Cited1-nucTagRFPT Construct Overview

Created 6 December 2010
Updated na

Gene Overview

nucTagRFPT

4.45 Kb

Cited1-002 - ENSMUST00000101336
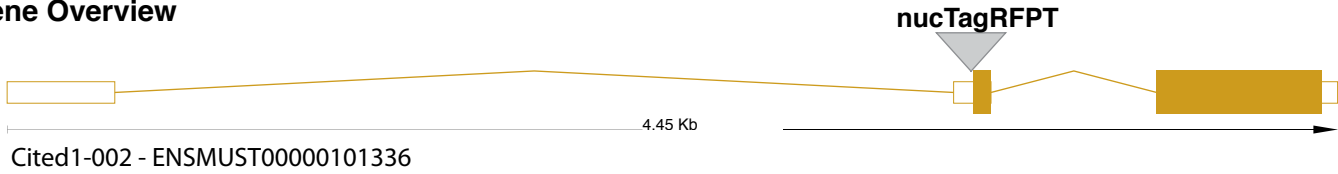
Design comments

There are three protein encoded transcripts reported for Cited1 that share a start ATG in the second exon. The ATG in Exon 2 was therefore targeted for insertion of the nucTagRFPT reporter cassette.
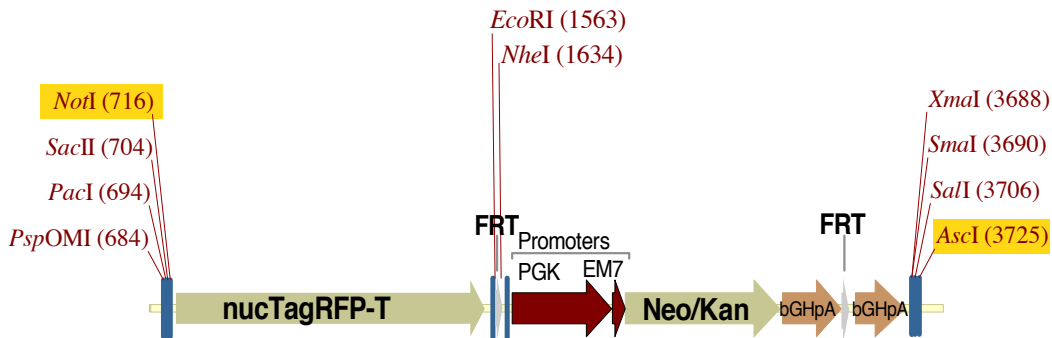
Target site in cDNA

cDNA for Cited1-002
Transcript length: 1090 bps
Translation length: 203 residues

```
   1 AGGGCTCTACGGGAGGCAGTGGGGGCTTAAGAGCCCGGTCCCCGCGCCGAGAACCAAGCG
  61 TGGTGGCTGTGATCGCCACCGCCCCGGAGTCCGGGCGCCGGAGCTGCGGGCCGGCAAGAG
 121 CGCGGGTCGGGGCAGCTCGGCGCTGCCCGGTGCGGTGCGGCGGCGCGGCGCGGCGCGGCG
 181 GTGCAGGCTTTTCCCAGGCGCCCTGGGGTCGCGTGGCCGCCCGTGCGGCTGGGGACTCTG
 241 AAGCGAGTGGCTAGCGGCGGCGGCGGCAGTGTGCGTGCCAAGGGGGCTCGGGGCTAGGTC
 301 GCTTCGTCCGTACCTCAGCTCCTGTGAGCTTTCCTCTCCCGGGCAAGCAGCTTCGCGGAG
 361 ACCAACAGGCCCAGCTGGCGGCATCAACTGCCACCGATTTATCGGACTTCTGCCCAGGCT
 421 CTGAAATGCCAACTATGTCGAGGCCTGCACTTGATGTCAAGGGTGGCACCACCTCTGGGA
 481 AGGAGGATGCCAACCAGGAGATGAACTCTCTGGCCTACTCCAACCTTGGAGTGAAGGATC
 541 GCAAGGCAGTGACTGTCCTGCACTACCCCGGGGTCACCGCAAATGGAGCCAAAGCCAACG
 601 GAGTTCCCACTAGCTCCTCTGGATCGACATCTCCAATAGGCTCTCCTACTGCCACCCCTT
 661 CTTCCAAACCCCCATCCTTCAACCTGCATCCTACCCCTCACCTGATGGCCAGCATGCAGC
 721 TTCAGAAGCTTAATAGCCAGTACCAAGGGGCTGCGGCTACTGCTGCTGCTGCCCCTCACTG
 781 GTGCAGGCCTACCAGGGGAGGAAGAGCCCATGCAAAACTGGGTCACCGCCCCTCTGGTAG
 841 TGGGAGGGTCTCCGGGATCTGTCTCTCCTCCTGCTGGTGCCCAGAGCCCTGCTCTCATTG
 901 ATTCTGACCCGGTGGATGAGGAGGTGCTGATGTCTCTGGTGGTTGAATTGGGGCTAGACC
 961 GAGCCAATGAGCCTTCCCGAGCTGTGGCTGGGGCAGAATGAGTTTGATTTCACTGCAGATT
1021 TTCCCTCTGGCTGCTGATGGCAAGTGCCCCTAAAAAAATGAGGAAAAAAAGCCACCGATT
1081 CTGTTGTAAA
```

