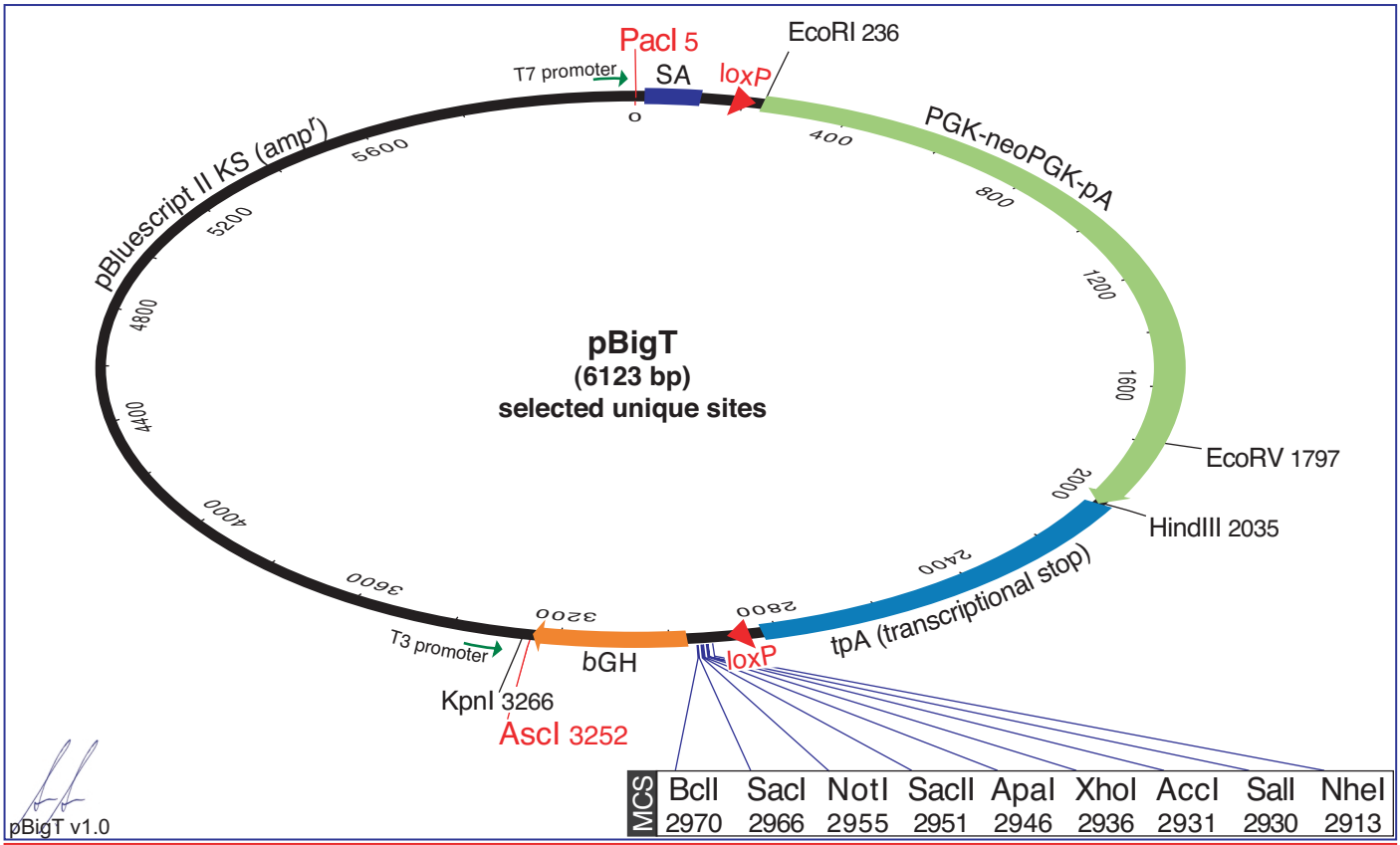

Description: pBigT is a vector to easily target the ubiquitously expressed ROSA26 locus with a cassette that allows the inducible expression of any gene from the ROSA26 locus. It is meant to be used with the plasmid ROSA26PA, which contains the genomic sequence with which to target the ROSA26 locus. Insert the gene you wish to express in the MCS, excise the entire inducible cassette with the rare cutters PacI and AscI, and clone it into the ROSA26 genomic sequence of pROSA26PA at the engineered PacI and AscI sites.

Warning: I and others who have used pBigT (and indeed, any plasmid with multiple *loxP* sites) have encountered problems with recombination in bacteria. You should perform rigorous diagnostic assays at all stages of making your targeting construct. In addition to diagnostic restriction digests, I suggest you sequence important regions of your finished targeting construct, such as the *loxP* sites.
This map is based on both deduced sequence as well as direct sequencing of the plasmid. The only regions whose sequence has been confirmed by direct sequencing are – the PacI and AscI sites, the splice acceptor, both *loxP* sies (including some flanking *neo* and *tpA* sequence), the MCS, and the bovine growth hormone pA signal.

Distribution: pBigT and it's derivatives can be freely distributed to anyone who would like to use them for research purposes, as long as you include this discription with the plasmid and send an email to plasmid@srinivas.org with the name and email of the person the plasmid was given to. If the use of pBigT or a derivative results in a publication, please cite the paper in which it is reported –

Srinivas, S., Watanabe, T., Lin, C.-S., Williams, C., Tanabe, Y., Jessell, T. and Costantini, F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Developmental Biology* 1:4

Background: I made the Big Tamale in the laboratory of Frank Costantini, Columbia University. Phil Soriano kindly provided all the key elements, including the Adenovirus splice acceptor, the *loxP* flanked PGK-neo/tpA cassette, and the ROSA26 genomic sequence. The sequence of the PGK-neo cassette was deduced from the sequence of pPNT, provided by Chris Paszty.

Updates: The latest update of this map (a pdf file in full technicolour with web links) and associated files, such as the actual sequence, can be downloaded from *http://www.srinivas.org*. Please send me your email address if you wish to be kept informed of new updates to the map. Your comments on the map and pBigT are very welcome.

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A man never stands as tall as when he stoops down to help a child: We scientists *do* have concerns outside of science. Please consider contributing to the education of illiterate children in India. '***Asha for Education***', a group of volunteers I have been actively involved with since 1994, works for this cause. Visit *http://www.ashanet.org* for more information.

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