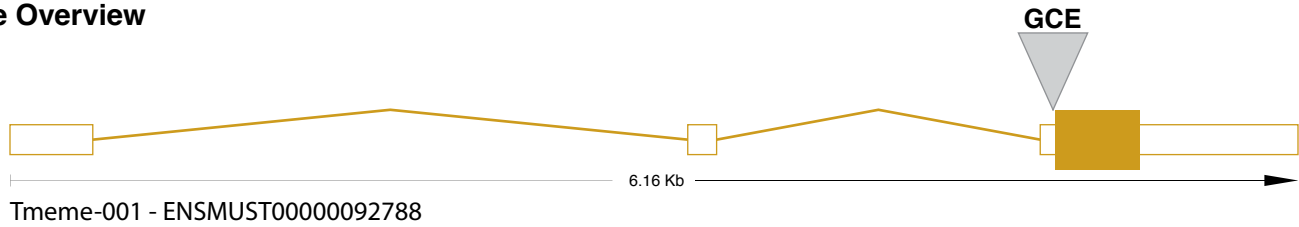


Tmem100-GCE Construct Overview

Created 21 July 2010

Updated 3 September 2010

Gene Overview



Design comments

There is a single transcript reported for Tmem100.

Target site in cDNA

cDNA for Tmem100-001

Transcript length: 1767 bps Translation length: 134 residues

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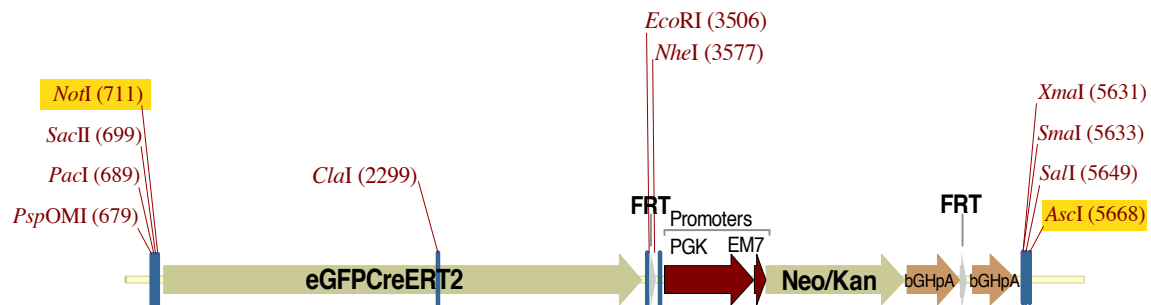
1 TCTGCAGCTTCACACTACAAAAGAGTGATATCGAAGACTAGGTTAGAGAAAAACATAAAA
61 AAGAATGCTTGCATAAGGATTTAAACGGGGAAAGAAAACAGGAGAGATTTTAGTGAC
121 TTTTTAAATAATGGGTTATGATGGATTACCTCCTGTAATACAGTGGATGGTGTGGCA
181 CGGAAAAAATAGAAAAGAAAAGAAAACGTAGAGTAAAAACACACAGCGAGTAAGGA
241 ATTTCCCTTACCAAATGTCTCCGGTCCCTAAATGGTCTGTAAAACCTTGCAGTACTT
301 TGTGTACGGTCCCTAGCATGGTATTTGCATCCCACTGTGGCCTCGCTGTTGGCCGCCG
361 CTTGCTGCTGCTCAGTCCACTTCTGGCTGAGAAAGAGGCAATCCCTGGTCTGCTCTTTT
421 ACCAATGCCAGTGGGTGACAGGCTCTTTCCAGAAAGTTGAACGGAGCTACGCTGGCAGA
481 TCCCTCTCTCCCAAGTCAAGTGGCTCTCTGGTAAATGGACTGCCTTTCTGTGACTTGA
541 TCCTGACCAGGCTTTCCAAATCTAGCCTGTGAAGCGAGATAAGAAAAATCCACAGAAGA
601 AAAAAAATGACCGAAGAATCCACAAAAGAGAACCTGGGAGCTCCAAAATCTCCACACC
661 TGTGCAATGGAGAAAACCCCAAGAGGGGAGTTGTGCTCACCACGGGACCTTGGTCAG
721 CGAGGTTGAGCTGATGGCCGCCACCGGGGGTGCCGAATCTCCTGTACCGCTGCATCAT
781 CCCCTTTCCTGGTGGTCTTCATCACTGGGATTTGTGTCACCGCTGTGGCTTACAGCTT
841 CAATTCCTAGGTTCCATCATCTCCATCTTCGGCTGGTCTTCTGCTCCCGACTGTTT
901 TTTACTAGCCTCCAGTGCCTTGTGCTGGAAGGTGAGACAAAAGGAACAAGAAAAGTCAAGAG
961 ACGCGAGAGTCAGACCGCTCTTGTGGTAAATCAGAGGTGCTTGTGTTGACTGAGT
1021 AAGAGCAAAACGCGAACGCTCACCCGCCACACTGCTCTAACTCAGTGAACATTCATCATAG
1081 GAAACCAAGCCCGTACCGGATGAGCTCAACTTTCGAATGAACACCAAAATGAAAAATAG
1141 GGCATAGAGAGGACTGAAAATGAGGGGTCAAGACGGTTCCCCCTTGGTAGGGAATAACTC
