GENE BRIDGES
THE RECOMBINEERING COMPANY

Red/ET Recombination

Cloning Without Restriction Enzymes

A Guide to Next Generation Cloning

TECHNOLOGY

Precise, High-Speed Cloning

with Red/ET Recombination

Inside this guide

Gene Bridges was founded in 2000 as a specialist DNA engineering company to commercialise Red/ET Recombination, the patented 'recombineering' technology developed at EMBL Heidelberg.

Red/ET Recombination permits the engineering of DNA in *E. coli* using homologous recombination mediated by phage protein pairs, either RecE/RecT or Redα/Redβ.

Recombineering with Red/ET differs from other DNA engineering technologies, such as cutting and pasting with restriction enzymes, PCR, DNA ligase, because it is

- **not limited by DNA size**
- **independent of restriction sites**

Any DNA molecule in *E. coli* of almost any size can be engineered at any site using Red/ET.

Recombineering therefore permits more DNA engineering freedom than any other technology and has become an essential component of the molecular biological tool kit.

Recombineering can easily be deployed alongside conventional DNA methodologies to give you time to do your research.

From its headquarters in Heidelberg, Gene Bridges provides recombineering licenses, services and products. This brochure provides an overview of the applications of Red/ET Recombination.

Red/ET Recombination

Why is Red/ET Recombination a superior approach to DNA engineering?

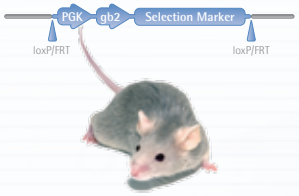
Red/ET Recombination allows a faster, more flexible and highly reliable modification of plasmids, BACs, or the *E. coli* genome than conventional cloning methods.

Red/ET exploits phage λ homologous recombination potential for *in vivo* genetic engineering in *E. coli*. Since Red/ET does not depend on restriction enzymes, ligation reactions or *in vitro* clean-up steps, it is highly applicable for the engineering of large DNA molecules.

Animal targeting constructs

Red/ET allows for the genetic engineering of tailor-made targeting constructs for animal models:

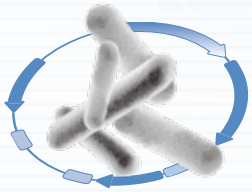
- conditional knock-out/knock-in
- promoter or reporter fusions
- exon swapping
- introduction of point mutations



E. coli strain modification

With Red/ET you can easily modify the *E. coli* genome:

- gene disruption, deletion or insertion
- reporter gene and tag integration
- promoter fine tuning
- introduction of point mutations



You can establish Red/ET Recombination in your laboratory by using Gene Bridges' kits or let our service facility in Heidelberg, Germany perform your project.

Red/ET at a glance

- only 50 bp of flanking sequence sufficient for recombination
- sequence independent
- precise at any position
- cloning without restriction enzymes
- no ligation reactions
- cloning of inserts up to 80 kb

Principle patents covering Red/ET Recombination

US Patent Nos. 6,355,412 and 6,509,156B by Stewart *et al.*

European Patent No. EP 1034260 B1

Japanese Patent No. EP 4139561

Patent Application PCT/EP98/07945, Novel DNA Cloning Method (ET).

U.S. Patent Application No. 09/350,830, Methods And Compositions For Directed Cloning and Sub-cloning Using Homologous Recombination.

Three Simple Steps with Red/ET

Three simple steps

Cells which express λ-derived *red* genes from plasmid pRed/ET promote base precise exchange of DNA sequences flanked by homology arms. The *in vivo* reaction is catalyzed by the exonuclease Redα and the DNA annealing protein Redβ.

