Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ9236	**Date of Submission:**	7/19/04
Lexicon Contract Name:	DNA131	**Mutation Type:**	x Standard Knock out
LexVision Name:	MAS238N1		☐ Conditional
Reference accessions:	ENSMUST00000015 996	**Is this gene X-linked?**	NO

Required Materials:
x pKOS clone DNA(s) __pKos 66_____
x Target Vector DNA __TV 66 IRES LacZMCI NEO_____
x Targeted ES Cell DNA __1D12_____
x Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' External**
Name of Probe:	**23/24**	**28/30**
Restriction Enzyme for Genomic Digest:	**EcoRI**	**EcoRI**
Predicted Wild-type Band (kb):	**Expected 19.5Kb, actual ~22kb**	**Expected 19.5Kb, actual ~22kb**
Predicted Mutant Band (kb):	**~11 kb**	**~6 kb**
Probe Size:	**391bp**	**417bp**

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:	1	5' Primer Name:	Neo3a
3' Primer Name:	2	3' Primer Name:	13
Predicted Wild-type Band (bp):	200bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	419bp

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Primer sequences:

Southern probes
DNA131 23 5' – GCCGTTGTTAGTCCTAGAGAAA
DNA131 24 5' – AAAGAAGTTCCTCTGGCAATTC
DNA131 28 5' – TTAAAGAAGCCTGAGCATGGTA
DNA131 30 5' – ATCTTAGAGCCTAGCTTAGCC

PCR Genotyping
DNA131-1 5' – AAGTTAACAAAGCTGGTAGGGC
DNA131-2 5' – TGGTCTCCTTCATAAGAACGGT
DNA131-13 5' – TTTACCTAGCCTACCCGTTACA
Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

AGCCTTCCACACCGTTCTTATGAAGGAGACCAAGTAGTTATAAGCTGCACAGGAAAAAATAATGGTGACATAAAGAGA
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Genomic Locus: ***(the deleted sequence represents nt13841-16811 in the sequence below)***

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<u>Selection Cassette</u>: IRES LACZ MCI NEO

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