



## Lexicon Genetics Incorporated – Genentech Project Materials

|                               |           |                               |                                      |
|-------------------------------|-----------|-------------------------------|--------------------------------------|
| <b>Genentech ID:</b>          | UNQ188    | <b>Date of Submission:</b>    | 1/14/05                              |
| <b>Lexicon Contract Name:</b> | DNA137    | <b>Mutation Type:</b>         | X Standard Knock out                 |
| <b>LexVision Name:</b>        | MEM458N1  |                               | <input type="checkbox"/> Conditional |
| <b>Reference accessions:</b>  | NM 133930 | <b>Is this gene X-linked?</b> | No                                   |

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**Required Materials:** X pKOS clone DNA(s) \_\_pKOS79\_\_\_\_\_  
 X Target Vector DNA \_\_pKOS79 FTV\_\_\_\_\_  
 X Targeted ES Cell DNA \_\_2E8\_\_\_\_\_  
 X Genomic Map

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

|                                        | <b><u>5' External</u></b> | <b><u>3' Internal</u></b> |
|----------------------------------------|---------------------------|---------------------------|
| Name of Probe:                         | <b>11 + 12</b>            | <b>Neo 2/5</b>            |
| Restriction Enzyme for Genomic Digest: | <b>EcoRI</b>              | <b>HindIII</b>            |
| Predicted Wild-type Band (kb):         | <b>9.3 Kb</b>             | -----                     |
| Predicted Mutant Band (kb):            | <b>4.9 Kb</b>             | <b>12.6 Kb</b>            |
| Probe Size:                            | <b>347 bp</b>             | <b>617 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:                                | DNA458-1 | 5' Primer Name:                          | Neo3a     |
| 3' Primer Name:                                | DNA458-3 | 3' Primer Name:                          | DNA458-16 |
| Predicted Wild-type Band (bp):                 | 333      | Predicted Wild-type Band (bp):           | None      |
| Predicted mutant band (bp)                     | none     | Predicted mutant band (bp)               | 223       |

| 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**

DNA458-11 5' – CCAGTATGAGCTGTTGACTG

DNA458-12 5' – CCATTCACTCTATGGGGAAC

Neo 2 5' – CCTCAGAAGAACTCGTCAAG

Neo 5 5' – GGCAGCGCGGCTATCGTG

**PCR Genotyping**

DNA458-1 5' – CGTAGATTAGGCTCTTTCACC

DNA458-3 5' – GCAGGTATGACACGGATG

DNA458-16 5' – CTTCCGCAGAGAATCTATCCAAC

Neo3a 5' – GCAGCGCATCGCCTTCTATC

**Genomic Sequence Deleted:**

ATGGCTCCACTGCCCCAAGGGGCTGGTCCCATCTCTGCTCTGGTGCTTGAGCCTGTTTCTGAGCCTCCCAGGACCTGTCTGGCTCCAACCCCTCTCCTC  
CTCCCCATCTTCTTCCCCGAGCTGAGCCCATCCGTGTATACCTGCCGGGCACCTGGTGAGCAACTTCAACAAGGTGGGTGCACTAATAGCCACGAGGG  
AGGGGGCCATTATTGGAGAGCAGGGAGGCTCTCTGCAAACTGCACTAGTGCTAGTCCCAGTGTGGGTGTGCTGGCTTTAACTGTATGGCAATTGGGTA  
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TCAGAACAAACAACAAAAACAAAAACAACTAATAAAAAATCCAAATGAATGTGTCTAGCTCTGTACTTAGATATTCCTAATTTCTTCCATATGAGG  
GCCTGGAGAGAACCATCCGGGACAACCTCGGGGTGGAAACACGGCCTGGGAGGAAGAGAAGTTGTCCAATACAAAGACAGGTAAAGAAGGTGGGG  
GCAG

**Genomic Locus:** *(the deleted sequence represents nt 9974 – 10666 in the sequence below).KOS79 (used to generate the target vector) represents nt 8198 – 15479 in the sequence below.*

[illegible]

[illegible]

CGTCTGCTGCTGCTGCATCATCTTCCTCCCAACAAGCTCCTGGCAACTCTAAATAGTCTCTCATCAAGCAGAAACTTGTGTGGCTTATCTGATTGCTTTGAT  
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**Selection Cassette:**

GGCCGCTCTAGAGGCCATAGCGGCCATTTAAATGGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCGCGCCCTCTCCCTC  
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**Targeted Locus:**

CACACACACATGGGCATGTGTGCACGCACACATGCACACAATTTTTTTTTAGCCTGCCTAGTTTATGTACAGAGCAGCCATGAGCACCTCTGATGTGT  
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[illegible]

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