



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ5923	Date of Submission:	9-29-04
Lexicon Contract Name:	DNA225	Mutation Type:	☒ Standard Knock out
LexVision Name:	MEM719N1		☐ Conditional
Reference accessions:	AK045373	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) KOS25 and KOS51
☐ Target Vector DNA pKOS51.TV
☐ Targeted ES Cell DNA 3D6
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	11+12	7+8
Restriction Enzyme for Genomic Digest:	SacI	XbaI
Predicted Wild-type Band (kb):	4.8 kb	9.4 kb
Predicted Mutant Band (kb):	6.5 kb	13.2 kb
Probe Size:	256 bps	168 bps

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:	DNA225-5	5' Primer Name:	Neo3a
3' Primer Name:	DNA225-6	3' Primer Name:	DNA225-6
Predicted Wild-type Band (bp):	204bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	262bp

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**

DNA225-7 5' – ATTACTACTATGGTCGTGGCT
 DNA225-8 5' – CTAGATAAAAAGGGCCAATGG
 DNA225-11 5' – CCAATCAATAATGCATGGCTACAT
 DNA225-12 5' – GCGCTCTTCCTAGACTCATCT

PCR Genotyping

DNA225-5 5' – CAGACAATGCCAAGCCTGCG
 DNA225-6 5' – AACAGAAGCACTTCCACAAGA
 Neo3a 5' – GCAGCGCATCGCCTTCTATCG

Genomic Sequence Deleted:

CATCATCCTAGGGGGGCTGTGATCAGCCTTCGGGCAGGGGACCCCCTCAACCTCACCTGCCACGCAGACAATGCCAA
GCCTGCGGCTTCCATCATCTGGCTACGTAAAGGAGAGGTCATCAATGGAGCCACCTACTCCAAGGTGAGCCTGAGGGA
GAGAATGGG

Genomic Locus: (the deleted sequence represents 20,016 bps to 20,180 bps in the sequence below)

GCAAGGGTGATGGGGAAGGTCAGGGAGTCTCCAAGTTGGCAGGGAAGAAGCCAGTCTCTCTCTGCCAGCCCACCGAGCC
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[illegible]

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Selection Cassette:

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