TECHNICAL PROTOCOL

FOR

FRT-amp-FRT

FRT flanked,
Ampicillin Selection Cassette

(A008)

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1 Eppendorf tubes + manual

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

Store tube at -20°C

Please read

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Short Description:

"FRT-amp-FRT" cassette is designed to allow ampicillin selection in prokaryotic cells.

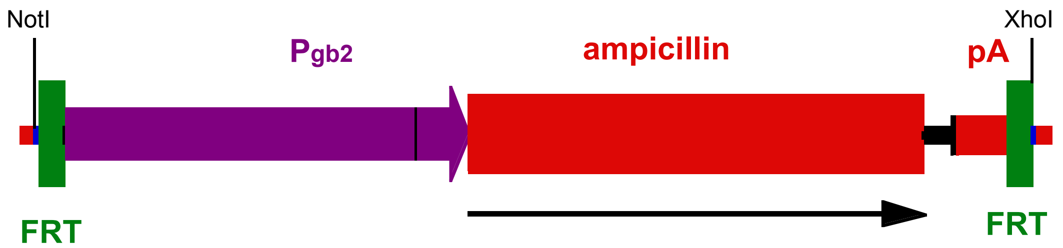
The prokaryotic promoter gb2 driving the gene for ampicillin resistance is a slightly modified version of the Em7 promoter; it mediates higher transcription efficiency than the generally used Tn5 promoter. A synthetic polyadenylation signal terminates the ampicillin expression. The cassette is flanked 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-amp-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-amp-FRT cassette" is not linear but plasmid based (3502 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 AATTAACCCTCACTAAAGGGC GGCCGC GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC ATTCTACCGG GTAGGGGAGG
  82 CGCTTTTCCC AAGGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT ACACAAGTGG CCTCTGGCTC
 162 GCACACATTC CACATCCACC GGTAGGCGCC AACCGGCTCC GTTCTTTGGT GGCCCCTTCG CGCCACCTTC TACTCCTCCC
 242 CTAGTCAGGA AGTTCCCCCC CGCCCCGCAG CTCGCGTCGT GCAGGACGTG ACAAATGGAA GTAGCACGTC TCACTAGTCT
 322 CGTGCAGATG GACAGCACCG CTGAGCAATG GAAGCGGGTA GGCCTTTGGG GCAGCGGCCA ATAGCAGCTT TGCTCCTTCG
 402 CTTTCTGGGC TCAGAGGCTG GGAAGGGGTG GGTCCGGGGG CGGGCTCAGG GGCGGGCTCA GGGGCGGGGC GGGCGCCCGA
 482 AGGTCCTCCG GAGGCCCGGC ATTCTGCACG CTTCAAAAGC GCACGTCTGC CGCGCTGTTC TCCTCTTCCT CATCTCCGGG
 562 CCTTTCGACC TGCAGC AGCACGTGTT GACAATTAAT CATCGGCATA GTATATCGGC ATAGTATAAT ACGACAAGGT
 638 GAGGAACTAA ACC atg agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt tgc
                 1▸Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala Phe Cys
 705 ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct gaa gat cag ttg ggt gca
  19▸Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala
 771 cga gtg ggt tac atc gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt cgc ccc gaa gaa
  41▸Arg Val Gly Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu
 837 cgt ttt cca atg atg agc act ttt aaa gtt ctg cta tgt ggc gcg gta tta tcc cgt gtt gac gcc
  63▸Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser Arg Val Asp Ala
 903 ggg caa gag caa ctc ggt cgc cgc ata cac tat tct cag aat gac ttg gtt gag tac tca cca gtc
  85▸Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val
 969 aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta tgc agt gct gcc ata acc atg agt
 107▸Thr Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
1035 gat aac act gcg gcc aac tta ctt ctg aca acg atc gga gga ccg aag gag cta acc gct ttt ttg
 129▸Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu
1101 cac aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc ata cca
 151▸His Asn Met Gly Asp His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro
1167 aac gac gag cgt gac acc acg atg cct gca gca atg gca aca acg ttg cgc aaa cta tta act ggc
 173▸Asn Asp Glu Arg Asp Thr Thr Met Pro Ala Ala Met Ala Thr Thr Leu Arg Lys Leu Leu Thr Gly
1233 gaa cta ctt act cta gct tcc cgg caa caa tta ata gac tgg atg gag gcg gat aaa gtt gca gga
 195▸Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly
1299 cca ctt ctg cgc tcg gcc ctt ccg gct ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
 217▸Pro Leu Leu Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg
1365 ggg tct cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt atc gta gtt atc tac
 239▸Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile Val Val Ile Tyr
1431 acg acg ggg agt cag gca act atg gat gaa cga aat aga cag atc gct gag ata ggt gcc tca ctg
 261▸Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu
1497 att aag cat tgg ta a GCGGGACTCT GGGGTTCGAA TAAAGACCGA CCAAGCGACG TCTGAGAGCT CCCTGGCGAA
 283▸Ile Lys His Trp
1572 TTCGGTACCA ATAAAAGAGC TTTATTTTCA TGATCTGTGT GTTGGTTTTT GTGTGCGGCG CG GAAGTTCCTATTCTCTAG
                           XhoI
1652 AAAGTATAGGAACTTCCTCGAGCCCTATAGTGAGTCGTATTA
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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.