# Supplementary Methods

## Generation of ultraconserved element null mice

Homologous arms were amplified by PCR (see primer tables below for each element) from DNA extracted from W4/129S6 mouse embryonic stem cells (Taconic) and cloned into the ploxPN2T vector. ploxPN2T was modified from pPN2T [1] by replacing the existing neomycin selection cassette with a neomycin selection cassette flanked by *loxP* sites for positive selection and two hsv-tk cassettes for negative selection. Constructs were linearized and 20 μg were electroporated into W4/129S6 mouse embryonic stem cells. The electroporated cells were selected under G418 (160 μg/ml) and 0.2 μM FIAU for a week. Colonies that survived were picked and expanded on 96-well plate and screened both by PCR with primers outside but flanking the homologous arm, and by Southern blot hybridization with probes targeting the vicinity of the homologous arms. To avoid potential regulatory effects due to the neomycin gene cassette, we next removed it by Cre-mediated recombination of LoxP sites in the ES cells. Clones that were correctly targeted were electroporated with 20 μg of the Cre recombinase-expressing plasmid TURBO-Cre (Obtained from Dr. Timothy J. Ley of the Embryonic Stem Cell Core at the Siteman Cancer Center, Washington University Medical School). Clones positive for Neo removal were screened both by PCR (primers and gels for each element below) and Southern blot hybridization (gel results below for each element), and checked for G418 sensitivity. Positive colonies were injected into C57BL/6J blastocyst stage embryos. Chimeric mice were subsequently crossed to C57BL/6J mice, generating agouti offspring that were heterozygous/hemizygous for the ultraconserved element deletion and were intercrossed to generate homozygous ultraconserved null mice.

