



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2914	Date of Submission:	9/29/03
Lexicon Contract Name:	DNA053	Mutation Type:	X Standard Knock out
LexVision Name:	ENZ015N1		☐ Conditional
Reference accessions:	ENSMUST00000034282, NM_030209	Is this gene X-linked?	no

Required Materials: X pKOS clone DNA(s) __pKOS 28_____
X Target Vector DNA __pKOS 28 Bgeo_____
X Targeted ES Cell DNA __1A1_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	53/54	55/56
Restriction Enzyme for Genomic Digest:	EcoRI	EcoRI
Predicted Wild-type Band (kb):	13.8	13.8
Predicted Mutant Band (kb):	5.6	9.2
Probe Size (bp):	194	173

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:	9	5' Primer Name:	Puro3a
3' Primer Name:	7	3' Primer Name:	50
Predicted Wild-type Band (bp):	137	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	582

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**

DNA053-53 5' – CATGTGACCATGAATGTTAG
 DNA053-54 5' – GCTCTCGCAAACCCCGCTGC
 DNA053-55 5' – TGGTCGGATCATGTCTCCAG
 DNA053-56 5' – CTTAGTCATGAAGGCCACTT

PCR Genotyping

DNA053-9 5' – TCTTCCAGCATATGCCGAGCTGC
 DNA053-7 5' – ATTTGCTCAGGGACTCCATGC
 Puro3a 5' – ATGACCCGCAAGCCCGGTGCC
 DNA053-50 5' – CAGTGACTCCGCCCATCAAG

Genomic Sequence Deleted:

AGGCTCTTTGGCTCTCTGTTTTATGAAAGTGTAAGTATAGCCAGTGATTCTTTGCATCAGAGACAGGAAACCCACCAGG
TAGCCTAAAGTCAGTGGGACGTTTAGACATAGGTCTCTCCTTAGCTCAATCTGGAACGCCCGAACGGACCGCTCTGAAT
GGACTCAGGACTTCAAAGACACTAAAACCACTTCTGGGAACTGGTACCGAAAGTCTCTGCCGTTCAGGGGTGAGCATG
CTCAGCTGGAAGGGCTTAAGCAGCCAGGATGAGTTCTTAAGCCATCCAGGAAAAGGATGTAGCTAAGGATAGCGGGGA
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GGAGTCCTAAAGGTCACC

Genomic Locus: *(the deleted sequence represents nt-3568-5320 in the sequence below)*

TGTGTCCAGGCTAGAACGAGGGCTCTCGCAAACCCCGCTGCCTTTAAATTTACCTCTCACATCTAGCCTTTATAGACAT
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Selection Cassette:

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G