

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Dkk3tm1Lex/Mmucd **MMRRC: 032271-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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

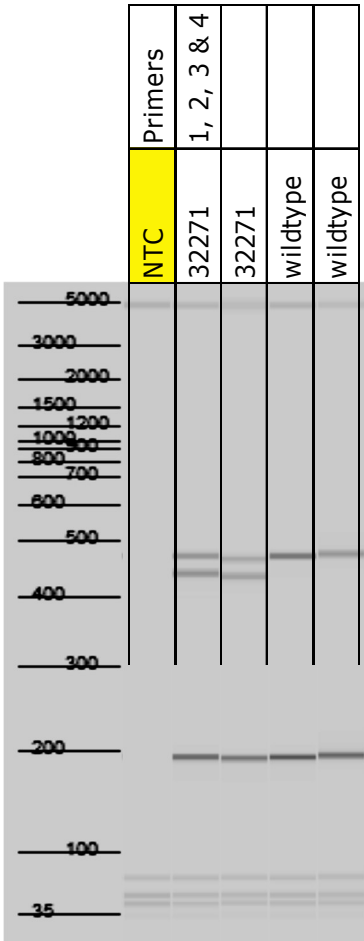
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA316-7	GTAGTGGTCACCACTGTTTC	Estimated Running Time: 90 min.		
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. DNA316-1	TCTCAACTACCCTCAGGAGGA	1 & 2	402	mutant
4. DNA316-2	ACTCCCTTTCTCATTCTCGCAC	3 & 4	159	wildtype
		1 & 2	438	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ258	Date of Submission:	8.11.05
Lexicon Contract Name:	DNA316	Mutation Type:	X Standard Knock out
LexVision Name:	SEC558N1		☐ Conditional
Reference accessions:	NM_015814	Is this gene X-linked?	No

Required Materials:

☐ pKOS clone DNA(s) __pKOS-9_____

☐ Target Vector DNA __pKOS-9TVPuro_____

☐ Targeted ES Cell DNA __1A4_____

☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	9/10	13/14
Restriction Enzyme for Genomic Digest:	HindIII	Acc65I
Predicted Wild-type Band (kb):	8.7 kb	17.8 kb
Predicted Mutant Band (kb):	4.4 kb	7.7 kb
Probe Size:	336 bp	327 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:	DNA316-1	5' Primer Name:	DNA316-7
3' Primer Name:	DNA316-2	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	159 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	402 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**

DNA316-9 5' – CCAGCAAGCTAACAAGCAAC
 DNA316-10 5' – CTGCTGTGTCATGTCCATAC
 DNA316-13 5' – GCTGGCTAAGATAACCCCTC
 DNA316-14 5' – CCCTTACCTTGTGAACTTCC

PCR Genotyping

DNA316-1 5' – TCTCAACTACCCCTCAGGAGGA
 DNA316-2 5' – ACTCCCTTTCTCATTCTCGCAC
 DNA316-7 5' – GTAGTGGTCACCACTGTTTC
 GT-IRES 5' – CCCTAGGAATGCTCGTCAAGA

CGCGGAACAAACATGCAGCGGCTCGGGGGTATTTTGCTGTGTACACTGCTGGCGGCGGCGGTCCCCACTGCTCCTGCTCCTTCCCCGACGGTCACTTGG
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[illegible]

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