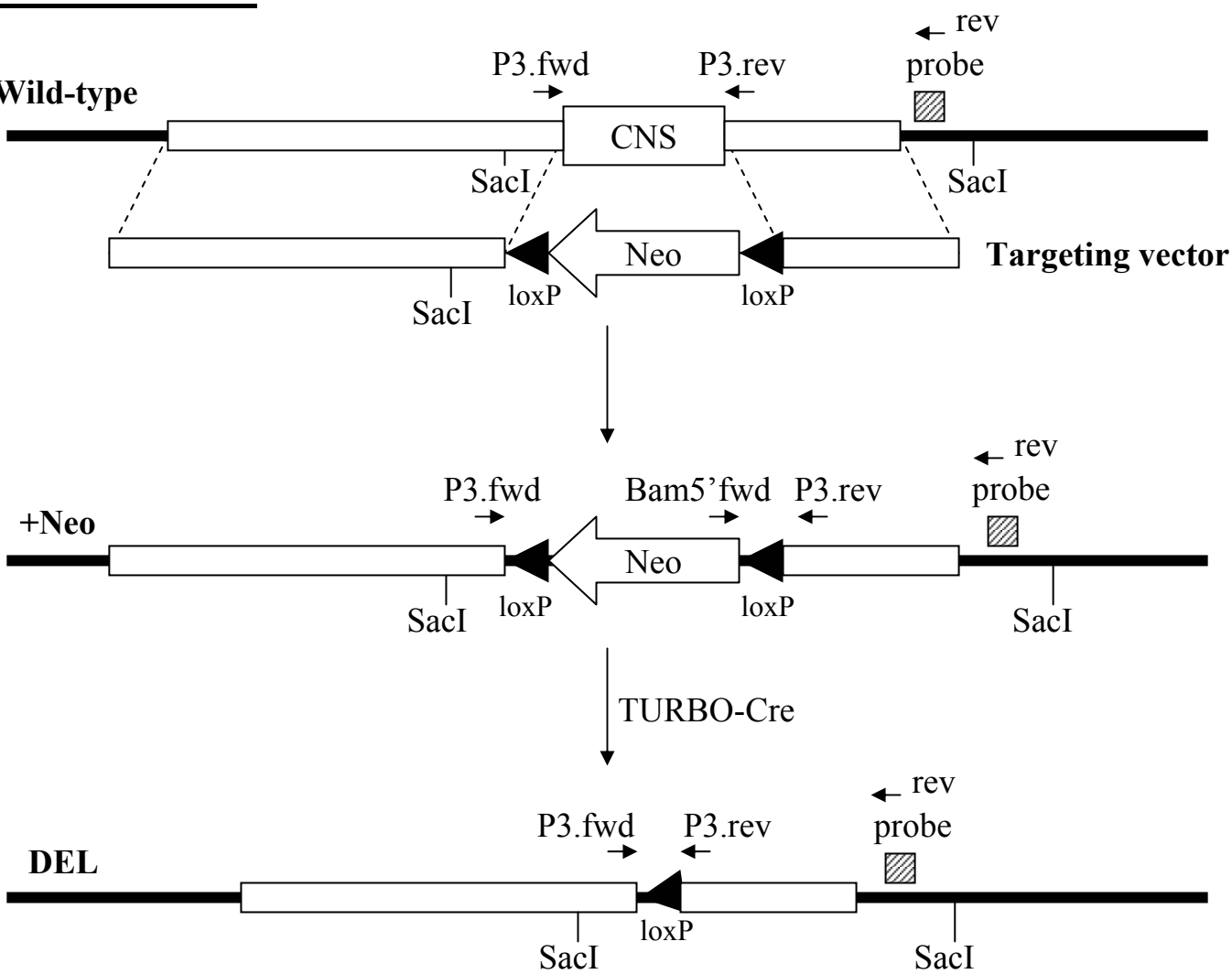
## Genotyping

Genomic DNA was extracted from a 1-cm section of tail that was incubated overnight in lysis buffer (containing 50mM Tris-HCl pH 8.0, 1mM EDTA, 1% SDS, 20mM NaCl and 1mg/ml Proteinase K) at 55 degrees Celsius. Genotyping was performed by PCR, with primers outside the deleted segments and within the targeting vector (see primer tables below for each element).

## Figure legend for genomic targeting and screening below.

Design of ultraconserved deletions displaying the wild-type, the Neo insertion (+Neo) and the Neo deletion (DEL) allele for each element (figures not drawn to scale). The points of integration of target vector homology arms are shown, primers (P2/P3.fwd and P2/P3.rev) used to screen embryonic stem (ES) cells by PCR for the presence of the Neo cassette and the Neo cassette deleted cells are indicated by arrows flanking the deleted segment. The probe used in the Southern hybridization for identification of embryonic stem cells carrying the deletion is shown as a striped box, together with the restriction sites. The sequence that was deleted, the expected DNA sizes for the Southern hybridization and PCR, the Southern hybridization and PCR gel results of each allele, and the primers that were used are shown following the figure.

# uc248 deletion

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## uc248 deleted sequence

gagtattaatacctgccatatcttaccgggcgaattgtggtttgaaaacactagcacaccactcagaggagctgggagtgggtgtcgtctgtgaggttacttt
cacttgtaaccctccccctccctgtccaatttctttgatgatttttcccataaatgcataagctgcagggacttagtagaagcaggccgcgccccctaaatgtg
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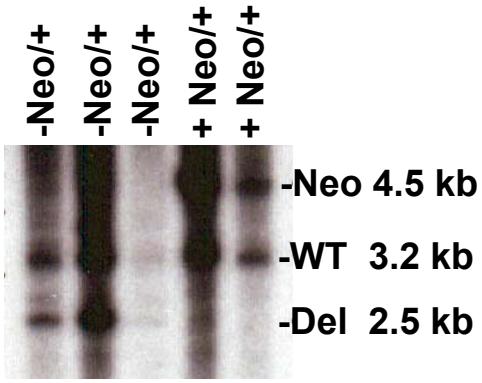
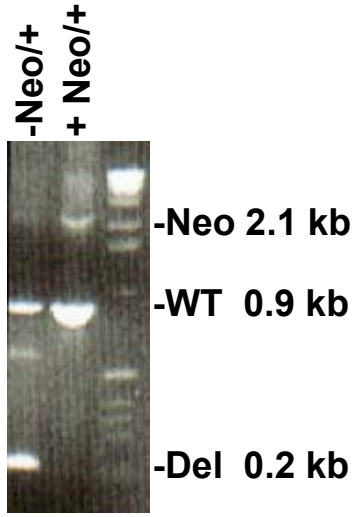
gattgctttgagaatgaaaatgtcccctaaaaacc

| Southern or PCR | Wild-type | Neo targeted | Neo deleted | Screen for |
|---|---|---|---|---|
| SacI digestion | 3247 bp | 4525 bp | 2573 bp | Both |
| PCR(Bam5'.fwd/rev) | No band | 1749 bp | No band | Neo targeted |
| PCR (P3.fwd+P3. rev) | 905 bp | 2183 bp | 231 bp | Neo removed |

