CSIR NET Life Science Unit 13

Cloning Technology

Gateway cloning technique is a cloning technique that removes many steps of cloning that can limit the success of cloning which normally occurs in traditional cloning method like restriction enzyme this enhance the workflow of cloning. some examples are like many restriction enzymes are not usable because they might cut within the gene of interest and disrupt the insert which makes the gene used less for downstream expression. So for low-efficiency recovery of recombinants from cloning large fragments is experience additional clean-up steps are also needed, time is wasted to screen colonies to find the clone of interest. All of the abovesaid steps take considerable time and effort, and no success is guaranteed. While In Gateway recombination cloning technology circumvents these cloning limitations which enable to access virtually any expression system.

The Gateway cloning method is an in vitro version of the integration and excision recombination reactions and this reaction takes place when lambda phage infects bacteria. In vivo, condition facilitation of these recombination reactions occurs by the recombination of attachment sites from the phage (attP) and the bacteria (attB). As a result of recombination reactions between the attP and attB sites, the phage integrates into the bacterial genome flanked by two new recombination sites. In specific certain conditions, the attL and attR sites can recombine which leads to the excision of the phage from the bacterial chromosome and the regeneration of attP and attB sites.

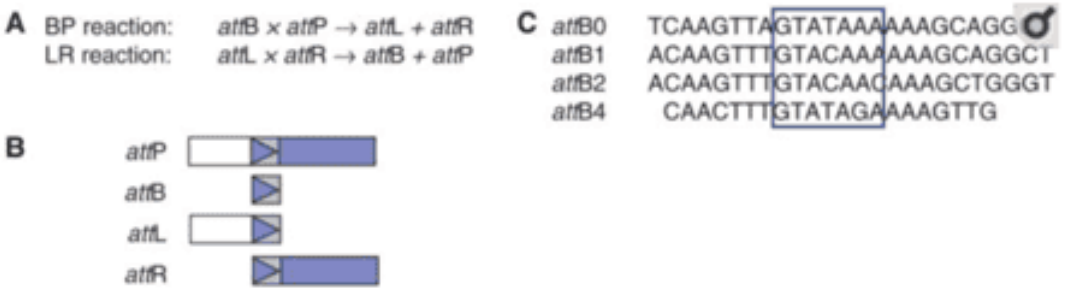
The Gateway cloning system is a recombinational cloning system that was developed for cloning multiple DNA fragments in parallel in a standardized manner and by using the same enzyme. This technique is purely based on the highly specific integration and excision reactions of bacteriophage λ into and out of the Escherichia coli genome. Because of the sites of much longer recombination sites (“att” sites) than restriction sites, they are extremely unlikely to occur by chance in DNA fragments. so, the same recombination enzyme can be used to robustly clone many different fragments of variable size in parallel reactions.

Basic Protocol of Gateway Cloning Technique:

1. Entry Clone Determination:

At the first step of cloning, it is determined how and where the experiment starts and entry of clone is done, as it contains the gene of interest or DNA fragment flanked by attL sequences, these sequences are then recombined with attR sequences for the creation of the desired expression in the clone. So at first Choose appropriate cloning vectors to create an Entry clone, or purchase a premade clone from our validated Ultimate ORF Clone Collection.

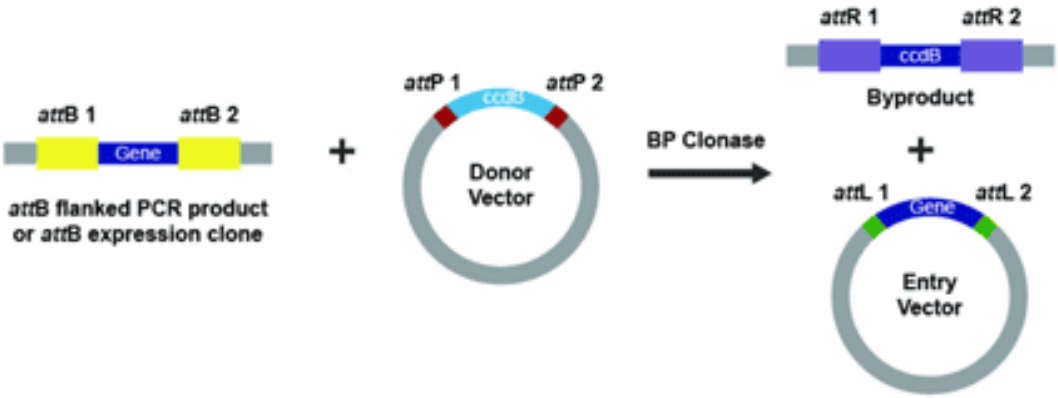
Generation of Entry Clone in Gateway Cloning



2. Reaction Mediation with Clonase Enzymes:

When the Entry clone is ready, the gene of interest is lucidly shuttled to a secondary plasmid, which is known as the Destination vector. This reaction is mediated by a particular enzyme which is known as the Clonase enzyme these enzyme contains the protein machinery which is necessary to excision the gene of interest from the Entry clone and integrate it into the Destination vector, then it becomes an expression clone.

