GENE BRIDGES
DNA ENGINEERING SPECIALISTS

Recombineering with Red®/ET®

Quick and Easy *E.coli* Gene Deletion Kit

Genome Engineering by Red/ET Recombination

- To knock-out genes on the E. coli chromosome in a base pair precise, specific, and faithful manner
- For permanent gene disruption in less than one week
- Enables marker-free gene disruption: Disruption of the gene by the supplied FRT-flanked kanamycin resistance marker cassette allows the subsequent removal of the selection marker by a FLP expression plasmid (available from Gene Bridges)
- Generate multiple knock-outs by either a repetitive insertion of the functional cassette or by combination with other available cassettes
- Strictly controlled recombination process due to an optimized design of the pRed/ET expression plasmid
- Fragments as large as at least 30kb can be replaced on the chromosome in one step
- Enables insertion of DNA fragments such as expression cassettes, reporter genes or ORFs from other organisms
- Positive controls are included to guide you through the protocol

Related Products:

Functional Cassette:	
A003:	loxP flanked, Pro- and Eukaryotic Kanamycin-Neomycin Selection Cassette (loxP-PGK-gb2-neo-loxP)
A006:	FRT flanked Chloramphenicol Selection Cassette (FRT-cm-FRT)
A007:	loxP flanked Chloramphenicol Selection Cassette (loxP-cm-loxP)
A008:	FRT flanked Ampicillin Selection Cassette (FRT-amp-FRT)
A009:	loxP flanked Ampicillin Selection Cassette (loxP-amp-loxP)

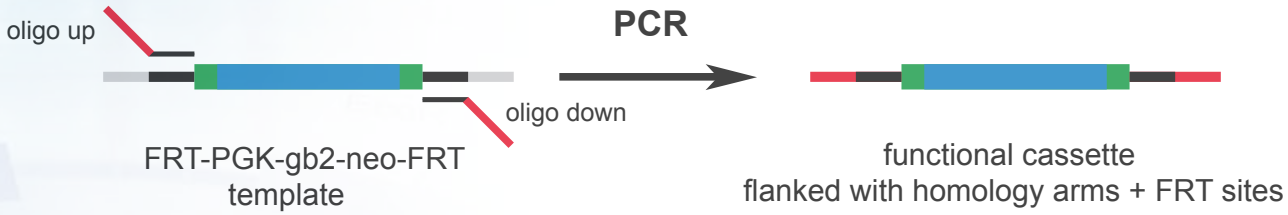
Prokaryotic Cre and FLP expression plasmids to remove the selection marker:	
A102:	FLP Expression plasmid: 705-Flp (cm resistance marker)
A103:	FLP Expression plasmid: 706-Flp (tet resistance marker)
A112:	Cre Expression plasmid: 705-Cre (cm resistance marker)
A113:	Cre Expression plasmid: 706-Cre (tet resistance marker)

Academic researchers can order Red/ET kits from our distributors. Commercial organizations require a commercial license for the use of Red/ET recombination. Please contact: licenses@genebridges.com.

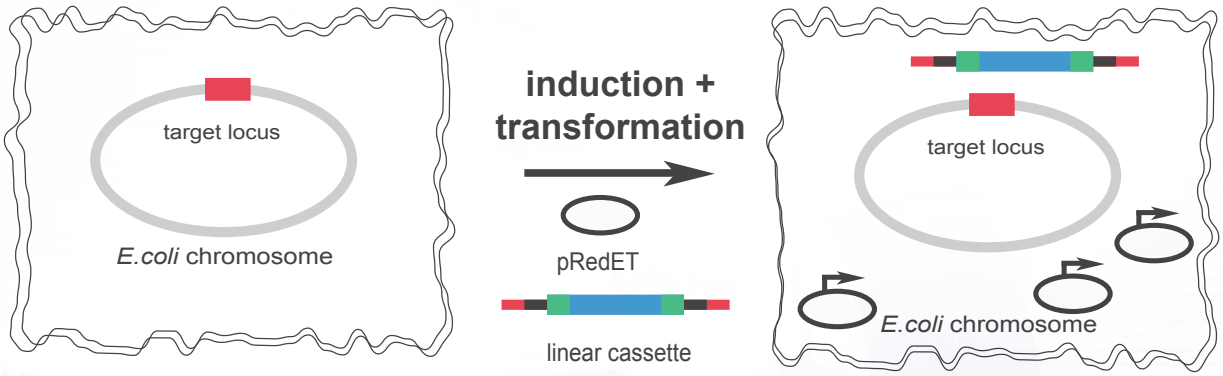
For further information visit our new website:
www.genebridges.com

Cloning Strategy – „Marker-free" gene disruption on the *E.coli* chromosome by Red®/ET® Recombination

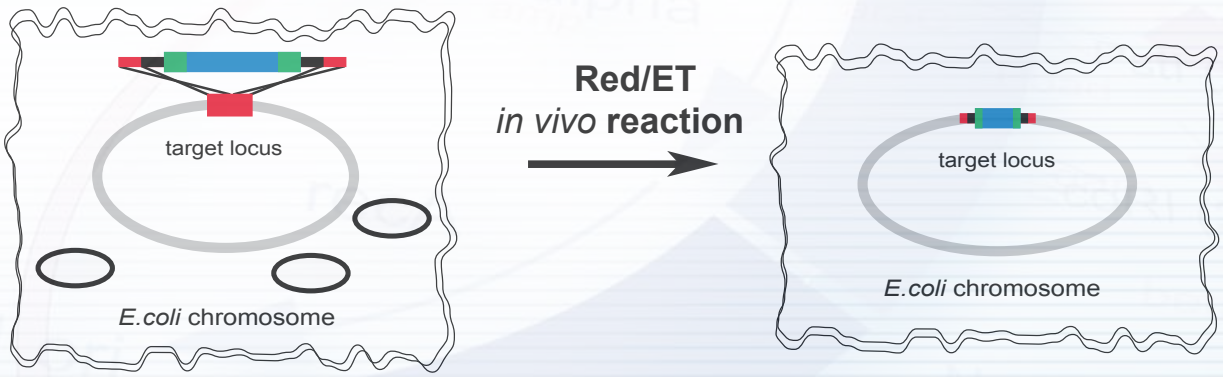
1. step: Generation of a PCR product from the functional cassette flanked with homology arms



2. step: Transformation of pRedET into the *E. coli* host, induction of the Red/ET recombination genes and subsequent transformation of the linear PCR proudct into the *E. coli* host.



3. step: Red/ET recombination inserts the functional cassette into the target locus



4. step: Transformation of FLP-expression plasmid into the *E. coli* host and removal of the selection marker by FLP recombination (optional)