



JENSEN: Construction of Il1rl2 cKO And mCherry KI Targeting Vector

Sections:

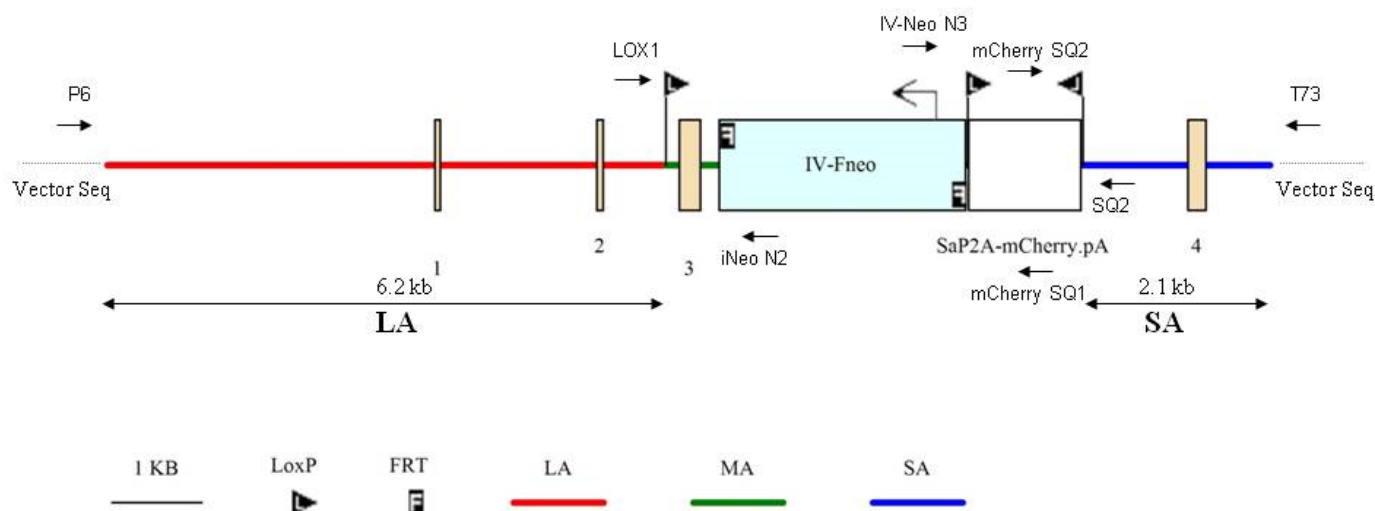
1. Vector Design Outline
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1. Vector Design Outline

A 9.4 kb region used to construct the targeting vector was first sub cloned from a positively identified C57BL/6 BAC clone (RP23-216C5) using a homologous recombination-based technique. The region is designed in such a way that the long homology arm (LA) extends ~6.2 kb 5' to the distal LoxP cassette and the short homology arm (SA) extends ~2.1 kb 3' to the inversion cassette. A FRT-flanked Neo cassette was inserted downstream of exon 3 and placed immediately upstream of the inversion cassette.

The targeting vector was confirmed by restriction analysis and sequencing after each modification step. The boundaries of the 2 homology arms were confirmed by sequencing with P6 and T73 primers that read through both sides of the backbone vector into the genomic sequences. IV-Neo N3 and iNeo N2 primers read from the selection cassette into the inversion cassette (IV-Neo N3) and the 3' end of the MA (iNeo N2). Primer JENS LOX1 was used to sequencing confirm the distal LoxP insertion. Primers mCherry SQ1-2 and JENS SQ2 were used to confirm the remaining sequence of the inversion cassette.

Targeting Vector For Il1rl2 cKO and mCherry Expression



Primers used for sequencing:

Primer P6:	5' - GAG TGC ACC ATA TGG ACA TAT TGT C	-3'
Primer T73:	5' - TAA TGC AGG TTA ACC TGG CTT ATC G	-3'
Primer iNeo N2:	5' - AGT ATG GCT TTC CTT CCC GAT GG	-3'
Primer IVNeo N3:	5' - TCT AAG GCC GAG TCT TAT GAG CAG	-3'
Primer JENS LOX1:	5' - AGG GAA GCT GTC TTT AGA ACC AAG C	-3'
Primer JENS SQ2:	5' - CAT TAC CTC TGG CAC ATG GAT TC	-3'
Primer mCherry SQ1:	5' - AGA CCG CCA AGC TGA AGG TGA C	-3'



Primer mCherry SQ2: 5'- AGT TCA TCA CGC GCT CCC ACT TG -3'



Backbone Vector Information

iTL cloning vector (~2.45 kb) derived from pSP72 (Promega) contains an ampicillin selection cassette for retransformation of the construct prior to electroporation. A FRT flanked Neomycin cassette was inserted into the gene as described in the project schematic. The targeting construct can be linearized with NotI prior to the electroporation of ES cells.

The total size of the targeting construct (including vector backbone and a DT cassette) is ~ 16.9kb.



