sources (Calue 1) singlet Siligents may be rere essentiating a GLP experter publish top as an indication, to the mamber of motiant mice generated position, 7, and 11% of mechanic pape sour-GPP publics (GP) 1 for more and a respectively (Kalsa *et al.* 2005 Kr (any *et al.* 2007).

In 8 arch of an alternative tool for high-throughput Securi gentline unitagenesis sueet in mice a 742 transition interfactual initiagent as system was gener-ated as the basis of a shuffar strategy used for the SB and use the new of a sound evaluating user of the an-interaction waves if likely et al. 2005, have stell 2005. In the present study, we use estillable frammative the zones are of the field composition wave in the gradina-nation of the field composition wave in the gradina-undagenesis in atoms. The results induces the poten-sities of the state of the study of the potenand use of this transposon system for a high-throughput, large-scale forward minagenesis where in the footset or roline

MATLEDUS AND METHODS

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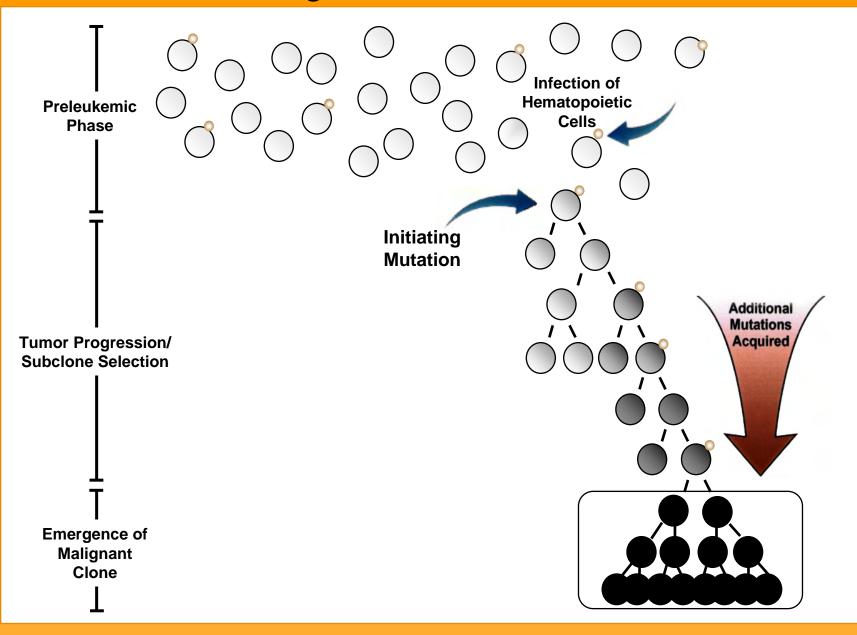
Mutagenesis with *Sleeping Beauty:* Inducing transposition of chromosomally-resident transposon vectors



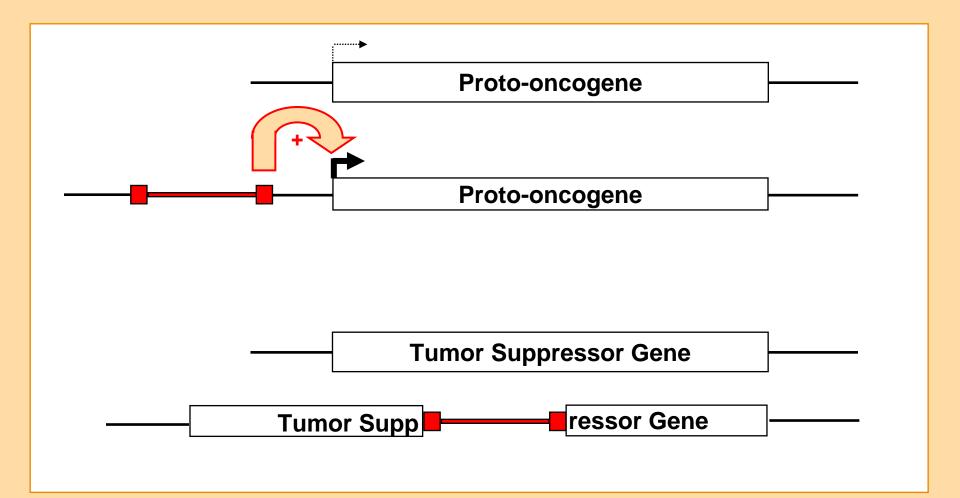
SB transposes efficiently in mouse somatic cells

