



## Lexicon Genetics Incorporated – Genentech Project Materials

|                               |           |                               |                                                        |
|-------------------------------|-----------|-------------------------------|--------------------------------------------------------|
| <b>Genentech ID:</b>          | UNQ16168  | <b>Date of Submission:</b>    | 2/1/05                                                 |
| <b>Lexicon Contract Name:</b> | DNA441N1  | <b>Mutation Type:</b>         | <input checked="" type="checkbox"/> Standard Knock out |
| <b>LexVision Name:</b>        | MAS261N1  |                               | <input type="checkbox"/> Conditional                   |
| <b>Reference accessions:</b>  | NM_027022 | <b>Is this gene X-linked?</b> | No                                                     |

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**Required Materials:**

- ✓ pKOS clone DNA(s)    \_\_pKOS-DNA441-42\_\_\_\_\_
- ✓ Target Vector DNA    \_\_pKOS-DNA441-TV42\_\_\_\_\_
- ✓ Targeted ES Cell DNA    \_\_1H6\_\_\_\_\_
- ✓ 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:                         | MAS261-34/35              | MAS261-36/37              |
| Restriction Enzyme for Genomic Digest: | ApaLI                     | SpeI                      |
| Predicted Wild-type Band (kb):         | 19.2 kb                   | 15.7 kb                   |
| Predicted Mutant Band (kb):            | 12.9 kb                   | 12.1 kb                   |
| Probe Size:                            | 257 bp                    | 231 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:                                | MAS261-5 | 5' Primer Name:                          | Neo3a    |
| 3' Primer Name:                                | MAS261-6 | 3' Primer Name:                          | MAS261-6 |
| Predicted Wild-type Band (bp):                 | 345 bp   | Predicted Wild-type Band (bp):           | none     |
| Predicted mutant band (bp)                     | none     | Predicted mutant band (bp)               | 288 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**

MAS261-34    5' – AAGTGTCTGTCCTAGAGTGG  
 MAS261-35    5' – ACTCACTGAACCATTTCATCG  
 MAS261-36    5' – AGAGGGTATCCAGAGTAGG  
 MAS261-37    5' – GAATCAGATTCTGACTGAGC

**PCR Genotyping**

MAS261-5    5' – GTGCTTGCAGCAGCTCAGG  
 MAS261-6    5' – ACATGTTTGGGAGCATCTCC  
 Neo3a        5' – GCAGCGCATCGCCTTCTATC

**Genomic Sequence Deleted:**

ATGGCAGCACCGATAAAGTTTCCATTTCGGCCCCAGAGGAGGTCAGCCCAGAGAGGCACGACCCCCAAAAAGGGGGCTTCGACGCTATCTATTGGAATT  
GAAAGAGAGCAATAAAGAGTTCTGGCTGTCAGGACACGCCGTGTTCAAGCTTCTGTCTTTGGTATACTGGAGTGGAGTCGATATGTTGTTGGGATTGGA  
GAGGTCCTCAGAAGTAGGGGAGAGTGCTTGCAGCAGCTCAGGTGTAATCTATTTTTCCACCCAGGGCTGCATGATCTCTGCACTAGACTACTTTGAAACA  
ATGCTCCCCACCTCGGTCTGATCCTGCTCAATCTGTATGGAAGCGCAATTCGCATATTCTTCATCTTCCTTAACACCTTAGCCATCAACAGATACATTC  
CCTTCGTCTTCTGGCCTATGCGCGTAAGTAAAGACTGTGGGGGAGGG

**Genomic Locus:** *(the deleted sequence represents nt 12346 – 12788 in the sequence below; KOS42 used to generate the target vector represents nt 5215 – 14782 in the contig below)*

[illegible]

ACCATGTGGTTGCTGGGATTTGAACCTCTGGACCATCGGAAGAGCAGTGGGGTGCTCTAACCCACTGAGCCATCTCACCAGCCCCATAAAGATTATTATA  
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### Selection Cassette

**Targeted Locus:**

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[illegible]