Even the most demanding tasks can be reduced to three basic steps:

1. Attachment of Homology Arms
2. Recombineering
3. Selection/Screening

Literature

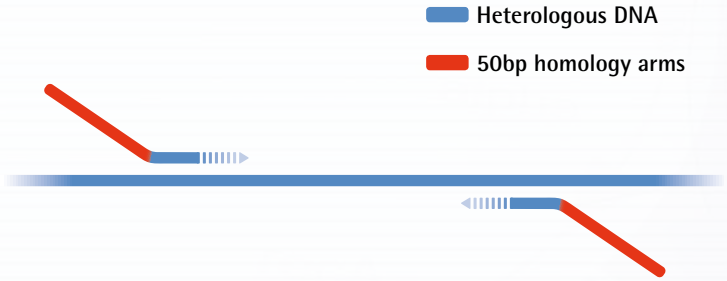
Zhang Y., Buchholz F., Muyrers J.P.P. and Stewart A.F. A new logic for DNA engineering using recombination in *E. coli*. **Nature Genetics** 20 (1998) 123-128.

Muyrers, J.P.P., Zhang, Y., Testa, G., Stewart, A.F. Rapid modification of bacterial artificial chromosomes by ET-recombination. **Nucleic Acids Res.** 27 (1999) 1555-1557.

Zhang Y., Muyrers J.P.P., Testa G. and Stewart A.F. DNA cloning by homologous recombination in *E. coli*. **Nature Biotechnology** 18 (2000) 1314-1317.

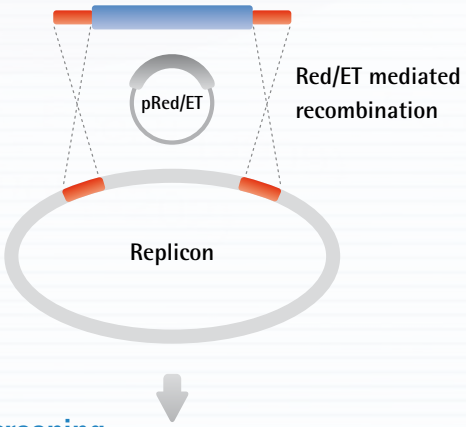
1. Attachment of homology arms

Red/ET Recombination requires linear DNA which is flanked by terminal homology stretches of only 50 bp. Thus, DNA homology arms for any given locus can easily be attached by PCR.



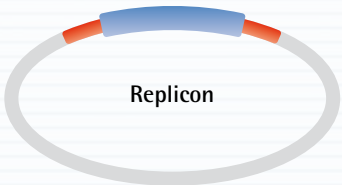
2. Recombineering

The insert is introduced into *E. coli* cells propagating the Red/ET expressing plasmid.



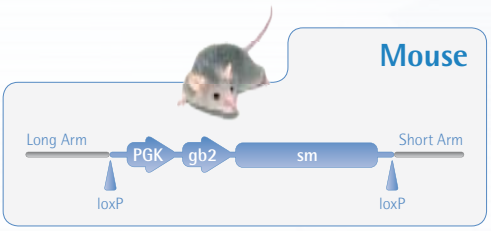
3. Selection/screening

Cells harboring the recombinant DNA are selected.



Animal Targeting Constructs

How can Red/ET Recombination help you to make tailored constructs for animal models?



At the cutting edge of DNA engineering, Gene Bridges' kits and cloning services fulfill the needs of our pharmaceutical, biotech, and academic clients for tailor-made targeting constructs.

Transgenic technologies are an essential component in the study of developmental biology and modelling genetic disorders. Red/ET Recombination enables *in vivo* DNA modifications irrespective of composition and size. Thus, Gene Bridges´ recombination kits open up exciting possibilities for the fast and reliable engineering of targeting constructs.

Use the modular system of our recombination kits and functional cassettes to prepare optimized targeting constructs. Alternatively, why not take advantage of our service facility in Heidelberg, Germany?

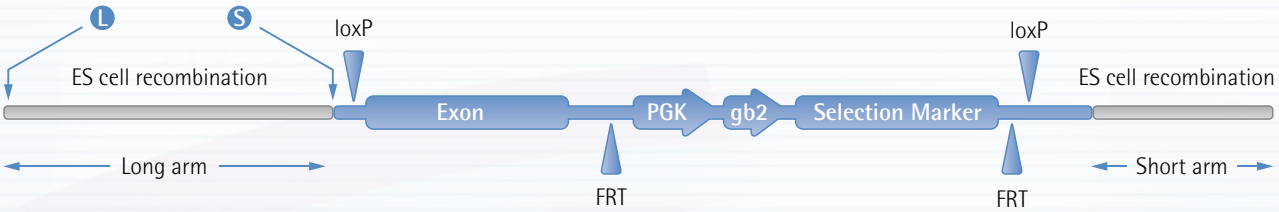
Our services include

- Assistance with project design and verification strategy
- Steps to improve efficiency for subsequent ES cell recombination or blastocyte microinjection
- Full documentation

Size of the constructs

Constructs can be prepared as high-copy plasmids with an overall size of up to 30 kb or low-copy plasmids with a size of up to 50 kb.