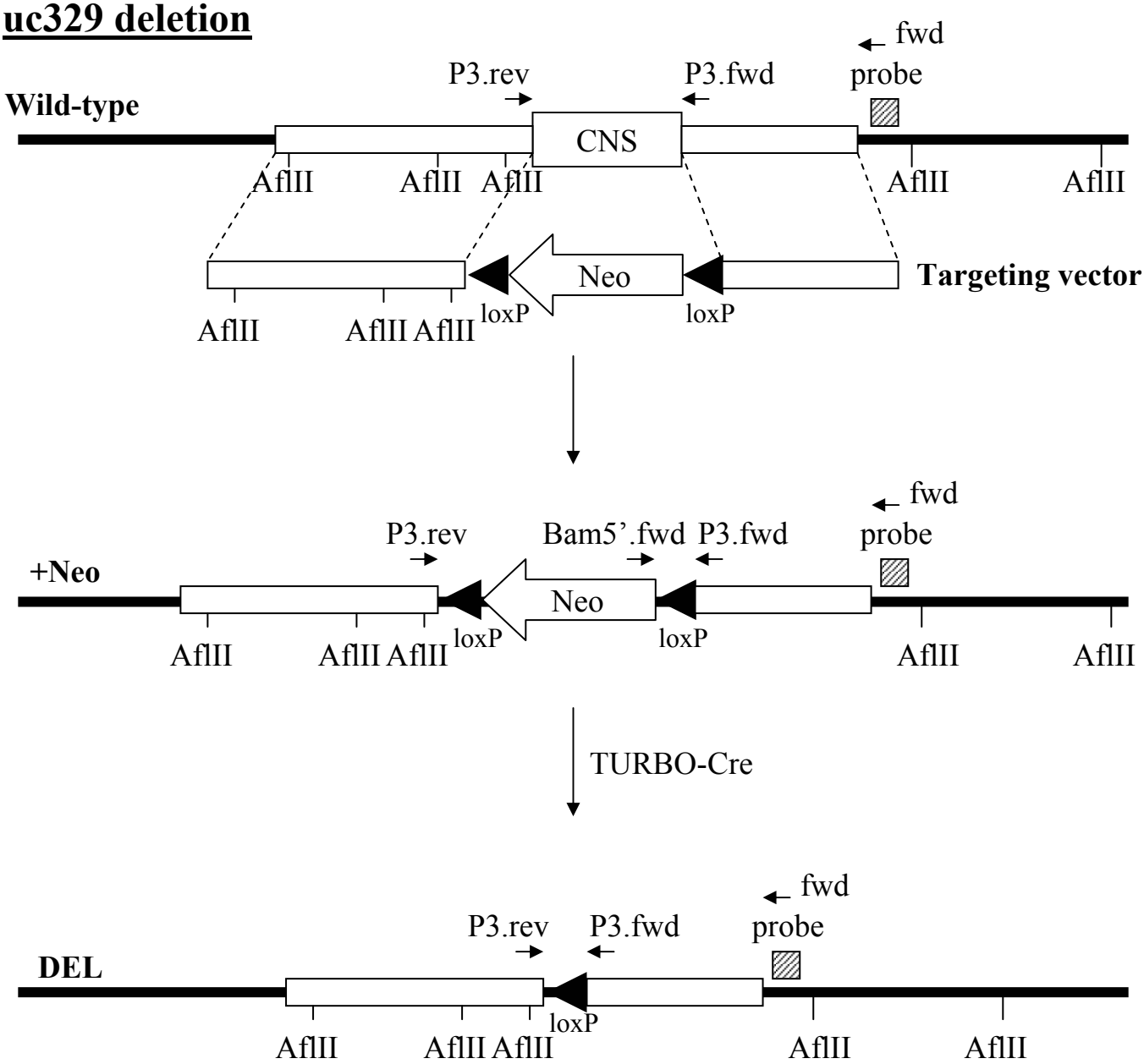



## uc248 Primers

| | Primers | Size (bp) |
|---|---|---|
| Long arm | 5':gaatgcggccgccagaactgagttgtcaggaaactgtct | 6039 |
| | 3':acgcgtcgacaagtggctgaaacaacgtcgtgctt | |
| Short arm | 5': ccatcggatcctggtgcagaactctgctgctctct | 1602 |
| | 3': atcggcaattgagcttgcgagggaattgagggtac | |
| Probe | 5': aagggtgggaaggaaagcggtcta | 311 |
| | 3': tttcctcctccctggcaggca | |
| Neo target screening | 5': ttggctggacgtaaactcctctt | Neo-1749<br>WT-none |
| | 3': tagaccgctttccttcccaccctt | |
| Neo removal | P3.fwd.: agcttccgatgttcacgttgt | Neo del-231<br>Neo-2183<br>WT-905 |
| | P3.rev: tggtcagggaaacagatcagg | |
| Genotyping | P3.fwd: agcttccgatgttcacgttgt | Del-231<br>WT-321 |
| | P3.rev: tggtcagggaaacagatcagg | |
| | 3': tcctctgagtggtgtgctagtgtt | |