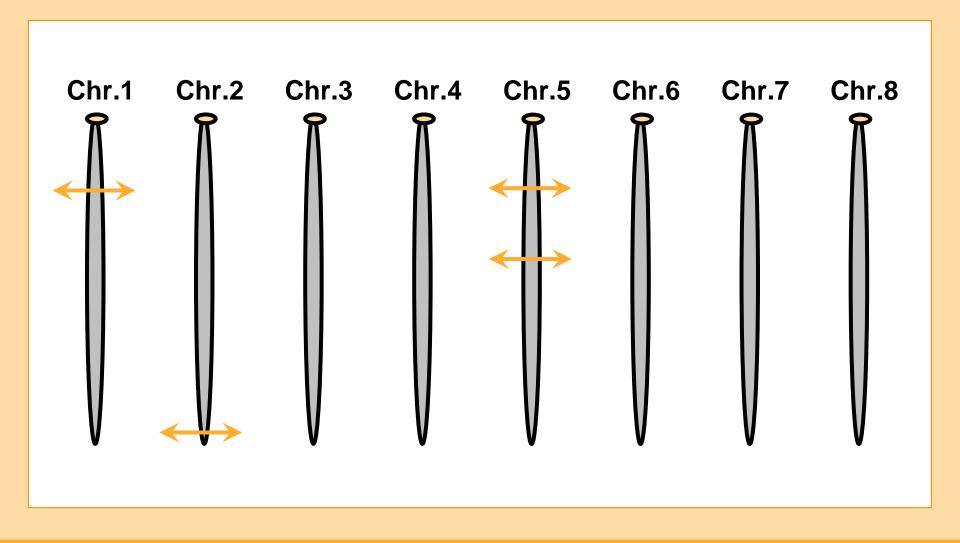
- Transposon excision marks about 1 in 15 cells in all tissues
- Transposon re-insertion sites cloned from somatic cells local hopping observed but insertions occur throughout genome
- Most transposon re-insertion events are rare within a given tissue (1 in 10,000 cells)
- Complexity of re-insertions is high and many, many insertion mutations probably occur in any tissue

