

Appendix

Table of Contents

Vector Report	1
ES Cell Report	10
Animal Report	18
Appendix Table S1	30
Appendix Table S2	30



Vector Report

Project: Mouse Uty Constitutive Knockin

1. Quote

TKK-171122-AMW-01

2. Gene

Uty

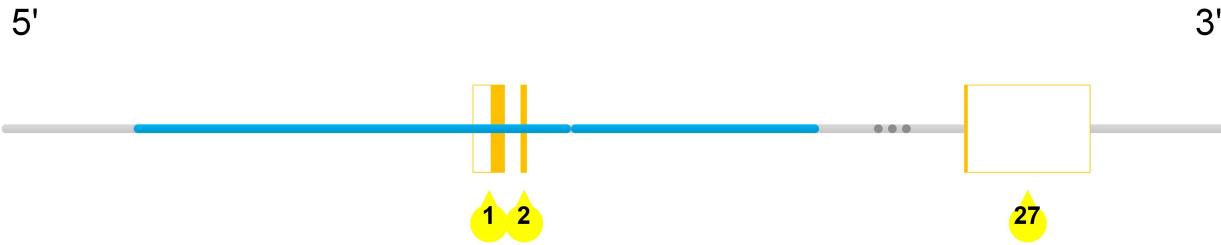
3. Objective

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.

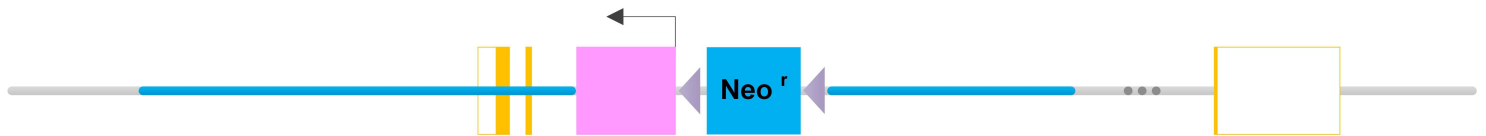
Wildtype allele



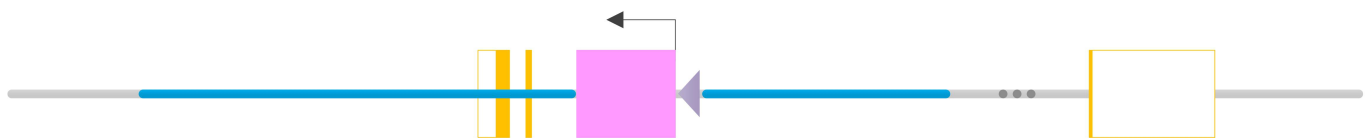
Targeting vector



Targeted allele



Constitutive KI allele (After Neo deletion)