1201 ATCTTTAACGACAAACTTAACCTAAGGGCTATTTCTAACACAGACAGAGGAATCAGCTT
1261 GCTTTCTGTTAAACGTTTAGGGAGTGTGGGAGATGCACAGCATTAAATAACAGTTGGT
1321 TCCATTTAGAAAGTACCAAGGGGAAAGTGGACAAATAATGAGAGCCCTGGCAAGTGGTG
1381 TTATAAAAACGTTCCACCAAAGCCTACATGGCTTGGCATTTCCACGTACCTAAGAAGT
1441 TCTGTTATATATATACATATATATTTTTTCCAAATAAGTTGATCTTTGGCCCCCCCCTTT
1501 TAAAAAATTTTCACTTTCAGTAACATCACTAGAGGTACTTTATTTTGAAGAATAGACTA
1561 ATATTTTTTATATTTTAAACAATGGACAATGTAGATGGTTGTAATGATATGTGACAAGAA
1621 AACAGAAATGTAGGTAACACAGATGACACAGGGACAGTTAAATTAATATGAAATAATCC
1681 AATCTAGCACCTTTGATGGCTTTTATACAAAAGTTCAAGTGTGCATTTCACTCAAAATAAT
1741 AAATGCTCATGGCTGCTGAAACTTAAA
  
```

Red bar = Left homology arm

Blue bar = Right homology arm

Reporter Cassette

A “GCE” reporter cassette (eGFP fused to tamoxifen inducible Cre-ERT2) was inserted into the consensus start ATG of the Tmem100 coding region. The Neo/Kan components were for selection in bacteria and removed with transient expression of Flpe-recombinase prior to microinjection.



Fragment of pCZV-GCE-FpNF-v2
4996 bp (molecule 7302 bp)

Tmem100-GCE Target Site Details

Created 3 September 2010
Updated 4 September 2010

Endogenous Targeting Site

Left homology arm
ctatctctct ccttttcttg gcacagctgt ttcaccctct atttcaactca gtgcgaaaga cccacacaaat ggagaatgtg aataagttag gcttgtgctc
gatagagaga gaaaaagacc cgtgtcgaca aagtgggaga taaagttagt cacgctttct ggggtgttta cctcttacac ttatctactc cgaacacgag

Left homology arm
ctgggtgacc tgggacagga ggggcctctc cacctctctg ctaccatttg cagggtggt gctgcatct tagctgatgt ctcccacctt cttcttcaca
gacccactgg accctgtcct ccccgagag gtggagagac gatggaaac gtcccgaacca cgacgtaga atcgactaca gaggtggaa gaagaagtgt

Exon 3
