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Commentary

Various Cloning Methods to Generate a Recombinant DNA

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DESCRIPTION

DNA cloning is the the most important step in recombinant DNA Technology and is the starting point for multiple inheritable engineering approaches to biotechnology. Large quantities of DNA are required for the process of genetic engineering. DNA cloning is a molecular biology approach that makes multiple identical clones of a piece of DNA, similar as a gene. There are four main ways in DNA cloning. In a typical cloning trial, a target gene is fitted into a ring-shaped piece of DNA called as plasmid. The plasmid is introduced into bacteria *via* a process called transformation, and bacteria carrying the plasmid are elected using antibiotics. Bacteria with the correct plasmid are used to make more plasmid DNA or in some cases, administered to express the gene and make protein. Various methods in DNA cloning:

Restriction enzyme based cloning

This is the traditional cloning approach where a gene of interest is fitted in to a vector by a cut and paste system. Restriction digestion of both gene of interest and vector was performed by using restriction enzymes that cut the gene at a specific sequence. Digestion with some enzymes evolves in a sticky end and some in blunt ends. After the digested fragments are cleaned up, the digested gene and vector are ligated to form a recombinant plasmid using DNA ligase enzyme. There are so many standardized protocols for this manner, but in some cases optimization of buffer is needed and time of restriction digestion also plays a major part. When using two restriction enzymes (double digestion) buffer used should be compatible for both the enzymes. However, gene and vector should be first digested with one enzyme followed by the other when there's no compatible buffer.

Polymerase Chain Reaction (PCR) Cloning

Polymerase chain reaction (PCR) cloning relies on a process called ligation, which is the approach of fitting a DNA fragment into a vector using DNA ligase. Ligation is important for this step because it's responsible for fitting the PCR product into a 'T-tailed' plasmid. The amplified PCR inserts contain an adenine residue at the 3' end of the DNA fractions. A 'T-tailed' plasmid vector has a single 3' deoxythymidine (T) at each end of the arms of a linearized plasmid. Thus, these PCR products can be ligated into 'T-tailed' vectors by using DNA ligase, and this step is followed by transformation. This approach can be choosed when the restriction enzymes aren't compatible or when there are findings of an internal restriction enzyme point in the DNA insert. One disadvantage of this manner is, it need a specific 'T-tailed' vector to perform PCR cloning. But 'T-tailed' vectors may not have helpful elements for the protein examination, similar as promoter region or protein tag.

Ligation Independent Cloning (LIC)

Ligation Independent Cloning (LIC) is performed by generating short sequences at the end of a DNA insert that match to the short sequences of a plasmid vector. Enzymes with 3' to 5' exonuclease activity chew 3' ends and induce cohesive ends between the DNA fraction and the linearized vector. The two substances are further combined for annealing step. During transformation, the host organism repairs the hacks on the recombinant DNA. The advantage of this manner is it won't produce any new restriction spots or unwanted sequences in the final DNA construct.

Recombinational cloning

This approach requires location-specific DNA recombinase enzymes, which exchange and recombine DNA pieces with particular recombination sites. The first step in this method is to fit a DNA fragment into an entry vector generating an entry clone. Another way to produce an entry clone is by exchanging and recombining a donor vector into an entry clone. After creating an entry clone, the succeeding step is to exchange and recombine the entry clone into a destination clone. The benefit of this approach is it can be used to place additional than five essentials into a single vector. It's generally used to identify protein-binding interlinkage or to optimize protein expression, purification and solubility. To perform this manner, a particular plasmid which has recombination sites are required.

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