2. Sequence Data Analysis

Sequencing Data from Targeting Construct

P6 primer sequencing data aligned with targeted allele sequence

Query	10449	AGC-ACAAACCTTTGTCCATGCTGCTCTACTTGAAGAATGCTCTGTAAGTTGCTATTAAG	10507
Sbjct	130	AGCTACAAACCTTTGTCCATGCTGCTCTACTTGAAGAATGCTCTGTAAGTTGCTATTAAG	189
Query	10508	GCTGCCAAATGGATAGCTTTTCTGTTTCTCTTGGCCCTCTGGATTTTCTTTCCCCTAGTT	10567
Sbjct	190	GCTGCCAAATGGATAGCTTTTCTGTTTCTCTTGGCCCTCTGGATTTTCTTTCCCCTAGTT	249
Query	10568	TTTAGATAGGCAGAACCAAGCCACCCTTTCCAAAGTCACTTGCTCCTCTCCCTGATAGTT	10627
Sbjct	250	TTTAGATAGGCAGAACCAAGCCACCCTTTCCAAAGTCACTTGCTCCTCTCCCTGATAGTT	309
Query	10628	TGCTTGGTGTGGTTACAGAAGGACAGTAGCTCTTCCGGTTCTTGGCTGACTACATTCCAC	10687
Sbjct	310	TGCTTGGTGTGGTTACAGAAGGACAGTAGCTCTTCCGGTTCTTGGCTGACTACATTCCAC	369
Query	10688	AGAGACTGTGCGGCTCTCTCACACACAGGGTTGCAGTGTGGGCACACACCTAGAAATCA	10747
Sbjct	370	AGAGACTGTGCGGCTCTCTCACACACAGGGTTGCAGTGTGGGCACACACCTAGAAATCA	429
Query	10748	CTGTGAGTGTGAGAGTCAAAACCGATGTCCCTTTGAAATGAATGCTTCTTTATTAGTTAAG	10807
Sbjct	430	CTGTGAGTGTGAGAGTCAAAACCGATGTCCCTTTGAAATGAATGCTTCTTTATTAGTTAAG	489
Query	10808	TATTAATGTTTAAAGCTTTACTTACAAGTACCTGGGCTATGGGACTGCCTTTGCTCCCG	10867
Sbjct	490	TATTAATGTTTAAAGCTTTACTTACAAGTACCTGGGCTATGGGACTGCCTTTGCTCCCG	549
Query	10868	ATTCATGCCAACCTTCATTAATGGCCAAGTGTGCTGAGACAGGAGCCAGGTCCTCTAACT	10927
Sbjct	550	ATTCATGCCAACCTTCATTAATGGCCAAGTGTGCTGAGACAGGAGCCAGGTCCTCTAACT	609
Query	10928	CCCAGATAACTAGTTACCCTTTCTAACCATCTTCTTTCTGCAGATGACATTGGAGAGGAA	10987
Sbjct	610	CCCAGATAACTAGTTACCCTTTCTAACCATCTTCTTTCTGCAGATGACATTGGAGAGGAA	669
Query	10988	TAAATGCAAGTCCTTTTATTTTGTATAAGGACATATGTCCGAGTTAAGTTTGAAAAGGTA	11047
Sbjct	670	TAAATGCAAGTCCTTTTATTTTGTATAAGGACATATGTCCGAGTTAAGTTTGAAAAGGTA	729
Query	11048	GCCCCAGGAGCATCAAGAGTAGATAAGGATTTAGGGTGTAAATGTACAAGCAGAATGAGT	11107
Sbjct	730	GCCCCAGGAGCATCAAGAGTAGATAAGGATTTAGGGTGTAAATGTACAAGCAGAATGAGT	789
Query	11108	TGCAGCACTGGTTGGCACCAGAACTTGCCAGCAGCTCCAGGGCCCTGTGACTAGGAGCCA	11167
Sbjct	790	TGCAGCACTGGTTGGCACCAGAACTTGCCAGCAGCTCCAGGGCCCTGTGACTAGGAGCCA	849
Query	11168	GCACCACTTGGAAGTGTGACGGGCAGATGGCTTCCTGCTTGCCAGGCTGGAAAGACCAGG	11227
Sbjct	850	GCACCACTTGGAAGTGTGACGGGCAGATGGCTTCCTGCTTGCCAGGCTGGAAAGACCAGG	908

Note: shaded sequence is from backbone vector

Query: Respective Targeted Allele Sequence

Sbjct: Sequencing data from Targeting Vector





JENS LOX1 primer sequencing data aligned with targeted allele sequence: genomic sequence is in plain text; 5' Loxp cassette is highlighted with LoxP site underlined

