

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Slc29a3tm1Lex/Mmucd **MMRRC: 032580-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (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:

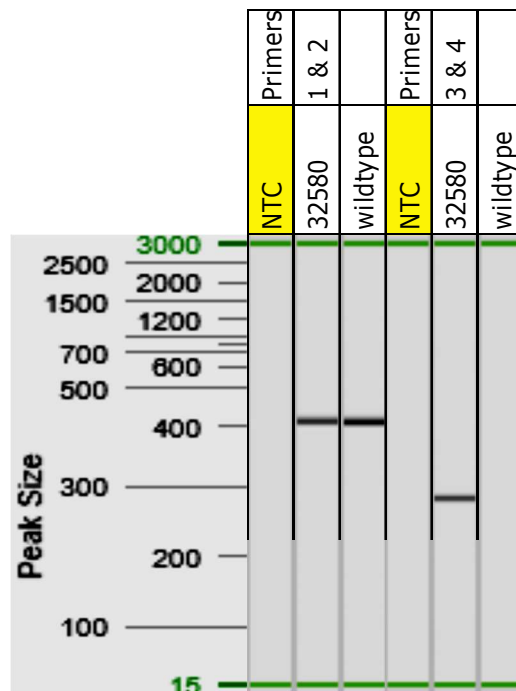
Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA426-5	GCCAGAGGATCGCTTCA	Estimated Running:Time: 90 min.		
2. DNA426-6	GACAACAGCAGGCAATCTAAA	Primer Combination	Band (bp)	Genotype
3. DNA426-22	ATGCAAATGGCCTCCCTCAAGTACC	3 & 4	284	Mutant
4. PuroJA	GAGGAAATTGCATCGCATTGTCT	1 & 2	415	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ717	Date of Submission:	3-24-05
Lexicon Contract Name:	DNA426A	Mutation Type:	X Standard Knock out
LexVision Name:	TRA350N1		☐ Conditional
Reference accessions:	NM_023596	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS9, pKOS12, pKOS40, pKOS91_____
 X Target Vector DNA __pKOS12TV_____
 X Targeted ES Cell DNA __2D8_____
 X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	15+16	17+19
Restriction Enzyme for Genomic Digest:	Bgl II	XmnI
Predicted Wild-type Band (kb):	11.2 kb	7.3 kb
Predicted Mutant Band (kb):	15.2 kb	11.9 kb
Probe Size:	358 bp	812 bp

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:	DNA426-5	5' Primer Name:	PuroJA
3' Primer Name:	DNA426-6	3' Primer Name:	DNA426-22
Predicted Wild-type Band (bp):	415 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	284 bp

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**

DNA426-15 5' – GAGATATGGGTGTTTGGGTAT
DNA426-16 5' – TCAAGGGAGTCCATCGG
DNA426-17 5' – AGGATACAGATCATCCATAGG
DNA426-19 5' – AATGAGTAAGTCCTGACTCCA

PCR Genotyping

DNA426-5 5' – GCCAGAGGATCGCTTCA
DNA426-6 5' – GACAACAGCAGGCAATCTAAA
DNA426-22 5' – ATGCAAATGGCCTCCCTCAAGTACC
PuroJA 5' – GAGGAAATTGCATCGCATTGTCT

Genomic Sequence Deleted:

GGTGGAGGTAGACAGCGGCACGCACAGTAAGTGCGGGTGCAGCGGCCCCCATGCTTGGCTGGGTCTTCCCTTCCCGAGG
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Genomic Locus: *(The deleted sequence represents nt12055-14461 in the sequence below. KOS12 used to generate the TV represents nt 9497-20154 in the sequence below.)*

[illegible]

[illegible]

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[illegible]

[illegible]

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Selection Cassette:

CGCTCTAGAGGCCATAGCGGCCATTTAAATGGCGCGCCGGATCCGAATTCCTCGAGGCTAGAACTAGCGATAAGCTTC
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