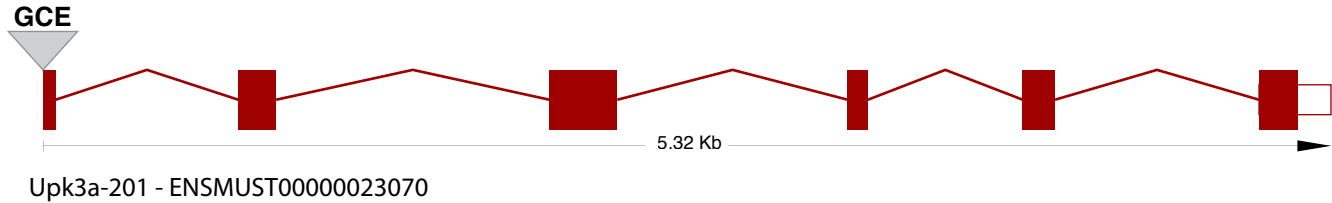


Upk3a-GCE Construct Overview

Created 30 August 2010
Updated 6 September 2010

Gene Overview



Design comments

There is a single transcript reported for Upk3a. The predicted start site ATG is at the beginning of the observed transcripts (i.e. no 5' UTR sequence). This ATG was selected for insertion of the reporter.

Target site in cDNA

cDNA for Upk3a-201

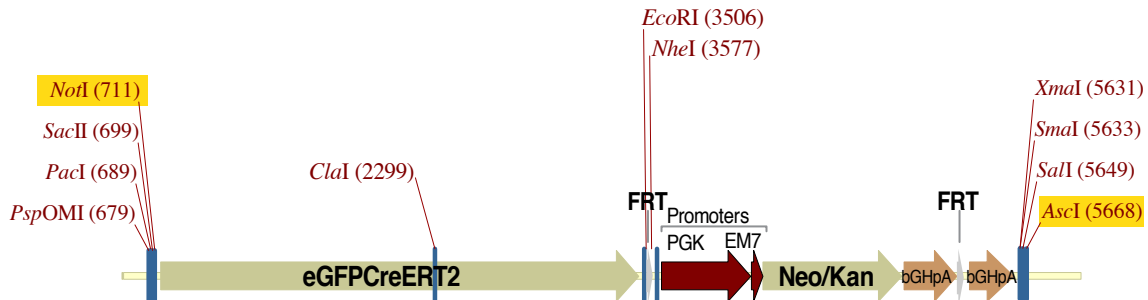
Transcript length: 1001 bps Translation length: 287 residues

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1  ATGCTCCTGCTCTGGGCCCTGCTGGCTCTCGGATGCCTGCGGTGTGGCTGGACTGTGAAC
61  CTCCAGCCCCAACTGGCCAGTGTGACCTTTGCCACCAACAACCCCTACCCTCACCACCGTG
121 GCCTTGAGAAGCCTCTGTGCATGTTTGATAGCTCAGAGCCACTCAGCGGCTCTTACGAG
181 GTTACCTCTATGCTATGGTCGACTCAGCCATGTCCAGGAATGTGCTGTACAGGACAGC
241 GCTGGCGTCCCACTGAGCACCCTTFCGGCAAACCAGGGTGGGAGGTGAGGCCCTAT
301 AAAGCTGCGGCCCTTTGACCTGACCCCTTGTGGTGACTTCCCAGCCTGGATGCTGTTGGA
361 GATGTGACCCAGGCTCAGAGATCCTGAACGCATACCTAGTCAGGGTGGGCAACAACGGG
421 ACCTGTTTTTGGGACCCCAACTTCCAGGGCTCTGCAACCCACCCCTGACAGCGGCCACT
481 GAGTACAGATTCAAGTATGTCCTGGTCAACATGTCCACAGGCTTGGTGCAGGACCAGACA
541 CTATGGTCAGATCCCATCTGGACCAACCGGCCCATCCCTACTCGGCCATCGACACGTGG
601 CCCGGCCGGCGGATGGAGGCATGATTGTCATCACGTCCATTTCTGGGCTCCCTGCCCTTC
661 TTCCTGCTCGTGGGTTTCGCTGGAGCCATCCTCAGCTTTGTGGACATGGGCAGTTCT
721 GATGGGAAATGACACAGACTCACAGATCACCCAGGAGGCTGTTCCCAAGACCTGGGG
781 ACTTCTGAGCCTTCCACTCATCTGTGAACCGGGGCCACCCCTAGACAGAGCAGAGGTG
841 TTCTCAGCAAGCTTCAAGACTGAAACAAGCCAAGCCCGGCCACCAAGCCATGGCCACTT
901 TCAACTTGGCCCTGTGGTGGCAGTGTGGTGTTTATGCTCTGACTGGACCAGATGTGA
961 AACATGACATCTTGGTCCAACCTCATGAAAAAGCTAAATAA
    
