How does the Red/ET Recombination system compare to other recombination systems, ie, Gateway, Cre-Lox, RecA/\(\chi\), RecA?

Gene Bridges’ proprietary Red/ET Recombination technology (e.g. Angrand et al 1999) has been published under several names:

- ET cloning/ ET recombination (e.g. Muyrers et al. 2000)
- GET recombination (e.g. Nefedov et al. 2000)
- Recombineering (e.g. Copeland et al. 2001)
- \(\lambda\) Red-mediated recombination (e.g. Murphy and Campellone 2003)

Red/ET Recombination is homologous recombination mediated by RecET or Red\(\alpha\beta\) and it utilizes short homology sequences to accomplish the recombination. Gateway and Cre-Lox systems are based on site-specific recombination. They have a different underlying recombination mechanism compared with Red/ET Recombination.

The RecA/\(\chi\) system is based on homologous recombination mediated by RecBCD in \textit{E.coli}. It is dependent on a short sequence called chi (\(\chi\)) present in the molecules. The homology sequences needed in RecA/\(\chi\) system are much longer than the ones used in Red/ET Recombination.

RecA mediated homologous recombination (Yang et al., Nature Biotechnology, 1997) is mediated by RecA alone and is dependent on longer homology sequences. The crucially longer homology sequences cannot be synthesized as oligonucleotides. Compared with Red/ET Recombination, it needs more conventional steps to pre-condition the recombinogenic molecules.

**Functionally?**

Any \textit{E.coli} strain can be converted in a Red/ET Recombination proficient strain. Seamless cloning is achieved, since Red/ET Recombination is not dependent on standard sequences like restriction sites, \textit{loxP}, FRT, att-sites,…

No site restriction – Red/ET functions at any point, independent of available restriction sites.

Red/ET can be applied with great ease to directly subclone or modify the bacterial chromosome resulting in designed bacterial strains.

Red/ET is one system for almost all applications: insertions, deletions from 1 bp up to 250 kb, substitutions, fusions, subcloning, BAC stitching.

Almost no size limitation.
Red/ET Recombination technology circumvents preparative DNA steps, since the DNA stays inside the bacterial cell.

Short sequence motives like restrictions sites, loxP, FRT, IRES can be introduced together with the actual DNA modification step.

**Efficiency of Red/ET Recombination?**

Recombination events per cell

2 end-mediated recombination event, due to introduction of linearized DNA

Absolute efficiency of recombination without selection can reach up to 5%

Recombination events vs background under selective conditions > 95%

**Fidelity (are open reading frames maintained when recombination occurs within coding sequence?**

Yes, since Red/ET Recombination is a nucleotide-precise cloning technology.

**Ease of use?**

Red/ET Recombination is an established technology based on standard procedures like generation of competent cells, PCR, DNA mini preparation, gel electrophoresis.

**Limitations and biases vs advantages**

Limitations/biases:

Red/ET cloning of repetitive elements, identical cassettes

The need for retransformation in case of high copy plasmids

Advantages:

unknown sequences can be cloned, only the sequence information of the homology arms is needed

Short sequence motives like restrictions sites, loxP, FRT, IRES can be introduced together with the actual DNA modification step
unlike site specific recombination no standard sequences will be introduced

What is the size range of sequence that can be cloned?
Cloning 1bp up to 250 kb; Subcloning size dependent on origin of replication; subcloning of 80 kb has been confirmed.

What is the length of homology required?
30-60 bp is recommended

Is electroporation required for cloning? Can high throughput cloning be done with chemically competent cells?
For a highly efficient performance electroporation is recommended resulting in an efficiency of $5 \times 10^5$ colonies per electroporation.

Chemically competent cells can be used for high throughput cloning, an efficiency of $10^3$ colonies per electroporation can be reached under such conditions. Additionally, there is a limitation regarding the size of DNA to be incorporated.

Can the system be used to shuttle sequence between vectors?
Yes, via Red/ET subcloning. The recipient vector needs to exert a different origin of replication than the donor vector. In pilot studies it was shown that Red/ET technology can be applied to shuttle between different vector sets.

Via Red/ET subcloning in two steps (preventing from PCR of recipient vector)

Has Gene Bridges tested the difference between efficiency of recombination where recombination-specific plasmids which are situated on a plasmid versus embedded in the bacterial chromosome?
A bacterial strain expressing Red/ET proteins directly from the chromosome like YZ2000 shows comparable efficiency to a bacterial strain electroporated with a Red/ET proficiency plasmid. In the latter case, the recombination system is only active for a short period of time so that the integrity of the DNA of interest is maintained. By applying Red/ET proficiency plasmids one can use any bacterial strains the lab is familiar with. This is an advantage especially when using bacterial BAC host strains or chromosomal modifications.
Has Gene Bridges isolated the recombination-specific proteins and demonstrated *in vitro* application?

Pilot studies have been undertaken, so far without success. Gene Bridges is studying the co-factors of Red/ET Recombination in *E.coli* to accomplish the goal of *in vitro* application.

Can the recombination-specific proteins function in eukaryotic cells? Can you provide examples of successful application in eukaryotic cells? How efficient is recET/Red in eukaryotic cells, if applicable?

Yes, please refer to our publication (BMC Biotechnology, 2003). Gene Bridges has also more indications though the data hasn´t been published yet. At this moment, we only can achieve a limited number of exercises in eukaryotic cells and the efficiency is still low.

In a traditional cloning situation, ie, linearized vector with homologous ends + insert with complimentary homologous ends, is there a difference in recombination efficiency when using a gel-isolated fragment and a PCR-generated fragment?

In theory, there is no impact on Red/ET performance using either an isolated DNA fragment or a PCR product. But a gel-isolated fragment exerts several disadvantages, such as EtBr staining, shearing and often a lower concentration. Normally, it shows a bit lower efficiency than a PCR-generated fragment.

A sometimes observed, higher efficiency after precipitation, maybe due to a higher DNA concentration that has been applied, to prevent from unsatisfying yield.

You once marketed several kits, including kits for standard cloning (and I believe, mutagenesis). It is noted that you now only market a BAC cloning kit. Is there a reason for this?

The applications of the kits have become more focused. Each kit includes a standard control experiment. The application of Red/ET in other fields can be accomplished with these kits as well (see PDF Document). Gene Bridges is developing several new kits with defined focuses. New kits are to be launched in the near future:

- Fragment cloning (applicable for isolated and PCR generated DNA)
- Targeting vector for conditional KO
- Modification of the bacterial chromosome
References and Patents


Patents