Reaction for Preparation of Vectors

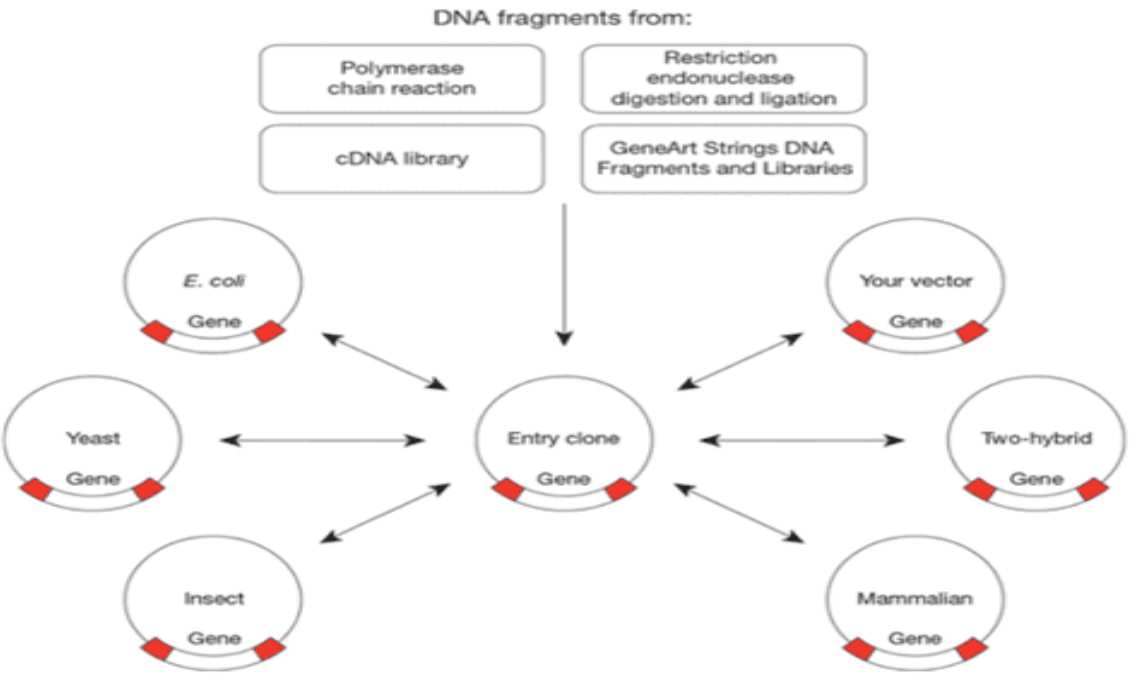


3. Destination Vector Selection:

When a cloned gene of interest or DNA fragment reaches into a Gateway vector, the process can shuttle it to as many Destination vectors as you require. their as many Destination vectors for application for example From proteins expression in *E. coli*, yeast, insect, or mammalian cells to RNAi studies, from crystallography to protein-protein interaction functional studies, And also for those applications which require a specialized or customized vector, the Gateway Vector Conversion System can convert any vector into a Gateway cloning–compatible vector.

Reaction of Gateway Cloning

Gateway Vector Technology Overview



Advantages and Disadvantages of Different Recombination-Based Cloning Methods

Cloning system(s)	Gateway	Univector Creator	In-Fusion, MAGIC, SEFC
Method Involve	In this technique λ-based BP and LR recombinases that use att sites, it is a Completely in vitro technique.	P1-based Cre recombinase that uses loxP sites, it is Completely in vitro technique.	The homologous recombination method is used
Advantages	In this technique, no gain/loss of nucleotides occurs during the transfer No digestion required Recombination-reversible Uses Entry clones Dozens of Destination vectors are available Multisite transfers possible Wide variety of recombination sites.	No gain/loss of nucleotides during transfer No digestion required Uses Entry clones	No recombination sites No enzyme purchase necessary, Multisite transfers possible Any vector can be used without conversion
Disadvantages	There is a Need to purchase enzymes Recombination sites may interfere with the experiment Need to make the desired vector compatible.	Need to purchase enzymes Recombination sites may interfere with the experiment Recombination irreversible Few Destination vectors are available Multisite transfers not possible Limited variety of recombination sites Need to make the desired	Occasional gain/loss of nucleotides during transfer Need to generate insert by either PCR or digest before recombination Do not use Entry clones.

vector
compatible