Left homology arm
gCTTGCATCC TGACCAGGCT TTCCAAATCT AGCCTGTGAA GCGAGATAAG AAAAATCCCA CAGAAGAAA AAAAATGACC GAAGAATCCA CAAAAGAGAA
cGAACGTAGG ACTGGTCCGA AAGGTTTAGA TCGGACACTT CGCTCTATTC TTTTtagggT GTCTTCTTTT TTTTACTGG CTTCCTAGGT GTTTCTCTT
Right homology arm

Exon 3
CCTGGGAGCT CAAAATCTC CCACACCTGT GACAATGGAG AAAAACCCTA AGAGGGAAGT TGTGGTCACC ACGGGACCCT TGGTCAGCGA GGTTCAGCTG
GGACCCTCGA GTTTTAGAG GGTGTGACA CTGTACCTC TTTTGGGGT TCTCCCTCA ACACCAGTGG TGCCCTGGGA ACCAGTCGCT CCAAGTCGAC

Right homology arm
Exon 3
ATGCCGCCA CCGGGGTGC CGAACTCTC TGCTACCGT GCATCATCC CTTTGGCGTG GTGGTCTCA TCACTGGGAT TGTGGTCACC GCTGTGGCTT
TACCGCGGT GGGCCACAG GCTTGAAGG ACGATGGCGA CGTAGTAGG GAAACGGCAC CACCAGAAGT AGTGACCCTA ACACCAGTGG CGACACCGAA

Right homology arm

Targeted Site - 5'

BstEII
CTGGGTGACC TGGGACAGGA GGGGCTCTC CACCTCTCTG CTACCATTTG CAGGGCTGGT GCTGCCATCT TAGCTGATGT CTCCACCTT CTTCTTCACA
GACCCACTGG ACCCTGTCTT CCCCAGGAGG GTGGAGAGAC GATGGTAAAC GTCCCGACCA CACGGTAGA ATCGACTACA GAGGTGGAA GAAGAAGTGT

Left homology arm
gCTTGCATCC TGACCAGGCT TTCCAAATCT AGCCTGTGAA GCGAGATAAG AAAAATCCCA CAGGTTGGC CGCCACCATG GTGAGCAAGG GCGAGGAGCT
cGAACGTAGG ACTGGTCCGA AAGGTTTAGA TCGGACACTT CGCTCTATTC TTTTtagggT GTCCAAACCG GCGGTGTGAC CACTCGTTCC CGTCTCTCGA

Left homology arm BstXI Kozak eGFPCreERT2
eGFPCreERT2
GTTACCGGG GTGGTCCCA TCCTGGTCGA GCTGGACGGC GACGTAACG GCCACAAGTT CAGCGTGTCC GCGAGGGCG AGGGCGATGC CACCTACGGC
CAAGTGCC CACCACGGGT AGGACCAGCT CGACCTGCCG CTGCATTTGC CCGTGTTCAG GTCGCACAGG CCGCTCCCG TCCCCTACG GTGGATGCCG

Targeted Site - 3'

BGH polyadenylation sequence
GGAAATGCA TGCATTGTC TGAGTAGGTG TCATTCCTATT CTGGGGGGTG GGGTGGGCA GGACAGCAAG GGGGAGGATT GGAAGACAA TAGCAGGCAT
