



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2500	Date of Submission:	5/12/05
Lexicon Contract Name:	DNA472	Mutation Type:	<input checked="" type="checkbox"/> Standard Knock out
LexVision Name:	SEC479N1		<input type="checkbox"/> Conditional
Reference accessions:	AK008922	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS-42_____
 X Target Vector DNA __pKOS-42TV_____
 X Targeted ES Cell DNA __2D2_____
 X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	17/18	Neo 2/5
Restriction Enzyme for Genomic Digest:	EcoRI	HpaI
Predicted Wild-type Band (kb):	8.3	-----
Predicted Mutant Band (kb):	5.9	11.3
Probe Size (bp):	311	609

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:	6	5' Primer Name:	Neo3a
3' Primer Name:	12	3' Primer Name:	16
Predicted Wild-type Band (bp):	384	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	267

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**

17 5' – CCGGGGGTGCAGGGGGTTTA
 18 5' – CAGGGGGTGGTGCTCAGGATA
 neo-2 5' – CCTCAGAAGAACTCGTCAAG
 neo-5 5' – GGCAGCGCGGCTATCGTG

PCR Genotyping

6 5' – TGGCATGTATGTGGCAAAAAGAGA
 12 5' – GAAGAGCCCCATCCCCACAGAGC
 16 5' – GTGTATAGGTGCAGGGTATGTG
 neo3a- 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

ATGCGACGCCCTCTGGCTGGGCTAGCCTGGCTGCTGTTGGCGCGGGCACCCGGGCGCTCCGGGAGGGTACCCGCATCTGGAGGGCGACGTGCGCTG
GCGCCGCTCTTCTCCTCACTTTTTCTGCGTGTGGACCTTGGTTCGGGTGCAGGGGACGCGTTGGCGCCAGGCCAGGACAGTGTGCTG
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CTTCTGTCTTCCAAGGGCCCTGAGTTTGTCTCCCA

Genomic Locus: (*The deleted sequence represents nt 8293-10583 in the sequence below. KOS42 used to generate the TV represents nt 5438-17586 in the sequence below.*)

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Selection Cassette:

GGCCTCCACAGGACAGGCAGACAGACAAGCAAGAAGAGTGTAGCCAGATAGAGACGTTGTTCAGGTTAGACAGCTCAGATCGTGCCCATGCGGGG
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