



## Lexicon Genetics Incorporated – Genentech Project Materials

<b>Genentech ID:</b>	UNQ828	<b>Date of Submission:</b>	11-10-04
<b>Lexicon Contract Name:</b>	DNA259	<b>Mutation Type:</b>	X Standard Knock out
<b>LexVision Name:</b>	SEC496N1		<input type="checkbox"/> Conditional
<b>Reference accessions:</b>	NM_175696	<b>Is this gene X-linked?</b>	No

**Required Materials:**

- pKOS clone DNA(s) \_\_pKOS29, pKOS69\_\_\_\_\_
- Target Vector DNA \_\_pKOS29TV\_\_\_\_\_
- Targeted ES Cell DNA \_\_1F2\_\_\_\_\_
- Genomic Map

**Southern Blot Analysis:**  
*External/Internal Probe Strategies*

	<b><u>5' External</u></b>	<b><u>3' Internal</u></b>
Name of Probe:	<b>12+13</b>	<b>3+14</b>
Restriction Enzyme for Genomic Digest:	<b>EcoRI</b>	<b>BamHI</b>
Predicted Wild-type Band (kb):	<b>7.0 kb</b>	<b>13.3 kb</b>
Predicted Mutant Band (kb):	<b>3.3 kb</b>	<b>6.7 kb</b>
Probe Size:	<b>302 bps</b>	<b>238 bps</b>

**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:	15	5' Primer Name:	Neo3A
3' Primer Name:	11	3' Primer Name:	11
Predicted Wild-type Band (bp):	415 bps	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	286 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**

12 5' – CTAAGTGAGCCCGTGTAGAC  
 13 5' – CATTCTCCCTGAAGCAACCC  
 3 5' – GTCGCAATTCTAGGTTTGGC  
 14 5' – CATGGAGGCAGGTACATTCCG

**PCR Genotyping**

15 5' – GACCTCTTTCCTTATGATGCACA  
 11 5' – CTTGGAGAGGATCTGACCAGC  
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

**Genomic Sequence Deleted:**

GTGCAGAATGTGGTAAGTTTGGCCCAGCAGAGGGAGCTTGCTTGGAGACCACTTTCCTGGGGGATTGGGCCCTTTCCT  
GCCTAGGCCAACTTGGGGCCTATTAAGGACTGGCCCCTTGGGAAGAGACAAAATGGGGCCAGGGTCTTCTAACACAC  
CTGCTCAAGCAGAGAAACCTGGAGGGAGGTACCCAAGACTCCTGGTGCAAACCTACCCTTTTCTACATGAGCCATCGC  
CTGGGTCTCACCTGCTTTTTCTCCCTTCCATAGGCTGCTCAACTGCTGTCCCAACTCCTCCCTCTCTGGCCACTGCTGT  
TGCTGTCCGTATTACCACCTGCTCAGGGCTCCTCCCATCGATCCCCACCAGCCCCAGCCCGCCCCCTGCGTCCGGGGT  
GGGCCCTCAGCCCCTCGCCATGTGTGTGTTTGGGAGCGGGCGCCTCCGCCAAGCCGATCCCCACGGGTCCCAAGATCAC  
GTCGGCAGGTTGTGCCAGGCACTGCCCCTCCTGCTACCCCATCAGGCTTTGAAGAGGGGGCCTCCCTCATCTCAGTACCC  
TTGGGCTATAGTGTGGGGTCCACAGTATCTCGGGAGGATGGAGGGGACCCCAACTCGGTCAATCCTGGATTTCTGCT  
CTGGACTATGGTTTTGCAGCCCCACATGGGCTGGCTACTCCGCACCCTAACTCAGACTCCATGCGGGATGACGGAGACG  
GGCTCATCCTTGGGGAAACACCTGCTACCTTGAGGCCCTTCTGTTTGGAGGGCGTGGAGAAGGTGAGTGAGCAAGGC  
TGGTAGGGATGTGAGGGATTGGGGGAGGGGGCGGGACTTGAAGACTATGATCTGCGGAAATTGAGGCTTTATCTGTGA  
GTGTCTCCAGCAAATGTGCCAGGTTCTAGGCTGGCAAAGGATGCAGAGGTGAAGTGTGCGTGCCTGCACTGTAAGGGGT  
TCAGGTCCACTGTGAAAACAGATGTGAGACACTTGGGGATGAGTCAACATAATAAACACCCTAACAGAAGCGCGAGC  
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GAGCTAGATGTTAAAAAACAGAATGGGGGCTGGTGAGATGGCTCAGTGGTTAAGAGCACTGACAGCTTCTCCTGAGTT  
CAAATCCCAGCAACCACATGGTGGCTCACACCCATCTGTAACGAGATCTGACGCCCTTCTGGAGTATCTGAAGACAG  
CTACAGTGTACTTACATATAATAAAAAATAAATAAATCTTTAAAAAAAACAAAAACAAAAACCAGAATGGGTATT  
CCAGGCAGGGGGAAGCCATGGAGGAAGGTCACTCACTACCAGGGAATGTGTTAGCACATATTGTGCTGGACTAGTG  
GAGGGAAGGCCATCTGATACCTTACCCATTTCTATACAGGAAGATAGTTCTGTCTGACCTTGGAGCCTCATCCTCAC  
CACATGTATGTGTAAGATTTGGTACATGTAATGTTTGGGAAAGTAGAGGAGCTGATAGCTCAGGAGAAAGTTGATTAT  
AGGCGAGGAGGGAGAGTCCAGAGGAGACAGGCAGTGTGGAGTCCAACGGCAACACTCGGCCACACCCACTTTGAACC  
TGCCAGGCCTCCCAACAGCCTTCTCCCTCTCCATCAGGTGTGGACCCTCAGCTTTATGTACGATCACCATATCCATC  
ATCATCGTTCCTGTGGCTACCGGCATCATCTTCAAGTTCTGGTAAGCCGGGGAGACGCCTGATGGCGTGGAAAGGCTAGG  
CACAGGGCAAGACCAAGGACAAGTTTCTCAGGAGTAGGAAACGATGAAGGGGAGAGCTTGGGGAAAGCTAACTGGAG  
TGGAGGAAGACTCGGGGAAATCAGTTCACGTTCTTCCAGAAAAAAATAACAAGGGCAGTGGGAGGGAAGGGAGGACT  
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CCTGTGGCCCTCCAAAAATGATCTAGAGGAAAGGAGAAGGAATAGTCTGACGGTCTCAGAAGCCAGTTTCTTGTGCTGT  
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GAAGACCTCTTTCCTTATGATGCACAAGCTGGGACCGTAGCCAGAAGCGGCGCAGGCCCTCAGGGCAGCAAGGTGCC  
TGAGGCAGGAGGAGAGTCAGCAACCACTGACAGACCTGTCTCCTGCTGGAGTCACTGTGCTGGGGGCTTTTGGGGACT  
CGCTACCCCCACCCCTGACCATGAGGAACCCCGAGGGGGACCCCGCCTGGGATGCCCCAGCCTAAGGGGGCTCCAG  
CCTTCCAGTTGAACCGGTGAGGGCGATGGGATGGGAGGGGCTGATGGGATGGGAGGGTGAGGAG

