TECHNICAL PROTOCOL

FOR

iCreERT2-FRT-neo-FRT

Ligand-inducible iCre with attached FRT flanked, Pro- and Eukaryotic Neomycin Selection Cassette

(A013)

Gene Bridges GmbH
Im Neuenheimer Feld 584
69120 Heidelberg, Germany
Tel + 49 (0)6221 13708 11
Fax + 49 (0)6221 13708 29
Email: contact@genebridges.com
www.genebridges.com

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CONTENTS

1 Eppendorf tube + manual

1. iCreERT2-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:

“iCreERT2-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 fused with the ligand-binding domain of the estrogen receptor (ER) to obtain a ligand-inducible recombinase.
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 iCreERT2-FRT-neo-FRT template encodes the neomycin/kanamycin resistance gene (aminoglycoside phosphotransferase) 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 “iCreERT2-FRT-neo-FRT template” is not linear but plasmid based (4185 bp 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.

iCreERT2

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ATG GTC TCC AAC CTG CTG ACT GTG CAC CAA AAC CTG CCT GCC CTC CCT GTG GAT GCC ACC TCT GAT GAA GTC
 M   V   S   N   L   L   T   V   H   Q   N   L   P   A   L   P   V   D   A   T   S   D   E   V
AGG AAG AAC CTG ATG GAC ATG TTC AGG GAC AGG CAG GCC TTC TCT GAA CAC ACC TGG AAG ATG CTC CTG TCT
 R   K   N   L   M   D   M   F   R   D   R   Q   A   F   S   E   H   T   W   K   M   L   L   S
GTG TGC AGA TCC TGG GCT GCC TGG TGC AAG CTG AAC AAC AGG AAA TGG TTC CCT GCT GAA CCT GAG GAT GTG
 V   C   R   S   W   A   A   W   C   K   L   N   N   R   K   W   F   P   A   E   P   E   D   V
AGG GAC TAC CTC CTG TAC CTG CAA GCC AGA GGC CTG GCT GTG AAG ACC ATC CAA CAG CAC CTG GGC CAG CTC
 R   D   Y   L   L   Y   L   Q   A   R   G   L   A   V   K   T   I   Q   Q   H   L   G   Q   L
AAC ATG CTG CAC AGG AGA TCT GGC CTG CCT CGC CCT TCT GAC TCC AAT GCT GTG TCC CTG GTG ATG AGG AGA
 N   M   L   H   R   R   S   G   L   P   R   P   S   D   S   N   A   V   S   L   V   M   R   R
ATC AGA AAG GAG AAT GTG GAT GCT GGG GAG AGA GCC AAG CAG GCC CTG GCC TTT GAA CGC ACT GAC TTT GAC
 I   R   K   E   N   V   D   A   G   E   R   A   K   Q   A   L   A   F   E   R   T   D   F   D
CAA GTC AGA TCC CTG ATG GAG AAC TCT GAC AGA TGC CAG GAC ATC AGG AAC CTG GCC TTC CTG GGC ATT GCC
 Q   V   R   S   L   M   E   N   S   D   R   C   Q   D   I   R   N   L   A   F   L   G   I   A
TAC AAC ACC CTG CTG CGC ATT GCC GAA ATT GCC AGA ATC AGA GTG AAG GAC ATC TCC CGC ACC GAT GGT GGG
 Y   N   T   L   L   R   I   A   E   I   A   R   I   R   V   K   D   I   S   R   T   D   G   G
AGA ATG CTG ATC CAC ATT GGC AGG ACC AAG ACC CTG GTG TCC ACA GCT GGT GTG GAG AAG GCC CTG TCC CTG
 R   M   L   I   H   I   G   R   T   K   T   L   V   S   T   A   G   V   E   K   A   L   S   L
GGG GTT ACC AAG CTG GTG GAG AGA TGG ATC TCT GTG TCT GGT GTG GCT GAT GAC CCC AAC AAC TAC CTG TTC
 G   V   T   K   L   V   E   R   W   I   S   V   S   G   V   A   D   D   P   N   N   Y   L   F
TGC CGG GTC AGA AAG AAT GGT GTG GCT GCC CCT TCT GCC ACC TCC CAA CTG TCC ACC CGG GCC CTG GAA GGG
 C   R   V   R   K   N   G   V   A   A   P   S   A   T   S   Q   L   S   T   R   A   L   E   G
ATC TTT GAG GCC ACC CAC CGC CTG ATC TAT GGT GCC AAG GAT GAC TCT GGG CAG AGA TAC CTG GCC TGG TCT
 I   F   E   A   T   H   R   L   I   Y   G   A   K   D   D   S   G   Q   R   Y   L   A   W   S
GGC CAC TCT GCC AGA GTG GGT GCT GCC AGG GAC ATG GCC AGG GCT GGT GTG TCC ATC CCT GAA ATC ATG CAG
 G   H   S   A   R   V   G   A   A   R   D   M   A   R   A   G   V   S   I   P   E   I   M   Q
GCT GGT GGC TGG ACC AAT GTG AAC ATT GTG ATG AAC TAC ATC AGA AAC CTG GAC TCT GAG ACT GGG GCC ATG
 A   G   G   W   T   N   V   N   I   V   M   N   Y   I   R   N   L   D   S   E   T   G   A   M
                                                 ERT2>
