



Vector Report

Quote: TKK-171122-AMW-01
Project: Mouse Uty Constitutive Knockin

-Confidential-

1. Quote

TKK-171122-AMW-01

2. Gene

Uty

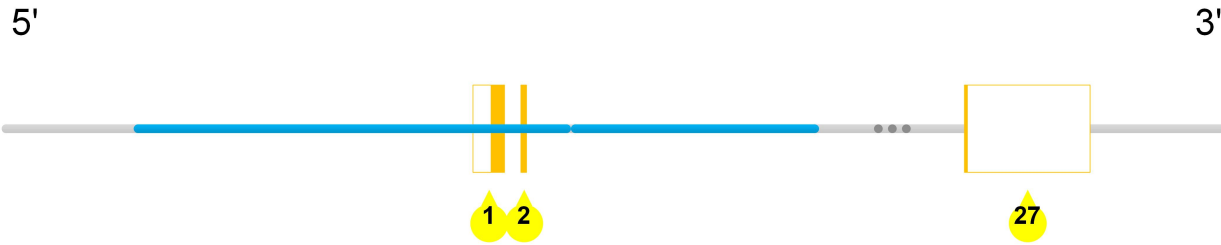
3. Objective

To create a mouse Uty constitutive knockin model in C57BL/6 mice

4. Summary

- The Uty gene (NCBI Reference Sequence: NM_009484.3) is located on mouse chromosome Y. Twenty-seven exons have been identified, with the ATG start codon in exon 1 and the TAA stop codon in exon 27.
- In the targeting vector, the KI sequence was inserted into intron 2 of mouse Uty gene in the reverse orientation.
- In the targeting vector, the Neo cassette was flanked by loxP sites. DTA will be used for negative selection.
- C57BL/6 ES cells will be used for gene targeting.

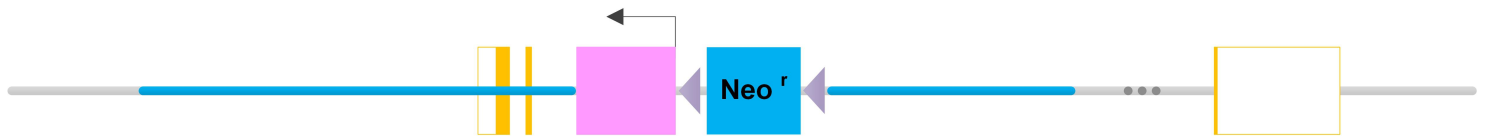
Wildtype allele



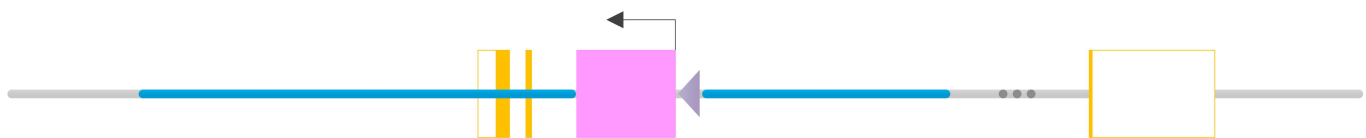
Targeting vector



Targeted allele



Constitutive KI allele (After Neo deletion)



Legends

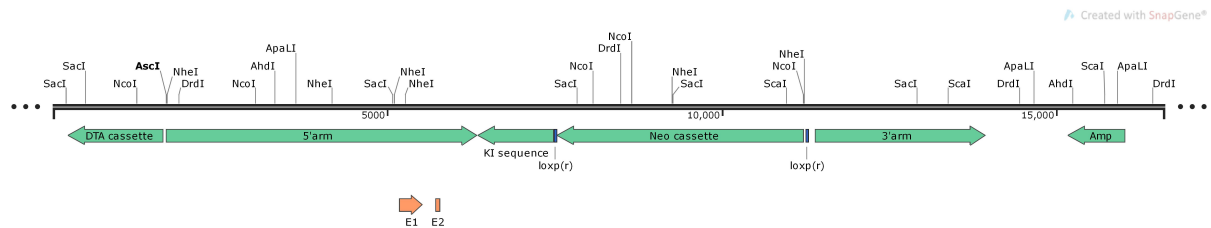


5. Method

Mouse genomic fragments containing homology arms (HAs) were amplified from BAC clone by using high fidelity Taq DNA polymerase, and were sequentially assembled into a targeting vector together with recombination sites and selection markers shown below.

Diagram

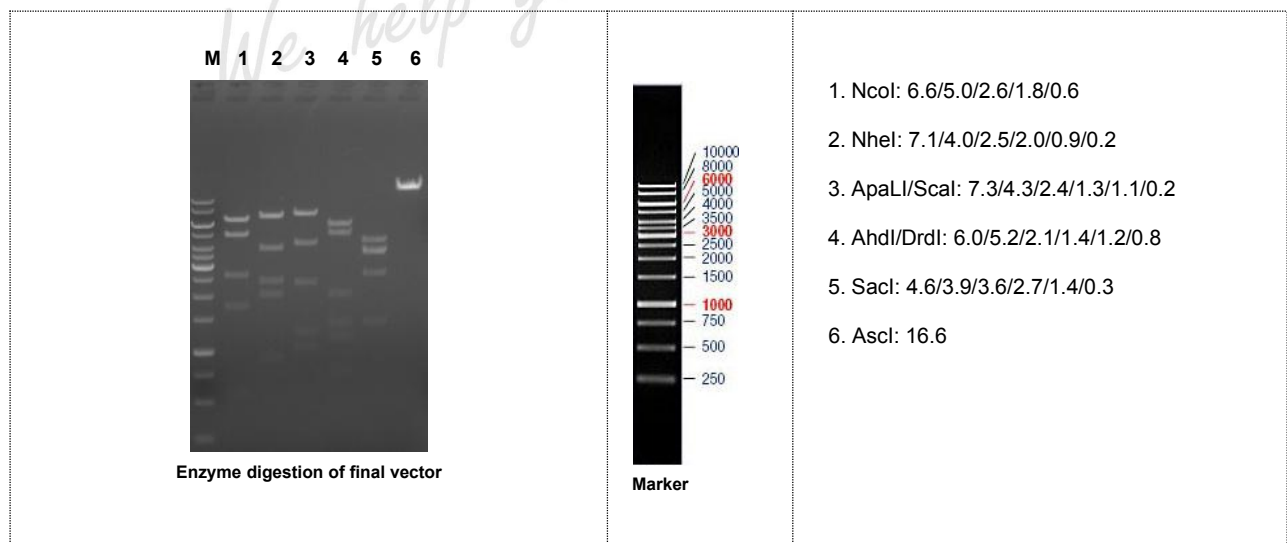
Linearized targeting vector



✂ Vector Linearization - AscI

6. Result

Your targeting vector was digested by restriction enzymes for confirmation purposes. Units below are all in kilo-base pair (kb).