Reporter Cassette

A 3xNLS modified TagRFP-T reporter was inserted at the ATG site of the Cited1 coding region selected. The Neo/Kan component is used for selection in bacteria and removed with transient expression of Flpe-recombinase prior to microinjection.

*Eco*RI (1563)
*Nhe*I (1634)
*Xma*I (3688)
*Sma*I (3690)
*Sal*I (3706)
*Not*I (716)
*Sac*II (704)
*Pac*I (694)
*Psp*OMI (684)
*Asc*I (3725)

FRT Promoters PGK EM7 FRT

nucTagRFP-T Neo/Kan bGHpA bGHpA

Fragment of pCZV-nucTagRFPT-FpNF-v2
3163 bp (molecule 5358 bp)

Cited1-nucTagRFPT Target Site Details

Created 4 December 2010
Updated 6 December 2010

Endogenous Targeting Site

```
                                              Left homology arm
tgtctctgct  cttgggccca  ggtgctctct  gttttgagtt  taactctatc  agcatccttg  tgtagctgaa  tggattcacc  ccgacttgga  attttctagt
acagagacga  gaacccgggt  ccacgagaga  caaaactcaa  attgagatag  tcgtaggaac  acatcgactt  acctaagtgg  ggctgaacct  taaaagatca

                                              Left homology arm
                                                                                          Exon 2
tctcccttca  cctttattat  ttcttctctt  ttacttgcag  ACCAACAGGC  CCAGCTGGCG  GCATCAACTG  CCACCGATTT  ATCGGACTTC  TGCCCAGGCT
agagggaagt  ggaaataata  aagaagagaa  aatgaacgtc  TGGTTGTCCG  GGTCGACCGC  CGTAGTTGAC  GGTGGCTAAA  TAGCCTGAAG  ACGGGTCCGA

 Left homology arm                             Exon 2
CTGAAATGCC  AACTATGTCG  AGGCCTGCAC  TTGATGTCAA  GGGTGGCACC  ACCTCTGGGA  AGGAGgtgag  ttttatcatt  ttacagatgg  agatactaag
GACTTTACGG  TTGATACAGC  TCCGGACGTG  AACTACAGTT  CCCACCGTGG  TGGAGACCCT  TCCTCcactc  aaaatagtaa  aatgtctacc  tctatgattc

                                                        Right homology arm
gcatggaaag  atggacttta  gggaaggagg  tcacgcacct  ggccttccta  ggtgtggcct  atagccgcac  tgcttgacca  gagaagatac  agcaggcttt
cgtacctttc  tacctgaaat  cccttcctcc  agtgcgtgga  ccggaaggat  ccacaccgga  tatcggcgtg  acgaactggt  ctcttctatg  tcgtcccgaa
                                                                                              PR-Cited1
                                              Right homology arm
```

