

## KanMX4 sequence used in serial or block PCR method

UP TAG PRIMER (70 bp)  
 Gene seq.-Uptag-AATCGAACGGAGCAGGGG

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1  TTAGCTTGCCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCAGAATACCCT
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+-----+
61  CCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTA
   -----+-----+-----+-----+-----+-----+-----+-----+
121 GCCCATACATCCCCATGTATAATCATTTCATCCATACATTTTGATGGCCGCACGGCGCG
   -----+-----+-----+-----+-----+-----+-----+-----+
181 AAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
   TTTGCGAGGGGAGTGTCT
   cp-N1
241 CGCGTTGAATTGTCCCCACGCCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCC
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
   GC
301 ACTGAGGTTCTTCTTTTCATATACTTCCTTTTAAAATCTTGCTAGGATACAGTTCTCACAT
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
   GAACGATCCTATGTCAAGAGTGTA
   cp-N10
361 CACATCCGAACATAAAACAACCATGGGTAAGGAAAAGACTCACGTTTCGAGGCCGCGATTA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
   G
421 N S N M D A D L Y G Y K W A R D N V G Q
   AATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGC TCGCGATAATGTCTGGGCAA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
481 S G A T I Y R L Y G K P D A P E L F L K
   TCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
541 H G K G S V A N D V T D E M V R L N W L
   CATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTG
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
601 T E F M P L P T I K H F I R T P D D A W
   ACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGG
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
661 L L T T A I P G K T A F Q V L E E Y P D
   TTACTCACCCTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATAT CCTGAT
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
721 S G E N I V D A L A V F L R R L H S I P
   TCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCCT
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
781 V C N C P F N S D R V F R L A Q A Q S R
   GTTTGTAAATGTCCTTTTACAGCGATCGCGTATTTTCGTC TCGCTCAGGCGCAATCACGA
   | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
   -----+-----+-----+-----+-----+-----+-----+-----+
  
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841 M N N G L V D A S D F D D E R N G W P V  
ATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTT  
-----+-----+-----+-----+-----+-----+-----+

**ACTAAAAGTACTGCTCGCATTACCAGCCGGACAA**  
cp-C1

E Q V W K E M H K L L P F S P D S V V T  
901 GAACAAGTCTGGAAAGAAAATGCATAAGCTTTTGCCATTCTCACC GGATT CAGT CGTCACT  
-----+-----+-----+-----+-----+-----+-----+

*HindIII*

**CTTGTT CAGACCT**  
cp-C3

H G D F S L D N L I F D E G K L I G C I  
961 CATGGTGATTTCTCACTTGATAACCTTATTTTGACGAGGGGAAATTAATAGTGTGATT  
-----+-----+-----+-----+-----+-----+-----+

*PflMI*

1021 D V G R V G I A D R Y Q D L A I L W N C  
GATGTTGGACGAGTCGGAAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACCTGC  
-----+-----+-----+-----+-----+-----+-----+

1081 L G E F S P S L Q K R L F Q K Y G I D N  
CTCGGTGAGTTTTCTCCTT CATTACAGAAACGGCTTTTTCAAAATATGGTATTGATAAT  
-----+-----+-----+-----+-----+-----+-----+

*ScaI*

1141 P D M N K L Q F H L M L D E F F \*  
CCTGATATGAATAAATTGAGTTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGTACTG  
-----+-----+-----+-----+-----+-----+-----+

1201 ACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCAATTTGTATAGTTTTTTTATATTGTAG  
-----+-----+-----+-----+-----+-----+-----+

1261 TTGTTCTATTTTAAATCAAATGT TAGCGTGATTTATATTTTTTTTTCGCCTCGACATCATCT  
-----+-----+-----+-----+-----+-----+-----+

1321 GCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGC  
-----+-----+-----+-----+-----+-----+-----+

1381 TGGTCGCTATACTGCTGTCGATTTCGATACTAACGCCCATCCAGTGT CGAAA 1433  
-----+-----+-----+-----+-----+-----+-----+

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← GCGGTAGGTCACAGCTTT-DNtag-Gene seq.  
DOWN TAG PRIMER (70 bp)