7. Sequence of the Final Targeting Vector

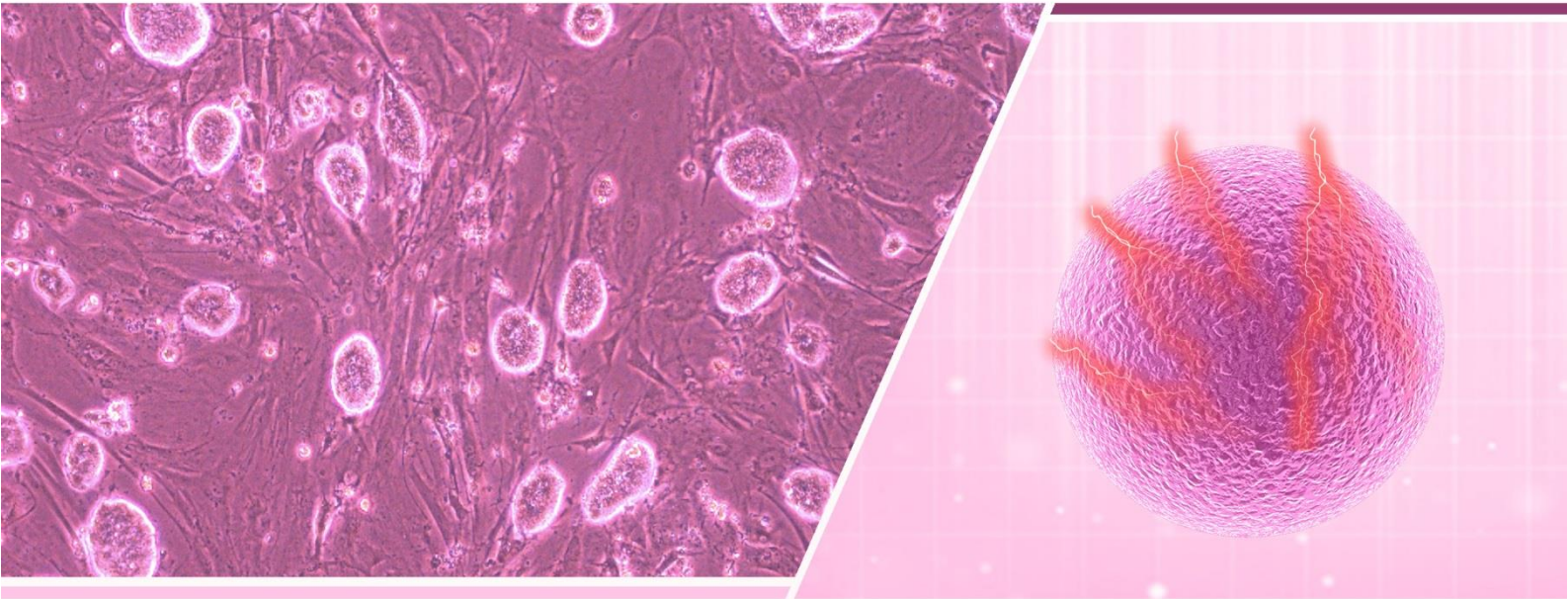
	Homology arms	KI region	loxP sites	Exons	Sequence confirmed regions					
1	CGCTTA	CAATTT	CCATTC	GCCATT	CAGGCT	GCGCAA	CTGTTG	GGAAGG	GCGATC	GGTGCG
61	GGCCTC	TTTCGT	ATTACG	CCAGCT	GGCGAA	AGGGGG	ATGTGC	TGCAAG	GCGATT	AAGTTG
121	GGTAAC	GCCAGG	GTTTTT	CCAGTC	ACGACG	TTGTAA	AACGAC	GGCCAG	TGAATT	GTAATA
181	CGACTC	ACTATA	GGGCGA	ATTGGA	GCTCCA	CCGCCC	GGGCTG	GTTCTT	TCCGCC	TCAGAA
241	GCCATA	GAGCCC	ACCGCA	TCCCCA	GCATGC	CTGCTA	TTGTCT	TCCCAA	TCCTCC	CCCTTG
301	CTGTCC	TGCCCC	ACCCCA	CCCCCC	AGAATA	GAATGA	CACCTA	CTCAGA	CAATGC	GATGCA
361	ATTTCC	TCATTT	TATTAG	GAAAGG	ACAGTG	GGAGTG	GCACCT	TCCAGG	GTCAAG	GAAGGC
421	ACGGGG	GAGGGG	CAAACA	ACAGAT	GGCTGG	CAACTA	GAAGGC	ACAGTC	GAGGCT	GATCAG
481	CGAGTG	CTAGGA	TCTGCA	TTCCAC	CACTGC	TCCCAT	TCATCA	GTTCCA	TAGGTT	GGAATC
541	TAAAAT	ACACAA	ACAATT	AGAATC	AGTAGT	TTAACA	CATTAT	ACACTT	AAAAAT	TTTATA
601	TTTACC	TTAGAG	CTTTAA	ATCTCT	GTAGGT	AGTTTG	TCCAAT	TATGTC	ACACCA	CAGAAG
661	TAAGGT	TCCTTC	ACAAAG	AGATCG	CCTGAC	ACGATT	TCCTGC	ACAGGC	TTGAGC	CATATA
721	CTCATA	CATCGC	ATCTTG	GCCACG	TTTTCC	ACGGGT	TTCAAA	ATTAAT	CTCAAG	TTCTAC
781	GCTTAA	CGCTTT	CGCCTG	TTCCCA	GTTATT	AATATA	TTCAAC	GCTAGA	ACTCCC	CTCAGC
841	GAAGGG	AAGGCT	GAGCAC	TACACG	CGAAGC	ACCATC	ACCGAA	CCTTTT	GATAAA	CTCTTC
901	CGTTCC	GACTTG	CTCCAT	CAACGG	TTCAGT	GAGACT	TAAACC	TAACTC	TTTCTT	AATAGT
961	TTCGGC	ATTATC	CACTTT	TAGTGC	GAGAAC	CTTCGT	CAGTCC	TGGATA	CGTCAC	TTTGAC
1021	CACGCC	TCCAGC	TTTTCC	AGAGAG	CGGGTT	TTCATT	ATCTAC	AGAGTA	TCCCGC	AGCGTC
1081	GTATTT	ATTGTC	GGTACT	ATAAAA	CCCTTT	CCAATC	ATCGTC	ATAATT	TCCTTG	TGTACC
1141	AGATTT	TGGCTT	TTGTAT	ACCTTT	TTGAAT	GGAAATC	TACATA	ACCAGG	TTTAGT	CCCGTG
1201	GTACGA	AGAAAA	GTTTTT	CATCAC	AAAAGA	TTTAGA	AGAATC	AACAAC	ATCATC	AGGATC
1261	CATGGC	ACGCGC	TTCTAC	AAGGCG	CTGGCC	GAAGAG	GTGCGG	GAGTTT	CACGCC	ACCAAG
1321	ATCTGC	GGCACG	CTGTTG	ACGCTG	TTAAGC	GGGTGC	CTGCAG	GGTCGC	TCGGTG	TTTCGAG
1381	GCCACA	CGCGTC	ACCTTA	ATATGC	GAAGTG	GACCTG	GGACCG	CGCCGC	CCCGAC	TGCATC
1441	TGCGTG	TTCGAA	TTCGCC	AATGAC	AAGACG	CTGGGC	GGGGTT	TGCTCG	ACATTG	GGTGGA
1501	AACATT	CCAGGC	CTGGGT	GGAGAG	GCTTTT	TGCTTC	CTCTTG	CAAAAC	CACACT	GCTCGA
1561	CATTGG	GTGGAA	ACATTC	CAGGCC	TGGGTTG	GAGAGG	CTTTTT	GCTTCC	TCTTGA	AAACCA
1621	CACTGC	TCGATT	TGTTAG	CAGCCT	CGAATC	AACCCG	GGCGAT	CCTAGG	CATGTA	GATCTA
1681	GCTGTC	GCGAAG	AGTGGC	GCGCCA	CCACCG	CCCAGC	TAGCTT	TCTTAA	ATTAAG	CAGTTT
1741	CTGTCA	GTGTTA	GGGATT	TTTACC	TGCAAT	TCCATC	ATATTT	GAATAC	TCTGTG	ACTGAG
1801	ACTGTT	AGGGTC	CAGTAA	TAGCCA	TACAAA	CCCCTC	AGAAGC	CAATCT	CAGTCA	TGTATC
1861	AATGGT	TTATTG	AACACA	TTCCTA	GACTGA	TTAGTC	AGGAGT	GGGGAT	CGAAAA	CTTCAA
1921	TAAGGC	ATTCTT	CAGAAC	CAATTT	TTATAT	GGGAAA	AACAAG	GACTTG	GTACAT	TTGCAA
1981	TGTTTA	TAACAT	TTGCAA	CATTTA	TGCTAT	CTAGAC	AAAGCA	GTGCTG	TCTGGC	CTTTAA
2041	GCTGAT	AGGCTA	TGTATA	GAGACA	AGGAGA	CAAGGA	GACCTC	CAAAGT	TCACAA	GGCTCA
2101	GTGAAA	AAGTAT	AACAAC	TTTTTT	TCAGGG	CAAGAA	ACAATA	GGTCAA	GTAAA	TTTATG
2161	AGCTAC	AAAATG	AAGATT	GAGGGA	ATATGG	CAGAAT	TTTAAT	ATTTAA	CAGAGA	AATGTC
2221	CTTAAA	CCCCCA	AAGAGT	AATTTA	AAAGGG	TTTTTA	TTATCA	AACACC	TTTTTC	TGTTTT
2281	AAGATT	CTCTAT	GTAGAG	TAGGTT	GCATTG	GACAAAT	TATCTT	TCTGGC	TTTACT	TACTGA
2341	GTTCTG	AAATTT	GAAAGG	ATTCAC	CATTAT	TTCTTT	GTGGCA	AATATT	TGTCAA	CCCCAG
2401	AACTCA	GCAGAT	ACAATA	GTGGAA	GAAATA	AGTCAG	CTCTTG	CTCCCT	CCCTTA	CCTCTA
2461	TTCTCT	AGCTCT	TTTGCT	CCCCTG	CTCCCC	CTCTCT	CCCCAT	CCCCTT	CCTTGC	TCTCTC
2521	TATGTG	GTCATG	GCTGCC	TTCTGT	TTCTCT	ACTATC	TCACAC	TCTTTG	CCTTTC	TACAAT
2581	AAATGC	CTTAAA	ACCAAA	AAATAA	AAAATA	AGAAAA	AAGTCA	GCAGAG	GAGAAAT	GAAAGG
2641	ATGGAC	TACTAC	TACTTC	AGAACT	GTTTAA	ACCTGA	GAATTT	TGAGGA	GAAAGA	TTGTTT
2701	AGTTTC	AACCCT	TACAAG	GGACTG	AGGTAC	ATATTT	TACATA	TTAAAG	CAGTCC	TGGCCT
2761	CCAGAT	TCTCCT	AGCATC	CCTCAG	TCCCAA	CCTGGC	ATTCTG	TCTCCA	AACCTA	AACTTT
2821	TCAGGT	CAGAGG	CTGGGC	TACCTT	TCCCCC	AGAAGC	TTCTCC	CTATAT	AATCCA	GACATT
2881	TGGACA	ATGACT	CCCCCC	CCCATG	TCCCCC	TCCTTT	TCCTTT	TCCTCC	TCTTCT	TCCTCT
2941	TCCTCC	CCACCT	TGTCTT	ATCCTC	TTTCTC	TCTCTC	TGTCTA	TCTCTC	TCTCTC	TCTTCT
3001	CCCTTC	TCTGTG	TACATG	AGCTTT	CTCTCT	CCATGG	TAACTT	CCCTGG	CCTCAG	TTATTG
3061	GGGCCA	GTGAAC	TTGCC	AAGAGC	AGCTTC	CCAATA	AGCCTG	CCTATA	TATAAA	ATATTA
3121	AAGACC	TGTTCC	ACAATA	TCCAAC	TAAAGC	AAGCTT	TATTTA	ACACTG	ACTAGG	AAGTTC
3181	AACACT	GACTAC	ATTCAT	GCCAGG	ATTCCA	AGAGAA	ATGGCT	TTGAAT	TAAAAT	CAGTCA
3241	TGTGAT	CATGCA	AAGAAA	CCTCTA	CAAATC	TATGAA	CTTTCC	ATATTT	GTCCAA	TTAGGG
3301	GTAAGC	ATACAT	CCTCAC	TGACTT	CCTGTC	TGCATA	TTTCTC	ACCTGT	GTGTGA	TCAAGC
3361	ATATCC	TGAGCA	CTTGGG	GCAACA	AACCTT	GCTATT	GAACATA	AAACAC	AACCTC	CAGTAT