```

Reporter Cassette

A "GCE" reporter cassette (eGFP fused to tamoxifen inducible Cre-ERT2) was inserted into the consensus start ATG of the Upk3a 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)

Upk3a-GCE Target Site Details

Created 6 September 2010
Updated 7 September 2010

Endogenous Targeting Site

Left homology arm

PL-Upk3a PstII

aggaccggac tgtatgcaaa tatctcaagg ggaggcttcg gattggctcg tttgagaggg gcggggcatg cccttgctgg tgacaggatga ggggagctgc
tcctggcctg acatacgttt atagagtcc cctccgaagc ctaaccaggc aaactctccc cgcccctgac gggaacgacc actgtccact cgcctcgacg

PstII Aval PspOMI Right homology arm

Left homology arm Exon 1 Right homology arm

agggagcagg tgcgcgttct caggcagagt gcatcgcgaa ggctcatctc gggcgATGCT CCTGCTCTGG GCCCTGCTGG CTCTCGGATG CCTGCGGTGT
tcctctgtcc acgcgcaaga gtccgtctca cgtagcgctt ccgagtagag cccgcTACGA GGACGAGACC CGGGACGACC GAGAGCCTAC GGACGCCACA

Right homology arm

Exon 1

GGCTGGAGta agccggggag aagacctcca gggcgtgtcc agggaggcct gaggggagtg gagacactga gtggcactag gaatctcatt tagggggttc
CCGACCTcat tcggccctc ttctggaggt cccgcacagc tccctccgga ctcccctcac ctctgtgact caccgtgac cttagatga atccccaaag

Right homology arm

aggaatagge gctgcatta gggacacaga tgttagcagc tgtagtaata gaaactgatg ggaatcccag cggccaccct tgggtggtcc tcaattctgt
tccttatccg cggacgtaat cctgtgtct acaatcgtcg acatcattat cttgtactac ctttagggtc ggggtggga acccgaccg agttaagaça

PR-Upk3a

Right homology arm

gatggttgtt gctaggaac a
ctaccaacaa cgatcccttg t

PR-Upk3a

Targeted Site - 5'

Left homology arm

AGGACCGGAC TGTATGCAAA TATCTCAAGG GGAGGCTTCG GATTGGTCCG TTTGAGAGGG GCggggcatg CCCTTGCTGG TGACAGGTGA GCGGAGCTGC
TCCTGGCCTG ACATACGTTT ATAGAGTTC CCTCCGAAGC CTAACCAGGC AAactctccc CGcccctgac GGGAACGACC ACTGTCCACT CGCCTCGACT