GTG AGG CTG CTC GAG GAT GGG GAC CTC GAG CCA TCT GCT GGA GAC ATG AGA GCT GCC AAC CTT TGG CCA AGC
 V   R   L   L   E   D   G   D   L   E   P   S   A   G   D   M   R   A   A   N   L   W   P   S
CCG CTC ATG ATC AAA CGC TCT AAG AAG AAC AGC CTG GCC TTG TCC CTG ACG GCC GAC CAG ATG GTC AGT GCC
 P   L   M   I   K   R   S   K   K   N   S   L   A   L   S   L   T   A   D   Q   M   V   S   A
TTG TTG GAT GCT GAG CCC CCC ATA CTC TAT TCC GAG TAT GAT CCT ACC AGA CCC TTC AGT GAA GCT TCG ATG
 L   L   D   A   E   P   P   I   L   Y   S   E   Y   D   P   T   R   P   F   S   E   A   S   M
ATG GGC TTA CTG ACC AAC CTG GCA GAC AGG GAG CTG GTT CAC ATG ATC AAC TGG GCG AAG AGG GTG CCA GGC
 M   G   L   L   T   N   L   A   D   R   E   L   V   H   M   I   N   W   A   K   R   V   P   G
TTT GTG GAT TTG ACC CTC CAT GAT CAG GTC CAC CTT CTA GAA TGT GCC TGG CTA GAG ATC CTG ATG ATT GGT
 F   V   D   L   T   L   H   D   Q   V   H   L   L   E   C   A   W   L   E   I   L   M   I   G
CTC GTC TGG CGC TCC ATG GAG CAC CCA GTG AAG CTA CTG TTT GCT CCT AAC TTG CTC TTG GAC AGG AAC CAG
 L   V   W   R   S   M   E   H   P   V   K   L   L   F   A   P   N   L   L   L   D   R   N   Q
GGA AAA TGT GTA GAG GGC ATG GTG GAG ATC TTC GAC ATG CTG CTG GCT ACA TCA TCT CGG TTC CGC ATG ATG
 G   K   C   V   E   G   M   V   E   I   F   D   M   L   L   A   T   S   S   R   F   R   M   M
AAT CTG CAG GGA GAG GAG TTT GTG TGC CTC AAA TCT ATT ATT TTG CTT AAT TCT GGA GTG TAC ACA TTT CTG
 N   L   Q   G   E   E   F   V   C   L   K   S   I   I   L   L   N   S   G   V   Y   T   F   L
TCC AGC ACC CTG AAG TCT CTG GAA GAG AAG GAC CAT ATC CAC CGA GTC CTG GAC AAG ATC ACA GAC ACT TTG
 S   S   T   L   K   S   L   E   E   K   D   H   I   H   R   V   L   D   K   I   T   D   T   L
ATC CAC CTG ATG GCC AAG GCA GGC CTG ACC CTG CAG CAG CAG CAC CAG CGG CTG GCC CAG CTC CTC CTC ATC
 I   H   L   M   A   K   A   G   L   T   L   Q   Q   Q   H   Q   R   L   A   Q   L   L   L   I
CTC TCC CAC ATC AGG CAC ATG AGT AAC AAA GGC ATG GAG CAT CTG TAC AGC ATG AAG TGC AAG AAC GTG GTG
 L   S   H   I   R   H   M   S   N   K   G   M   E   H   L   Y   S   M   K   C   K   N   V   V
CCC CTC TAT GAC CTG CTG CTG GAG GCG GCG GAC GCC CAC CGC CTA CAT GCG CCC ACT AGC CGT GGA GGG GCA
 P   L   Y   D   L   L   L   E   A   A   D   A   H   R   L   H   A   P   T   S   R   G   G   A
TCC GTG GAG GAG ACG GAC CAA AGC CAC TTG GCC ACT GCG GGC TCT ACT TCA TCG CAT TCC TTG CAA AAG TAT
 S   V   E   E   T   D   Q   S   H   L   A   T   A   G   S   T   S   S   H   S   L   Q   K   Y
TAC ATC ACG GGG GAG GCA GAG GGT TTC CCT GCC ACA GCT TGA TGAAGATCTGAGCTCCCTGGCGGAATTCGGATCCAGATC
 Y   I   T   G   E   A   E   G   F   P   A   T   A
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                                                                                    FRT
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GCC GTC GGG CAT GCG CGC CTT GAG CCT GGC GAA CAG TTC GGC TGG CGC GAG CCC CTG ATG CTC TTC GTC CAG
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GAA TGG GCA GGT AGC CGG ATC AAG CGT ATG CAG CCG CCG CAT TGC ATC AGC CAT GAT GGA TAC TTT CTC GGC
AGG AGC AAG GTG AGA TGA CAG GAG ATC CTG CCC CGG CAC TTC GCC CAA TAG CAG CCA GTC CCT TCC CGC TTC
AGT GAC AAC GTC GAG CAC AGC TGC GCA AGG AAC GCC CGT CGT GGC CAG CCA CGA TAG CCG CGC TGC CTC GTC
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                                                                    <neoR
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                                        <gb2
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                                                                    <PGK    FRT
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ACTTTCTAGAGAATAGGAACTTCGCGGCGCCCTTTAGTGAGGGTTAATT
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