

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Il22tm1Lex/Mmucd **MMRRC: 032401-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

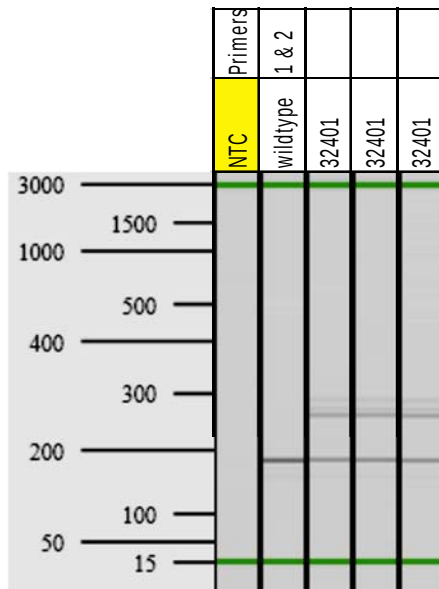
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 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	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA460-40	CTCAGACCTCTACAGACAATCATC	Estimated 9 min.		
2. DNA460-22	CAGCTGGCGGCCAAAGTCCC	Primer	Band (bp)	Genotype
		1 & 2	268	mutant
		1 & 2	196	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

[file:///Users/ttang/Desktop/lexicon/Project Materials/2003-Q4/HR/UNQ3099/UNQ3099_genotyping.xls](file:///Users/ttang/Desktop/lexicon/Project%20Materials/2003-Q4/HR/UNQ3099/UNQ3099_genotyping.xls).

Genentech ID:	UNQ3099	Date of Submission:	11/3/03
Lexicon Contract Name:	DNA460	Mutation Type:	☐ Standard Knock out
LexVision Name:	SEC249C1		☒ Conditional
Reference accessions:	NM_016971	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) pKOS.4
☐ Target Vector DNA
X Targeted ES Cell DNA 1A3
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	DNA460-43/44	DNA460-59/60
Restriction Enzyme for Genomic Digest:	EcoR I	Pst I
Predicted Wild-type Band (kb):	20	8.3
Predicted Mutant Band (kb):	12	7
Probe Size (bp):	513	434

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:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:	DNA460-40	5' Primer Name:	DNA460-40
3' Primer Name:	DNA460-22	3' Primer Name:	DNA460-41
Predicted Wild-type Band (bp):	196	Predicted Wild-type Band (bp):	2200
Predicted mutant band (bp)	268	Predicted mutant band (bp)	374

Primer sequences:**Southern probes**

DNA460-43 5' – CACTAAGCTTGAAGCAGGG
 DNA460-44 5' – AGGCAACAGTTCACCTGGT
 DNA460-60 5' – CACGGGACAGCTGAAATACAT
 DNA460-59 5' – ACAGGAAGCGTGAAATCTACAAT

PCR Genotyping

DNA460-40 5' – CTCAGACCTCTACAGACAATCATC
 DNA460-41 5' – GATACAGGTGCAGCTAAGCGAG
 DNA460-22 5' – CAGCTGGCGGCCAAAGTCCC

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Genomic Sequence Deleted:

TCAACTGTTGACACTTGTGCGATCTCTGATGGCTGTCTGCGAGAAATCTATGAGTTTTTCCCTTATGGGGACTTTGGCCGCCAGCTGCCTGCTTCTCATT
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Genomic Locus: (the deleted sequence represents nt-3795-6808--in the sequence below)

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Selection Cassette: pLFNeo LoxC

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Targeted Locus:

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[illegible]