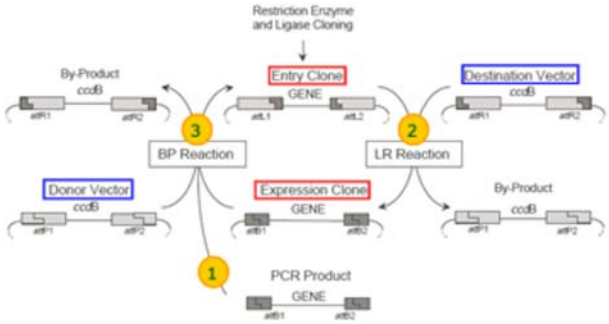
BxSeqTools' Ultimate Molecular Cloning Guides - Gateway Cloning

Gateway is a cloning method based upon the site specific recombination of lambda bacteriophage.



Gateway Cloning construct design with BxSeqTools - Get Started Now

Advantages of using BxSeqTools for Gateway cloning design:

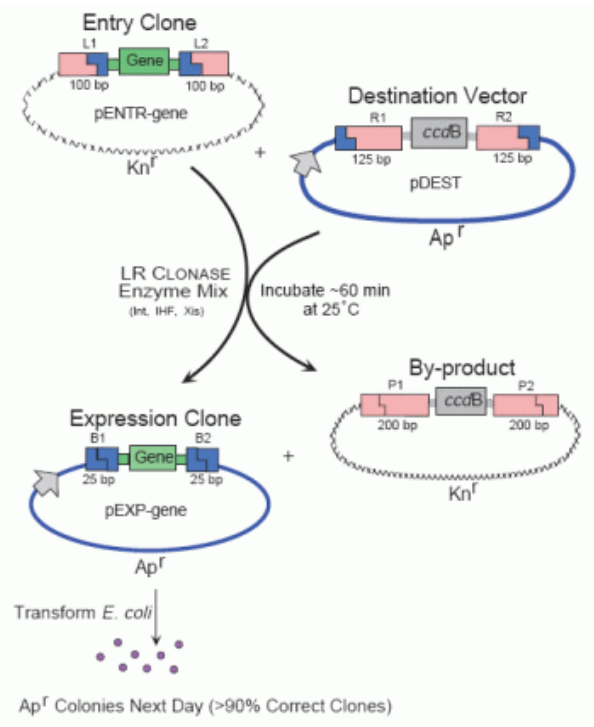
1. Search and retrieve sequences automatically (**No need to go to GenBank!**)
2. Design PCR primers with terminal attB sites
3. Clone PCR fragments into an Entry Clone via the BP Reaction.
4. Perform LR recombination reaction with selected Entry Clone and Destination Vector.
5. Perform BP recombination reaction with selected Expression Clone and Donor Vector.
6. Automatically generate cloning constructs: either Entry Clone or Expression Clone (**No more sequence copy-and-paste!**)
7. Save constructs in database for long term storage and data sharing (**Teamwork !**)

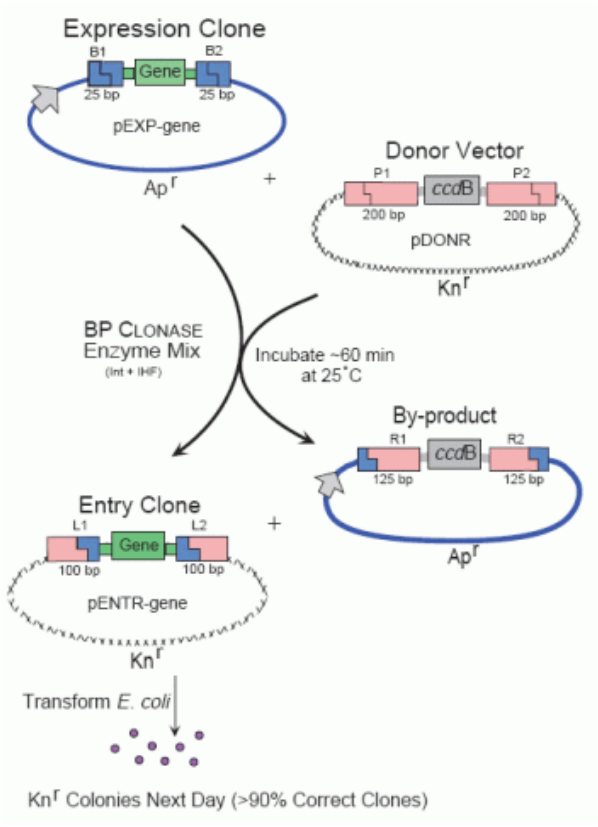
What is Gateway Cloning?

Gateway is a cloning method based upon the site specific recombination of lambda bacteriophage.