Legends

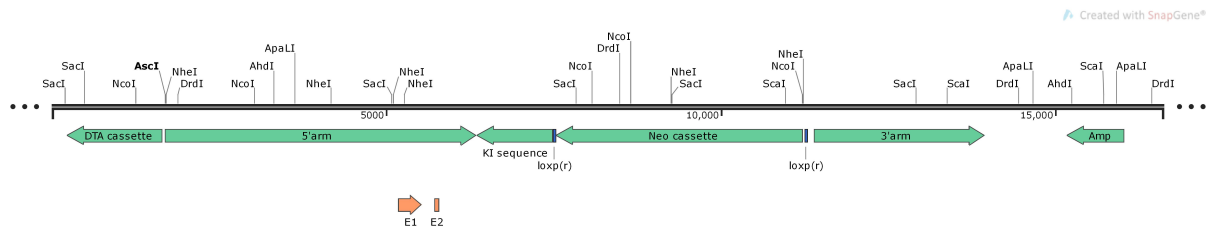


5. Method

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

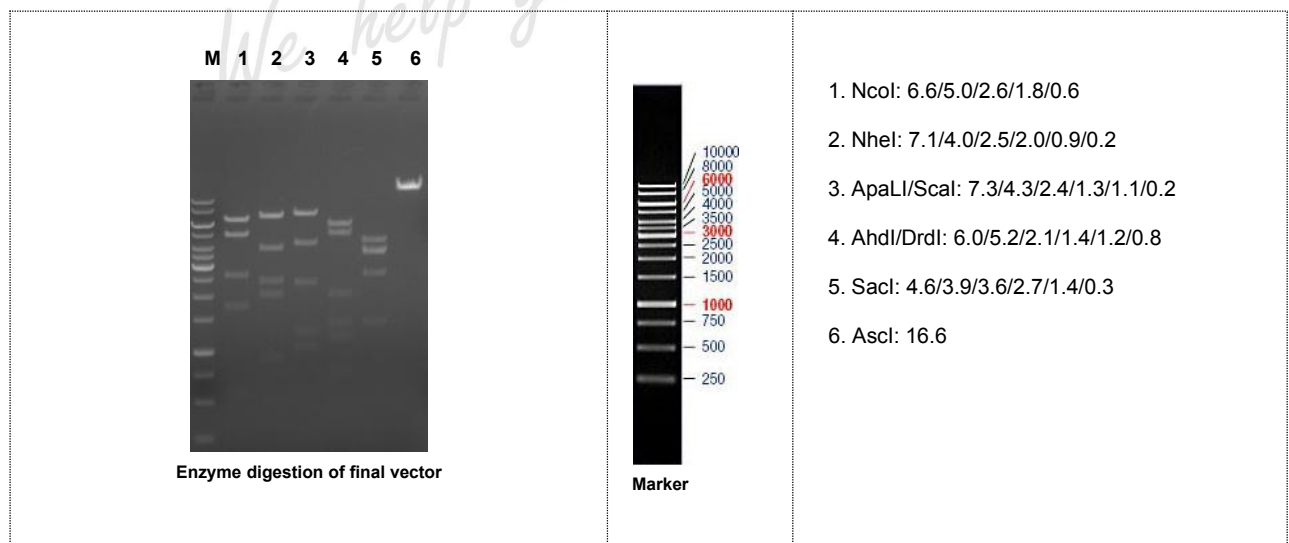
Linearized targeting vector



✂ 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

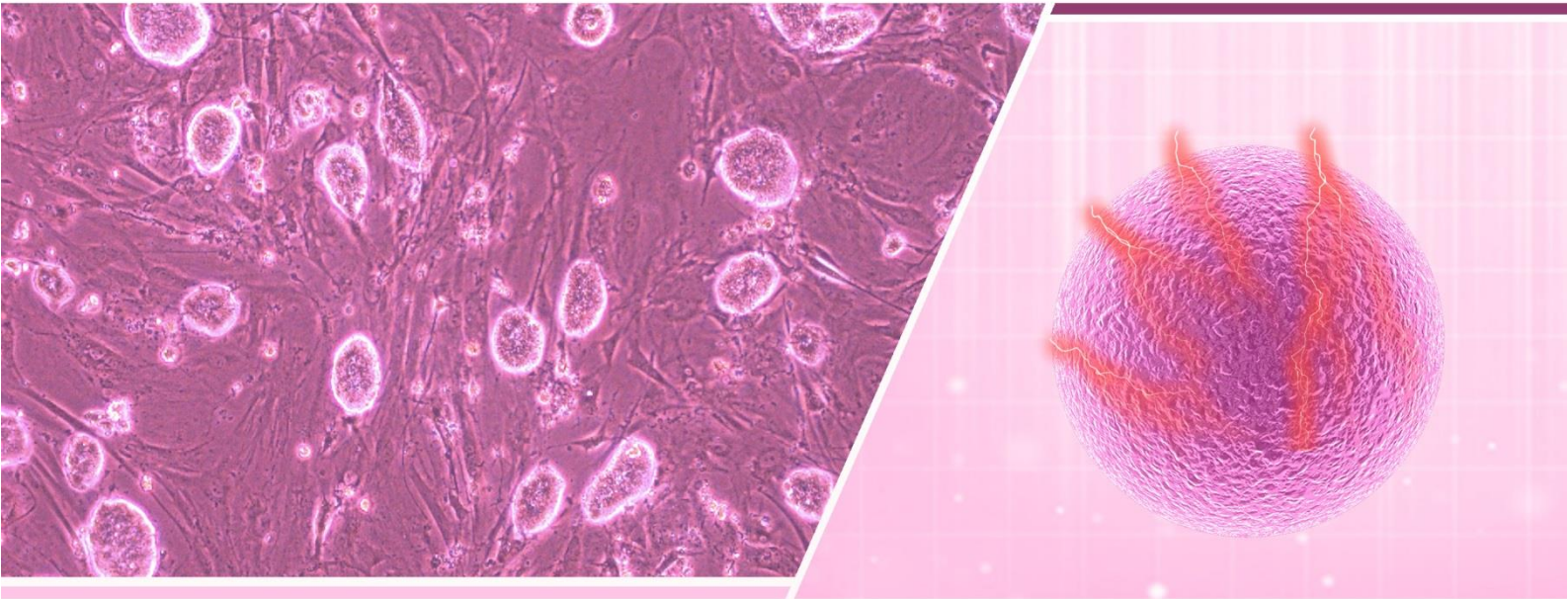
	Homology arms	KI region	loxP sites	Exons	Sequence confirmed regions					
1	CGCTTA	CAATTT	CCATTC	GCCATT	CAGGCT	GCGCAA	CTGTTG	GGAAGG	GCGATC	GGTGCG
61	GGCCTC	TTCGCT	ATTACG	CCAGCT	GGCGAA	AGGGGG	ATGTGC	TGCAAG	GCGATT	AAGTTG
121	GGTAAC	GCCAGG	GTTTTT	CCAGTC	ACGACG	TTGTAA	AACGAC	GGCCAG	TGAATT	GTAATA
181	CGACTC	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	ACAAAT	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	TTCGAG
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	TGGGTG	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	ATTCCT	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	AAAAAA	AGAAAA	AAGTCA	GCAGAG	GAGAAAT	GAAAGG
2641	ATGGAC	TACTAC	TACTTC	AGAACT	GGTTAA	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	GAACCA	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	CGACTC	GGTGCC
7141	ACTTTT	TCAAGT	TGATAA	CGGACT	AGCCTT	ATTTTA	ACTTGC	TATTTT	TAGCTC	TAAAC
7201	CGATTC	GCCCGG	TGGCAG	TGGGTG	TTTCGT	CCTTTC	CACAAG	ATATAT	AAAGCC	AAGAAA
7261	TCGAAA	TACTTT	CAAGTT	ACGGTA	AGCATA	TGATAG	TCCATT	TTAAAA	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	CTTCGT	ATAGCA	TACATT	ATACGA	AGTTAT	ATTAAG	GGTTCC	GCAAGC	TCTAGT	CGAGCC
7561	CCAGTC	GGTTCT	TTCCGC	CTCAGA	AGCCAT	AGAGCC	CACCGC	ATCCCC	AGCATG	CCTGCT
7621	ATTGTC	TTCCCA	ATCCTC	CCCCTT	GCTGTC	CTGCCC	CACCCC	ACCCCC	CAGAAT	AGAATG
7681	ACACCT	ACTCAG	ACAATG	CGATGC	AATTTT	CTCATT	TTATTA	GGAAA	GACAGT	GGGAGT
7741	GGCACC	TTCCAG	GGTCAA	GGAAGG	CACGGG	GGAGGG	GCAAAC	AACAGA	TGGCTG	GCAACT
7801	AGAAGG	CACAGT	CGAGGC	TGATCA	GCGAGC	TCTAGA	GAATTG	ATCCCC	TCAGAA	GAAGTC
7861	GTCAAG	AAGGCG	ATAGAA	GGCGAT	GCGCTG	CGAATC	GGGAGC	GGCGAT	ACCGTA	AAGCAC
7921	GAGGAA	GCGGTC	AGCCCA	TTTCGC	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	GGTCAC	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	CCCCGG	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
8521	CGCTGA	CAGCCG	GAACAC	GGCGGC	ATCAGA	GCAGCC	GATTGT	CTGTTG	TGCCCA	GTCATA
8581	GCCGAA	TAGCCT	CTCCAC	CCAAGC	GGCCGG	AGAACC	TGCGTG	CAATCC	ATCTTG	TTCAAT
8641	GGCCGA	TCCCAT	GGTTTA	GTTCTT	CACCTT	GTCGTA	TTATAC	TATGCC	GATATA	CTATGC
8701	CGATGA	TTAATT	GTCAAC	AGGCTG	CAGGTC	GAAAGG	CCCGGA	GATGAG	GAAAGG	GAGAAC
8761	AGCGCG	GCAGAC	GTGCGC	TTTTGA	AGCGTG	CAGAAT	GCCGGG	CCTCCG	GAGGAC	CTTCGG
8821	GCGCCC	GCCCCG	CCCCTG	AGCCCC	CCCCTG	AGCCCC	CCCCCG	GACCCA	CCCCTT	CCCAGC
8881	CTCTGA	GCCAG	AAAGCG	AAGGAG	CAAAGC	TGCTAT	TGGCCG	CTGCCC	CAAAGG	CCTACC
8941	CGCTTC	CATTGC	TCAGCG	GTGCTG	TCCATC	TGCACG	AGACTA	GTGAGA	CGTGCT	ACTTCC
9001	ATTTGT	CACGTC	CTGCAC	GACGCG	AGCTGC	GGGGCG	GGGGGG	AACTTC	CTGACT	AGGGGA
9061	GGAGTA	GAAGGT	GGCGCG	AAGGGG	CCACCA	AAGAAC	GGAGCC	GGTTGG	CGCTA	CCGGTG
9121	GATGTG	GAATGT	GTGCGA	GGCCAG	AGGCCA	CTTGTTG	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	TATTTG	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	TTCATC	ATCCAG	GTTACG	GATATA	GTTTAT
9541	GACAA	ATTTAC	ATTGGT	CCAGCC	ACCAGC	TTGCAT	GATCTC	CGGTAT	TGAAAC	TCCAGC
9601	GCGGGC	CATATC	TCGCGC	GGCTCC	GACACG	GGCATC	GTGTCC	AGACCA	GGCCAG	GTATCT
9661	CTGACC	AGAGTC	ATCCTT	AGCGCC	GTAATC	CAATCG	ATGAGT	TGCTTC	AAAAAT	CCCTTC
9721	CAGGGC	GCGAGT	TGATAG	CTGGCT	GGTGGC	AGATGG	CGCGGC	AACACC	ATTTTT	TCTGAC
9781	CCGGCA	AAACAG	GATGTT	ATTCGG	ATCATC	AGCTAC	ACCAGA	GACGGA	AATCCA	TCGCTC
9841	GACCAG	TTTAGT	TACCCC	CAGGCT	AAGTGC	CTTCTC	TACACC	TGCGGT	GCTAAC	CAGCGT
9901	TTTCGT	TCTGCC	AATATG	GATTAA	CATTCT	CCCACC	GTCAGT	ACGTGA	GATATC	TTTAA
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	ATTTAA	CTTGCA	CCATGC	CGCCCA	CGACCG	GCAAAC	GGACAG	AAGCAT	TTTCCA
10381	GGTATG	CTCAGA	AAACGC	CTGGCG	ATCCCT	GAACAT	GTCCAT	CAGGTT	CTTGCG	AACCTC
10441	ATCACT	CGTTGC	ATCGAC	CGGTAA	TGCAGG	CAAATT	TTGGTG	TACGGT	CAGTAA	ATTGGA
10501	CACCTT	CCTCTT	CTTCTT	GGGCAT	GGCCGC	AGGAAA	GCAGAG	CCCTGA	AGCTCC	CATCAC
10561	CGGCCA	ATAAGA	GCCAAG	CCTGCA	GTGTGA	CCTCAT	AGAGCA	ATGTGC	CAGCCA	GCCTGA
10621	CCCCAA	GGGCCC	TCAGGC	TTGGGC	ACACTG	TCTCTA	GGACCC	TGAGAG	AAAGAC	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
10921	GCCATT	CCGCTG	ATTCTG	CTCTTC	CAGCCA	CTGGCT	GACCGG	TTGGAA	GTACTC	CAGCAG
10981	TGCCTT	GGCATC	CAGGGC	ATCTGA	GCCTAC	CAGGTC	CTTCAG	TACCTC	CTGCCA	GGGCTT
