TECHNICAL PROTOCOL

FOR

iCre-FRT-neo-FRT

Codon improved Cre (iCre) with attached FRT flanked, Pro- and Eukaryotic Neomycin Selection Cassette

(A012)

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CONTENTS

1 Eppendorf tube + manual

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

Store tube at -20℃

Please read

The products listed in this manual are for research purposes only. They are not designed for diagnostic or therapeutic use in humans, animals or plants. The Red®/ET® recombination technology is the intellectual property of Gene Bridges GmbH.

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Short Description:

“iCre-FRT-neo-FRT” PCR template is designed to facilitate the insertion of such a functional cassette into targeting constructs by Red/ET recombination.

A codon-improved version of P1 bacteriophage derived Cre-recombinase (Shimshek et al. 2002) is located upstream of the neomycin/kanamycin resistance gene (aminoglycoside phosphotransferase). Mammalian codon usage was applied for the altered Cre version (iCre). By introducing silent base mutations the high CpG content of the prokaryotic coding sequence was reduced, thereby reducing the chances of epigenetic silencing in mammals (Cohen-Tannoudji et al., 2000).

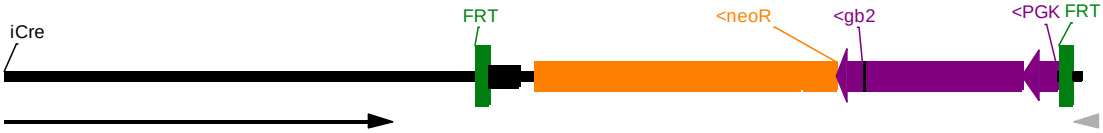
The iCre-FRT-neo-FRT template encodes the neomycin/kanamycin resistance gene which combines a prokaryotic promoter (gb2) for kanamycin resistance in *E.coli* with a eukaryotic promoter (PGK) for neomycin resistance in mammalian cells.

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 phosphoglycerate kinase gene (PGK) is used as eukaryotic promoter. A synthetic polyadenylation signal terminates the kanamycin/neomycin transcription. The cassette is flanked by FRT sites for later excision by Flp-recombinase.

Using the provided PCR template one can easily create an iCre-FRT-neo-FRT cassette flanked by homology arms to insert the cassette by Red/ET recombination into the vector of choice. The template can easily be used to generate targeting constructs mediated by a single Red/ET Recombination step.

The “iCre-FRT-neo-FRT template” is not linear but plasmid based (3446bp in size). Due to its R6K origin it can’t replicate in most of the frequently used *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.



iCre

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ATG GTC TCC AAC CTG CTG ACT GTG CAC CAA AAC CTG CCT GCC CTC CCT GTG GAT
Met Val Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp
GCC ACC TCT GAT GAA GTC AGG AAG AAC CTG ATG GAC ATG TTC AGG GAC AGG CAG
Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg Gln
GCC TTC TCT GAA CAC ACC TGG AAG ATG CTC CTG TCT GTG TGC AGA TCC TGG GCT
Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg Ser Trp Ala
GCC TGG TGC AAG CTG AAC AAC AGG AAA TGG TTC CCT GCT GAA CCT GAG GAT GTG
Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val
AGG GAC TAC CTC CTG TAC CTG CAA GCC AGA GGC CTG GCT GTG AAG ACC ATC CAA
Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln
CAG CAC CTG GGC CAG CTC AAC ATG CTG CAC AGG AGA TCT GGC CTG CCT CGC CCT
Gln His Leu Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro
TCT GAC TCC AAT GCT GTG TCC CTG GTG ATG AGG AGA ATC AGA AAG GAG AAT GTG
Ser Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val
GAT GCT GGG GAG AGA GCC AAG CAG GCC CTG GCC TTT GAA CGC ACT GAC TTT GAC
Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp
CAA GTC AGA TCC CTG ATG GAG AAC TCT GAC AGA TGC CAG GAC ATC AGG AAC CTG
Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn Leu
GCC TTC CTG GGC ATT GCC TAC AAC ACC CTG CTG CGC ATT GCC GAA ATT GCC AGA
Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala Arg
ATC AGA GTG AAG GAC ATC TCC CGC ACC GAT GGT GGG AGA ATG CTG ATC CAC ATT
Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu Ile His Ile
GGC AGG ACC AAG ACC CTG GTG TCC ACA GCT GGT GTG GAG AAG GCC CTG TCC CTG
Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu
GGG GTT ACC AAG CTG GTG GAG AGA TGG ATC TCT GTG TCT GGT GTG GCT GAT GAC
Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp
CCC AAC AAC TAC CTG TTC TGC CGG GTC AGA AAG AAT GGT GTG GCT GCC CCT TCT
Pro Asn Asn Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser
GCC ACC TCC CAA CTG TCC ACC CGG GCC CTG GAA GGG ATC TTT GAG GCC ACC CAC
Ala Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His
CGC CTG ATC TAT GGT GCC AAG GAT GAC TCT GGG CAG AGA TAC CTG GCC TGG TCT
Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser
GGC CAC TCT GCC AGA GTG GGT GCT GCC AGG GAC ATG GCC AGG GCT GGT GTG TCC
Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser
ATC CCT GAA ATC ATG CAG GCT GGT GGC TGG ACC AAT GTG AAC ATT GTG ATG AAC
Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met Asn
TAC ATC AGA AAC CTG GAC TCT GAG ACT GGG GCC ATG GTG AGG CTG CTC GAG GAT
Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu Leu Glu Asp
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CGT CGT GGC CAG CCA CGA TAG CCG CGC TGC CTC GTC CTG CAG TTC ATT CAG GGC
ACC GGA CAG GTC GGT CTT GAC AAA AAG AAC CGG GCG CCC CTG CGC TGA CAG CCG
GAA CAC GGC GGC ATC AGA GCA GCC GAT CGT CTG TTG TGC CCA GTC ATA GCC GAA
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