**Genomic Locus: (The deleted sequence represents nt5487-8217-in the sequence below. KOS29 used to generate the TV represents nt 2836-12899 in the sequence below.)**

GTGCTCCACCACTGACTCACGCTTCGAGCTTATCACGTGATGGCTCACTGTCTCTCTACTAAATTGTAAGTCACGAGGA  
TTGCGGTGACTTTGTTTTGTTCTTCACTGTATGCCCTGGTCCAGCGCCCTACCCAGCTTGTAGTTAATGTTCAAAAAAT  
ATTTGCTGAGGTCTGGGTGTGGTGTGCTGCATTGCCGTAATTCCCAACACTTAAGGAAATAAAGACAGGAGGTGAGGGGT  
CAAGGTTACCCCTCAGCTACATAGTGAATTCAAGGCCAGGCCAGACCATATGAGACTCTGTCTCACATAAAATAAACATTT  
GTTGAAAGGAATAAAGAACAGTAAAAATAATTGATCATGATGCAAAGCTTCAAGGCACTGTTTTTTCATTTGAT  
CAACGCGGTCTCTCGAGGTTACAATGATCCTCCGAGGTTACAATGAGTCCACTAGAGCTTATCCAGGCTCCCTGGGA  
GCCTAGGCAGTGGTGGGAGTTGTCCAAGCTTCCCTGCAATGCCCGACAGCTGTGCTTTCCTATGAAGTGCAGTGTCTTT  
TCCTGCCACTGGGAGACACTGGGAGGGAGGGCATCTCACTGACTTGGAAATGAAAGGCTGTGAGGGACAGGCAAGAAG  
CAGAGTAGCAGCTGCCTGGCCGCTGGTGGATGCTGGTCTCAACAGCAGGCTTCTTCCCTGGGACGGAGAGCCGGAGTC  
ACGAGGGTGCTAAGGGGCAGAAGGAGGAGGATGGATGGCCAGGAGGGGTGCGTCTAGGGGCAGAGCGAAGAGATGA  
GGGAATGCAAGCAGACGGAGAGGGAAAGCAGGGGATGCAAGGGAGAGAATGAACAGCGTCCAGTGTGGGCGGGGGG  
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CAGAGGGCGATGGCGGAGAAAAGATAAGGAAACACGGATGAAATGAGGACCCCTTTAGATTTTGGTCCAGAGAGCCG  
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GGGACGACAGGGAGTCTGCTTTGGCAGAAGCACTGTTATGAAGGGGGCACACACTGGTGGCTTTTCCATTGACTTATT  
TAGGGTGTGCAGTGCATGTGCTCGCATATGGCGGTGAGGAGCAACGTTGTAGGAAGGGGTTGGTTCTCTCCCCC  
CCTGCGGGTCTTAAGTATCCAAGGTAGCTTGTACGTTTGTGACGTTTGTGACGTTATCTCACCGGCCCTTGAATATTT

