The pWIZ vector for Transgenic RNAi

The pWIZ plasmid (White Intron Zipper) is a *Drosophila* transgenic vector designed to express double-stranded RNA as a snapback hairpin, and it is derived from the pUAST transformation plasmid. This offers the advantages of the GAL4/UAS modular expression system to express dsRNA with cell- and temporal-specificity. To construct an RNAi-silencing transgene from pWIZ, a fly gene fragment is cloned into pWIZ to form an inverted repeat, with the repeats placed upstream and downstream of the second intron of the *white* gene (Figs. 1 and 2). The second intron of the *white* gene bears all features of a consensus *Drosophila* intron, and it can be spliced in heterologous tissues (Mount et al. 1992; Guo et al. 1993). The intron is flanked by *Eco*RI, *Bgl*II, *Not*I, *Xho*I, *Spe*I and *Avr*II sites on the 5' side, and by *Nhe*I, *Mlu*I and *Xba*I sites on the 3' side. The entire cassette is downstream of the UAS enhancer-promoter and upstream of the SV40 transcription termination site. The *Avr*II and *Nhe*I sites in pWIZ conform to the consensus sequences for 5' and 3' splice sites, respectively. Thus, a DNA fragment inserted into the *Avr*II site, and again into the *Nhe*I site, forms a competent exon-intron-exon structure. Moreover, the *Spe*I, *Avr*II, *Nhe*I, and *Xba*I sites are unique in pWIZ, providing convenient cloning sites for gene fragments.

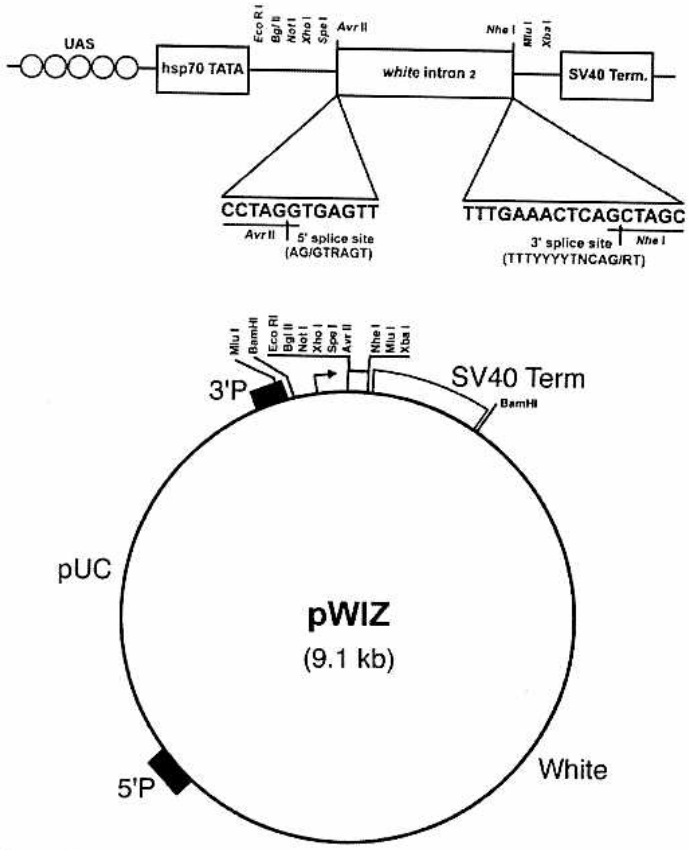

Figure 1. Schematic representation of the pWIZ vector. The sequences at the junction of the 5' and 3' splice sites in the vector are highlighted, and arrows indicate the 5' and 3' splice sites. The consensus sequences for 5' and 3' splicing are shown in parentheses: /, the splice site; R, purine; Y, pyrimidine; N, any base.

In deciding what portion of a gene to express as a snapback RNA, you should consider several criteria. One, the size of the DNA fragment for insertion should be between 500 and 1000 base pairs in length, as this triggers stronger silencing than shorter fragments. Two, the DNA fragment should preferably be a single complete exon from the target gene, since exons often contain sequences that facilitate the processing of transcripts. It is known that exonic splicing enhancers (ESEs) are prevalent in many genes where they play an important role in splice-site identification, particularly through recruitment of U2AF to 3' splice sites (Cartegni et al, 2002). Avoid using exons that are known to be alternatively spliced, since these might contain silencing sequences that repress or restrict splicing. Three, the fragment should not have sequences in the antisense (3'-5') orientation that match a 5' consensus splice site. This is important to prevent splicing from cryptic sites within the first repeat if it is present in a construct with 3'-5' / 5'-3' (coding strand) orientation. Four, it is preferable to place the inverted repeats in a 3'-5' / 5'-3' (coding strand) orientation since any ESEs within the downstream repeat will then be competent to stimulate 3' splice-site utilization. Five, ensure that the fragment does not contain any restriction sites used in the PCR primers.

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GTGAGTTTCTATTCGCAGTCGGC
TGATCTGTGTGAAATCTTAATAA
AGGGTCCAATTACCAATTTGAAA
CTCAG
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Figure 2. DNA sequence of the second intron of the white gene that is present as a spacer/linker in pWIZ.