3421	TCTGCA	GTTATT	TGTCCT	TAGGTA	AGTGTA	GCATAC	ACATTA	ACTTTA	AAGAAT	TCCAG
3481	AGCTGA	TTTTTT	GTTTGT	TTGTTT	GTTTGT	TTGTTT	GTTTGT	TTTTGA	GAGAAA	GTTTTT
3541	CTGTGT	AGCCCT	GGCTGT	CCTGGA	ACTCAC	TCTGTA	GACCAG	GCTTCC	TCAAAC	TCAGAA
3601	ATCCAC	CTGCCT	CTGCCT	CCCAAG	TGCTGG	GATTAA	AGGCGT	GCACCA	CCACGG	CTGGCT
3661	CTGACT	TTTTTT	TTTTTT	AAGAGT	TACTTT	ATTTAT	ATGAGT	ACACTG	TTACTG	TTTTCA
3721	GACACC	CCAGAA	GAGGGC	ATTGGA	TCCCAT	TACAGA	TGGTTG	TGAACC	ACCATG	TTGGTG
3781	GTGGGA	ATTGAA	CTCAGG	ACCTCT	GGAAGA	GCAGTC	AGTGCT	CTTAAC	CATACC	ATGCAG
3841	TTAGTA	TCTGAG	AGTAAA	GTAGCA	GAAGCT	ATACAC	CGTCTG	AGAATC	AATTCT	TGTAGT
3901	GTAGGC	TTCTGG	AGTCCC	AGAAAC	TGCTTT	TCAAGT	TAAATT	GGGTGC	AGGGAG	GGAGGG
3961	GGAGAA	TAGGTC	TAATTG	TTTCTG	AAACCT	TTTGGC	AGAAGG	GTAAAA	GATAAG	TCAGAT
4021	TGCCTG	TTTTTT	GCATCT	TATGGC	TTCCAG	GTCTGC	AAAGTG	TTTTAG	AAGCTT	CCTAGA
4081	ATTTTG	TTATGG	AAGCTT	TTGAAA	AGACTT	AGACAT	GTTTTG	TGGACC	TGGTTT	GGGGGT
4141	AGGGAA	TTTACT	TGAAGA	GGGATT	AGGTAG	TGTTTG	CTAGCC	AGTCTT	CAATAC	AATTCA
4201	AGTTTA	AGGTCA	TCTTAG	ATTTAT	AAATAT	TTGTGT	TGGAGA	CTTGTG	ATACTG	GTTTCT
4261	GAGATG	GGGAAC	TAGGTT	TCCTTG	TGAAGG	TCCAGG	CCCAGT	AAGTAA	TGGTAA	TACAAG
4321	TCAATT	CAAAAC	TCCTGA	GAACAG	CAAAAT	TGGAAC	TTGTCT	TATTAT	ATTTTT	TATTAT
4381	TGTATT	ATATAT	AAATGA	ATGCTC	CTCTTA	CAAAGT	GTTGGA	TCATAT	TTACAA	ATTTTA
4441	GCTACA	ACAACC	AAAAGG	TCAAAA	GAAATG	GTATTG	TTCTCA	AATGGT	TACTGC	ATCTTC
4501	AAAATG	CAATGA	GGGATT	CCCCCG	TCCTTC	CCCCCC	CCCCGC	CCCCTC	ACGTCA	CTCCTT
4561	GCCCCC	GCAGGG	ATAACC	TTTGTG	AGGGAC	TGTTCA	TTCACT	ATGATC	TGTTGT	TTAAGG
4621	ACAAAG	TCATGT	GACTTT	TATAAA	GTTTTT	TTGGTT	TGATGT	GGTTGA	GTGGAT	GGGTAA
4681	TTAATG	AGTTTA	GCTATG	GTGGTG	ACAAAA	AAACAC	GACCCA	GTTACT	CATTGA	GGATGA
4741	TTTCAA	TTTTAG	CTGAAT	ACATTT	TGTTTT	CCCAAC	GTGTGT	GTCATT	ACTTCA	AGATCT
4801	TAATTT	TAAAGT	TTGCTA	GAATTA	GTATTA	AGGAGC	ACCGAT	TCTGGG	AACTTA	ACAGAA
4861	GGTGT	CTGTAA	GGCAAG	AGGAAA	ATTTTT	TTTTTT	AACTAT	TTGGTC	TCTAAT	TGGCTT
4921	TCATGT	GCCCTA	AAAACC	GATGTT	TGATGT	ATTTTT	TCTTAG	TGCTCT	TTGTC	CTTGTG
4981	AGATCA	GTATAG	CAATTA	AATGAA	AGGGTC	TGGATA	TCTAGT	GTGGAA	TAGAAA	AGAGTT
5041	ACAAAG	CATACC	TAAAAC	TAATTT	CACTGT	GGTCGG	TAGGTA	GAGCTC	TTAGGC	TGACAT
5101	CACGAG	CTAGCG	AAGTAG	CAAGTT	TAGCTG	CACTGG	TGATGA	CGCAAG	TCAACA	AAAGTC
5161	ACGTGG	GATAAA	ACGGTT	GCTTCC	ATTGGA	AAGCCT	GGCTTT	AGGGAG	AGGTTA	TGGACG
5221	TTACGT	GCGGTT	CACACT	GACGGT	ATTATA	ACAACT	TTATGC	GGATGC	TAGCGA	AGTTTG
5281	ATATTC	TAAGAA	CTTCGC	TTAGGT	TCTGCA	ACAATG	AGTTCT	TCTTGC	GTTTAC	CATCTG
5341	CGTGGT	AATGTG	ACCAGA	TAGCCT	CTGCCG	CTTTCT	CCTTTG	CCAGTT	TCCATG	AAATCT
5401	TACGGA	TTATCT	CTCACT	ACTGCT	GCCCTA	GGTAAT	GAGGAA	AAGAAA	ATGGCG	GCGGAA
5461	AAGGCT	AGAGGC	GAGGGC	GAAGAG	GGGTCC	TTCAGT	CTCACA	GTCGAA	GAGAAG	AAGGCG
5521	CTTTGT	GGATTA	GATAGG	TACGCA	CTGCTA	ATACTT	ACAGGT	TGTCTT	TCTCTC	TCCGGG
5581	TTCAAA	AGACTC	TCCAGG	AGGCCT	GACTGC	ATCTTG	CTTGCG	GTGACG	ACTCGC	TTTCGC
5641	CTAGCA	CTGTTA	ACAATG	TTTTGG	GAGATT	TTTTAC	TGATGT	CTTTCT	GTGATC	ATTAAC
5701	AAGAGC	CATGTA	TTTCTT	CCACAG	CAGCTT	CTTCGG	GTTCTT	GACCCG	ATGCAA	AGATGG
5761	CGCCAA	GATGAA	GACGCT	GTTGAA	CAAGGT	CAGCAA	CTGTTT	AAACAT	AATTAC	TTATAT
5821	TTTTCT	CCTAAG	CTTTTC	ACTGTT	CCAAGC	CTGGAG	CCTCTC	CCTACT	CTGAAA	GTGCTC
5881	ATTATA	GCTTTT	AGGGAT	CTTTGA	AACTTT	TTTTTT	CCTTCC	CCGTTT	CTCCTT	TCTTTG
5941	CTTACT	TTCTGT	ATAGTT	TGAGGC	CCTGCC	TCAGCA	TACAGC	CCAGAG	CACGAG	CTTATT
6001	TGCAGT	TTTTCT	GAGGTC	AGCTAT	ATCCTC	CAGTTA	CTCATC	TCAAGT	TATGAG	AAGACG
6061	CTTGGT	TGTTGC	AGCCTC	TTGTGA	TATACA	CACGTT	TTATTA	TTTTCC	CATAAG	GACAAA
6121	GTTATA	GGTAAG	GAGATA	AAGAGT	TTCCGT	ACTTAA	AAAGAA	TTTTTT	ACGATG	CAGTAG
6181	TAATAC	TATGCA	GAAATA	ATACTG	AATTCC	AGTTTG	GAATGT	TAATTT	TTTTTT	TTTTTT
6241	ACTTCC	TTGTAG	TTTATT	TTCCAG	AGAGTG	AGAGAG	CACACG	GCATTT	CCTTTG	TGAAAC
6301	TTTGCA	TTGTCA	TTTAGT	TATTGG	CTGCTG	GTATCA	ATCAAG	ATGGTG	GCTTAA	GACTGA
6361	CGGGCA	CCGGAG	CCAATT	CCCCTT	CCTTTC	AAGACC	TGGTAC	CAAAAA	AGCACC	GACTCG
6421	GTGCCA	CTTTTT	CAAGTT	GATAAC	GGACTA	GCCTTA	TTTTAA	CTTGCT	ATTTCT	AGCTCT
6481	AAAACT	TTTGAG	GCTCAT	CTCTGC	AGGTGT	TTTCGT	CCTTCC	ACAAGA	TATATA	AAGCCA
6541	AGAAAT	CGAAAT	ACTTTC	AAGTTA	CGGTAA	GCATAT	GATAGT	CCATTT	TAAAAC	ATAATT
6601	TTAAAA	CTGCAA	ACTACC	CAAGAA	ATTATT	ACTTTC	TACGTC	ACGTAT	TTTGTA	CTAATA
6661	TCTTTG	TGTTTTA	CAGTCA	AATTAA	TTCCAA	TTATCT	CTCTAA	CAGCCT	TGTATC	GTATAT
6721	GCAAAT	ATGAAG	GAATCA	TGGGAA	ATAGGC	CCTCTT	CTAGAA	AAAAAG	CACCGA	CTCGGT
6781	GCCACT	TTTTCA	AGTTGA	TAACGG	ACTAGC	CTTATT	TTAACT	TGCTAT	TTCTAG	CTCTAA
6841	AACATC	TACAAG	ATTACG	GTCTGG	GTGTTT	CGTCCT	TTCCAC	AAGATA	TATAAA	GCCAAG
6901	AAATCG	AAATAC	TTTCAA	GTTACG	GTAAGC	ATATGA	TAGTCC	ATTTTA	AAACAT	AATTTT
6961	AAAACT	GCAAAC	TACCCA	AGAAAT	TATTAC	TTTCTA	CGTCAC	GTATTT	TGTACT	AATATC
7021	TTTGTG	TTTACA	GTCAAA	TTAATT	CCAATT	ATCTCT	CTAACA	GCCTTG	TATCGT	ATATGC

