

The following donor insert was inserted into the PCR2.1 vector (Invitrogen).

BamHI and EcoRI restriction sites

Homology arms (all underlined sequence, regardless of color)

TCOF1 exon 1

Puromycin resistance (alternative Blast^R is below)

GSG linker

P2A ribosomal skipping sequence

Destabilization domain

3XGGGS linker

TCOF1 start codon

GGATCC

CAGCAATTCGGGTGTCTTTATGCCTCAGCTTAGATATCTCTCTGGGGTTCGGTGCTCC
TCCTTGGGGCGCCCATAGGCTGCATCTGTCCCTGACAAGGCACACAAGATATTGAACTA
TAGTTGCTTGGTTACGGACAGTCGTCTGTGAGCTTTGAGGGCAACTGCCATGTATTA
TGCATCTTTTGAATCCCCCTAGCCTAGCCGGGTGCTAGCCCAAGTTAGGTGCTTATTAC
AGATTTGTTTCAGTTAGCGAATGAATCAATGAATAAACTGGCAATTCGTGTCTGGTTCAT
GGGCACCAAAAACCGTGCGAAATGGATAAATGAGCAGGTAATGGCAGTACAAAAGTTCGG
ATCTAGAGAAAGATCCAGATTAGGGAGTTCAGAGATGAGTAAAACGCAGACCCAGCCAT
CAGAGAATTAACAAGGAAGATAAATCTCTTCAAGAAGCCAGCCGGAAGGATTAAGAGAG
AGTCGTCCAAGTCCCGGCCGGCCCCGGGGGCGGGTCCCTGTCTAGCCCCGCATGCTCCG
CGCCAATGGGCGGTATTGTTGATCACGAGTCTCCGCCCCCTTCGCCTTGAGGGCGGGG
CCGGCCACTCCCGGAGAGGGGACTACGTTTCCCGCGCGCCGCGCGGCCGGAGC**GAAAG**
AGGAGCCGGAAGTGGGGCGCGGAGGCTTAAGGGCGCGAGGGAAGTGGCGGGCGGGGAC
TAAGCGGGGCGGTGCAGGTAGCCGGCCGGCCGGGGGTTCGCGGGT

ATGACCGAGTACAAGCCCACGGTGCGCCCTCGCCACCCGCGACGACGTCCCCAGGGCCGT
ACGCACCCCTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGCCACACCGTCGATCCGG
ACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCGCGTCGGGCTC
GACATCGGCAAGGTGTGGGTTCGCGGACGACGGCGCCGCGGTGGCGGTCTGGACCACGCC
GGAGAGCGTCGAAGCGGGGGCGGTGTTTCGCCGAGATCGGCCCGCGCATGGCCGAGTTGA
GCGGTTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCACCGGCC
AAGGAGCCCGCGTGGTTCCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGCAAGGG
TCTGGGCAGCGCCGTGCTGCTCCCCGGAGTGGAGGCGGCCGAGCGCGCCGGGGTGCCCC
CCTTCCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTCACC
GTCACCGCCGACGTCGAGGTGCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAGCC
CGGTGCC

GGATCCGGA

GCCACGAACTTCTCTCTGTTAAAGCAAGCAGGAGACGTGGAAGAAAACCCCGTCCC

ATGGGAGTGCAGGTGGAAACCATCTCCCCAGGAGACGGGCGCACCTTCCCCAAGCGCGG
CCAGACCTGTGTGGTGCACCTACACCGGGATGCTTGAAGATGGAAAGAAAGTCGATTCTT
CCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGGAGGTGATCCGAGGC
TGGGAAGAAGGGGTTGCCAGATGAGTGTGGGTTCAGAGAGCCAAACTGACTATATCTCC
AGATTATGCCTATGGTGCCACTGGGCACCCAGGCATCATCCACCACATGCCACTCTCG
TCTTCGATGTGGAGCTTCTAAAACCGGAA

GGT GGA GGT GGA TCT GGT GGA GGT GGA TCT GGT GGC GGC GGT TCA
ATGGCCGAGGCCAGGAAGCGGCGGGAGCTACTTCCCCTGATCTACCACCATCTGCTGCG
GGCTGGCTATGTGCGTTCGGCGCGGGAAGTGAAGGAGCAGAGCGGCCAGGTAAGCGTTC
GTGGGCCGTGTGCGAGGGCCGCGTGCAAGATGTGGAGATCAGCGGCCCGCGGCCCGCGC
CCCGTCCCCAGGCGACCCGGCAGGCGCCCGGAGCCGGGTCCCAGTGCTCGACGGCGC
GGCCAGGGGTACCGGAGGAGCCGCAATCTCTGCCTTCCCAGTGCAGTTCAGTTCCCCT
GGCCTCACTTTCCTCATCCGGGCAGTGGGTTGGGCCAGAAAGTGTGCTCGCAGGGGCC
GAACTTTGTAATTCCCGCTTCCCTTACCTCCACGCCCTTCCCTGGTAGCGGGTATTTTAA
GTTTCCTAAGTCTCCCGCCACGTGGCTAGGCTCTGCGCGGCCCCCCCTGGGGCAAGGAG
GTTGCTGCGAGTCTCGGGGGTGGATTGCGACCAGCCCTCGGGGGAGCGCCTGGAAAAG
GGACCCTACAGCCTCTTAGTTGAGCTGGAAGTACAGGCCCTTAGCGATTGTCTGTACTGA
GACGGTCAGCTTTGGTACTCGAACTCGTGGTACAGTCATTTCTTACTTGATTAATTCAA
CTGTTAATCCGGTACTCAGGGTGCTTCCCTGGTGTTTGAATGGTGAGCACCATCTCTCAG
CCCTCCTGGAGCTTACACTCTAGTAAGGTAGACAAATCAAAGAATCACAAAAGTGACTA
TATGCTTAGCACCGCAATAAGGGCCGGCCAAAAGGGC
GAATTC

Blasticidin resistance:

ATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTTGAAAGAGCAACGGCTACAAT
CAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACG
GCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGAACTC
GTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGAT
CGGAAATGAGAACAGGGGCATCTTGAAGCCCTGCGGACGGTGCCGACAGGTGCTTCTCG
ATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGTT
GGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGC