inGenious Targeting Laboratory

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NRFT: Construction of Conditional Targeting Vector

Sections:
1. Vector Design Outline
2. Sequence Data Analysis
3. Backbone sequence
4. Cassette Sequences

1. Vector Design Outline

A 11.4 kb region used to construct the targeting vector was first sub cloned from a positively identified C57BL/B6 BAC clone (RPCI23 336N23) BAC clone. The region was designed such that the long homology arm (LA) extends about ~7kb 5' to exon 4. The short homology arm (SA) starts 3' to exon 5 and is ~1.6 kb long. The loxP/FRT flanked Neo cassette is inserted on the 3' side of exon 5 and the single loxP site is inserted on the 5' side of exon 4. The target region is 2843 bps and includes exons 4-5.

The targeting vector is confirmed by restriction analysis after each modification step. P6 and T7 primers anneal to the vector sequence and read into the 5' and 3' ends of the BAC sub clone. N1 and N2 primers anneal to the 5' and 3' ends of the Neo cassette and sequence the SA and LA, respectively.

BAC Subclone and confirmation
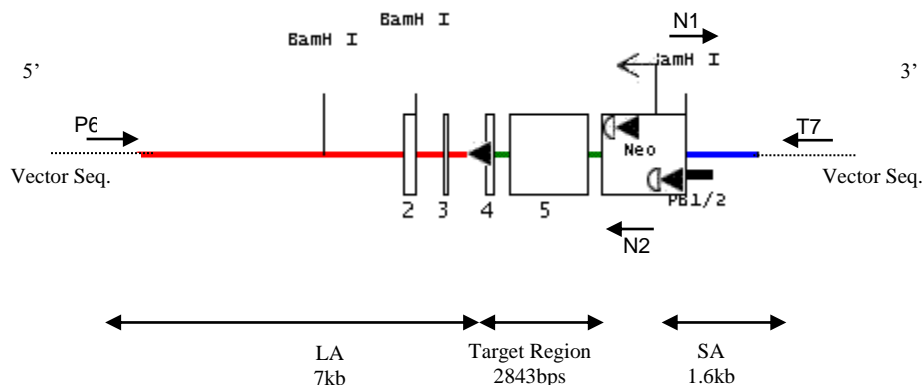
PCR primers used for sequencing:
```
Primer P6 5'-GAG TGC ACC ATA TGG ACA TAT TGT C-3'
Primer T7 5'-TAA TGC AGG TTA ACC TGG CTT ATC G-3'
Primer N1 5'-TGC GAG GCC AGA GGC CAC TTG TGT AGC-3'
Primer N2 5'-TTC CTC GTG CTT TAC GGT ATC G-3'
Primer Lox 5'-GAC CTG TAT GGG CTG GTG AGA TG-3'
```

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Backbone Vector Information

The BAC was sub cloned into a ~2.4kb backbone vector (pSP72, Promega) containing an ampicillin selection cassette for retransformation of the construct prior to electroporation. A pGK-gb2 loxP/FRT Neo cassette was inserted into the gene as described in the project schematic. The targeting construct can be linearized using NotI prior to electroporation into ES cells.

The total size of the targeting construct (including vector backbone and Neo cassette) is ~12.7kb.