7081 AAATAT GAAGGA ATCATG GGAAAT AGGCC TCGGAT CCAAAA AAGCAC GCACTC GGTGCC
 7141 ACTTTT TCAAGT TGATAA CGGACT AGCCTT ATTTTA ACTTGC TATTTT TAGCTC TAAAC
 7201 CGATTG GCCCGG TGGCAG TGGGTG TTTTCG CTTTTC CACAAG ATATAT AAAGCC AAGAAA
 7261 TCGAAA TACTTT CAAGTT ACGGTA AGCATA TGATAG TCCATT TTAATA CATAAT TTTAAA
 7321 ACTGCA AACTAC CCAAGA AATTAT TACTTT CTACGT CACGTA TTTTGT ACTAAT ATCTTT
 7381 GTGTTT ACAGTC AAATTA ATTCCA ATTATC TCTCTA ACAGCC TTGTAT CGTATA TGCAAA
 7441 TATGAA GGAATC ATGGGA AATAGG CCCTCA CGCGTG CGGCCG CCTCGA GGGACC TAATAA
 7501 CTTTCG ATAGCA TACATT ATACGA AGTTAT ATTAAG GGTTC GCAAGC TCTAGT CGAGCC
 7561 CCAGCT GGTTC TCCCGC CTCAGA AGCCAT AGAGCC CACCGC ATCCCC AGCATG CCTGCT
 7621 ATTGTC TTCCCA ATCCCT CCCCTT GCTGTC CTGCCC CACCCC ACCCCC CAGAAT AGAATG
 7681 ACACCT ACTCAG ACAATG CGATGC AATTTT CTCATT TTATTA GGAAAG GACAGT GGGAGT
 7741 GGCACC TTCCAG GGTCAA GGAAGG CACGGG GGAGGG GCAAAC AACAGA TGGCTG GCAACT
 7801 AGAAGG CACAGT CGAGGC TGATCA GCGAGC TCTAGA GAATTG ATCCCC TCAGAA GAACTC
 7861 GTCAAG AAGGCG ATAGAA GGCGAT GCGCTG CGAATC GGGAGC GGCGAT ACCGTA AAGCAC
 7921 GAGGAA GCGGTC AGCCCA TTCGCC GCCAAG CTCTTC AGCAAT ATCACG GGTAGC CAACGC
 7981 TATGTC CTGATA GCGGTC CGCCAC ACCCAG CCGGCC ACAGTC GATGAA TCCAGA AAAGCG
 8041 GCCATT TTCCAC CATGAT ATTCGG CAAGCA GGCATC GCCATG GGTAC GACGAG ATCATC
 8101 GCCGTC GGGCAT GCGCGC CTTGAG CCTGGC GAACAG TTCGGC TGGCGC GAGCCC CTGATG
 8161 CTCTTC GTCCAG ATCATC CTGATC GACAAG ACCGGC TTCCAT CCGAGT ACGTGC TCGCTC
 8221 GATGCG ATGTTT CGCTTG GTGGTC GAATGG GCAGGT AGCCGG ATCAAG CGTATG CAGCCG
 8281 CCGCAT TGCATC AGCCAT GATGGA TACTTT CTCGGC AGGAGC AAGGTG AGATGA CAGGAG
 8341 ATCCTG CCCCAG CACTTC GCCCAA TAGCAG CCAGTC CCTTCC CGCTTC AGTGAC AACGTC
 8401 GAGCAC AGCTGC GCAAGG AACGCC CGTCGT GGCCAG CCACGA TAGCCG CGCTGC CTCGTC
 8461 CTGCAG TTCATT CAGGGC ACCGGA CAGGTC GGTCTT GACAAA AAGAAC CGGGCG CCCCTG
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 8581 GCCGAA TAGCCT CTCCAC CCAAGC GGCCGG AGAAC TGCGTG CAATCC ATCTTG TTCAAT
 8641 GGCCGA TCCCAT GGTTTA GTTCTT CACTTT GTGCTA TTATAC TATGCC GATATA CTATGC
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 8761 AGCGCG GCAGAC GTGCGC TTTTGA AGCGTG CAGAAT GCCGGG CCTCCG GAGGAC CTTCGG
 8821 GCGCCC GCCCG CCCCTG AGCCCG CCCCTG AGCCCG CCCCCG GACCCA CCCCTT CCCAGC
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 9001 ATTTGT CACGTC CTGCAC GACGCG AGCTGC GGGGCG GGGGGG AACTTC CTGACT AGGGGA
 9061 GGAGTA GAAGGT GCGCGG AAGGGG CCACCA AAGAAC GGAGCC GGTGG CGCCTA CCGGTG
 9121 GATGTG GAATGT GTGCGA GGCCAG AGGCCA CTTGTG TAGCGC CAAGTG CCCAGC GGGGCT
 9181 GCTAAA GCGCAT GCTCCA GACTGC CTTGGG AAAAGC GCCTCC CCTACC CGGTAG AATTTT
 9241 GACGAC CTGCAG CCAAGC TAGCTT CGCGAG CTCGAC CGAACA AACGAC CCAACA CCCGTG
 9301 CGTTTT ATTCTG TCTTTT TATTGC CGCTCA GCTTTA CAGTGA CAATGA CGGCTG GCGACT
 9361 GAATAT TAGTGC TTACAG ACAGCA CTACAT ATTTTC CGTCGA TGTTGA AATCCT TTCTCA
 9421 TATGTC ACCATA AATATC AAATAA TTATAG CAATCA TTTACG CGTTAA TGGCTA ATCGCC
 9481 ATCTTC CAGCAG GCGCAC CATTGC CCCTGT TTTACT ATCCAG GTTACG GATATA GTTCAT
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 9601 GCGGGC CATATC TCGCGT GGCTCC GACACG GGCAT GTGTCC AGACCA GGCCAG GTATCT
 9661 CTGACC AGAGTC ATCCCT AGCGCC GTAAAT CAATCG ATGAGT TGCTTC AAAAAT CCCTTC
 9721 CAGGGC GCGAGT TGATAG CTGGCT GGTGGC AGATGG CGCGGC AACACC ATTTTT TCTGAC
 9781 CCGGCA AAACAG GTAGTT ATTCGG ATCATC AGCTAC ACCAGA GACGGA AATCCA TCGCTC
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 9901 TTTTCG TCTGCC AATATG GATTAA CATTCT CCCACC GTCAGT ACGTGA GATATC TTTAAC
 9961 CCTGAT CCTGGC AATTTT GGCTAT ACGTAA CAGGGT GTTATA AGCAAT CCCCAG AAATGC
 10021 CAGATT ACGTAT ATCCTG GCAGCG ATCGCT ATTTTC CATGAG TGAACG AACCTG GTCGAA
 10081 ATCAGT GCGTTC GAACGC TAGAGC CTGTTT TGCACG TTCACC GGCATC AACGTT TTCTTT
 10141 TCGGAT CCGCCG CATAAC CAGTGA AACAGC ATTGCT GTCACT TGGTCG TGGCAG CCCGGA
 10201 CCGACG ATGAAG CATGTT TAGCTG GCCCAA ATGTTG CTGGAT AGTTTT TACTGC CAGACC
 10261 GCGCGC CTGAAG ATATAG AAGATA ATCGCG AACATC TTCAGG TTCTGC GGGAAA CCATTT
 10321 CCGGTT ATTCAA CTTGCA CCATGC CGCCCA CGACCG GCAAAC GGACAG AAGCAT TTTCCA
 10381 GGTATG CTCAGA AAACGC CTGGCG ATCCCT GAACAT GTCCAT CAGGTT CTTGCG AACCTC
 10441 ATCACT CGTTGC ATCGAC CCGTAA TGCAGG CAAATT TTGGTG TACGGT CAGTAA ATTGGA
 10501 CACCTT CCTCTT CTTCTT GGGCAT GGCCGC AGGAAA GCAGAG CCCTGA AGCTCC CATCAC
 10561 CCGCCA ATAAGA GCCAAG CCTGCA GTGTGA CCTCAT AGAGCA ATGTGC CAGCCA GCCTGA
 10621 CCCCCA GGGCCC TCAGGC TTGGGC AACTG TCTCTA GGACCC TGAGAG AAAGC ATACCC
 10681 ATTTCT GCTTAG GGCCCT GAGGAT GAGCCC AGGGGT GGCTTG GCACCT AAGCAA AGGACA

