



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ719	Date of Submission:	1/12/05
Lexicon Contract Name:	DNA376	Mutation Type:	☒ Standard Knock out
LexVision Name:	MEM756N1		☐ Conditional
Reference accessions:	NM_177735	Is this gene X-linked?	NO

Required Materials:

- ✓ pKOS clone DNA(s) __pKOS-8_____
- ✓ Target Vector DNA __pKOS-8TVneo_____
- ✓ Targeted ES Cell DNA __2H12_____
- ✓ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	17/18	21/22
Restriction Enzyme for Genomic Digest:	EcoRI	EcoRV
Predicted Wild-type Band (kb):	8.1	5.4
Predicted Mutant Band (kb):	5.2	7.8
Probe Size (bp):	385	299

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:	3	5' Primer Name:	Neo3a
3' Primer Name:	4	3' Primer Name:	14
Predicted Wild-type Band (bp):	400	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	203

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' – CGTGGGCACCGAAGTGAC
 18 5' – AGGCCTGGTGTTATAGCTCAGT
 21 5' – GGAAGAGTTGGCCCAGG
 22 5' – GGGCAGAGAGGCTAAAGACAGG

PCR Genotyping

3 5' – GGCCACAGGTTGAGCACTTAAC
 4 5' – ATGCATACCCGTCTCCAAAG
 14 5' – GGCTCTGTGGCATCATTGG
 neo3a 5' – GCAGCGCATCGCCTTCTATC

[illegible]

AATTCTGAGGATTTTGTGGGAATTTTGCATTTTAAATCACCTGCAGCACCCCCACCCTACCCCCACTCCCCACCCCAACCCCTCTCCTAGAAATCCA
CCATAAAAATTCATCTAGAAATGGACACCCTGGTGCCATTAGGTTGTATGTGCCTAGAGATTATGTGATTTAATTTCAAGAAATACACAAGTATATATATA
TATATTTATTTTGTATTTTCAACACGAGGATTTCTTGTATAGCCCTGATTGTCTCTGGAACCTCATAGAAATGGACACCCTGGTCCATTAGGTTGTATG
TGCCTTGAAGTTATGTGATTTAAATTAAGAAGACACAAACCATTTGTTTGTGTTTGGAGCAGGGTTCTCTGTGTAGCCCTGGCTGAGCTGGAGC
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[illegible]

[illegible]

Targeted Locus:

[illegible]

TCATGAGTTCAATTCTCAGCAACCATACGATGGCTCAGAACCATCTGTAATGGGATTCAATGCCCTCTTCTGGTGTGTCTGAAGAGTGCTCCCATACATT
AAATAAGCAAATAATTCTTTAAAAAAAATGAAAGAATCAAGACATAGTCATGCCGTGGGCCAGCCTGAGCTAGCTTATCCTTCAACTGAGACTGTTT
TCCCAGGTTATCCATCACAGTTCCAAGGCCCTCCCTCCTCCCTTGGACCATCCCCAAGAATGCCACTCAGTTGTGACTCTATGGAGAGATTAAACCAG
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GCAGACAGAGGCAGGACTCTGGG