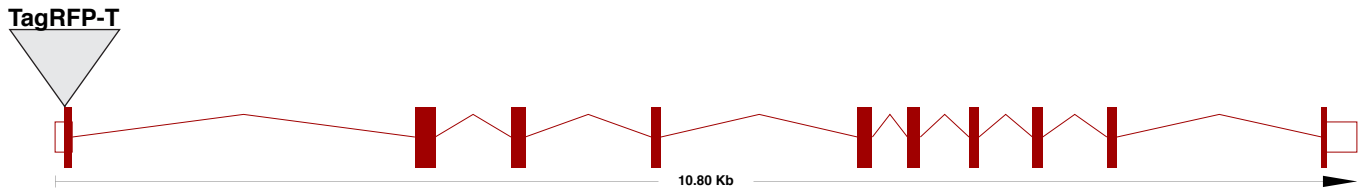


Akr1b7-TagRFP-T BAC construct details

Created 2 March 2010
Updated 23 March 2010

Gene Overview



Design comments

There is a single reported transcript for Akr1b7 with a single upstream ATG that is in frame with the reported open reading frame of the protein. This ATG site was selected for targeting the TagRFP-T reporter. In this construct, the endogenous Kozak sequence was left intact in the amplification primers used in creating the BAC targeting cassette.

Homology Arm Primers:

Akr1b7RFPL
AGAGAAAGCAGGCATTTTCATCTGCTCACTCAGAGAACTCTCTGCAGCAACCATGGTGCTTAAGGGCGAAGA

Akr1b7bGHR
CAGGCCACAAGGGCATCTTGGCTTTGGTACTGAGTCCACGAAGGTGGCCATAGAGCCCACCGCATCC

Target site in cDNA

cDNA fpr Akr1b7-001

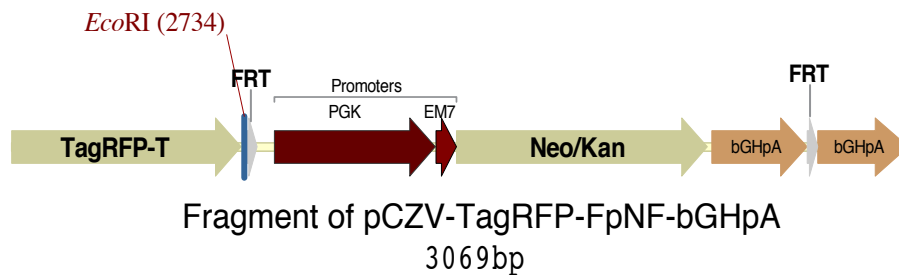
Transcript length: 1281 bps, Translation length: 316 residues

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ATAAAAATGTCACCAGCCTCCTTGTAGAGAAAGCAGGCATTTTCATCTGCTCACTCAGAGA
ACTCTCTGCAGCAACCATGGCCACCTTTCGTGGAACCTCAGTACCAAGCCAAGATGCCCT
TGTGGCCTGGCACCTGGAAGTCTTCCCAGGCCAAGTCAAGGAAGCCGTGAAGGGCCG
CATTGATGCTGGGTATCGCCACATGACTGTGCCTATGTGATCACAATGAGAATGAGGT
GGGAGAAGCTATCCAAGAGAAGATCAAAGAGAATGCTGTGAAGCGGAGGATCTCTTCAT
CGTCAGCAAGCTGTGGGCCACATTCCTTTGAGAAAAGCCTGGTGAAGAAAAGCCTTCCAGAA
CACCTCTCGGATCTGAAGCTGGACTATCTGGACCTGTATCTGGTCCACTGGCCACAGGG
ATTTCAAGCTGGGAATGCTTTATACCCAAGGCAATAAAGGCAAGTTCTCTGAGTAA
ATCCACATTTCTGGATGCTGGGAGGCCATGGAGGAACCTGGTGGACCAGGGGCTGGTGAA
AGCTCTGGGCATCTCCAACCTCAACCACTTCCAGATTGAAAGGCTCCTGAACAAGCCTGG
ACTAAAACATAAGCCAGTGACCAACCAAGATTGAGAGCCACCTTATCTCACCAGGAA
ACTGATCCAATACTGTCAATCCAAGGGCATCGCTGTTACAGCCTACAGTCCCCTGGGCTC
CCCAGACAGGCCTTATGCCAAGCCAGAAGACCCCGTAGTAATGGAGATTCCCAAGATCAA
AGAGATTGCTGCAAAACACAAGAAAACAGTAGCTCAGGTTCTGATTCCGGTTCATGTCCA
AAGGAATGTGGTGTATCCCAAGTCTGTGACACCTCACGCATACAGGAGAACCTGCA
GGTCTTCGACTTCCAGTTGAGTGAGGAGGACATGGCTGCCATTCTCAGTTCACACAGGAA
CTGGAGGGCCTGTGACCTGTGGATGCAAGGACTGAAGAGGACTATCCTTTCCACAGGAA
ATACTGAGGTCCACTTGTCTGATGAGATCCGTGATGATAGATTCTCTCGTCTCTCAA
ATCAACCTGGATGATAGCTACCATCTGGCAATGCAGGATTTAAATTTTGTGTCATCAA
GGAGCAAGAAAATGTTCCAGAGAAACGAAGGGCAACGGTTCTCAGTACGAGGGCTGAGC
CATTTTGTGTCATCAGGAGCATGTAGTATGGTAACACTGAAGATACAATGATAGAACAA
AAAAAAGCAATAATAG
    