Basic targeting construct



For efficient ES cell recombination, flanking homology arms can be extended to 5 kb (short arm) and 10 kb (long arm). Appropriate restriction sites for linearization (L) and screening (S) can easily be incorporated.

Animal Targeting Constructs

Custom service work flow for targeting constructs

1. Provide us with the gene name (NCBI Acc. No.) and kind of modification.
2. We develop an *in silico* strategy and provide you with the electronic data for cross checking.
3. We order the appropriate BAC clone based on the mouse strains C57/BL6 or 129Sv.
4. We clone ≤18kb of the modified allele into a high-copy vector backbone, providing large (>5kb) homology arms for an efficient ES cell recombination. Constructs ≥20kb are available as low copy plasmids upon request.
5. We confirm integrity of the final targeting construct by sequencing.
6. You receive an *E. coli* glycerol stock harboring the construct and a detailed report.

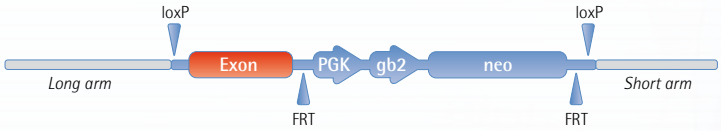
Gene Bridges contact:

+49 6221 13708 11

info@genebridges.com

Conditional knock-out targeting constructs

Analyse gene function by flanking an essential exon with loxP sites for subsequent excision by Cre recombinase.

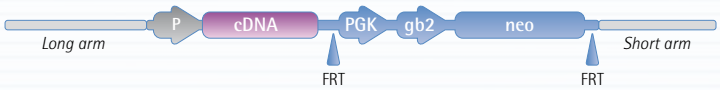


Reporter constructs

Analyse gene expression by a reporter gene fused to the native promotor without introducing additional nucleotides which may affect the expression pattern.

Promoter fusion constructs

Fuse your cDNA to the promoter of interest, without introducing additional nucleotides which may affect the expression pattern.



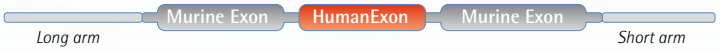
Animal Targeting Constructs

Targeting constructs introducing point mutations

Study the effects of SNPs in your animal model and insert single base pair mutations at any position.

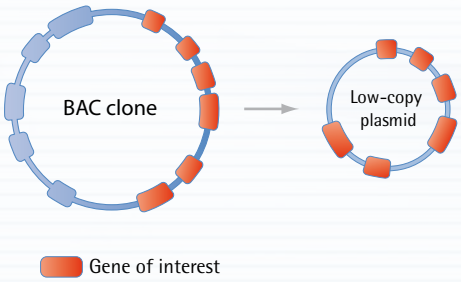
Targeting constructs to humanize animal models

Replace a given exon with the human counterpart to study the influence of your drug on a human-derived allele.



Optimized transgene constructs

Transfer a whole genomic locus up to 50 kb from a BAC clone into a low copy plasmid. Unwanted flanking sequences are removed yielding optimized constructs.



Transgenes are generally more reliably expressed if the intron-exon boundaries are preserved in the transgene construct.

E. coli Strain Modifications

Custom service work flow for *E. coli* strain optimization

1. Provide us with a reference sequence file and your *E. coli* strain*.
2. We will help to optimize the project design and provide you with electronic data for cross checking.
3. We confirm clone integrity by sequencing.
4. You receive an *E. coli* glycerol stock and a detailed report.

Gene Bridges contact:

+49 6221 13708 11

info@genebridges.com

*common *E. coli* strains can be provided by Gene Bridges.

How can Red/ET Recombination help you to optimize *E. coli* strains?

