Sequence of Act79B with 2K upstream/downstream

Breakpoints of Act79B[F3-3] deletion

GTCAGACAGCAAGGGTGCAAGGGAAAATTGAGCAGTACCAAAAGCGCAGAATGGAGGTAA ATCTGTCTATATTTACTTGAAGAAATCTATGTGTAATCTATTAATATACAACCGAAAAAT AACCAAAATATGATTTCCAAAAGGGCACTCTTATTTGTTGATCGAAACAAGACCTTCTAA GAACTAATTAAGACTTACTTAGCACTGATTACAAATACTATCTGCCCACTTAATTGATCC GATCCCATGCCACATTTAGCTGTGAACTGAAAAAGTTGCAATATATCGAAGTATTCAGAG CTCCTCCACCCCTGGATGCGGTTATCTACTGAGTCGGAGGAACCACAAGTCCCTGCTGGC GGGCATTATTTTTACCTCTTTCAACTACTCGACGCGCGTACGACCAATGATACGTGGTTG GTGTCCGTACCTGATCCGCAAGGTGGTCACGTAATGCTCCGCAGTTGGCGAGCACTTGGC AAAGTTAACGGGCCAAAGTTGGAGCTCAGAAGTTTGCTCCTTTGGGGAATGCACTCGCTG CAGTAAAAGCAGAGTGCATGCACCTCCTCGGATGGGGCACACTTAATTAGAATATCGGGC ATTAATCTTATGGCTAATGCCACGATGAGATCGAGCGATATCGGTGGACAGGCGCAAAAT TGCTGTTGGCCGCATGCACCGCTGCGCCAAACCCCCCAACCACTTTCCTTTGGGCTCACA GGGCTATGTGCCGTGCATAGCCGTACAAAATTATTTGATTCTATTTTTTCAATTTGATTT CAATGAAATCCACACGACAGCGTCAACATTTATAAGATGTATAAACGCAAGTGCTTTGGC TGCATTTTTGAGTGTGACAACATTCGGGCCACACCATCTATGGACTGAGTGGGGGCCCAG AAGAGCATTGGCACCAATTTCTCTTACTCGAGCAGGGAAAAGCTGCCTACAAGACCAAGA TAAAGAACTTGGGGGAAAGGCTGGCCACTACATTGCATTGTTCACGGGTTGTTCTTGTTG CTGCGCCGCCGTTTTGCGGGGGTTTATTTATATAAAAACTTAAAAATATATCTACAGATG CCTATACAAGTCCTGGCGCATATTTCGATATGTTCTCTCCTCTGTTCTTTGTCGAAGTCA TAGCAAAGTTGGCAGCCAGACATAATTTAGCGAAAACGAAATGAAACTAAGTGATAAATG GTATTTGGGAATGGAGCAAATTGAATACTCTGAACGAACGCAAAATCCCGATAATGCTCT GACTAATACGACTTAGGGCCTGATGGAAATAAATTATTCTATCAAAAAACGGAGAGCCTA TTGTCAGAGCTTAAATCGAAGCTCGTTAGATAAATCCATTTGTTAGCTTTGGCTTAACAC ATTCTGTCATTTGATGCttaaattaaaagtaattggaatacccaattttgctatttgtca aaaaaatatatctattgtgtatatttgggactattgccattctaaaacaatatataaaat aaatataaatatttatattatgtatgtatatttatgactattgccattctaaaacaatat ataaatatcaaaactatctagcctaagcccagcgttccaataaattatttattttcccaa atttcacaagcttataagtgtgtgcggattaaaattctaacaatataacaagacttacaa cttacaaaacaacttattttatattgaaatctagtaccaatttagttgctctaagttgtg gcttaactagggttctttaattcgtaatccaacttgttgccgtaggcgtaatacccgaaa tcagaacacttttgtgaaatcgaaatgatgtgcatccgaccaccctccccggaaacgcct gatccccagccagcgttgcatat**cgcggaattcatcaacatgttactagatgaacaattg ttcgagatgacagggacatgggcgtggggccggcggggcgggacagaacttatttaaatg**