# uc329 deletion

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## uc329 deleted sequence

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gctttgtggtaaataatcaattcaaaccagcaccgagtctctctttctgtaattctaaaaacccattaaattatattgttgggaggaaacaatagctgtgctaatc
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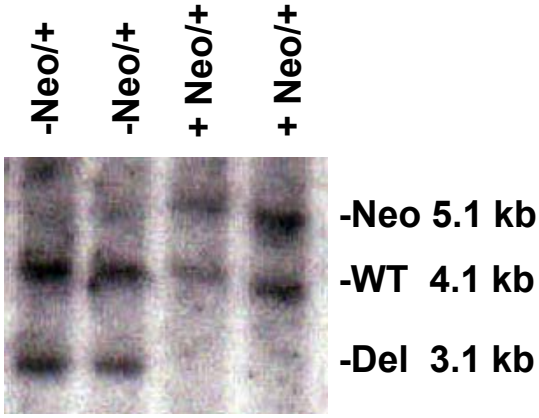
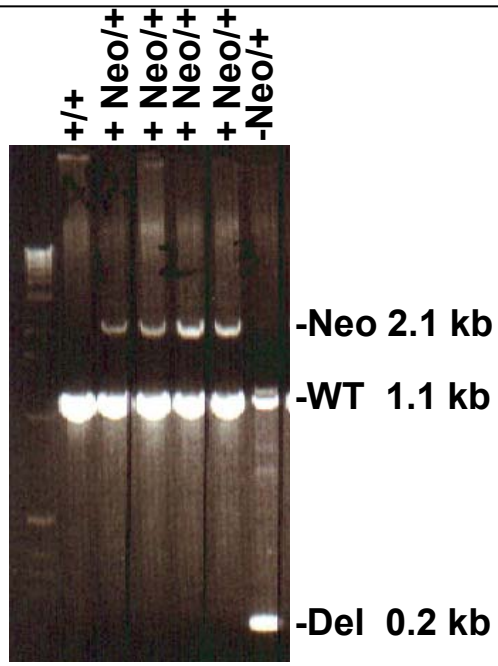
at