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2. Sequence Data Analysis

Sequencing Data from BAC Sub Clone and Targeting Construct

P6 sequencing data aligned with genomic sequence

```
Query  19422  AGTGAGCCATAGGCCTGTCTTCTGCCTTTGGAGTCTTTGTTGAAAAGCAGATGGCATTAT  19481
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  135    AGTGAGCCATAGGCCTGTCTTCTGCCTTTGGAGTCTTTGTTGAAAAGCAGATGGCATTAT  194

Query  19482  ATTGTCTACCATGTAATGCAGTGTGGGACAGGAACCTTCGAGAAGTAAATGGGCAAATAT  19541
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  195    ATTGTCTACCATGTAATGCAGTGTGGGACAGGAACCTTCGAGAAGTAAATGGGCAAATAT  254

Query  19542  TTAGAAATGCAACAGTCATAAGTAAGATAGATTTAAAAGACACATCTGCCTGGAGTCATT  19601
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  255    TTAGAAATGCAACAGTCATAAGTAAGATAGATTTAAAAGACACATCTGCCTGGAGTCATT  314

Query  19602  TATTACTACAAGTAATACTGCAGGTATTAATAAATAAAGTGTTTATTTATTAGGCAGTAA  19661
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  315    TATTACTACAAGTAATACTGCAGGTATTAATAAATAAAGTGTTTATTTATTAGGCAGTAA  374

Query  19662  GACCACAGAGTAGGGCTTTGTAAATGAAGGTTAATTATGCAGCTGGTTATTCAACTGTTA  19721
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375    GACCACAGAGTAGGGCTTTGTAAATGAAGGTTAATTATGCAGCTGGTTATTCAACTGTTA  434

Query  19722  AGGTTTGGGCATTCCTTCAGTGCACTCACAGACGCTGCCAGCCCTCCCCTGGGTGACTTT  19781
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435    AGGTTTGGGCATTCCTTCAGTGCACTCACAGACGCTGCCAGCCCTCCCCTGGGTGACTTT  494

Query  19782  CAGACTGTAGAACATGAGTCTTGTTTTCTATCTGGTTTTATTCAAAAATACTTAGGCATT  19841
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495    CAGACTGTAGAACATGAGTCTTGTTTTCTATCTGGTTTTATTCAAAAATACTTAGGCATT  554

Query  19842  GTCTGTTGACAGGGGAGCTAAACCAGGTTTACTTTCTAGGTGAATAATATATATTCAGCT  19901
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555    GTCTGTTGACAGGGGAGCTAAACCAGGTTTACTTTCTAGGTGAATAATATATATTCAGCT  614

Query  19902  GGGCATCATAGGAGATATAGGATGGGCACTGTAGCCAAGTGTTCTTCACTCTCGATGTTC  19961
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  615    GGGCATCATAGGAGATATAGGATGGGCACTGTAGCCAAGTGTTCTTCACTCTCGATGTTC  674

Query  19962  TAGTAACGAATGTGTGCATGAAGAAGAGAATGCCGAGAAGCAAAGGGTAAACTAAGGTGG  20021
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  675    TAGTAACGAATGTGTGCATGAAGAAGAGAATGCCGAGAAGCAAAGGGTAAACTAAGGTGG  734

Query  20022  GTATTTGGGGTGTGCGTACACCTTATGCAACCCCAGCATTTGGGAGCTTGAGTG-AGG-A  20079
               |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||| ||| |
Sbjct  735    GTATTTGGGGTGTGCGTACACCTTATGCAACCCCAGCATTTGGGAGCTKGAGTGGAGGGA  794

Query  20080  GGAGGAGGATGCATCTG-AGGTCAGCC   20105
               ||||||||||||||||| ||||||||||
Sbjct  795    GGAGGAGGATGCATCTGGAGGTCAGCC   821

Query: Genomic Sequence from ENSEMBL Database
Sbjct : Sequencing data from BAC sub clone
```

T7 sequencing data aligned with genomic sequence

```
Query  29986  TCATCTCCGGAGAGCCGGGATTAAAAGCGTGTGCCACCATTTTGTAATGGTGGAAAAACT  30045
               |||| |||| |||||||||||||||||||| |||||||||||| || | ||||||
Sbjct  872    TCATTTCCGAAGAGCCGGGATTAAAAGCGTGTG-CACCATTTTGTAAT-GTKG-AAAACT  816
```

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```
Query  30046  AGCTCACTTAATGTGGATAGGTTGAGACTGATTAGACATGTAGAGGGAATAAAAACAAAG  30105
              |||||||||||||  |||| | ||||||||||||||||||||||||||||||||||||||
Sbjct  815    AGCTCACTTAATG-KGATA-G-TGAGACTGATTAGACATGTAGAGGGAATAAAAACAAAG  759

Query  30106  GCCCTAAAATTGGACAATGAACATTTCttttttttggggggggtggggtggggtggggAG  30165
              | ||||||||||||||||||||||||| |||||||||||||||| |||| |||| ||||||
Sbjct  758    G-CCTAAAATTGGACAATGAACATTTC-TTTTTTGGGGGGGGT-GGGT-GGGT-GGGGAG  704

Query  30166  GTTCGAGACAAGGTTTCTCTGTATAGCCCTGGCTGTCCTGGAACCCACTTTGTAGACCAG  30225
              ||||||||||||||||||||||||| |||||||||||||||||||| |||||||||||||
Sbjct  703    GTTCGAGACAAGGTTTCTCTGTATAG-CCTGGCTGTCCTGGAACCCAC-TTGTAGACCAG  646