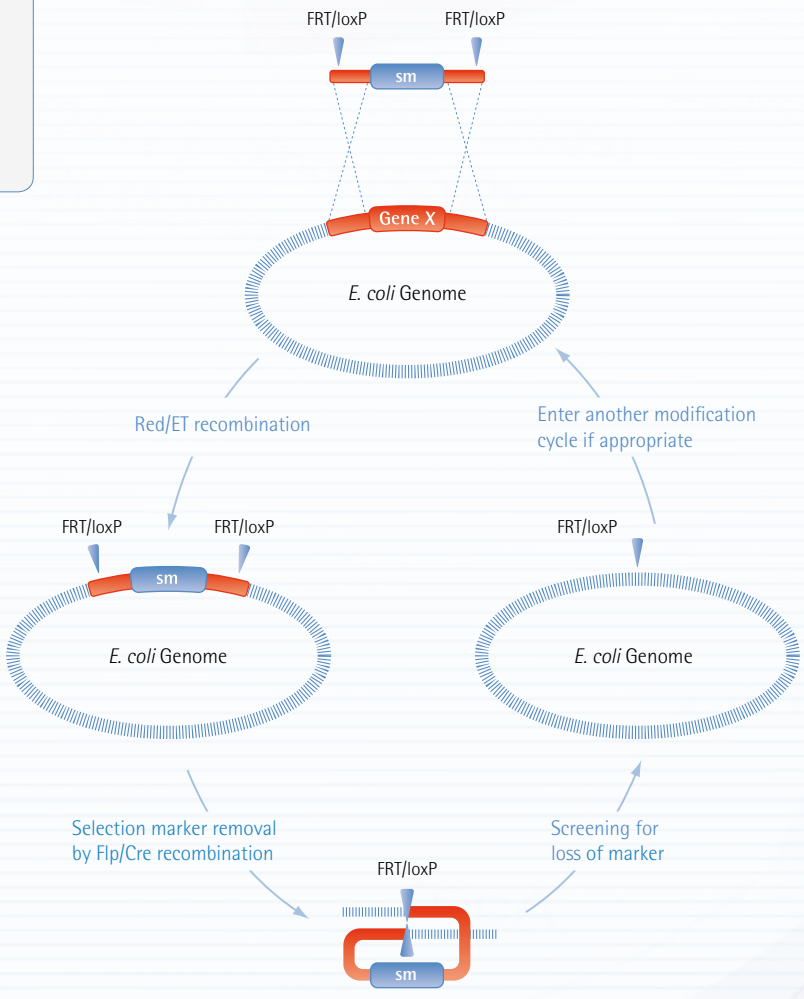
Escherichia coli is frequently used as model organism and functions as microbial factory in biotechnology. Recombination with Red/ET enables a defined and rapid access to chromosomal modifications:

- Gene disruption, deletion, insertion, modification
- Reporter gene or tag integration
- Promoter fine tuning

Use the modular system of our recombination kits and functional cassettes to prepare optimized *E. coli* strains or use our service facility in Heidelberg, Germany.

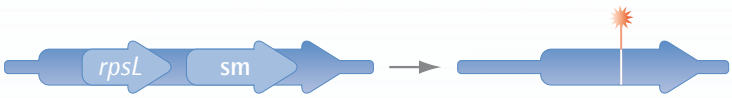
Markerless knock-out of *E. coli* genes

In combination with Flp/Cre technology multiple markerless genome modifications can be achieved.



E. coli Strain Modifications

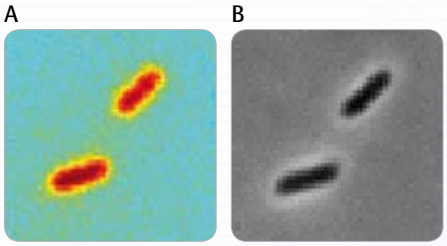
Seamless modifications



Insert point mutations or other seamless modifications by employing a selection - counterselection cassette (*rpsL*-sm).