```

Red bar = Left homology arm
Blue bar = Right homology arm

Reporter Cassette



Akr1b7-TagRFP-T Target Site Details

Created 2 March 2010
Updated 21 March 2010

Endogenous Targeting Site

```
1 tttaatcttt gtttacgatg atcagtgtgg cacaagattg acatgaagtt cctgttctca tgccccaacc cttggctgtg gctgcttgcc aatgtggtaa
aaattagaaa caaatgctac tagtcacacc gtgttctaac tgtacttcaa ggacaagagt acggggttgg gaaccgacac cgacgaacgg ttacaccatt

Homologous Primer L
5' UTR of Akr1b7
101 gagcccgcct cctttatcca ggacATAAAA ATGTCACCCAG CCTCCTTGTA GAGAAAGCAG GCATTTCATC TGCTCACTCA GAGAACTCTC TGCAGCAACC
ctcgggcgga ggaatataggt cctgTATTTT TACAGTGGTC GGAGGAACAT CTCTTTCGTC CGTAAAGTAG ACGAGTGAGT CTCTTGAGAG ACGTCGTTGG

Akr1b7 coding
201 ATGGCCACCT TCGTGGAACT CAGTACCAAA GCCAAGATGC CCCTTGTGGG CCTGGGCACC TGGAAAgTga gtgtgcagtc ttgggacacc tgtgccttt
TACCGGTGGA AGCACCTTGA GTCATGGTTT CGGTTCCTACG GGAACACCC GGACCCGTGG ACCTTCcact cacacgtcag aacctgtgag acgacggaaa

Homologous Primer R
301 gaggggaggt ctggacattt tctttctgtg gcagagcadc tatctgcctt gggtttgata aggccagcat taccactgtg tgcagctgc tttgggttgc
ctcccctcca gacctgtaa agaaaagaca cgtctcgtag atagacggaa cccaaactat tccggtcgtg atagtggaca acagctcagc aaaccaacg
```

Targeted Site - 5'

```
1 tttaatcttt gtttacgatg atcagtgtgg cacaagattg acatgaagtt cctgttctca tgccccaacc cttggctgtg gctgcttgcc aatgtggtaa
aaattagaaa caaatgctac tagtcacacc gtgttctaac tgtacttcaa ggacaagagt acggggttgg gaaccgacac cgacgaacgg ttacaccatt

Homology primer L
5' UTR of Akr1b7
101 gagcccgcct cctttatcca ggacATAAAA ATGTCACCCAG CCTCCTTGTA GAGAAAGCAG GCATTTCATC TGCTCACTCA GAGAACTCTC TGCAGCAACC
ctcgggcgga ggaatataggt cctgTATTTT TACAGTGGTC GGAGGAACAT CTCTTTCGTC CGTAAAGTAG ACGAGTGAGT CTCTTGAGAG ACGTCGTTGG

