



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ904	Date of Submission:	12/9/04
Lexicon Contract Name:	DNA234	Mutation Type:	<input checked="" type="checkbox"/> Standard Knock out
LexVision Name:	SEC515N1		<input type="checkbox"/> Conditional
Reference accessions:	BM453823	Is this gene X-linked?	NO

Required Materials: pKOS clone DNA(s) __pKOS-54, 21_____
 Target Vector DNA __21TVneo_____
 Targeted ES Cell DNA __3C7_____
 Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	IRES 8/9	26+27
Restriction Enzyme for Genomic Digest:	AccI	Bgl I
Predicted Wild-type Band (kb):	-----	8.3
Predicted Mutant Band (kb):	8.4	3.4
Probe Size:	263 bp	365 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:	5	5' Primer Name:	Neo3a
3' Primer Name:	6	3' Primer Name:	8
Predicted Wild-type Band (bp):	488 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	431 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**

IRES-8 5' – AGGAAGCAGTTCCTCTGGAA

IRES-9 5' – CACATGTAAAGCATGTGCACC

26 5' – CCATGGATCCAGGAAGACAATA

27 5' – TGAAGTGTCTGGGCTTGGGTA

PCR Genotyping

5 5' – AGAAGGGACTTAAGGCAATATGAG

6 5' – AATTGGCACTGCTTTCTATATCT

8 5' – CCCACCAAGAACATATGAGTTTGA

neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

ATGGCGAGACTCGGGCTGCTTCTCCTCTGCTGCTGCCCTGCCACCACACTTCTCCTCAGTGTGCATGGCCAGACACTGCACAGGGCACCATTGGCAAAC
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GGGTGGGGCTATCACATCAAAGGCAGCATCTGGGTGCTACCGGAATCCCGAGGTATCAACACTTCTGAAGCAAG

Genomic Locus: (The deleted sequence represents nt 7119-9168 in the sequence below. KOS21 used to generate the TV represents nt 4534-11852 in the sequence below.)

CCAGAGGGTCTTTGGGTAAAGATATGAAGGTGAAGCCTAGATTGTGTGAAGCCCAAAAATGTTAGAGATGCCAGAGTCATAGGATAGGAACTA
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Selection Cassette: LacZ/Neo

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Targeted Locus:

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