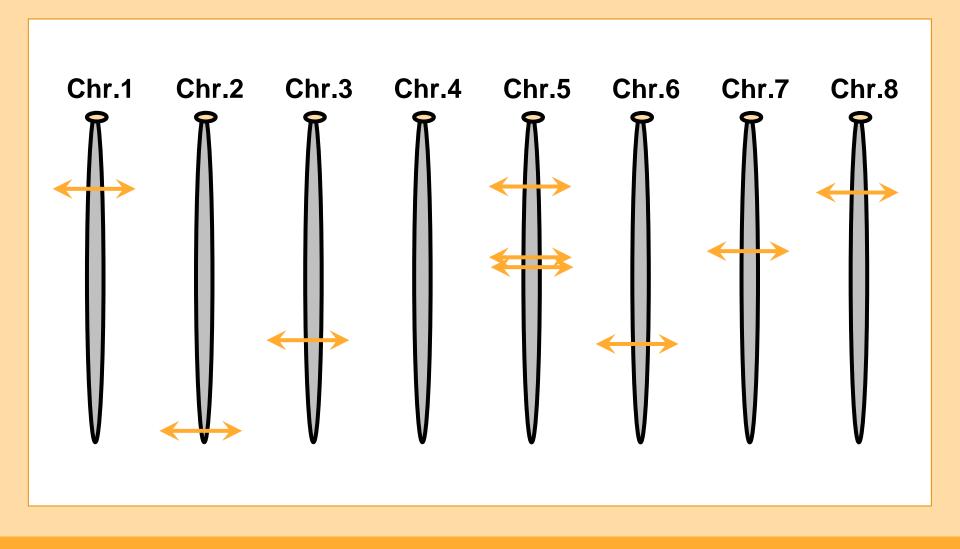
Retrovirally induced leukemia

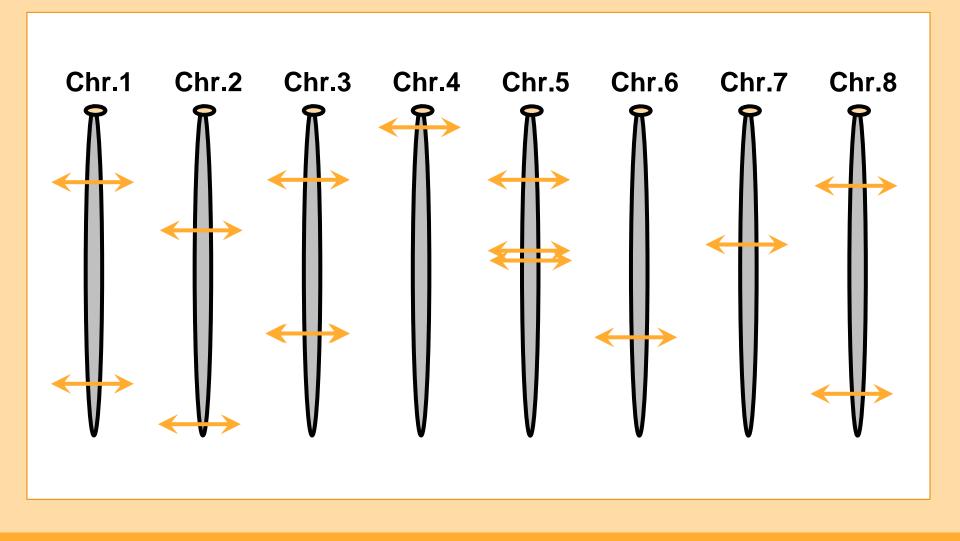


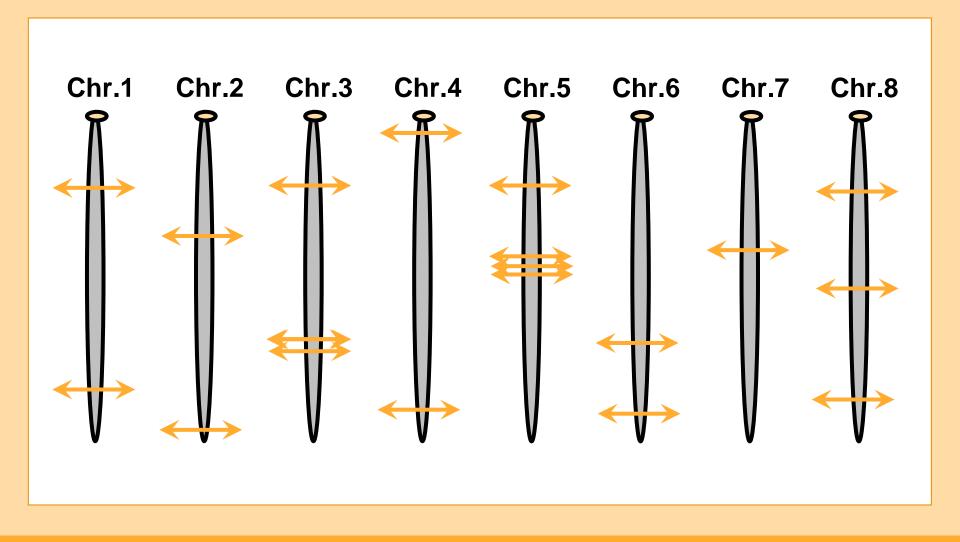
Could we mimic retroviral insertional mutagenesis?