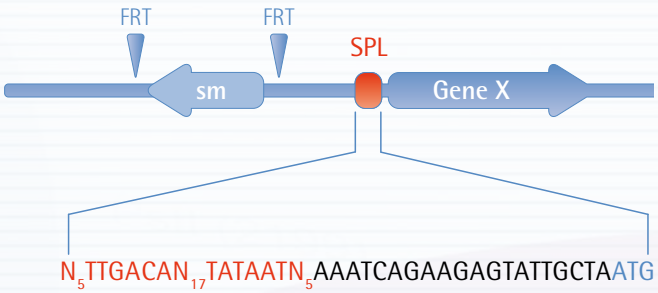
Reporter gene or tag integration

Use a chromosomal reporter strain to analyse single copy gene expression.

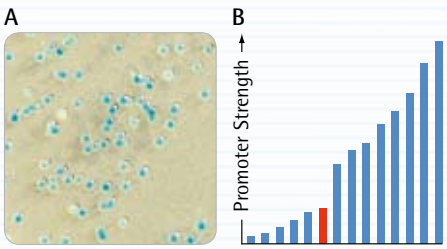


Protein tagging in *E. coli* cells achieved by chromosomal fusion of *GeneX* with *cfp*, encoding for cyan fluorescent protein. **A.** Fluorescence microscopy. **B.** Dark field microscopy. Pictures provided by Stavans Lab, Weizmann Institute of Science, Israel.

Promoter fine tuning



Optimize gene expression by fusion of a synthetic promoter library (SPL) to the gene of interest.



Analysis of the activity of *pgi-lacZ* under control of a synthetic promoter library. **A.** Transformants appear white to dark blue in the presence of X-gal. **B.** Promoter activity of a subset of SPL clone. Red: native promoter. (Biotechniques, Vol 45, No 3, 2008).

Large Fragment Cloning

Selected BAC resource centres

BPRC

BACPAC Resource Center at Children's Hospital Oakland Research Institute
http://bacpac.chori.org

Geneservice

http://www.geneservice.com

AGI

Arizona Genomics Institute
http://www.genome.arizona.edu

CUGI

Clemson University Genomics Institute
http://www.genome.clemson.edu

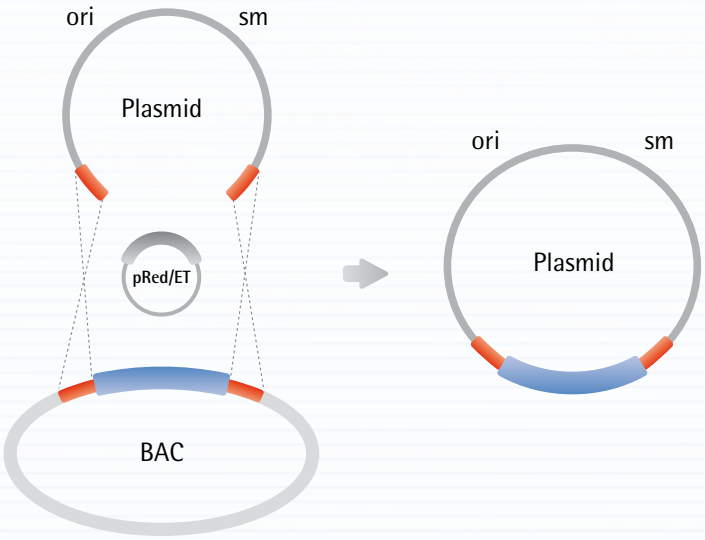
How can you take advantage of large BAC libraries?

Genome projects for more than 400 eukaryotic organisms are currently either running or finished. For the majority of these projects, annotated large insert BAC (bacterial artificial chromosome) libraries are available.

Red/ET Recombination makes this valuable source easily accessible. Large genomic fragments of any sequence can be cloned into plasmids by gap repair. The method is not restrained by the general fidelity and amplicon size limitations of PCR.

Advantages of BAC subcloning

- Fast and simple cloning of large fragments
- Cloning size up to 30 kb for high-copy plasmids
- Cloning size up to 80 kb for low-copy plasmids
- Cloning independent of restriction sites
- Flanking of the cloned fragment with functional cassettes or restriction sites easily possible



A plasmid backbone which contains an origin of replication (ori) and a selectable marker (sm) is PCR-amplified with primers introducing homology arms (red).

