

TECHNICAL PROTOCOL  
FOR  
**FRT-PGK-gb2-neo-FRT-  
loxP**

FRT flanked,  
Pro- and Eukaryotic Neomycin  
Selection Cassette plus loxP site

(A004)

## CONTENTS

### 1 Eppendorf tubes + manual

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

**Store tube at -20°C**

### Please read

The products listed in this manual are for research purposes only. They are not designed for diagnostic or therapeutic use in humans, animals or plants. The Red<sup>®</sup>/ET<sup>®</sup> recombination technology is the intellectual property of Gene Bridges GmbH.

### Conditions of use

3.1 Purchaser will not manufacture, copy, reproduce, transmit, distribute, sell, lease, transfer, or improve upon the MATERIALS without prior written consent from GENE BRIDGES.

3.2 All MATERIALS related to the technology must be purchased from GENE BRIDGES or its authorized distributors. Use of any of the stated products from a source other than GENE BRIDGES will exempt GENE BRIDGES from any and all liabilities and warranties.

3.3 All MATERIALS purchased by research organizations, universities and other non-profit organizations may not be used for any commercial purpose. These MATERIALS are to be used for research purposes only. The MATERIALS may not be used to provide a commercial or non-commercial service, of any kind.

3.4 The purchase of MATERIALS by a private consumer is neither intended nor permitted.

## Short Description:

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

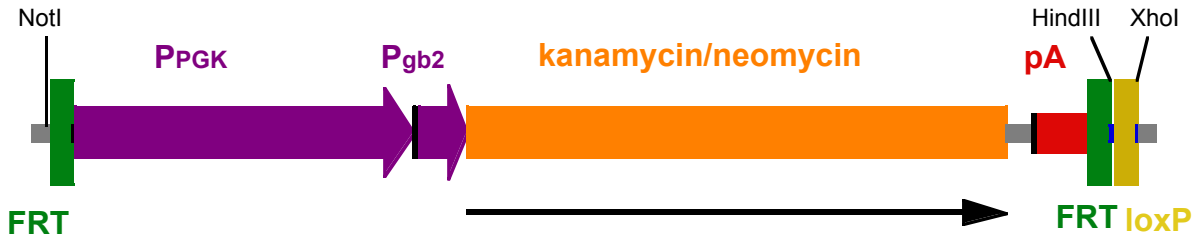
It combines a prokaryotic promoter (gb2) for expression of kanamycin resistance in *E.coli* with a eukaryotic promoter (PGK) for expression of neomycin resistance in mammalian cells. The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency 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 *NotI* and *XhoI* sites flank the cassette for convenient cloning with restriction sites.

Using the provided PCR template one can easily create a FRT-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<sup>®</sup>/ET<sup>®</sup> Recombination.

The “FRT-PGK-gb2-neo-FRT-loxP cassette” 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.



NotI

```

1  AATTAACCCCTCACTAAAGGCGGCGCCGGAAGTTCCTATTCTCTAGAAAAGTATAGGAACCTTC ATTCCTACCGG GTAGGGGAGG
82  CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT ACACAAGTGG
152 CCTCTGGCTC GCACACATTC CACATCCACC GGTAGCGCC AACC GGCTCC GTTCTTTGGT GGCCCTTCG
222 CGCCACCTTC TACTCCTCCC CTAGTCAGGA AGTTC CCCCC CGCCCCGAG CTGCGTCTGT GCAGGACGTG
292 ACAAAATGAA GTAGCACGTC TACTAGTCT CGTGCAGATG GACAGCACCG CTGAGCAATG GAAGCGGGTA
362 GGCCTTTGGG GCAGCGGCCA ATAGCAGCTT TGCTCCTTCG CTTTCTGGGC TCAGAGGCTG GGAAGGGGTG
432 GGTCCGGGGG CGGGCTCAGG GGCGGGCTCA GGGCGGGGC GGGCGCCCGA AGTCTCTCCG GAGGCCCGGC
502 ATTCTGCACG CTCAAAAAGC GCACGTCTGC CGCGCTGTTC TCTCTTCTCT 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 GTC 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 GCGGACTCTGGGGTTCAATAAAGA
    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 TGTGTGTGG TTTTGTGTG CGGCGG GAAGTTCCTATTCTCTAGAAAAGTATAGGAACCTTCAAGCTT ATAACTTCGT
                                                                                               XhoI
1627 ATAGCATACA TTATACGAAG TTAT CTCGAGCCCTATAGTGAGTCGTATTA
    
```

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