Gateway

① “Gateway attB1, and attB2” sequences are added to the 5’, and 3’ end of a gene fragment, respectively, using gene specific PCR primers and PCR amplification;

② the PCR amplification products are then mixed with special plasmids called Gateway “Donor vectors” (Invitrogen nomenclature) and the proprietary “BP Clonase” enzyme mix. The enzyme mix catalyzes the recombination and insertion of the attB-sequence-containing PCR product into the attP recombination sites in the Gateway Donor vector. Once the cassette is part of the target plasmid, it is called an "Entry clone" in the Gateway nomenclature, and recombination sequences are referred to as the Gateway “attL” type.

③ Gateway BP reaction: PCR-product with flanking attB sites (this step can also use other methods of DNA isolation) + Donor vector containing attP sites + BP clonase

④ Gateway Entry clone, containing attL sites, flanking gene of interest

⑤ Gateway LR reaction:
Entry clone containing attL sites + Destination vector containing attR sites, and promoters and tags + LR clonase

⑦ Expression clone containing attB sites, flanking gene of interest, ready for gene expression.