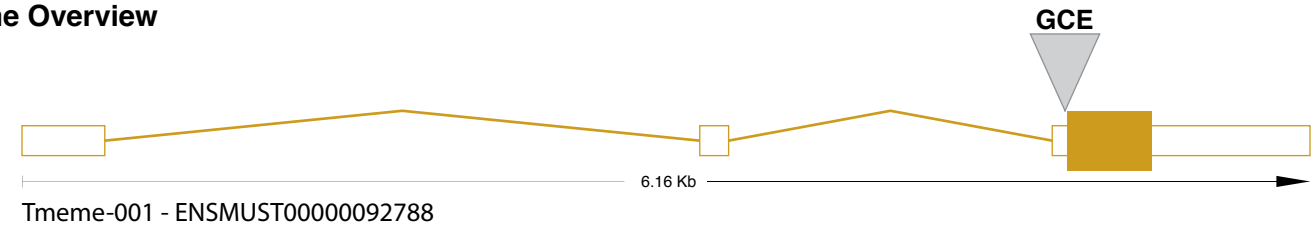
Tmem100-GCE Construct Overview

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 TCTGCAGCTTCACACTACAAAAGAGTGATATCGAAGACTAGGTTAGAGAAAACCATAAAA
  61 AAGAATGCTTGCATAAGGATTTTAAACGGGGGAAAGAAAACAGGAGAGATTTTTAGTGAC
 121 TTTTTAAATAATTGGGTTATGATGGATTCACTCCTGTAATTACAGTGGATGGTGTGGGCA
 181 CGGAAAAAAAAATAGAAAAGAAAAGGAAAACGTAGAGTAAAATACAACAGCGAGTAAGGA
 241 ATTTCCTTTCACCAAATTGTCTCCGGTCCCTTAATGGTCTGTAAATCTTGCTGAGTACTT
 301 TGTGTACGGTCCCTAGCATGGTGATTTGCATCCCACTGTCGGCCTCGCTGTTGGCCGCCG
 361 CTTGCTGCTGTCTCAGTCCACTTCTGGCTGAGAAAGAGGCAATCCCTGGTCTGTCCTTTT
 421 ACCAATGCCCAGTGGGTGACAGGCTCTTTCCCAGAAGTTGAACGGAGCTACGCTGGCAGA
 481 TCCCTCTCTCCCAAGTCAAGTGGCCTCTCTGGTAATGGACTGCCTTTCTGTGAGCTTGCA
 541 TCCTGACCAGGCTTTCCAAATCTAGCCTGTGAAGCGAGATAAGAAAAATCCCACAGAAGA
 601 AAAAAAAATGACCGAAGAATCCACAAAAGAGAACCTGGGAGCTCCAAAATCTCCCACACC
 661 TGTGACAATGGAGAAAAACCCCAAGAGGGAAGTTGTGGTCACCACGGGACCCTTGGTCAG
 721 CGAGGTTCAGCTGATGGCCGCCACCGGGGGGTGCCGAACTCTCCTGCTACCGCTGCATCAT
 781 CCCCTTTGCCGTGGTGGTCTTCATCACTGGGATTGTGGTCACCGCTGTGGCTTACAGCTT
 841 CAATTCCCATGGTTCCATCATCTCCATCTTCGGCCTGGTCCTTCTGTCCTCCGGACTGTT
 901 TTTACTAGCCTCCAGTGCCTTGTGCTGGAAGGTGAGACAAAGGAACAAGAAAGTCAAGAG
 961 ACGCGAGAGTCAGACCGCTCTTGTGGTAAATCAGAGGTGCTTGTTTGCTTAAGACTGAGT
1021 AAGAGCAAACGCGAACGCTCACCCGCCCACACTGCTCTAACTCAGTGAACTCATTCATAG
1081 GAAACCAGCCCGGTACCGGATGAGCTCAACTTTCGAATGAACTACCAAAATGAAAAATAG
1141 GGCATAGAGAGGACTGAAAATGAGGGGTCAAGACGGTTCCCCCTTGGTAGGGAATAACTC
1201 ATCTTTAACGACAAACTTAACCCTAAGGGCTATTTCTAACACAGACAGAGGAATCAGCTT
1261 GCTTTTCCTGTTAAACGTTTAGGGAGTGTGGGAGATGCACAGCATTAAATAACAGTTGGT
1321 TCCATTTAGAAAGTACCCAAGGGAAGAATGGACAAATAATGAGAGCCCTGGCAAGTGGTG
1381 TTATAAAAACGTTCCACCAAAAGCCTACATTGGCTTGGCATTCCCACGTACCTAAGAAGT
1441 TCTGTTATATATATACATATATATTTTTTCCAATAAGTTGATTCTTTGCCCCCCCCTTTT
1501 TAAAAGAATTTTCACTTTCAGTAACATCACTAGAGGTACTTTATTTTGAAGAATAGACTA
1561 ATATTTTTTATATTTTAACAATGGACAATTGTAGATGGTTGTAATGATATGTCAGAAGAA
1621 AACAGAAATGTAGGTAACACAGATGACACAGGGACAGTTAAATTAATATTGAAATAATCC
1681 AATCTAGCACCTTTGATGGCTTTTATACAAAAGTTCAGTGTGCATTTCACTCAAAATAAT
1741 AAATGCTCATGGCTGCTGAAACTTAAA
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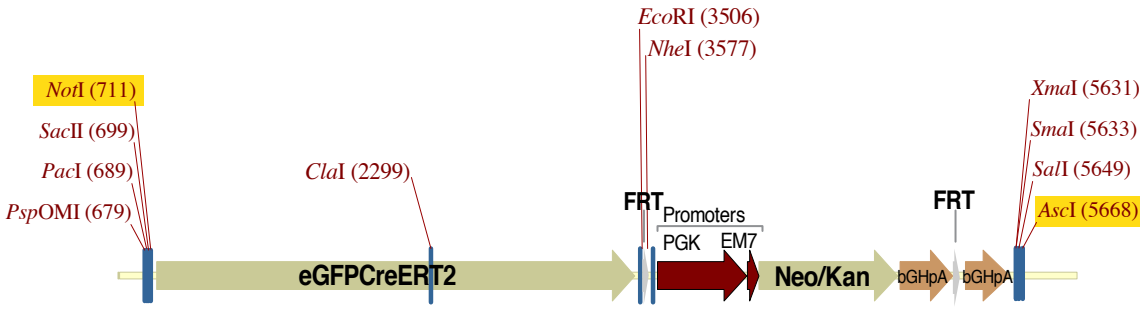
Red bar = Left homology arm
Blue bar = Right homology arm