**Cagctgc|**cggagcgcataacGAATCACTCTGATCGCTGTCGCTGTTGGATTTACACGTCG TGAGTGTAGTCTTGTCCGCCCATCCGAAATCCGTAACCCGCATAAGGGATAACCGATCTG TCTGTATCCTTGTAGTGCCGCCCGCACCAAACTAACCAAACATGTGTGACGAAGAAGCAT CAGCCCTGGTCGTAGACAACGGCTCCGGCATGTGCAAGGCCGGATTCGCCGGAGACGACG CGCCCCGCGCCGTATTCCCCTCGATCGTAGGCCGTCCCCGTCACCAGGGCGTGATGGTGG GTATGGGTCAGAAGGACTGCTACGTGGGCGACGAGGCGCAGAGCAAGCGCGGTATCCTGT CGCTGAAGTACCCCATCGAACACGGCATTATCACCAACTGGGATGACATGGAGAAGGTCT GGCACCACACCTTCTACAACGAGCTGCGTGTGGCCCCCGAGGAGCACCCCGTTCTGCTGA CCGAGGCTCCCTTGAACCCCAAGGCCAACCGCGAGAAGATGACCCAGATCATGTTCGAGA CGTTCAACTCCCCGGCCATGTACGTGGCCATCCAGGCCGTGCTCTCCCTGTACGCCTCCG GCCGTACCACCGGTATCGTCCTGGACTCCGGTGACGGTGTCTCCCACACCGTGCCCATCT ATGAGGGCTATGCCCTGCCCCACGCCATCCTTCGTCTAGATCTGGCCGGTCGCGATCTAA CCGACTACCTGATGAAGATCCTCACCGAGCGCGGCTACAGCTTCACCACCACCGCCGAGC GCGAGATTGTGCGCGACATCAAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAGG AGATGGCCACTGCCGCCGCCTCCACCTCCCTGGAGAAGTCTTACGAGCTGCCCGATGGCC AGGTAATCACCATCGGCAACGAGCGCTTCCGCACCCCGGAGGCCCTCTTCCAGCCATCGT TCCTGGGCATGGAGTCCTGCGGCATCCACGAGACCGTCTACCAGTCCATCATGAAGTGCG ACGTGGACATCCGCAAGGATCTGTATGCCAACAATGTGCTGTCTGGCGGCACTACCATGT ATCCAGgtgcgtagtcttaattaattaggaccataaagttcagaggaaattcttccgagg gaatgggatcaaaactatgcgggatacttaaaaaaaaaaacaagtgttactttatacatt catttggcagagaggaaatctttaaataataaagcctaaatatttagctgagctttgtaa taacagttaaaaaaaatcttatggaaagtagtattacaaaaaaaaaaaaaagaattcacc taatgggttatatcctttccctatatctcatattcatgcatgctattattaaaatgtcat gtaatgagtacaccaaag**c|tcctcggttctgtagcaccactaatggattctatttctgtc ctcttcagGTATCGCTGACCGTATGCAAAAGGAAATCACCGCACTTGCCCCGTCCACCAT CAAGATCAAGATCATCGCCCCGCCAGAGCGCAAGTACTCCGTCTGGATCGGTGGCTCCAT CCTGGCTTCGTTGTCCACCTTCCAGCAGATGTGGATCTCCAAGCAGGAGTATGACGAGTC CGGTCCCGGCATCGTCCACCGCAAGTGCTTCTAAGCATCCAAGCCACCCAAACCAGATCA ACATCTCCTCGAGCGCGGCCCTGGTGTTTGTCTCCAGCGTAAGACATCCGACCAGGCGCC GGCGCCAAGGGTGAGGACGCAGTTCAGTGAAAAGTATCTTTAAATTACATTTAGTTGATG AAGAAGTTTTAACATAGATAGAGAAAACAAGAGAAAGAGACGAGAAAAGACCGGAAACGA GCGGCAACGC**CTTCTTTTTTTATTATTATTGCTATTTTTATTTGATTTGACTCGGAATTT GTACTGCTTGACTCGCTTGCCAGCGCGTAAAGTGGCCAAAACAATCGGTTGTACGTACAA