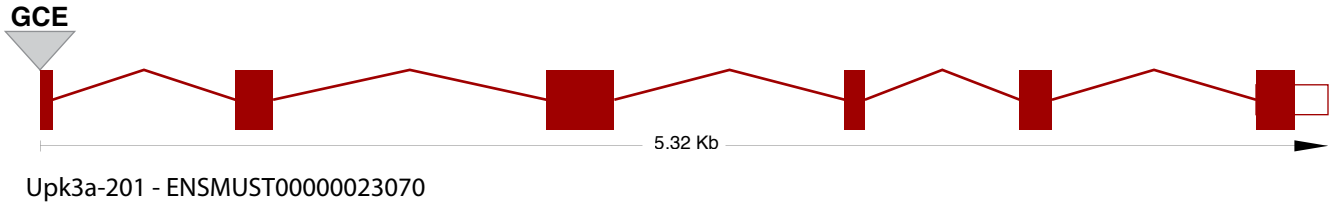
Upk3a-GCE Construct Overview

Created 30 August 2010
Updated 6 September 2010

Gene Overview



Upk3a-201 - ENSMUST00000023070

Design comments

There is a single transcript reported for Upk3a. The predicted start site ATG is at the begining 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 CTCCAGCCCCAACTGGCCAGTGTGACCTTTGCCACCAACAACCCTACCCTCACCACCGTG
121 GCCTTGGAGAAGCCTCTGTGCATGTTTGATAGCTCAGAGCCACTCAGCGGCTCTTACGAG
181 GTTTACCTCTATGCTATGGTCGACTCAGCCATGTCCAGGAATGTGTCTGTACAGGACAGC
241 GCTGGCGTCCCACTGAGCACCACTTTCCGGCAAACCCAGGGTGGGAGGTCAGGCCCCTAT
301 AAAGCTGCGGCCTTTGACCTGACCCCTTGTGGTGACTTGCCCAGCCTGGATGCTGTTGGA
361 GATGTGACCCAGGCCTCAGAGATCCTGAACGCATACCTAGTCAGGGTGGGCAACAACGGG
421 ACCTGTTTTTGGGACCCCAACTTCCAGGGCCTCTGCAACCCACCCCTGACAGCGGCCACT
481 GAGTACAGATTCAAGTATGTCCTGGTCAACATGTCCACAGGCTTGGTGCAGGACCAGACA
541 CTATGGTCAGATCCCATCTGGACCAACCGGCCCATCCCCTACTCGGCCATCGACACGTGG
601 CCCGGCCGGCGGAGTGGAGGCATGATTGTCATCACGTCCATTCTGGGCTCCCTGCCCTTC
661 TTCCTGCTCGTGGGTTTCGCTGGAGCCATCATCCTCAGCTTTGTGGACATGGGCAGTTCT
721 GATGGGGAAATGACACACGACTCACAGATCACCCAGGAGGCTGTTCCCAAGACCCTGGGG
781 ACTTCTGAGCCTTCCTACTCATCTGTGAACCGGGGCCCACCCCTAGACAGAGCAGAGGTG
841 TTCTCCAGCAAGCTTCAAGACTGAAACAAGCCAAGCCCCGGCACCAAGCCATGGCCACTT
901 TTCAACTTGGCCCTGTGGTTGGCAGTGATGGTGTTTATGCTCTGACTGGACCAGATGTGA
961 AACATGACATCTTGGTCCAACCTCATGAAAAAGCTAAATAA
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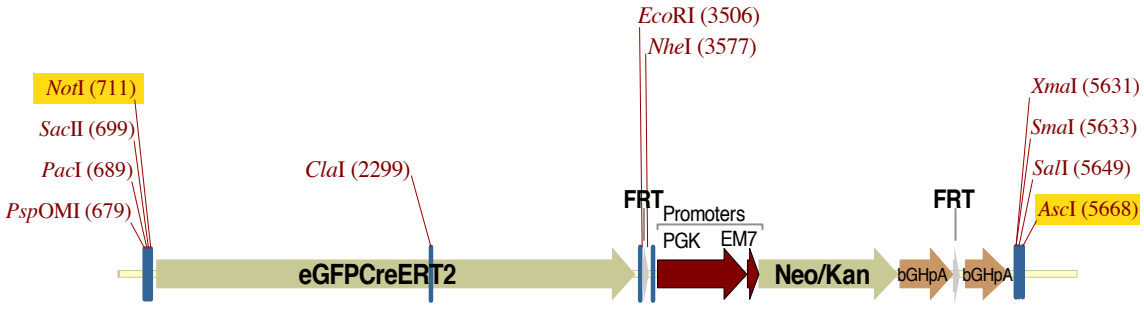
Reporter Cassette

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.