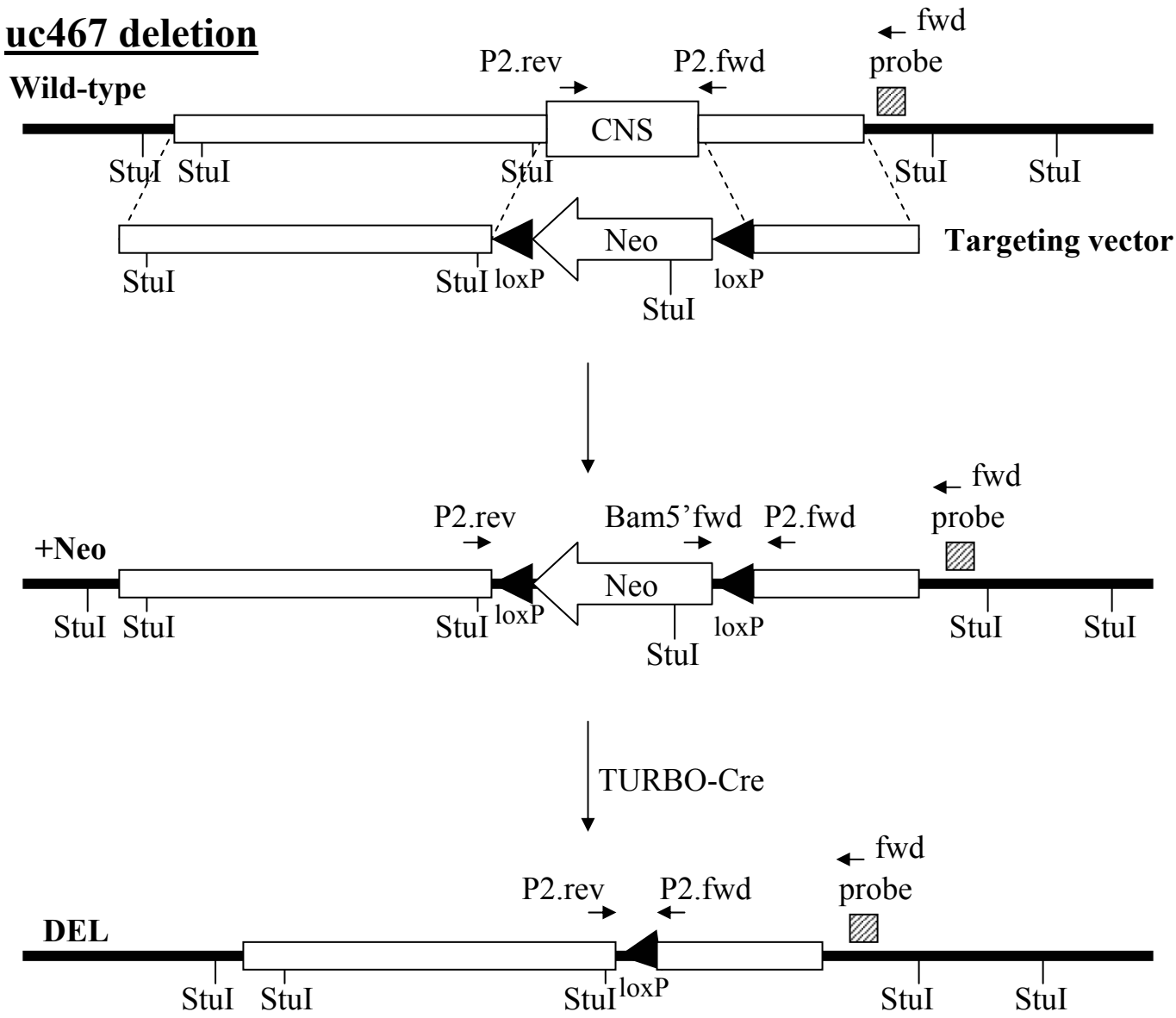
| Southern or PCR | Wild-type | Neo targeted | Neo deleted | Screen for |
|---|---|---|---|---|
| AflII digestion | 4106 bp | 5097 bp | 3145 bp | Both |
| PCR(Bam5'.fwd/fwd) | No band | 2003 bp | No band | Neo targeted |
| PCR (P3.fwd + P3.rev) | 1191 bp | 2182 bp | 230 bp | Neo deleted |




## uc329 Primers

| | Primers | Size (bp) |
|---|---|---|
| Long arm | 5': gaatgcggccgcgagctgctcatgtatgactgagct | 5047 |
| | 3': gtagctcgagcagcaggataaggcagaaattagg | |
| Short arm | 5': ccatcggatccactgacagaaaggcaggctagg | 1870 |
| | 3': ccatcgaattcctcccacaccgttctgtgatt | |
| Probe | 5': ggctattgttataaaaagaggttagcac | 256 |
| | 3': ctctgtgtggtcatttctattccc | |
| Neo target screening | 5':gctatgctggacagacctcagtctt | Neo-2003<br>WT-none |
| | 3': ttggctggacgtaaactcctctt | |
| Neo removal | 5':acgtactttcccatgctttcc | Neo del-230<br>Neo-2182<br>WT-1191 |
| | 3': gatagctctcctgtgagtcagtagg | |
| Genotyping | 5':acgtactttcccatgctttcc | Del-230<br>WT-320 |
| | 3': gatagctctcctgtgagtcagtagg | |
| | 3': acagagcgtgattagcacag | |

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## uc467 deleted sequence

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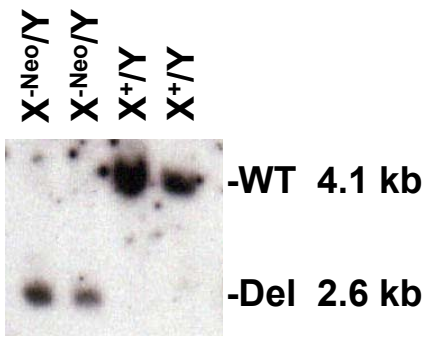
tctatactgtatgtcgatggaaaaagtagcaacaaaatattgatct

| Southern or PCR | Wild-type | Neo targeted | Neo deleted | Screen for |
|---|---|---|---|---|
| StuI digestion | 4188 bp | 2991 bp | 2669 bp | Both |
| PCR(Bam5'.fwd/fwd) | No band | 2149 bp | No band | Neo targeted |
| PCR (P2.fwd + P2.rev) | 1746 bp | 2179 bp | 227 bp | Neo deleted |