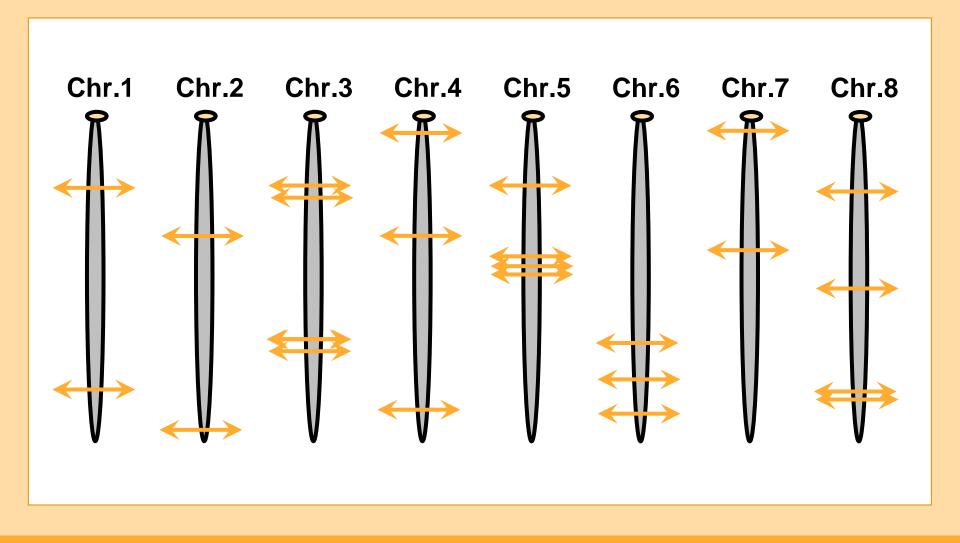


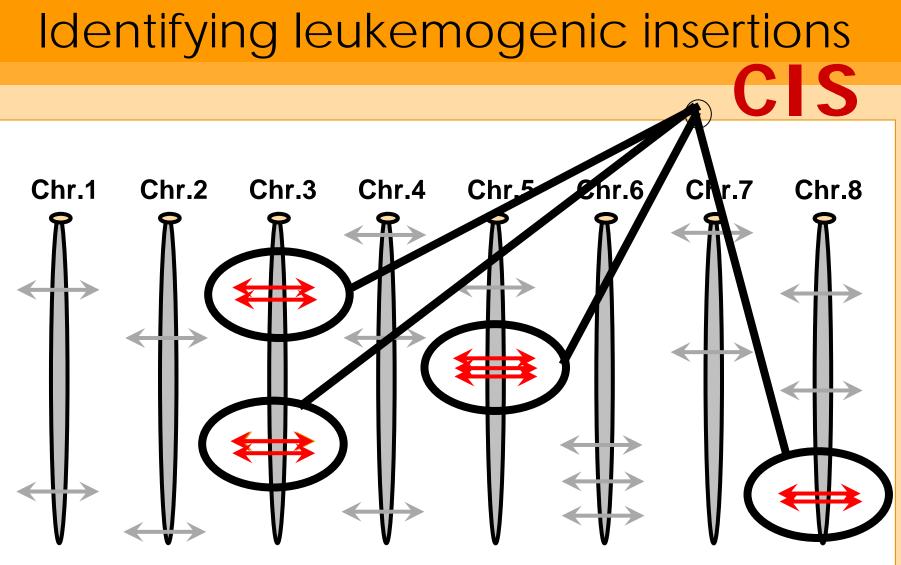






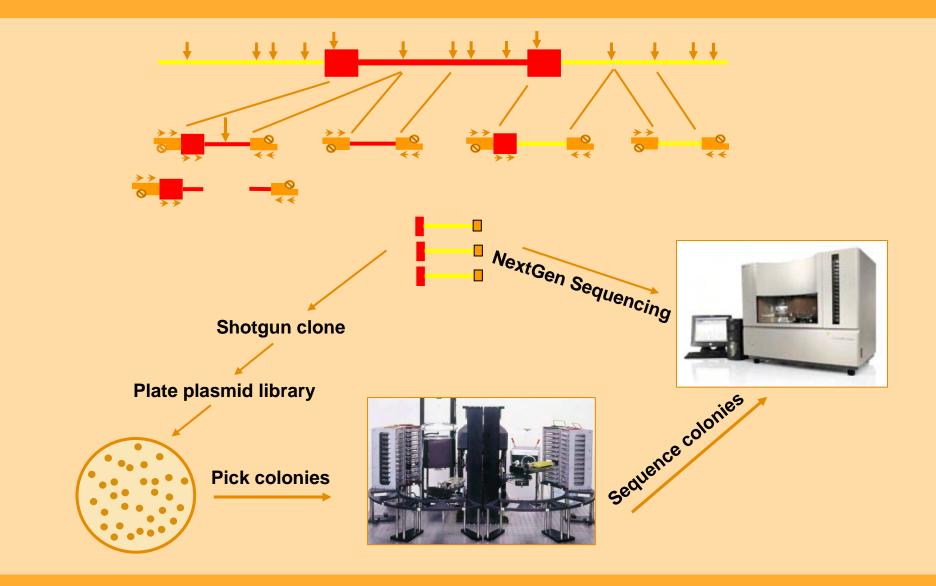




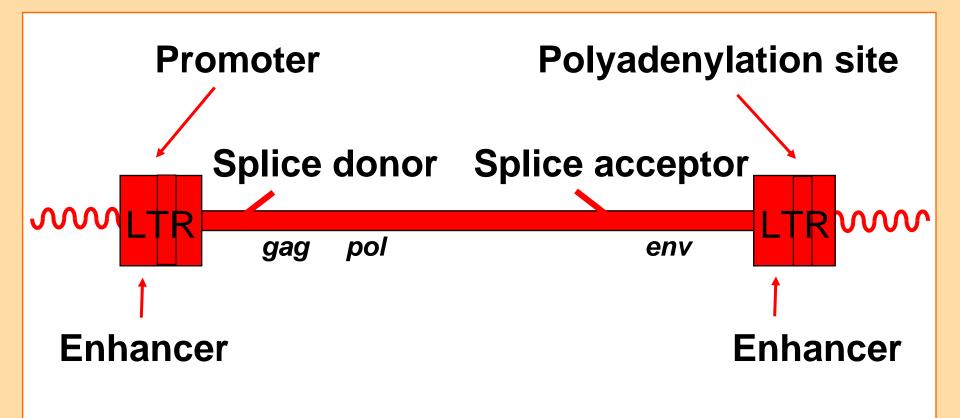


CIS = common integration site

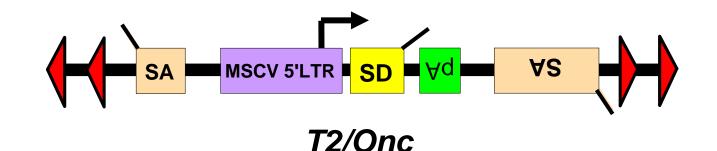
Shotgun cloning strategy using ligation-mediated PCR



Proviruses are potent somatic mutagens



Mimicking retroviral mutagenesis using an "insertionally oncogenic" SB transposon

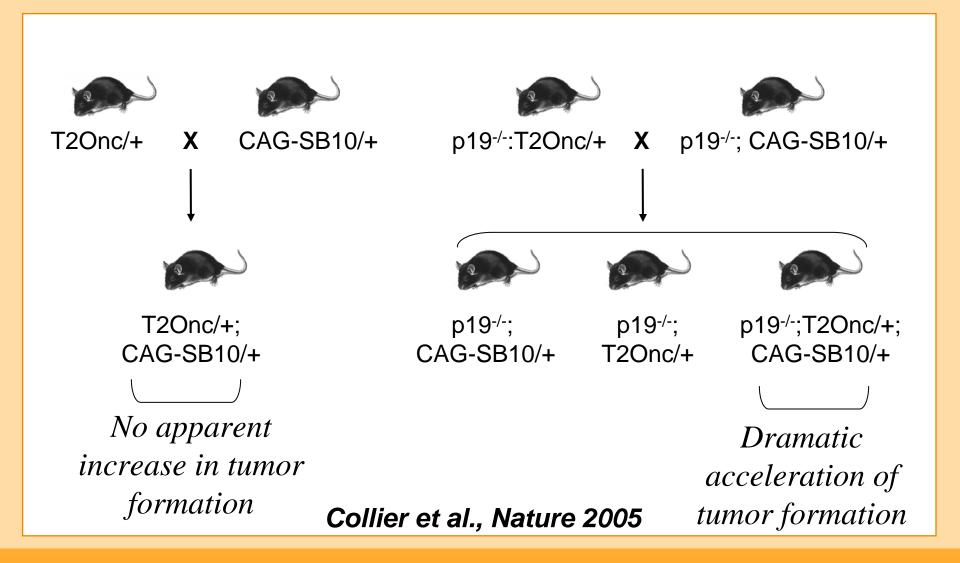


Types of mutations that can be induced:

- C-terminal truncations (both orientations)
- N-terminal truncations
- Promoter/enhancer insertions