11041	GGAGCA	GCCAGC	CTGCAA	CACCTG	CCTGCC	AAGCAG	AGTGAC	CACTGT	GGGCAC	AGGGGA
11101	CACAGG	GTGGGG	CCCACA	ACAGCA	CCATTG	TCCACT	TGTCCC	TCACTA	GTAATA	GAACTC
11161	TAGGGT	TGCGGG	GGGTGG	GGGAGG	TCTCTG	TGAGGC	TGGTAA	GGGATA	TTTGCC	TGGCCC
11221	ATGGAG	CTAGCT	TGGCTG	GACGTA	AACTCC	TCTTCA	GACCTA	ATAACT	TGTTAT	AGCATA
11281	CATTAT	ACGAAG	TTATAT	TAAGGG	TTATTG	AATATG	ATCGGA	ATTGGG	CTGCAG	GAATTC
11341	GATAGC	TTGGCT	GCAGGT	CGACGT	ACGTAG	CAAGCT	TGATGG	GCCCTG	GTACCG	AAATAG
11401	AGGGAG	GAGAGG	GGAAAT	GAAACA	GTTTTA	GAAAGG	AAACAG	GGTAGT	TTTACC	AAGAAA
11461	ATATTA	AACCTA	GCAAGT	ACAGGT	TAATGA	AAAGTA	TTCCCC	ATTACT	TAAAAG	GAGAAAT
11521	TATCAA	GTCTTT	TTAAAC	CTTTAA	AAAAAG	ATTCTT	AGCCGG	GCGGGG	TGGCAC	ATGCCT
11581	TTAATC	CCAGCA	CTTGGG	AGGCAG	AGGCAG	GCAGAT	TTCTGA	GTTTGA	GGCCAG	TCTGGT
11641	CTACAG	AGTGAG	TTCCAG	GACAGC	CAGGGC	TACACA	GAGATA	CCCTGT	CTCAAA	AAAAAA
11701	AAAAAA	ATTCTT	AAAGGT	AACATA	TTTAGA	TACTTG	TAAACC	ATAGCA	AAAAAA	GCACAG
11761	ATCTGT	GACATA	CATACT	TGTTAC	CATTAT	ATAGTT	TCCGTT	CTAATG	AAAATC	AGTGAT
11821	TATAAA	CTTGAT	AATTTT	ATACCA	CCAATG	AAGTAT	TAATAG	TATTCT	TTTTTT	GTTTTT
11881	GGAGAC	AGGGTT	TCTCAG	TATAGC	TCTGGT	TGCTAT	ACAGTT	AATAGT	GTTCTT	AACATA
11941	TATGTG	TATATA	TAAATT	TATGTA	TGTATG	TATATA	TATATA	TACATA	TATATA	TATGCA
12001	TATTAT	GCACAC	ATAATT	TTTGGT	TTGGTT	TGGTTG	TGAGCC	TAGCCT	TTAAGG	ACTGAG
12061	TCCAAG	ATTTAA	ACACTC	TTCAGG	TGATAT	GTATAG	AAACAT	ATTTCT	GTTTAC	TAGTAG
12121	GTTGCT	AGGTTT	CTGGTG	TACAGT	GGGTAA	TTTTAA	ATTTGA	TGTTAA	CCTCCA	CACTCT
12181	TTTAAG	ATTTCA	TGGTAA	AAGCTT	AAAGAA	ATGATT	TTGTCT	AGTTTT	TATTTT	TATTTT
12241	TTAATT	TTTTTT	ATTTTT	TTGGTT	TTTGGG	GACAGG	GTTTCT	CTGTAT	AGCTAT	GGCTGT
12301	CCTGGA	ACTCAC	TTTGTA	GACCAG	CTGGGC	CTCAAA	CTCAGA	AATCTG	CTGTCT	TCTCCC
12361	TCCTGA	GTGCTG	GGATTA	AAGGCA	TGCACC	ACCACG	CCTGAC	GTGGTT	TTTTAA	GATATA
12421	ATTATT	TAACTA	TATCTT	AGCATT	TCTTTT	TTTTTT	TTAGAG	TAGTAA	TTCTTA	TATTAT
12481	AAAAAA	GACTTG	GTGTTG	AATGTA	TTTTTT	GAGTGA	CTACAA	CAACTA	TGTGTG	ATCCTG
12541	TTTGTC	CTAGAA	CTCACA	GACAGA	AATATC	ACTTTC	TCTGCT	TTCTCA	TTGCTC	ATTGCT
12601	AAGATT	AAAGCC	CTGTTT	TCCTAA	AACTGG	CTTCTT	TTAAAT	TTAATA	ATTTTG	TATATA
12661	TTCATG	TCTGGA	AGTTAT	AGGACA	AATTGT	GGAAGT	CAGTTT	TCTTCT	TCTATC	ATGTCA
12721	GGGCTG	GAGGCA	AGTCCT	TTTACT	AGCTAA	GCCACT	TCCTTG	AACTTT	CAGTTT	ATGTGT
12781	ATAGTG	TTTCTA	CTGCTT	GTAGGT	CTATTC	CACTTG	AGTGTC	TGGTTC	TACTAA	GGAGTT
12841	TAGAAG	AGACAG	ACCTAT	GGAACT	GGAATT	AGTGAA	GGTTGT	GAGCTT	CACTAA	TCAAAC
12901	CAGTGT	TCTCTT	GCAGAG	CTCTTT	AGTTTA	TTTAAG	TAGCAT	ATATTT	TTCAGG	TGCTTT
12961	GTAATA	TCAATT	TATATG	TTAGTA	AACTGT	TTTCAA	ATATTG	AAATAA	CAGGTA	TTTTTT
13021	AACTAA	TTGCAA	CAGCAT	TTTAAA	AATGAG	AAGTTA	AAAACA	AAATAT	GAGAAA	CTATCA
13081	AGTGCC	ACTGTT	CTTAGA	ACATTT	ATGAGT	TGTGCC	TTTATT	AAACAT	TGAGTA	ATATGT
13141	ATTTTT	GTTTTA	GATCAG	TCTCAG	CCTTTC	TAATGC	AGTTTC	TCATGT	TGTGAT	GACCCC
13201	CAACCA	TAAAAT	TATTTT	TGTTGT	TATTCA	TAACTA	ATTTTG	GTGCTG	TTATGA	ATCATT
13261	ATGTAA	ATATCT	GAATAG	TTTATT	AGTGAC	CAAAAA	GGTATT	TTGACC	CATAGG	TTGAGA
13321	ACCATT	ATTTTA	GACACT	GTCTTA	GTCTAT	ATTTTG	TTGCC	AGAATA	GCTTFA	AATTTG
13381	AAGAAG	TACTCA	TCTCTC	AGCCTC	CAAGTA	TTGAGA	TTTCAG	GCATGA	GCCACT	ACTCTT
13441	TAACAG	TTATTA	AAATGT	ATTACA	ATTATA	ATTTAA	AATGAC	AAGTAT	TAAAAA	CTGAAA
13501	TATAGG	GCTGGT	GAGATG	GCTCAG	TGGGTA	AGAGCA	CCCGAC	TGCTCT	TCCGAA	GGTCCA
13561	GAGTTC	AAATCC	CAGCAA	CCACAT	GGTGGC	TCACAA	CCATCC	ATAACG	AGATCT	GACTCC
13621	CTCTTC	TGGAGT	GTCTGA	AGACAG	CTACAG	TGTACT	TACATA	TAATAA	ATAAAT	CTTTAA
13681	AAAAAA	AAAAAC	AAAAAA	AAACCT	GAAATA	TATAAA	GGGCAA	TTGATG	GATGGT	GGTGGG
13741	ATATCC	CTTTAT	CCTAGC	ACTGTG	CAGAGG	CAGTTG	GGTCTC	TTATAA	GTTTGA	GGCCAG
13801	CCTGAT	CTACAT	AGCAAG	TTTCAG	GACAGC	AAGATT	AGACAG	AGAAAC	CTGCCT	CAAAAA
13861	AGGACA	ATTTGT	TTTTTA	GCCTGA	CAGTGG	TGGCAT	ACACCT	TTAAAC	CCAGCA	CTTGGG
13921	GGACAT	AGGCAG	ATAGAT	CTTTGG	CGGCCG	CGTACC	AGCTTT	TGTTCC	CTTTAG	TGAGGG
13981	TTAATT	TCGAGC	TTGGCG	TAATCA	TGGTCA	TAGCTG	TTTCCT	GTGTGA	AATTGT	TATCCG
14041	CTCACA	ATTCCA	CACAAC	ATACGA	GCCGGA	AGCATA	AAGTGT	AAAGCC	TGGGGT	GCCTAA
14101	TGAGTG	AGCTAA	CTCACA	TTAATT	GCGTTG	CGCTCA	CTGCCC	GCTTTC	CAGTCG	GGAAAC
14161	CTGTCT	TGCCAG	CTGCAT	TAATGA	ATCGGC	CAACGC	GCGGGG	AGAGGC	GGTTTG	CGTATT
14221	GGGCGC	TCTTCC	GCTTCC	TCGCTC	ACTGAC	TCGCTG	CGCTCG	GTCGTT	CGGCTG	CGGCGA
14281	GCGGTA	TCAGCT	CACTCA	AAGGCG	GTAATA	CGGTTA	TCCACA	GAATCA	GGGGAT	AACGCA
14341	GGAAG	AACATG	TGAGCA	AAAGGC	CAGCAA	AAGGCC	AGGAAC	CGTAAA	AAGGCC	GCGTTG

