Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ224	**Date of Submission:**	
Lexicon Contract Name:	DNA032	**Mutation Type:**	X Standard Knock out
LexVision Name:	MEM650N1		☐ Conditional
Reference accessions:	NM_008411	**Is this gene X-linked?**	

Required Materials:
X pKOS clone DNA(s) __pKOS78_____
X Target Vector DNA __MEM650-78-TV2puro_____
X Targeted ES Cell DNA __3F10_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' Internal	**3' External**
Name of Probe:	**19/20**	**21/22**
Restriction Enzyme for Genomic Digest:	**Pst I BamHI**	**Hind III**
Predicted Wild-type Band (kb):	**5.3 kb 5.6 kb**	**5.8 kb**
Predicted Mutant Band (kb):	**8.0 kb 1.8 kb**	**11.2 kb**
Probe Size:	**331 bp**	**536 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:	MEM650-3	5' Primer Name:	MEM650-25
3' Primer Name:	MEM650-6	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	657 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	497 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
MEM650-19 5' – CACGTGAGATTAGAACAAGAC
MEM650-20 5' – GGCAAGTGTGTTGAGTGTG
MEM650-21 5' – GCATCTCTAAGTTGTGTTTCTG
MEM650-22 5' – CAGAAAGGTGATATTCTGCAC

PCR Genotyping
MEM650-3 5' – CAGCTGAATTCTACAGAGGC
MEM650-6 5' – CCTTCTGCAAATTCATCTGTC
MEM650-25 5' – CTGAGCCCTGTGCAGAATCGG
GT-IRES 5' – CCCTAGGAATGCTCGTCAAGA

Genomic Sequence Deleted:

TGGAGGTCACTGGAAGGCTGTTCATCTGGGCTATTTTAGCTGTCTCCTGTGGGGCACAGCTGAATTCTACAGAGGCTGAAGGTAAGAAAATTGAACCA
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Genomic Locus: *(the deleted sequence represents nt 11982-16108 in the sequence below. KOS78 used to generate the TV represents nt 10479 - 19098 in the sequence below.)*

ACTGAACAGTGAAACAACATGAAGCTGTTGACTATTAAGATTCAAGCACACATTCAGTGACACCGGTAGAGGCTCACCCTTCATGGTTGAACAGCTAC
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Selection Cassette: IRES/bGEO/Puro

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Targeted Locus: (selection cassette represented by nt 11994-17267)

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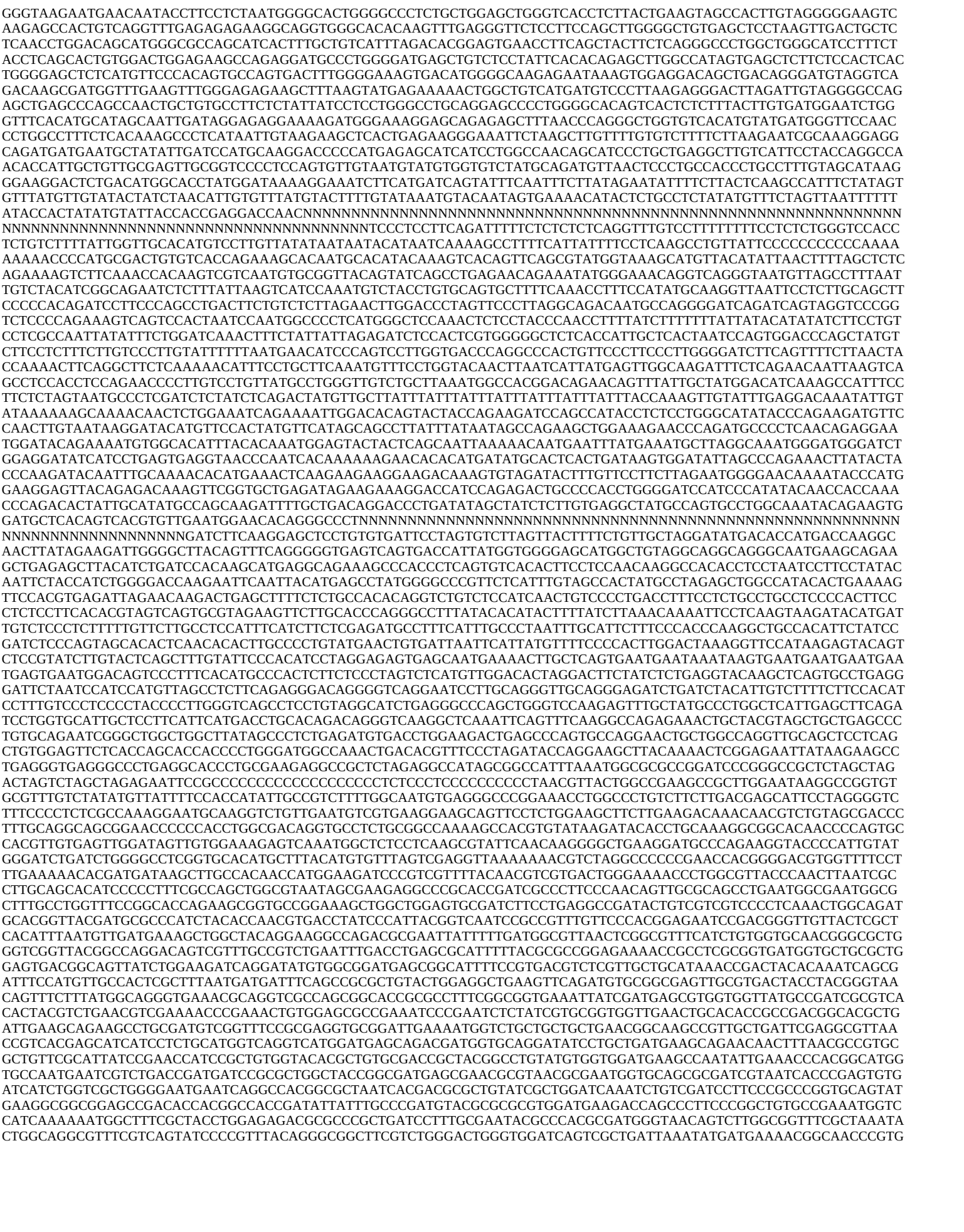

