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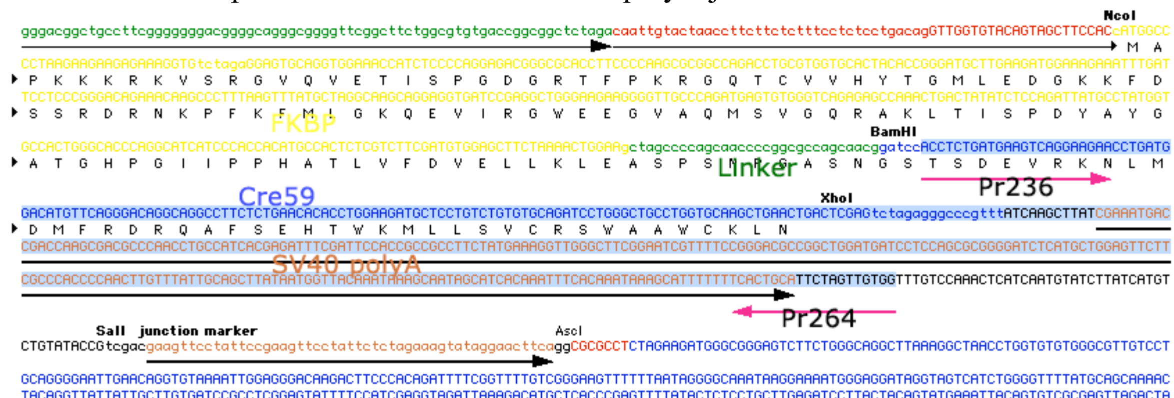
http://ifrjr.nord.univ-mrs.fr/-Brue-and-Herman-group-protocols-

GENOTYPING ROSA-DICRE MICE

I. GENOTYPING ROSA-DICRE MICE USING PRIMERS SPECIFIC FOR THE DICRE CONSTRUCT (PLOS ONE, 2007:2(12), E1355)

PRINCIPLE: Amplification of the Cre59-SV40 polyA junction



PCR PROCEDURE

Primers :

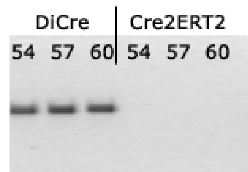
Target	Primer pair	Amplicon size (bp)
Rosa-DiCre DNA (iCre59-SV40pA)	Pr236 *acctctgatgaagtcaggaagaa* Pr264 *ccacaactagaatgcagtga*	411 bp

Mix:

	Volumes (µl) for 1 reaction	Volumes (µl) for 25 reactions
10x Taq Platinium buffer (w/o $MgCl_2$)	2	50
dNTP, 10 mM stock	0,4	10
Primers, 10 pmol/µl stock	(0,4 + 0,4)	(10 +10)
$MgCl_2$ 50 mM	1,2 (3mM final)	30
BSA, 10 mg/ml (Biolabs)	1	25
Taq Platinium (Invitrogen, ref 10966034)	0,08	2
H2O, qsp 19 µl	13,52	340

Put together at RT, distribute 19 µl in each PCR tube.
Add 1 µl target DNA prepared by the HotShot procedure (Truett et al., Biotechniques 29:52-54, 2000).
Do PCR, using 57°C for annealing, 30 secs elongation time and 35 cycles.
Run a 2% agarose gel loaded with 10 µl of the reaction.

EXPECTED RESULTS



Exemple with three different annealing temperatures, with DiCre DNA (positive band) and a negative control (Cre2ERT2 DNA)

POSSIBLE ALTERNATIVES

Use the same PCR mix and conditions, but using either of the following primer pairs:

Target	Primer pairs	Amplicon size (bp)
FRB-Cre60 junction #1	Pr109 *ttaatggaggcccaagagtg* Pr235 *gcatccacattctcctttctg*	344 bp
FRB-Cre60 junction #2	Pr280 *ttggggaaaggaacgtgaaagg* Pr279 *aggtgctgttggatggtcttca*	355 bp

II. GENOTYPING ROSA-DICRE MICE USING THE GENERIC PRIMERS OF P. SORIANO, DETECTING THAT THERE IS AN INSERT (ANY) WITHIN THE ROSA26 LOCUS.

NOTES:

1. This procedure allows the **identification of homozygous vs. heterozygous vs. WT animals**.
2. It works when insertion has been done using P.Soriano's targeting construct.
3. The conclusion is based on the absence of the 603 bp band corresponding to the WT locus => don't forget to have a WT or a heterozygous control (to test that PCR works OK)

Perform 2 separate PCR reactions (multiplexing is hazardous due to competition between primers), using the following primer pairs:

Target	Primer pairs	Amplicon size (bp)
Wild-type Rosa26 locus	Pr306 ggagcgggagaaatggatatg Pr307 aaagtcgctctgagttgttat	603
Rosa26 locus with insert	Pr307 (as above) Pr305 gcgaagagtttgtcctcaacc	325

Use PCR mix and conditions as above, but set annealing at 60°C.