10741 CTGGGG CTCAGC TGGCAG CAAAGT GACCAG GATGCT GAGGCT TTGACC CAGAAG CCAGAG
10801 GCCAGA GGCCAG GACTTC TCTTGG TCCCAG TCCACC CTCACT CAGAGC TTTACC AATGCC
10861 CTCTGG ATAGTT GTCGGG TAACGG TGGACG CCACTG ATTCTC TGGCCA GCCTAG GACTTC
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11161 TAGGGT TGCGGG GGGTGG GGGAGG TCTCTG TGAGGC TGGTAA GGGATA TTTGCC TGGCCC
11221 ATGGAG CTAGCT TGGCTG GACGTA AACTCC TCTTCA GACCTA **ATAACT TCGTAT AGCATA**
11281 **CATTAT ACGAAG TTATAT** TAAGGG TTATTG AATATG ATCGGA ATTGGG CTGCAG GAATTC
11341 GATAGC TTGGCT GCAGGT CGACGT ACGTAG CAAGCT TGATGG GCCCTG GTACCG AAATAG
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14281 GCGGTA TCAGCT CACTCA AAGGCG GTAATA CGGTTA TCCACA GAATCA GGGGAT AACGCA
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14821 TGGTGG CCTAAC TACGGC TACACT AGAAGA ACAGTA TTTGGT ATCTGC GCTCTG CTGAAG
14881 CCAGTT ACCTTC GGAAAA AGAGTT GGTAGC TCTTGA TCCGGC AAACAA ACCACC GCTGGT
14941 AGCGGT GTTTTT TTTGTT TGCAAG CAGCAG ATTACG CGCAGA AAAAAA GGATCT CAAGAA
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16501 GTCTAT TCTTTT GATTTA TAAGGG ATTTTG CCGATT TCGGCC TATTGG TTAAAA AATGAG
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ES Cell Report

Quote: TTK-171122-AMW-01
Project: Mouse Uty Constitutive Knockin

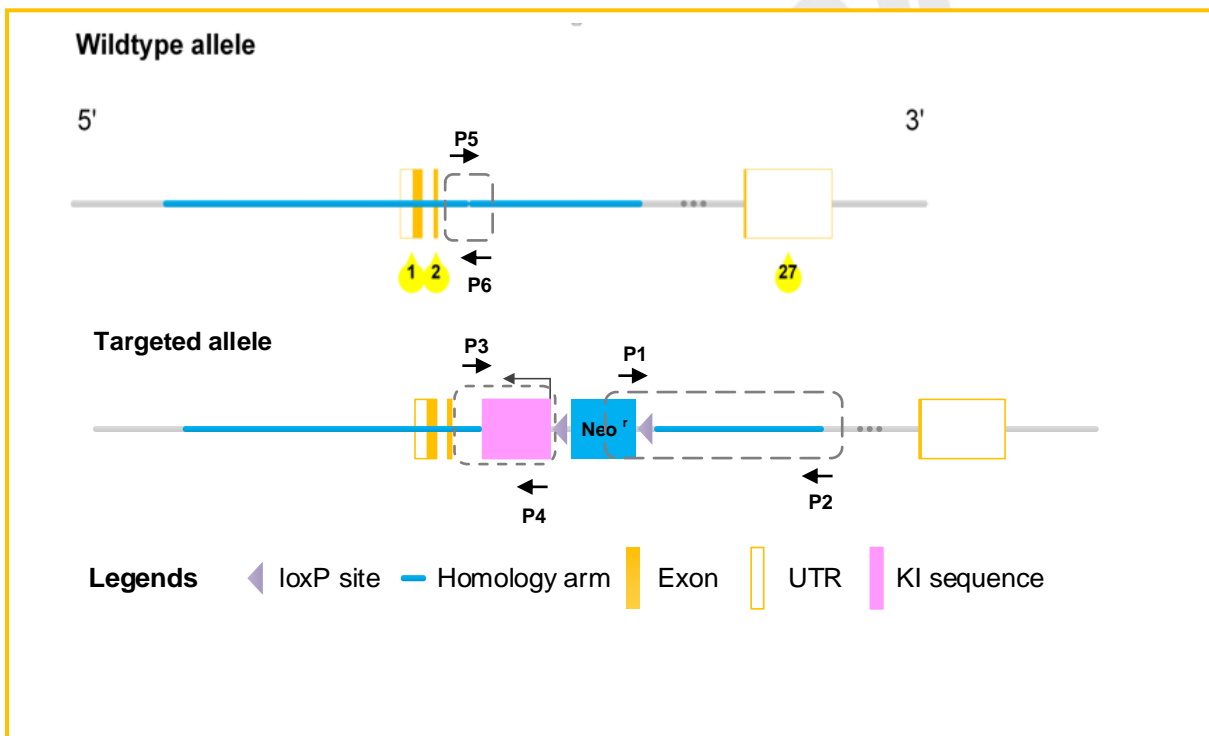
-Confidential-

1. Summary

The Uty targeting construct was linearized by restriction digestion with *Ascl*, followed by phenol/chloroform extraction and ethanol precipitation. The linearized vector was transfected into C57BL/6 ES cells according to cyagen's standard electroporation procedures. The transfected ES cells were subject to G418 selection (200 µg/mL) 24 hours post electroporation. 188 G418 resistant clones were picked and amplified in 96-well plates. Two copies of 96-well plates were made, one copy was frozen down and stored at -80°C and the other copy of the 96-well plates was used for DNA isolation and subsequent PCR screening for homologous recombination. The PCR screening identified 4 potential targeted clones, from among which 4 were expanded and further characterized by Southern blot analysis. All of the four expanded clones were confirmed to be correctly targeted.

1.1. PCR Screening

Regions in the following diagram were selected for PCR screening.



1.1.1. 3'arm PCR

Primers for 3'arm PCR:

Neo-F (P1): 5'-CAGCACCATTTGCCACTTGTCC-3'

3'arm-R (P2): 5'-CACAGGTCTGACTCAAAAAACGAC-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: ~3.0 kb

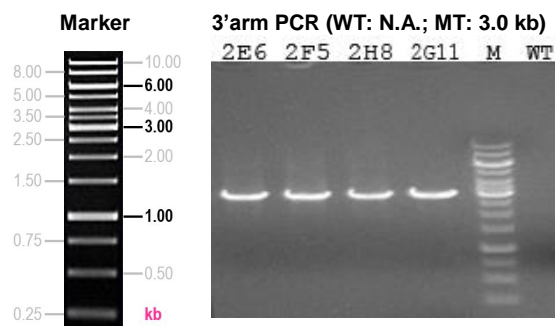
Reaction Mix:

Component	x1
ES cell genomic DNA	2.0 μ l
Forward primer(10 μ M)	0.8 μ l
Reverse primer(10 μ M)	0.8 μ l
dNTPs(2.5 mM)	2.4 μ l
5X LongAmp Taq Reaction	4.0 μ l
LongAmp Taq DNA Polymerase	1.2 μ l
ddH ₂ O	8.8 μ l
Total	20.0 μ l

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	60 °C	30s	
Extension	65 °C	50s/kb	
Additional extension	65 °C	10min	

PCR Result:



1.1.2. KI PCR

The potentially targeted clones were further screened by PCR for the presence of the KI site.