Query  30226  AAATCTGCCTGCTCTGCCTCACGAGTGCTGGGATTAAAGGCGTGCACCACCACGCCCGGC  30285
              ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  645    AAATCTG-CTGCTCTGCCTCACGAGTGCTGGGATTAAAGGCGTGCACCACCACGCCCGGC  587

Query  30286  TTGAACATTTCTTTAAAACAAAAATCCTTACTGCCATTGTAAAAGTTGGGTGCTGGGTAT  30345
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586    TTGAACATTTCTTTAAAACAAAAATCCTTACTGCCATTGTAAAAGTTGGGTGCTGGGTAT  527

Query  30346  GGCGCTACACTCTGGAGGAAGTATTATCTATCTACATTCAAACCCAGCCTCTGCAGTCCC  30405
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526    GGCGCTACACTCTGGAGGAAGTATTATCTATCTACATTCAAACCCAGCCTCTGCAGTCCC  467

Query  30406  AAGGCTACGTAATGGGATTTGAGGAAAGGGGTCCTGAGATGCACTTAAGAATTGTGTATG  30465
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466    AAGGCTACGTAATGGGATTTGAGGAAAGGGGTCCTGAGATGCACTTAAGAATTGTGTATG  407

Query  30466  TCAATAAGCATAGACTTGCATATTTTCCCTCTCAAGTCCTATCGATACACTAGGACCAAC  30525
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406    TCAATAAGCATAGACTTGCATATTTTCCCTCTCAAGTCCTATCGATACACTAGGACCAAC  347

Query  30526  CTTGTACTTACTAGGCCTGGCACTAGACTGAACCTGTTGCCAGTCATTTGTTAGAGAATG  30585
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346    CTTGTACTTACTAGGCCTGGCACTAGACTGAACCTGTTGCCAGTCATTTGTTAGAGAATG  287

Query  30586  AATGGCCTCCAGCCACTCATTGAGCTCTCAGCCTTATCTCATCCATAAGATGGAAACACC  30645
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286    AATGGCCTCCAGCCACTCATTGAGCTCTCAGCCTTATCTCATCCATAAGATGGAAACACC  227

Query  30646  CAGAAGTGGATACATTGTTCATCATCTTCAAAACTTATTTTTGGTGACATGAAAAACTAG  30705
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226    CAGAAGTGGATACATTGTTCATCATCTTCAAAACTTATTTTTGGTGACATGAAAAACTAG  167

Query  30706  TAAGAGAATATGATGTATGACTGAAGGGTTGAGTCAAAATTGGACCTAGGATTCATGAGA  30765
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166    TAAGAGAATATGATGTATGACTGAAGGGTTGAGTCAAAATTGGACCTAGGATTCATGAGA  107

Query  30766  GGCTTGGTCCTCTGGGGGCATGGTAATGATGGAAGATTCTAGGAGACACTGTTCCAAAT  30824
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106    GGCTTGGTCCTCTGGGGGCATGGTAATGATGGAAGATTCTAGGAGACACTGTTCCAAAT  48
```

Query: Genomic Sequence from ENSEMBL Database
Sbjct : Sequencing data from BAC sub clone

N1 sequencing data aligned with genomic sequence

```
Query  29285  CTGCTGTACAGTACTGTGGCACAGAGAACAAAACCTGTAACCTCCCTGTGTTCTTAGAAG  29344
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  217    CTGCTGTACAGTACTGTGGCACAGAGAACAAAACCTGTAACCTCCCTGTGTTCTTAGAAG  276