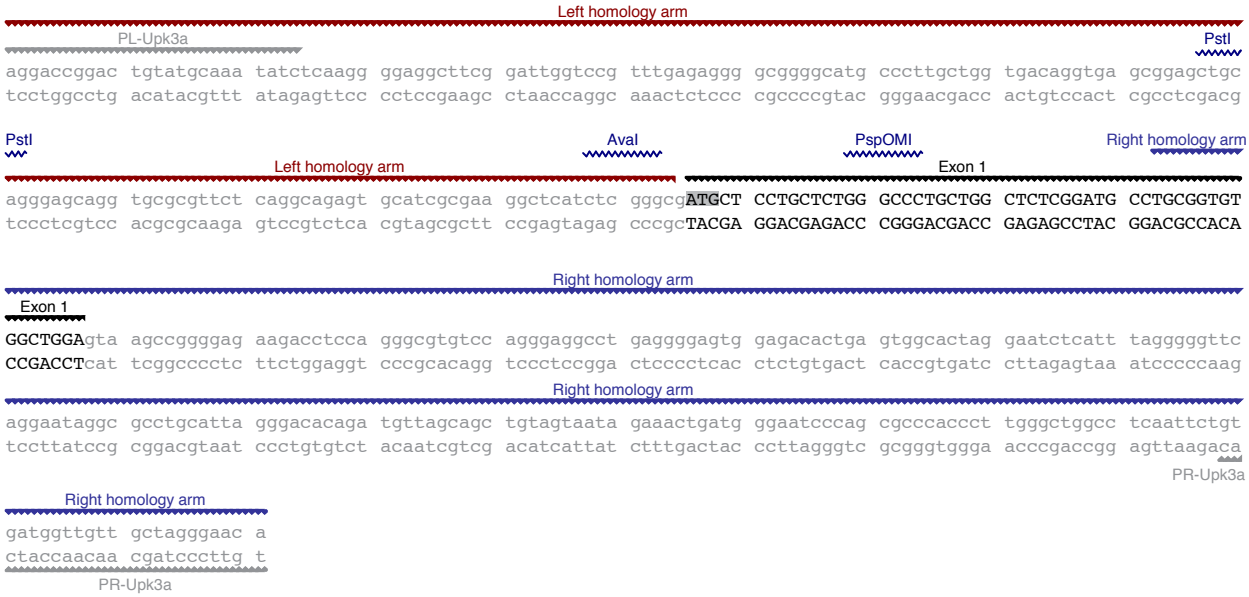


Fragment of pCZV-GCE-FpNF-v2
4996 bp (molecule 7302 bp)

Upk3a-GCE Target Site Details

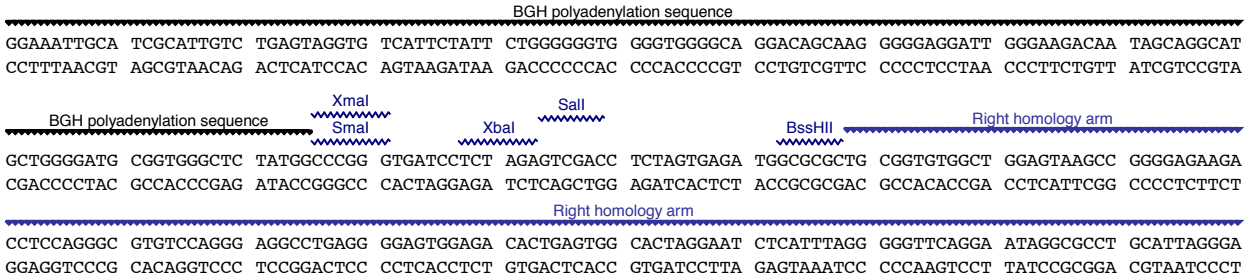
Created 6 September 2010
Updated 7 September 2010

Endogenous Targeting Site



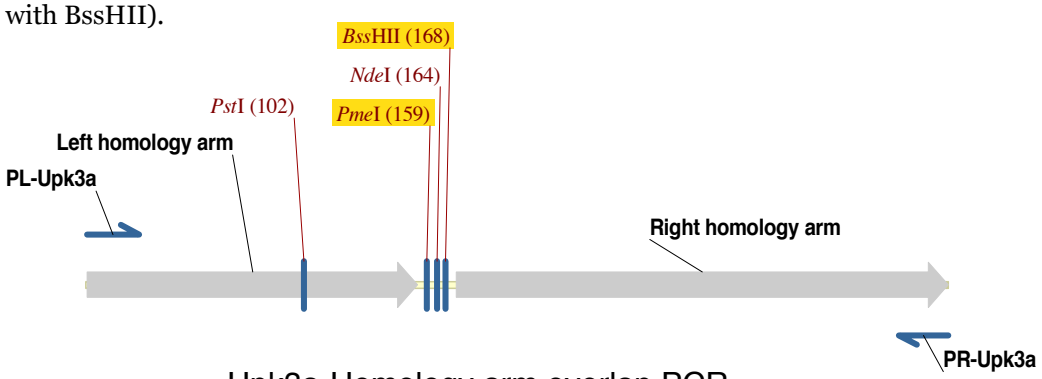
Targeted Site - 5’

