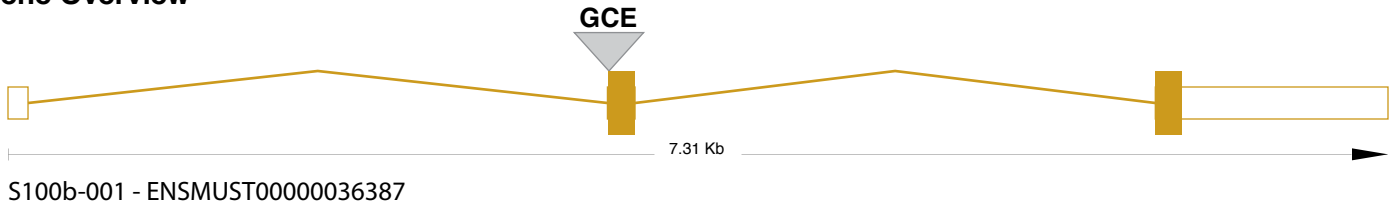


S100b-GCE Construct Overview

Created 30 August 2010
Updated 6 September 2010

Gene Overview



Design comments

There is a single transcript reported for S100b. The predicted start site ATG is in the second exon and was used for insertion of the reporter.

Target site in cDNA

cDNA for S100b-001

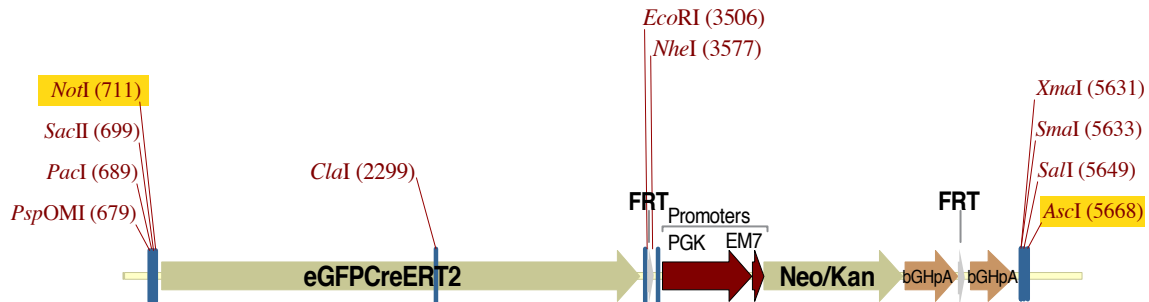
Transcript length: 1484 bps Translation length: 92 residues

