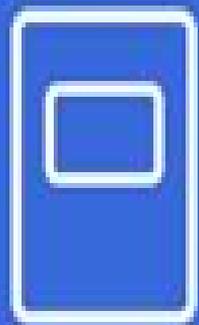


MOLECULAR CLONING

Tutorial: Gateway Cloning



TUTORIAL

Gateway Cloning

Learn how to simulate Gateway[®] cloning in Gateway[®] Primer, including how to automatically design attB-flanked oligonucleotide primers, simulate BP and LR reactions, and also simulate Multiple Gateway cloning.

Introduction

Gateway[®] Primer provides all of the tools required for primer design and in silico simulation of Gateway[®] cloning.

Thermo Fisher Scientific describe their Gateway[®] Technology as "a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda to provide a rapid and highly efficient way to move a gene of interest into multiple vector systems." See the [Thermo Fisher Scientific website](#) for a full description of Gateway[®] cloning.

To express a gene of interest using Gateway the following steps are taken:

1. Generate an entry clone by performing a BP recombination reaction between a pDONR[®] vector and an attB-PCR product or expression clone.
2. Generate the desired expression clone by performing an LR recombination reaction between the entry clone and a Gateway[®] destination vector.
3. Introduce your expression clone into the host system of choice for expression of your gene of interest.

In this tutorial we will learn how to:

- Design Gateway-compatible oligonucleotide primers to amplify a sequence of interest
- Simulate PCR to create a Gateway-compatible PCR product
- Simulate recombination of PCR product with a donor vector via a "BP" reaction to create an Entry vector
- Simulate recombination of an Entry vector with a Destination vector via an "LR" reaction to create an Expression vector
- Simulate multiple Gateway cloning

Tutorial Structure

The first three exercises in this tutorial cover the steps required for simulation of single vector Gateway cloning.

The fourth and fifth exercises cover simulation of multiple Gateway cloning.

Gateway Cloning Handbook

CH Cherryholmes



Gateway Cloning Handbook:

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