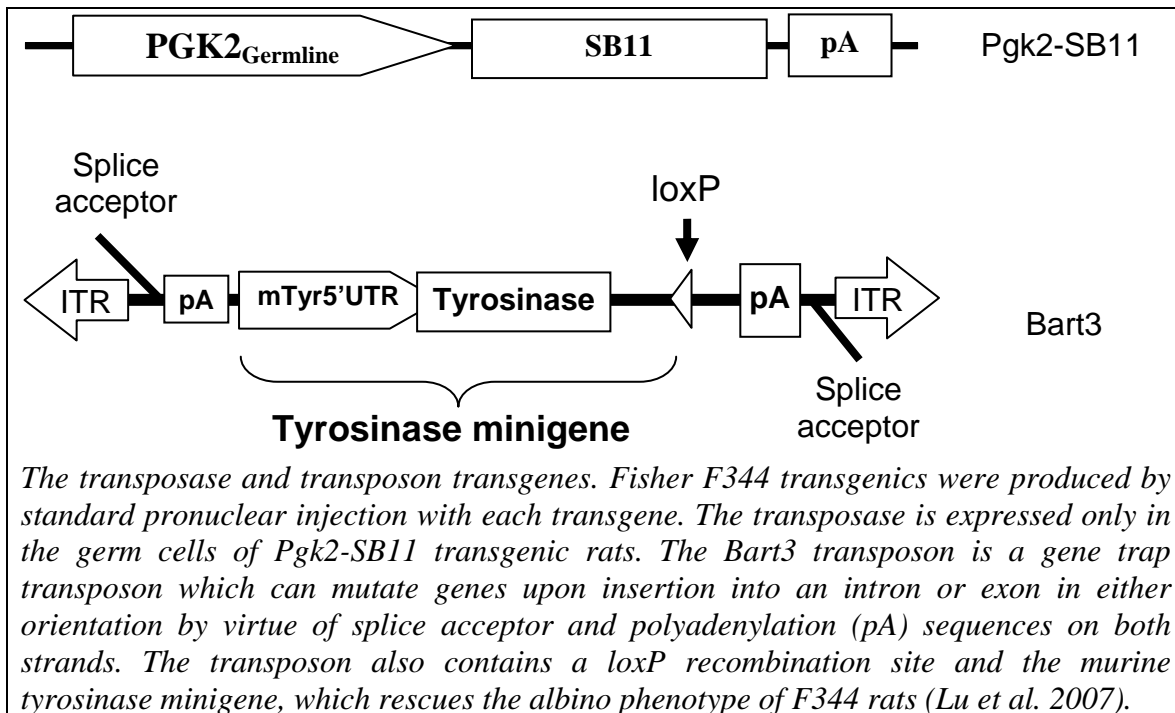


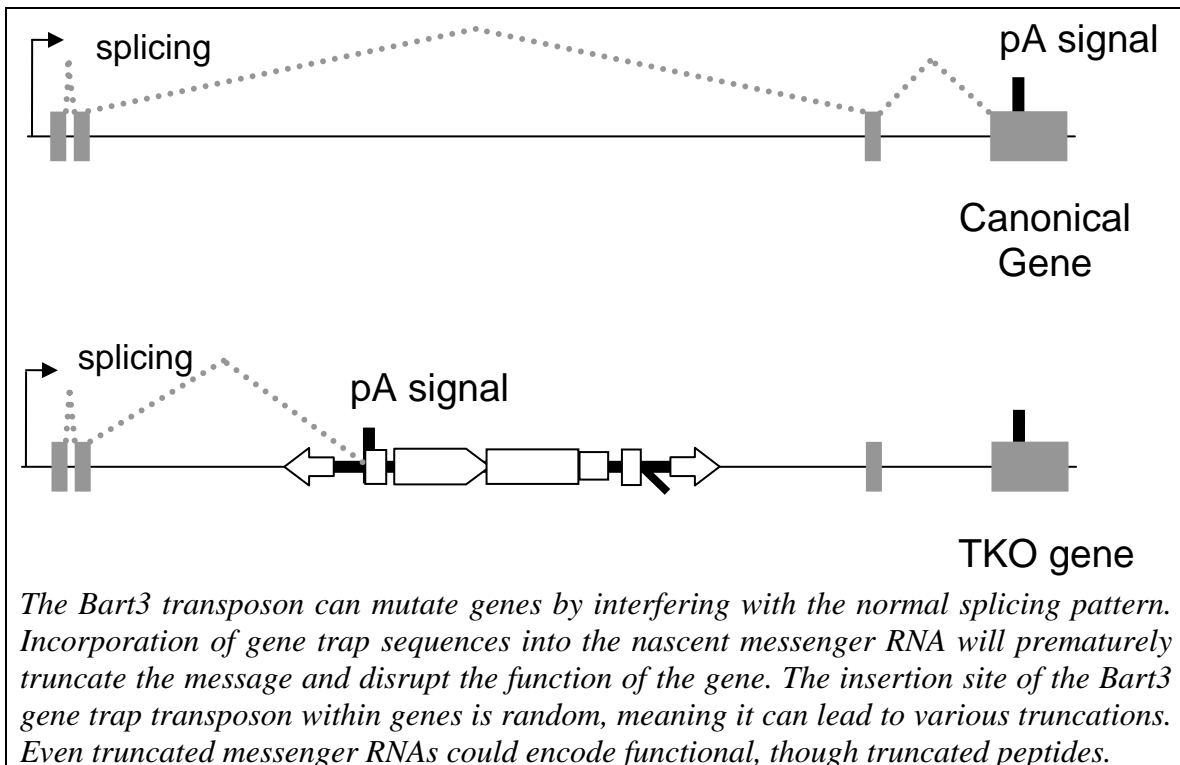
## Sleeping Beauty Primer

The *Sleeping Beauty* (SB) transposon is a cut-and-paste transposable element which was reconstructed from ancient teleost fish sequences in 1997 [1] and has since been used as a tool for gene delivery [2] and mutagenesis [3-7] in laboratory vertebrates. The SB transposon system consists of two parts - 1) a **transposon**, which is defined as any genetic cargo flanked by two inverted terminal repeats (ITRs) of ~230 bp, and 2) a catalytic DNA recombinase, called a **transposase**, which can recognize and bind the ITR sequences of the transposon and catalyze transposition from a chromosome site to a new position in the host genome. SB transposons belong to the Tc1/*mariner* superfamily of transposable elements and always insert into TA dinucleotides. We have now developed the SB system for insertional gene trap mutagenesis in rats for the purpose of mutating genes and creating mutant strains [7].

SB gene trap mutagenesis in rats is described by Lu *et al.* 2007 and is accomplished with two independent strains of Fisher F344 transgenic rats [7]. The first strain of rat, called the P<sub>gk2</sub>-SB11 rat, expresses the SB transposase under the control of the germ cell-specific phosphoglycerate kinase 2 (P<sub>gk2</sub>) promoter. The transposase is only expressed and can catalyze transposition in developing gametes. The second strain carries multiple copies of the Bart3 gene trap transposon at a single position on a rat chromosome called the *donor site*.