14401 CTGGCG TTTTTC CATAGG CTCCGC CCCCCT GACGAG CATCAC AAAAAT CGACGC TCAAGT
14461 CAGAGG TGGCGA AACCCG ACAGGA CTATAA AGATAC CAGGCG TTTCCC CCTGGA AGCTCC
14521 CTCGTG CGCTCT CCTGTT CCGACC CTGCCG CTTACC GGATAC CTGTCC GCCTTT CTCCCT
14581 TCGGGA AGCGTG GCGCTT TCTCAT AGCTCA CGCTGT AGGTAT CTCAGT TCGGTG TAGGTC
14641 GTTCGC TCCAAG CTGGGC TGTGTG CACGAA CCCCCC GTTCAG CCCGAC CGCTGC GCCTTA
14701 TCCGGT AACTAT CGTCTT GAGTCC AACCCG GTAAGA CACGAC TTATCG CCACTG GCAGCA
14761 GCCACT GGTAAC AGGATT AGCAGA GCGAGG TATGTA GGCGGT GCTACA GAGTTC TTGAAG
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
15001 GATCCT TTGATC TTTTCT ACGGGG TCTGAC GCTCAG TGGAAC GAAAAC TCACGT TAAGGG
15061 ATTTTG GTCATG AGATTA TCAAAA AGGATC TTCACC TAGATC CTTTTA AATTAA AAATGA
15121 AGTTTT AAATCA ATCTAA AGTATA TATGAG TAAACT TGGTCT GACAGT TACCAA TGCTTA
15181 ATCAGT GAGGCA CCTATC TCAGCG ATCTGT CTATTT CGTTCA TCCATA GTTGCC TGACTC
15241 CCCGTC GTGTAG ATAACT ACGATA CGGGAG GGCTTA CCATCT GGCCCC AGTGCT GCAATG
15301 ATACCG CGAGAC CCACGC TCACCG GCTCCA GATTTA TCAGCA ATAAAC CAGCCA GCCGGA
15361 AGGGCC GAGCGC AGAAGT GGTCCCT GCAACT TTATCC GCCTCC ATCCAG TCTATT AATTGT
15421 TGCCGG GAAGCT AGAGTA AGTAGT TCGCCA GTTAAT AGTTTG CGCAAC GTTGTT GCCATT
15481 GCTACA GGCATC GTGGTG TCACGC TCGTCG TTTGGT ATGGCT TCATTC AGCTCC GGTTC
15541 CAACGA TCAAGG CGAGTT ACATGA TCCCCC ATGTTG TGCAAA AAAGCG GTTAGC TCCTTC
15601 GGTCCCT CCGATC GTTGTC AGAAGT AAGTTG GCCGCA GTGTTA TCACTC ATGGTT ATGGCA
15661 GCACTG CATAAT TCTCTT ACTGTC ATGCCA TCCGTA AGATGC TTTTCT GTGACT GGTGAG
15721 TACTCA ACCAAG TCATTC TGAGAA TAGTGT ATGCGG CGACCG AGTTGC TCTTGC CCGGCG
15781 TCAATA CGGGAT AATACC GCGCCA CATAGC AGAACT TTAAAA GTGCTC ATCATT GGAAAA
15841 CGTTCT TCGGGG CGAAAA CTCTCA AGGATC TTACCG CTGTTG AGATCC AGTTCG ATGTAA
15901 CCCACT CGTGCA CCCAAC TGATCT TCAGCA TCTTTT ACTTTC ACCAGC GTTCTT GGTGTA
15961 GCAAAA ACAGGA AGGCAA AATGCC GCAAAA AAGGGA ATAAGG GCGACA CGGAAA TGTGTA
16021 ATACTC ATACTC TTCCTT TTTCAA TATTAT TGAAGC ATTTAT CAGGGT TATTGT CTCATG
16081 AGCGGA TACATA TTTGAA TGTATT TAGAAA AATAAAA CAAATA GGGGTT CCGCGC ACATTT
16141 CCCCCG AAAGTG CCACCT GACGCG CCCTGT AGCGGC GCATTA AGCGCG GCGGGT GTGGTG
16201 GTTACG CGCAGC GTGACC GCTACA CTTGCC AGCGCC CTAGCG CCCGCT CCTTTC GCTTTC
16261 TTCCCT TCCTTT CTCGCC ACGTTC GCCGGC TTTCCC CGTCAA GCTCTA AATCGG GGGCTC
16321 CCTTTA GGGTTC CGATTT AGTGCT TTACGG CACCTC GACCCC AAAAAA CTTGAT TAGGGT
16381 GATGGT TCACGT AGTGGG CCATCG CCCTGA TAGACG GTTTTT CGCCCT TTGACG TTGGAG
16441 TCCACG TTCTTT AATAGT GGACTC TTGTTC CAAACT GGAACA ACACCT AACCTT ATCTCG
16501 GTCTAT TCTTTT GATTTA TAAGGG ATTTTG CCGATT TCGGCC TATTGG TTAAAA AATGAG
16561 CTGATT TAACAA AAATTT AACGCG AATTTT AACAAA ATATTA A