Gain of function or loss of function mutations

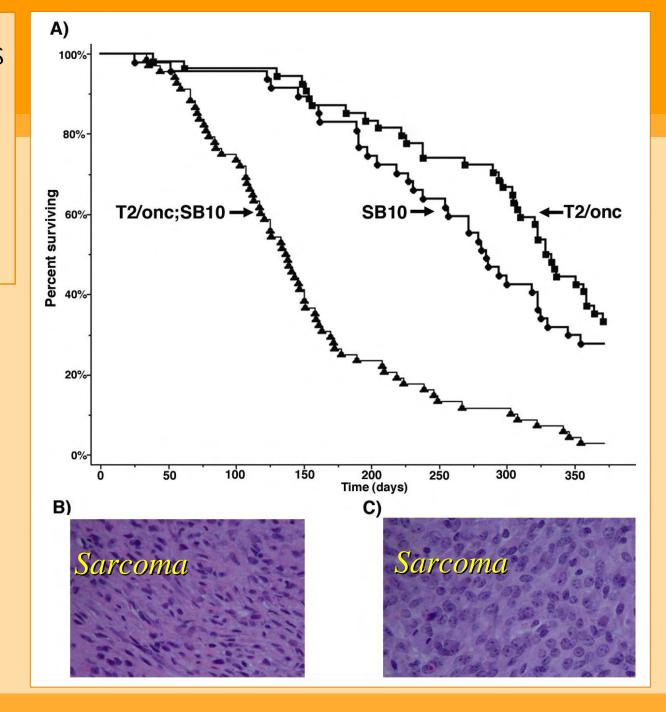
CAGGS-SB10 plus T2/Onc transgenes do not cause highlypenetrant tumor formation on a wild-type background, but accelerate cancer in *p19Arf-/-* mice



CAGGS-SB10 plus T2/Onc accelerates tumor formation on the *p19Arf*-/background

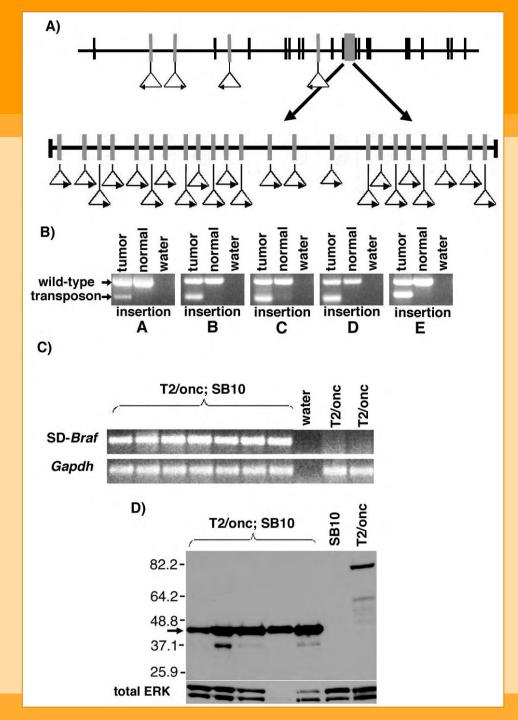
•Results similar with two independent T2/Onc lines (one on Chr. 1 and one on Chr 15)

Primarily sarcomas



Braf gene T2/Onc insertions

- •Tumor specific
- •Produce chimeric transcript
- •C-terminal "kinase domain only" peptide

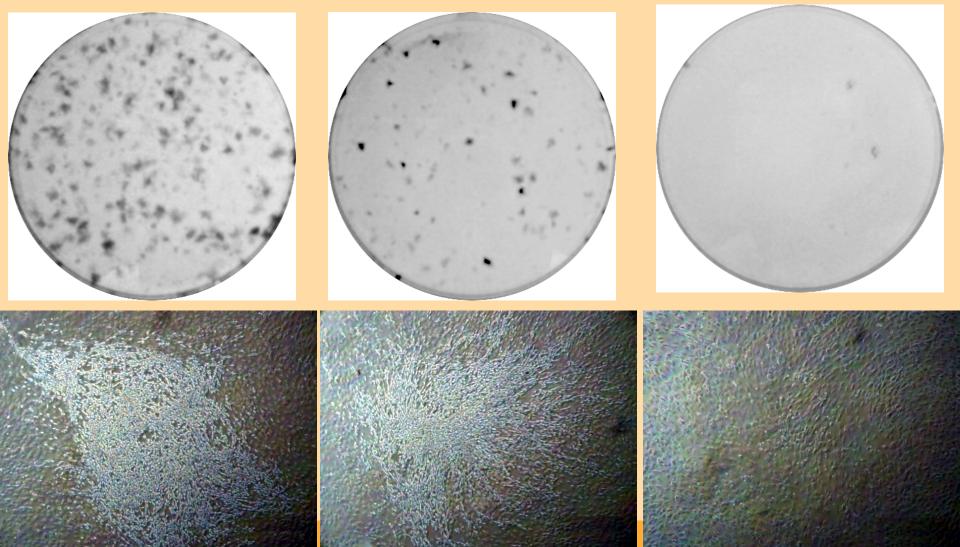


The cloned T2/Onc - *Braf* fusion transforms NIH 3T3 cells

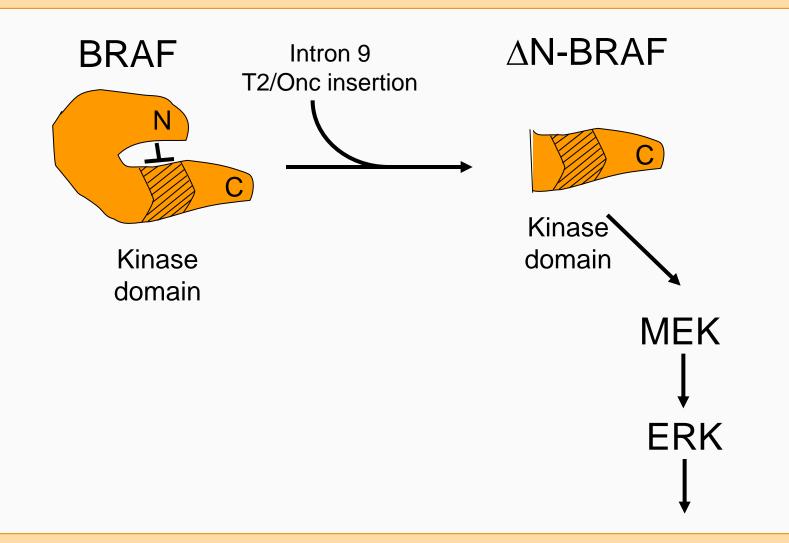
NRAS

T-Braf (Fwd)

