

**Additional information for Lox-Stop-Lox TOPO  
(Provided by: Jacks lab)**

pBS.DAT-LoxStop

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Improved Stopper is different from Lakso et al PNAS, 1992. 89:6232-6 in several ways:

1. A tetrameric tandem array of SV40 polyA is used – idea from Phil Soriano’s work.

\*\*Note that tetramer can undergo recombination to produce shorter sequences. To check size of polyA, double digest with BamH1 + EcoR1 and probe with SV40 polyA – full length tetrameric polyA is 3.85 kb; recombinants will be shorter by increments of 0.85 kb.

2. Adenoviral strong splice acceptor typically used in gene trap vectors is fused upstream of the his3 stuffer fragment to prevent splicing around the stopper (in the case that transcription isn’t completely silenced).

3. PGK-puro added to the 5’ end in direction opposite of the target gene, to allow selection in ES cells, primary cells, etc.

4. Mutant splice donor site on 3’ end different from Lakso (in case transcription note totally squelched).

5. The stopper was designed to fit neatly into genomic Sal1 or Xho1 sites. After cre-recombinase excision of the stopper, a LoxP and a repeat of the Sal or Xho site will only be left in the endogenous loci.

6. In vitro, stopper works as well as Lakso using a luciferase assay. The role of the SA and mutant splice donor sites have not been rigorously tested in this assay.

7. In vivo, LSL-KrasG12D and LSL-p53R270H and LSL-p53R172H mice have been successfully made. LSL-KrasG12D mice have no phenotype when aged 3 years. LSL-p53 mice have a phenotype similar to p53<sup>+/-</sup> mice.

8. Experienced labs have substantial difficulty cloning with this stopper. I advocate putting the stopper in as the LAST step in a targeting strategy, into an intronic Sal or Xho site, if possible. I then perform colony lifts to find potential positive clones, and restriction map for proper orientation.

Best of luck,  
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