nature | **methods**

Transposon-mediated genome manipulation in vertebrates

Zoltán Ivics, Meng Amy Li, Lajos Mátés, Jef D Boeke, Andras Nagy, Allan Bradley & Zsuzsanna Izsvák

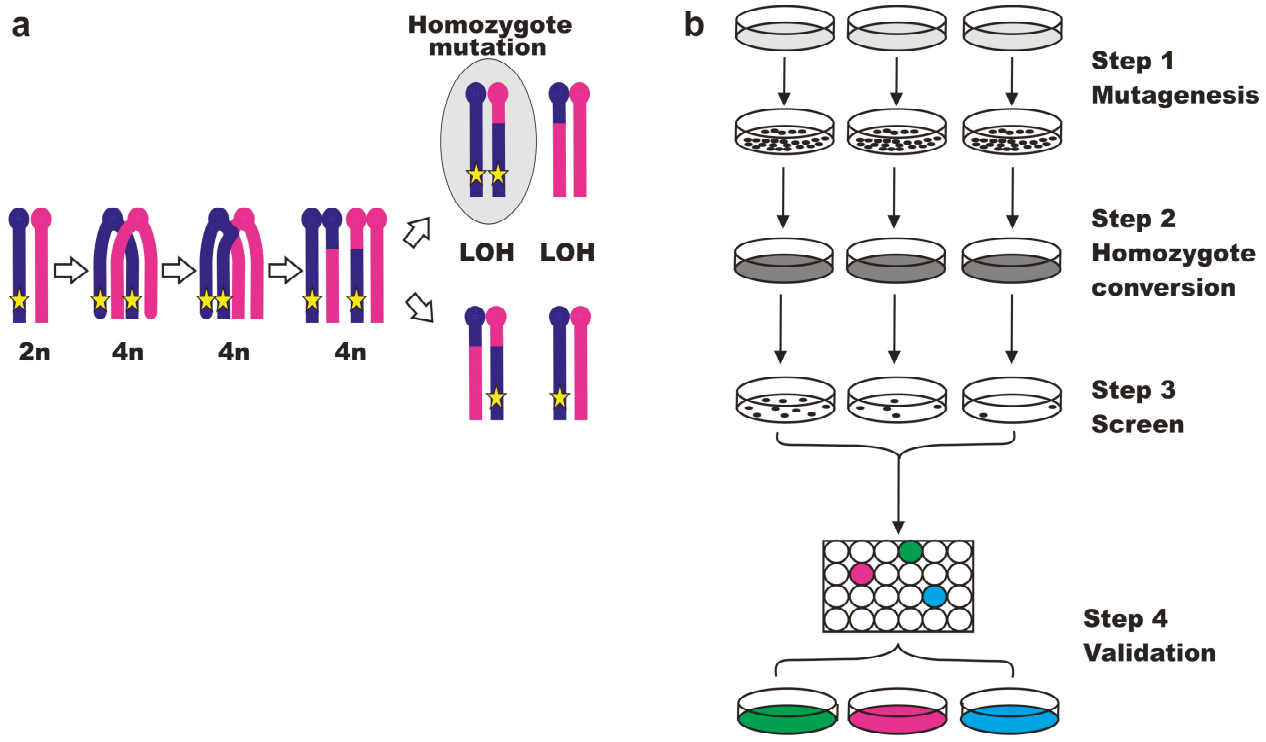
Supplementary figures and text:

Supplementary Figure 1. Recessive genetic screens in *Blm*-deficient ES cells.

Supplementary Figure 2. Synthetic L1/*ORFeus* transgene and progeny retrotransposon insertions.

Supplementary Figure 3. Somatic mutagenesis in the mouse with transposable elements.

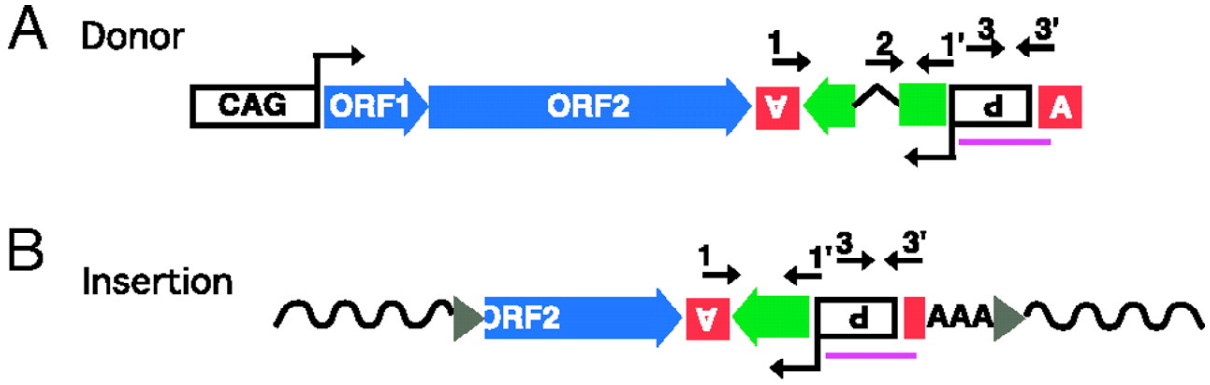
Supplementary Figure 1. Recessive genetic screens in *Blm*-deficient ES cells.



(**a**) Non-sister mitotic homologous recombination gives rise to homozygote mutants. The star represents the integration site of an intertional mutagen. The genotype highlighted in grey contains a bi-allelic mutation. (**b**) Flow chart for recessive genetic screens using a *Blm*-deficient ES cells system.

