



TECHNICAL PROTOCOL

FOR

FRT-PGK-gb2-neo-FRT

**FRT flanked,
Pro- and Eukaryotic
Neomycin Selection Cassette**

(A002)

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1 Eppendorf tubes + manual

1. FRT-PGK-gb2-neo-FRT: PCR template (50 ng/μl, 20μl)
2. This manual

Store tube at -20°C

Please read

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Short Description:

“FRT-PGK-gb2-neo-FRT” template is designed to allow neomycin/kanamycin selection in prokaryotic and eukaryotic cells.

The FRT-PGK-gb2-neo-FRT template encodes the neomycin/kanamycin resistance gene which combines a prokaryotic promoter (gb2) for expression of kanamycin resistance in *E.coli* with a eukaryotic promoter (PGK) for expression of neomycin resistance in mammalian cells.

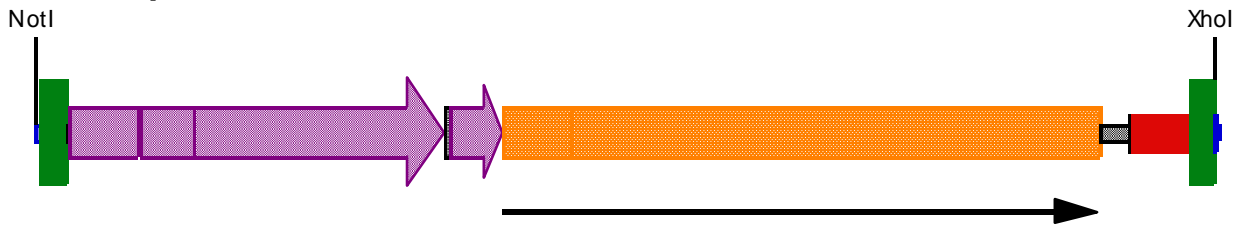
The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the normally used Tn5 promoter. The promoter of the mouse Phosphoglucokinase gene (PGK) is used as eukaryotic promoter. A synthetic polyadenylation signal terminates the kanamycin/neomycin expression. The cassette is flanked by FRT sites for later excision by FLP-recombinase.

Using the provided PCR template one can easily create a FRT-PGK-gb2-neo-FRT cassette flanked by any restriction sites to clone the cassette into the vector of choice. The restriction sites can be introduced by adding the corresponding sequence in the PCR primer. The template can easily be used to generate targeting constructs mediated by a single Red/ET Recombination step.

The “FRT-PGK-gb2-neo-FRT template” is not linear but plasmid based (3446bp in size). Due to its R6K origin it can not replicate in most of the *E. coli* strains. The PCR product can therefore be used directly for downstream applications without any further purification.

At least 20 PCR reactions can be performed using 1µl per reaction as template.

Map: FRT-PGK-gb2-neo-FRT template



NotI

1 AATTAACCTCACTAAAGG GCGGCCGC GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC ATTCTACCGG GTAGGGGAGG
82 CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT ACACAAGTGG CCTCTGGCCT
162 CGCACACATT CCACATCCAC CGGTAGGCGC CAACCGGCTC CGTTCTTTGG TGGCCCTTC GCGCCACCTT CCACTCCTCC
242 CCTAGTCAGG AAGTTCOCCC CGGCCCGCA GCTCGCGTGG TGCAGGACGT GACAAATGGA AGTAGCACGT CTCACTAGTC
322 TCGTGCAGAT GGACAGCACC GCTGAGCAAT GGAAGCGGGT AGGCCTTTGG GGCAGCGGCC AATAGCAGCT TTGCTCCTTC
402 GCTTTCTGGG CTCAGAGGCT GGGAAGGGGT GGGTCCGGGG GCGGGCTCAG GGGCGGGCTC AGGGCGGGG CGGGCGCCCG
482 AAGTCTCTCC GGAGCCCGG CATTCTGCAC GCTTCAAAG CGCACGTCTG CCGCGCTGTT CTCCTCTTCC TCATCTCCGG
562 GCCTTTGCAG C TGCAGC AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT ACGACAAGGT
639 GAGGAACTAA ACC ATG GGA TCG GCC ATT GAA CAA GAT GGA TTG CAC GCA GGT TCT CCG GCC GCT TGG
1 Met Gly Ser Ala Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp
706 GTG GAG AGG CTA TTC GGC TAT GAC TGG GCA CAA CAG ACG ATC GGC TGC TCT GAT GCC GCC GTG TTC
19 Val Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Oys Ser Asp Ala Ala Val Phe
772 CGG CTG TCA GCG CAG GGG CGC CCG GTT CTT TTT GTC AAG ACC GAC CTG TCC GGT GCC CTG AAT GAA
41 Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu
838 CTG CAG GAC GAG GCA GCG CGG CTA TCG TGG CTG GCC ACG ACG GGC GTT CCT TGC GCA GCT GTG CTC
63 Leu Gln Asp Glu Ala Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Oys Ala Ala Val Leu
904 GAC GTT GTC ACT GAA GCG GGA AGG GAC TGG CTG CTA TTG GGC GAA GTG CCG GGG CAG GAT CTC CTG
85 Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu Val Pro Gly Gln Asp Leu Leu
970 TCA TCT CAC CTT GCT CCT GCC GAG AAA GTA TCC ATC ATG GCT GAT GCA ATG CCG CGG CTG CAT ACG
107 Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr
1036 CTT GAT CCG GCT ACC TGC CCA TTC GAC CAC CAA GCG AAA CAT CGC ATC GAG CGA GCA CGT ACT CGG
129 Leu Asp Pro Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala Arg Thr Arg
1102 ATG GAA GCC GGT CTT GTC GAT CAG GAT GAT CTG GAC GAA GAG CAT CAG GGG CTC GCG CCA GCC GAA
151 Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu Glu His Gln Gly Leu Ala Pro Ala Glu
1168 CTG TTC GCC AGG CTC AAG GCG CGC ATG CCC GAC GGC GAG GAT CTC GTC GTG ACC CAT GGC GAT GCC
173 Leu Phe Ala Arg Leu Lys Ala Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala
1234 TGC TTG CCG AAT ATC ATG GTG GAA AAT GGC CGC TTT TCT GGA TTC ATC GAC TGT GGC CGG CTG GGT
195 Oys Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Oys Gly Arg Leu Gly
1300 GTG GCG GAC CGC TAT CAG GAC ATA GCG TTG GCT ACC CGT GAT ATT GCT GAA GAG CTT GGC GGC GAA
217 Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu
1366 TGG GCT GAC CGC TTC CTC GTG CTT TAC GGT ATC GCC GCT CCC GAT TCG CAG CGC ATC GCC TTC TAT
239 Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe Tyr
1432 CGC CTT CTT GAC GAG TTC TTC TGA GCGGGACTCTGGGGTTCGAATAAAGACCGACCAAGCGAC GTC TGA
261 Arg Leu Leu Asp Glu Phe Phe
1501 GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTTAT TTTCATGATC TGTGTGTTGG TTTTGTGTG CGGCGCG GAA
XhoI
1581 GTTCTATTCTCTAGAAAGTATAGGAACTTC C TCGAGCCCTATAGTGAGTCGTATTA

Please take into consideration that the sequence given above does not reflect the complete plasmid but refers to the functional cassette.