T-Braf (Rev)



T2/Onc activation of Braf

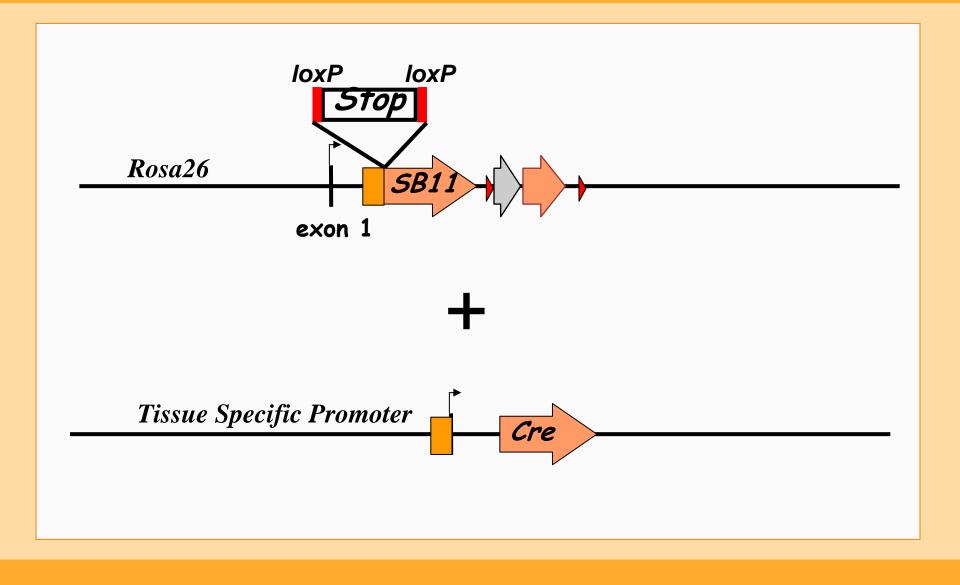


Can SB somatic mutagenesis cause cancer in other tissues?

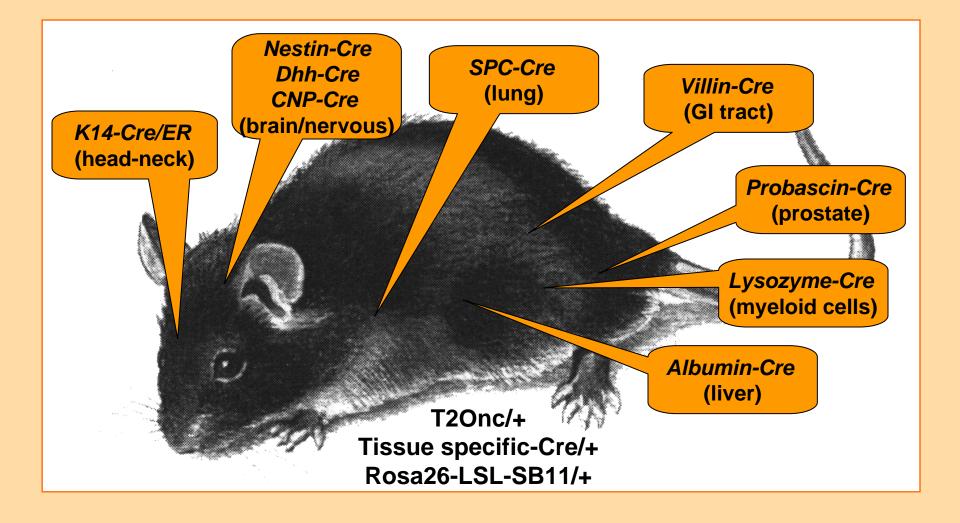
• Common epithelial derived cancers?

 Copeland/Jenkins lab generated knockin allele to express SB11 transposase from the *Rosa26* locus using a loxPflanked "STOP" cassette

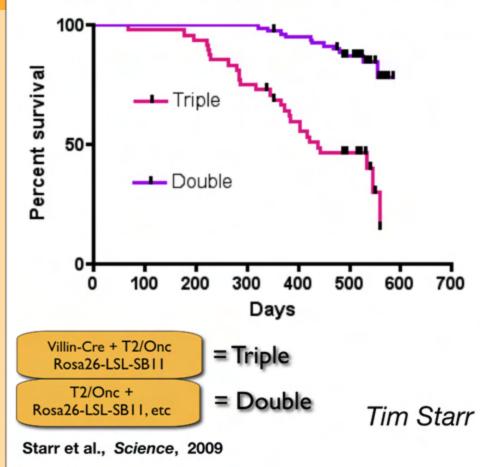
A conditional Rosa26-SB11 transposase knockin allele