Query  29345  TGGCATTCTAAGAGGGCTAGGAAGCATACTAAGGAAgggggggggggATTGAGAAAACCA  29404
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  277    TGGCATTCTAAGAGGGCTAGGAAGCATACTAAGGAAGGGGGGGGGGGGATTGAGAAAACCA  336
```

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Query  29405  GTTTTTaaaaaaaGCTGGTCGTAATGGCTTACATCTTCAATTGCTGAGCTCTGTAGACAA  29464
               ||||||         |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337    GTTTTTAAAAAAAGCTGGTCGTAATGGCTTACATCTTCAATTGCTGAGCTCTGTAGACAA  396

Query  29465  GGCTACCTTTGAACTCTGGTGCAGAGTCTGGCAAATCTGTGAGTTGCCCAGCCTGGTCTA  29524
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397    GGCTACCTTTGAACTCTGGTGCAGAGTCTGGCAAATCTGTGAGTTGCCCAGCCTGGTCTA  456

Query  29525  CATAGAGAGTTCCAGGACAATCAGAGCTATGTAGAAAGAGACCCTGGGTCAACAAGTCCA  29584
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457    CATAGAGAGTTCCAGGACAATCAGAGCTATGTAGAAAGAGACCCTGGGTCAACAAGTCCA  516

Query  29585  AATAAACACTAGTGAAAATCATCACAACGTATAGGAGCTTGCAACATCTGAAGGTATAGG  29644
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517    AATAAACACTAGTGAAAATCATCACAACGTATAGGAGCTTGCAACATCTGAAGGTATAGG  576

Query  29645  TTGATATGCTAATCAGCTCCACAGAGCACAGCTGAGCAGGCTGCACAGAGGCTGGAAGTG  29704
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  577    TTGATATGCTAATCAGCTCCACAGAGCACAGCTGAGCAGGCTGCACAGAGGCTGGAAGTG  636

Query  29705  ATGGGAAGAAGAGCGTTATGAAAAGCACAGGCATGGGCAGTCTTAAAAAGGGGGGATCTG  29764
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  637    ATGGGAAGAAGAGCGTTATGAAAAGCACAGGCATGGGCAGTCTTAAAAAGGGGGGATCTG  696

Query  29765  AGGTT-GAAGTGGGCTATGAGACTGGTCCTCTGGTCAGTGAACCTGAACCCGAGCAGCCC  29823
               ||||| |||||||||||||||| |||||||||||||||||||| |||||||| |  || ||
Sbjct  697    AGGTTTGAAGTGGGCTATGAAACTGGTCCTCTGGTCAGTGA-CCTGAACCCAA-CASCC-  753

Query  29824  AGGTGAGGGGTGGGACGAGAGAGACCAGTTGTTCCATATATACATCTCATACATCTCTTT  29883
               || |||||||| |||||||| ||| |||| |||||||| |||| ||||||| ||| ||||||
Sbjct  754    AG-TGAGGGGTGAGACGAAAGAAACCA-TTGTTC-ATATWTACATCTC-TAC-TCTCTTT  808

Query  29884  CCAACCCCCT-CTTGGTTTTCAAGGCAGG   29911
               ||| |||| | ||||||||||||||||||
Sbjct  809    CCA-CCCC-TYCTTGGTTTTCAAGGCAGG   835
```

Query: Genomic Sequence from ENSEMBL Database
Sbjct : Sequencing data from Targeting Vector

N1 Raw sequencing data (LoxP highlighted in yellow, FRT underlined)

```
TGCCAAGTGCCCAGCGGGGCTGCTAAGCGCATGTCCAGACTGCCTTGGGAAAAGCGCCTCCCCTACCCGGT
AGAATGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGTTCGAACATAACTTCGTATAGCATACATTAT
ACGAAGTTATGGTACCTGCAGAATTCATGCATAAGCTTGGATCCGTTCTTCGGACGCCTCGTCAACACCGT
ACGCTGCTGTACAGTACTGTGGCACAGAGAACAAAACCTGTAACCTCCCTGTGTTCTTAGAAGTGGCATTC
TAAGAGGGCTAGGAAGCATACTAAGGAAGGGGGGGGGGGATTGAGAAAACCAGTTTTTAAAAAAAGCTGGT
CGTAATGGCTTACATCTTCAATTGCTGAGCTCTGTAGACAAGGCTACCTTTGAACTCTGGTGCAGAGTCTG
GCAAATCTGTGAGTTGCCCAGCCTGGTCTACATAGAGAGTTCCAGGACAATCAGAGCTATGTAGAAAGAGA
CCCTGGGTCAACAAGTCCAAATAAACACTAGTGAAAATCATCACAACGTATAGGAGCTTGCAACATCTGAA
GGTATAGGTTGATATGCTAATCAGCTCCACAGAGCACAGCTGAGCAGGCTGCACAGAGGCTGGAAGTGATG
GGAAGAAGAGCGTTATGAAAAGCACAGGCATGGGCAGTCTTAAAAAGGGGGGATCTGAGGTTTGAAGTGGG
CTATGAAACTGGTCCTCTGGTCAGTGACCTGAACCCAACASCCAGTGAGGGGTGAGACGAAAGAAACCATT
GTTCATATWTACATCTCTACTCTCTTTCCACCCCYCTTGGTTTTCAAGGCAGGTCYYTTCTGCAACCYTG
TKGTCCTAAAATCTCCCAAAAAACTGSTTTTA
```

