TECHNICAL PROTOCOL

FOR

FRT-PGK-gb2-hygro-FRT

FRT flanked,
Pro- and Eukaryotic
Hygromycin Selection Cassette

(A010)

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CONTENTS

1 Eppendorf tubes + manual

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

Store tube at -20°C

Please read

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Short Description:

“FRT-PGK-gb2-hygro-FRT” template is designed to allow hygromycin selection in prokaryotic and eukaryotic cells.

The FRT-PGK-gb2-hygro-FRT template encodes the hygromycin resistance gene which combines a prokaryotic promoter (gb2) for expression in *E.coli* with a eukaryotic promoter (PGK) for expression 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 hygromycin expression. The cassette is flanked by FRT sites for later excision by FLP-recombinase.

Using the provided PCR template one can easily create a FRT-PGK-gb2-hygro-FRT 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 “FRT-PGK-gb2-hygro-FRT template” is not linear but plasmid based (3643bp 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.

We recommend growing the recombined cells between 4 and 24h under non-selective conditions before streaking the culture on plates conditioned with 100 µg/ml hygromycin plus the antibiotics for the selective marker of the target vector (e.g. 15 µg/ml chloramphenicol for BAC clones).

At least 20 PCR reactions can be performed using 1µl per reaction as template.

Map: FRT-PGK-gb2-hygro-FRT cassette


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                                                                NotI
   1 AATTAACCCTCACTAAAGGGCGGCCGCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCATTCTACCGGGTAGGGGAGG
  82 CGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACA
 170 TTCCACATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCGTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTC
 258 CCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTG
 346 AGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGGTGG
 434 GTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTTCAAAAG
 522 CGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCAGCAGCACGTGTTGACAATTAATCATCGGCATAG
 610 TATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACC ATG AAA AAG CCT GAA CTC ACC GCG ACG TCT GTC
                                               1 Met Lys Lys Pro Glu Leu Thr Ala Thr Ser Val
 685 GAG AAG TTT CTG ATC GAA AAG TTC GAC AGC GTC TCC GAC CTG ATG CAG CTC TCG GAG GGC GAA GAA
  12 Glu Lys Phe Leu Ile Glu Lys Phe Asp Ser Val Ser Asp Leu Met Gln Leu Ser Glu Gly Glu Glu
 751 TCT CGT GCT TTC AGC TTC GAT GTA GGA GGG CGT GGA TAC GTC CTG CGG GTA AAT AGC TGC GCC GAT
  34 Ser Arg Ala Phe Ser Phe Asp Val Gly Gly Arg Gly Tyr Val Leu Arg Val Asn Ser Cys Ala Asp
 817 GGT TTC TAC AAA GAT CGT TAT GTT TAT CGG CAC TTT GCA TCG GCC GCG CTC CCG ATT CCG GAA GTG
  56 Gly Phe Tyr Lys Asp Arg Tyr Val Tyr Arg His Phe Ala Ser Ala Ala Leu Pro Ile Pro Glu Val
 883 CTT GAC ATT GGG GAA TTC AGC GAG AGC CTG ACC TAT TGC ATC TCC CGC CGT GCA CAG GGT GTC ACG
  78 Leu Asp Ile Gly Glu Phe Ser Glu Ser Leu Thr Tyr Cys Ile Ser Arg Arg Ala Gln Gly Val Thr
 949 TTG CAA GAC CTG CCT GAA ACC GAA CTG CCC GCT GTT CTG CAG CCG GTC GCG GAG GCC ATG GAT GCG
 100 Leu Gln Asp Leu Pro Glu Thr Glu Leu Pro Ala Val Leu Gln Pro Val Ala Glu Ala Met Asp Ala
1015 ATC GCT GCG GCC GAT CTT AGC CAG ACG AGC GGG TTC GGC CCA TTC GGA CCG CAA GGA ATC GGT CAA
 122 Ile Ala Ala Ala Asp Leu Ser Gln Thr Ser Gly Phe Gly Pro Phe Gly Pro Gln Gly Ile Gly Gln
1081 TAC ACT ACA TGG CGT GAT TTC ATA TGC GCG ATT GCT GAT CCC CAT GTG TAT CAC TGG CAA ACT GTG
 144 Tyr Thr Thr Trp Arg Asp Phe Ile Cys Ala Ile Ala Asp Pro His Val Tyr His Trp Gln Thr Val
1147 ATG GAC GAC ACC GTC AGT GCG TCC GTC GCG CAG GCT CTC GAT GAG CTG ATG CTT TGG GCC GAG GAC
 166 Met Asp Asp Thr Val Ser Ala Ser Val Ala Gln Ala Leu Asp Glu Leu Met Leu Trp Ala Glu Asp
1213 TGC CCC GAA GTC CGG CAC CTC GTG CAC GCG GAT TTC GGC TCC AAC AAT GTC CTG ACG GAC AAT GGC
 188 Cys Pro Glu Val Arg His Leu Val His Ala Asp Phe Gly Ser Asn Asn Val Leu Thr Asp Asn Gly
1279 CGC ATA ACA GCG GTC ATT GAC TGG AGC GAG GCG ATG TTC GGG GAT TCC CAA TAC GAG GTC GCC AAC
 210 Arg Ile Thr Ala Val Ile Asp Trp Ser Glu Ala Met Phe Gly Asp Ser Gln Tyr Glu Val Ala Asn
1345 ATC TTC TTC TGG AGG CCG TGG TTG GCT TGT ATG GAG CAG CAG ACG CGC TAC TTC GAG CGG AGG CAT
 232 Ile Phe Phe Trp Arg Pro Trp Leu Ala Cys Met Glu Gln Gln Thr Arg Tyr Phe Glu Arg Arg His
1411 CCG GAG CTT GCA GGA TCG CCG CGG CTC CGG GCG TAT ATG CTC CGC ATT GGT CTT GAC CAA CTC TAT
 254 Pro Glu Leu Ala Gly Ser Pro Arg Leu Arg Ala Tyr Met Leu Arg Ile Gly Leu Asp Gln Leu Tyr
1477 CAG AGC TTG GTT GAC GGC AAT TTC GAT GAT GCA GCT TGG GCG CAG GGT CGA TGC GAC GCA ATC GTC
 276 Gln Ser Leu Val Asp Gly Asn Phe Asp Asp Ala Ala Trp Ala Gln Gly Arg Cys Asp Ala Ile Val
1543 CGA TCC GGA GCC GGG ACT GTC GGG CGT ACA CAA ATC GCC CGC AGA AGC GCG GCC GTC TGG ACC GAT
 298 Arg Ser Gly Ala Gly Thr Val Gly Arg Thr Gln Ile Ala Arg Arg Ser Ala Ala Val Trp Thr Asp
1609 GGC TGT GTA GAA GTA CTT GCC GAT AGT GGA AAC CGA CGC CCC AGC ACT CGT CCG AGG GCA AAG GAA
 320 Gly Cys Val Glu Val Leu Ala Asp Ser Gly Asn Arg Arg Pro Ser Thr Arg Pro Arg Ala Lys Glu
1675 TAG GTTTCCCTGCCACAGTCTGAGAGCTCCCTGGCGAATTCGGTACCAATAAAAGAGCTTTATTTTCATGATCTGTGTGTTGGTTTT
                                                                XhoI
1762 TGTGTGCGGCGCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCTCGAGCCCTATAGTGAGTCGTATTA
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