

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6N.129S5-Sost^{tm1Lex}/Mmucd MMRRC: 032597-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
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:

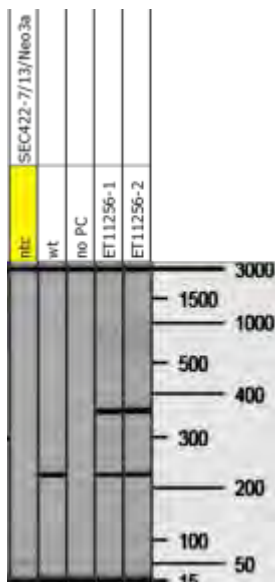
- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust

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. SEC422-7	GGAGGGCGCAAGTGTATACAT	Argarose: 1.5% V: 90		
2. SEC422-13	TTGTGGATGAGTCTCACATGG	Estimated Running:Time: 90 min.		
3. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
		1 and 2	223 bp	Wild-type
		2 and 3	356 bp	Mutant



Lexicon's original protocol below.



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2976	Date of Submission:	9-29-04
Lexicon Contract Name:	DNA057	Mutation Type:	☒ Standard Knock out
LexVision Name:	SEC422N1		☐ Conditional
Reference accessions:	NM_024449	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) [KOS36](#)
☐ Target Vector DNA [pKOS36.TV.1](#)
☐ Targeted ES Cell DNA [1F8](#)
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	1+2	16+17
Restriction Enzyme for Genomic Digest:	BclI	StuI
Predicted Wild-type Band (kb):	12.6 kb	6.4 kb
Predicted Mutant Band (kb):	5.3 kb	4.7 kb
Probe Size:	467 bps	316 bps

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:	SEC422-7	5' Primer Name:	Neo3a
3' Primer Name:	SEC422-13	3' Primer Name:	SEC422-13
Predicted Wild-type Band (bp):	223bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	356bp

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**

SEC422-1 5' – TTCCAAGGACAGATCGCATCAT
 SEC422-2 5' – GCCACCTGCCTCGACTTGTA
 SEC422-16 5' – GACCATGAATGTTCTCGTAACG
 SEC422-17 5' – GGGTGTTCTTATGAAAGCTAA

PCR Genotyping

SEC422-7 5' – GGAGGGCGCAAGTGTATACAT
 SEC422-13 5' – TTGTGGATGAGTCTCACATGG
 Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

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Genomic Locus: (the deleted sequence represents 20,460 bps to 24,618 bps in the sequence below)

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[illegible]

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Selection Cassette:

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