Targeted Site - 5'

```
                                              Left homology arm
ttttgagttt  aactctatca  gcatccttgt  gtagctgaat  ggattcaccc  cgacttggaa  ttttctagtt  ctcccttcac  ctttattatt  tcttctcttt
aaaactcaaa  ttgagatagt  cgtaggaaca  catcgactta  cctaagtggg  gctgaacctt  aaaagatcaa  gagggaagtg  gaaataataa  agaagagaaa

                                              Left homology arm                           Kozak      3XNLS-Tag-RFP
tacttgcagA  CCAACAGGCC  CAGCTGGCGG  CATCAACTGC  CACCGATTTA  TCGGACTTCT  GCCCAGGCTC  TGAAGTTTGG  CCGCCACCAT  GGTGCACGTG
atgaacgtcT  GGTTGTCCGG  GTCGACCGCC  GTAGTTGACG  GTGGCTAAAT  AGCCTGAAGA  CGGGTCCGAG  ACTTCAAACC  GGCGGTGGTA  CCACGTGCAC

                                                        3XNLS-Tag-RFP
GATCCAAAAA  AGAAGAGAAA  GGTAGATCCA  AAAAAGAAGA  GAAAGGTAGA  TCCAAAAAAG  AAGAGAAAGG  TACACGTGAG  CATGGTGTCT  AAGGGCGAAG
CTAGGTTTTT  TCTTCTCTTT  CCATCTAGGT  TTTTTCTTCT  CTTTCCATCT  AGGTTTTTTC  TTCTCTTTCC  ATGTGCACTC  GTACCACAGA  TTCCCGCTTC
```

Targeted Site - 3'

```
                                              bGHpA
GGAAATTGCA  TCGCATTGTC  TGAGTAGGTG  TCATTCTATT  CTGGGGGGTG  GGGTGGGGCA  GGACAGCAAG  GGGGAGGATT  GGGAAGACAA  TAGCAGGCAT
CCTTTAACGT  AGCGTAACAG  ACTCATCCAC  AGTAAGATAA  GACCCCCCAC  CCCACCCCGT  CCTGTCGTTC  CCCCTCCTAA  CCCTTCTGTT  ATCGTCCGTA

                                       XmaI                                 SalI
              bGHpA                    SmaI             XbaI                                      BssHII
GCTGGGGATG  CGGTGGGCTC  TATGGCCCGG  GTGATCCTCT  AGAGTCGACC  TCTAGTGAGA  TGGCGCGCGA  AATGCCAACT  ATGTCGAGGC  CTGCACTTGA
CGACCCCTAC  GCCACCCGAG  ATACCGGGCC  CACTAGGAGA  TCTCAGCTGG  AGATCACTCT  ACCGCGCGCT  TTACGGTTGA  TACAGCTCCG  GACGTGAACT
                                                                                            Right homology arm

TGTCAAGGGT  GGCACCACCT  CTGGGAAGGA  Ggtgagtttt  atcatttac  agatggagat  actaaggcat  ggaaagatgg  actttaggga  aggaggtcac
ACAGTTCCCA  CCGTGGTGGA  GACCCTTCCT  Ccactcaaaa  tagtaaatg  tctacctcta  tgattccgta  cctttctacc  tgaaatccct  tcctccagtg
                                              Right homology arm
```