Left homology arm Kozak eGFPCreERT2

AGGGAGCAGG TGCgcgttct CAGGCAGAGT GCATcgcgaa GGctcatctc GGGCGTTTGG CCGCCACCAT GGTGAGCAAG GGCAGGAGC TGTTCACCG
TCCTCTGTCC ACGGCAAGA GTCCGTCTCA CGTAGCGCTT CCGAGTAGAG CCGCAAACC GGCGTGGTA CCACTCGTTC CCGTCCTCCG ACAAGTGGCC

eGFPCreERT2

GGTGGTGCC ATCCTGGTCG AGCTGGACGG CGACGTAAAC GGCCACAAGT TCAGCGTGC CGGCGAGGGC GAGGGCGATG CCACCTACGG CAAGCTGACC
CCACCACGG TAGGACCAGC TCGACTGCC GCTGCATTTC CCGGTGTTCa AGTCGCACAG GCCCTCCCG CTCCCCTAC GGTGGATGCC GTTCGACTGG

Targeted Site - 3'

BGH polyadenylation sequence

GGAAATTGCA TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG GGGGAGGATT GGAAGACAA TAGCAGGCAT
CCTTTAACGT AGCGTAACAG ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CTTGTCTTC CCCCTCCTAA CCTTCTGTT ATCGTCCGTA

BGH polyadenylation sequence Xmal SmaI XbaI Sall BssHII Right homology arm

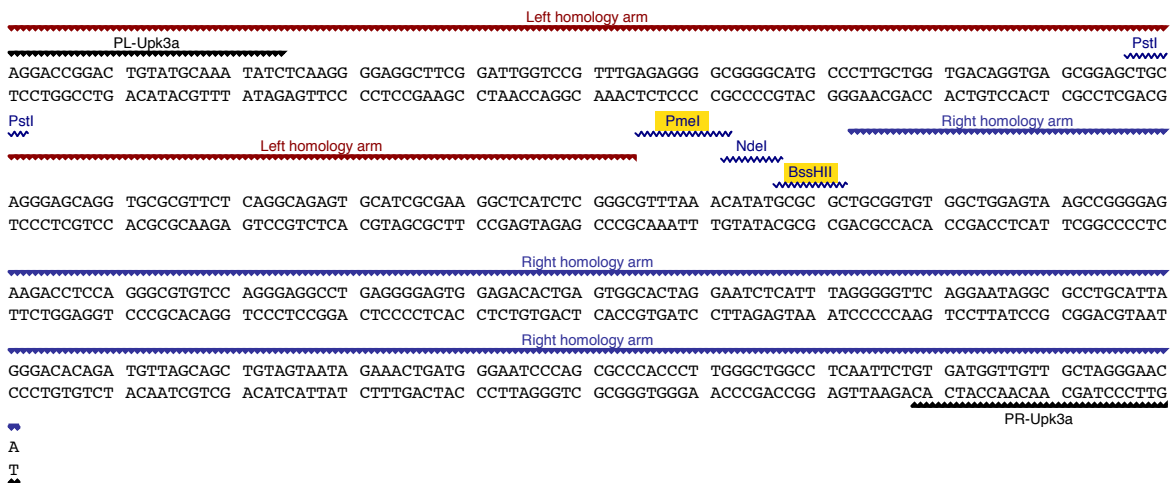
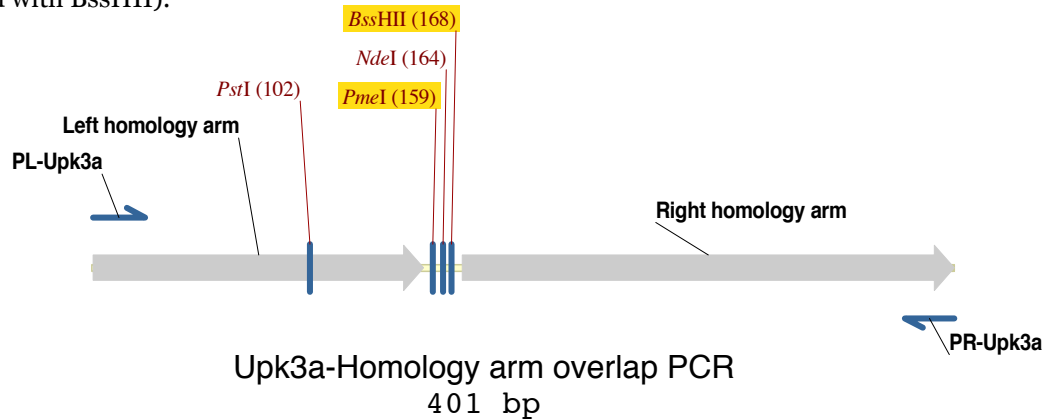
GCTGGGGATG CCGTGGGCTC TATGGCCCGG GTGATCCTCT AGAGTCGACC TCTAGTGAGA TGGCGCGCTG CCGTGTGGT GGAGTAAGCC GGGGAGAAGA
CGACCCCTAC GCCACCCGAG ATACCGGGCC CACTAGGAGA TCTCAGCTGG AGATCACTCT ACCGCGGCAC GCCACACCGA CCTCATTGCG CCCCTCTTCT

Right homology arm

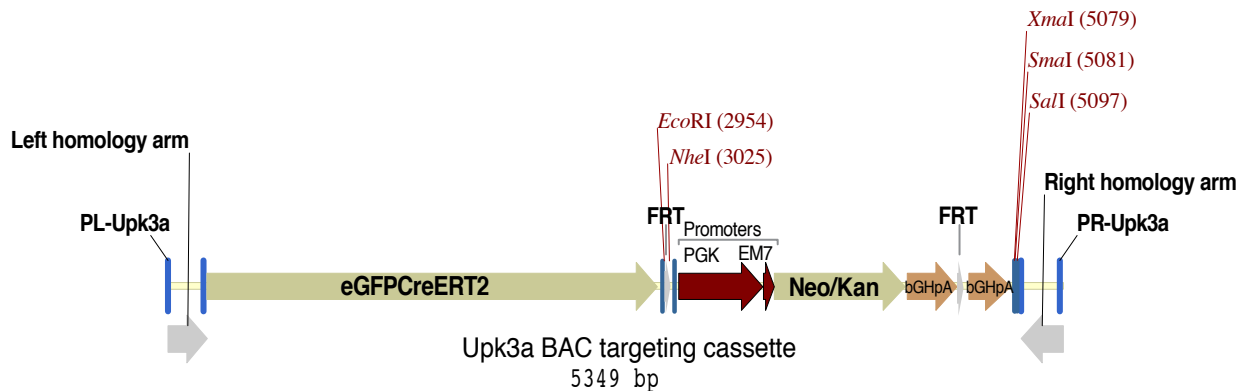
CCTCCAGGGC GTGTCCAGG AGGCCTGAGG GGAGTGGAGA CACTGAGTGG CACTAGGAAT CTCATTAGG GGGTTCAGGA ATAGGCGCCT GCATTAGGGA
GGAGTCCCG CACAGGTCC TCCGGACTCC CCTCACCTCT GTGACTCACC GTGATCCTTA GAGTAAATCC CCCAAGTCTT TATCCGCGGA CGTAATCCCT

BAC targeting cassette for Upk3a

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



Upk3a-GCE BAC Transgene

Created 3 September 2010
Updated 4 September 2010

BAC clone RP23-235E13 was targeted by recombineering with the Upk3a-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.

