



## Lexicon Genetics Incorporated – Genentech Project Materials

|                               |           |                               |                                      |
|-------------------------------|-----------|-------------------------------|--------------------------------------|
| <b>Genentech ID:</b>          | UNQ832    | <b>Date of Submission:</b>    | 4-6-05                               |
| <b>Lexicon Contract Name:</b> | DNA431A   | <b>Mutation Type:</b>         | X Standard Knock out                 |
| <b>LexVision Name:</b>        | TRA354N1  |                               | <input type="checkbox"/> Conditional |
| <b>Reference accessions:</b>  | NM_134100 | <b>Is this gene X-linked?</b> | No                                   |

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**Required Materials:** X pKOS clone DNA(s) \_\_pKOS1\_\_\_\_\_  
 X Target Vector DNA \_\_pKOS1.TV\_\_\_\_\_  
 X Targeted ES Cell DNA \_\_2D7\_\_\_\_\_  
 X Genomic Map

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

|                                        | <u>5' External</u> | <u>3' External</u> |
|----------------------------------------|--------------------|--------------------|
| Name of Probe:                         | 16+17              | 10+11              |
| Restriction Enzyme for Genomic Digest: | EcoR I             | Nde I              |
| Predicted Wild-type Band (kb):         | 15.0 kb            | 12.8 kb            |
| Predicted Mutant Band (kb):            | 12.0 kb            | 6.5 kb             |
| Probe Size:                            | 468 bp             | 432 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:                                | DNA431A-19 | 5' Primer Name:                          | Neo3a      |
| 3' Primer Name:                                | DNA431A-18 | 3' Primer Name:                          | DNA431A-18 |
| Predicted Wild-type Band (bp):                 | 242 bp     | Predicted Wild-type Band (bp):           | none       |
| Predicted mutant band (bp)                     | none       | Predicted mutant band (bp)               | 352 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**

DNA431-10    5' – TAGGGAATGGAATGCACAGC  
 DNA431-11    5' – CACAGCTGAGTTTATCAGGC  
 DNA431-16    5' – TACTGCAAGAGGAGACAAGC  
 DNA431-17    5' – CCCATTTACTCTTGTCACGG

**PCR Genotyping**

DNA431-18    5' – AACTGAGGTGGGTACAGGAGAGGACAAGTG  
 DNA431-19    5' – ACTTTGATCCTCCAGGACAAGG  
 Neo3A        5' – GCAGCGCATCGCCTTCTATC

**Genomic Sequence Deleted:**

GGCCCATCATGCTGGTGACTGCCTATTTGTCTTTTGTGGGCTCCTGGCCTCCTGCCTGGGTCTGGAGCTGTCAAGATGC  
AGAGCCAGGCCCCCTGGAAGAGCTTGCAGCAACCCCTCCTTCCTTCAGTTTCAACTAGACTTCTATCAAGTCTACTTTCT  
GGCCCTGGCAGCGGACTGGCTCCAAGCTCCCTACCTGTATAAACTCTATCAGCATTACCACTTCTCGGAGGGTCAGATT  
GCTATCCTCTACGTCTGTGGCCTTGCCCTCCACGGTCTCTTTGGCCTAGTGGCCTCCTCCCTTGTGGACTGGCTGGGTCTG  
TAAGAAGTCTTGTGTCCTCTTCTCCCTCACTTACTCTCTATGTTGCATAACCAAGCTCTCTCAGGACTACTTCGTGCTGCT  
GGTGGGCCGAGCACTCGGTGGGCTGTCCACAGCTCTCCTCTTCTCAGCCTTTGAGGCCTGGTACATCCACGAGCACGTG  
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CCGAGACAGAGCAGGCCGGGGTTCTCAATTGGTTCAGAGTGGCCCTGCATTTGCTGGCCTGTCTAGGGCTCCTTGTCTCT  
CCACGACAGCGATCGGAAAACAGGCCACCAGGAATATGTTTAGCATCTGCTCTGCCGTATGTTGACGACTCTGCTGGC  
AGTGGCAGGACTCTTCACCGTGGTGAGACACGACGCTGAGTTGCGGGTGCCCTCACCCACCGGGGAGCCCTACGCCCC  
TGAACCTTTGATCCTCCAGGACAAGGGGTCTGAGATGGGCTCTGGAACACACCCACCTAC

**Genomic Locus: (The deleted sequence represents nt 12349-13758 in the sequence below. KOS1 used to generate the TV represents nt 7389-16306 in the sequence below.)**

[illegible]

GAAGGCTACCTACCAGGAGCCCCCTCCGTCTCTGGGCGTGGACCTCCAGCTACCTCTCCCTGCTTGCCCTGTCCCCCTA  
CTGCCTGTGCCTGTCCCCTGTCCAGCTGCCCCCTCTCCCATGTTCTTGCACTGCTTGCCCTCAGATGCCGCCGCTGCT  
ACCTGCCCTTCTGTCCAGGCTCCCTGGCCACCCCTGCACGTGTTCTTGCCCTCCTTGCCCTGCGTGTCTCCCTCAATG  
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#### **Selection Cassette:**

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