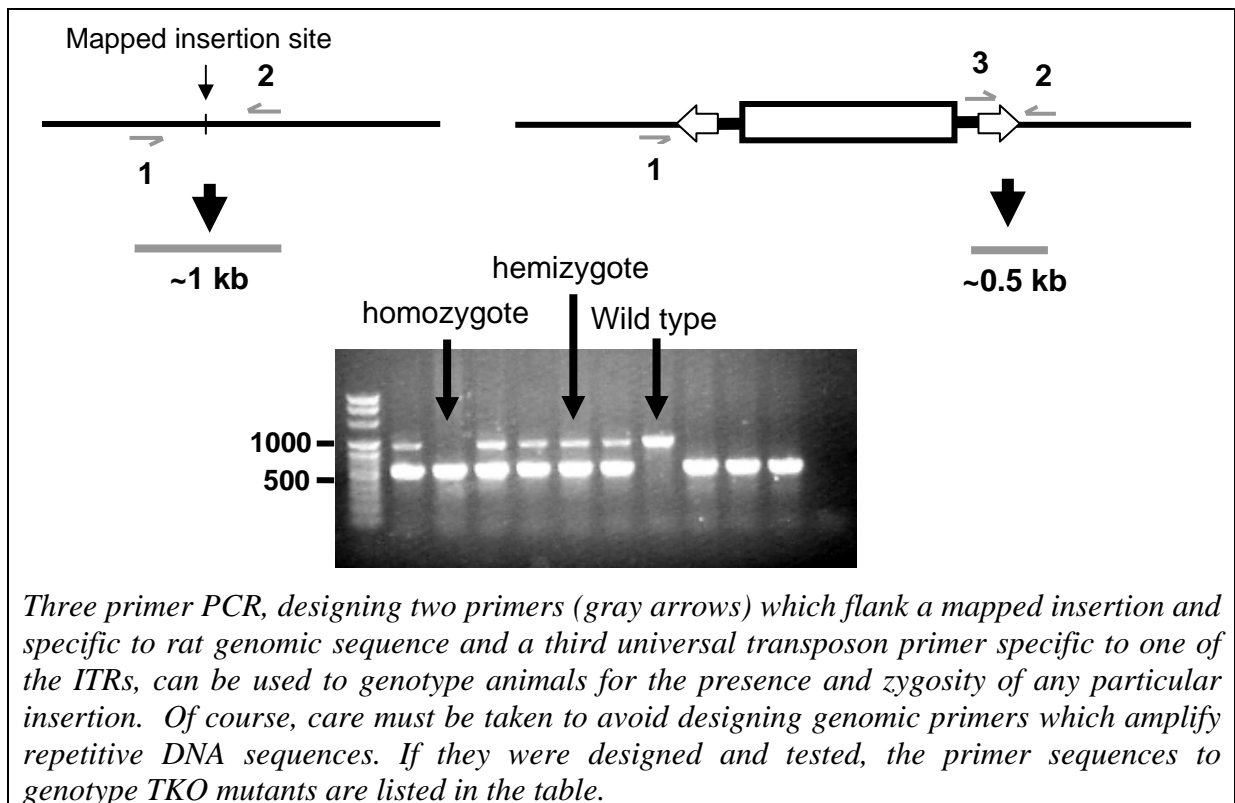
SB transposon-mediated gene knockout (TKO) is accomplished by breeding the P<sub>gk2</sub>-SB11 and Bart3 transgenic rats together to generate bigenic animals. In the developing sperm of bigenic males, one or more transposons “jump” from the donor site to new positions in the sperm genome. These mutant sperm fertilize wild-type F344 embryos by outcross to generate mutant offspring. Like chemical mutagenesis with ENU, transposon insertion is essentially random, though more insertions occur on the donor chromosome than other chromosomes. In practice, we observe ~1.2 insertions per gamete in offspring of bigenic males [7] and identify insertions in known rat genes (Refseq or Ensembl genes with validated homology to human and/or mouse genes and/or supporting EST information) in about 5% of these offspring. A TKO mutant, therefore, harbors a copy of the Bart3 transposon within its transcription unit. The splice acceptor sequences will intercept splicing from the nearest upstream exon and incorporate gene trap sequences including a premature polyA sequence.



The TKO mutants listed were identified by cloning the insertion sites of Bart3 transposons in the offspring of bigenic males using PCR techniques and sequencing [7]. The mutant animals may harbor more than one transposable element on one or more chromosomes. Because transposons can jump locally to the same chromosome as the donor site, mutations on the donor site chromosome can be linked to other transposon-

induced mutations [4]. Conversely, jumps to other chromosomes are not likely to be tightly genetically linked to any other mutations and should be able to be segregated in a single outcross generation.

We can identify carriers of individual insertions by three-primer PCR because we know the precise location of the transposon insertion in each case. This protocol can also distinguish hemizygous and homozygous carriers among intercross generations.



As with any mutant generated by the PGA, animals should be independently genotyped and verified for the presence of the mutation by PCR or Southern blot analysis. Also, because Bart3 insertion into an intron of a gene does not directly disrupt the coding sequence, assays should be performed to ensure that the gene is entirely disrupted. Splicing around the Bart3 gene trap sequences may occur in some instances, leading to full-length, functional gene messenger RNAs and peptides. RT-PCR using primers specific to flanking exons, or protein assays should be used to confirm the true level of gene disruption for any mutation.

