



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ576	Date of Submission:	11.24.04
Lexicon Contract Name:	DNA353	Mutation Type:	☒ Standard Knock out
LexVision Name:	MEM740N1		☐ Conditional
Reference accessions:	ENSMUST00000042591, AK016183 NM_144539	Is this gene X-linked?	No

Required Materials:

- ☒ pKOS clone DNA(s) __KOS33, 34, 7 _____
- ☒ Target Vector DNA __TV7-1_____
- ☒ Targeted ES Cell DNA __3C6_____
- ☒ Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	18+19	22+23
Restriction Enzyme for Genomic Digest:	XbaI	NcoI
Predicted Wild-type Band (kb):	5.8 kb	10.1 kb
Predicted Mutant Band (kb):	4.1 kb	5.7 kb
Probe Size:	274 bp	178 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:	DNA353-17	5' Primer Name:	Neo3a
3' Primer Name:	DNA353-26	3' Primer Name:	DNA353-27
Predicted Wild-type Band (bp):	246bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	407bp

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**

DNA353-18 5' – CTACTCACATCTGGTGAC
DNA353-19 5' – GTATCCTTGTCACCTTCTG
DNA353-22 5' – GAAGAAGCCAAGATGCTGTC
DNA353-23 5' – GCACACAGTGCTTAGTAGC

PCR Genotyping

DNA353-17 5' – GAATGGGAGCAAGATGGTAG
DNA353-26 5' – CTGCTTGGCTCTTCTCTAC
Neo3a 5' – GCAGCGCATCGCCTTCTATC
DNA353-27 5' – GGGTAGCCTCAAACAAAG

Genomic Sequence Deleted:

CAGCTTCTGGAACCTCTGAAGAAGGTGGCCGGTGCCCTTGATGGATCTGTGACATTCACCTCTGAATATCACTGAAATAAAGGTTGACTATGTTGTATGGA
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AGATCCCCAAAGTGGAAGAAGCT

Genomic Locus: (The deleted sequence represents nt 12685-17644 in the sequence below. KOS7 used to generate the TV represents nt 11036-21998 in the sequence below.)

AACCCCTTCTGGGTCAATTTATATGATTAGGCTCTTCCACCTCTCCATTCTGTTTGGCAAGAATTTAGGAATCAAAATTACAATGTTTTCTAATAACTA
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Selection Cassette: LacZ/Neo

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