

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

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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-neo-loxP



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1  AATTAACCCTCACTAAAGG GCGGCCGCAT AACTTCGTAT AGCATAACATT ATACGAAGTT ATATTCTACC
70  GGTAGGGGA GGCGCTTTTC CCAAGGCAGT CTGAGCATG CGCTTTAGCA GCCCCGCTGG GCACCTGGCG
140 CTACACAAGT GGCCTCTGGC CTCGCACACA TTCCACATCC ACCGGTAGGC GCCAACCGGC TCCGTTCTTT
210 GGTGGCCCCG TCGCGCCACC TTCTACTCCT CCCCTAGTCA GGAAGTTCCC CCCGCCCCG CAGCTCGCGT
280 CGTGCAGGAC GTGACAAATG GAAGTAGCAC GTCTACTAG TCTCGTGAG ATGGACAGCA CCGCTGAGCA
350 ATGGAAGCGG GTAGGCCTTT GGGGCAGCGG CCAATAGCAG CTTTGCTCCT TCGCTTTCTG GGCTCAGAGG
420 CTGGAAGGG GTGGGTCCGG GGGCGGGCTC AGGGGCGGGC TCAGGGGCGG GCGGGGCGCC CGAAGGTCTT
490 CCGGAGGCC GGCATTCTGC ACGCTTCAA AGCGCACGTC TGCCGCGCTG TTCTCCTCTT CCTCATCTCC
560 GGGCCTTTTC ACC TGCAGC AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT
629 ACGACAAGGT GAGGAATAA ACC ATG GGA TCG GCC ATT GAA CAA GAT GGA TTG CAC GCA GGT TCT
      1 Met Gly Ser Ala Ile Glu Gln Asp Gly Leu His Ala Gly Ser
694 CCG GCC GCT TGG GTG GAG AGG CTA TTC GGC TAT GAC TGG GCA CAA CAG ACG ATC GGC TGC TCT
      15 Pro Ala Ala Trp Val Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser
757 GAT GCC GCC GTG TTC CGG CTG TCA GCG CAG GGG CGC CCG GTT CTT TTT GTC AAG ACC GAC CTG
      36 Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr Asp Leu
820 TCC GGT GCC CTG AAT GAA CTG CAG GAC GAG GCA GCG CGG CTA TCG TGG CTG GCC ACG ACG GGC
      57 Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly
883 GTT CCT TGC GCA GCT GTG CTC GAC GTT GTC ACT GAA GCG GGA AGG GAC TGG CTG CTA TTG GGC
      78 Val Pro Cys Ala Ala Val Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly
946 GAA GTG CCG GGG CAG GAT CTC CTG TCA TCT CAC CTT GCT CCT GCC GAG AAA GTA TCC ATC ATG
      99 Glu Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met
1009 GCT GAT GCA ATG CGG CGG CTG CAT ACG CTT GAT CCG GCT ACC TGC CCA TTC GAC CAC CAA GCG
      120 Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro Ala Thr Cys Pro Phe Asp His Gln Ala
1072 AAA CAT CGC ATC GAG CGA GCA CGT ACT CGG ATG GAA GCC GGT CTT GTC GAT CAG GAT GAT CTG
      141 Lys His Arg Ile Glu Arg Ala Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu
1135 GAC GAA GAG CAT CAG GGG CTC GCG CCA GCC GAA CTG TTC GCC AGG CTC AAG GCG CGC ATG CCC
      162 Asp Glu Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala Arg Met Pro
1198 GAC GGC GAG GAT CTC GTC GTG ACC CAT GGC GAT GCC TGC TTG CCG AAT ATC ATG GTG GAA AAT
      183 Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys Leu Pro Asn Ile Met Val Glu Asn
1261 GGC CGC TTT TCT GGA TTC ATC GAC TGT GGC CGG CTG GGT GTG GCG GAC CGC TAT CAG GAC ATA
      204 Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile
1324 GCG TTG GCT ACC CGT GAT ATT GCT GAA GAG CTT GGC GGC GAA TGG GCT GAC CGC TTC CTC GTG
      225 Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe Leu Val
1387 CTT TAC GGT ATC GCC GCC CCC GAT TCG CAG CGC ATC GCC TTC TAT CGC CTT CTT GAC GAG TTC
      246 Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe Tyr Arg Leu Leu Asp Glu Phe
1450 TTC TGA GCGGACTCTGGGGTTTCGAATAAAGACCGACCAAGCGAC GTC TGA GAGCTCCCTG GCGAATTCGG
      267 Phe
1521 TACCAATAAA AGAGCTTTAT TTTCATGATC TGTGTGTGG TTTTGTGTG CGGCGCGATA ACTTTCGTATA
      XhoI
1591 GCATACAITTA TACGAAGTTA TC TCGAGCCCTATAGTGAGTCGTATTA
    
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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.