Homology primer L
TagRFP-T Coding region (start)
201 ATGGTGTCTA AGGGCGAAGA GCTGATTAAG GAGAACATGC ACATGAAGCT GTACATGGAG GGCACCGTGA ACAACCACCA CTCAAGTGC ACATCCGAGG
TACCACAGAT TCCCCTTCTT CGACTAATTC CTCTTGTACG TGTAATTCGA CATGTACCTC CCGTGGCACT TGTTGGTGGT GAAGTTCACG TGTAGGCTCC

TagRFP-T Coding region (start)
301 GCGAAGGCAA GCCCTACGAG GGCACCCAGA CCATGAGAAT CAAGTGGTC GAGGGCGGCC CTCTCCCTTT CGCCTTCGAC ATCCTGGCTA CCAGCTTCAT
CGCTTCCGTT CGGGATGCTC CCGTGGGTCT GGTACTCTTA GTTCCACCAG CTCCC GCCG GAGAGGGGAA GCGGAAGCTG TAGGACCGAT GGTGGAAGTA
```

Targeted Site - 3'

```
bGHpA from TagRFP-T
TGTTGTTTGC CCCTCCCCCG TGCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCTT TTCTTAATAA AATGAGGAAA TTGCATCGCA TTGCTGTAGT
ACAACAACG GGGAGGGGGC ACGGAAGGAA CTGGGACCTT CCACGGTGGT GGTGACAGGA AAGATTATT TTACTCTTT AACGTAGCGT AACAGACTCA

bGHpA from TagRFP-T
AGGTGTCATT CTATTCTGGG GGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG
TCCACAGTAA GATAAGACCC CCCACCCAC CCCGCTCTGT CGTTCCTTCT CTAACCTT CTGTTATCGT CCGTACGACC CCTACGCCAC CCAGATACC

Right Homology Arm
GCCACCTTCG TGGAACTCAG TACCAAAGCC AAGATGCCCT TTGTGGGCTT GGCACCTGG AAGgtgagt tgcagtcttg ggacacctgc tgcctttgag
CGGTGGAAGC ACCTTCAGTC ATGTTTCGG TTCTACGGG AACACCCGGA CCCGTGGACC TTCcactcac acgtcagaac cctgtggagc acggaactc

Right Homology Arm
Intron 1
gggaggtctg gacattttct tttctgtgca gagcatctat ctgccttggg ttgtgataag ccagcattat cacctgttgt cagctgcttt gggttgcaga
ccctccagac ctgtaaaaga aaagacacgt ctgtagata gacggaaccc aaactattcc ggtcgttaata gtggacaaca gtgcagcaga cccaactctc

Intron 1
TagRFP-T
gagaggtctg gacattttct tttctgtgca gagcatctat ctgccttggg ttgtgataag ccagcattat cacctgttgt cagctgcttt gggttgcaga
ccctccagac ctgtaaaaga aaagacacgt ctgtagata gacggaaccc aaactattcc ggtcgttaata gtggacaaca gtgcagcaga cccaactctc
```

Reporter 5' end

```
ACATGTGTGC TGGGCCACG CGGCCAGATC TGAGCTCGCG GCCCGGATAT CGCTAGCATG ACTGGTGGAC AGCAAATGGG TCGGGATCTG TACGACGATG
TGTACACAG ACCCGGGTGC GCCGGTCTAG ACTCGAGCGC CGGCCTTATA GCGATCGTAC TGACCACCTG TCGTTTACCC AGCCCTAGAC ATGCTGTCTAC

BamHI PspOMI NotI Sacl EagI NheI
ACGATAAGGA TCCGATGGTG TCTAAGGGCG AAGAGCTGAT TAAGGAGAAC ATGCACATGA AGCTGTACAT GGAGGGCACC GTGAACAACC ACCACTTCAA
TGCTATTCTT AGGCTACCAC AGATTCCCAG TTCTCGACTA ATTCTCTTGG TACGTGTACT TCGACATGTA CCTCCCCTGG CACTTGTGGT TGGTGAAGTT
```