CCTTTAACGT AGCGTAACAG ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCT CTTGTCTGTT CCCCCTCTAA CCCTCTGTG ATCGTCCGTA

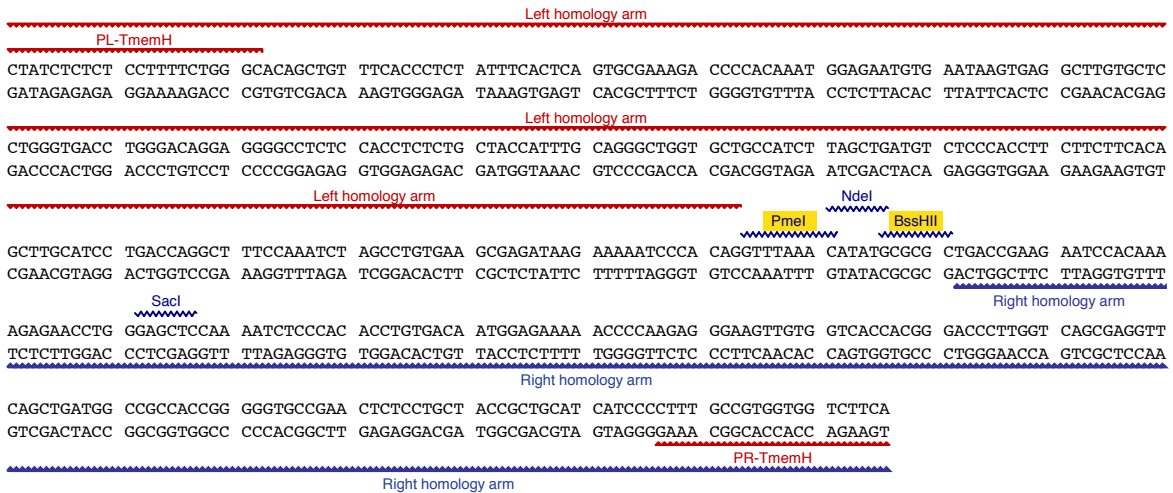
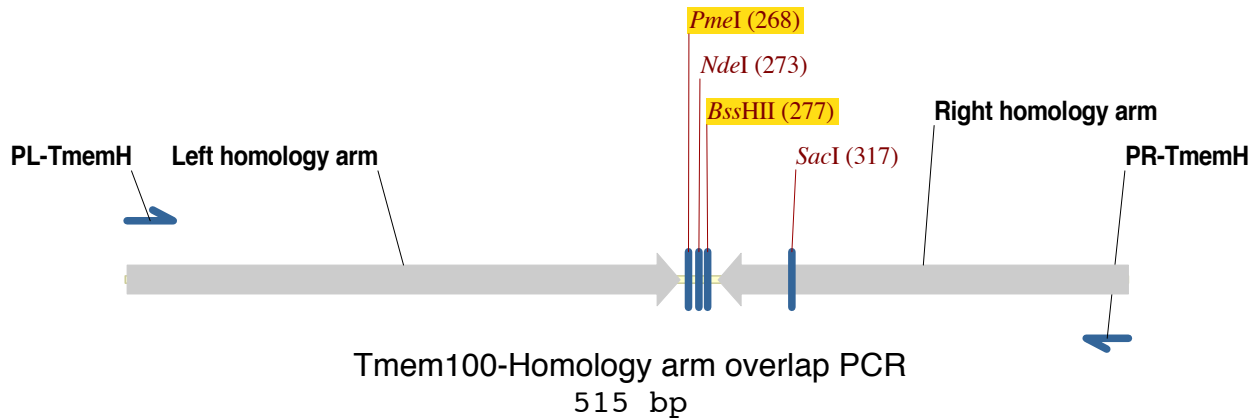
BGH polyadenylation sequence SmaI XbaI Sall BssHII SacI
GCTGGGGATG CCGTGGGCTC TATGGCCCGG GTGATCCTCT AGAGTCGACC TCTAGTGAGA TGGCGCGCTG ACCGAAGAAT CCACAAAAGA GAACCTGGGA
CGACCCCTAC GCCACCCGAG ATACCCGGCC CACTAGGAGA TCTCAGCTGG AGATCACTCT ACCGCGCGAC TGGCTTCTTA GGTGTTTTCT CTGGACCCCT

Right homology arm
GCTCCAAAT CTCCACACC TGTGACAATG GAGAAAACC CCAAGAGGA AGTTGTGGT ACCACGGGAC CTTTGGTCAG CGAGGTTTCA CTGATGGCCG
CGAGGTTTTA GAGGGTGTGG ACACGTTPAC CTCTTTTTTG GGTCTCCCT TCAACACCAG TGGTGCCTG GGAACCAGTC GCTCCAAGTC GACTACCCGG

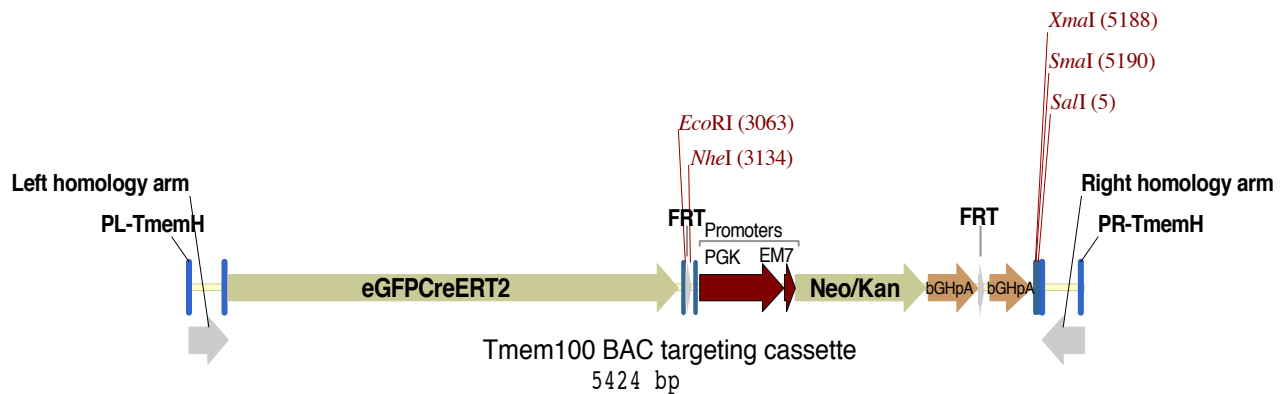
Right homology arm

BAC targeting cassette for Tmem100

The homologous arms for recombineering were created by overlap-PCR. The resulting product, cloned into a shuttle vector (not shown), contained the Left and Right homology arms joined by a polylinker sequence introduced into the overlap primers. This polylinker sequence included *PmeI* and *BssHII* restriction sites for subsequent cloning of the GCE (v2) reporter cassette into the center location using *NotI* (blunt fill-in) and *AscI* (compatible end with *BssHII*).



Reporter + Arms



Tmem100-GCE BAC Transgene

Created 3 September 2010
Updated 4 September 2010

BAC clone RP23-183L17 was targeted by recombineering with the Tmem100-GCE targeting construct. The genomic context of the GCE reporter is shown below. The BAC and the target gene are highlighted in yellow. Flanking primers and construct primers are highlighted in the lower schematic.