Primers for KI PCR:

KI-F (P3): 5'-CTCAAGTTATGAGAAGACGCTTGG-3'

KI-R (P4): 5'-TCGACTAGAGCTTGCGGAACCCT-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: ~1.5 kb

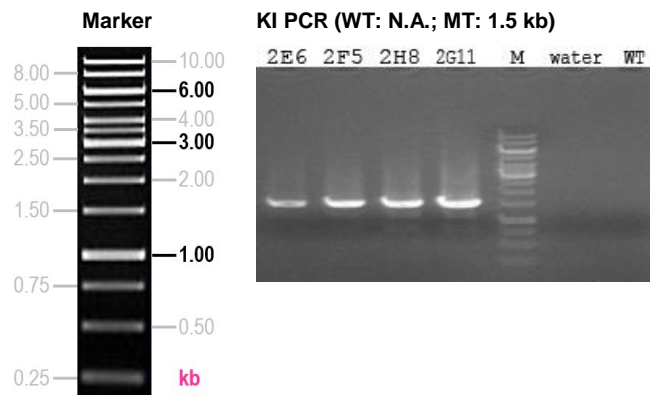
Reaction Mix:

Component	x1
ES cell genomic DNA	2.0 μ l
Forward primer(10 μ M)	0.8 μ l
Reverse primer(10 μ M)	0.8 μ l
dNTPs(2.5 mM)	2.4 μ l
5X LongAmp Taq Reaction	4.0 μ l
LongAmp Taq DNA Polymerase	1.2 μ l
ddH ₂ O	8.8 μ l
Total	20.0 μ l

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	60 °C	30s	
Extension	65 °C	50s/kb	
Additional extension	65 °C	10min	

PCR Result:



1.1.3. WT PCR

The potentially targeted clones were further screened by PCR for the presence of the WT site.

Primers for WT PCR:

WT-F (P5): 5'-CTCAAGTTATGAGAAGACGCTTGG-3'

WT-R (P6): 5'-TTCATTAACCTGTACTTGCTAGG-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: ~406 bp

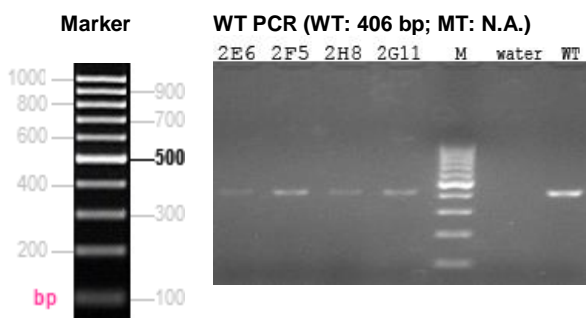
Reaction Mix:

Component	x1
ES cell genomic DNA	1.5 µl
Forward primer(10 µM)	1.0 µl
Reverse primer(10 µM)	1.0 µl
P112 Taq DNA Polymerase	12.5 µl
ddH ₂ O	9.0 µl
Total	25.0 µl

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30 s	33 x
Annealing	60 °C	30 s	
Extension	72 °C	30 s	
Additional extension	72 °C	5 min	
storage temperature	25 °C		

PCR Result:



1.2. Result

Samples 2E6, 2F5, 2H8 and 2G11 have been confirmed as potentially targeted ES clones.

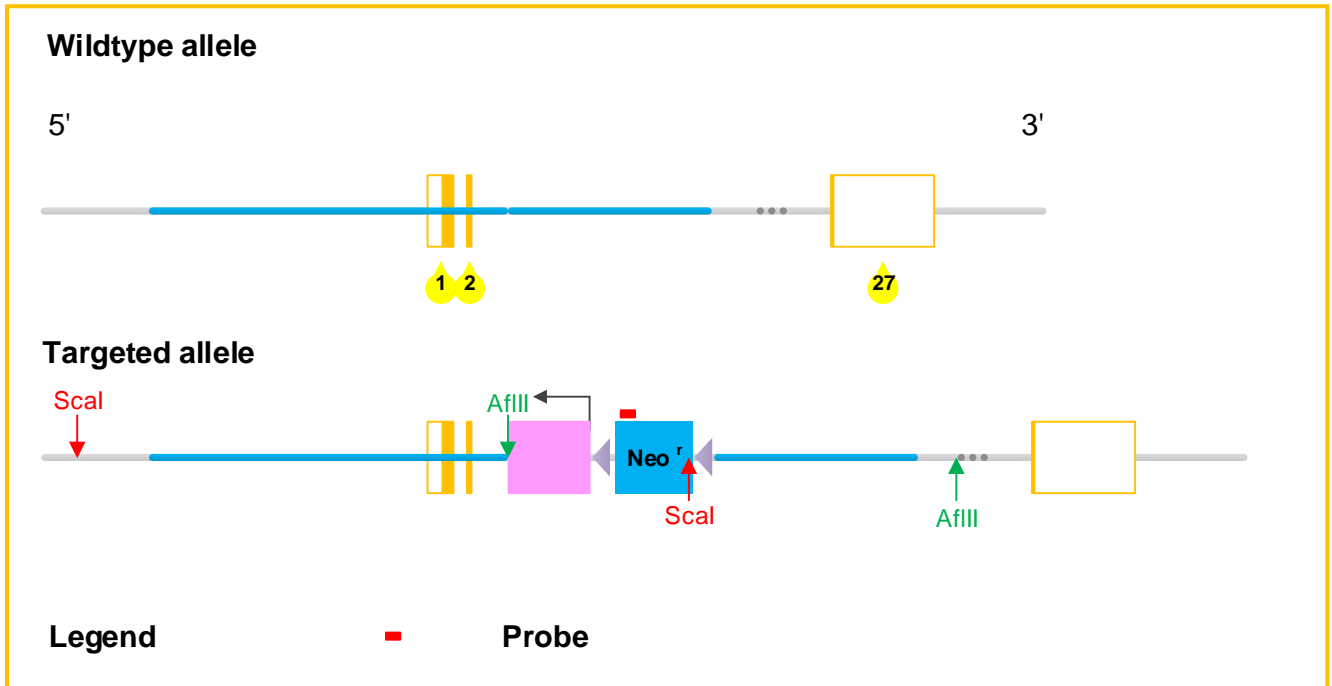


1.3. Southern Blot Analysis

The positive clones (2E6, 2F5, 2G11 and 2H8) from PCR screening were expanded and further characterized by Southern blot analysis. The Southern strategy is shown in the diagram below. The genomic DNA was digested with either *Scal* or *AflIII*, and hybridized using a Neo probe. The Neo probe is expected to detect the following DNA fragment from targeted allele in the Southern analysis: ~11.46 kb (with *Scal* digestion) and ~13.06 kb (with *AflIII* digestion).

Diagram:

Regions in the following diagram were selected for Southern blot.

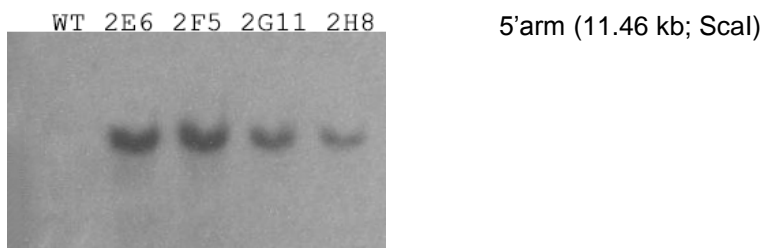


Expected Fragment Sizes for Southern Blot:

Neo Probe (containing 5'arm)-11.46 kb-*Scal*
Neo Probe (containing 3'arm)-13.06 kb-*AflIII*

Result:

All of the four ES clones (2E6, 2F5, 2G11 and 2H8) were confirmed correct by Southern blot analysis.





