



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ633	Date of Submission:	2-3-04
Lexicon Contract Name:	DNA507	Mutation Type:	x Standard Knock out
LexVision Name:	ENZ279N1		☐ Conditional
Reference accessions:	BC031544	Is this gene X-linked?	NO

Required Materials: X pKOS clone DNA(s) pKOS31
X Target Vector DNA pKOS31.TV
X Targeted ES Cell DNA 2D4
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	24/44	41/43
Restriction Enzyme for Genomic Digest:	BamH1	BamH1
Predicted Wild-type Band (kb):	13.5 kb	13.5 kb
Predicted Mutant Band (kb):	4.5 kb	8.4 kb
Probe Size:	351 bp	551 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:	20	5' Primer Name:	Neo3A
3' Primer Name:	49	3' Primer Name:	49
Predicted Wild-type Band (bp):	200 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	295 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**

24 5' – TTGGCTTCATTTTGTACCTGG
 41 5' – GCAAGTTTTTGTCCATGGGG
 43 5' – ATGAGGGAAGTGAATCTGGG
 44 5' – TCCGTTATCGAAGTTGTTTCC

PCR Genotyping

20 5' – ACAGGAAGTACTGGACAAGG
 49 5' – AGAAAAGGAGGCTGCAAACC
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

CTTATGGCCAAACAGAATGCACAGGTGGATGTTCAATTACATCACCTGGGGACTGGACAGCAGGTGGGTTTTCCATTG
CTAGTTGGGAGAAGTCTTCTAGCTGACCCATGGCTTAGGAATGTAAATAATTCTGGTGTCTGGATGAGTGTGGTAAAT
GGCAAACCTCAAGTCTTGCTTTTCATCCTTAGAGTCAGTCACATAAACATCTTAGAAACGTGCTTGCTCTAGGGTTGCTTAG
CCCTGAATATATGCTATATTCTAGGTCATGTTGGGACTCCAGTGGCTTGCAATTTTGTAAGCTGGAAGATGTGGCTGA
CATGAACTACTTTTCAGTAAACAACGAAGGCGAGGTGAGTAGGTCATGTCCAGCACTCAGGTAATTGACGTGGAGAAG
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GGGACATTGGTTCGCTGGCTTCCAGTGGGTATTT

Genomic Locus: (the deleted sequence represents nt 10903-11646 -in the sequence below)

[illegible]

GTGTTCCCTTCTTGGAGTAGTCTCCAAAATTTCCAGCCATTCTGAAGCTTGTTTGAGGACAGAAGCACTAACATCTCAATA
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Selection Cassette:

CGCTCTAGAGGCCATAGCGGCCGGATCCTCGAGGCGCGCCGGATCCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGA
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