



## Lexicon Genetics Incorporated – Genentech Project Materials

|                               |           |                               |                                      |
|-------------------------------|-----------|-------------------------------|--------------------------------------|
| <b>Genentech ID:</b>          | UNQ536    | <b>Date of Submission:</b>    | 11-11-04                             |
| <b>Lexicon Contract Name:</b> | DNA324    | <b>Mutation Type:</b>         | X Standard Knock out                 |
| <b>LexVision Name:</b>        | MEM764N1  |                               | <input type="checkbox"/> Conditional |
| <b>Reference accessions:</b>  | NM_029537 | <b>Is this gene X-linked?</b> | No                                   |

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**Required Materials:** X pKOS clone DNA(s)   \_\_pKOS13\_\_\_\_\_  
X Target Vector DNA   \_\_pKOS13 TV\_\_\_\_\_  
X Targeted ES Cell DNA   \_\_1H6\_\_\_\_\_  
X Genomic Map

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

|                                        | <u><b>5' External</b></u> | <u><b>3' External</b></u> |
|----------------------------------------|---------------------------|---------------------------|
| Name of Probe:                         | <b>11+15</b>              | <b>13+14</b>              |
| Restriction Enzyme for Genomic Digest: | <b>EcoRV</b>              | <b>Bgl I</b>              |
| Predicted Wild-type Band (kb):         | <b>16.4 kb</b>            | <b>7.3 kb</b>             |
| Predicted Mutant Band (kb):            | <b>14.5 kb</b>            | <b>3.3 kb</b>             |
| Probe Size:                            | <b>401 bp</b>             | <b>288 bp</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:                                | DNA324-3 | 5' Primer Name:                          | Neo3A    |
| 3' Primer Name:                                | DNA324-4 | 3' Primer Name:                          | DNA324-4 |
| Predicted Wild-type Band (bp):                 | 279 bp   | Predicted Wild-type Band (bp):           | none     |
| Predicted mutant band (bp)                     | none     | Predicted mutant band (bp)               | 230 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**

DNA324-11    5' – AAAGCCAGTGTAGGACTACC  
 DNA324-15    5' – CTTGTCCACTGTTGTTTCC  
 DNA324-13    5' – TGCAATGGTCCCTTGTTAGC  
 DNA324-14    5' – CTAAGTACAGGCTGTGTTCC

**PCR Genotyping**

DNA324-3    5' – GTGAGAAGAGGTTTCAGTCG  
 DNA324-4    5' – GTCCAGTGGAGTGTTCTGT  
 Neo3A        5' – GCAGCGCATCGCCTTCTATC

### **Genomic Sequence Deleted:**

CCCCCTGGAAGCATGGAGACTGTGGTGATCGTCGCCATAGGTGTGCTGGCCACCATTTTCCTGGCCTCATTTGCTGCTTT  
GGTGGTGGTCTGTAGGCAGCGTTACTGCCGGCCTCGAGACCTACTACAGCGTTATGATTCCAAGTGAGTAGACTGTGAT  
GGGGTGGGGGTGGGGCAGGGAGCTGGGGGGCTGACAACAGGAAGCCCGAGGGACAGAAGGAATTTGGAGCTGGCCGC  
TTCTGCCCAACTTATTCTCTCTTAAGCGGTTCTGTCAGGCCACACTTCATACTATAAAGACTCCAGAGCGTAAGAACATT  
CCTTAAGCATCCCAGCATCCCAGAGGCACCTCTTTCTCTGTTTCATTAGGTCATGGAGAAGTCACTGAACGTGTGCACCTG  
ACACGAAAGCCCTGACTTTGCGATGCTAGCTGCCTCATTTGGCAGAGGGATTAGCCAAAGCCCTTACCAGCCAAAACC  
CTGGGAACGGACATCTGACACTTAGCCACACACCTAAGCAGCAGGTGTTCCAGATCATGCAGCCCCAGCACAGGGGTG  
CCTGTCTCCTTGACAGATTTCTTCTTCATCATGCGTTGTTGTGTGAAATTGATTTTGTAAAGTAAGACCAGTGTAGGGA  
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GCTGTGAAGGTGGACATAGTGTCTCAGAGGGGTGATGCTGTACACACGTTATAGAGCATAGGTAGGCCTGGGTGAA  
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GTCTGTGTATAGCCTTGCTGTCTAGAACTTGTTCTCTAGACCAGGCTGGCCTCGAACTCACATTGAGATCTTCCCG  
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TCTGCCCTCTCCAGCAGTTCAGCTTGTTTTGCTTCATTTGCGTGTGGTCTGCCTTCCTGACCCAGTGTTTAGCTGTGTG  
CCCTTGCTTGTGTGTGTGGGCACATTACAGTTAGATCTGTACCCAGGGCAAGCATTCTGTCTCTACCTCTGTCACTATA  
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AAGATCTAAATTATAGCAGTGGCTGCGGTGAGAAGAGGTTCACTCGGAGTCACTCATCGGAACTCTTAACGGTGGCT  
CTCCCTTCCAGGCCCATCGTGGACCTCATTGGTGCTATGGAGACGCAGTCTGAACCTCCGAGCTGGAGCTGGATGATG  
TCGTCATCACCAACCCCCACATCGAGGCCATCCTGGAGAATGAGGACTGGATCGAAGATGCCTCGTAAGGCCACAGGA  
AGCTTC

**Genomic Locus:** *(The deleted sequence represents nt 10001-11824 in the sequence below. KOS13 used to generate the TV represents nt 3754-13159 in the sequence below.)*

GGATGTGGCACTAAACTAGAAATGAGCGAGGCAGGCTGGGCAATCTGTCTGTAGAGCGCTCCCAGCAGTCATTTTAAAA  
GGTTTAAGAATCTCTGCCAAGAGGATATTTCTCATTCTCCCAGTTGTTGTGGTATGACCGACCTGATGATTGTCAAGGG  
ACACCCCTGAAGAGGGTCTCTATGTCTTTACTTCAGCTAAGTGAAGTGAAGGAAATGCAAACCTTGACCCCTAGCCCA  
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### **Selection Cassette:**

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### **Targeted Locus:**

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