N2 sequencing data aligned with genomic sequence

```
Query  28626  CACTTTATAAGTAACTATGCAGAATTATAGCCAAAGCTAGTATAGCAATAATATGAAACT  28685
               |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  892    CACTTTWTAAGTAACTATGCAGAATTATAGCCAAAGCTAGTATAGCAATAATATGAAAC-  834
```

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```
Query  28686  TTACAAAGCATTAAAGTCTCAATGTTGAATCAGTTTCATTTTAACTCTCAAGTTAATTTC  28745
              ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  833    TTACAAAGCATTAAAGTCTCAATGTTGAATCAGTTTCA-TTTAACTCTCAAGTTAATTTC  775

Query  28746  TTAGGCACCATTTGGGAGAGTTTCTGTTTAAGTGTAAATACTACAGAACTTATTTATACT  28805
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  774    TTAGGCACCATTTGGGAGAGTTTCTGTTTAAGTGTAAATACTACAGAACTTATTTATACT  715

Query  28806  GTTCTCACTTGTTACAGTCATAGACTTATATGACATCTGGCTAAAAGCAAACTATTGAAA  28865
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  714    GTTCTCACTTGTTACAGTCATAGACTTATATGACATCTGGCTAAAAGCAAACTATTGAAA  655

Query  28866  ACTAACCAGACCACTATACTTTTTTATATACTGTATGAACAGGAAATGACATTTTTATAT  28925
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  654    ACTAACCAGACCACTATACTTTTTTATATACTGTATGAACAGGAAATGACATTTTTATAT  595

Query  28926  TAAATTGTTTAGCTCATAAAAATTAAAAGGAGCTAGCACTAATAAAAGAATATCATGACT  28985
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  594    TAAATTGTTTAGCTCATAAAAATTAAAAGGAGCTAGCACTAATAAAAGAATATCATGACT  535

Query  28986  TAAACTACTTTGGACTTTTTGAATTTATTCACACTATTTTCCATAGGACAATCACTCATT  29045
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  534    TAAACTACTTTGGACTTTTTGAATTTATTCACACTATTTTCCATAGGACAATCACTCATT  475

Query  29046  TACCACATTTGGTTATTTTACATTTTCAAAATGGGTTTGAAAATACAGAGGCATTTTATA  29105
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  474    TACCACATTTGGTTATTTTACATTTTCAAAATGGGTTTGAAAATACAGAGGCATTTTATA  415

Query  29106  GCCATGTGTGGCAGTCCATGATTTTTATTCCCGACATTCAGGAGGCAGAAGCAGGCAGAT  29165
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414    GCCATGTGTGGCAGTCCATGATTTTTATTCCCGACATTCAGGAGGCAGAAGCAGGCAGAT  355

Query  29166  CCCTGGGCTCCAGGACGGCCAAGGCTACATGAGAGCTTGTCTCAAGAAAGACAAACCCTT  29225
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  354    CCCTGGGCTCCAGGACGGCCAAGGCTACATGAGAGCTTGTCTCAAGAAAGACAAACCCTT  295

Query  29226  TCTATACTAAACGTTAGCTAGGATTGTCAAGGAGATGGTATATATCCACAATGGTATGC  29284
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  294    TCTATACTAAACGTTAGCTAGGATTGTCAAGGAGATGGTATATATCCACAATGGTATGC  236
```
Query: Genomic Sequence from ENSEMBL Database
Sbjct : Sequencing data from Targeting Vector

N2 Raw sequencing data (LoxP highlighted in yellow, FRT underlined)

TCGGCTCCGATTCGCAGCGCATCGCCTTCTATCGTCTTCTTGACGAGWTCTTCTGAGCGGGACTCTGGGGT
TCGAATAAAGACCGACCAAGCGACGTCTGAGAGCTCCCTGATATCAGATCTAC<mark>ATAACTTCGTATAATGTA</mark>
<mark>TGCTATACGAAGTTAT</mark>CCGCGGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCTAGACTAGTACGC
GTGTACACTTAAGCCGGCGTACGCATACCATTGTGGATATATACCATCTCCTTGACAATCCTAGCTAACGT
TTAGTATAGAAAGGGTTTGTCTTTCTTGAGACAAGCTCTCATGTAGCCTTGGCCGTCCTGGAGCCCAGGGA
TCTGCCTGCTTCTGCCTCCTGAATGTCGGGAATAAAAATCATGGACTGCCACACATGGCTATAAAATGCCT
CTGTATTTTCAAACCCATTTTGAAAATGTAAAATAACCAAATGTGGTAAATGAGTGATTGTCCTATGGAAA
ATAGTGTGAATAAATTCAAAAAGTCCAAAGTAGTTTAAGTCATGATATTCTTTTATTAGTGCTAGCTCCTT
TTAATTTTTATGAGCTAAACAATTTAATATAAAAATGTCATTTCCTGTTCATACAGTATATAAAAAAGTAT
AGTGGTCTGGTTAGTTTTCAATAGTTTGCTTTTAGCCAGATGTCATATAAGTCTATGACTGTAACAAGTGA
GAACAGTATAAATAAGTTCTGTAGTATTTACACTTAAACAGAAACTCTCCCAAATGGTGCCTAAGAAATTA
ACTTGAGAGTTAAATGAAACTGATTCAACATTGAGACTTTAATGCTTTGTAAGTTTCATATTATTGCTATA
CTAGCTTTGGCTATAATTCTGCATAGTTACTTAWAAAGTGG

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LoxP Raw sequencing data (LoxP highlighted in yellow; KpnI, ScaI, MscI EcoRI. Cassette is 68bps)