To construct a snapback transgene, the fly DNA fragment is inserted twice into pWIZ, with inserts in

opposite orientation on either side of the intron (Fig. 3). The simplest means to insert the DNA is as a PCR fragment. The system is designed so that a single PCR fragment derived from only two PCR primers can be inserted on either side of the intron. This is because *Spe*I, *Avr*II, *Nhe*I and *Xba*I sites are all ligation-compatible with each other. Consequently, restriction sites compatible with *Avr*II and *Nhe*I sites should be engineered in the PCR primers at their 5' ends. The resulting PCR product will then have *Avr*II- and *Nhe*I-compatible ends after appropriate restriction digestion. For efficient digestion, we add an extra four nucleotides to the 5' side of each primer restriction site.

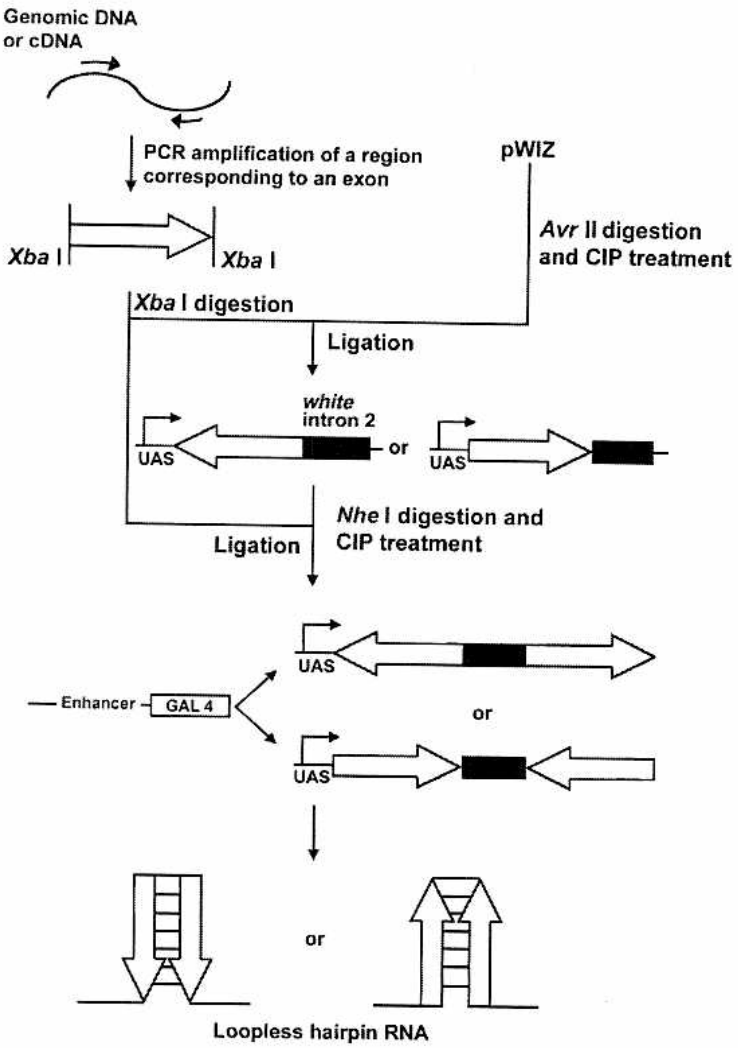

Figure 3. A typical procedure for making a RNAi construct using the pWIZ vector. A DNA fragment corresponding to a Drosophila target gene is amplified by PCR. A restriction site (SpeI, AvrII, NheI, or XbaI) compatible with AvrII and NheI should be present at the 5' end of each PCR primer. In the figure, an XbaI site is present at each end of a PCR-amplified exon for cloning. The PCR product is inserted reiteratively in two ligation steps into the AvrII and NheI sites of pWIZ. CIP: calf intestinal phosphatase used to dephosphorylate the 5' ends of vector DNA prior to ligation. Recombinants are selected in the desired orientation, such that after the second ligation step, inserts are in opposite orientation on either side of the white intron. Tail-tail repeats are preferred (see text) though head-head repeats could be used.

The snapback construct is made by two sequential insertions of the same PCR product into the *Avr*II and *Nhe*I sites of pWIZ (Fig. 3). The PCR product is digested with the appropriate enzyme and ligated into the *Avr*II site of pWIZ. After a clone with the desired orientation of the insert is selected, the same PCR product is ligated into the *Nhe*I site of the pWIZ derivative, and recombinants with the insert in opposite orientation to the first are screened and selected. We normally propagate the pWIZ parental plasmid and the first recombinant plasmid in a *DH5alpha* strain of *E. coli*. Host bacterial strains we have used for transformation and propagation of the final repeat-construct plasmid are either *DH5alpha* or *SURE* (Stratagene).

Some repeat-construct plasmids are highly stable in *DH5alpha* whereas others require using the *SURE* strain. Generally, plasmid instability is not a problem with pWIZ constructs in *SURE* cells. If it is, you can try harvesting small liquid cultures of transformed bacteria before they reach saturation growth, and purifying the plasmid DNA from these limited samples. Take care in looking for deletion variants of an unstable plasmid, which show up as multiple smaller bands when the plasmid DNA is run on an agarose gel. If you still are having problems with instability, try cloning a different fragment (portion) of the gene.

From **Lee, Y.S. and Carthew, R.W. (2003) Making a better RNAi vector for *Drosophila*: Use of intron spacers. *Methods* 30, 322-329.**

Other references:

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