

TECHNICAL PROTOCOL  
FOR  
**PGK-gb2-neo**  
Pro- and Eukaryotic  
Neomycin Selection Cassette  
(A001)

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### 1 Eppendorf tubes + manual

1. PGK-gb2-neo: 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.

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## Short Description:

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

The PGK-gb2-neo 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.

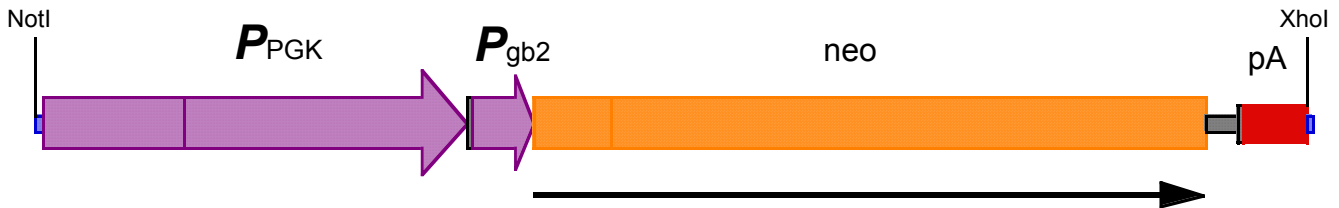
The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the normally used Tn5 promoter. The promoter of the mouse Phosphoglucokinase gene (PGK) is used as eukaryotic promoter. A synthetic polyadenylation signal terminates the kanamycin/neomycin expression.

Using the provided PCR template one can easily create a PGK-gb2-neo 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 “PGK-gb2-neo template” is not linear but plasmid based (3377bp 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: PGK-gb2- neo-template



NotI

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1  GCGGCCGC ATTCTACCGG GTAGGGGAGG CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC
79  ACTTGGCGCT ACACAAGTGG CCTCTGGCTC GCACACATTC CACATCCACC GGTAGGCGCC AACCGGCTCC GTTCTTTGGT
159 GGGCCCTTCG CGCCACCTTC CACTCCTCCC CTAGTCAGGA AGTTCCCCCC CGCCCCGAG CTGCGTCTGT GCAGGACGTG
239 ACAAATGGAA GTAGCAGTTC TACTAGTCT CTGTCAGATG GACAGCACCG CTGAGCAATG GAAGCGGGTA GGCCTTTGGG
319 GCAGCGGCCA ATAGCAGCTT TGCTCCTTCG CTTTCTGGGC TCAGAGGCTG GGAAGGGGTG GGTCCGGGGG CGGGCTCAGG
399 GCGGGGCTCA GGGGCGGGG GGGCGCCGA AGGTCTCCG GAGGCCCGG ATTCTGCACG CTTCAAAGC GCACGTCTGC
479 CGCGCTGTTT TCCTCTTCCT CATCTCCGGG CTTTTCGACC TGCAGC AGCACGTGTT GACAATTAAT CATCGGCATA
555 GTATATCGGC ATAGTATAAT ACGACAAGGT GAGGAACTAA ACC ATG GGA TCG GCC ATT GAA CAA GAT GGA TTG
                               1 Met Gly Ser Ala Ile Glu Gln Asp Gly Leu
628 CAC GCA GGT TCT CCG GCC GCT TGG GTG GAG AGG CTA TTC GGC TAT GAC TGG GCA CAA CAG ACG ATC
11 His Ala Gly Ser Pro Ala Ala Trp Val Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile
694 GGC TGC TCT GAT GCC GCC GTG TTC CGG CTG TCA GCG CAG GGG CGC CCG GTT CTT TTT GTC AAG ACC
33 Gly Cys Ser Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr
760 GAC CTG TCC GGT GCC CTG AAT GAA CTG CAG GAC GAG GCA GCG CGG CTA TCG TGG CTG GCC ACG ACG
55 Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala Ala Arg Leu Ser Trp Leu Ala Thr Thr
826 GGC GTT CCT TGC GCA GCT GTG CTC GAC GTT GTC ACT GAA GCG GGA AGG GAC TGG CTG CTA TTG GGC
77 Gly Val Pro Cys Ala Ala Val Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly
892 GAA GTG CCG GGG CAG GAT CTC CTG TCA TCT CAC CTT GCT CCT GCC GAG AAA GTA TCC ATC ATG GCT
99 Glu Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met Ala
958 GAT GCA ATG CCG CGG CTG CAT ACG CTT GAT CCG GCT ACC TGC CCA TTC GAC CAC CAA GCG AAA CAT
121 Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro Ala Thr Cys Pro Phe Asp His Gln Ala Lys His
1024 CGC ATC GAG CGA GCA CGT ACT CGG ATG GAA GCC GGT CTT GTC GAT CAG GAT GAT CTG GAC GAA GAG
143 Arg Ile Glu Arg Ala Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu Glu
1090 CAT CAG GGG CTC GCG CCA GCC GAA CTG TTC GCC AGG CTC AAG GCG CGC ATG CCC GAC GGC GAG GAT
165 His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala Arg Met Pro Asp Gly Glu Asp
1156 CTC GTC GTG ACC CAT GGC GAT GCC TGC TTG CCG AAT ATC ATG GTG GAA AAT GGC CGC TTT TCT GGA
187 Leu Val Val Thr His Gly Asp Ala Cys Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly
1222 TTC ATC GAC TGT GGC CGG CTG GGT GTG GCG GAC CGC TAT CAG GAC ATA GCG TTG GCT ACC CGT GAT
209 Phe Ile Asp Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp
1288 ATT GCT GAA GAG CTT GGC GGC GAA TGG GCT GAC CGC TTC CTC GTG CTT TAC GGT ATC GCC GCT CCC
231 Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro
1354 GAT TCG CAG CGC ATC GCC TTC TAT CGC CTT CTT GAC GAG TTC TTC TGA GCGGGACTCTGGGGTTCAATAAA
253 Asp Ser Gln Arg Ile Ala Phe Tyr Arg Leu Leu Asp Glu Phe Phe
1426 GACCGACCAAGCGAC GTC TGA GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTTAT TTTCATGATC
                               XhoI
1497 TGTGTGTTGG TTTTGTGTG CGGCGCG CTCGAG
    
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Please take into consideration that the sequence given above does not reflect the complete plasmid but refers to the functional cassette.