3'arm (13.06 kb; AflIII)





Animal Report

Quote: TKK-171122-AMW-01
Project: Mouse Uty Constitutive Knockin

-Confidential-

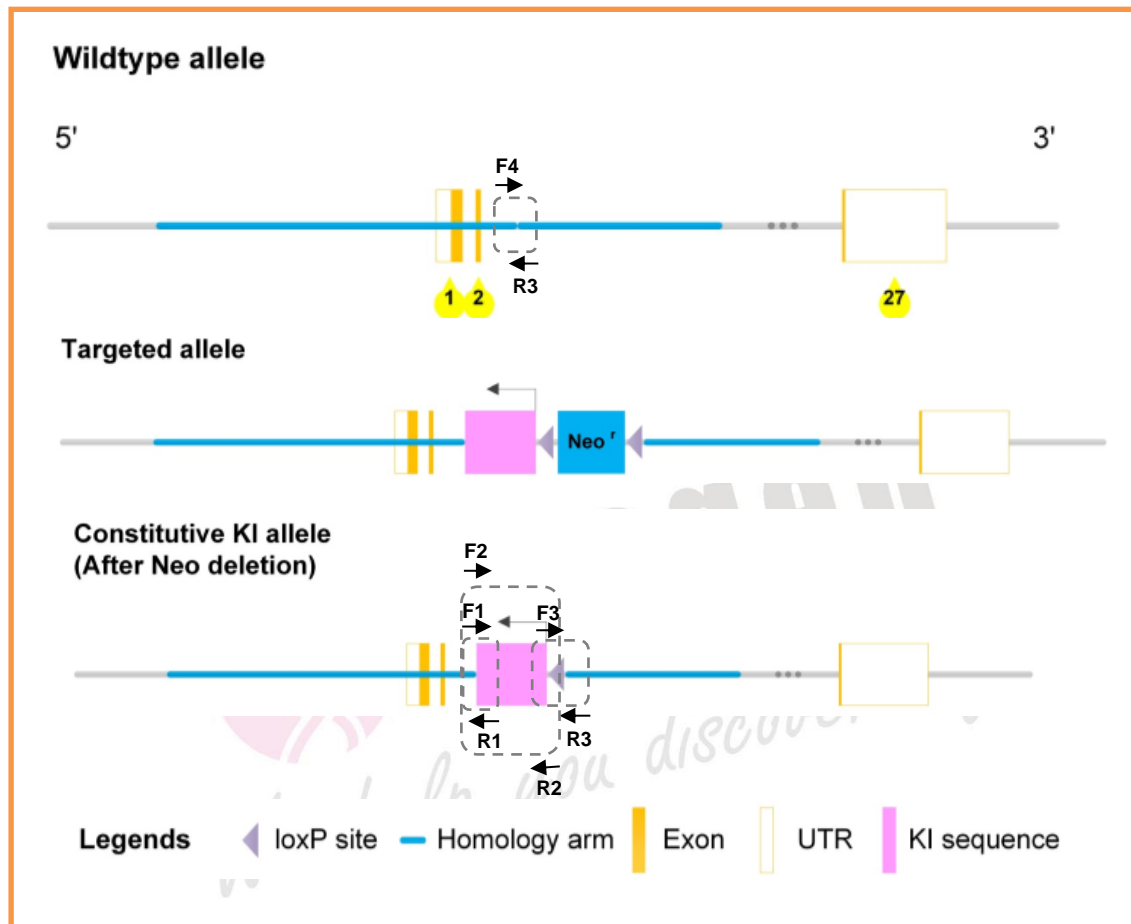
1. Animal Generation

Targeted ES cell clone 2E6 was injected into C57BL/6 albino embryos, which were then re-implanted into CD-1 pseudo-pregnant females. Founder animals were identified by their coat color, their germline transmission was confirmed by breeding with C57BL/6 females and subsequent genotyping of the offspring. The Neo cassette is self-deleted in germ cells so the offspring were Neo cassette-free. Eight male heterozygous targeted mice were generated from clone 2E6 as final deliverables for this project.

2E6 ESC, F1 mice	
8 Males ♂	-
D.O.B: 09-03-2018	
Mouse ID: 1, 2, 3, 4, 7, 8, 9, 10	



1.1. Genotyping Strategy



1.2. KI PCR

Primers for KI PCR:

KI-F (F1): 5'-TGCTGGTATCAATCAAGATGGTGG-3'

KI-R (R1): 5'-AAGGACGAAACACCTGCAGAGAT-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: 194 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	9.0 µl
Total	25.0 µl

Cycling Condition:

Quote: TKK-171122-AMW-01

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	62 °C	35s	
Extension	72 °C	35s	
Additional extension	72 °C	5 min	

1.3. KI1 PCR

Primers for KI1 PCR:

KI1-F (F2): 5'-GGTATCAATCAAGATGGTGGCTT-3'

KI1-R (R2): 5'-ATGTATGCTATACGAAGTTATTAGGTC-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: 1.2 kb

Reaction Mix:

Component	x1
DNA	1.5 µL
Forward primer (10 µM)	0.6 µL
Reverse primer (10 µM)	0.6 µL
dNTPs (2.5 mM)	1.8 µL
5X LongAmp Taq Reaction	3.0 µL
LongAmp Taq DNA Polymerase	0.9 µL
ddH ₂ O	6.6 µL
Total	15.0 µL

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	60 °C	30s	
Extension	65 °C	50s/kb	
Additional extension	65 °C	10min	

1.4. Neo deletion PCR

Primers for Neo deletion PCR:

Neo-del-F (F3): 5'-GGCAGTGGGTGTTTCGTCCTT-3'

Neo-del-R (R3): 5'-AACTGTTTCATTTCCCCTCTCCTC-3'

Forward1 (F4): 5'-GGTAAGGAGATAAAGAGTTTCCGTAC-3'

Expected PCR Product:

Wildtype: 257 bp

Targeted: 448 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl

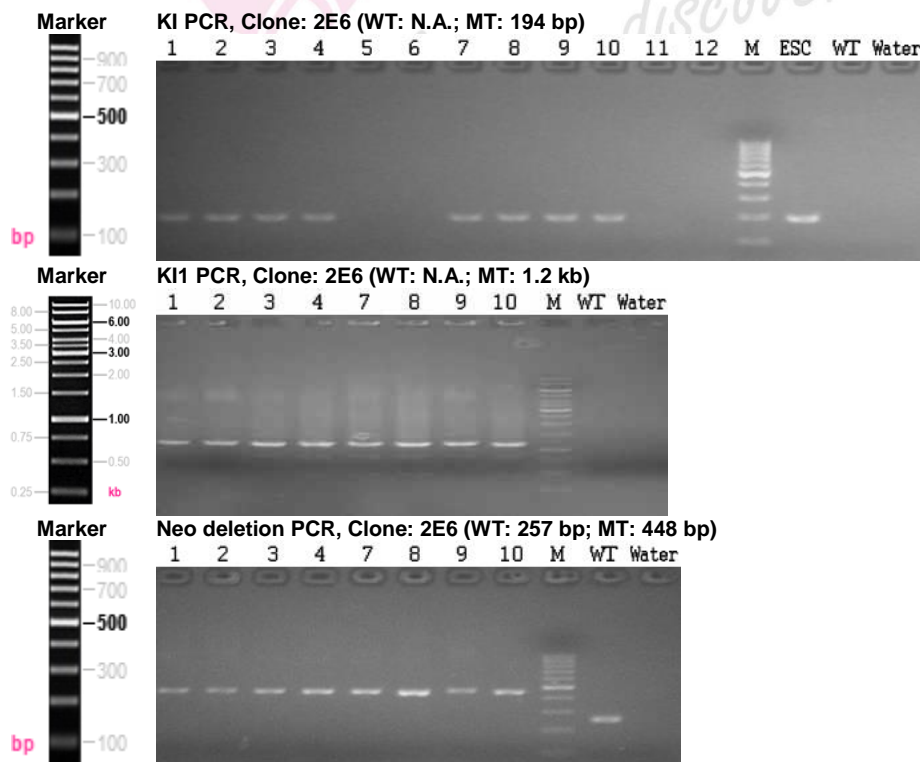
Neo-del-F (F3) (10 µM)	1.0 µl
Neo-del-R (R3) (10 µM)	1.5 µl
Forward1 (F4) (10 µM)	0.5 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	8.0 µl
Total	25.0 µl

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	62 °C	35s	
Extension	72 °C	35s	
Additional extension	72 °C	5 min	

1.5. Result:

Eight pups (1#, 2#, 3#, 4#, 7#, 8#, 9# and 10#) from clone 2E6 were identified positive by PCR screening for KI, KI1 and Neo deletion, the positive pups were reconfirmed by PCR screening for KI1 and Neo deletion.