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 is used for selection in bacteria and removed with transient expression of Flpe-recombinase prior to microinjection.

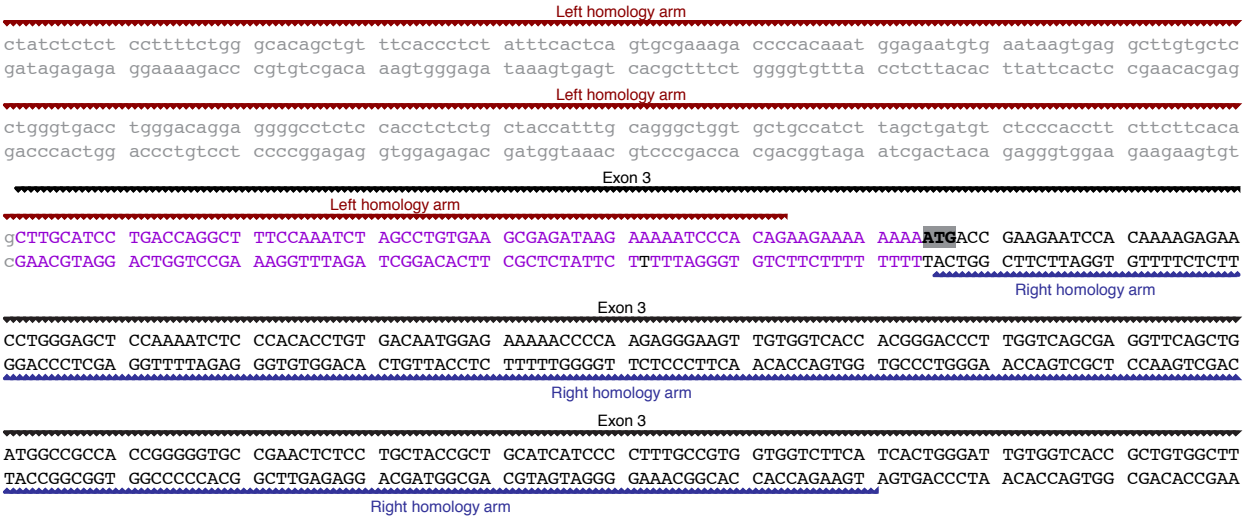


Fragment of pCZV-GCE-FpNF-v2
4996 bp (molecule 7302 bp)