Query	16421	TCTGACTGCAAGTATAGGCAGGAAAACGACCAGGTTGGTTCTATGTTGAGACAGAGACTA	16480
Sbjct	13	TCTGNCTGC-AGTATAGGCAGG-AAACGACCAGGTTGGTTCTATGTTGAGACAGAGACTA	70
Query	16481	AGGGCAGAAGAGTGAACAGATACAGACACAGGGGCCGTGGTTCTGTGGAGGGTCCTGGGG	16540
Sbjct	71	AGGGCAGAAGAGTGAACAGATACAGACACAGGGGCCGTGGTTCTGTGGAGGGTCCTGGGG	130
Query	16541	AAGCCTTGTCCTCACTATACATTGAAGGCTTAGCATTATGCTTCTAAATGACTGGGCT	16600
Sbjct	131	AAGCCTTGTCCTCACTATACATTGAAGGCTTAGCATTATGCTTCTAAATGACTGGGCT	190
Query	16601	AGTTTGGGGAAACACCCCAAATAA <u>CTTCGTATAATGTATGCTATACGAAGTTATGT</u> ACAA	16660
Sbjct	191	AGTTTGGGGAAACACCCCAAATAA <u>CTTCGTATAATGTATGCTATACGAAGTTATGT</u> ACAA	250
Query	16661	ACTCCTAAATGCAATTTACATTGACCAGAGCAAAATATTCCTAAAAAGAATAATGACT	16720
Sbjct	251	ACTCCTAAATGCAATTTACATTGACCAGAGCAAAATATTCCTAAAAAGAATAATGACT	310
Query	16721	CCAGTTTCTGCCttttattttattttattttattttattttattttattgttttC	16780
Sbjct	311	CCAGTTTCTGCCTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTGTTTC	370
Query	16781	CTTTCCTTCCCCAGATACGTGTGAGGACATTTTATGCACAATGTGATAATTCAGAGGG	16840
Sbjct	371	CTTTCCTTCCCCAGATACGTGTGAGGACATTTTATGCACAATGTGATAATTCAGAGGG	430
Query	16841	CCAGCCTTTTCCTTTCAACTGCACATACCCGCCAGAAACAAACGGGGCAGTAAATCTGAC	16900
Sbjct	431	CCAGCCTTTTCCTTTCAACTGCACATACCCGCCAGAAACAAACGGGGCAGTAAATCTGAC	490
Query	16901	ATGGTACAAAACACCTAGCAAAAGCCCAGTATCTAACAACAGACACCTTAGAGTTCACCA	16960
Sbjct	491	ATGGTACAAAACACCTAGCAAAAGCCCAGTATCTAACAACAGACACCTTAGAGTTCACCA	550
Query	16961	GGACCAGACCTGGATCTTGTTTCTTCCATTGACACTGGAGGACTCCGGTATCTATCAGTG	17020
Sbjct	551	GGACCAGACCTGGATCTTGTTTCTTCCATTGACACTGGAGGACTCCGGTATCTATCAGTG	610
Query	17021	TGTTATAAGGTAAGTCCTTCATTTAAAGTGGAATAA-TTCCCAAGTCTCCCTCTCCATT	17079
Sbjct	611	TGNTATAANGNAAGTCCTTCATTTAAAGTGGNACTAATTTCCCAAGTCTCCCTCTCCATT	670

Query: Respective Targeted Allele Sequence
Sbjct: Sequencing data from Targeting Vector



iNeoN2 primer sequencing data aligned with targeted allele sequence. genomic sequence is in plain text; Neo sequence is in red text with FRT site underlined;

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Query 16762  attttatatttattgttttCCTTTCCTTCCCCAGATACGTGTGAGGACATTTTATGCACA 16821
            |||
Sbjct 661    ATTTTATTTTA-NGTTTTCCTTTCNTTCCCCAGATACGTGTGAGGACATTTTATGCACA 603

Query 16822  ATGTGATAATTTTCAGAGGGCCAGCCTTTTCCTTCAACTGCACATACCCGCCAGAAACAA 16881
            |||
Sbjct 602    ATGTGATAATTTTCAGAGGGCCAGCCTTTTCCTTCAACTGCACATACCCGCCAGAAACAA 543

Query 16882  ACGGGGCAGTAAATCTGACATGGTACAAAACACCTAGCAAAAGCCCAGTATCTAACAACA 16941
            |||
Sbjct 542    ACGGGGCAGTAAATCTGACATGGTACAAAACACCTAGCAAAAGCCCAGTATCTAACAACA 483

Query 16942  GACACCTTAGAGTTCACCAGGACCAGACCTGGATCTTGTTTCTTCCATTGACACTGGAGG 17001
            |||
Sbjct 482    GACACCTTAGAGTTCACCAGGACCAGACCTGGATCTTGTTTCTTCCATTGACACTGGAGG 423

Query 17002  ACTCCGGTATCTATCAGTGTGTTATAAGGTAAGTCCTTCATTTAAAGTGGAACATAATTCC 17061
            |||
Sbjct 422    ACTCCGGTATCTATCAGTGTGTTATAAGGTAAGTCCTTCATTTAAAGTGGAACATAATTCC 363