TGAAAGGGGAGAGCATGCTATGGTTTAGCTTTCAAAATATTACCGGTAATTGTAAAGAGTATTTTGTAAAAGATGAGG  
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GAACCGGAAATCAAAACACCTTTAGCCTGAACAACAAGGAGTTGTCATGTCCTAGGATGAAGAGCTCGGGACACAAGA  
GGGTGGGGGTGGGATGGCTCAAGCCATCTGCTTGAGTCACATTAGGTTTGCCTGGCTATTAGACATCGGACGGAGTGG  
ATATGTCAAGTGGGCGGTTGGACAAATGAGTCTGGCACTCATTTGTAAATTTAGGACTCACTGACATTTACAGAGTGT  
TAAGCCCTGGGACTGGATGAGATCACTGGACAAAGAATGTGGAAAGAAAAGAAGGCCAAGGACTAAGCCCTAGGAGC  
GCCAACATGTCGAGGTCAGAACCAGGAAGACAGTAAAGAACAGCCAGAGGGTTGGAGGGGAGCCAGGAAAGTGTGG  
TTTCTAGAAGTTTGGGGCGCAGGGTTGGGGGGGTCATGCAGGGAGAAGGGTTAGGTATGAATGGGTAGGGTCAAAG  
GAATTTTAAAAAAGATTTATTTATTTATTTATATGTAAGTACACTGTAGCTGTCTTTAGACACTCCAGAAGAGGGAGTCA  
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TGTTTGGGAGCGGGCGCTCCGCCAAGCCGATCCCCACGGGTCCAAGATCACGTCCGCAGGTTGTGCCAGGCACTGC  
CCCTCTGCTACCCCATCAGGCTTTGAAGAGGGGCCCTCCCTCATCTCAGTACCCTTGGGCTATAGTGTGGGGTCCACA





GTCTCTCCGGAAGCCCGGGAACGCTCTCCTGATGCTCAGCTTCAGCCCTTTAACACACGCTGCCTTCACGTCCGGTTTG  
GGGTCACATCCACCGAGCGCTCCACCGGTCCCCGGGTAAGGAGCTACTTCCGCCCTTACGTGTCGTAACCTC

**Selection Cassette:**

CGCTCTAGAGGCCATAGCGGCCATTTAAATGGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGA  
ATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGT  
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CTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGT  
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CCGCCCAGCGTCTTGTCAATTGGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCCGATA  
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CACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATG  
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GCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCTTGGCGCAGCTGTGCTCGACGTTGTCACTGA  
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AGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTTCGACCACCAAGCGAAA  
CATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGG  
CTCGCGCCAGCCGAAGTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGAT  
GCCTGCTTGGCCAATATCATGGTGGAAAATGGCCGCTTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACC  
GCTATCAGGACATAGCGTTGGTACCCCGTATATTGCTGAAGAGCTTGGCGGCAATGGGCTGACCCGTTCTCCTGCT  
TTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTACGAGTCTTCTGAGGGGATCGGCAAT  
AAAAAGACAGAATAAAACGCACGGGTGTTGGGTGCTTTGTTTCGGATCCGAATTCCTCGAGGGCGCGCCATTTAAATGG  
CCAGCGAGGCCGGTACCCAATTTCGCCCTATAG

**Targeted Locus:**

GATCATAGTGAAATGAGGAATTAATCTGCTGGGGGCACTCCAATTTGCGTATGCAGTCTTCTCTCCCACCCCTCTTTGGT  
CCTCTCACCCACGGTCTTGAGCTGCCTTTCCACCTTTGCCAGAGTTGGGGACTCAAATGTATGGATGACTCAGAATCTCA  
GACTGCTTGGGGGTGAGAAATCATGAGGTCTGTGAAGCTGCGCTTAGGTAGAGGGGAAGCTTACCATCACTACCCAAA  
GGAGGTTGCGTCCCCCAACCCCTCCATCCCTGCTCCTTTCCTAACCTTGACCTGGACAAAAGCTGAGCTCGCAGC  
GTAGCAAACGAAGGGTTAAGTCGTGCGTGGCCTACAGACGTGAGAAGGAGAGGGCGGAGACCAAGTGGTCTAGTCGC  
CCGCCGAACCCAGCCGAGCCCGGCCGAGCCAGCCAGCCGAGCCGAAGCGAGCGTCTCCCGCCGCCGCCGCCGCCG  
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CGCCCAACCGTTTCTGCGACCCCGAGCCCGCGTAGCTCCGAGCGCCTGCAGTCGTGCGCGCCGCCACACCAGGCATG  
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