1.6. Suggested Breeding and Genotyping Assay for Tissue-specific knockin Mice Generation

Step 1: Inter-cross heterozygous loxP-flanked mice to generate homozygous loxP-flanked mice

Primers for targeted allele:

Neo-del-F (F3): 5'-GGCAGTGGGTGTTTCGTCCTT-3'

Neo-del-R (R3): 5'-AACTGTTTCATTTCCCCTCTCCTC-3'

Forward1 (F4): 5'-GGTAAGGAGATAAAGAGTTTCCGTAC-3'

Wildtype: 257 bp

Homozygotes: 448 bp

Heterozygotes: 448 bp/257 bp





Animal Report

Quote: TKK-171122-AMW-01
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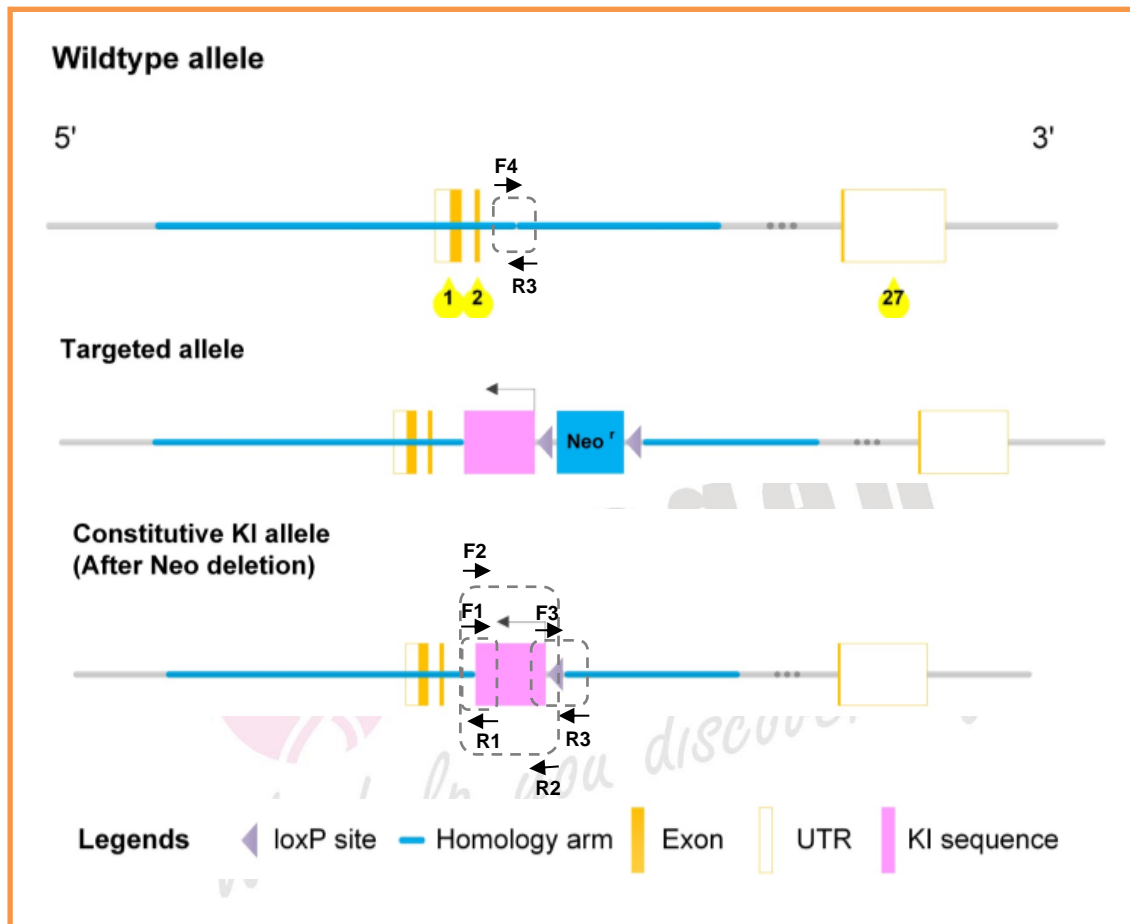
1. Animal Generation

Targeted ES cell clone 2H8 was injected into C57BL/6 albino embryos, which were then re-implanted into CD-1 pseudo-pregnant females. Founder animals were identified by their coat color, their germline transmission was confirmed by breeding with C57BL/6 females and subsequent genotyping of the offspring. The Neo cassette is self-deleted in germ cells so the offspring were Neo cassette-free. Four male heterozygous targeted mice were generated from clone 2H8 as final deliverables for this project.

2H8 ESC, F1 mice	
4 Males ♂	-
D.O.B: 09-02-2018	
Mouse ID: 1, 2, 3, 4	



1.1. Genotyping Strategy



1.2. KI PCR

Primers for KI PCR:

KI-F (F1): 5'-TGCTGGTATCAATCAAGATGGTGG-3'

KI-R (R1): 5'-AAGGACGAAACACCTGCAGAGAT-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: 194 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	9.0 µl
Total	25.0 µl

Cycling Condition:

Quote: TKK-171122-AMW-01

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	62 °C	35s	
Extension	72 °C	35s	
Additional extension	72 °C	5 min	

1.3. KI1 PCR

Primers for KI1 PCR:

KI1-F (F2): 5'-GGTATCAATCAAGATGGTGGCTT-3'

KI1-R (R2): 5'-ATGTATGCTATACGAAGTTATTAGGTC-3'

Expected PCR Product:

Wildtype: N.A.

Targeted: 1.2 kb

Reaction Mix:

Component	x1
DNA	1.5 µL
Forward primer (10 µM)	0.6 µL
Reverse primer (10 µM)	0.6 µL
dNTPs (2.5 mM)	1.8 µL
5X LongAmp Taq Reaction	3.0 µL
LongAmp Taq DNA Polymerase	0.9 µL
ddH ₂ O	6.6 µL
Total	15.0 µL

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	60 °C	30s	
Extension	65 °C	50s/kb	
Additional extension	65 °C	10min	

1.4. Neo deletion PCR

Primers for Neo deletion PCR:

Neo-del-F (F3): 5'-GGCAGTGGGTGTTTCGTCCTT-3'

Neo-del-R (R3): 5'-AACTGTTTCATTTCCCCTCTCCTC-3'

Forward1 (F4): 5'-GGTAAGGAGATAAAGAGTTTCCGTAC-3'

Expected PCR Product:

Wildtype: 257 bp

Targeted: 448 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl

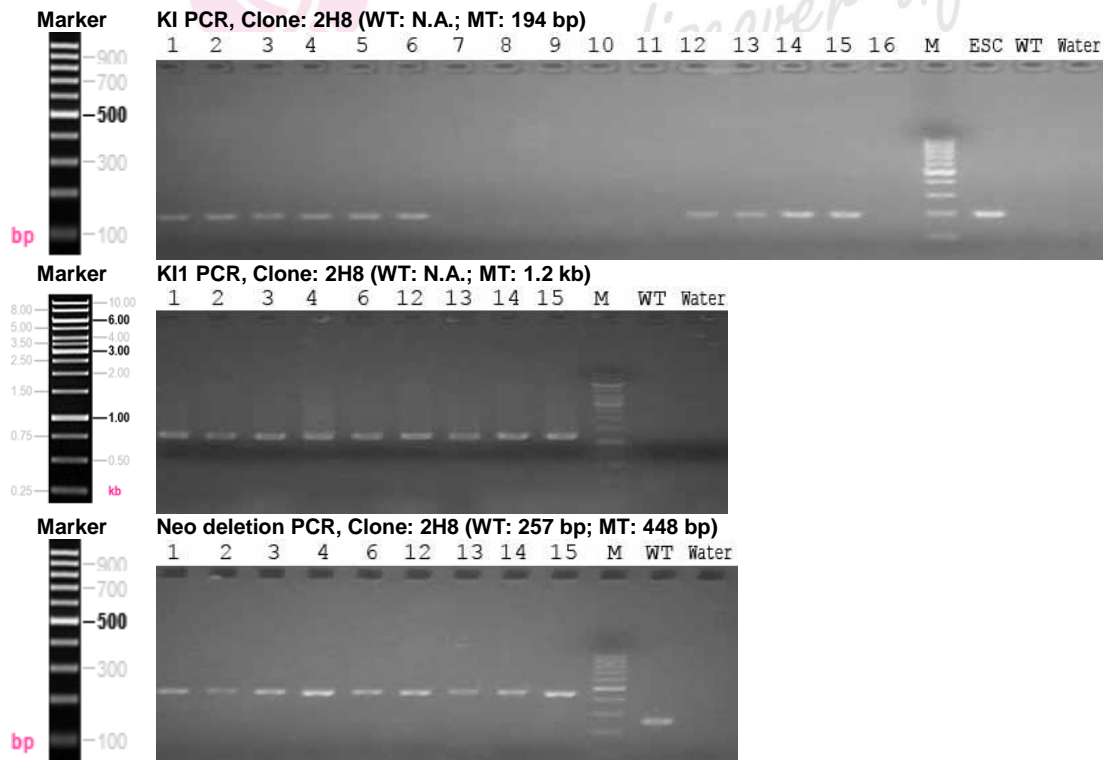
Neo-del-F (F3) (10 µM)	1.0 µl
Neo-del-R (R3) (10 µM)	1.5 µl
Forward1 (F4) (10 µM)	0.5 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	8.0 µl
Total	25.0 µl

Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	33 x
Denaturation	94 °C	30s	
Annealing	62 °C	35s	
Extension	72 °C	35s	
Additional extension	72 °C	5 min	

1.5. Result:

Four pups (1#, 2#, 3# and 4#) from clone 2H8 were identified positive by PCR screening for KI, KI1 and Neo deletion, the positive pups were reconfirmed by PCR screening for KI1 and Neo deletion.



1.6. Suggested Breeding and Genotyping Assay

Step 1: Inter-cross heterozygous targeted mice to generate homozygous targeted mice

Primers for targeted allele:

Neo-del-F (F3): 5'-GGCAGTGGGTGTTTCGTCCTT-3'

Neo-del-R (R3): 5'-AACTGTTTCATTTCCCCTCTCCTC-3'

Forward1 (F4): 5'-GGTAAGGAGATAAAGAGTTTCCGTAC-3'

Wildtype: 257 bp

Homozygotes: 448 bp

Heterozygotes: 448 bp/257 bp