ES Cell Report

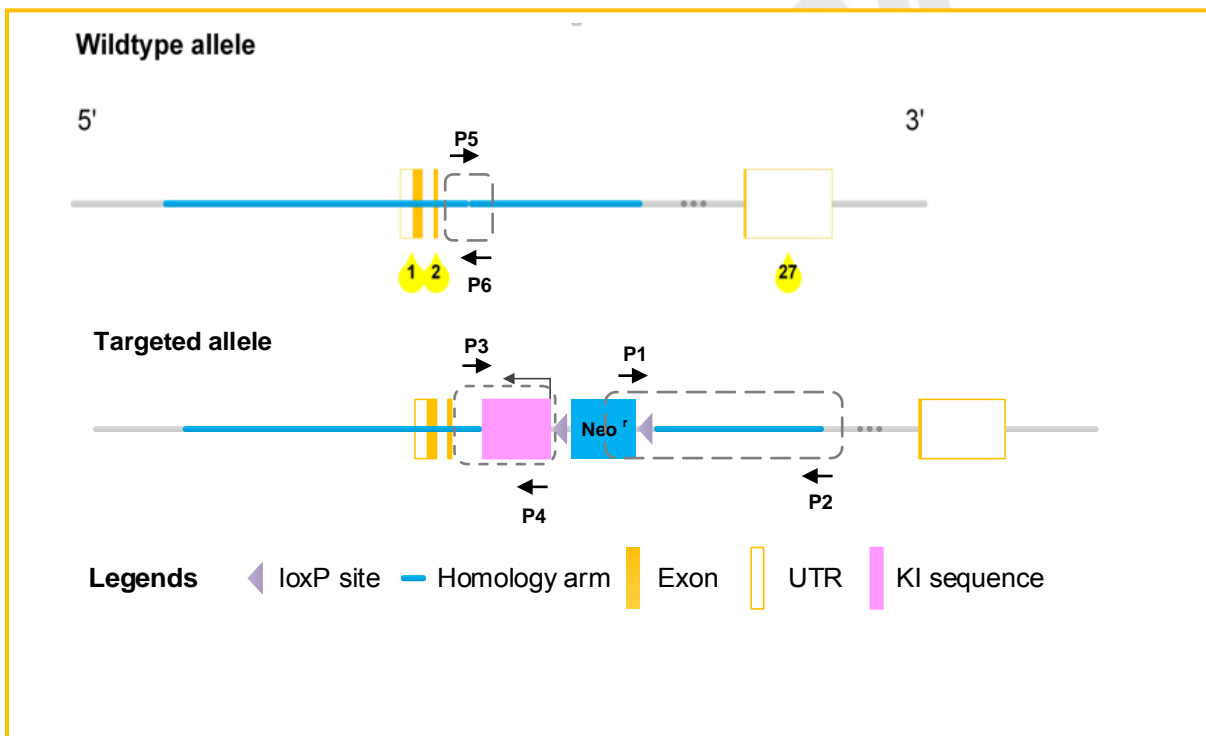
Project: Mouse Uty Constitutive Knockin

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

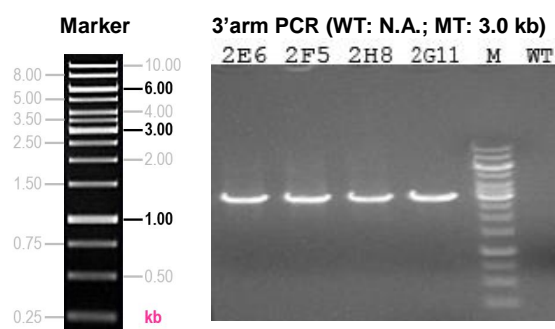
Reaction Mix:

