



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2964	Date of Submission:	October 27, 2003
Lexicon Contract Name:	DNA055	Mutation Type:	X Standard Knock out
LexVision Name:	ENZ221N1		☐ Conditional
Reference accessions:	NM_139305	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __pKOS18 and pKOS85_____
 ☐ Target Vector DNA __pKOS18TV_____
 ☐ Targeted ES Cell DNA __2G6_____
 ☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	15+16	13+14
Restriction Enzyme for Genomic Digest:	Bgl II	BamHI
Predicted Wild-type Band (kb):	6.5 kb	15.4 kb
Predicted Mutant Band (kb):	12.2 kb	12.3 kb
Probe Size:	216 bps	169 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:	23	5' Primer Name:	Neo3A
3' Primer Name:	22	3' Primer Name:	22
Predicted Wild-type Band (bp):	573 bps	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	366 bps

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**

15 5' – GAGAACCTATACAGTGCCAG
 16 5' – GGCTGACTCACAGTGCCC
 13 5' – CAAGAACACCTAGCAGTGTG
 14 5' – CACACCTCTGCTCCAGCAG

PCR Genotyping

23 5' –GTGATGAGTGAAATGATGGACC
 22 5' –CTTACTGTGTAACCCTGACC
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

TCCCTTGGGTTAGAGGATCTATCGACTCCCAAGGCTCCTGAGCACAGTCAAGGTTCCACGGGGATGAAAAAGGTAAG
TGGTCATTAAC TTCCAAATCTAGGCTCAGGTAAGTTATGCCTTGCTCTCCCCACTCCCCTCTCTTCCCCGAAATGCTA
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Genomic Locus: (the deleted sequence represents nt 8599-11618 in the sequence below)

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[illegible]

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Selection Cassette:

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