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Short Commentary Open Access

Recombineering Recovery – Large DNA Cloning by Closing the Circle *In Vivo*

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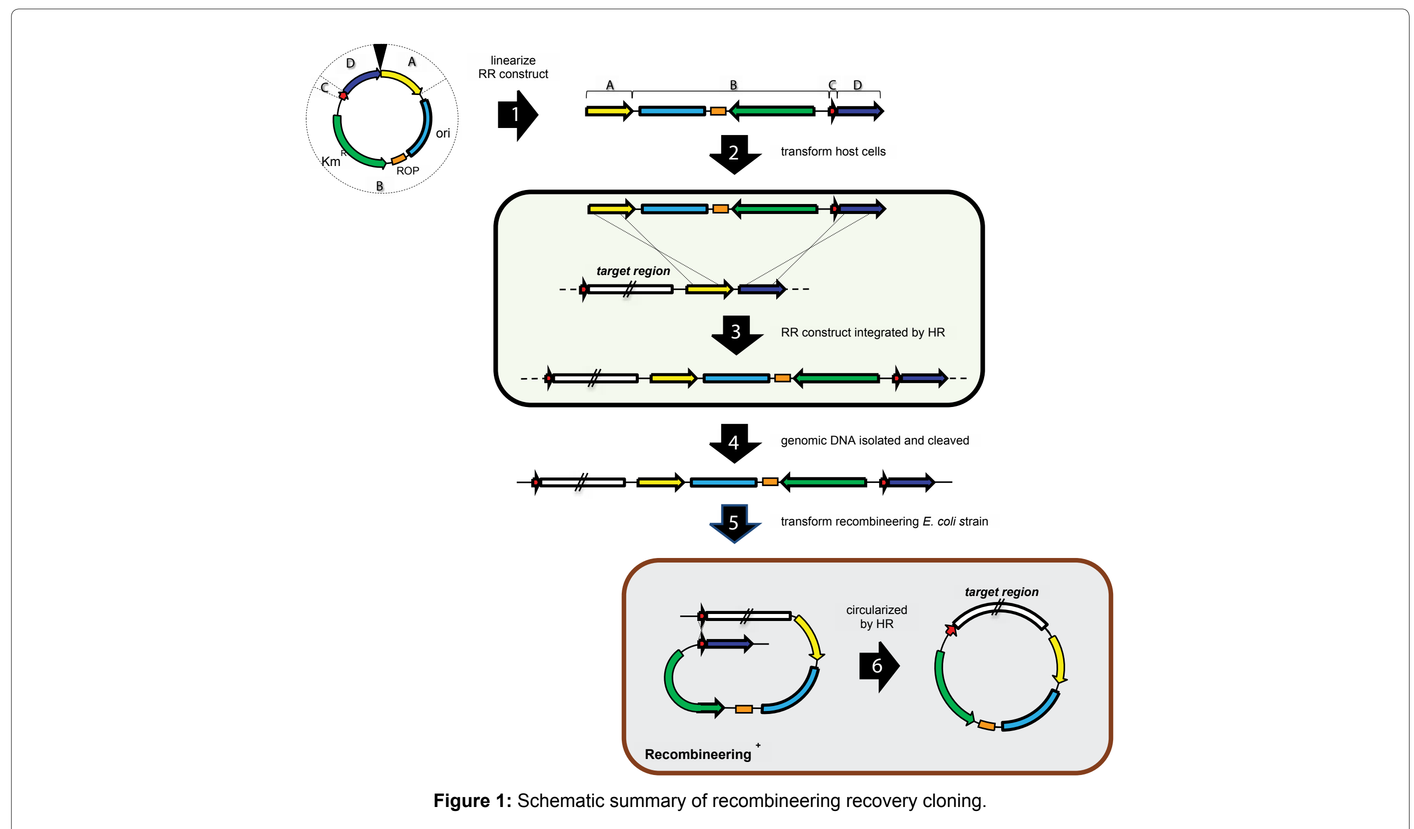

Figure 1: Schematic summary of recombineering recovery cloning.

Short Commentary

In order to study metabolic pathways and utilize them for biotechnology, it is frequently necessary to clone gene clusters that can be tens of kilobases in size. Traditional library construction, followed by screening and sub-cloning, is time consuming and costly. PCR amplification and assembly is more precise, quicker and cheaper, but the DNA polymerases used have an inherent error rate and incorrect fragment pairing can occur. Total gene synthesis can be rapid, but again sequence verification is necessary.

An alternative procedure for cloning large error-free DNA fragments, named recombineering recovery (RR), has recently been reported in FEBS letters [1]. This method was used to precisely clone a copper resistance gene cluster (6 genes, ~8.2 kb) from the freshwater cyanobacterium *Synechocystis* sp. PCC 6803. RR utilizes homologous recombination (HR) to (i) insert a cloning vector adjacent to the gene(s) of interest in the genome of the desired species, and (ii) recover this plasmid plus the target sequences from isolated transformant DNA by circularization in a recombineering strain of *Escherichia coli* (Figure 1).

RR appears to be highly efficient and should be applicable to species that can be transformed by HR, which currently include numerous prokaryotes, yeasts, filamentous fungi, *Dictyostelium*, single-celled protozoan parasites, cultured vertebrate cell lines and the moss *Physcomitrella patens*. A likely application of RR is the targeted knockout and cloning of gene clusters responsible for the production of economically important secondary metabolites.

Since DNA cloned by RR is synthesized by the host DNA replication machinery, it should be free from errors. Furthermore, the cloned DNA can be tailored for future applications by rational design of the transformation construct. A vector with a tightly repressed promoter would permit regulated expression of potentially harmful gene products. In addition, different plasmid replicons could be used to control the copy number of a cloned fragment, or allow recovery in other bacterial species or yeast.

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