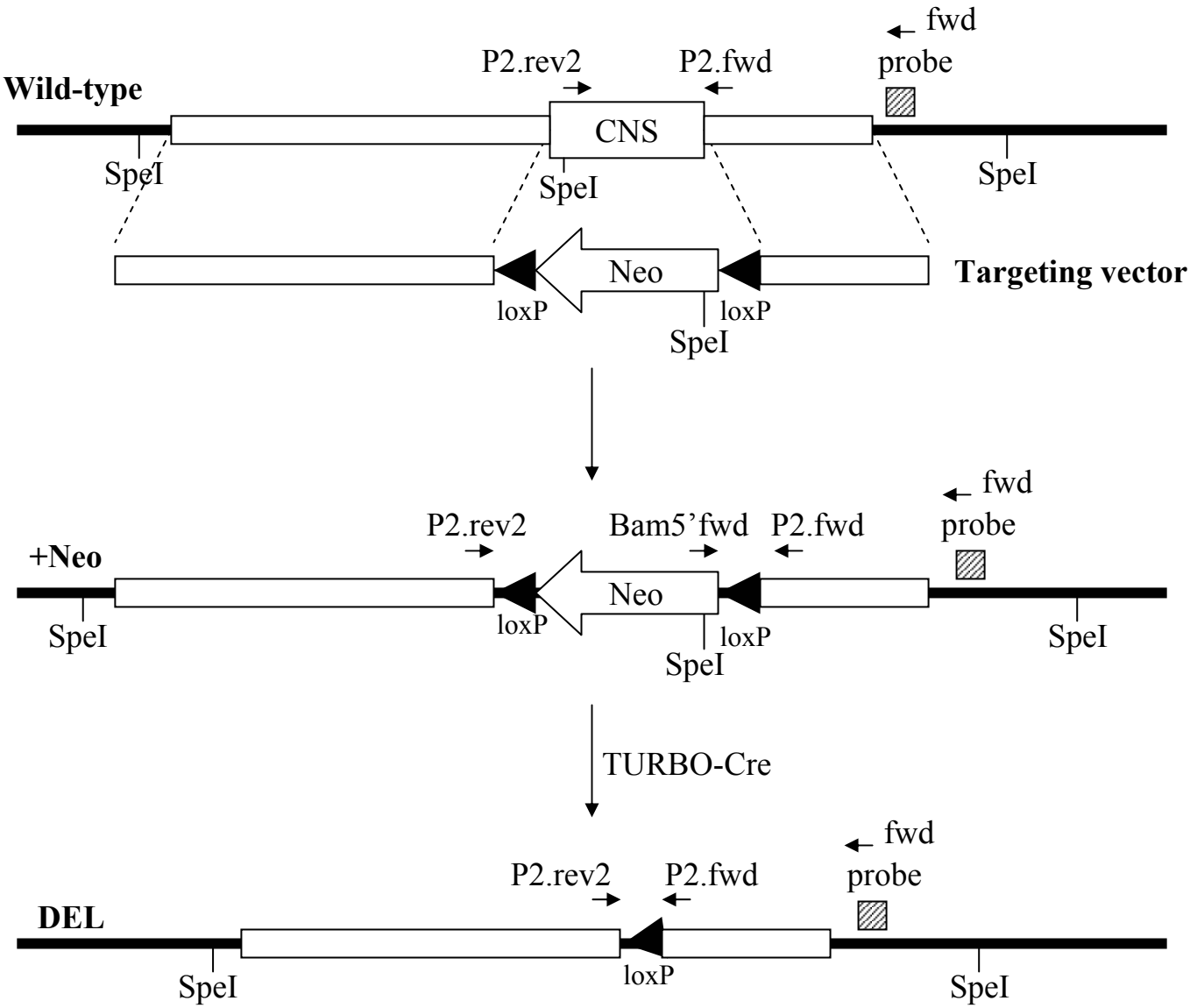




## uc467 Primers

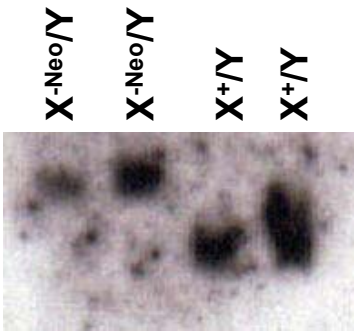
| | Primers | Size (bp) |
|---|---|---|
| Long arm | 5': gaatgcggccgcgttcatgcgttagtgtgtttaagtgtggta | 5261 |
| | 3': gtagctcgagtcaatgagccatctctgtcatgaaactcag | |
| Short arm | 5': ccatcggatcccaccctctgctcttttccaaacttcc | 2021 |
| | 3': ccatcgaattcactgttacctggtttctgtcaatattctacc | |
| Probe | 5': agaggacatagctcctgatgtgtttac | 326 |
| | 3': tatgatgtggcctgtgacagcttatc | |
| Neo target screening | 5': gggcatccatgttaggctttacact | Neo-2149<br>WT-none |
| | 3': ttggctggacgtaaactcctctt | |
| Neo removal | 5': tacttgaagcaaatggaggtgcaatg | Neo del-227<br>Neo-2179<br>WT-1746 |
| | 3': ctaggcctttggtagagttgatccat | |
| Genotyping | 5': tacttgaagcaaatggaggtgcaatg | Del-227<br>WT-348 |
| | 3': ctaggcctttggtagagttgatccat | |
| | 3': ttctgtccagtatggttgacagtact | |

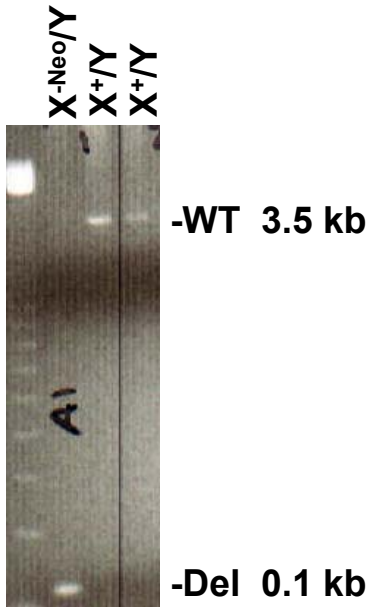
# uc482 deletion




**-Figure legend on page 2.**

| Southern or PCR | Wild-type | Neo targeted | Neo deleted | Screen for |
