GENE BRIDGES
DNA ENGINEERING SPECIALISTS

TECHNICAL PROTOCOL

FOR

FRT-cm-FRT

FRT flanked,
Chloramphenicol Selection Cassette

(A006)

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CONTENTS

1 Eppendorf tubes + manual

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

Store tube at -20°C

Please read

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Short Description:

“FRT-cm-FRT” cassette is designed to allow chloramphenicol selection in prokaryotic cells.

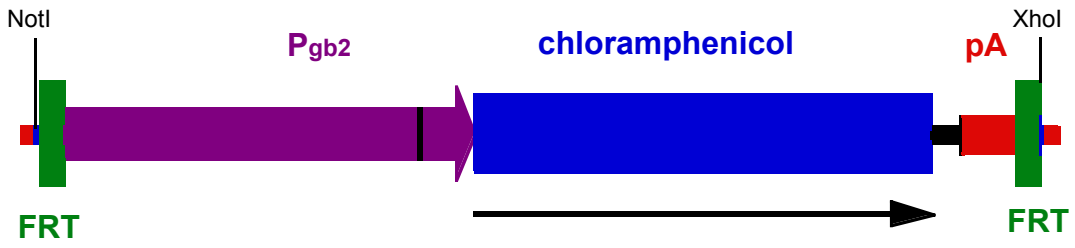
The prokaryotic promoter gb2 driving the gene for chloramphenicol resistance is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the generally used Tn5 promoter. A synthetic polyadenylation signal terminates the chloramphenicol expression. The cassette is flanked by FRT sites for later excision by Flp-recombinase. Unique *Not*I and *Xho*I sites flank the cassette for convenient cloning with restriction sites.

Using the provided PCR template one can easily create a FRT-cm-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 engineer the *E. coli* genome by Red®/ET® Recombination.

The “FRT-cm-FRT cassette” is not linear but plasmid based (3302 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.

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                                                NotI
   1 AATTAACCCTCACTAAAGG GCGGCCGC GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC ATTCTACCGG
  72 GTAGGGGAGG CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT
 142 ACACAAGTGG CCTCTGGCCT CGCACACATT CCACATCCAC CGGTAGGCGC CAACCGGCTC CGTTCTTTGG
 212 TGGCCCCTTC GCGCCACCTT CCACTCCTCC CCTAGTCAGG AAGTTCCCCC CCGCCCCGCA GCTCGCGTCG
 282 TGCAGGACGT GACAAATGGA AGTAGCACGT CTCACTAGTC TCGTGCAGAT GGACAGCACC GCTGAGCAAT
 352 GGAAGCGGGT AGGCCTTTGG GGCAGCGGCC AATAGCAGCT TTGCTCCTTC GCTTTCTGGG CTCAGAGGCT
 422 GGGAAGGGGT GGGTCCGGGG GCGGGCTCAG GGGCGGGCTC AGGGGCGGGG CGGGCGCCCG AAGGTCCTCC
 492 GGAGGCCCGG CATTCTGCAC GCTTCAAAAG CGCACGTCTG CCGCGCTGTT CTCCTCTTCC TCATCTCCGG
 562 GCCTTTCGAC C TGCAGC AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT
 629 ACGACAAGGT GAGGAACTAA ACC ATG GAG AAA AAA ATC ACT GGA TAT ACC ACC GTT GAT ATA TCC
                           1  Met Glu Lys Lys Ile Thr Gly Tyr Thr Thr Val Asp Ile Ser
 694 CAA TGG CAT CGT AAA GAA CAT TTT GAG GCA TTT CAG TCA GTT GCT CAA TGT ACC TAT AAC CAG
  15  Gln Trp His Arg Lys Glu His Phe Glu Ala Phe Gln Ser Val Ala Gln Cys Thr Tyr Asn Gln
 757 ACC GTT CAG CTG GAT ATT ACG GCC TTT TTA AAG ACC GTA AAG AAA AAT AAG CAC AAG TTT TAT
  36  Thr Val Gln Leu Asp Ile Thr Ala Phe Leu Lys Thr Val Lys Lys Asn Lys His Lys Phe Tyr
 820 CCG GCC TTT ATT CAC ATT CTT GCC CGC CTG ATG AAT GCT CAT CCG GAA TTC CGT ATG GCA ATG
  57  Pro Ala Phe Ile His Ile Leu Ala Arg Leu Met Asn Ala His Pro Glu Phe Arg Met Ala Met
 883 AAA GAC GGT GAG CTG GTG ATA TGG GAT AGT GTT CAC CCT TGT TAC ACC GTT TTC CAT GAG CAA
  78  Lys Asp Gly Glu Leu Val Ile Trp Asp Ser Val His Pro Cys Tyr Thr Val Phe His Glu Gln
 946 ACT GAA ACG TTT TCA TCG CTC TGG AGT GAA TAC CAC GAC GAT TTC CGG CAG TTT CTA CAC ATA
  99  Thr Glu Thr Phe Ser Ser Leu Trp Ser Glu Tyr His Asp Asp Phe Arg Gln Phe Leu His Ile
1009 TAT TCG CAA GAT GTG GCG TGT TAC GGT GAA AAC CTG GCC TAT TTC CCT AAA GGG TTT ATT GAG
 120  Tyr Ser Gln Asp Val Ala Cys Tyr Gly Glu Asn Leu Ala Tyr Phe Pro Lys Gly Phe Ile Glu
1072 AAT ATG TTT TTC GTC TCA GCC AAT CCC TGG GTG AGT TTC ACC AGT TTT GAT TTA AAC GTG GCC
 141  Asn Met Phe Phe Val Ser Ala Asn Pro Trp Val Ser Phe Thr Ser Phe Asp Leu Asn Val Ala
1135 AAT ATG GAC AAC TTC TTC GCC CCC GTT TTC ACC ATG GGC AAA TAT TAT ACG CAA GGC GAC AAG
 162  Asn Met Asp Asn Phe Phe Ala Pro Val Phe Thr Met Gly Lys Tyr Tyr Thr Gln Gly Asp Lys
1198 GTG CTG ATG CCG CTG GCG ATT CAG GTT CAT CAT GCC GTT TGT GAT GGC TTC CAT GTC GGC AGA
 183  Val Leu Met Pro Leu Ala Ile Gln Val His His Ala Val Cys Asp Gly Phe His Val Gly Arg
1261 ATG CTT AAT GAA TTA CAA CAG TAC TGC GAT GAG TGG CAG GGC GGG GCG TAAGCGGGACTCTGGGGTT
 204  Met Leu Asn Glu Leu Gln Gln Tyr Cys Asp Glu Trp Gln Gly Gly Ala •••
1328 CGAATAAAGACCGACCAAGCGAC GTC TGA GAGCTCCCTG GCGAATTCGG TACCAATAAA AGAGCTTTAT
                                                                      XhoI
1397 TTTCATGATC TGTGTGTTGG TTTTTGTGTG CGGCGCG GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC C TCGAG
1474 CCCTATAGTGAGTCGTATTA
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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.