

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6N.129S5-Nrrostm1Lex/Mmucd **MMRRC:** 032441-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA477.73	GCCTCTTACACAACTTCATTGCAC	Estimated Running Time: 90 min.		
2. GT IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. DNA477.79	CGGCTGACTCCTTGTAGTTC	1 & 2	447	mutant
		1 & 3	475	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ3030	Date of Submission:	3.9.05
Lexicon Contract Name:	DNA477	Mutation Type:	X Standard Knock out
LexVision Name:	SEC309N1		☐ Conditional
Reference accessions:	NM_146069.2	Is this gene X-linked?	NO

Required Materials: ☐ pKOS clone DNA(s) n/a
 X Target Vector DNA DNA477.FTV.PURO F6
 X Targeted ES Cell DNA 1D2
 X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	75+76	77+78
Restriction Enzyme for Genomic Digest:	EcoRV	XmaI
Predicted Wild-type Band (kb):	24.1 kb	27.7 kb
Predicted Mutant Band (kb):	12.0 kb	16.5 kb
Probe Size:	194 bp	186 bp

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:	DNA477.73	5' Primer Name:	DNA477.73
3' Primer Name:	DNA477.79	3' Primer Name:	GT IRES
Predicted Wild-type Band (bp):	475 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	447 bp

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**

DNA477.75	5' – GCCTTCCCAGGACTGTTGTTTAC
DNA477.76	5' – TGTCTATCACATCGGTTAGCTCTT
DNA477.77	5' – TGGGCTGAGAACAGATAAGA
DNA477.78	5' – TCCTTATTTGGCATTTAATCAGTA

PCR Genotyping

DNA477.73	5' – GCCTCTTACACAACCTTCATTGCAC
DNA477.79	5' – CGGCTGACTCCTTGTAAGTTC
GT IRES	5' – CCCTAGGAATGCTCGTCAAGA

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Selection Cassette: Beta Geo Puro

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Targeted Locus:

[illegible]

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