GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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STRAIN NAME: **C.129S5-*Il22*$^{tm1.1Lex}$/Mmucd** **MMRRC:** **032402-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) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (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 OF REACTION:	**25.000 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (ºC)	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 (↓1ºC/cycle)	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

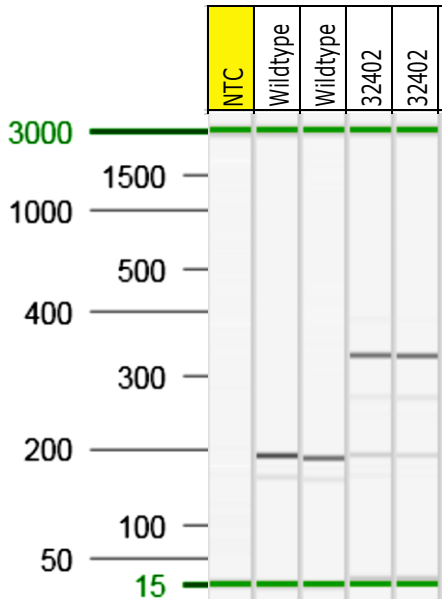
Name	Nucleotide Sequence (5' - 3')
1. DNA460-40	CTCAGACCTCTACAGACAATCATC
2. DNA460-41	GATACAGGTGCAGCTAAGCGAG
3. DNA460-22	CAGCTGGCGGCCAAAGTCCC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	374	Mutant
1 & 3	196	Wild-type



PCR protocol developed by MMRRC at University of California, Davis

Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ3099	**Date of Submission:**	11/3/03
Lexicon Contract Name:	DNA460	**Mutation Type:**	☐ Standard Knock out
LexVision Name:	SEC249C1		**X** 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

	5' External	**3' External**
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:

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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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