```
AGTCAGWTGGTAGAGTACCSGACTGCTCTTCTGAAGGTCCGGAGTTCAATCCCAGCAACCRCAAGGTGGCT
CACAACATCCATAACAAGATCTGACTCCCTCTTCTGGAGTGTCTGAAGACAGCTACAGTGTACTTACATAT
AATAAATAAATAAATCTTTAAAAAAAAAAATAAGGCCTGTAAACTACAAGTCCATTTTACTGTATAGCTGG
AAACAGGAATCAGAATAATTTTCCCAGTACTTGGCCAGGTACCATAACTTCGTATAGCATACATTATACGA
AGTTATGAATTCGTCGCCACCGTGGAAACTGGATATAGGTATATAAAATATTTTGACTAGTAAAGAACAAC
TATTAATCAGCATTTGGATTAAAAAAATCTTAATCTGTTGTTTGAAGCATTCTGCTAGATATTATGGGTAC
AGATTAAGTCCTAATGAATGTTTTTATCCATTTTGAAGTCTGCCTTTAAATACATGGAGTGAAATAACCTA
GGAGTGTATTAATATGGAGTCACTGGGAGGAGGAAATGTTTCATTTTATAAAAGCAGCCTGAGAGCTGTAG
GCCCTGCTGCTGTCTGTTCTTCATGCCTTGGTTCTCACTCACATGAATCAATGTCACGTCAATCTTGGGCT
TTCTTCACTTGCATTTCAGTCGCTTGCCCTGGATATCCCCAGCCACGCTGAARGTTCARTCTTCACTGCCC
CTCATCACGCCCAGTCCCTCAATAGCTCTCTGGAGGCAGCCATGACTGATTTAGCAGCATAGAGCAAGACA
TGGAGCAAGTTTGGCAGAGCTATTTTCCATTCCCGATTACAGGTAAGAGAGCTCCTAGGAGTGTGCTGTTT
TCTGCRGGCCCTTTTAARTTAKTCATCCT
```

NB/ Raw sequencing data is aligned with the genomic sequence predicted from the ENSEMBL database.

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3. Backbone Sequence

3'end of BAC subclone joins here
ATCGATGATATCAGATCTGCCGGTCTCCCTATAGTGAGTCGTATTAATTTCGATAAGCCAGGTTAACCTGC
ATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACT
GACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATC
CACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
AGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGT
CAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC
TCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTC
ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCC
CCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTT
ATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCT
TGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT
ACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGT
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TACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATACCTGCAGGCG**CGCCATTTAAATGCG**
GCCGC
5' end of BAC subclone joins here

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4. Cassette Sequence

LoxP/FRT PGK-gb2-Neo Cassette (5'-3')

LoxP sites are <mark>highlighted in yellow</mark>
FRT sites are ***Bold/Italics***
N1 and N2 sequencing primers <u>are underlined</u>
ATG and **STOP** codons are indicated

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CCTAGGCGTACGGTGTTGACGAGGCGTCCGAAGAACGGATCCAAGCTTATGCATGAATTCTGCAGGTACCA
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TGTACACTTAAGCCGGCGTACG
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