Two types of recombination sites have been engineered to recombine uniquely between the two ends of a source sequence and a host vector. The sites have also been engineered to allow the recombination reactions to be completely reversible using two sets of catalytic proteins. These two reactions are called **LR** and **BP**, so named because of the recombination sites involved in each reaction: **LR recombines attL and attR sites, BP recombines attB and attP sites.** The recombined sites transform by swapping opposite halves into the opposite set, so attL and attR become attB and attP and vice versa. Vectors engineered to contain these sites have been given the names: **Entry Clone**, **Expression Clone**, **Donor Vector**, and **Destination Vector**.

LR reaction	BP reaction
Takes a sequence of interest in a transcriptionally silent Entry Clone, recombines it with an antibiotic resistant Destination Vector to produce a transcriptionally active Expression Clone.	Takes a sequence of interest from an Expression Clone, recombines it with a Donor Vector to produce an Entry Clone for further cloning.





How Gateway Technology Works?

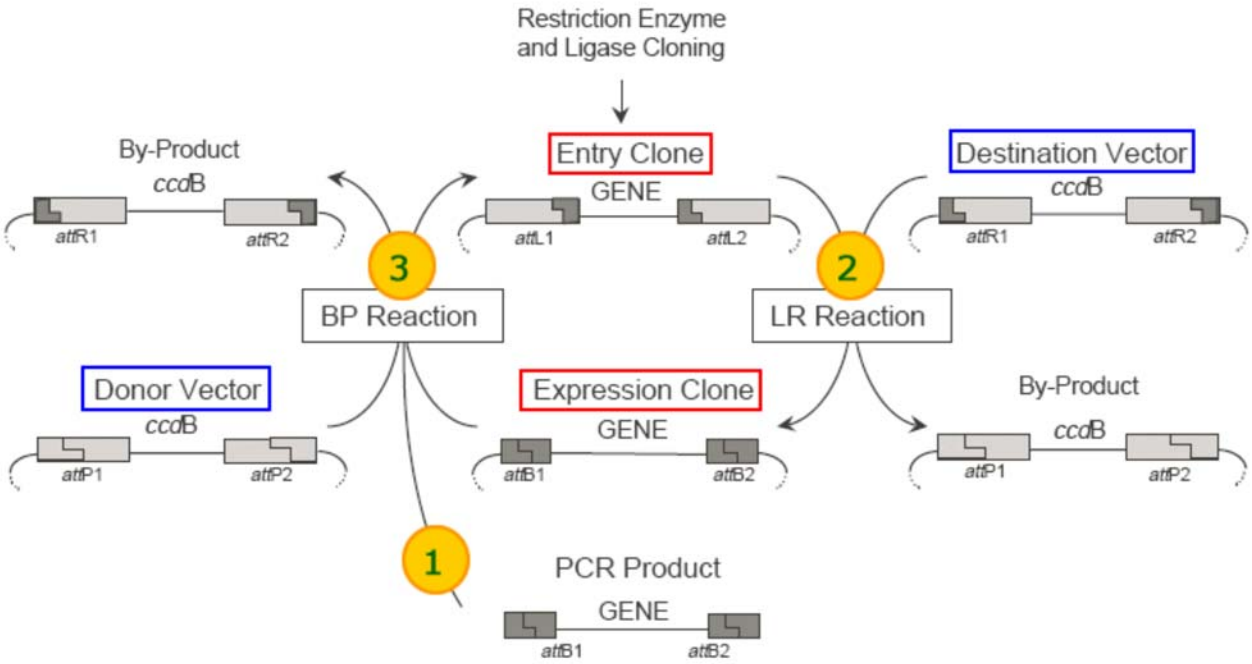
Gateway Technology uses lambda phage-based site-specific recombination instead of restriction endonuclease and ligase to insert a gene of interest into an expression vector. The DNA recombination sequences (*att*L, *att*R, *att*B, and *att*P) and the LR and BP Clonase enzyme mixtures that mediate the lambda recombination reactions are the foundation of Gateway Technology. Transferring a gene into a destination vector is accomplished in just two steps:

Step 1: Clone the gene of interest into an entry vector:

- Using restriction endonucleases and ligase.
- Starting with a cDNA library prepared in an attL Entry Vector.
- Using an Expression Clone from a library prepared in an attB expression vector via the BP Reaction.
- **Recombinational cloning of PCR fragment with terminal attB sites, via the BP Reaction.**

Step 2: Mix the ***Entry Clone*** containing the gene of interest *in vitro* with the appropriate Gateway expression vector (***Destination Vector***) and Gateway LR Clonase enzyme mix. Site-specific recombination between the *att* sites generates an ***Expression Clone*** and a by-product.

Step 3: Mix the ***Expression Clone*** or *PCR products flanked by attB sites* with ***Donor Vector*** to generate new Entry Clone, via BP Reaction.


Restriction Enzyme
and Ligase Cloning
By-Product
ccdB
attR1
attR2
Entry Clone
GENE
attL1
attL2
Destination Vector
ccdB
attR1
attR2
3
BP Reaction
2
LR Reaction
Donor Vector
ccdB
attP1
attP2
Expression Clone
GENE
attB1
attB2
By-Product
ccdB
attP1
attP2
1
PCR Product
GENE
attB1
attB2