TAAAAACCAATAACCCATGTGaatcgaacccaagtcgaacgtcacaggagcagcaggaat gcgtggtggggaaacttttttaattaggcctcgacgaatgtcaatcaggcacttgccggc caacttttattctctaacagctgccatggcaatcccccagtacgggtatctgtatctgta tctgtatctgtatctgtatctgtgcatctatccccccgcatgctcttctgtcacattttc acaaacccagatggattttccacacgaagagaggttgcgaggaatgacttctggaggact gaagtcggaaatacaaggagcagcgacagtcttaactggagcccaactgctcacagaact tttcgccaacataaatgtttgtgctcaacttttggctgcaaacttttgaggtttcgccaa actatgcataaaccaggtgccgaaaactgttgcttaattagttagggccaagcttgctgg agtcgccaaagaaaagagtctaactgggtttattggtctaatgtctgctgctaagtcgtc agtaacacggaaactcttctaatgactagggagcacgtgcattcttgatgtttttgggca cccaaaatcttgccccaaaaccttgccaattatgattttagagatgcagtcctggggatt ctggcacgtgtagatatcaattgaacttttgttccatggtccttgcaccaacgccactgc tcagcggtccatgggtcattggatccaagtgcggttcattatccgccgtccagccaggga gagcggaaacagagaaattagtgcggcacgttggcgtacaactaaatgtttggcggactg gctggtccgaaatgatagtaaaggcctggctggaaatcgctactcaatcaatcagtggtc gcagcaggtcaacaaatttggccataaagccctaaaactgaggggttaatagctaatggg tcgatttggcccacactcagagaagtcactagaatgcacatttatgattcctggctaaat ttttatatatttctgtggttaagttaacttatgtgagaaagaacaattctttaaccaata tcacagttaacattaataataacatttttgcagataattttagtaaaagtggaaatagag tggagctaaaattttttcttcttttagtgcagaataccaggcggccgaaaagtgggccaa aatccgttctctggtttcgtattgttatgcttgcagcacttgtctgtacatcaaattgaa tttaattacagcccggccagggttttcgtgctcccccggagtcatccacattctcggagt aatgctgcgtcgattgtttgtttgtatgtcggtttcagtttgcggaaatggttttgcatt ttgcatttgctgccattgcaactgcaactgcaacaccgagctgcaagttctgtgcaagtt ctgtttattttatgctaatttaatacactcgccctatcagccggcccattgataccatta taatttggtgttaattccgaaggcaaatacttcaatgtatttgccattttcattatgcag atttgcccaggcgcaaaacaaacaagctggaattaaagcccaaagttaacgagttatctc tcgtctgccgagagctgtgggcgtgagttgtatgggaaaagggttggaggcccacaaaaa cttggacttgatggtcgatggttttgacaagctaacaaattgttttccctggctggaggt gcaccacatgcggacaggaaattaataaataaaagggctgcaccatgataccagacccct ccatcttcatcaacctaggcgttttcctcacatcctgcttgccacctaatagccctcccc cgcatcctttgagcttaactgaccgccgcagacctcctcttaattgaggcgactctatca acatcttggccatcactatcaatttccgtcggctgctgtaaatttaaatttatacgcctg ccactgacacgctcccaaaaatggaaaaactttagcacacg

