



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ185	Date of Submission:	7-12-05
Lexicon Contract Name:	DNA136A	Mutation Type:	X Standard Knock out
LexVision Name:	MEM606N1		☐ Conditional
Reference accessions:	NM_029720	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS30_____
X Target Vector DNA __pKOS30.TV_____
X Targeted ES Cell DNA __1D9_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	10+11	20+21
Restriction Enzyme for Genomic Digest:	Nde I	Acc651
Predicted Wild-type Band (kb):	7.6 kb	19.5 kb
Predicted Mutant Band (kb):	8.4 kb	16.0 kb
Probe Size:	464 bp	385 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:	DNA136A-23	5' Primer Name:	DNA136A-upper
3' Primer Name:	DNA136A-22	3' Primer Name:	GT Ires
Predicted Wild-type Band (bp):	198 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	434 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**

DNA136A-10	5' – GTTCTCTAGAAGAACCAGCC
DNA136A-11	5' – CTTCTGGTACAGATGTGAGC
DNA136A-20	5' – GAGTTTGGCTTCTGAATCCG
DNA136A-21	5' – AAGGATGAGAGGACCTTTGC

PCR Genotyping

DNA136A-22	5' – ACTTCAGAAAGCCCATACTGCC
DNA136A-23	5' – TTCTTCAGCTTGCAGAGGAACG
DNA136A-upper	5' – TCTCATTGGTGGCTTCTCGTG
GT Ires	5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

CCGGGACGCCCTCCCGCCATGCACCTGCTGCTTGACAGCCGCGTTCGGGCTGCTGCTGCTGCTGCCGCCGCCGGGGCCGTAGCCTCCCGGAAGCCGACG
ATGTGCCAGAGATGCCGGACGCTGGTGGACAAGTTCAACCAGGTGGGCGGGCGGCCGGGTCCGGGGCAGCGCGCTGGGGCAGGGGGCTGGCGCGG
GGTTCACGGTACGCCATCGAGCTGGTGCCACCTCCACTACAGGGGATGGCCAACACGGCCAGGAAGAATTCCGTGGCGGCACACGGCGTGGGAAG
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ACACACACTGAAAGCGTGTCTTCCAGGCACCTACGGGCCAGCTGCAAGGTACACCCCCCCCCCCCCCCCCCTCCGGGACATCCGAGCCT
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CTTGGGTGATGGAGAGGCTGTGGGGAGCAGCC

Genomic Locus: *(The deleted sequence represents nt 11406-13909 in the sequence below. KOS30 used to generate the TV represents nt-9821-19485 in the sequence below.)*

CTGAGAATAGCCCAACACTCCACCAAGTCTCTGGGACTGGCTCAGCCTCACTCCCTCCCTTTCCCAAGACAGACTATGGTTGGCGGGAGGGAGGAGTA
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[illegible]

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Selection Cassette:

CGCTCTAGAGGCCATAGCGGCCGATCTCTGTGGCGCGCGGATCCGAATTCCTCGAGGCTAGAACTAGCGATAAGCTTCGAGCGGGATCAATTCCGC
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GCTGGACAGACAGTTCATCTGGGCATGAAGAAACATTAGAAAAAATCAGGACACAGGTTGAG