THE RECOMBINEERING COMPANY
GENE BRIDGES

TECHNICAL PROTOCOL

FOR

loxP-PGK-gb2-neo-loxP

loxP flanked,
Pro- and Eukaryotic
Neomycin Selection Cassette

(A003)

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CONTENTS

1 Eppendorf tubes + manual

1. loxP-PGK-gb2-neo-loxP: PCR template (50 ng/µl, 20µl)
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®/ET® recombination technology is the intellectual property of Gene Bridges GmbH.

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Short Description:

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

The loxP-PGK-gb2-neo-loxP 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.

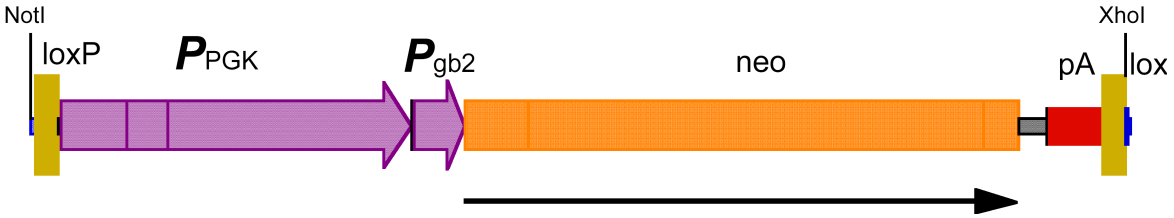
The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the normally used Tn5 promoter. The promoter of the mouse Phosphoglucokinase gene (PGK) is used as eukaryotic promoter. A synthetic polyadenylation signal terminates the kanamycin/neomycin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

Using the provided PCR template one can easily create a loxP-PGK-gb2-neo-loxP 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 “loxP-PGK-gb2-neo-loxP 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: loxP-PGK-gb2-loxP cassette

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      NotI
   1  GCGGCCGCAT AACTTCGTAT AGCATACATT ATACGAAGTT ATATTCTACC GGGTAGGGGA GGCGCTTTTC
  71  CCAAGGCAGT CTGGAGCATG CGCTTTAGCA GCCCCGCTGG GCACTTGGCG CTACACAAGT GGCCTCTGGC
 141  CTCGCACACA TTCCACATCC ACCGGTAGGC GCCAACCGGC TCCGTTCTTT GGTGGCCCCG TCGCGCCACC
 211  TTCTACTCCT CCCCTAGTCA GGAAGTTCCC CCCCGCCCCG CAGCTCGCGT CGTGCAGGAC GTGACAAATG
 281  GAAGTAGCAC GTCTCACTAG TCTCGTGCAG ATGGACAGCA CCGCTGAGCA ATGGAAGCGG GTAGGCCTTT
 351  GGGGCAGCGG CCAATAGCAG CTTTGCTCCT TCGCTTTCTG GGCTCAGAGG CTGGGAAGGG GTGGGTCCGG
 421  GGGCGGGCTC AGGGGCGGGC TCAGGGGCGG GGCGGGCGCC CGAAGGTCCT CCGGAGGCCC GGCATTCTGC
 491  ACGCTTCAAA AGCGCACGTC TGCCGCGCTG TTCTCCTCTT CCTCATCTCC GGGCCTTTCG ACC TGCAGC
 560  AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT ACGACAAGGT GAGGAACTAA
 630  ACC ATG GGA TCG GCC ATT GAA CAA GAT GGA TTG CAC GCA GGT TCT CCG GCC GCT TGG GTG
        1 Met Gly Ser Ala Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val
 690  GAG AGG CTA TTC GGC TAT GAC TGG GCA CAA CAG ACG ATC GGC TGC TCT GAT GCC GCC GTG
  20 Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser Asp Ala Ala Val
 750  TTC CGG CTG TCA GCG CAG GGG CGC CCG GTT CTT TTT GTC AAG ACC GAC CTG TCC GGT GCC
  40 Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr Asp Leu Ser Gly Ala
 810  CTG AAT GAA CTG CAG GAC GAG GCA GCG CGG CTA TCG TGG CTG GCC ACG ACG GGC GTT CCT
  60 Leu Asn Glu Leu Gln Asp Glu Ala Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro
 870  TGC GCA GCT GTG CTC GAC GTT GTC ACT GAA GCG GGA AGG GAC TGG CTG CTA TTG GGC GAA
  80 Cys Ala Ala Val Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
 930  GTG CCG GGG CAG GAT CTC CTG TCA TCT CAC CTT GCT CCT GCC GAG AAA GTA TCC ATC ATG
 100 Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met
 990  GCT GAT GCA ATG CGG CGG CTG CAT ACG CTT GAT CCG GCT ACC TGC CCA TTC GAC CAC CAA
 120 Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro Ala Thr Cys Pro Phe Asp His Gln
1050  GCG AAA CAT CGC ATC GAG CGA GCA CGT ACT CGG ATG GAA GCC GGT CTT GTC GAT CAG GAT
 140 Ala Lys His Arg Ile Glu Arg Ala Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp
1110  GAT CTG GAC GAA GAG CAT CAG GGG CTC GCG CCA GCC GAA CTG TTC GCC AGG CTC AAG GCG
 160 Asp Leu Asp Glu Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala
1170  CGC ATG CCC GAC GGC GAG GAT CTC GTC GTG ACC CAT GGC GAT GCC TGC TTG CCG AAT ATC
 180 Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys Leu Pro Asn Ile
1230  ATG GTG GAA AAT GGC CGC TTT TCT GGA TTC ATC GAC TGT GGC CGG CTG GGT GTG GCG GAC
 200 Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu Gly Val Ala Asp
1290  CGC TAT CAG GAC ATA GCG TTG GCT ACC CGT GAT ATT GCT GAA GAG CTT GGC GGC GAA TGG
 220 Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp
1350  GCT GAC CGC TTC CTC GTG CTT TAC GGT ATC GCC GCC CCC GAT TCG CAG CGC ATC GCC TTC
 240 Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe
1410  TAT CGC CTT CTT GAC GAG TTC TTC TGA GCGGGACTCTGGGGTTCGAATAAAGACCGACCAAGCGAC GTC
 260 Tyr Arg Leu Leu Asp Glu Phe Phe
1479  TGA GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTTAT TTTCATGATC TGTGTGTTGG
                                                                      XhoI
1542  TTTTTGTGTG CGGCGCGATA ACTTCGTATA GCATACATTA TACGAAGTTA TC TCGAG
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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.