

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6;129S5-Prss22^{tm1Lex}/Mmucd **MMRRC:** 032534-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.275
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
Primer 3. (stock concentration is 20μM)	0.5
Primer 4. (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.

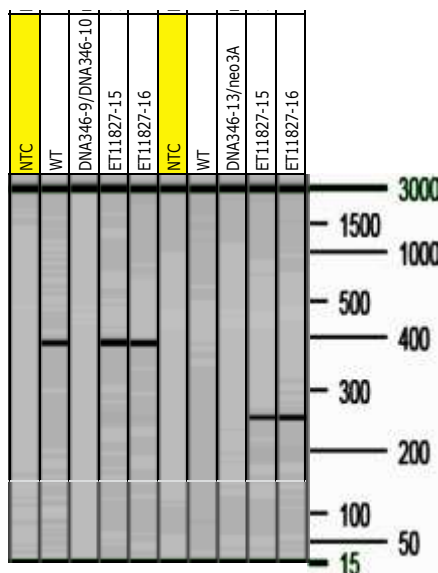
Strategy:

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')	Agarose: 1.5% V: 90		
1. DNA346-10	CTCCCACTCCCTTAACCTTT	Estimated Running Time: 90 min.		
2. DNA346-9	ACAGGTGGTGGAGCAGCGAACAGT	Primer Combination	Band (bp)	Genotype
3. DNA346-13	CACTTATTCCAGTCCCCAAAT	1 & 2	406	Wild-type
4. Neo3a	GCAGCGCATCGCCTTCTATC	3 & 4	266	Mutant





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ302	Date of Submission:	1-21-2005
Lexicon Contract Name:	DNA346	Mutation Type:	X Standard Knock out
LexVision Name:	PRT348N1		☐ Conditional
Reference accessions:	NM_133731, ENSMUST00000041649	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS58_____
X Target Vector DNA __TV58_____
X Targeted ES Cell DNA __3E5_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	14/15	16/17
Restriction Enzyme for Genomic Digest:	BglII	XbaI
Predicted Wild-type Band (kb):	~10.8	4.9
Predicted Mutant Band (kb):	~14.2	9.0
Probe Size:	553	606

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:	DNA346-9	5' Primer Name:	Neo3a
3' Primer Name:	DNA346-10	3' Primer Name:	DNA346-13
Predicted Wild-type Band (bp):	406	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	266

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**

DNA346-14 5' – CCAACCTTGCCACACATTACC
DNA346-15 5' – GGCCATTTGTACTATCGTCTG
DNA346-16 5' – AGCCCAGACAAACATTAGTCA
DNA346-17 5' – CATGCCCTCCGTGATGG

PCR Genotyping

DNA346-9 5' – ACAGGTGGTGGAGCAGCGAACAGT
DNA346-10 5' – CTCCCCTCCCTTAACCTTT
DNA346-13 5' – CACTTATTCCAGTCCCCAAAT
Neo3a 5' – GCAGCGCATCGCCTTCTATC

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Genomic Sequence Deleted:

[illegible]

Genomic Locus: *(The deleted sequence represents nt--8098--9958--in the sequence below. KOS 58 used to generate the TV represents nt-3617--12717--- in the sequence below.)*

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Selection Cassette:

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