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

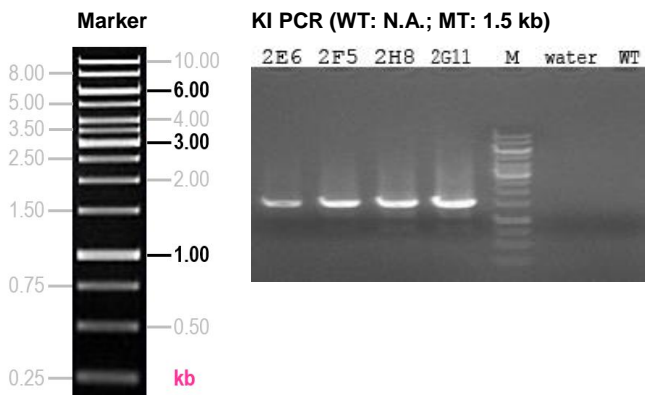
Reaction Mix:

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

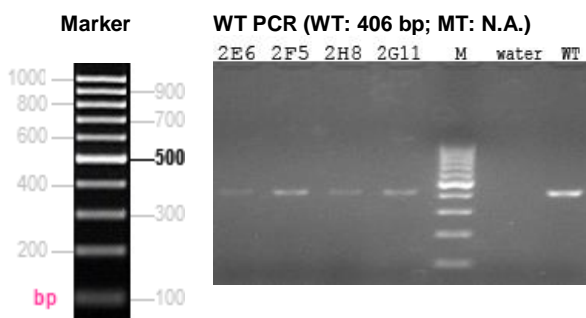
Reaction Mix:

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.

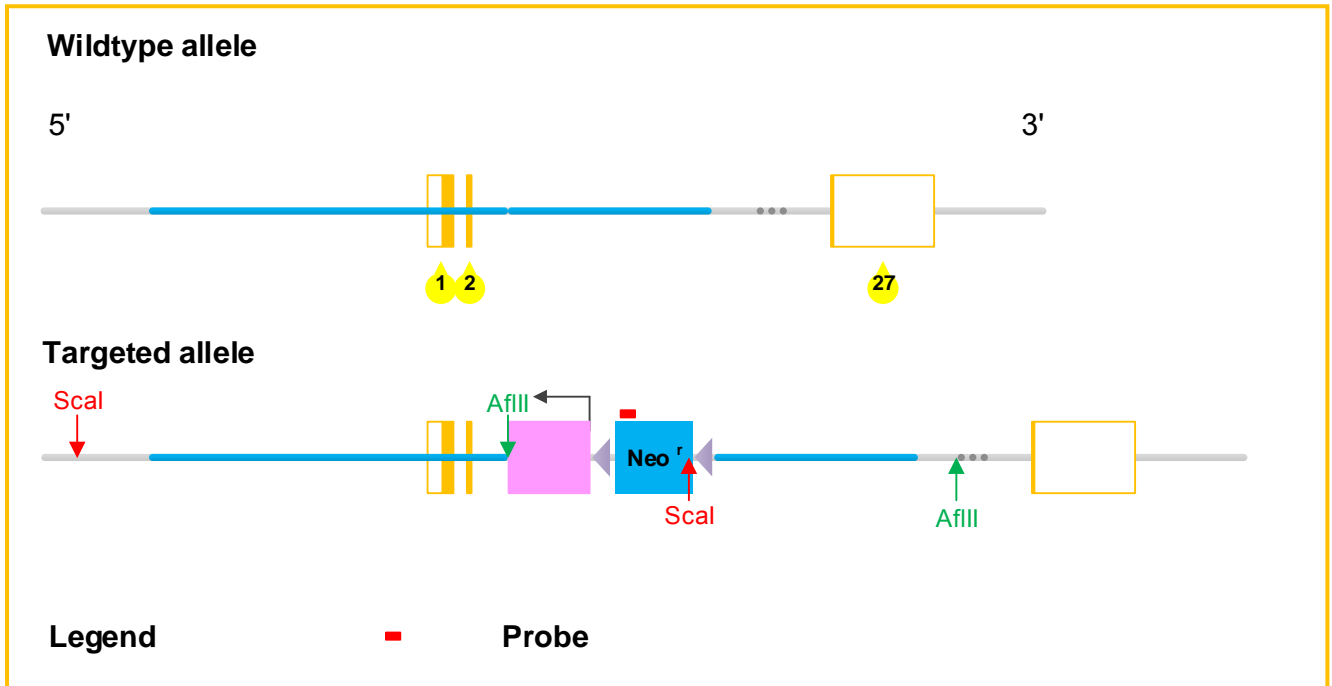


1.3. Southern Blot Analysis

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.

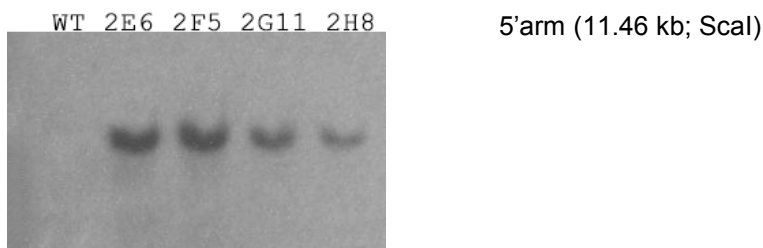


Expected Fragment Sizes for Southern Blot:

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)