Nature Methods: doi: 10.1038/nmeth.1332

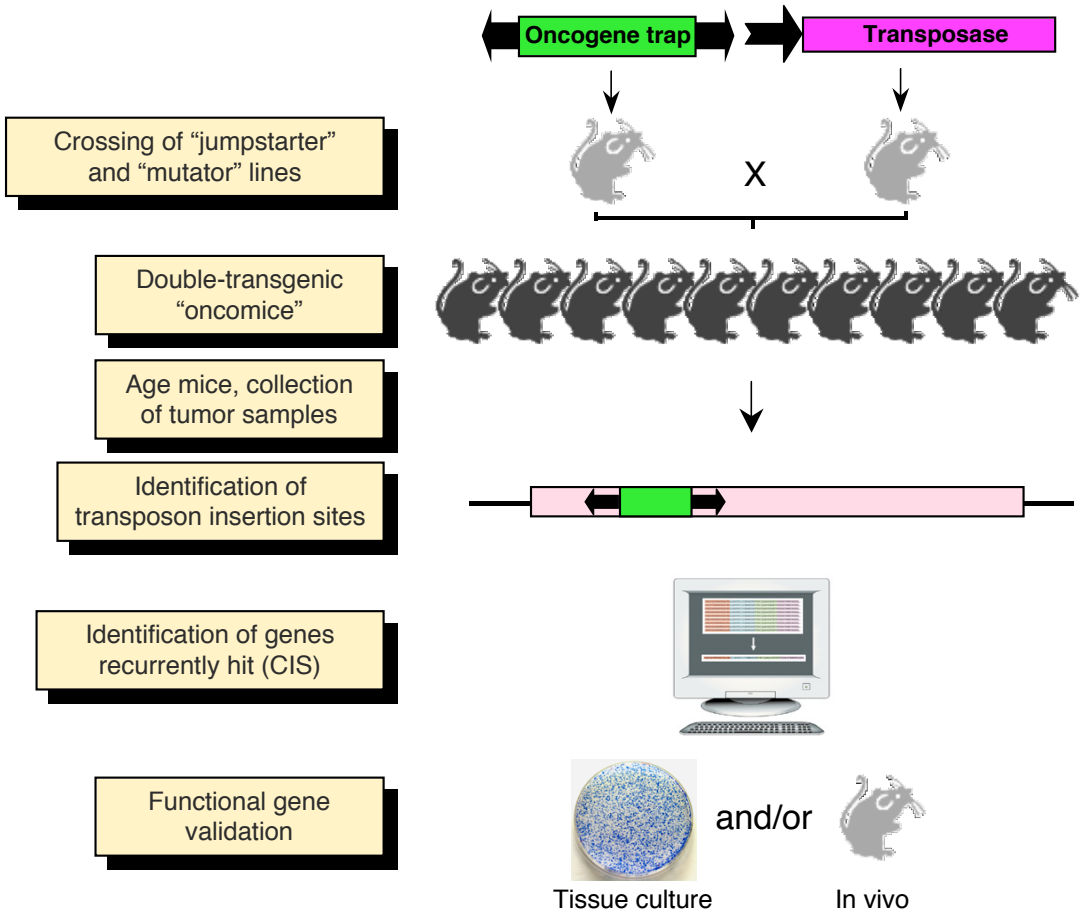
Supplementary Figure 2. Synthetic L1/*ORFeus* transgene and progeny retrotransposon insertions.



(**a**) The transgene construct or donor element consists of the following sequence elements from 5' to 3': (i) a composite CMV IE enhancer/modified chicken β-actin promoter, designated "CAG". (ii) synthetic L1 ORF1, ORF2 and 5' portion of 3'UTR. (iii) Herpes simplex virus thymidine kinase poly(A) signal (boxed inverted letter A) in antisense orientation to polyadenylate gfp mRNA. (iv) gfp (green block arrow), a modified version of EGFP coding sequence. The gfp ORF is in antisense orientation relative to L1 and interrupted by intron 2 of human β-globin gene, which is in sense orientation relative to L1; gfp serves as a "retrotransposition indicator gene". (v) Rous sarcoma virus LTR promoter in antisense orientation relative to L1, which drives gfp transcription (boxed inverted P for promoter). (vi) β-globin poly(A) signal (boxed upright letter A). Numbered arrows above the diagram represent locations of genotyping PCR primers. Region used to generate Southern blotting probes is indicated (purple line). (**b**) Structure of a representative progeny element. A typical progeny insertion is 5' truncated, intronless, ends in a poly(A) tail (AAA) and is flanked by target site duplications (gray triangles) and target genomic DNA sequences (wavy solid lines). Primers 1 and 1' (intron flanking primers) amplify a longer product when derived from the donor element (A) than from the progeny insertions (B); product length differs by the length of the intron. Primers 2 and 1' (primer 2 is the "intron spanning" primer that spans the splice junction give rise to a product only from progeny retrotransposition events. Primers 3 and 3' are control primers that give rise to products of constant length for donor and progeny elements.

Nature Methods: doi: 10.1038/nmeth.1332

Supplementary Figure 3. Somatic mutagenesis in the mouse with transposable elements.



Breeding of "jumpstarter" and "mutator" stocks induces transposition in the soma of double-transgenic animals ("oncomice"). In case of tissue-specific screens, a third genotype containing a tissue-specific Cre allele has to be crossed in. The crosses can be made either in wild-type or in specific cancer-predisposed genetic backgrounds. Transposition in somatic cells leads to random insertional mutations, and animals are aged for tumor development. Transposon insertions are cloned from genomic DNA isolated from tumor samples, and are subsequently mapped and annotated with respect to mutagenized genes. Those genes repeatedly mutated in multiple, independent tumors are designated as common insertion sites or CIS. These candidate cancer genes are functionally validated.

Nature Methods: doi: 10.1038/nmeth.1332