Query 17062  CAAGTCTCCCTCTCCATTTCTAAAGACAACCTTTTAAAGAAAATGGGTATTATTGGCCTTT 17121
            |||
Sbjct 362    CAAGTCTCCCTCTCCATTTCTAAAGACAACCTTTTAAAGAAAATGGGTATTATTGGCCTTT 303

Query 17122  ATTTTGAGACTTGTTATATAGCCTAGACAAGTTTCCAAGTCTTCTTAAAGTACTGAAA 17181
            |||
Sbjct 302    ATTTTGAGACTTGTTATATAGCCTAGACAAGTTTCCAAGTCTTCTTAAAGTACTGAAA 243

Query 17182  GGACAGGTGTGAACCACCACAACCAGCCGCTACACACCACAACCAGCTGCTACACAAATG 17241
            |||
Sbjct 242    GGACAGGTGTGAACCACCACAACCAGCCGCTACACACCACAACCAGCTGCTACACAAATG 183

Query 17242  CGTACGTTCGTGGGATTGTGTCCGTGTCGCGAAGTTCCTATACTTTCTAGAGAATAGGAA 17301
            |||
Sbjct 182    CGTACGTTCGTGGGATTGTGTCCGTGTCGCGAAGTTCCTATACTTTCTAGAGAATAGGAA 123

Query 17302  CTTCCCGCGGTTGTAAGTTCTCCAGATCTAGAGTAAGACAGAAGTCTGGAAGACATGGGA 17361
            |||
Sbjct 122    CTTCCCGCGGTTGTAAGTTCTCCAGATCTAGAGTAAGACAGAAGTCTGGAAGACATGGGA 63

Query 17362  GTGAGTTGTGAGGTGATCCAGGAAGAGACCTTCTGCAATCCAGTGACCA 17410
            |||
Sbjct 62    GTGAGTTGTGAGGTGATCCAGGAAGAGACNT-CTGCA-TCCAGNGANCA 16

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Query: Respective Targeted Allele Sequence
 Sbjct: Sequencing data from Targeting Vector

IV-NeoN3 primer sequencing data aligned with targeted allele sequence: Neo sequence is in **red text** with FRT site underlined; Lox71 site highlighted in **yellow**; Sa.**P2A**-mCherry.pA inversion cassette is in **blue text**.

Query	19781	GGACGCTCGCTGCGCCCTTCGTCTGACGTGGCAGCGCTCGCCGTGAGGAGGGGGGCGCCC	19840
Sbjct	27	GGACGCTCGCTGCGCCCTTCGTCTGACGTGGCAGCGCTCGCCGTGAGGAGGGGGGCGCCC	86
Query	19841	GCGGGAGGCGCCAAAACCCGGCGCGGAGGCCGCATGCTCCAGACTGCCTTGGGAAAAGCG	19900
Sbjct	87	GCGGGAGGCGCCAAAACCCGGCGCGGAGGCCGCATGCTCCAGACTGCCTTGGGAAAAGCG	146
Query	19901	CCTCCCCTACCCGGTAGAATGAAGTTCCTATACTTTCTAGAGAATAGGAACCTCGTTGGT	19960
Sbjct	147	CCTCCCCTACCCGGTAGAATGAAGTTCCTATACTTTCTAGAGAATAGGAACCTCGTTGGT	206
Query	19961	ACCGTACGCAATTGTACCGTTCGTATAATGTATGCTATACGAAGTTATCTAGCGCGCCTC	20020
Sbjct	207	ACCGTACGCAATTGTACCGTTCGTATAATGTATGCTATACGAAGTTATCTAGCGCGCCTC	266
Query	20021	GACTCTAGCCCCCTCGACTAGAGGATCGAGCCCCAGCTGGTTCCTTCCGCCTCAGAAGCC	20080
Sbjct	267	GACTCTAGCCCCCTCGACTAGAGGATCGAGCCCCAGCTGGTTCCTTCCGCCTCAGAAGCC	326
Query	20081	ATAGAGCCACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTG	20140
Sbjct	327	ATAGAGCCACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTG	386
Query	20141	TCCTGccccaccccacccccAGAAATAGAATGACACCTACTCAGACAATGCGATGCAATT	20200
Sbjct	387	TCCTGCCCCACCCACCCCCAGAAATAGAATGACACCTACTCAGACAATGCGATGCAATT	446
Query	20201	TCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACG	20260
Sbjct	447	TCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACG	506
Query	20261	GGGGAGGGGCAAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCAGCGA	20320
Sbjct	507	GGGGAGGGGCAAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCAGCGA	566
Query	20321	GCTCACTTGTACAGCTCGTCCATGCCGCCGGTGGAGTGGCGGCCCT-CGGCGCGT-TCGT	20378
Sbjct	567	GCTCANTNGTANNGCTCGTACATGNGGTGGTGGAGTGGCGGCCCTACGGCGCGTNTCGT	626

Query: Respective Targeted Allele Sequence
Sbjct: Sequencing data from Targeting Vector



mCherry SQ1 primer sequencing data aligned with targeted allele sequence: Sa. **P2A-mCherry**. pA inversion cassette is in **blue** text.