 **cyagen**
We help you discover life



Animal Report

Project: Mouse Uty Constitutive Knockin

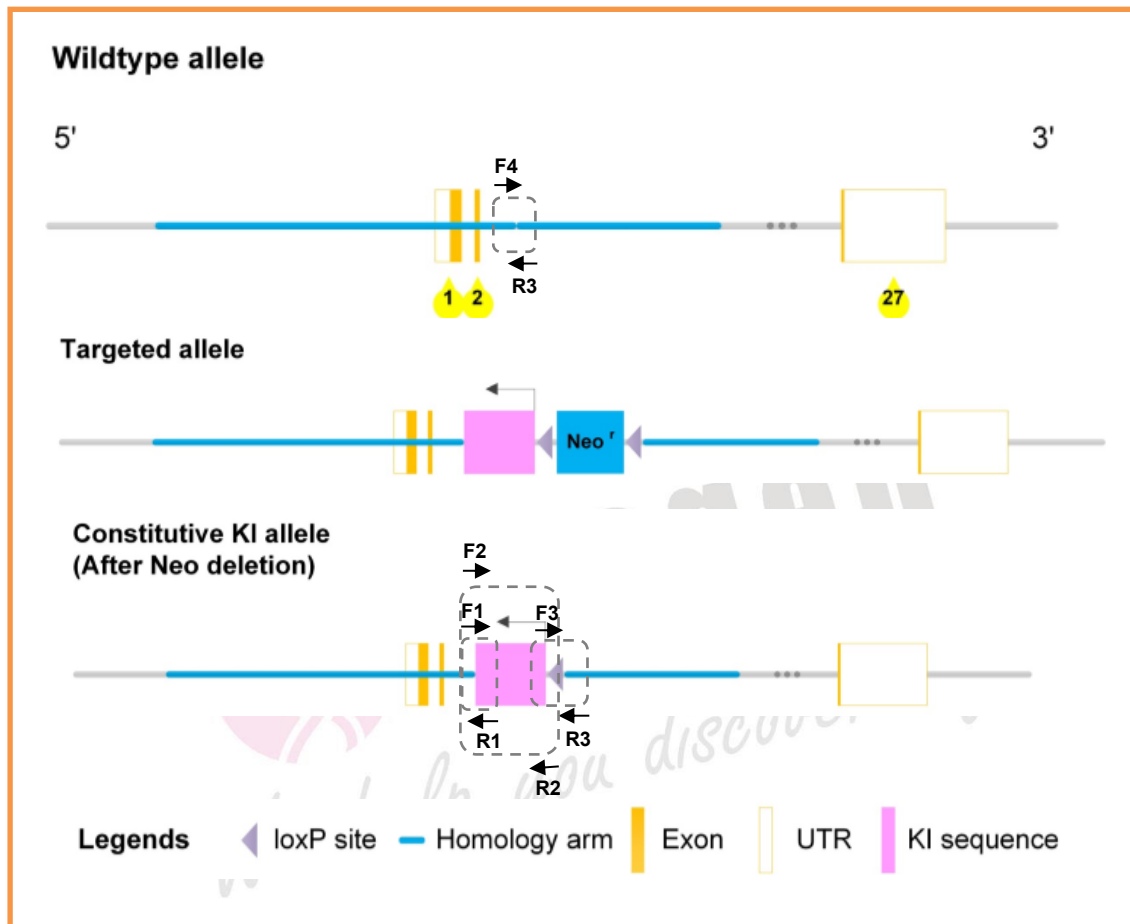
1. Animal Generation

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:

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	×1
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	×1
Mouse genomic DNA	1.5 µl

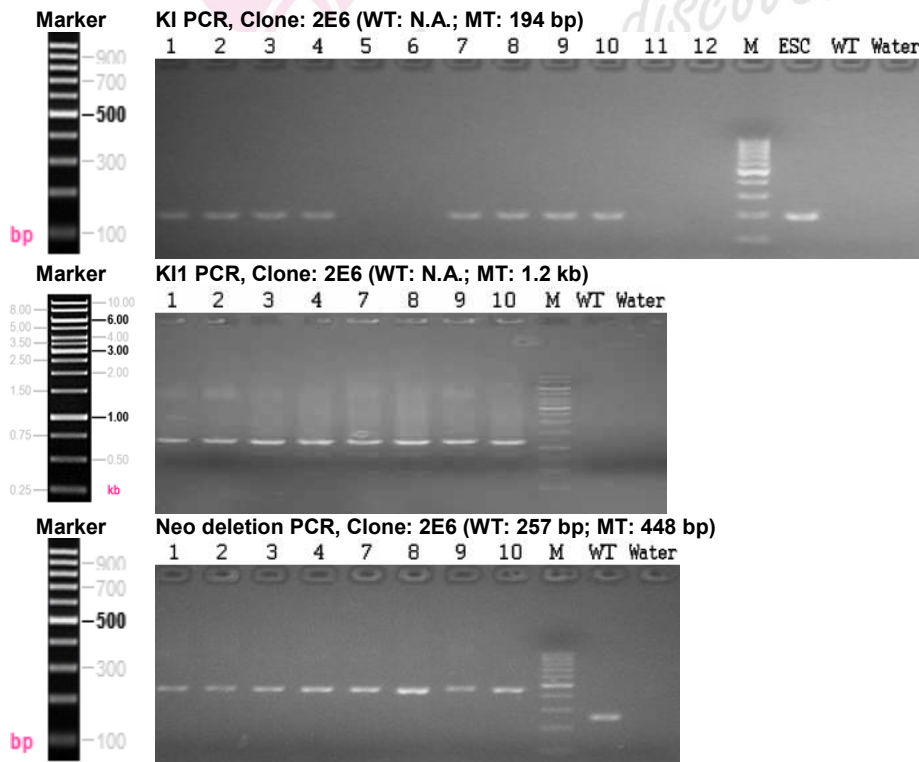
Neo-del-F (F3) (10 µM)	1.0 µl
Neo-del-R (R3) (10 µM)	1.5 µl
Forward1 (F4) (10 µM)	0.5 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	8.0 µl
Total	25.0 µl