Red/ET Recombination Kits

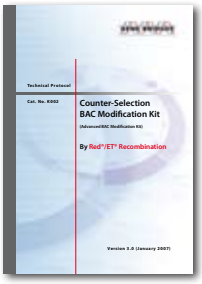
Establish Red/ET Recombination in your lab - Gene Bridges offers a wide range of kits, modular cassettes and plasmids.

Quick & Easy BAC Modification Kit

Cat.No. K001

Developed for deletion of BAC fragments and all types of basic modifications. Functional elements: Tn5-*neo*^{R}.

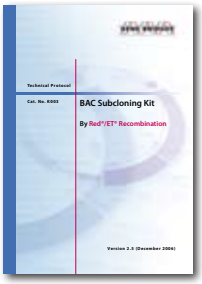
Counter Selection BAC Modification Kit



Cat.No. K002

Developed for the insertion of point mutations in BAC clones or the *E. coli* genome. Functional elements: Positive/negative selection marker cassette *rpsL-neo*^{R}.

BAC Subcloning Kit



Cat.No. K003

Developed to transfer fragments up to 30 kb from BACs or the *E. coli* chromosome. Functional elements: Linear vector ColE1 ori-*amp*^{R}

Kit Contents

- pRed/ET expression plasmid
- Suitable control experiments
- Detailed manual, maps, sequences

Academic researchers can order Gene Bridges kits, cassettes and plasmids from our website:

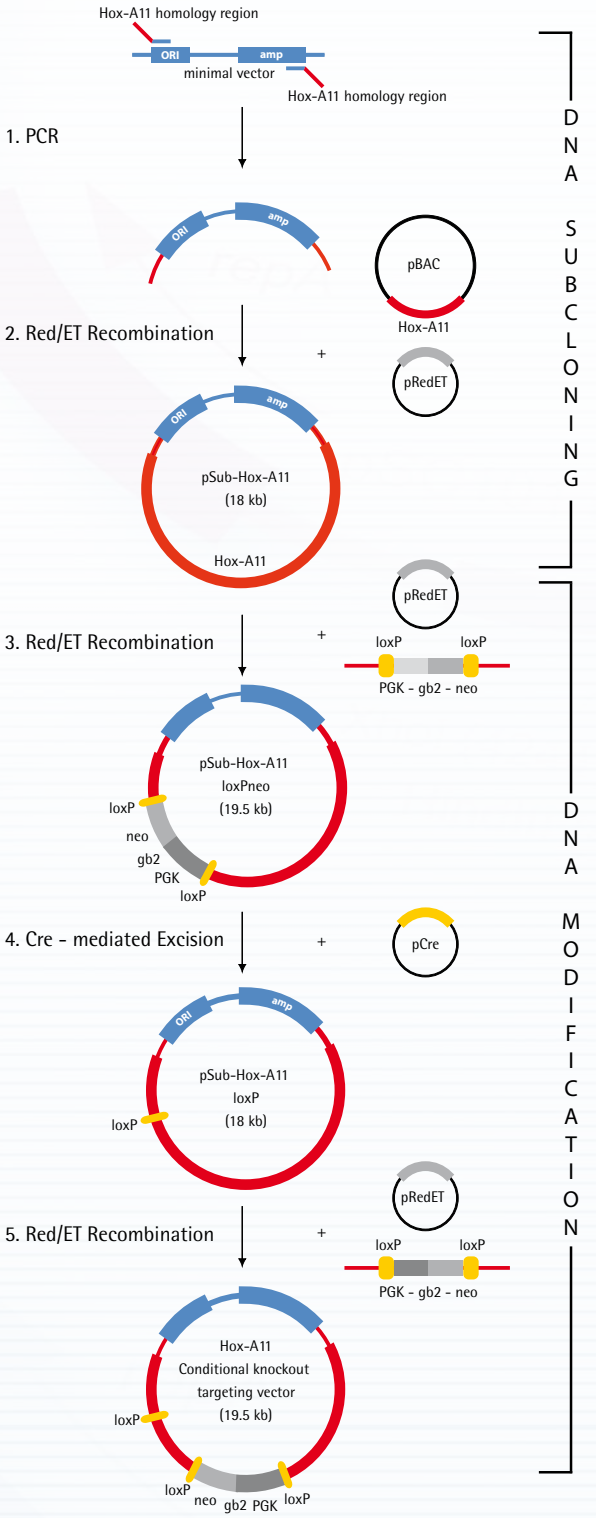
www.genebridges.com

or from our regional distributors. Commercial organisations require a license from Gene Bridges to use the Red/ET Recombination technology. Please contact:

licensing@genebridges.com

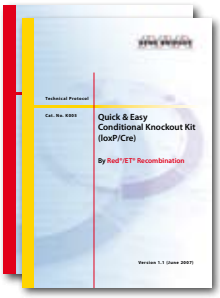
Red/ET Recombination Kits

Constructing conditional knock-outs



Construction of a conditional knock-out targeting vector for the murine homeobox protein Hox-A11 using the BAC Subcloning Kit and the Quick & Easy Conditional Knock-Out Kit (loxP/cre).

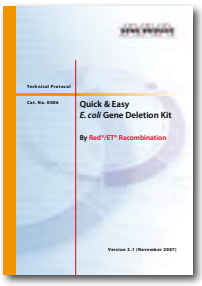
Quick & Easy Conditional Knock-Out Kit



Cat.No. K004 (loxP), K005 (FRT)

Developed for the insertion of FRT or loxP sites, respectively into high-copy plasmids. Flp or Cre expression plasmid included.
Functional elements:
loxP-PGK-gb2-neo-loxP (K004)
FRT-PGK-gb2-neo-FRT (K005)

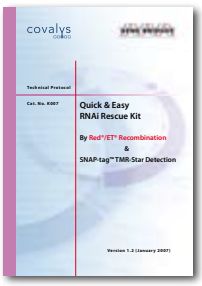
Quick & Easy *E. coli* Gene Deletion Kit



Cat.No. K006

Developed for the deletion of *E. coli* genes. Markerless modification possible in combination with expression plasmid A104 or A105 (see page 14).

Quick & Easy RNAi Rescue Kit



Cat.No. K007

Developed to insert a SNAP-tag cassette into a BAC clone to confirm the specificity of an RNAi-based loss-of-function (LOF) phenotype. Determine the transfection efficiency by the integrated SNAP-tag casette.

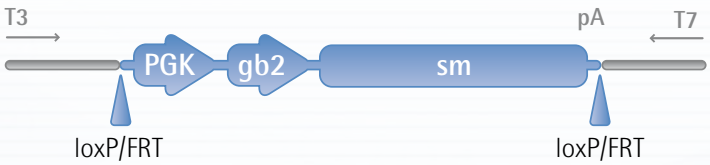
Selection Cassettes

Increase your flexibility by using additional selection marker cassettes optimized for Red/ET recombination

Cassette Features

- Selection cassettes are driven by eukaryotic (PGK) and prokaryotic (gb2) promoters, respectively
- Selection markers flanked by loxP or FRT-sites can be removed in a Cre or Flp recombination step where appropriate
- Due to a modular architecture, selection cassettes can be PCR-amplified with master primers
- Zero background because cassettes are encoded by suicide plasmids to avoid false positive clones