Tissue specific transposon SB mutagenesis



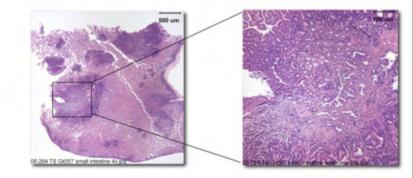
Accelerated Death in Triple Versus Double Transgenics (Villin-Cre)

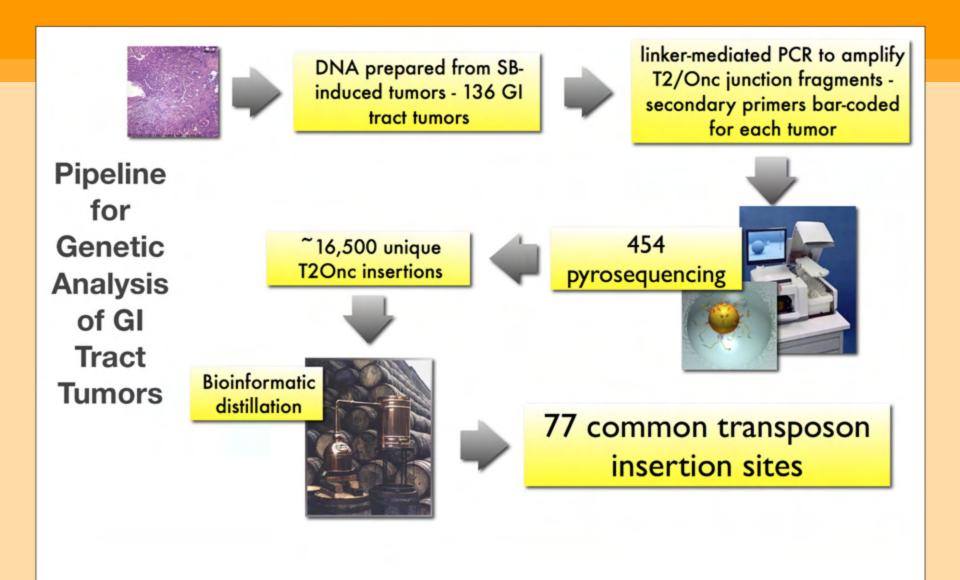


Triple transgenic mice developed GI tract tumors

•All had small intestine tumors (adenomas and some adenocarcinomas)

•25% also had tumor(s) in colon





Mutation of Human CRC Genes in SB-Induced GI Tract Tumors

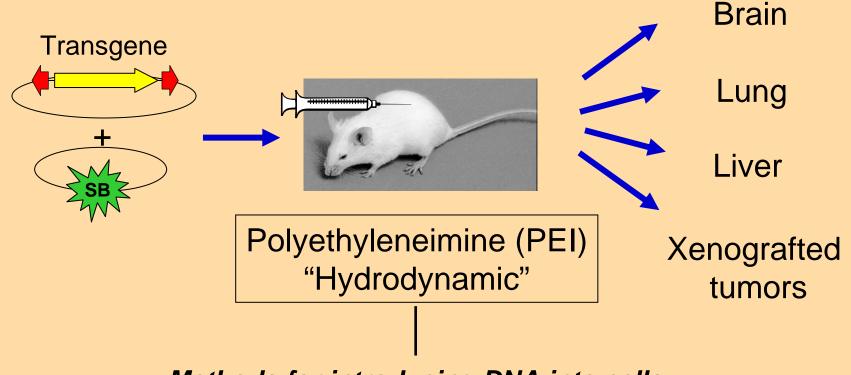
| Gene | Notes | | |
|--------|----------------------------------------------------------------------|--|--|
| Арс | TSG mutated in >80% colorectal cancer (CRC) | | |
| Bmpr1a | TSG mutated in CRC, encodes BMP receptor | | |
| Smad4 | TSG mutated in CRC, TGFb signal transducer | | |
| Fbxw7 | TSG mutated in CRC, F-box protein | | |
| Cdk8 | Oncogene amplified in CRC, Cyclin dependent kinase 8 | | |
| Pten | TSG mutated in CRC, PTEN | | |
| Dcc | TSG mutated in CRC, "deleted in colon cancer" | | |
| Nsd1 | TSG mutated in Sotos syndrome | | |
| Notch1 | Oncogene activated in cancer | | |
| Pi3kr1 | Oncogene activated in cancer, p85alpha PI3K subunit | | |
| Tcf12 | Transcription factor, mutated in chondrosarcoma | | |
| | Mutated in human colorectal cancer Mutated in other human cancers | | |

| CIS-Associated Genes are Altered in Human Cancer | | | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------------|--|--|
| •47% of those examined have one or more non- | SB Screen | Re-seq Screen | | |
| silent somatic mutations in human tumor (COSMIC database) [p<0.05] | Smad4 Uhrf2 Apc | SMAD4 UHRF2 APC | | |
| •Significant number show copy loss or gene amplification in a study of ~150 human colorectal cancer by array comparative genome hybridization (Scott Powers at CSHL) [p<0.05] | Cntn4 Cutl1 Fbxw7 Pten Add3 Ankrd11 | CNTN4 CUTL1 FBXW7 PTEN ADD3 ANKRD11 | | |
| •14% have somatic mutations in human colorectal cancer (Wood et al, 2008) and 5% were CAN genes suggested to be drivers for human colorectal cancers [p<0.005] | Dcc Dnahc1 Dstn Gpbp1 Ppp1r12a Rreb1 Wac | DCC DNAH1 DSTN GPBP1 PPP1R12A RREB1 WAC | | |

