

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Enpp6tm1Lex/Mmucd **MMRRC: 031628-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.05
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
Primer 3. (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.

Strategy:

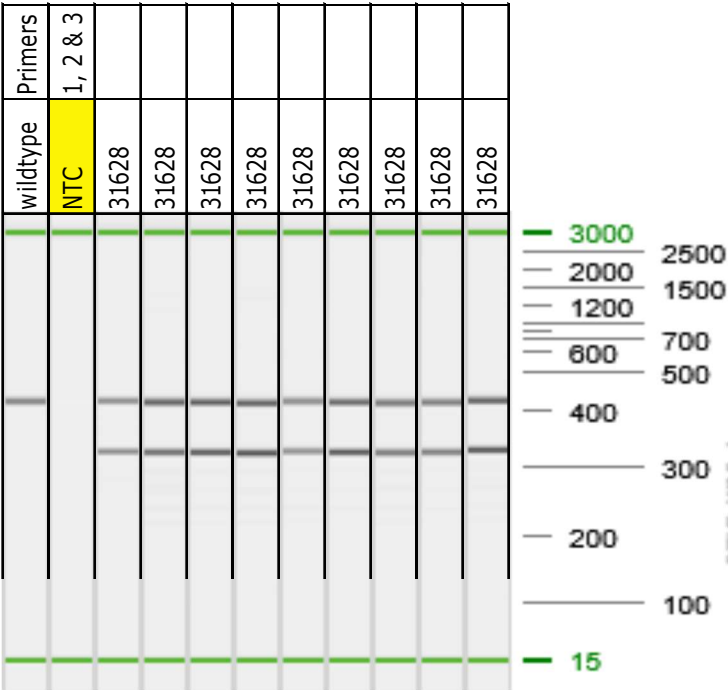
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA012-2	AGCATGGCAGCAAAGCTCTG	Estimated Running:Time: 90 min.		
2. DNA012-4	GTCTGAAATAGGTCCTTGGG	Primer Combination	Band (bp)	Genotype
3. Neo3a	GCAGCGCATCGCCTTCTATC	1 & 2	435	Wildtype
		2 & 4	338	mutant

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ1889	Date of Submission:	1/15/04
Lexicon Contract Name:	DNA012	Mutation Type:	X Standard Knock out
LexVision Name:	PHD225N1		☐ Conditional
Reference accessions:	NM_177304 ENSMUST00000039840	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS13_____
X Target Vector DNA __FTV 13_____
X Targeted ES Cell DNA __1H12_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	DNA012-15/ DNA012-16	Neo 2/5
Restriction Enzyme for Genomic Digest:	EcoRI	BglII, ApaI, XmaI
Predicted Wild-type Band (kb):	5.6kb	none
Predicted Mutant Band (kb):	3.2kb	19.8kb, 20.8kb, 18.9kb
Probe Size:	681bp	609bp

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:	DNA012-2	5' Primer Name:	Neo3a
3' Primer Name:	DNA012-4	3' Primer Name:	DNA012-4
Predicted Wild-type Band (bp):	435bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	338bp

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**

DNA012-15 5' – CCACTCTTGTTCTGACTGG
 DNA012-16 5' – AGAAAATGTGGACACGTTGTG
 Neo2 5' – CCTCAGAAGAAGCTCGTCAAG
 Neo5 5' – GGCAGCGCGGCTATCGTG

PCR Genotyping

DNA012-2 5' – AGCATGGCAGCAAAGCTCTG
 DNA012-4 5' – GTCTGAAATAGGTCCTTGGG
 Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GGCAGCAAAGCTCTGGACCTTCCTGCTGGGCTTTGGGCTCAGCTGGGTGTGGCCGGCTTCTGCCACCGGAAGCTCCTG
GTGTTGCTCCTGGATGGTTTTTCGCTCAGACTACATCAGTGAGGATGCTCTAGCATCCTTGCTGGCTTCAGAGAGATTGT
GAACAGAGGGCGTCAAAGTGGATTACTTGACTCCAGACTTTCCAGCCTCTCCTATCCCAATTACTACACCCTCATGACT
GGTGAGTACCTA

Genomic Locus: (the deleted sequence represents nt-8361 to 8610 in the sequence below)

CACTGAGACAGAATGAAACAGACATCACATACAGGGGAGGGGGCTTCTTTACATAAAATTATCTACTCCATACTCAAAA
TAACCCTCCAGGCAACACCCATTTACAGAGAGGTAAGCTGAAGCGTGGAAAAGAATTCAAGTAGCACAAACCAAGTG
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Selection Cassette:

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