Recovery of Tn insertions

Restriction digests

Digest 5-10 μl DNA with excess (20 units) restriction enzyme in 20 μl reaction volume. Chose enzymes which do not cut within *mariner* transposon, but which cut well in mycobacterial DNA preps. Enzymes which have worked well for me are *Bam*HI, *Pst*I, *Sal*I, and *Sst*I. After 2 hour digest, heat inactivate for 20 minutes at 80^{o}C. Run ½ of sample on Southern blot. Save rest of sample for ligation and inverse PCR.

Recovery of insertion sites

After heat inactivation (20 minutes at 80^{o}C), self ligate 1μl of this digest was using 1 unit T4 DNA ligase in a 20 μl reaction volume , over night at 16 ^{o}C. PCR using 1 μl of this ligation mix in a 50 μl reaction volume using Clonetech Advantage® GC cDNA PCR kit (1.5 M GC melt). PCR parameters: 94 ^{o}C x 1 minute followed by 30 cycles of 94 ^{o}C x 30 seconds, 68 ^{o}C for 5 minutes). Primers used direct DNA synthesis outward from within the kanamycin resistance gene portion of the mariner transposon. Primers are CGCATCTTCCCGACAACGCAGACCGTTCG (my primer 821A) and TAATCGCGGCCTCGAGCAAGACGTTTCCCG (my primer 822A). Predicted PCR product should be 800 bp shorter than the band seen on Southern blot. For best results, PCR from the digest that gives the lowest MW band on Southern blot.

Typical 50μl reaction contains

821A primer (40 μMolar)	1 μl
822A primer (40 μMolar)	1 μl
Ligation mix	1 μl
10 mMol dNTP mix	1 μl
5 x reaction buffer	10 μl
5 molar GC melt	15 μl
H_2O	20 μl