



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ648	Date of Submission:	01/15/05
Lexicon Contract Name:	DNA425	Mutation Type:	☒ Standard Knock out
LexVision Name:	ENZ716N1		☐ Conditional
Reference accessions:	ENSMUST00000025076, NM_026092	Is this gene X-linked?	no

Required Materials: ☒ pKOS clone DNA(s) __pKOS-27_____
☒ Target Vector DNA __pKOS-27TVneo_____
☒ Targeted ES Cell DNA __1F8_____
☒ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>	<u>3' Internal</u>
Name of Probe:	19+22	7+8	7+8
Restriction Enzyme for Genomic Digest:	NcoI	HindIII	NcoI
Predicted Wild-type Band (kb):	15.2 kb	4.0 kb	15.2 kb
Predicted Mutant Band (kb):	11.0 kb	8.3 kb	4.5 kb
Probe Size (bp):	316 bp	424 bp	424 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:	DNA425-27	5' Primer Name:	Neo3a
3' Primer Name:	DNA425-9	3' Primer Name:	DNA425-9
Predicted Wild-type Band (bp):	299 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	430 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**

DNA425-7	5' – TTTTGATAGAGCTAGGGATTGAC
DNA425-8	5' – CCAGTTTTTCAGCAGGAATAGAA
DNA425-19	5' – CAGCTATGAGTTATGCCTTG
DNA425-22	5' – ACATATTTTGA CTGTGATGG

PCR Genotyping

DNA425-9	5' - TTCTATTCCTGCTGAAA ACTGG
DNA425-27	5' - GAATCACTGCCATGTTGCCTG
Neo3a	5' - GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GGATGAAGTCTGTTGGTGTCTTCGCCCTAATTATCAGCTTCAGCATAGTCGCGAGAATCCAAAATCTACACTCGATGTAAACTGGCAAAAATCTTTGCAA
AGGCCGGCCTGGACAATTACGGGGGATTTGCCCTTGGAACTGTGAGATTCTCCCAAGCCTACAGCCTCACTCCACTTTTGAGTGTGGGTGTCTGCTA
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TGCACCTTTCTTACCCTTCTTCTTCCCATAGCCCTTGTTCGCCATTGCTATGGTCTGTACTCATGACCACAGGGAGGGGAAAAGAACAGCGATGTCACC
CATCCAGTGTGTGGAGTAGTCTAAGCTTCTCTTCCACATGAGATTGAGCTATTGGCTACTGTGCAGGTTCTGGTGTGGCACCTGATCCGTGACACAT
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ATTCTTTCCTCTCATTTAGGGCTCTGTCATGGCATAGCTGAGAGCCACTACAACTACAGCCGAGAATGTCCTGGAGGTGGAAGCATGACTATGG
CATTTTCCAGATAAACAGTTTCACATGGTGCAGAAATGCAAGAAAGCATCAGAAGAATCACTGCCATGTTGCCGTCTCAGGTACTGTTT

Genomic Locus: (The deleted sequence represents nt12229 – 13013 in the sequence below. KOS-27 used to generate the TV represents nt 4609 – 15676 in the sequence below.)

TTTCCCCAGTATGAAGGTTTCATACTTTGCTCATGAAGTATCTCACATTACCACAACATGCCTGTGCAGAACTAAGAACTCAGTGTGGGTGCAGAGGCCT
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[illegible]

Selection Cassette:

Targeted Locus:

TTTCCCCAGTATGAAGGTTTCATACATTGCTCATGAAGTATCTCACATTACCACAACATGCGCTGTCAGAACTAAGAACTCAGTGTGGGTGCAGAGGCCT
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[illegible]