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1  CACACCCAGTTCTCTGCAGGAAGAATAAGAAGCTGTTCTGTCTACACTCCTGTTACTC
61  GGACACTGAAGCCAGAGAGGACTCCAGCAGCAAAGGTGACCAGGAGCTCCCGGGATGTCC
121 GAGCTGGAGAAGCCATGGTTGCCCTCATTGATGCTTCCACAGTACTCCGGGCGAGAG
181 GGTGACAAGCACAAGCTGAAGAAGTCAGAACTGAAGGAGCTTATCAACACAGACTCTCT
241 CACTTCCTGGAGGAAATCAAGGAGCAGGAAGTGGTGACAAAGTATGAGAGCGCTGGAC
301 GAAGATGGGGATGGGGAGTGTGACTTCCAGGAGTTCATGGCCTTCGTGCCATGGTGACC
361 ACGCCTGCCATGAGTCTTTGAACATGAGTGAGACCAAAAAAAAAAAAAAAAAAAAAAAA
421 AAGTAGCTGAGCTGTTCTGCAACAGACATGAGGGTCACAAGAGGAGGCACAGCAAAGCC
481 TCATGGGCTCGAAGGAGCTGAGCTCTCTAGACACACATAACTAATTAGGAAGCTTGGTTT
541 GCTTTGGGGATGAAACCCCAACCCCTTCCAGGGGCTGCTTAAAGTTAGCACATCATGA
601 TTTCTGCTAGGCTAGGCATTCCTGAGCTGACTGGTCCAGGAAGTGTGGTAAGGGTC
661 ACTTAGGGGATGAGATCAACGCTCTGTTGATAAAACACGTTGCTGACCACCATGCCCC
721 TGTAGAAGAGTCACCTGTAAGAAATCAAGGCAGACTACCAACAGTACTTCCGTCAGTCAGC
781 TTTCTTAGATGTATGCTTCCCATACATCTACAGACCCACAGCTGGATCTGTGCCACCCG
841 AAGAGGTTGCTCATCTTACACCTGCTTGGCCATCTGCCAGCAAATGATGCTCCAGAAA
901 GTAGACAGACTGATCGCCTACACCTTCCCGAGCTCAGAGGAGCACAGCCACACTAAAGT
961 CTCCTTCCACAGTGTGCAGTGATTCGTTACTAAGTAAATATTTAATGCCAACAGTTCT
1021 AACTGGGAAACAATTAGATCTCACAACAACTAAGTATGAACATTTCTAAGTCCAGGACCGAGA
1081 ATCAAAATCTGCTCGGCAGACTTCTCCTTCCAGGATGATCGCTTTGTTCTGGAGGACA
1141 GAGGAGGGGAATGGCCAGAGTCTTTTCTAGTTAGTTGATTTCTCCTTGCTCCCTCTGCT
1201 GTCATAATGTGAGTGGCTGCGGAAGTTGAGATTCACAGGACGAGCTGATGGGGAATTA
1261 GGGTCTCGAGGTGTCTTTAGCTGCTGGGAGCCATGTCTGACCTGCTGGTCCCTAGG
1321 GCCTGCTTAGCCCTTGGTGAGGCTGAGAGTCGAGGACTGTGGGAAGCTGGACTTGAGGCT
1381 TTCTAACTTGCATATTTCAAATAGCGACGTTTAAATAATCCAAGTTACATTTAAATAAAA
1441 ACTGCATTGCAAATATTAAGCCATTCTAGGAGAATTTCTAAA
    
