

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-Itln1^{tm1Lex}/Mmucd **MMRRC:** 032412-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	11.275
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 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 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:

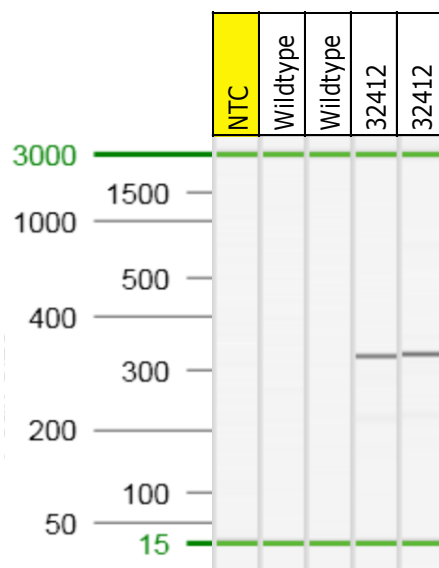
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. DNA050-8	GAGTAGTTGAAGGAGAAGC
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA

Electrophoresis Protocol:

Argarose: 1.5% V: 90		
Estimated Running Time: 90 min.		
Primer Combination	Band (bp)	Genotype
1 & 2	320	Mutant





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2789	Date of Submission:	10/12/04
Lexicon Contract Name:	DNA050	Mutation Type:	X Standard Knock out
LexVision Name:	SEC448N1		☐ Conditional
Reference accessions:	NM_010584; ENSMUST00000043094	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS 11_____
X Target Vector DNA __TV11-Bgeo_____
X Targeted ES Cell DNA __1G12_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>	<u>3' Internal</u>
Name of Probe:	16/17	14/15	Neo 2/5
Restriction Enzyme for Genomic Digest:	HpaI	EcoRI	SpeI
Predicted Wild-type Band (kb):	19.0	8.4	none
Predicted Mutant Band (kb):	11.1	5.4	16
Probe Size:	254 bps	228 bps	605 bps

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:	DNA050-1	5' Primer Name:	DNA050-8
3' Primer Name:	DNA050-2	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	298	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	331

Primer sequences:**Southern probes**

DNA050-14 5' – GCTGTAACCAGTGTTTCTAATG
DNA050-15 5' – GTTCAGTATTACATCCAGTGAC
DNA050-16 5' – GTAAC TTCAAAGGAGCCATG
DNA050-17 5' – TGCTCAATACCACTGGTGC
Neo-2 5' - CCTCAGAAGA ACTCGTCAAG
Neo-5 5' - GGCAGCGCGGCTATCGTG

PCR Genotyping

DNA050-1 5' – CAAGTCTCTGCACTGCAGACCT
DNA050-2 5' – ACCTGTTGGTGTCCAGGTTCTC
DNA050-8 5' – GAGTAGTTGAAGGAGAAGC
GT-IRES 5' – CCCTAGGAATGCTCGTCAAGA

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Selection Cassette: IRESBGeo-PGKPuro

Targeted Locus:

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