

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UCDAVIS
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530-754-MMRRC

NAME OF PCR: B6;129S5-Reg3g^{tm1Lex}/Mmcd MMRRC # 032538-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	10.275
10x Buffer (15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20μM) UNQ429-6	0.5
Primer 2 (stock concentration is 20μM) UNQ429-7	0.5
Primer 3 (stock concentration is 20μM) UNQ429-R	0.5
Primer 4 (stock concentration is 20μM) Neo3a	0.5
Taq Polymerase 5Units/μL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	} 40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: UNQ429-6	CTT CCA GTG AAG TCT AAA TCT CC
2: UNQ429-7	GAG TGG GGT CAT GGA GCC CAA TCC AAG
3: UNQ429-R	GAT GCT AGG TGG ATT ATG CCA TCA AC
4: Neo3a	GCA GCG CAT CGCCTT CTA TC

Electrophoresis Protocol:

% Agarose: 1.5 V: 90

Estimated Running Time (min): 90

Primers	Expected Bands	Genotype
1 and 2	182 bp	Wild-type
3 and 4	355 bp	Mutant



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ429	Date of Submission:	8.22.05
Lexicon Contract Name:	DNA482	Mutation Type:	X Standard Knock out
LexVision Name:	SEC588N1		☐ Conditional
Reference accessions:	NM_011036	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __pKOS-60, 12_____
 ☐ Target Vector DNA __pKOS12 FTV Neo_____
 ☐ Targeted ES Cell DNA __1D6_____
 ☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	26/27	20/21
Restriction Enzyme for Genomic Digest:	ScaI	PvuII
Predicted Wild-type Band (kb):	9.5	8.6
Predicted Mutant Band (kb):	12.5	4.1
Probe Size (bp):	515	211

PCR Strategies:

For standard knockouts, give wildtype and mutant-specific strategies

For conditionals, give 5' loxP and cre-excision strategies

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	6	5' Primer Name:	28
3' Primer Name:	7	3' Primer Name:	GTIres
Predicted Wild-type Band (bp):	182	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	495

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:**Southern probes**

26 5' – GCCCTTTCCTTAATACTCTTA
 27 5' – ATGCCAACTGAGAAACCTAAT
 20 5' – CTCTCCACTTCTGATAACCTGC
 21 5' – GCCATAAGCTGGCAAATGCACTGTAG

PCR Genotyping

6 5' – CTTCCAGTGAAGTCTAAATCTCC
 7 5' – GAGTGGGGTCATGGAGCCCAATCCAAG
 28 5' – CTGCCCACCATCCTTAAC
 GTIres 5' – CCCTAGGAATGCTCGTCAAGA

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[illegible]

Selection Cassette: LacZ/Neo

Targeted Locus:

[illegible]

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