

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: B6.129S5-*Rtn4*^{tm1Lex}/Mmcd **MMRRC #** 032548-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) UNQ330-17	0.5
Primer 2 (stock concentration is 20μM) UNQ330-18	0.5
Primer 3 (stock concentration is 20μM) UNQ330-R2	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	} 40x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: UNQ330-17	CTG CAG TAC CTC TAC CTA CAA G
2: UNQ330-18	CTG ATG AGG AAC CTCG GAA CTT C
3: UNQ330-R2	GGA ATG TCA CCT CAC TTC CATATG TG
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	385 bp	Wild-type
3 and 4	288 bp	Mutant



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ330	Date of Submission:	1.6.04
Lexicon Contract Name:	DNA067	Mutation Type:	x Standard Knock out
LexVision Name:	SEC458N1		☐ Conditional
Reference accessions:	NM_022982	Is this gene X-linked?	No

Required Materials:

- x pKOS clone DNA(s) __ KOS37_____
- x Target Vector DNA __TV37-1_____
- x Targeted ES Cell DNA __1D2_____
- x Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	3+4	15+16
Restriction Enzyme for Genomic Digest:	XbaI	BstEII
Predicted Wild-type Band (kb):	14.7kb	9.9kb
Predicted Mutant Band (kb):	9.6kb	13.5kb
Probe Size:	346bp	243bp

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:	-17	5' Primer Name:	Neo3a
3' Primer Name:	-18	3' Primer Name:	UNQ330-R2
Predicted Wild-type Band (bp):	385bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	288bp

Primer sequences:

Southern probes

SEC458N1-3 5' – CCTCAAACCCATGTGCAACCC
SEC458N1-4 5' – GGAGTAGGCGACGCAAGAG
SEC458N1-15 5' – TCACATTTGGGTCTAGCCT
SEC458N1-16 5' – GCATACTATGTGTGGCCTACTT

PCR Genotyping

SEC458N1-10 5' – TCCACCCCATCATGTTTACAGG
SEC458N1-17 5' – CTGCAGTACCTCTACCTACAAG
SEC458N1-18 5' – CTGATGAGGAACCTCGGAACTTC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

Neo3a 5' – GCAGCGCATCGCCTTCTATC
UNQ330-R2 GGAATGTCACCTCACTTCCATATGTG

Genomic Sequence Deleted:

GCTGGCATGGGTGTTATGGCTACAGGCCTGGAGGGTAGCAACACCATGCCCTGGTGCTTGTGTGTGCTACAATGAGCCCAAGGTAAC
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Genomic Locus: 10kb flanking the deletion (the deleted sequence represents nt 12285-14003 in the sequence below)

CCCTGGGCATCCTCCAGAAGCCATCTCTACAGCCACACAAAGTCTGCCATTTGCCTATAGTGGGCGTCTAGTGTATGCT
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[illegible]

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Selection Cassette: LacZ/Neo

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[illegible]

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