Query	20124	AATCCTCCCCCTTGCTGTCCTGccccacccccccccccAGAATAGAATGACACCTACTCA	20183
Sbjct	732	AATCCTCCCCCTTGCTGTCNTGCCCCACCCACCCCCAGAAATAGAATGACACCTACTCA	673
Query	20184	GACAATGCGATGCAATTTCTCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCA	20243
Sbjct	672	GACAATGCGATGCAATTTCTCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCA	613
Query	20244	GGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAAGGCACAG	20303
Sbjct	612	GGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAAGGCACAG	553
Query	20304	TCGAGGCTGATCAGCGAGCTCACTTGTACAGCTCGTCCATGCCGCCGGTGGAGTGGCGGC	20363
Sbjct	552	TCGAGGCTGATCAGCGAGCTCACTTGTACAGCTCGTCCATGCCGCCGGTGGAGTGGCGGC	493
Query	20364	CCTCGGCGCGTTTCGTACTGTTCCACGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCCA	20423
Sbjct	492	CCTCGGCGCGTTTCGTACTGTTCCACGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCCA	433
Query	20424	ACTTGATGTTGACGTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGCCCTTGTAGGTGG	20483
Sbjct	432	ACTTGATGTTGACGTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGCCCTTGTAGGTGG	373
Query	20484	TCTTGACCTCAGCGTCGTAGTGGCCGCCGTCTTCAGCTTCAGCCTCTGCTTGATCTCGC	20543
Sbjct	372	TCTTGACCTCAGCGTCGTAGTGGCCGCCGTCTTCAGCTTCAGCCTCTGCTTGATCTCGC	313
Query	20544	CCTTCAGGGCGCCGTCTCGGGGTACATCCGCTCGGAGGAGGCCTCCCAGCCCATGGTCT	20603
Sbjct	312	CCTTCAGGGCGCCGTCTCGGGGTACATCCGCTCGGAGGAGGCCTCCCAGCCCATGGTCT	253
Query	20604	TCTTCTGCATTACGGGGCCGTTCGAGGGGAAGTTGGTGCCGCGCAGCTTCACCTTGTAGA	20663
Sbjct	252	TCTTCTGCATTACGGGGCCGTTCGAGGGGAAGTTGGTGCCGCGCAGCTTCACCTTGTAGA	193
Query	20664	TGAACTCGCCGTCCTGCAGGGAGGAGTCTGGGTACCGGTACACGCGCCGCTCCTCGA	20723
Sbjct	192	TGAACTCGCCGTCCTGCAGGGAGGAGTCTGGGTACCGGTACACGCGCCGCTCCTCGA	133
Query	20724	AGTTCATCAGCGCTCCCACTTGAAGCCCTCGGGGAAGGACAGCTTCAAGTAGTCGGGGA	20783
Sbjct	132	AGTTCATCAGCGCTCCCACTTGAAGCCCTCGGGGAAGGACAGCTTCAAGTAGTCGGGGA	73
Query	20784	TGTCGGCGGGGTGCTTCACGTAGGCCTTGGAGCCGTACATGAAGTGAAGGGACAGG	20839
Sbjct	72	TGTCGGCGGGGTGCTTCACGTAGGCCTTGGAGCCGTACATGAAGTGAAGGG-ACAGG	18

Query: Respective Targeted Allele Sequence
Sbjct: Sequencing data from Targeting Vector



mCherry SQ2 primer sequencing data aligned with targeted allele sequence: Sa.P2A-mCherry.pA inversion cassette is in blue text; Lox66 cassette sequence is highlighted in yellow with Lox66 site underlined; genomic sequence is in plain text.