|---|---|---|---|---|
| SpeI digestion | 5830 bp | 3079 bp | 7626 bp | Both |
| PCR(Bam5'.fwd/fwd) | No band | 1936 bp | No band | Neo targeted |
| PCR (P2.fwd + P2.rev2) | 3596 bp | 2096 bp | 144 bp | Neo deleted |




## uc482 Primers

| | primers | Size (bp) |
|---|---|---|
| Long arm | 5': gaatgcggccgctgggaacaaatctgatgtgaacttagaggc | 3119 |
| | 3': gtagctcgagccagctcatttggaagaagctgacaagtcca | |
| Short arm | 5': ccatcggatcctctgtcttagtttgaggttctgtaaatagt | 1730 |
| | 3': atcggggtacctccattagtttgaatgctaaccagaaag | |
| Probe | 5': cagagaaatacatatattgctgtttctc | 255 |
| | 3': gagacctttatttttatttacccctcta | |
| Neo target screening | 5': ggaagtggggtatggcattagag | Neo-1936<br>WT-none |
| | 3': ttggctggacgtaaactcctctt | |
| Neo removal | 5':actatttacagaacctcaaactaagacaga | Neo del-144<br>Neo-2096<br>WT-3596 |
| | 3': tggacttgtcagcttcttccaaatgag | |
| Genotyping | 5':actatttacagaacctcaaactaagacaga | Del-144<br>WT-305 |
| | 3': tggacttgtcagcttcttccaaatgag | |
| | 3': ttattgctttcatgtgcctccacc | |