Basic functional cassette

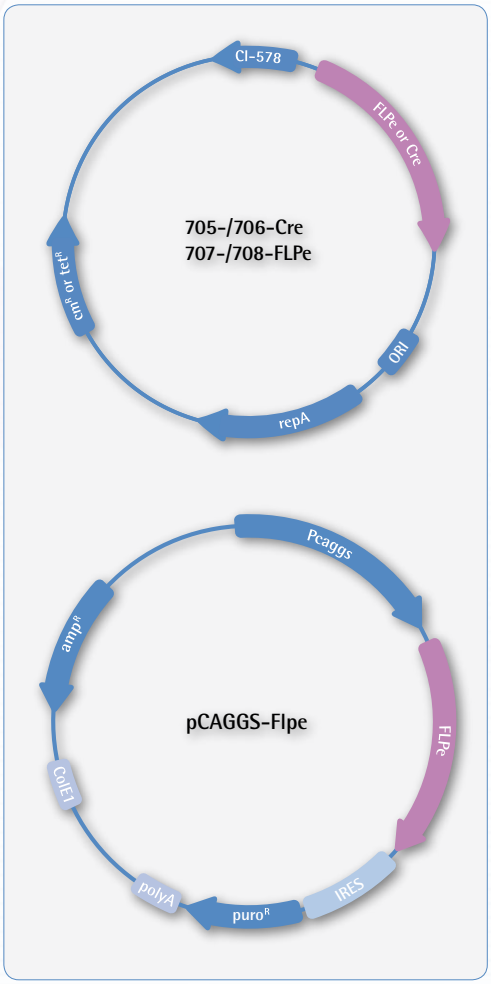


Cassettes overview

Cat.No.	Cassette	*E. coli* Selection	Mammalian Selection
A001	PGK-gb2-neo	kan^R	neo^R
A002	FRT-PGK-gb2-neo-FRT	kan^R	neo^R
A003	loxP-PGK-gb2-neo-loxP	kan^R	neo^R
A004	FRT-PGK-gb2-neo-FRT-loxP	kan^R	neo^R
A005	loxP-FRT-PGK-gb2-neo-FRT	kan^R	neo^R
A006	FRT-gb2-cm-FRT	cm^R	-
A007	loxP-gb2-cm-loxP	cm^R	-
A008	FRT-gb2-amp-FRT	amp^R	-
A009	loxP-gb2-amp-loxP	amp^R	-
A010	FRT-PGK-gb2-hygro-FRT	hyg^R	hyg^R
A011	loxP-PGK-gb2-hygro-loxP	hyg^R	hyg^R

PGK : Eukaryotic promoter
gb2 : Prokaryotic promoter
FRT : Flp recognition target site
loxP : Cre recognition target site
neo : Neomycin
kan : Kanamycin
cm : Chloramphenicol
amp : Ampicillin
hyg : Hygromycin

Recombinase Expression Plasmids



Optimized Cre and FLPe* expression plasmids for an efficient site-specific recombination.

Prokaryotic expression plasmids Flpe and Cre

- For the removal of FRT or loxP flanked DNA
- Gene expression and plasmid propagation are tightly controlled by temperature
- Available with various antibiotic resistance markers
- Compatible with ColE1 based plasmids (e.g. pUC, pBS or pBR322 derivative, RK2, R6K, cosmid, P1 and BAC)

*Flpe is a more thermostable derivative of wild type Flp displaying an enhanced activity at 37°C (Buchholz *et al.* Nature Biotechnology, 16:657-662 (1998)).

Cat.No.	Plasmid / Recombinase	Selection
A104	707-FLPe	tet^R
A105	708-FLPe	cm^R
A112	705-Cre	cm^R
A113	706-Cre	tet^R

Eukaryotic expression plasmid for FLPe

- For the removal of FRT-flanked DNA, e.g. neomycin resistance markers in mammalian cells.

Cat.No.	Plasmid / Recombinase	Selection
A201	pCAGGS-FLPe (Academia)	puro^R, amp^R
A202	pCAGGS-FLPe (Industry)	puro^R, amp^R

*Flpe is a more thermostable derivative of wild type Flp displaying an enhanced activity at 37°C (Buchholz *et al.* Nature Biotechnology, 16:657-662 (1998)).

The use of this product is governed by the terms and conditions of the pCAGGS-FLPe Material Transfer Agreement.

puro : Puromycin
CAGGS : Eukaryotic promoter
CI-578 : Prokaryotic promoter

FAX to Gene Bridges

Dear Customer,

for detailed information please use the form below and fax to:

FAX: +49 (0)6221 13708 29

I am interested in:

- ☐ Animal Targeting Constructs
- ☐ *E.coli* Modification
- ☐ Gene Bridges Services
- ☐ Commercial licenses

My questions or remarks:

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Name. . . . . .

Company/Institution . . . . . .

Building/Room. . . . . .

Street. . . . . City. . . . . .

State . . . . . Country. . . . . .

ZIP/Post Code. . . . . .

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