```

Reporter Cassette

A "GCE" reporter cassette (eGFP fused to tamoxifen inducible Cre-ERT2) was inserted into the ATG of the S100b coding region. The Neo/Kan component 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)

S100b-GCE Target Site Details

Created 3 September 2010
Updated 6 September 2010

Endogenous Targeting Site

Left homology arm

PL-S100b BamHI

gaaacttcgc tctgtagcct atggggacat agaagggaca ggggtggcct gggcaaaaga ggatgaacc tggtcagcgt ggatcccagg atgcctctcc
ctttgaagcg agacatcgga taccocctgta tcttccctgt cccaccggga cccgttttct cctacttggg accagtcgca cctaggggtcc tacggagagag

Left homology arm

PstI

ctggtaggcc tctotacccc tcactgtgagc tgaggcctga gaacaacgta ttaagctctc caaagaacag ggtagaaaao atgaaaacgt atccttcctc
gaccatccgg agagatgggg agtagactcg actccggact cttgttgcac aattcgagag gtttcttgtc ccatcttttg tactttttgca taggaaggag

Left homology arm

Exon 2

SmaI XmaI Aval NcoI

PstI

tgcagCTCCC GGCATGTCGG AGCTGGAGAA GGCCATGGTT GCCCTCATTT ATGTCTTCCA CCAGTACTCC GGGCGAGAGG GTGACAAGCA CAAGCTGAAG
acgtcGAGGG CCGTACAGGC TCGACCTCTT CCGGTACCAA CGGGAGTAAC TACAGAAGGT GGTCATGAGG CCCGCTCTCC CACTGTTCGT GTTCGACTTC

Right homology arm

Exon 2

SacI

AAGTCAGAAC TGAAGGAGCT TATCAACAAC GAGCTCTCTC ACTTCCTGGA Ggtaagagac catctctgag ctgcactaga aatggctcct atgtctgctc
TTCAGTCTTG ACTTCCTCGA ATAGTTGTTG CTCGAGAGAG TGAAGGACCT Ccattctctg gtagagactc gacgtgatct ttaccaggaac tacagacgag

Right homology arm

PR-S100b

ctgtctctgt atccagaccc tgggttgcct
gacagagaca taggtctggg acccaacgaa

PR-S100b
Right homology arm

Targeted Site - 5'

Left homology arm

CTGGTAGGCC TCTTACCCC TCATCTGAGC TGAGGCTTGA GAACAACGTA TTAAGCTCTC CAAAGAACAG GGTAGAAAAC ATGAAAACGT ATCCTTCCTC
GACCATCCGG AGAGATGGGG AGTAGACTCG ACTCCGGACT CTTGTTGCAT AATTCGAGAG GTTCTTGTGC CCATCTTTTG TACTTTTGCA TAGGAAGGAG

Left homology arm Kozak eGFPCreERT2

tgcagCTCCC GGCCTCCACC ATGCTGAGCA AGGGCGAGGA GCTGTTACC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCAGCGTAA ACGGCCACAA