Query	20787	CGGCGGGGTGCTTCACGTAGGCCTTGGAGCCGTACATGAACTGAGGGGACAGGATGTCCC	20846
Sbjct	19	CGGCGGGGTGCTTCACGTAGGCCTTGGAGCCGTACATGAACTGAGGGGACAGGATGTCCC	78
Query	20847	AGGCGAAGGGCAGGGGGCCACCCTTGGTCACCTTCAGCTTGGCGGTCTGGGTGCCCTCGT	20906
Sbjct	79	AGGCGAAGGGCAGGGGGCCACCCTTGGTCACCTTCAGCTTGGCGGTCTGGGTGCCCTCGT	138
Query	20907	AGGGGCGGCCCTCGCCCTCGCCCTCGATCTCGAACTCGTGGCCGTTTACGGAGCCCTCCA	20966
Sbjct	139	AGGGGCGGCCCTCGCCCTCGCCCTCGATCTCGAACTCGTGGCCGTTTACGGAGCCCTCCA	198
Query	20967	TGTGCACCTTGAAGCGCATGAACTCCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGC	21026
Sbjct	199	TGTGCACCTTGAAGCGCATGAACTCCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGC	258
Query	21027	TCACCATAGGTCCAGGGTTCTCCTCCACGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAG	21086
Sbjct	259	TCACCATAGGTCCAGGGTTCTCCTCCACGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAG	318
Query	21087	TAGCTCCGCTTCCGTCCTCACACGTATCTGGGGAAGGAAAGGaaaacaataaaaataaaaat	21146
Sbjct	319	TAGCTCCGCTTCCGTCCTCACACGTATCTGGGGAAGGAAAGGAAACAATAAAATAAAAT	378
Query	21147	aaaataaaaataaaaataaaaataaaaAGGCAGAAAACCTGGAGTCATTATTTCTTTTAGG	21206
Sbjct	379	AAAATAAAATAAAATAAAATAAAATAAAGGCAGAAAACCTGGAGTCATTATTTCTTTTAGG	438
Query	21207	GAATATTTTGTCTCTGGTCAATGTGAAATTGCATTTAGGAGTTGTACCGTTCGTATAGCA	21266
Sbjct	439	GAATATTTTGTCTCTGGTCAATGTGAAATTGCATTTAGGAGTTGTACCGTTCGTATAGCA	498
Query	21267	TACATTATACGAAGTTATACGCGTAGGGCAGCCTTGAAGTGGCAGGAGATGACGTCCAGT	21326
Sbjct	499	TACATTATACGAAGTTATACGCGTAGGGCAGCCTTGAAGTGGCAGGAGATGACGTCCAGT	558
Query	21327	GAGCACTTTTGAAAGCAGAACAGCTTTAGAAATAACTTTTGGAGCACAGATTGCACCATC	21386
Sbjct	559	GAGCACTTTTGAAAGCAGAACAGCTTTAGAAATAACTTTTGGAGCACAGATTGCACCATC	618
Query	21387	TTCTTCATTGAAGTTTGTAGCCAGACCAGCTTACAGAGAGAGGTGTTTATCAATGAGAT	21446
Sbjct	619	TTCTTCATTGAAGTTTGTAGCCAGACCAGCTTACAGAGAGAGGTGTTTATCAATGAGAT	678
Query	21447	TGCTGCCATTTCCGAAGATGGCTAATGGTTTTGGAAAACCTGGCTGATCCAGACCCAA-CT	21505
Sbjct	679	TGCTGCCATTTCCGAAGATGGCTAATGGTTTTGGAAAACCTGGCTGATCCAGACCCAACT	738

Query: Respective Targeted Allele Sequence
Sbjct: Sequencing data from Targeting Vector



JENS SQ2 primer sequencing data aligned with targeted allele sequence: Sa.**P2A**-mCherry.pA inversion cassette is in **blue** text; Lox66 cassette sequence is highlighted in **yellow** with Lox66 site **underlined**; genomic sequence is in plain text.

Query	20806	GGCCTTGGAGCC-GTACATGAACTGAGGGG-ACAGGATGTCCAGGCCAAGGGCAGGGG	20863
Sbjct	775	GGCCTTGGAGCCCGTACATGAACTGAGGGGACAGGATGTCCAGGCCAAGGGCAGGGG	716
Query	20864	CCACCCTTGGTCACCTTCAGCTTGGCGGTCTGGGTGCCCTCGTAGGGGCGGCCCTCGCCC	20923
Sbjct	715	CCACCCTTGGTCACCTTCAGCTTGGCGGTCTGGGTGCCCTCGTAGGGGCGGCCCTCGCCC	658
Query	20924	TCGCCCTCGATCTCGAACTCGTGGCCGTTACGGAGCCCTCCATGTGCACCTTGAAGCGC	20983
Sbjct	657	TCGCCCTCGATCTCGAACTCGTGGCCGTTACGGAGCCCTCCATGTGCACCTTGAAGCGC	598
Query	20984	ATGAACTCCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGCTCACC <u>CAT</u> AGGTCCAGGG	21043
Sbjct	597	ATGAACTCCTTGATGATGGCCATGTTATCCTCCTCGCCCTTGCTCACCATAGGTCCAGGG	538
Query	21044	TTCTCTCCACGCTTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCGTCC	21103
Sbjct	537	TTCTCTCCACGCTTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCGTCC	478
Query	21104	TCACACGTATCTGGGAAGGAAAGGaaaacaataaaaataaaaataaaaataaaaataa	21163
Sbjct	477	TCACACGTATCTGGGAAGGAAAGGAAAACAATAAAATAAAATAAAATAAAATAAAATAA	418
Query	21164	aaaaataaaaGGCAGAAAACCTGGAGTCATTATTCTTTTAGGGAATATTTTGCTCTGGT	21223
Sbjct	417	AATAAAATAAAGGCAGAAAACCTGGAGTCATTATTCTTTTAGGGAATATTTTGCTCTGGT	358
Query	21224	CAATGTGAAATTGCATTTAGGAGTTTG <u>TACCGTTCGTATAGCATACATTATACGAAGTTA</u>	21283
Sbjct	357	CAATGTGAAATTGCATTTAGGAGTTTGACCGTTCGTATAGCATACATTATACGAAGTTA	298
Query	21284	<u>TACGCGT</u> AGGGCAGCCTTGAAGTGGCAGGAGATGACGTCCAGTGAGCACTTTTGAAAGCA	21343
Sbjct	297	TACGCGTAGGGCAGCCTTGAAGTGGCAGGAGATGACGTCCAGTGAGCACTTTTGAAAGCA	238
Query	21344	GAACAGCTTTAGAAATAACTTTTGAGACACAGATTGCACCATCTTCTTCATTGAAGTTT	21403
Sbjct	237	GAACAGCTTTAGAAATAACTTTTGAGACACAGATTGCACCATCTTCTTCATTGAAGTTT	178
Query	21404	AGCCAGACCAGCTTACAGAGAGAGGTGTTTTATCAATGAGATTGCTGCCATTTCCGAAG	21463
Sbjct	177	AGCCAGACCAGCTTACAGAGAGAGGTGTTTTATCAATGAGATTGCTGCCATTTCCGAAG	118
Query	21464	ATGGCTAATGGTTTTTGAAAACCTGGCTGATCCAGACCCAACTAGATCATTCAAACACATC	21523
Sbjct	117	ATGGCTAATGGTTTTTGAAAACCTGGCTGATCCAGACCCAACTAGATCATTCAAACACATC	58
Query	21524	TGATAAGGAATCAGCTTAAGGCAGACATTTGAAAGCTAGCAAATCTGGG	21572
Sbjct	57	TGATAAGGAATCAGCTTAAGGCAGACATT-GAAAGCTAGNAAANCTGGG	10