BAC targeting cassette for Cited1

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. The nucTagRFPT reporter was cloned into the PmeI and BssHII sites of the homology arm PCT product. To accomplish this, the nucTagRFPT was digested with NotI (then blunted) and AscI (compatible overhang with BssHII).
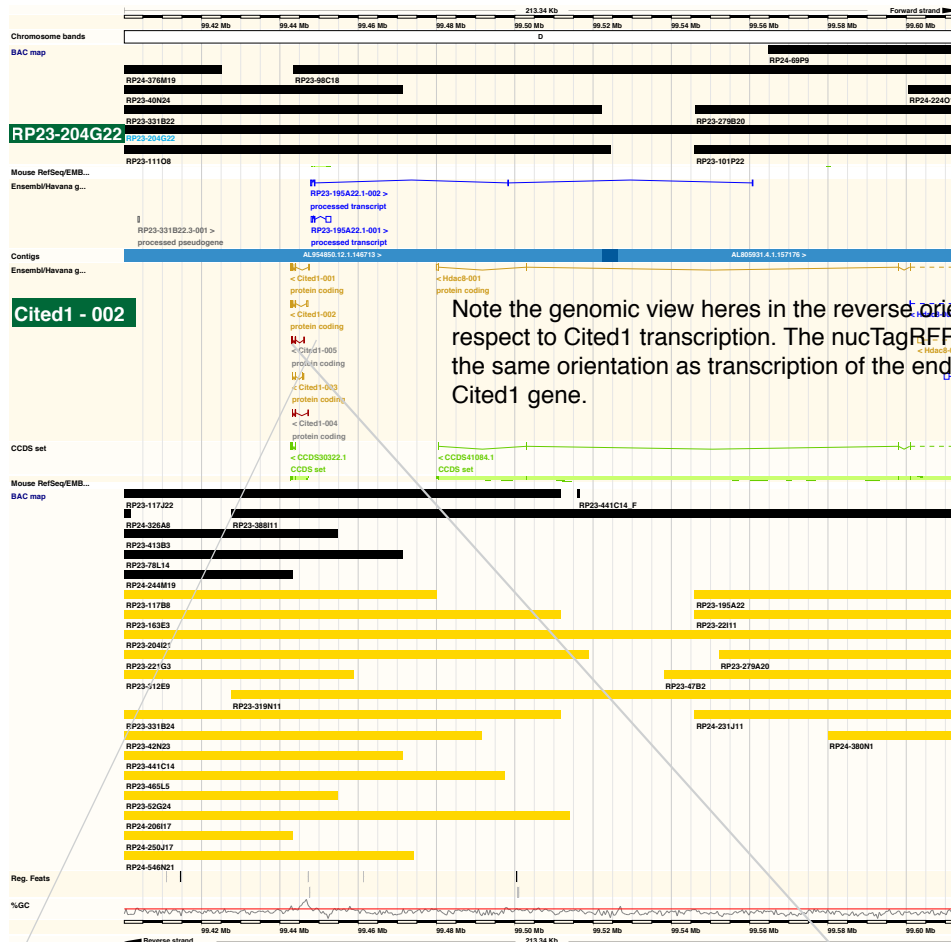