Primers tested:

Set 1:

5’3’

Act79B Forward Primer:

ACTTGTTGCCGTAGGCGTAA

5’3’

Reverse Primer

\*GCGTTGCCGCTCGTTTCC

Set 2:

5’3’

Forward Primer Act79B:

\*CCCGGAAACGCCTGATCC​

5’3’

Reverse Primer Act79B:

CTGGTTTGGGTGGCTTGG

Blue=first set of primers

Purple=second set of primers

Green=target sequence

\*=**Primers that worked**:

Forward Primer (set 2) \*CCCGGAAACGCCTGATCC​

Reverse Primer (set 1) \*GCGTTGCCGCTCGTTTCC

Sequencing of PCR product from DNA line F3-3

F3-3F:

NNNNNNNNNNNNNNNTCNTCANNTGTTACTAGATGACAATTGTTCGAGATGACAGGGNCATGGGCGTGGGGCCGGNGGGG

CGGGACAGAACTTATTTAAATGCAGCTGCTCCTCGGTTCTGTAGCACCACTAATGGATTCTATTTCTGTCCTCTTCAGGT

ATCGCTGACCGTATGCAAAAGGAAATCACCGCACTTGCCCCGTCCACCATCAAGATCAAGATCATCGCCCCGCCAGAGCG

CAAGTACTCCGTCTGGATCGGTGGCTCCATCCTGGCTTCGTTGTCCACCTTCCAGCAGATGTGGATCTCCAAGCAGGAGT

ATGACGAGTCCGGTCCCGGCATCGTCCACCGCAAGTGCTTCTAAGCATCCAAGCCACCCAAACCAGATCAACATCTCCTC

GAGCGCGGCCCTGGTGTTTGTCTCCAGCGTAAGACATCCGACCAGGCGCCGGCGCCAAGGGTGAGGACGCAGTTCAGTGA

AAAGTATCTTTAAATTACATTTAGTTGATGAAGAAGTTTTAACATAGATAGAGAAAACAAGAGAAAGAGACGAGAAAAGA

CCGGAAANNNNNNGGCAACNN

F3-3R:

NNNNNNNNNNNNNNGNNNNNCTATCTATGTNNNNCTTCTTCNTCAACTAAATGTAATTTAAAGATACTTTTCNCTGAACT

GCGTCCTCACCCTTGGCGCCGGCGCCTGGTCGGATGTCTTACGCTGGAGACAAACACCAGGGCCGCGCTCGAGGAGATGT

TGATCTGGTTTGGGTGGCTTGGATGCTTAGAAGCACTTGCGGTGGACGATGCCGGGACCGGACTCGTCATACTCCTGCTT

GGAGATCCACATCTGCTGGAAGGTGGACAACGAAGCCAGGATGGAGCCACCGATCCAGACGGAGTACTTGCGCTCTGGCG

GGGCGATGATCTTGATCTTGATGGTGGACGGGGCAAGTGCGGTGATTTCCTTTTGCATACGGTCAGCGATACCTGAAGAG

GACAGAAATAGAATCCATTAGTGGTGCTACAGAACCGAGGAGCAGCTGCATTTAAATAAGTTCTGTCCCGCCCCGCCGGC

CCCACGCCCATGTCCCTGTCATCTCGAACAATTGTTCATCTAGTAACATGTTGATGAATTCCGCGATATGCAACGCTGGC

TGGGGATCAGGNNN

Original Act79B sequence

***Bold/italicized***=targets

Blue=sequence of PCR product

Red=deleted region

gatccccagccagcgttgcatatcgcggaattcatcaacatgttactagatgaacaattg ttcgagatgacagggacatgggcgtggggccggcggggcgggacagaacttatttaaatg