Targeted Site - 3’

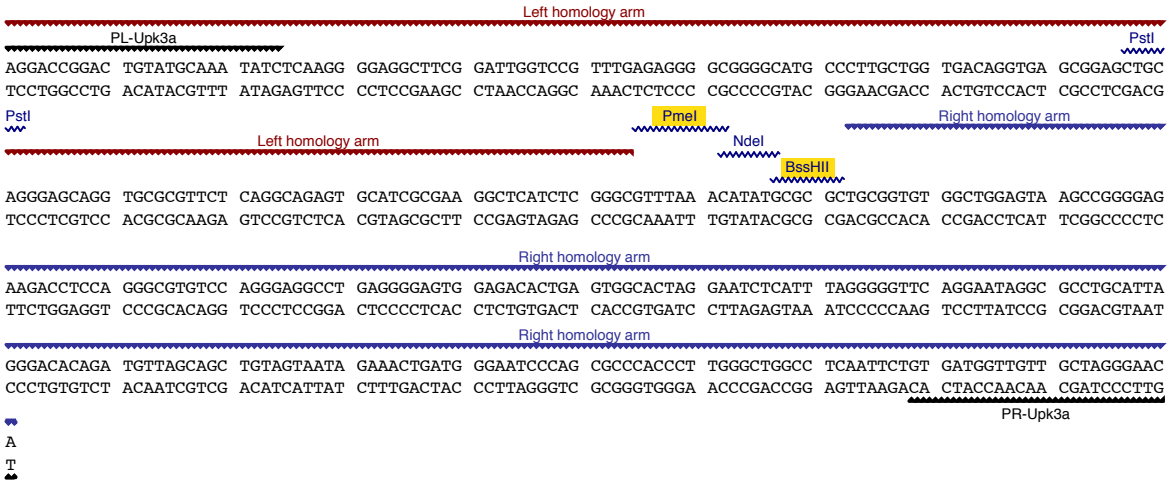


BAC targeting cassette for Upk3a

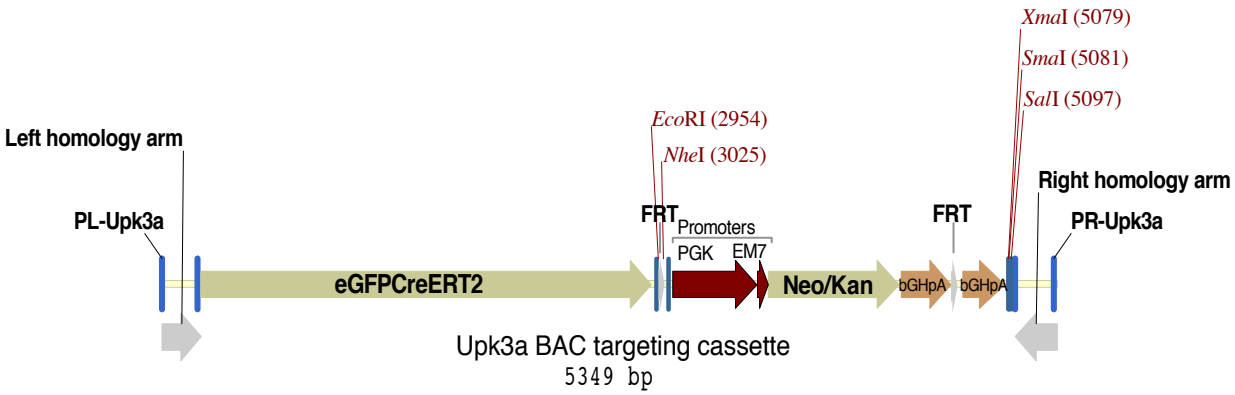
The homologous arms for recombineering were created by overlap-PCR. The resulting product, cloned into a shuttle vector (not shown), contained the Left and Right homology arms joined by a polylinker sequence introduced into the overlap primers. This polylinker sequence included PmeI and BsshII restriction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



Upk3a-Homology arm overlap PCR
401 bp



Reporter + Arms



Upk3a BAC targeting cassette
5349 bp

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.