

Constructing PB-SV40 (T/t) Transgene (PB-Tag)

To construct the PB-SV40 (T/t) Transgene (PB-Tag) a SalI site was first added to each end of the minimal rat probasin promoter (-426/+28 fragment; rPB) HindIII fragment by PCR. The resulting SalI fragment was ligated into SalI digested pBluescript II SK- vector so that the orientation was facing the EcoRV site. Then the SV40 fragment was isolated by digesting pBR322:SV40 with BglI (GeneBank Acc# V01380, position 1/5243) and converting this to a blunt end. The SV40 early genes were liberated by digestion with BamHI (position 2534). Note that the V01380 sequence in Genebank is the Reverse Compliment. The BglI (blunted) – BamHI fragment of SV40 was then ligated into the EcoRV (blunt) and BamHI digested vector carrying the rPB promoter to generate PB-Tag. The EcoRV/BglI site was destroyed but the BamHI site was regenerated.

The first 20 bases of the SV40 fragment immediately after the BglI/EcoRv fusion:

5' CGCCTCGGCC TCTGAGCTAT 3'

The last 20 bases of the SV40 fragment up to and including the BamHI site:

5' ATCTTATCAT GICTGGATCC 3'