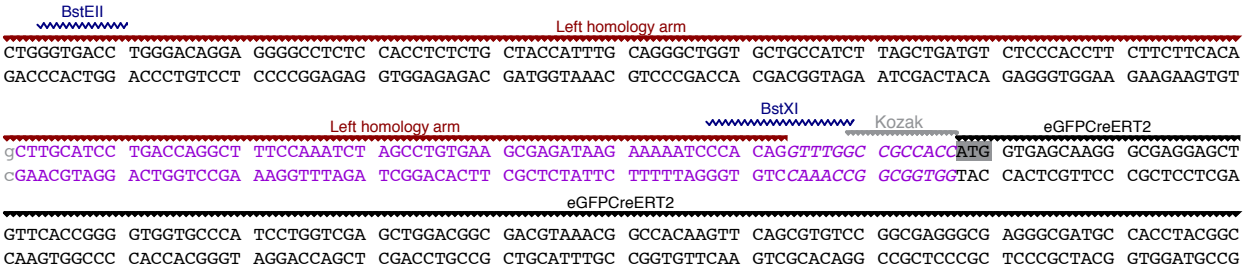
Tmem100-GCE Target Site Details

Created 3 September 2010
Updated 4 September 2010

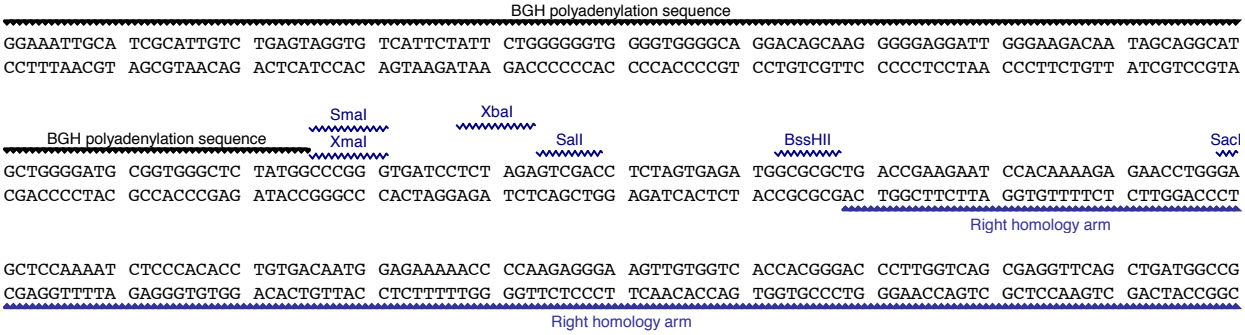
Endogenous Targeting Site



Targeted Site - 5’

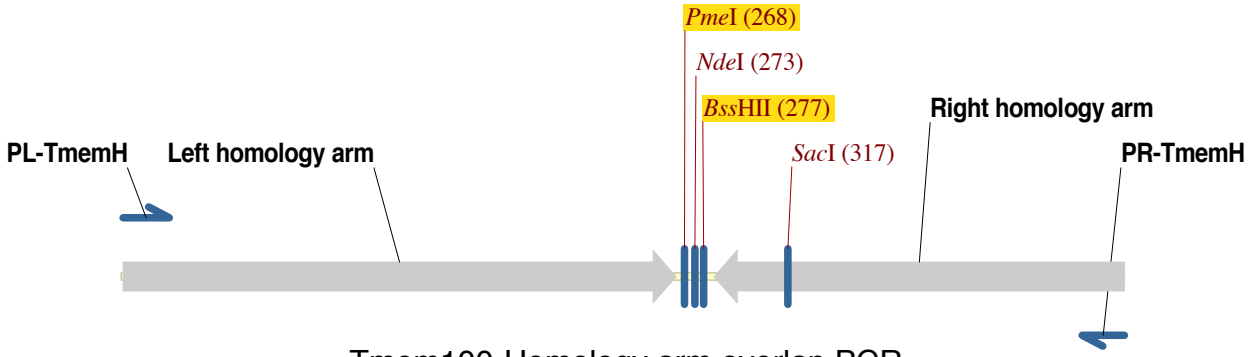


Targeted Site - 3’