Comparative Genomics: CIS Genes Likely to be Drivers of CRC

| Gene Symbol | Mutated in human cancer* | Amplified or deleted in human CRC† | Aberrantly expressed in human CRC‡ | Known human cancer gene§ |
|-------------|-----------------------------|---------------------------------------|------------------------------------------|-----------------------------|
| ANKRD11 | x | х | x | |
| APC | х | | х | x |
| BMPR1A | х | х | x | х |
| DSTN | х | x | x | |
| EVII | х | | x | х |
| FBXW7 | х | | | х |
| GPBP1 | х | x | x | |
| NOTCHI | х | x | x | х |
| NSD1 | х | | х | х |
| PPP1R12A | х | | x | |
| PTEN | х | x | x | х |
| RREBI | х | x | x | |
| SMAD4 | х | x | x | х |
| TCF12 | х | х | х | х |
| TNPO3 | x | х | х | |

Somatic cell gene transfer: *SB* can be used for longterm gene transfer and expression in many settings

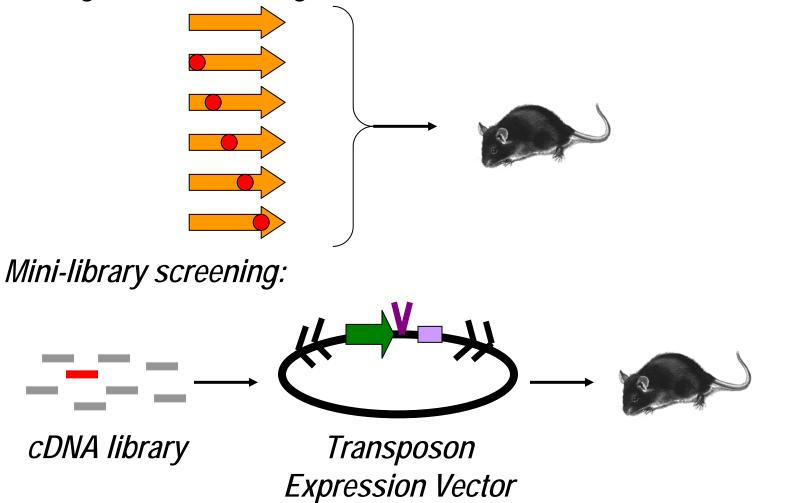


Methods for introducing DNA into cells

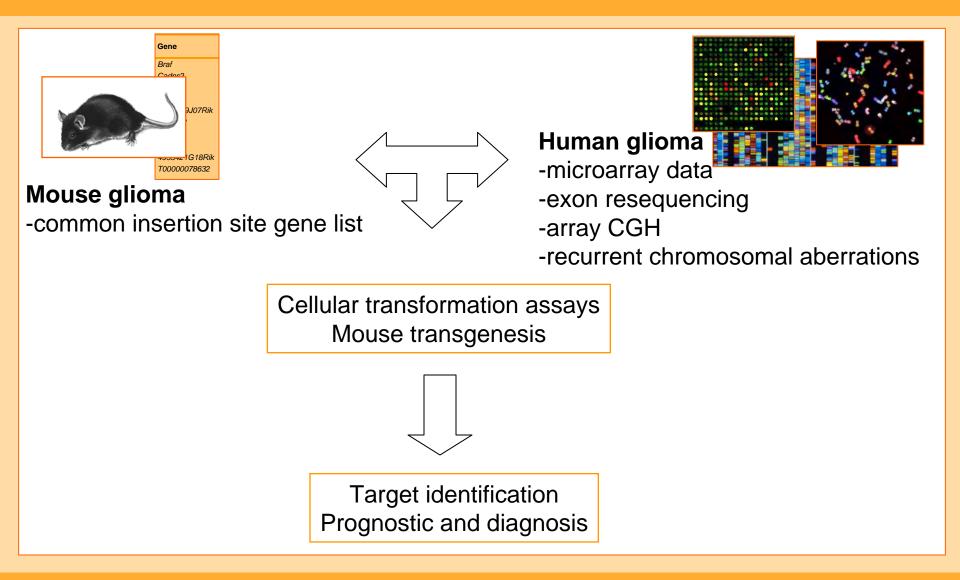
Yant et al., 2000; Beleur et al., 2003; Ohlfest et al., 2004 and others.

Can SB be used to deliver activated oncogenes to soma for creating cancer models?

Testing of mutant oncogenes:



Ultimately what do we want to do with these cancer models?



Conclusions and future directions

- Chromosomally resident SB vectors transpose in mouse soma
- T2/Onc SB vector + SB transposase transgenes can accelerate or initiate tumor formation in cancer predisposed (*Arf-/-*) or wild-type mice
- SB-induced tumor development due to insertional mutagenesis, allows identification of common sites of transposon insertion and associated cancer genes lymphoma, sarcoma, brain tumors, carcinomas also
- SB can integrate transposons containing oncogenes into genomes of somatic cells to model tumorigenesis in vivo

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Paul Marker

Scott Fahrenkrug Dan Carlson

Scott Mclvor Andy Wilber Joel Franzen

Perry Hackett Karl Clark Jason Bell

Ekker Lab Darius Balciunas **Production of transgenic mice** Sandra Horn Cesilie Granum

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Histopathology Nicole Kirchoff Cathy Carlson

Cytogenetics LeAnn Oseth Betsey Hirsch

Sequencing David Adams Allan Bradley Tony Cox