TECHNICAL PROTOCOL

FOR

FRT-PGK-gb2-neo-FRT-loxP template

(Version 1.0)

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CONTENTS

1 Eppendorf tubes + manual

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

Store tube at -20°C

Please read

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Short Description:

"PGK-gb2-neo-FRT-loxP" template is designed to allow kanamycin/neomycin selection in prokaryotic and eukaryotic cells, respectively.

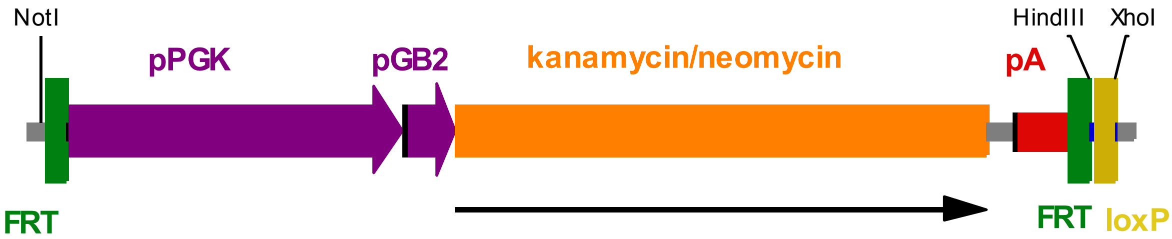
It combines a prokaryotic promoter (gb2) for expression of kanamycin resistance in *E.coli* with a eukaryotic promoter (PGK) for expression of neomycin resistance in mammalian cells. The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the generally used Tn5 promoter. The promoter of the mouse Phosphoglucokinase gene (PGK) is used as the eukaryotic promoter. A synthetic polyadenylation signal terminates the kanamycin/neomycin expression. The cassette is flanked by FRT sites for later excision by Flp-recombinase. An additional single loxP site is located at the 3' end of the cassette. Unique *Not*I and *Xho*I sites flank the cassette for convenient cloning with restriction sites.

Using the provided PCR template one can easily create a PGK-gb2-neo-FRT-loxP cassette flanked by any other 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 Red®/ET® Recombination.

The "PGK-gb2-neo-FRT-loxP template" is not linear but plasmid based (3485 bp in size). Due to its R6K origin the plasmid cannot replicate in most *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 1 µl per reaction as template.

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                          NotI
   1  AATTAACCCTCACTAAAGGGCGGCCGCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC ATTCTACCGG GTAGGGGAGG
  82  CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT ACACAAGTGG
 152  CCTCTGGCTC GCACACATTC CACATCCACC GGTAGGCGCC AACCGGCTCC GTTCTTTGGT GGCCCCTTCG
 222  CGCCACCTTC TACTCCTCCC CTAGTCAGGA AGTTCCCCCC CGCCCCGCAG CTCGCGTCGT GCAGGACGTG
 292  ACAAATGGAA GTAGCACGTC TCACTAGTCT CGTGCAGATG GACAGCACCG CTGAGCAATG GAAGCGGGTA
 362  GGCCTTTGGG GCAGCGGCCA ATAGCAGCTT TGCTCCTTCG CTTTCTGGGC TCAGAGGCTG GGAAGGGGTG
 432  GGTCCGGGGG CGGGCTCAGG GGCGGGCTCA GGGGCGGGGC GGGCGCCCGA AGGTCCTCCG GAGGCCCGGC
 502  ATTCTGCACG CTTCAAAAGC GCACGTCTGC CGCGCTGTTC TCCTCTTCCT CATCTCCGGG CCTTTCGACC TGCAGC
 578  AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT ACGACAAGGT GAGGAACTAA ACC ATG
                                                                                   1 Met
 654  GGA TCG GCC ATT GAA CAA GAT GGA TTG CAC GCA GGT TCT CCG GCC GCT TGG GTG GAG AGG CTA
   2  Gly Ser Ala Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val Glu Arg Leu
 717  TTC GGC TAT GAC TGG GCA CAA CAG ACA ATC GGC TGC TCT GAT GCC GCC GTG TTC CGG CTG TCA
  23  Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser Asp Ala Ala Val Phe Arg Leu Ser
 780  GCG CAG GGG CGC CCG GTT CTT TTT GTC AAG ACC GAC CTG TCC GGT GCC CTG AAT GAA CTG CAG
  44  Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln
 843  GAC GAG GCA GCG CGG CTA TCG TGG CTG GCC ACG ACG GGC GTT CCT TGC GCA GCT GTG CTC GAC
  65  Asp Glu Ala Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val Leu Asp
 906  GTT GTC ACT GAA GCG GGA AGG GAC TGG CTG CTA TTG GGC GAA GTG CCG GGG CAG GAT CTC CTG
  86  Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu Val Pro Gly Gln Asp Leu Leu
 969  TCA TCT CAC CTT GCT CCT GCC GAG AAA GTA TCC ATC ATG GCT GAT GCA ATG CGG CGG CTG CAT
 107  Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His
1032  ACG CTT GAT CCG GCT ACC TGC CCA TTC GAC CAC CAA GCG AAA CAT CGC ATC GAG CGA GCA CGT
 128  Thr Leu Asp Pro Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala Arg
1095  ACT CGG ATG GAA GCC GGT CTT GTC GAT CAG GAT GAT CTG GAC GAA GAG CAT CAG GGG CTC GCG
 149  Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu Glu His Gln Gly Leu Ala
1158  CCA GCC GAA CTG TTC GCC AGG CTC AAG GCG CGC ATG CCC GAC GGC GAG GAT CTC GTG GTG ACC
 170  Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr
1221  CAT GGC GAT GCC TGC TTG CCG AAT ATC ATG GTG GAA AAT GGC CGC TTT TCT GGA TTC ATC GAC
 191  His Gly Asp Ala Cys Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp
1284  TGT GGC CGG CTG GGT GTG GCG GAC CGC TAT CAG GAC ATA GCG TTG GCT ACC CGT GAT ATT GCT
 212  Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala
1347  GAA GAG CTT GGC GGC GAA TGG GCT GAC CGC TTC CTC GTG CTT TAC GGT ATC GCC GCT CCC GAT
 233  Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp
1410  TCG CAG CGC ATC GCC TTC TAT CGC CTT CTT GAC GAG TTC TTC TGA GCGGGACTCTGGGGTTCGAATAAAGA
 254  Ser Gln Arg Ile Ala Phe Tyr Arg Leu Leu Asp Glu Phe Phe •••
1481  CCGACCAAGCGAC GTC TGA GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTTAT TTTCATGATC
                                                                    HindIII
1550  TGTGTGTTGG TTTTTGTGTG CGGCGCG GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAAGCTT ATAACTTCGT
                                 XhoI
1627  ATAGCATACA TTATACGAAG TTAT CTCGAGCCCTATAGTGAGTCGTATTA
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