**KanMX4 sequence used in gene synthesis method**

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1      GCCGGGTCACCCGGCCAGCGACATGGAGGCCAGAATACCCTCCTTGACAGTCTTGACGT
-----+-----+-----+-----+-----+-----+-----+
61     GCGCAGCTCAGGGGCATGATGTGACTGTGCGCCGTACATTTAGCCATACATCCCCATGT
-----+-----+-----+-----+-----+-----+-----+
121    ATAATCATTGTCATCCATACATTTTGGATGGCCGCACGGCGGAAGCAAAAATTACGGCTC
-----+-----+-----+-----+-----+-----+-----+
181     CTGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCA
-----+-----+-----+-----+-----+-----+-----+
      TTTGCGAGGGGAGTGTCTGC
      cp-N1
241    CGCCGCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCA
-----+-----+-----+-----+-----+-----+-----+
301     TATACTTCCTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACA
-----+-----+-----+-----+-----+-----+-----+
      GAACGATCCTATGTCAAGAGTGTAG
      cp-N10
361     M G K E K T H V S R P R L N S N M D A
      NcoI
ACCATGGGTAAGGAAAAGACTCACGTTTCGAGGCCGCGATTAAATTCCAACATGGATGCT
-----+-----+-----+-----+-----+-----+-----+
421     D L Y G Y K W A R D N V G Q S G A T I Y
      NruI
GATTTATATGGGTATAAATGGGCTCGCGATAATGTGCGGCCAATCAGGTGCGACAATCTAT
-----+-----+-----+-----+-----+-----+-----+
481     R L Y G K P D A P E L F L K H G K G S V
      ClaI
CGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTT
-----+-----+-----+-----+-----+-----+-----+
541     A N D V T D E M V R L N W L T E F M P L
GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTT
-----+-----+-----+-----+-----+-----+-----+
601     P T I K H F I R T P D D A W L L T T A I
CCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCGATC
-----+-----+-----+-----+-----+-----+-----+
661     P G K T A F Q V L E E Y P D S G E N I V
      EcoNI      SspI
CCCGGCAAAACAGCATTCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTT
-----+-----+-----+-----+-----+-----+-----+
721     D A L A V F L R R L H S I P V C N C P F
GATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTTCGATTCCTGTTTGTAAATTGCCTTTT
-----+-----+-----+-----+-----+-----+-----+
781     N S D R V F R L A Q A Q S R M N N G L V
      PvuI
AACAGCGATCGCGTATTTCTGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTT
-----+-----+-----+-----+-----+-----+-----+

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841 D A S D F D D E R N G W P V E Q V W K E  
GATGCGAGTGATTTTGTGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAA  
-----+-----+-----+-----+-----+  
**ACTAAACTACTGCTCGCATTACCGACCGACAACCTTGTTTCAGACCT**  
**cp-C1 cp-C3**

*HindIII*  
901 M H K L L P F S P D S V V T H G D F S L  
ATGCATAAGCTTTTGCCATTCTCACC GGATT CAGTCGTCAC T CATGGTGATTCTCACTT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

961 D N L I F D E G K L I G C I D V G R V G  
GATAACCTTATTTTGTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGA  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

*pflMI*  
1021 I A D R Y Q D L A I L W N C L G E F S P  
ATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAAC TGCCTCGGTGAGTTTTCTCCT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

1081 S L Q K R L F Q K Y G I D N P D M N K L  
TCATTACGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

*ScaI*  
1141 Q F H L M L D E F F \*  
CAGTTTCATTTGATGCTCGATGAGTTTTTCTAAATCAGTACTGACAATAAAAAGATTCTTG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

1201 TTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAA  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

1261 ATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCAGATGCGAAGTTAA  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

1321 GTGCGCAGAAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

1381 CGATTGATACTAACGC 1397  
-----+-----