



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2783	Date of Submission:	1-10-05
Lexicon Contract Name:	DNA197	Mutation Type:	X Standard Knock out
LexVision Name:	MEM706N1		☐ Conditional
Reference accessions:	AA030296	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __pKOS11, pKOS80____
☐ Target Vector DNA __pKOS11TV____
☐ Targeted ES Cell DNA __2D7____
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	14+15	18+19
Restriction Enzyme for Genomic Digest:	Avr II	Hind III
Predicted Wild-type Band (kb):	12.8 kb	13.1 kb
Predicted Mutant Band (kb):	9.6 kb	10.3 kb
Probe Size:	373 bp	331 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:	DNA197-21	5' Primer Name:	Neo3A
3' Primer Name:	DNA197-22	3' Primer Name:	DNA197-22
Predicted Wild-type Band (bp):	301 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	425 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**

DNA197-14 5' – AGGCGACTGCATGTTAAAGG
 DNA197-15 5' – TATTGGACATGGTAAAAGGCC
 DNA197-18 5' – CACATACTGTAATCTTCCAGC
 DNA197-19 5' – TCTGTCTGAACACTTCCTGG

PCR Genotyping

DNA197-21 5' – CCCCACCTTCATCTAAGTGC
 DNA197-22 5' – AAGGGTAGAGTTAGGGATTCC
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

CGATGAGCGGCGCCTGCTCGAGCTACGTGAGCGCTGAGCAGGAGGTGGTGC GCGGCTTCAGCTGCCCCGCTGCCAGGGG
GCGAAGCGGCTGCTGTCTTCTGCTGTGGCTTCCGCGACCACAAGTACTGCTGCGACGACCCGCACAGCTTCTTCCCCTA
CGAGCACAACCTACATGTGGTGGCTCAGGTACAGGCCCTGGCTCTAACCTCACCTTGAACCCCTTCTCCCATCTTATCCC
ACTAGCCCAACCGCAAGCCAGCCTCTTGAAGCCCCACCTTCATCTAAGTGCCCTTCCCTCAAATCATCCTTAC

Genomic Locus: (The deleted sequence represents nt10928-11238 in the sequence below. KOS11 used to generate the TV represents nt-3965-13156 in the sequence below.)

AATGCACTAGACAGAAGTTTGATTTACACTCAGGAGAGGATGGCCTGGATGACTTGAACTTCATCATGTAGTCACAAC
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Selection Cassette:

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GGGTAATGTTGAGGGAGTCAGGGATACGTAGTGAGTTCCAGGCCAGCC