### ***Selected References***



tctcctcaactttcttcaaaattaatagactacattttccccacagaaattctctataggaacttcaatttttttatttaaccaggaaaa  
catatttaacatacttagctataataattaagattaaattgtgataaaaacttattttgacagtgggtggaaactgtcctattaaattac  
catatcattccaattttatcctcagaaaatgccaatagatgatcaataaataatttgcagtatacttattgcattcccagctatagctaat  
atagattgcattaagactgtcttagggtagacaatagtcaaacagaattggatagggcatattcaattctgtagctaccaaatgtgt  
atacaagggaaaaatgtgtaaatgaatacttacatagaaagagcaggactgggtataagattgtcatgtaataaagttgggtggaga  
cttgagtacattgcagaggaataaagagagctttaatttaagagaatcagtcctatgaagaaaacaagacagcaaaactacact  
tcagtcattctctcatgaggctctacagatttgaaaatgagcatgggaaagnccttagaaaatgagcatgggaaagcccttagaa  
agagtgatgaggattttctganaattttgctaagatgcctgatagtagaacaagggaagaaaaagttgtaattaagtttaacagg  
agaagctggattccacctgcaactaggtgggaaagagttacaaaaggcccatgtagctacaagtaatacaaaaaaatcc  
ccaggggaaacgaggtcctgtgtaacttactatgggctttaagagaagaaaaacaaaaaggactaagactctgaaggaaatc  
atgtgagagcttcttattccagcaagagacaaaatctccatggaaacttctttctgcaccacacactgttctttctacctcgca  
aggctgccttgaatctcaagagaatcctatggagcagtcacacattttaaatactgaaacaaacctaggggaagaaaagaagc  
agagcaggctgacattccagcattatcaggaaagcaatgatttcttagattccgcagccccagtggtcagataaacggtttctc  
aaaccttcaacttcttctctacagtataaataaagaaccactcagcttttagtgaagcagcatagagaagggagttccaaa  
gagacgtttgtttctgaccattctcattatccttcttcatggagcagtgtattcaaacatccagtaagtccattactcactccaca  
ttttatgagcaaaataataaaagagagatagagtaagaatgaaggag  
agagagagagagagaggaag  
agaggtgtttcttaactagaaactttatgcattgaagcagttcacaaaataacaaagtaacaaagtaagatatctttggaataatca  
attcaagataatcaaggaaaaatgagaggcaactattttagactgattactttataaataaataagctcagcttagccagatataa  
gcaatattctgagtctgaagaaaaattttgacaaaatgagttctataatgttattgtctacttatgatcttaatacaacaggcttgt  
attcagaatctagatgtttcatgacctttatcataagagatgatgtattcttgatactacttctcatttgcaaattccaattattattaattc  
atatcaattagaataatatacttcttcaattagttacctcactatgggctatgtacaaactccaagaaaaagttagtcatgtgctttg  
cagaagataaaagcttagtgaaaacaggctgagagtatttgatgtaagaaggggagtggttatataggtcttagccaaaacatgt  
gatagtcactccaggggtgctggaaaagaagtctgtgacactcattaacctattggtgcagattttgtatgatctaaaggaatgttc  
ttggctgtttgtattgccttctgtggagttccagatctctgatggccatttctctcagcctgtgcctcctctaagaactgttgcaa  
aagaatgctgccaccatggatgggtgatgggagtcctcgcggccagcttcaggcagaggttctgccaggatccttctgtc  
cagtgaccatctggacctcagttcccttcaaaaggggtgatgaccgtgagtcctggccctctgtgtttataataggacctgcc  
agtgtcaggcaacttcatgggttcaactcggaaactgtaagttggattgggggccaattgtacagagaagcgagcttgc  
aftagaagaacattttgattgagtgctccgaaaagaataagttctttcttacctcactttagcaaaacatactatcagctcagctc  
atgtcatccccacaggcacctatggccaaatgaacaatgggtcaacacctatgtaataatgatacaacatctacgacctttgtat  
ggatgcattactatgtgtcaaggacacactgcttgggggctctgaaatagggaggacattgatttggccatgaagcaccagg

