

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: B6N.129S5-Hamp^{tm1Lex}/Mmucd **MMRRC:** 032358-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
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
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:

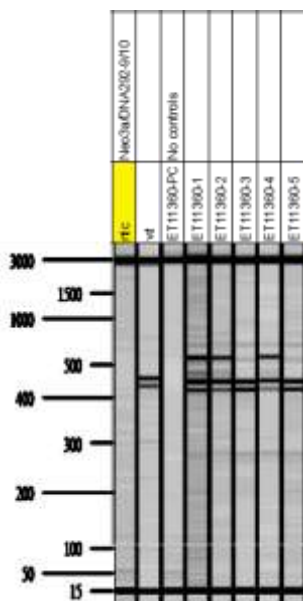
- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust 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:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. DNA292-9	TTGCACGGGGAAGAAAGCAG	Argarose: 1.5% V: 90		
2. DNA292-10	GACCTGTAAACCCAGCTCAG	Estimated Running Time: 90 min.		
3. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
		1 and 2	429	Wild-type
		2 and 3	543	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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