

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-Mfsd2a^{tm1Lex}/Mmucd **MMRRC:** 032467-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.

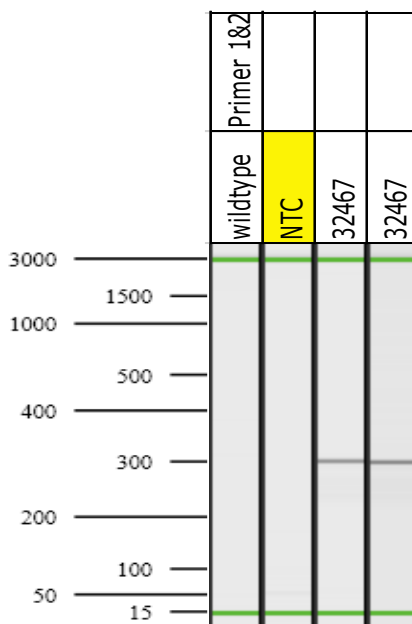
Strategy:

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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA314-5	CACTTCCTAAAGCCTTACTTC	Estimated Running:Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA314-1	CCTGGTTTGCTAAGTGCTAGC	1 & 2	301	Mutant
4. DNA314-2	GTTCACTGGCTTGGAGGATGC	3 & 4	210	Wild-type



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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ300	Date of Submission:	10-11-04
Lexicon Contract Name:	DNA314	Mutation Type:	x Standard Knock out
LexVision Name:	MEM757N1		☐ Conditional
Reference accessions:	XM_131683, AK006096	Is this gene X-linked?	no

Required Materials:

- ☐ pKOS clone DNA(s) kos30
- ☐ Target Vector DNA DNA314-FTV30
- ☐ Targeted ES Cell DNA 2E10
- ☐ Genomic Map

Southern Blot Analysis:
 External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	8 + 9	12 + 13
Restriction Enzyme for Genomic Digest:	PstI	Acc65I
Predicted Wild-type Band (kb):	13.3	11.7
Predicted Mutant Band (kb):	15.8	7.3
Probe Size:	266 bp	292 bp

PCR Strategies:

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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:	DNA314-1	5' Primer Name:	Neo3a
3' Primer Name:	DNA314-2	3' Primer Name:	DNA314-5
Predicted Wild-type Band (bp):	210	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	301

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

DNA314-8 5' – CTAGAGGGACTGAAATGCTG
DNA314-9 5' – CCTGGGATCTTGCTGATATG
DNA314-12 5' – GTTTAAGATCCAGGTGGGAG
DNA314-13 5' – ACACGACACCAGAAGCCTG

PCR Genotyping

DNA314-1 5' – CCTGGTTTGCTAAGTGCTAGC
DNA314-2 5' – GTTCACTGGCTTGGAGGATGC
DNA314-5 5' – CACTTCCTAAAGCCTTACTTC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

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AGCAAGGAGTGTCTGTTGACCGGGAGTGTAGGAGAAAGTCTGAAGCCCCCTTGCACACCCCTACAATGGGTTTGGCCCAAGAGAGGCGCGG

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