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

GENOTYPING CRE2ERT2 MICE

I. GENOTYPING CRE2ERT2 MICE USING PRIMERS SPECIFIC FOR THE ICRE (GENESIS, IN PRESS)

PRINCIPLE: Amplification of a portion of the iCre coding sequence

PCR PROCEDURE

Primers :

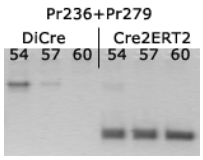
Target	Primer pair	Amplicon size (bp)
Cre2ERT2 DNA (iCre19-92)	Pr236 *acctctgatgaagtcaggaagaa* Pr279 *aggtgctgttggatggtcttca*	221 bp

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°-60°CC for annealing, 30 secs elongation time and 35 cycles.
Run a 2% agarose gel load with 10 µl of the reaction.

EXPECTED RESULTS



Exemple with three different annealing temperature, with DiCre DNA (negative control) and Cre2ERT2 DNA (positive control)

POSSIBLE ALTERNATIVES

Use the same PCR mix and conditions, but using the following primer pair:

Target	Primer pairs	Amplicon size (bp)
iCre sequence	Pr236 *acctctgatgaagtcaggaagaa* Pr235 *gcatccacattctcctttctg*	326 bp

II. GENOTYPING CRE2ERT2 MICE USING THE GENERIC PRIMERS OF P. SORIANO, DETECTING THAT THERE IS AN INSERT (ANY) WITHIN THE ROSA26 LOCUS.

NOTE: 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.