

Cre Transgene (lckCre or CD4Cre, primers are within the Cre cDNA) PCR

Cre 531-
5' CGA TGC AAC GAG TGA TGA GG 3'

Cre 819-
5' GCA TTG CTG TCA CTT GGT CGT 3'

positive cre band is 250bp, negative cre has no band.

94 degrees 1min
58 degrees 1min
72 degrees 1min
30 cycles

(If you wish to design your own primers you can download the sequence for the 5' end of **lckCre** or **CD4Cre**)

Dnmt 2lox/1lox PCR

3 arrows in Fig 1 of Lee Paper indicate the location of the primers for PCR. Reading left to right The first arrow is exon1.1 primer. 2nd arrow is anti-lox2 and the 3rd arrow is 3'loxBam120.

Three primers were used in a single reaction to detect Dnmt2lox vs Dnmt1lox allele: exon1.1, antilox2, 3'loxBam120. Primer sequences (5'-3') are as follows: exon1.1: ggg cca gtt gtg tga ctt gg; anti lox2: tga acc tct tcg agg gacc; 3'loxBam120: atg cat agg aac aga tgt gtgc

(94 4', 94 1', 55 1', 72 1') 30 cycles

2lox 175 bp (product of exon 1.1 and anti-lox2)
1 lox 255 bp (product of exon 1.1 and 3'loxBam120)

Dnmt 2lox/wt/n PCR

exon 1.1 5' GGG CCA GTT GTG TGA CTT GG 3'
exon 1.2 5' ACA GTA GGA CAC ACA GGG GC 3'

(94 1', 58 1', 72 1') 30 cycles

2lox = 140 bp
wt or n = 123 bp

To distinguish between n and wt allele, use MT2 primer in place of exon 1.2: MT2 5' CTG TCC GAC TTG CTT CTC CTG 3' Same PCR program, wt band will be 120 bp and no product for n.

Dnmt 2lox/1lox/n Southern

Digest with Bgl II/Spe I
Wt= 4kb
n= 2.5kb
1 lox=1.9kb
2 lox=1.6kb

Probe is generated by PCR as a fragment of 500 bp using primers
MT Apa I 5' ACT GGA GTT ACC GAT GCT GG 3'
MT Sph I 5' TCT ATG AAC TGT GTG CCT GG 3'

LckBcl transgene PCR

bcl5'-
5' TTC AGT GAC CTG ACA TCC CA 3'

bcl3'
5' TCC ACA AAA GTA TCC CAG CC 3'

Annealing temp of 58 degrees and 30 cycles.

Bcl positive = 255 bp band
Bcl negative = no band.