

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Susd4^{tm1Lex}/Mmucd MMRRC # 032614-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 (50-200ng/μL) 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')
1. 32614-koF	CACCTTCGAGGTCACAGCCTCTG
2. GT-IRES-R2	CCCTAGGAATGCTCGTCAAGAAGAC

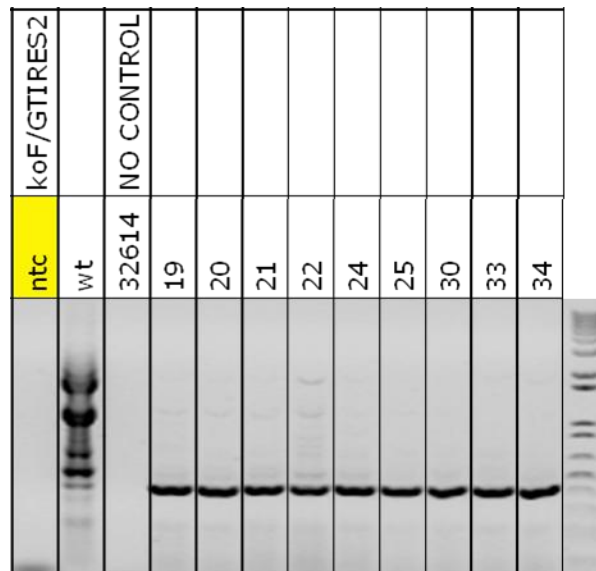
Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	414 bp	KO

Please note: MMRRC-UCD developed this protocol with different primers than those provided on the donor protocol below. The donor's protocol did not work in our hands.





\Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ196	Date of Submission:	11/23/04
Lexicon Contract Name:	DNA024	Mutation Type:	X Standard Knock out
LexVision Name:	MEM649N1		☐ Conditional
Reference accessions:	NM_144796	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) ___pKOS 54_____
X Target Vector DNA ___TV 54 puro_____
X Targeted ES Cell DNA ___1A8_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	14/15	16/17
Restriction Enzyme for Genomic Digest:	BglII	SfiI
Predicted Wild-type Band (kb):	10.8 kb	>40 kb
Predicted Mutant Band (kb):	15.9 kb	9.8 kb
Probe Size:	533 bp	600 bp

PCR Strategies:

For standard knockouts, give wildtype and mutant-specific strategies

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	MEM649-5	5' Primer Name:	MEM649-10
3' Primer Name:	MEM649-2	3' Primer Name:	GT Ires
Predicted Wild-type Band (bp):	259	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	579

Primer sequences:**Southern probes**

MEM649-14 5' – ATCCTCTTGGCACGGCTCTA
 MEM649-15 5' – GACTGGCCTTCTTCTGTATCAG
 MEM649-16 5' – GCCCAGTTACCCTTTATCTAT
 MEM649-17 5' – TAGAATGTTAGTGTGCGAATGACT

PCR Genotyping

MEM649-5 5' – CTGTGGTTTCAACTGGCGCTGTG
 MEM649-2 5' – GCTGCCGGTGGGTGTGCGAACCTA
 MEM649-10 5' – TCGGGCTGTCCACTGGGCTCTAGG
 GT Ires 5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

CGACTCGTGCCCGACAGGTGCAGGCGCGCCGGCTTCCCATTGGCCACGGCGTCCTCCTCCGCCCACGCC
 ATCCCCTTACCCCTGCTACCGGCGCTGGCTGGGACCCGCGGAGGCGCGTGCAAGGCGCGCGCCCTGC
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 CCGTGATCCTGTGGTTTCAACTGGCGCTGTGCTTTGGCCCTGCACAGCTCACGGGTGGTGAGTGACCCC
 GCTGTGGTGGGGGGAGCTTTGAATGGCCCAGCAGGGAGGG

Genomic Locus: *(The deleted sequence represents nt-5707-7045--in the sequence below. KOS54 used to generate the FTV represents nt 4519-13055 in the contig below.)*

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Selection Cassette:

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