Cagctg***ccggagcgcataacGAATCACTC***TGATCGCTGTCGCTGTTGGATTTACACGTCG TGAGTGTAGTCTTGTCCGCCCATCCGAAATCCGTAACCCGCATAAGGGATAACCGATCTG TCTGTATCCTTGTAGTGCCGCCCGCACCAAACTAACCAAACATGTGTGACGAAGAAGCAT CAGCCCTGGTCGTAGACAACGGCTCCGGCATGTGCAAGGCCGGATTCGCCGGAGACGACG CGCCCCGCGCCGTATTCCCCTCGATCGTAGGCCGTCCCCGTCACCAGGGCGTGATGGTGG GTATGGGTCAGAAGGACTGCTACGTGGGCGACGAGGCGCAGAGCAAGCGCGGTATCCTGT CGCTGAAGTACCCCATCGAACACGGCATTATCACCAACTGGGATGACATGGAGAAGGTCT GGCACCACACCTTCTACAACGAGCTGCGTGTGGCCCCCGAGGAGCACCCCGTTCTGCTGA CCGAGGCTCCCTTGAACCCCAAGGCCAACCGCGAGAAGATGACCCAGATCATGTTCGAGA CGTTCAACTCCCCGGCCATGTACGTGGCCATCCAGGCCGTGCTCTCCCTGTACGCCTCCG GCCGTACCACCGGTATCGTCCTGGACTCCGGTGACGGTGTCTCCCACACCGTGCCCATCT ATGAGGGCTATGCCCTGCCCCACGCCATCCTTCGTCTAGATCTGGCCGGTCGCGATCTAA CCGACTACCTGATGAAGATCCTCACCGAGCGCGGCTACAGCTTCACCACCACCGCCGAGC GCGAGATTGTGCGCGACATCAAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAGG AGATGGCCACTGCCGCCGCCTCCACCTCCCTGGAGAAGTCTTACGAGCTGCCCGATGGCC AGGTAATCACCATCGGCAACGAGCGCTTCCGCACCCCGGAGGCCCTCTTCCAGCCATCGT TCCTGGGCATGGAGTCCTGCGGCATCCACGAGACCGTCTACCAGTCCATCATGAAGTGCG ACGTGGACATCCGCAAGGATCTGTATGCCAACAATGTGCTGTCTGGCGGCACTACCATGT ATCCAGgtgcgtagtcttaattaattaggaccataaagttcagaggaaattcttccgagg gaatgggatcaaaactatgcgggatacttaaaaaaaaaaacaagtgttactttatacatt catttggcagagaggaaatctttaaataataaagcctaaatatttagctgagctttgtaa taacagttaaaaaaaatcttatggaaagtagtattacaaaaaaaaaaaaaagaattcacc taatgggttatatcctttccctatatctcatattcatgcatgctattattaaaatgtcat

Gtaatgagtaca***ccaaagctcctcggttctgtagcacc***actaatggattctatttctgtc ctcttcagGTATCGCTGACCGTATGCAAAAGGAAATCACCGCACTTGCCCCGTCCACCAT CAAGATCAAGATCATCGCCCCGCCAGAGCGCAAGTACTCCGTCTGGATCGGTGGCTCCAT CCTGGCTTCGTTGTCCACCTTCCAGCAGATGTGGATCTCCAAGCAGGAGTATGACGAGTC CGGTCCCGGCATCGTCCACCGCAAGTGCTTCTAAGCATCCAAGCCACCCAAACCAGATCA ACATCTCCTCGAGCGCGGCCCTGGTGTTTGTCTCCAGCGTAAGACATCCGACCAGGCGCC GGCGCCAAGGGTGAGGACGCAGTTCAGTGAAAAGTATCTTTAAATTACATTTAGTTGATG AAGAAGTTTTAACATAGATAGAGAAAACAAGAGAAAGAGACGAGAAAAGACCGGAAACGA GCGGCAACGCCTTCTTTTTTTATTATTATTGCTATTTTTATTTGATTTGACTCGGAATTT GTACTGCTTGACTCGCTTGCCAGCGCGTAAAGTGGCCAAAACAATCGGTTGTACGTACAA TAAAAACCAATAACCCATGTGaatcgaacccaagtcgaacgtcacaggagcagcaggaat