Query: Respective Targeted Allele Sequence
Sbjct: Sequencing data from Targeting Vector

**T73 primer sequencing data aligned with respective targeted allele sequence**

```
Query 23100 ACA-CAAATTCCTG-CCTAAGAAAGACTCCTC-AGGGC-TATCTGTGTTT-TGTTACTCA 23154
          ||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 298 ACACCAAATTCCTTGCCCTAAGAAAGNNTCCTCCAGGGCTTATCTGNGTTCTTGTTACTCA 239

Query 23155 -CCAAACCTTGTCCTCCAGCCATGGCTGCTGCTATCAACTACTGCCACCCTCCTCCCGCCA 23213
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 238 CCAAACCTTGTCCTCCAGCCATGGCTGCTGCTATCAACTACTGCCACCCTCCTCCCGCCA 179

Query 23214 CTCCCCACCTCAGTATTTGAAAACCTCAGTTGTATCTTAGCCTCTTCTCTTTGAAATGTGA 23273
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 178 CTCCCCACCTCAGTATTTGAAAACCTCAGTTGTATCTTAGCCTCTTCTCTTTGAAATGTGA 119

Query 23274 ACAGTTTCCCATGCTCCTCTTTCTTCATCCTGTGTTTGCTTTAACCTGGCCTTCTCTCCT 23333
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 118 ACAGTTTCCCATGCTCCTCTTTCTTCATCCTGTGTTTGCTTTAACCTGGCCTTCTCTCCT 59

Query 23334 CAAGGCTCAGCTAGACACT 23352
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 58 CAAGGCTCAGCTAGACACT 40
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Query: Respective Targeted Allele Sequence

Sbjct: Sequencing data from Targeting Vector



Backbone Vector Sequence

3' end of BAC subclone joins here

catCGATGATGGGCCACATTGG CCTCGACGATATCGCGATCGCCGATAAGCCAGGTTAACCTGCATTAACGCGCCGTCGACGCGGCGCG
TTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
AGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAA
AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCGTCCGCTTACCGGATACCTGT
CCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTG
GGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGGTAAGACACGACTT
ATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACT
ACGGCTACACTAGAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGC
AAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT
CTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTTTGGTTCATGAGATTATCAAAAAAGGATCTTCACCTAGA
TCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC
ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGAAGGGCCG
AGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATA
GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGC
AGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGGTACT
CAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAAATACCGCGCCACATAGCAGA
ACTTTAAAAGTGCTCATCATTTGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACC
CACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAA
AGGGAATAAGGGCGACACGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATG
AGCGGATACATATTTGAATGTATTTAGAAAAATAAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGA
AACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACC
TCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTGCGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTGCTTAGAACG
CGGCTACAATTAATACATAACCTTATGTATCATAACATACGATTTAGGTGACACTATAGAACTCGATGCGGCCCTTCAGGCGCGCCA