Cited1 homology arm overlapPCR
486 bp

Reporter + Arms

Cited1 BAC targeting cassette
3486 bp
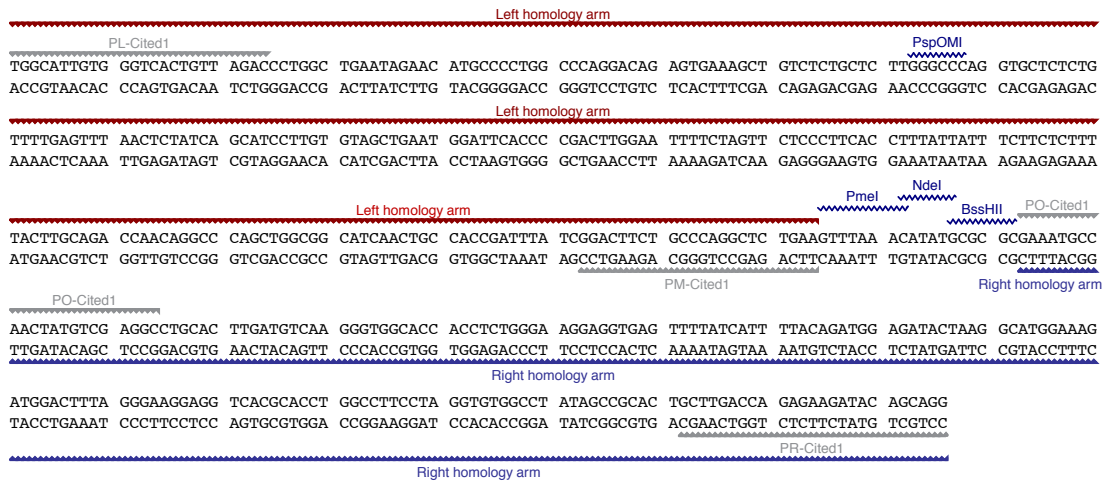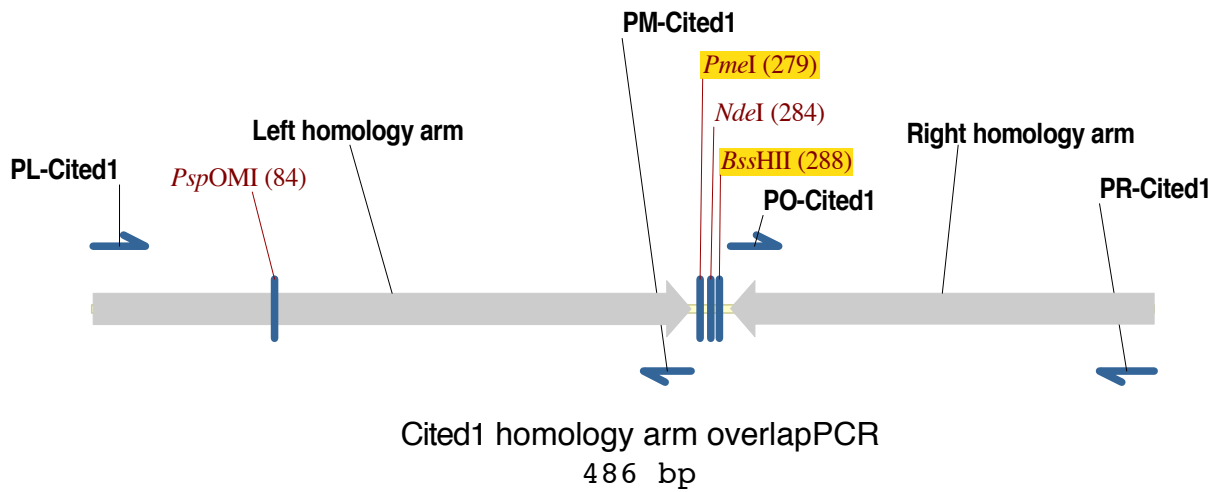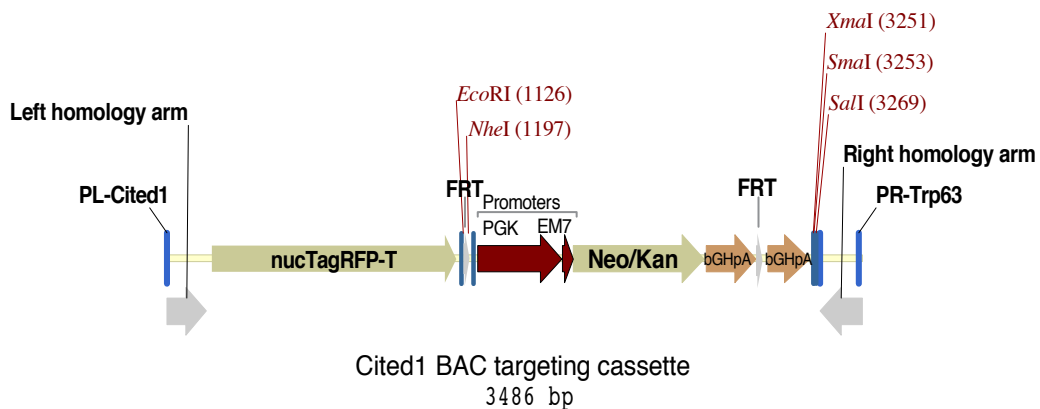
Cited1-TagRFPT BAC Transgene

Created 2 December 2010
Updated 6 December 2010

BAC clone RP23-204G22 was targeted by recombineering with the Cited1-nucTagRFPT BAC targeting construct. The genomic context of Cited1 and the TagRFPT reporter is shown below. The BAC and the target gene are highlighted in dark green. Flanking primers are highlighted with yellow in the lower schematic.

Note the genomic view heres in the reverse orientation with respect to Cited1 transcription. The nucTagRFPT is placed in the same orientation as transcription of the endogenous Cited1 gene.

Cited1-nucTagRFPT BAC construct
214565 bp