

## GATEWAY<sup>®</sup> AND TOPO CLONING

### Introduction to Gateway Cloning

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Gateway Cloning Technology is Invitrogen's universal cloning system that provides a rapid and highly efficient route to protein expression, functional analysis and cloning/subcloning of DNA segments. This system is based on the well-characterized bacteriophage lambda-based site-specific recombination system ( $\text{attL} \times \text{attR} \leftrightarrow \text{attB} \times \text{attP}$ ). In overview, Gateway is a 2-step cloning process; in the first step a sequence of interest is inserted into an Entry Clone. The second step transfers the sequence into a variety of  $\text{attB}$ -containing Expression Clones that can be propagated and expressed in a range of host cells for a given experiment.

TOPO Technology is a fast, efficient way to clone. The key to TOPO Cloning is the enzyme DNA topoisomerase I, whose biological role is to cleave and rejoin DNA during replication. To harness this activity, vectors are linearized and each end is conjugated with topoisomerase on the 3' phosphate. This enables fast ligation of DNA sequences with compatible ends. After 5 minutes at room temperature, enzyme is released, the ligation is complete and the recombinant molecule is ready for transformation into *E. coli*. Many Invitrogen expression vectors are adapted for one-step TOPO Cloning of PCR products in both directional and non-directional formats. Other vectors contain  $\text{att}$  recombination sequences exterior to the TOPO cloning sites so that cloned inserts are ready for entry into the Gateway system.

Vector NTI Advance 10 offers an interlocking set of computational tools for planning Gateway or TOPO cloning experiments from beginning to end. These allow you to simulate recombinant strategy, as well as to validate design of your reagents before proceeding to primer ordering, bench-top operations, etc. Steps necessary to identify and screen transformants, expedite purification, fuse an insert with vector tags, etc. can be identified and modifications made before

# Topo Gateway Cloning Manual

**J Elliott**



## **Topo Gateway Cloning Manual:**

Immerse yourself in heartwarming tales of love and emotion with Explore Love with is touching creation, Experience Loveis Journey in **Topo Gateway Cloning Manual** . This emotionally charged ebook, available for download in a PDF format ( PDF Size: \*), is a celebration of love in all its forms. Download now and let the warmth of these stories envelop your heart.

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