gtttctgccttggcacagacttttctgttattgtgggaacaagaattcgagaactaactgggatgagaacttcaactgttccact  
gggaltggagagatgcagaaaactgtgacatttgacagatgagtacttgggaggtcgtcacctgaaaaactaacttactcag  
cccagcatccttctctcctcctggcagatcattttagcagatcagaagagtataatagccatcaggttttatgcgatggaacact  
gagggaccactattacgfaatcctggaaccatgacaaagccaaaacccccaggctccatctcagcagatgtggaattttgtct  
gagttgaccagatgaatcgtgatcaatggatagaactgccaatttcagctttagaacacactggaaggatttgcagtcact  
cacaggatagcagatccttctcaaagtagcatgcacaatgccttacatatttatgaatggaacaatgcccaagtacagggat  
cggccaacgatcccattttctctcaccatgcttttggacagatttttgacaatggctgcaaggcaccgcccttttggaa  
gtttaccagaagccaatgcacatatcgccataacagagactttacatggtcctttcataccgctctatagaatggtgattctt  
cataacatccaaggatctgggatagctacagctacctcaagagtcagatccaggcttttacagaaattatattgagccttactg  
gaacaagccagtcgtatctggccatggcttctggggcagcactggtgggagctgttattgctgcagctctctctgggcttagcag  
taggctatgccttcagaagaagaagaagaagcaacccccaggaggaaaggcagccactcctcatggacaaagacgacta  
ccacagcttctgtatcagagccatctgtgacatcctaggaacagagtgaggactgaaaggtttacactcactcagcctatttgtt  
ggtgttctacaaatftaaactagtataaaacatagaccatagctgtttggctttttcagaccatgtttttcctaagctcagtttcta  
agaaatgactgggatttgctaaaatataatataataaataactactaataagctaaataaaattcctcttccccctaaaaaaa  
aaaaaaaaaaaaaaaaaaggaaattcctgcaggttaaacgaattcgttaaacataactcgtataatgtatgctatacgaagta  
aattctttcggacttttgaaggatggtggtgggggaaggattcgaacctcgaagtcgatgacggcagatttagagctgctccc  
ttggccgcccgggaacccaccacaggtaatgctttactggcctgctccctatcgggaagcggggcgcacatatacaaatga  
cgcgccgtgtaaagtgttacgttgagaaagaatttcacacaaaaaccaacacacagatgtaatgaaataaagatattttattgc  
ggccccgggaattcgcggccgccccgggcccgggtcagttatctatcagcgtctggtacttgggtgtaggggcagcgttcttcc  
ttgtggaacgggctggtacccagttggccacggggttgcctggaggggacaaacacgggcaagggtcagtaaagttccctcca  
gctcaggacctctgcatcccaccctaggttagtcaagctcccaaaccaataaattgaacctgaattggacacattttgctttag  
gcatctctcttctcttttgcacccagccccctccctataccctcctgttcagctccagcacctaaaactgtagtaaaatftaa  
gctaccaaagctcccacagctctggtccttagcttcaagagtagtaattcgagaatatacctataaaaagtccaaattcccaaccatt  
cataggccataaacgcttccctctctgtttttattttatagcaaggtccctcctccccactcctcctctctgccccctcca  
tctcacagacacgaccaagtcctgagagtcctgaacctcacttctttccagtaacctctccggctcagggcgcgatccatgg  
aattcactagtgcgcggcggcgtctagatggccagatctaagcttgggaaggctactcgaatgtttgaccaagttaacaat  
ftaaaggcaatgctaccaataactaattgagtgatgtaaaacttctgaccactgggaatgtgatgaaagaataaaagctgaaatg  
aatcattctctactattattctgatatttcacattctaaataaagtggtgatcctaactgacctaaagacagggaattttactaggat  
taaatgtcaggaattgtgaaaaagtgtttaaatgtatttggctaagggtgatgtaaaactccgacttcaactgTANNNNNN  
NNNNNNNNNNNNNNNNNNNNNGENOMICSEQUENCE