

GATEWAY[®] AND TOPO CLONING

Introduction to Gateway Cloning

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 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 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

DJ Losen



Topo Gateway Cloning Manual:

Whispering the Techniques of Language: An Mental Quest through **Topo Gateway Cloning Manual**

In a digitally-driven world wherever displays reign supreme and immediate conversation drowns out the subtleties of language, the profound secrets and emotional nuances concealed within phrases frequently move unheard. However, set within the pages of **Topo Gateway Cloning Manual** a captivating literary value pulsing with natural thoughts, lies an extraordinary journey waiting to be undertaken. Penned by an experienced wordsmith, this marvelous opus attracts visitors on an introspective journey, lightly unraveling the veiled truths and profound influence resonating within the cloth of each and every word. Within the emotional depths with this emotional evaluation, we will embark upon a sincere exploration of the book is primary styles, dissect their interesting publishing style, and succumb to the strong resonance it evokes deep within the recesses of readers hearts.

http://www.frostbox.com/About/virtual-library/Documents/wv_polo_20engine.pdf

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