acgtcGAGGG CCGCGGTGG TACCACCTCGT TCCCCTCTCT CGACAAGTGG CCCACCACG GGTAGGACCA GCTCGACCTG CCGCTGCATT TGCCGGTGT

eGFPCreERT2

GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCTGAAAGTT CATCTGCACC ACCGGCAAGC TGCCCGTGCC CTGGCCACC
CAAGTCGCAC AGGCCCTCC CGCTCCCGCT ACGGTGGATG CCGTTCGACT GGGACTTCAA GTAGACGTGG TGGCCGTTTC ACGGGCACGG GACCGGGTGG

Targeted Site - 3'

BGH polyadenylation sequence

GAAATGCA TCGCATGTC TGAGTAGGT TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG GGGGAGGATT GGAAGACAA TAGCAGGCAT
CCTTTAACGT AGCGTAACAG ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CCGTCTGTTT CCCCTCTTAA CCCTCTGTT ATCGTCCGTA

BGH polyadenylation sequence Sall S100b coding region

XmaI SmaI

GCTGGGGATG CCGTGGGGTC TATGGCCCGG GTGATCCTCT AGAGTCGACC TCTAGTGAGA TGGCGCGGG ATGTCCGAGC TGGAGAAGGC CATGGTTGCC
CGACCCCTAC GCCACCCGAG ATACCGGGCC CACTAGGAGA TCTCAGCTGG AGATCACTCT ACCGCGCCCC TACAGGCTCG ACCTCTTCCG GTACCAACGG

Right homology arm

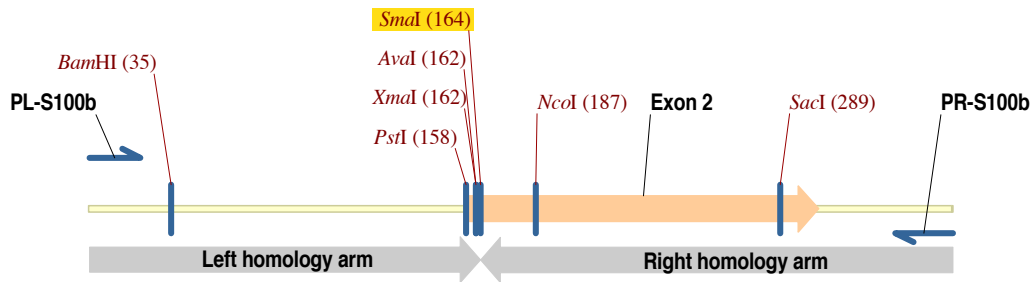
S100b coding region

CTCATTTGAT TCTTCCACCA GTACTCCGGG CGAGAGGGTG ACAAGCACAA GCTGAAGAAG TCAGAACTGA AGGAGCTTAT CAACAACGAG CTCTCTCACT
GAGTAACTAC AGAAGGTGGT CATGAGGCC GCTCTCCAC TGTTTCGTGT CGACTTCTTC AGTCTTGACT TCCTCGAATA GTTGTGCTC GAGAGAGTGA

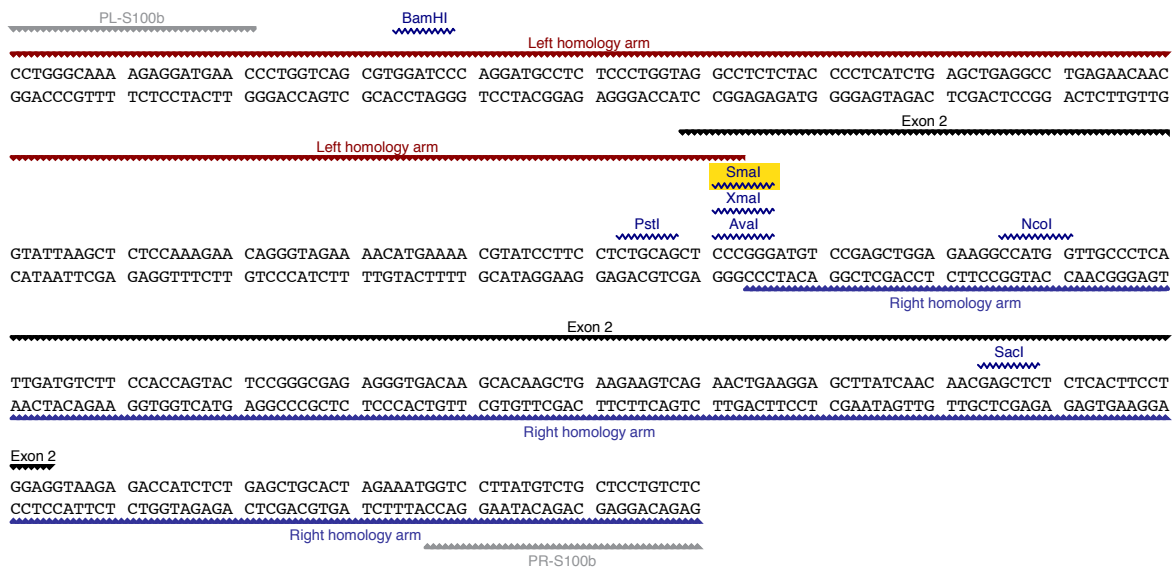
Right homology arm

BAC targeting cassette for S100b

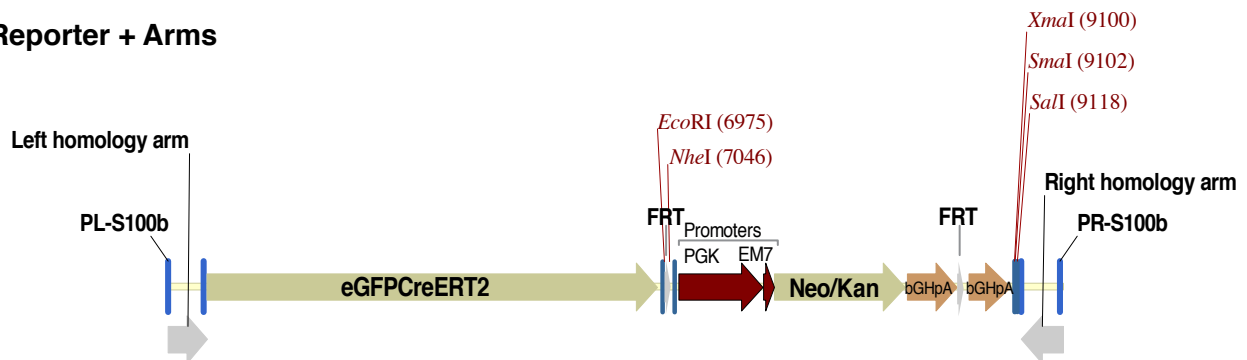
The homologous arms for recombineering were created by PCR. The resulting product, cloned into a shuttle vector (not shown), contained the Left and Right homology arms. A *SmaI* site located at the start ATG was used for cloning of the GCE (v2) reporter cassette into the center location using *NotI* (blunt fill-in) and *AscI* (blunt fill-in).



S100b-Homology arm overlap PCR
360 bp



Reporter + Arms



S100b BAC targeting cassette
5321 bp (shown of 14268)

S100b-GCE BAC Transgene

Created 3 September 2010
Updated 6 September 2010

BAC clone RP23-213P2 was targeted by recombineering with the S100b-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.

