

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

FRT flanked,
Pro- and Eukaryotic Neomycin
Selection Cassette plus loxP site
2nd version

(A005)

CONTENTS

1 Eppendorf tubes + manual

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

Store tube at -20°C

Please read

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Short Description:

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

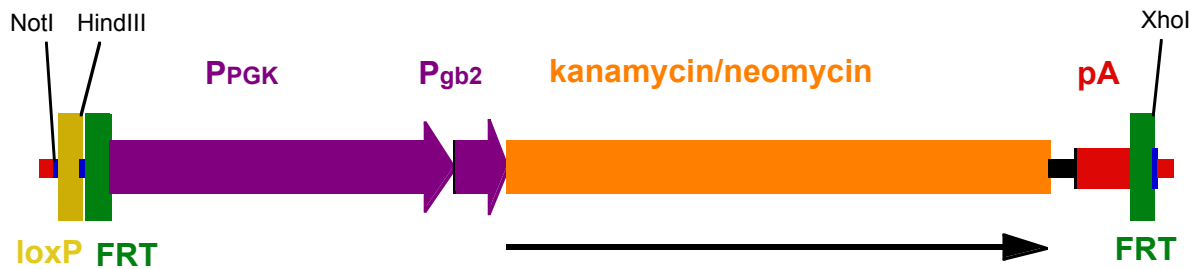
It combines a prokaryotic promoter (gb2) for expression of kanamycin resistance in *E.coli* with a eukaryotic promoter (PGK) for expression of neomycin resistance in mammalian cells. The prokaryotic promoter gb2 is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency 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 5' 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 loxP-FRT-PGK-gb2-neo-FRT 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 “loxP-FRT-PGK-gb2-neo-FRT 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 HindIII

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1  AATTAACCCCTACTAAAGG CCGGCCGCAT AACTTCGTAT AATGTATGCT ATACGAAGTT AT AAGCTTGAAGTTCCT
77  ATTCTCTAGAAAAGTATAGGAACTTC ATTCTACCGG GTAGGGGAGG CGCTTTTCCC AAGGCAGTCT GGAGCATGCG
152 CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT ACACAAGTGG CCTCTGGCCT CGCACACATT CCACATCCAC
222 CGGTAGGCGC CAACCGGCTC CGTTCCTTGG TGGCCCTTC GCGCCACCTT CCACTCTCC CCTAGTCAGG
292 AAGTTCCCCC CCGCCCCGCA GCTCGCGTCG TGCAGGACGT GACAAATGGA AGTAGCACGT CTCACTAGTC
362 TCGTGCAGAT GGACAGCACC GCTGAGCAAT GGAAGCGGT AGGCCTTTGG GGCAGCGGCC AATAGCAGCT
432 TTGCTCCTTC GCTTTCGGG CTCAGAGGCT GGAAGGGGT GGGTCCGGG GCGGGCTCAG GGGCGGGCTC
502 AGGGGCGGGG CGGGCGCCG AAGGTCCTCC GGAGGCCCG CATTCTGCAC GCTTCAAAG CGCACGCTG
572 CCGCGTGTT TCCTCTTCC TCATCTCCG GCCTTTCGAC CTGCAGCAGC ACGTGTGAC AATTAATCAT
642 CCGCATAGTA TATCGGCATA GTATAATACG ACAAGGTGAG GAACTAAACC ATG GGA TCG GCC ATT GAA CAA
                                     1 Met Gly Ser Ala Ile Glu Gln
713 GAT GGA TTG CAC GCA GGT TCT CCG GCC GCT TGG GTG GAG AGG CTA TTC GGC TAT GAC TGG GCA
8 Met Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val Glu Arg Leu Phe Gly Tyr Asp Trp Ala
776 CAA CAG AC G ATC GGC TGC TCT GAT GCC GCC GTG TTC CCG CTG TCA GCG CAG GGG CGC CCG
29 Met Gln Gln Thr Ile Gly Cys Ser Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro
836 GTT CTT TTT GTC AAG ACC GAC CTG TCC GGT GCC CTG AAT GAA CTG CAG GAC GAG GCA GCG CGG
49 Met Val Leu Phe Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala Ala Arg
899 CTA TCG TGG CTG GCC ACG ACG GGC GTT CCT TGC GCA GCT GTG CTC GAC GTT GTC ACT GAA GCG
70 Met Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val Leu Asp Val Val Thr Glu Ala
962 GGA AGG GAC TGG CTG CTA TTG GGC GAA GTG CCG GGG CAG GAT CTC CTG TCA TCT CAC CTT GCT
91 Met Gly Arg Asp Trp Leu Leu Leu Gly Glu Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala
1025 CCT GCC GAG AAA GTA TCC ATC ATG GCT GAT GCA ATG CGG CGG CTG CAT ACG CTT GAT CCG GCT
112 Met Pro Ala Glu Lys Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro Ala
1088 ACC TGC CCA TTC GAC CAC CAA GCG AAA CAT CGC ATC GAG CGA GCA CGT ACT CGG ATG GAA GCC
133 Met Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala Arg Thr Arg Met Glu Ala
1151 GGT CTT GTC GAT CAG GAT GAT CTG GAC GAA GAG CAT CAG GGG CTC GCG CCA GCC GAA CTG TTC
154 Met Gly Leu Val Asp Gln Asp Asp Leu Asp Glu Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe
1214 GCC AGG CTC AAG GCG CGC ATG CCC GAC GGC GAG GAT CTC GTC GTG ACC CAT GGC GAT GCC TGC
175 Met Ala Arg Leu Lys Ala Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys
1277 TTG CCG AAT ATC ATG GTG GAA AAT GGC CGC TTT TCT GGA TTC ATC GAC TGT GGC CGG CTG GGT
196 Met Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu Gly
1340 GTG GCG GAC CGC TAT CAG GAC ATA GCG TTG GCT ACC CGT GAT ATT GCT GAA GAG CTT GGC GGC
217 Met Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly
1403 GAA TGG GCT GAC CGC TTC CTC GTG CTT TAC GGT ATC GCC GCT CCC GAT TCG CAG CGC ATC GCC
238 Met Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala
1466 TTC TAT CGC CTT CTT GAC GAG TTC TTC TGA GCGGGACTCTGGGGTTCGAATAAAGACCGACCAAGCGAC GTC
259 Met Phe Tyr Arg Leu Leu Asp Glu Phe Phe
1538 TGA GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTAT TTTCATGATC TGTGTGTTGG TTTTGTGTG
                                     XhoI
1611 CCGCGCG GAAGTTCCTATTCTCTAGAAAAGTATAGGAACTTC C 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.