GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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STRAIN NAME: **B6;129S5-*Hsd17b13*^{tm1Lex}/Mmucd** **MMRRC:** **032367-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) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	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 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

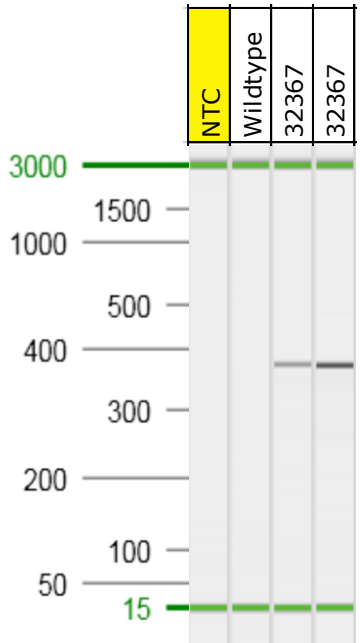
Name	Nucleotide Sequence (5' - 3')
1. DNA081-19	CTGGGCCAAACTTTTTAGACAC
2. Neo3a	GCAGCGCATCGCCTTCTATC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2	380	Mutant



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ497	**Date of Submission:**	7/28/04
Lexicon Contract Name:	DNA081	**Mutation Type:**	X Standard Knock out
LexVision Name:	ENZ717N1		☐ Conditional
Reference accessions:	**BC019427**	**Is this gene X-linked?**	NO

Required Materials:
X pKOS clone DNA(s) __KOS-38_____
X Target Vector DNA __ENZ717.38TV.1_____
X Targeted ES Cell DNA _ 1D12___
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' External**
Name of Probe:	**DNA081-13/14**	**DNA081-15/16**
Restriction Enzyme for Genomic Digest:	**BglI**	**NcoI**
Predicted Wild-type Band (kb):	**17.6 kb**	**7.5 kb**
Predicted Mutant Band (kb):	**11.6 kb**	**4.5 kb**
Probe Size:	**391 bp**	**323 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:	**DNA081-17**	5' Primer Name:	**Neo3a**
3' Primer Name:	**DNA081-18**	3' Primer Name:	**DNA081-19**
Predicted Wild-type Band (bp):	**457 bp**	Predicted Wild-type Band (bp):	**none**
Predicted mutant band (bp)	**none**	Predicted mutant band (bp)	**366 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
DNA081-13 5' – GTTGTTAGAACGAGGCCTAAG
DNA081-14 5' – GTTGCTCCACTGATTTCTCTC
DNA081-15 5' – GAGTGTAGAGAGAATGAATCC
DNA081-16 5' – GCCTGCAGATCTGTATGGAG

PCR Genotyping
DNA081-17 5' – GGCGTCATCATCTACTCCTACCTG
DNA081-18 5' – TGGCTCCTGCGTCTCTTGTTATCA
DNA081-19 5' - CTGGGCCAAACTTTTTAGACAC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

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Genomic Sequence Deleted:

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Genomic Locus: ***(the deleted sequence represents nt10286-15090 -in the sequence below)***

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Selection Cassette:

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Targeted Locus:

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