# uc482 deleted sequence

aaagaagtaacaaactttgtaaaaaatagaactattcttctctctctgcatatggtttaatattttccctgaagtaaaactttcagtctgtgaccttgagaaacccagaaagtaaatgcaaacagtacaggacggaaggtcacccatcctctctactactttccataaagggtatcaaatattcatccgcacaggtacatgtcttcgtaataaggcagatgggaactaaagcaaaggcaactccaaatgggggtggaggcacatgaaagcaataattttgaatctttgacatttgttttgaaactaccttactaccctataccctttaccatctgtctgttatccctcccaacacccacctctgtcttcccacagggctccattgtctgcagttcctgtttctcaggggcggagctgtgggaggcagccatgggtgccataataggcacctgcggctagcacaatgggctgtgggtgcttgtgggagagacagattgatgtattgtgcatgggggaggagggctgtcacttgaatccatgtgaacccaggctttaggggcaagggagattgacagcttttgtaggttgcatttctttttcaaagcttggctttaatagacagtaagtttggggccccaggctttcactcccataattctaacagggcaatcacgagcattcagcatttctttttaccccccttgttccgcattaaatgatttcagtaaaggaatgtgcagcattaattagaatttgttagcagataccaaggggaaaagtttcttcacaacaactataggaggtgtcatttgtacaaagacttttttgtttttctttccccattacattatcactgcaatatgtaaggtagtgattcttttcatttaaattaagtggaaagctactaaatcttgttttattctccaaaaagaataaggtccttaacacttatctttttaacttctttcatttcctaaggttggtgtttacttttgctttcagtccaacccatttactggtctgtgaggttgtgtatccagttttgtgtaccattgagctcttacaattgcaggcagcccactgtactgatatagaaagattgtttccagggaaaaatggccacaaataatattggaaaacattgatataccagtccctttttagtaatggtacatcaagaagtgccaaaaattagagtcatttaggttcatatcctagtgttttcaaaagccattttaaccttacccataacatcaaagttatggaaggatggggcagtggccaaagaatgtttttttttttcctatagaaaactcattccgaaactacgtaaaaaattttaagcatcatcctcacactcgcatagcaccattttcttacagttagtaaaaaggatctctctactttgtgtctatgttttattctttgacatctatctagtatgaaatctaatgtcagatagacatgtcctataagcatgttcttcagtgtttggaaaactgatgaaacgttattaacatttttgatgtgattcttctagcatctctcctgtataaaatcatttcccaccaatgggaagagttgaaggctaattgaattagatttttttcatttaattcttttacttaaatgtgtcaggacagcagtgaaaaatattaagtctatcctgaattctcagagagaaaatattcccagagttgtctaactctgtctataacaatttggtgcaatggctttaaaagtagtggggtgataataattagggtgtgtgacagaaatttcagtgttggattcaacatttttactgtgcagggcatgaaaattgccaagtgggggctagtcagctctaagtactaattaactaattaatcaggtgctgaaaccaagtgaatggaggataccagccagacaactcaagcccaagcagagggttgcactctttaaaagacactgcactctttcttcttggtttttaacccaatcaggaatgagagagaggcggggggaggggctaggtgggggtgggtgggttatggtacaagcttttcacttaggaaaaagtaagataaaaacatgtgcctctgataatgctgtaaggcgccctgagtaggtctgtggctgactttccagtcaagaagtgtttttggtttggtgggagctggaggtatagagaattctgtcttcctggggtttatgtctataatacatacatatatatcatatacatcatatacatctacatctacatgtatacatgtaggagaatgagaaacatgtctcttggggagctgatcttaggtcttttgcctcatccttcgttttatgtaaatgagaagcgtccaattacacatatggaaaaaaggtttaatgaatcgtgaaaagcctggtttgtagagaaagggcacagtctcttctccttatttccctcctcctcctcctcctcctcctcctcctcctcctcctctacctcctgttcatccacctcccttacttctccctcttgaccttcagatttcatttagaaacatgtatttcttttcaggatgatgatgctcatcatttttttcacattctaattttaatacttaagaattatcaaacactttcctagaagattgaaatttatatttgtaaggttttctggatacttaaaatattctacactagaaactacaaaattctgattttcctcccctaaatttgaaaaaaaaaaaagattacatgtctgttcctcccccctttgatgttattcttggctcaactttaatttcatgttttgtttgttctggaattgcttgcagtgaaaatgggttgcaacattagaaaggaactaatactgagtccaaagaaaagacagtcccccataacttgcagcttagtttggtattgcattttaactagctggaattttagggtgttggaaattaggcaattcttattttacttttcttttcaaaaagagtccgaattttctgctgcagtagaaagttcttttttagttttcagttattttctttctccttttcttaatgttggtgtcctgagtagttggaagtggatcacacagaaccccttaccatcccaagttttaaaatgatacaaacctttacagtaagactaaggagatcagtgctatgtctggccatccctctcagaatctgagaagtatgcaactagttccaaagaagtattgtgagagaacagcagggcgcttcgcaatcatgaccttcaagtctttctcagagaccttcaagtcttcctcagagacaaggttcctcactgggcatctggggaaactgaggcctaagaggacgagaaaggagcagttacctttctccctcgacaaaatactgaccgaattatgccacgtgtttcttctaagtgtagaagccaaagggtccatatcgcctgtctagttcactaaagggacgggtcttccactctacccctcagcctccctaatcagcaccccagatgtgaaaaacaacagaaagttgctcccctaatcctcagtgacaacacggggctgtaaaatttca

## References

1. Paszty C, Mohandas N, Stevens ME, Loring JF, Liebhaber SA, et al. (1995) Lethal alpha-thalassaemia created by gene targeting in mice and its genetic rescue. Nat Genet 11: 33-39.