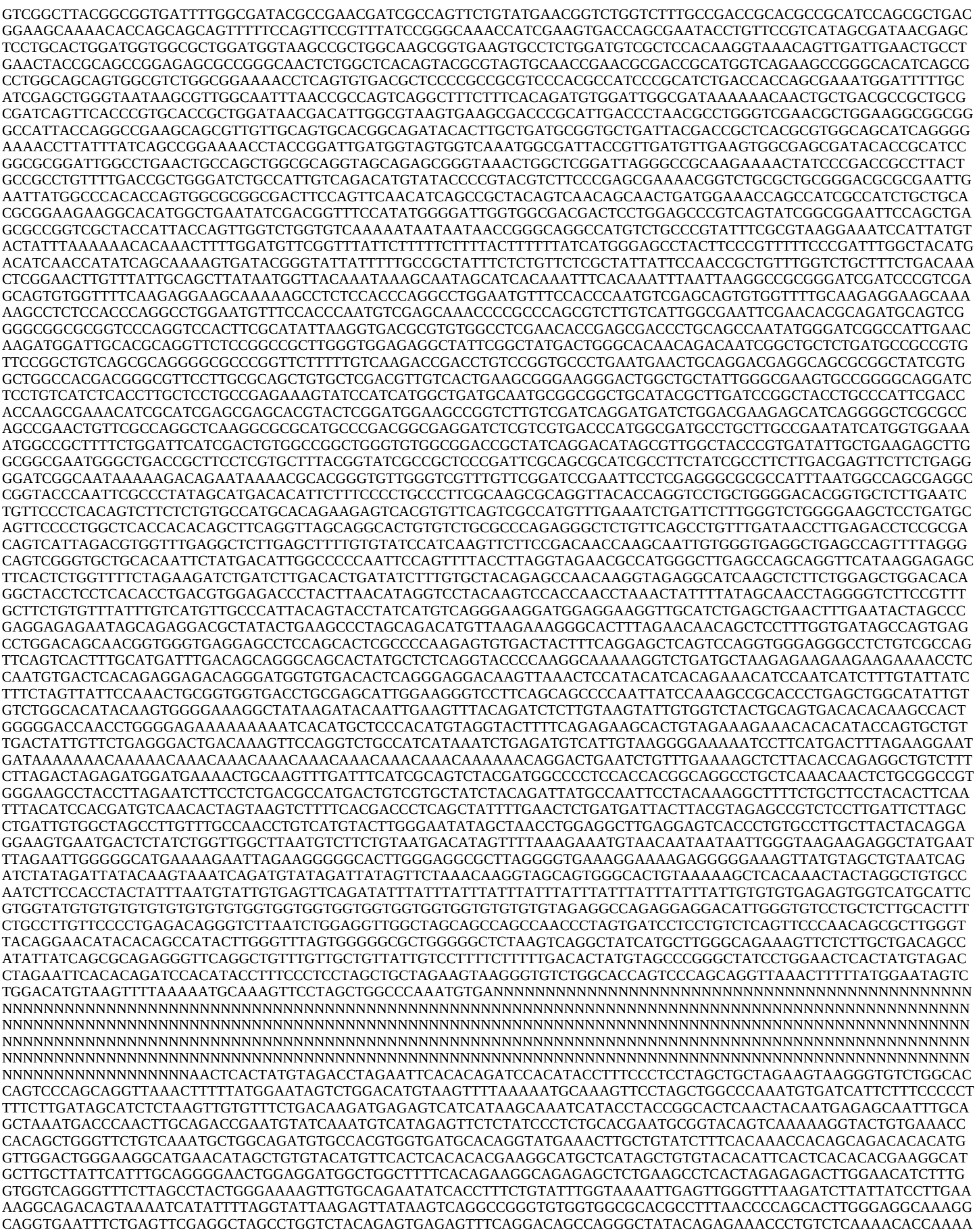

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