BAC targeting cassette for Tmem100

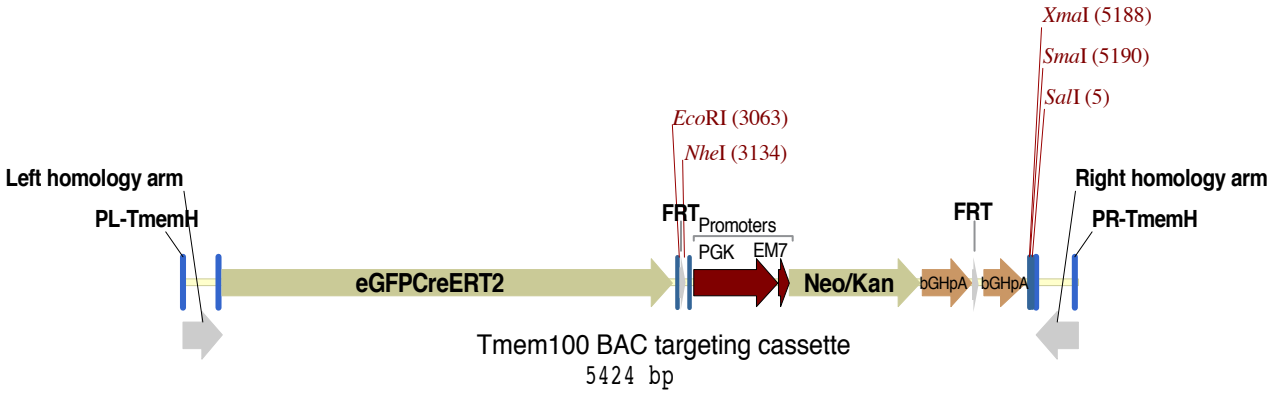
The homologous arms for recombineering were created by overlap-PCR. The resulting product, cloned into a shuttle vector (not shown), contained the Left and Right homology arms joined by a polylinker sequence introduced into the overlap primers. This polylinker sequence included PmeI and BsshII restriction 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).



Tmem100-Homology arm overlap PCR
515 bp

Left homology arm
PL-TmemH
CTATCTCTCT CCTTTTCTGG GCACAGCTGT TTCACCCTCT ATTTCACTCA GTGCGAAAGA CCCCACAAAT GGAGAATGTG AATAAGTGAG GCTTGTGCTC
GATAGAGAGA GGAAAAGACC CGTGTCGACA AAGTGGGAGA TAAAGTGAGT CACGCTTTCT GGGGTGTTTA CCTCTTACAC TTATTCACTC CGAACACGAG
Left homology arm
CTGGGTGACC TGGGACAGGA GGGGCCTCTC CACCTCTCTG CTACCATTTG CAGGGCTGGT GCTGCCATCT TAGCTGATGT CTCCCACCTT CTTCTTCACA
GACCCACTGG ACCCTGTCCT CCCCGGAGAG GTGGAGAGAC GATGGTAAAC GTCCCGACCA CGACGGTAGA ATCGACTACA GAGGGTGGAA GAAGAAGTGT
Left homology arm
NdeI
PmeI
BssHII
GCTTGCATCC TGACCAGGCT TTCCAAATCT AGCCTGTGAA GCGAGATAAG AAAAATCCCA CAGGTTTAAA CATATGCGCG CTGACCGAAG AATCCACAAA
CGAACGTAGG ACTGGTCCGA AAGGTTTAGA TCGGACACTT CGCTCTATTC TTTTTAGGGT GTCCAAATTT GTATACGCGC GACTGGCTTC TTAGGTGTTT
Right homology arm
SacI
AGAGAACCTG GGAGCTCCAA AATCTCCCAC ACCTGTGACA ATGGAGAAAA ACCCCAAGAG GGAAGTTGTG GTCACCACGG GACCCTTGGT CAGCGAGGTT
TCTCTTGGAC CCTCGAGGTT TTAGAGGGTG TGGACACTGT TACCTCTTTT TGGGGTTCTC CCTTCAACAC CAGTGGTGCC CTGGGAACCA GTCGCTCCAA
Right homology arm
CAGCTGATGG CCGCCACCGG GGGTGCCGAA CTCTCCTGCT ACCGCTGCAT CATCCCCTTT GCCGTGGTGG TCTTCA
GTCGACTACC GGCGGTGGCC CCCACGGCTT GAGAGGACGA TGGCGACGTA GTAGGGGAAA CGGCACCACC AGAAGT
PR-TmemH
Right homology arm

