Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ1825	Date of Submission:	10-11-05
Lexicon Contract Name:	DNA135A	Mutation Type:	X Standard Knock out
LexVision Name:	MEM668N1		☐ Conditional
Reference accessions:	ENSMUST00000024792	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s)   __pKOS52, pKOS36_____
                         X Target Vector DNA   __pKOS36TV_____
                         X Targeted ES Cell DNA __1E2_____
                         X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' internal	3' External
Name of Probe:	**16+17**	**4+5**
Restriction Enzyme for Genomic Digest:	**EcoRI**	**EcoRI**
Predicted Wild-type Band (kb):	**13.6**	**13.6**
Predicted Mutant Band (kb):	**7.9**	**5.2**
Probe Size:	**613 bp**	**455 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:	DNA135-5'	5' Primer Name:	Neo3a
3' Primer Name:	DNA135-3'	3' Primer Name:	DNA135-9
Predicted Wild-type Band (bp):	124	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	468

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
DNA135-16   5' – CACAAACATGCACCAACATCCTG
DNA135-17   5' – GTTCATGGTGATTCTTAGCTCAAG
DNA135-4   5' – CACAAGGGCTATGCACTCAC
DNA135-5   5' – GTCGATCTGTCTGCTGATGG

PCR Genotyping
DNA135-5'   5' – TAGCAGGTAAGGTGCAGATGTAGC
DNA135-3'   5' – ATAGAAGCAGGCATGGTTCCTAGC
DNA135-9   5' –GATAGTATAGGAGAGTTGGAG
Neo3a   5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

TGCCCAGCCATGGACTGCTACCTGCTGCTGCTGCTGCTGCTCCTGGGACTAGCAGGTAAGGTGCAGATGTAGCCACGCC
TCCTTCACCAGTATCACAGATGCCATAGCTACCCCCCTCTGGATCCCCAAGTCCCTATGTTGGGGAGTGGGCTAGGAAC
CATGCCTGCTTCTATCCAACCCTCACTCCCACATCTTTTCCTTCTGCAGGCCAAGGCTCAGCTGACAGTCATCCCGAGGT
GCTACAGGCACCGGTGGGGTCATCCATTCTAGTGCAGTGCCACTACCGGCTCCAGGATGTGAGGGCTCTCAAGGTGTGG
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TTCCTCACTGACCTGGGTGGGGGGCTCCTGCAGGTGGAAATGGTGACCCTGCAGGAGGAGGACACAGGGGAGTATGGT
TGTGTGGTGGAGGGAGCGGCAGGACCCCAGACCCTGCATAGGGTCTCCCTGTTGGTTCTTCCACCAGGTGAGTTACC

Genomic Locus: *(The deleted sequence represents nt11397-11946-in the sequence below. KOS36 used to generate the TV represents nt 7655-15866 in the sequence below.)*

CGCCTTGAAGCACTGGGGGAGACGCAAGGCCTGGTGTCGGCAGCTGGGTGAGGAGGGCCCATGCCAGCGTGTGGTGAG
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Selection Cassette:

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Targeted Locus:

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