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

Project: Mouse Uty Constitutive Knockin

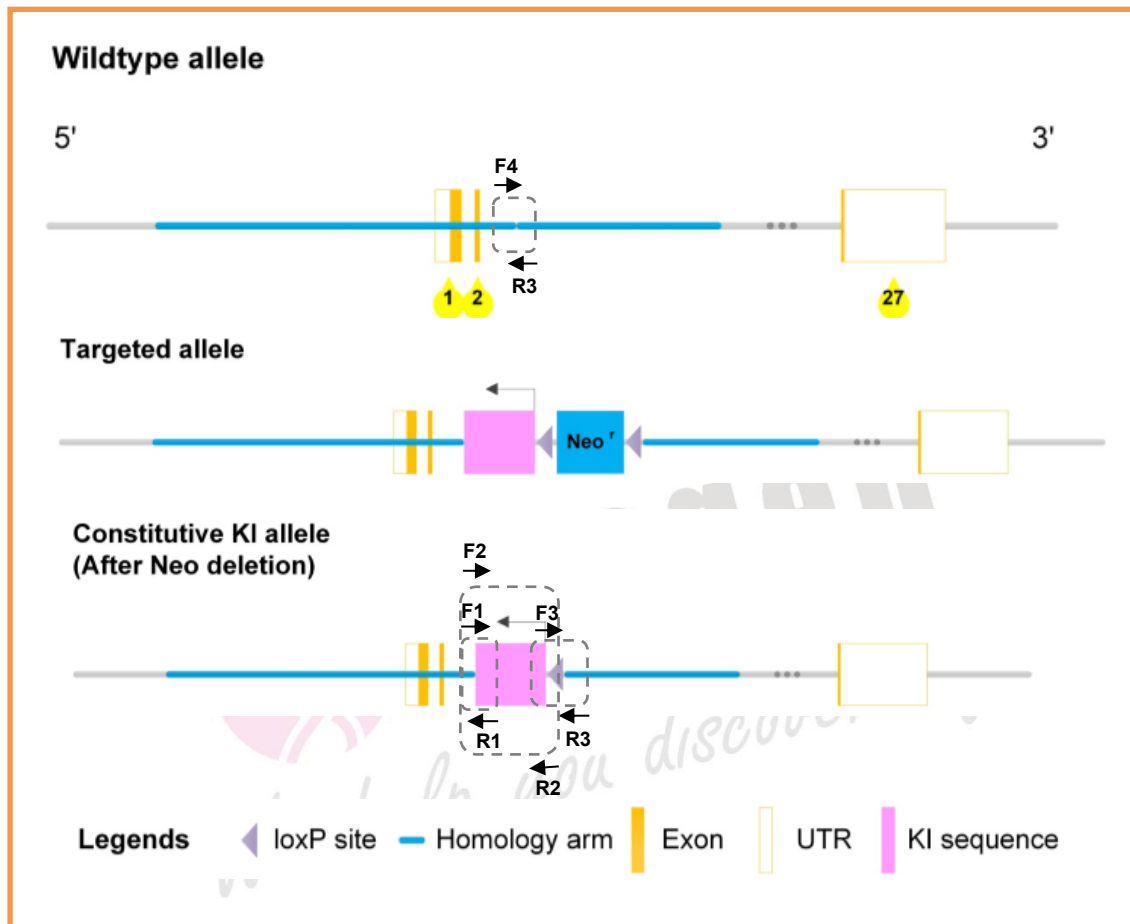
1. Animal Generation

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:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30s	33 x
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	×1
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	
Denaturation	94 °C	30s	33 x
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	×1
Mouse genomic DNA	1.5 µl

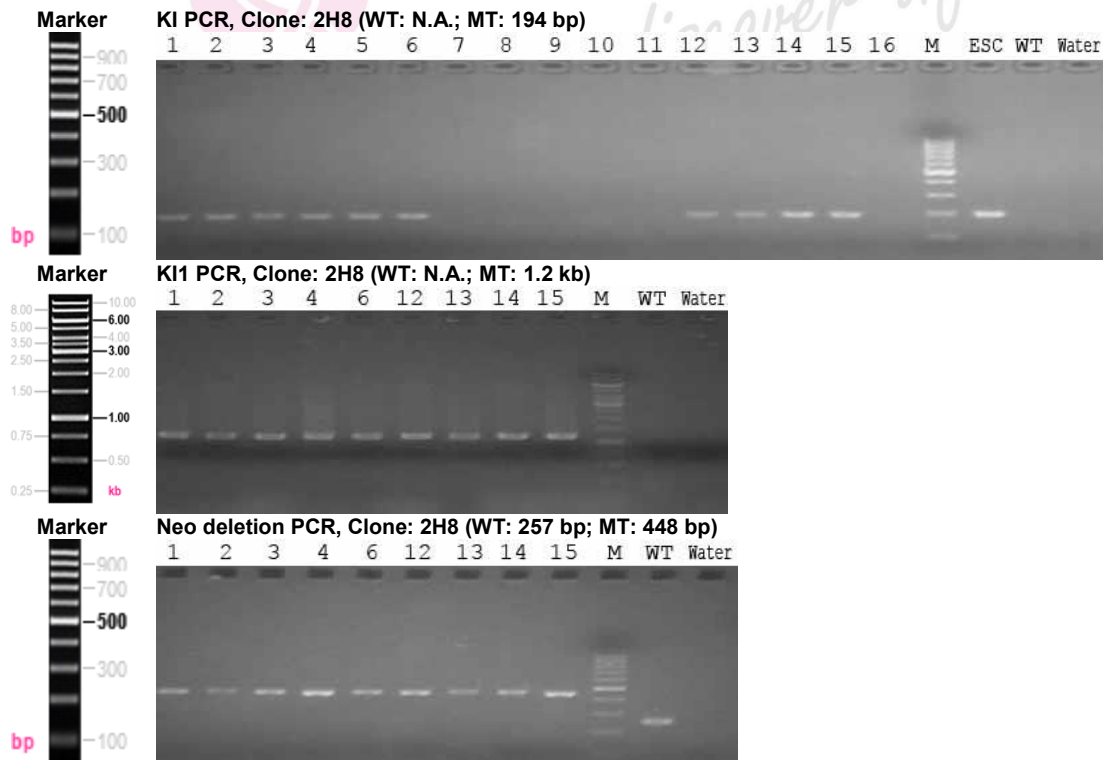
Neo-del-F (F3) (10 µM)	1.0 µl
Neo-del-R (R3) (10 µM)	1.5 µl
Forward1 (F4) (10 µM)	0.5 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	8.0 µl
Total	25.0 µl

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'-AACTGTTTCATTTCCCCTCCTC-3'

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

Wildtype: 257 bp

Homozygotes: 448 bp

Heterozygotes: 448 bp/257 bp



Appendix Table S1. Oligonucleotides used in this study

Oligonucleotides	5'→3'
Y forward	GGCAGTGGGTGTTTCGTCCTT
Y reverse	AACTGTTTCATTTCCCCTCTCCTC
Y-WT forward	GGTAAGGAGATAAAGAGTTTCCGTAC
Cas9-M forward	ACACCAGCACCAAAGAGGTG
Cas9-M reverse	GTAGGTCAGGGTGGTCACGA
Cas9-WT forward	AAGGGAGCTGCAGTGGAGTA
Cas9-WT reverse	CCGAAAATCTGTGGGAAGTC
IY573F	GTTCCCGATTGCAGCGTGCC
IY573R	CATCACAGAATGGAGATGGC
IY574F	GGCTACCAGAACAGACTGAA
IY574R	ATACAGGCAACCAGGCTCAC
IY575F	CCACACACACATGCCATAGC
IY575R	CTGGAGAGGCTATCTTAACTGG
RG287F	GACTTGCAGATACATCCCTTC
RG285R	CCAGAGTTTCCAAAAGGCTC

Appendix Table S2. PCR set-ups

Reaction	Oligonucleotides used
Y-locus genotyping	Y forward + Y reverse
Sex determination	Y-WT forward + Y reverse
<i>Cas9</i> -locus genotyping	Cas9-M forward + Cas9-WT forward + Cas9-WT reverse
<i>Atp5b</i> amplification	IY573F + IY573R
<i>Casp8</i> amplification	IY575F + IY575R
<i>Cdc20</i> amplification	IY574F + IY574R
<i>Atp5b</i> sequencing	IY573F
<i>Casp8</i> sequencing	RG285R
<i>Cdc20</i> sequencing	RG287F