TTTAAATGCGGCCGCACTCAGGATGTCCCTGAAGct

5' end of BAC subclone joins here



3. Cassette Sequences

FRT flanked IV-UBS-Neo Cassette (3'-5')

FRT sites are ***Bold/Italics***

ATG and **STOP** codons are indicated

GTACGTTTCGTGGGATTGTGTCCGTGTGCGC***GAAGTTCCTATACTTTCTAGAGAATAGGAAC******TTCC***CGCGGTTGTAAGTTCTCCAGATCTA
GAGTAAGACAGAAGTCTGGAAGACATGGGAGTGAGTTGTCAGGTGATCCAGGAAGAGACCTTCTGCAATCCAGTGACCAATTAATTACA
GCAGAAAGGACCATCGGGAAGGAAAGCCATACTCTCCAGGAACGTCATTAGTCGGGATCTTCAGTTGCTACAAGAAGCAGATGTCAAAC
GGCCTTCCCCTAACCATGTGAGAAAGTGAGCTTTCACTGGCCCGGGTGTGAAGTGATTCTAATGGAATAAATGGATTTGCTAAGGAATAG
TTTCCTCAGAAAGAAATCCTGGGAGCAAGTGGGGAAGCTGACTCAGCAAAACAGAGCTGTTTCTTGAGGACGATGCCAATAGCAATCAT
TTGACCAAACTGAAGTGCCGTCAGGAGGCATGAGGATCTGATATCAGGGAGCTCTCAGACGTCGCTTGGTCGGTCTTTATTTCGAACCC
CAGAGTCCCGC***TCAG***GAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAA
GCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCTTGATAGCGGTCCGCCACACCCAGCCGGC
CACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATATTTCGGCAAGCAGGCATCGCCATGGGTACAGACGAGATCCTCGCCG
TCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTTCGGCTGGCGCGAGCCCTGATGCTCTTCGTCCAAATCATCCTGATCGACAAGACC
GGCTTCCATCCGTGTCCGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCC
GCATTGCATCAGCCATGATGGATACTTTCTCGGCCGGAGCAAGATGAGATGACAGGAGATCCTGCCCGGCACTTCGCCCAATAGCAGC
CAGTCCCTTCCCCTTCCTGTCGACACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCTGTCGCGCAGCCACGATAGCCGCGCTGCCTCGTC
CTGCAATTCAATCAAGGCACCGGATAGGTCTGACAAAAAGAACCGGGCGCCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAG
AGCAGCCGATCGTCTGTTGTGCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCA
ATGGCCGATCC***CAT***GGTTTAGTTCTCACCTTGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACACGTCTAAC
AAAAAGCCAAAAACGGCCAGAATTTAGCGGACAATTTACTAGTCTAACACTGAAAATTACATATTGACCCAAATGATTACATTTCAA
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ATAAAACCGACCAAAGAACTGACGCCTCACTTATCCCTCCCCTCACCCGAGGTCCGGCGCCTGTCGATTCAGGAGAGCCTACCCTAGG
CCCGAACCCCTGCGTCTGCGACGGAGAAAAAGCCTACCGCACACCTACCGGCAGGTGGCCCCACCTGCATTATAAGCCAAACAGAACGGG
TGACGTCACGACACGACGAGGGCGCGCGCTCCCAAAGTTACGGGTGCACTGCCCAACGGCACCGCCATAACTGCCGCCCCCGCAACAGA
CGACAAACCGAGTTCTCCAGTCAGTGACAACTTCACGTACGGGTCCCAGATGGTGCCCCAGCCCATCTCACCCGAATAAGAGCTTTC
CCGCATTAGCGAAGGCCCTCAAGACCTTGGGTTCTTGCCGCCCACCATGCCCCCACCTTGTTTCAACGACCTCACAGCCCGCCTCACAA
GCGTCTTCCATTCAAGACTCGGGAACAGCCGCCATTTTGCTGCGCTCCCCCAACCCCAAGTTTCAGGGCAACCTTGCTCGCGGACCCAG
ACTACAGCCCTTGGCGGTCTCTCCACACGCTTCCGTCCCACCGAGCGGCCCGGGCGGCCACGAAAGCCCCGGCCAGCCAGCAGCCCGCT
ACTACCAAGTGACGATCACAGCGATCCACAAACAAGAACTGCGACCCAAATCCCGGCTGCGACGGAAGTACTGTGCCACACCCGGCG
CGTCCTTATATAATCATCGGCGTTACCCGCCCCACGGAGATCCCTCCGCAGAATCGCCGAGAAGGGACTACTTTTCTCGCCTGTTCCG
CTCTCTGGAAGAAAACCAAGTGCCCTAGAGTCAACCAAGTCCCGTCTTAAATGTCTTCTGCTGATACTGGGGTTCTAAGGCCGAGTC
TTATGAGCAGCGGGCCGCTGTCTGAGCGTCCGGGCGGAAGGATCAGGACGCTCGCTGCGCCCTTCGTCTGACGTGGCAGCGCTCGCCG
TGAGGAGGGGGCGCCCGGGAGGCGCCAAACCCGGCGCGGAGGCCGCATGCTCCAGACTGCCCTGGGAAAAGCGCCTCCCCTACCC
GGTAGAAT***GAAGTTCCTATACTTTCTAGAGAATAGGAAC******TTCC***GTTGGTACCGTACGCAATTG