Reporter + Arms



Tmem100 BAC targeting cassette
5424 bp

Tmem100-GCE BAC Transgene

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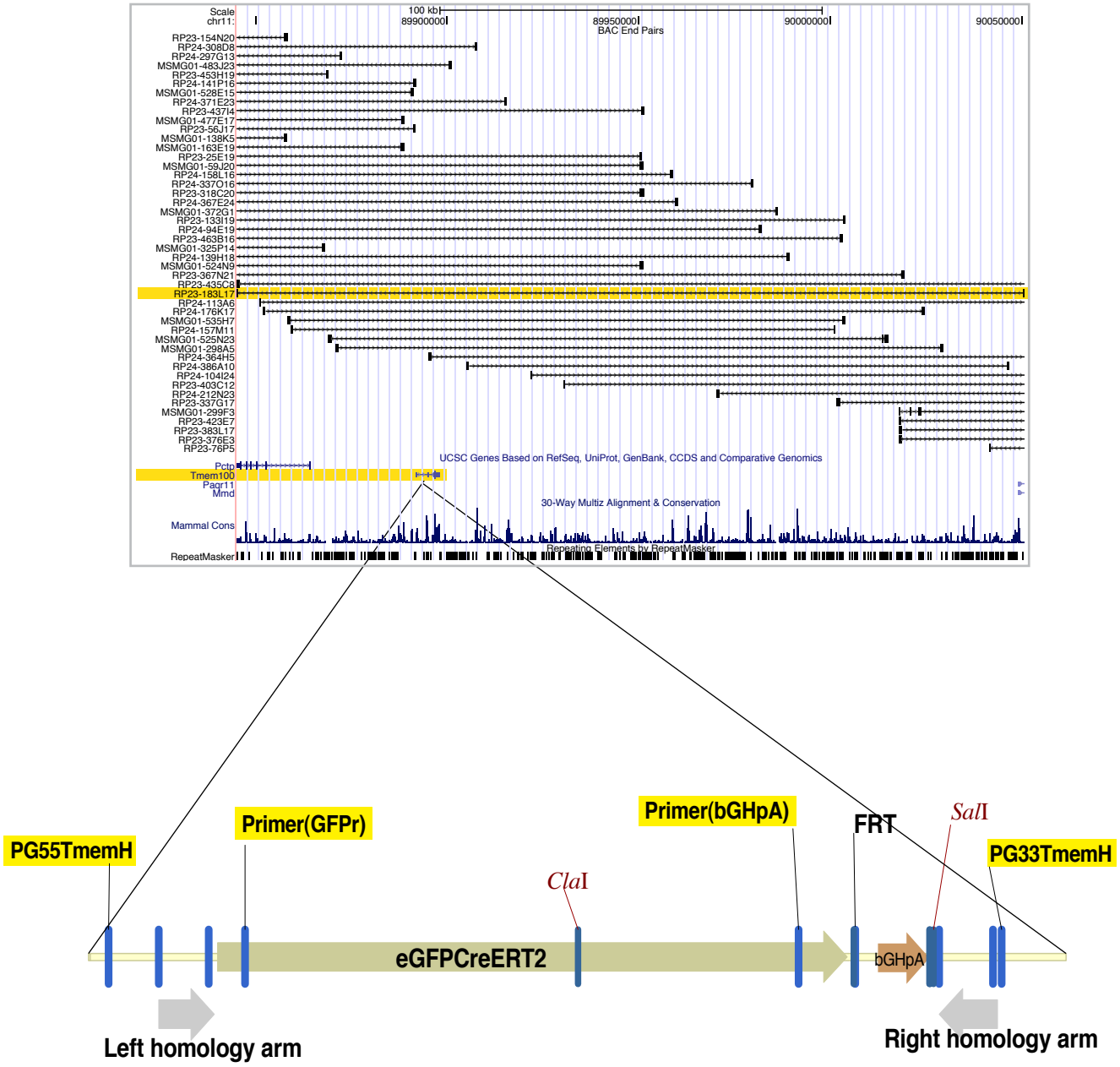
BAC clone RP23-183L17 was targeted by recombineering with the Tmem100-GCE targeting construct. The genomic context of the GCE reporter is shown below. The BAC and the target gene are highlighted in yellow. Flanking primers and construct primers are highlighted in the lower schematic.