



## Lexicon Genetics Incorporated – Genentech Project Materials

|                               |           |                               |                                      |
|-------------------------------|-----------|-------------------------------|--------------------------------------|
| <b>Genentech ID:</b>          | UNQ643    | <b>Date of Submission:</b>    | 8.22.05                              |
| <b>Lexicon Contract Name:</b> | DNA448A   | <b>Mutation Type:</b>         | X Standard Knock out                 |
| <b>LexVision Name:</b>        | MSC346N2  |                               | <input type="checkbox"/> Conditional |
| <b>Reference accessions:</b>  | NM_177840 | <b>Is this gene X-linked?</b> | No                                   |

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**Required Materials:**

☐ pKOS clone DNA(s)    \_\_pKOS/56\_\_\_\_\_  
☐ Target Vector DNA    \_\_pKOS-56TVNeo\_\_\_\_\_  
☐ Targeted ES Cell DNA    \_\_2E2\_\_\_\_\_  
☐ Genomic Map

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

|                                        | <u>5' External</u> | <u>3' Internal</u> |
|----------------------------------------|--------------------|--------------------|
| Name of Probe:                         | 14/15              | Neo 2/5            |
| Restriction Enzyme for Genomic Digest: | XbaI               | XbaI               |
| Predicted Wild-type Band (kb):         | 13.7 kb            | ---                |
| Predicted Mutant Band (kb):            | 9.1 kb             | 8.3 kb             |
| Probe Size:                            | 236 bp             | 609 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:                                | DNA448-5 | 5' Primer Name:                          | Neo3a      |
| 3' Primer Name:                                | DNA448-6 | 3' Primer Name:                          | DNA448-rev |
| Predicted Wild-type Band (bp):                 | 157 bp   | Predicted Wild-type Band (bp):           | none       |
| Predicted mutant band (bp)                     | none     | Predicted mutant band (bp)               | 524 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**

DNA448-14    5' – GCCTGGCAACACATTTTCATG  
 DNA448-15    5' – CAGGCTATGACACTGCCTG  
 Neo-2        5' – CCTCAGAAGAACTCGTCAAG  
 Neo-5        5' – GGCAGCGCGGCTATCGTG

**PCR Genotyping**

DNA448-5    5' – TAGCACCACAGCATCCTGGC  
 DNA448-6    5' – GAGAAGACACGTTGTGGCAGG  
 DNA448-rev    5' – TGACCTCCCCACTAGACCAACTGT  
 Neo3a        5' – GCAGCGCATCGCCTTCTATC

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[illegible]

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[illegible]

**Selection Cassette:**

GGCGCTCTAGAGGCCATAGCGGCCCTTTAAATGGCGCGCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCGCCCCCTCTCCCTC  
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