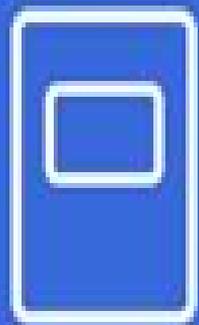


# MOLECULAR CLONING

# Tutorial: Gateway Cloning



TUTORIAL

## Gateway Cloning

Learn how to simulate Gateway<sup>®</sup> cloning in Gateway<sup>®</sup> Primer, including how to automatically design attB1/attR oligonucleotide primers, simulate BP and LR reactions, and also simulate Multiple Gateway cloning.

### Introduction

Gateway<sup>®</sup> Primer provides all of the tools required for primer design and in silico simulation of Gateway<sup>®</sup> cloning.

Thermo Fisher Scientific describe their Gateway<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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

**Sebastian Brünink**



## Gateway Cloning Handbook:

**Handbook of Biochemistry and Molecular Biology** Roger L. Lundblad, Fiona Macdonald, 2018-06-14 Edited by renowned protein scientist and bestselling author Roger L Lundblad with the assistance of Fiona M Macdonald of CRC Press this fifth edition of the Handbook of Biochemistry and Molecular Biology gathers a wealth of information not easily obtained including information not found on the web Presented in an organized concise and simple to use format this popular reference allows quick access to the most frequently used data Covering a wide range of topics from classical biochemistry to proteomics and genomics it also details the properties of commonly used biochemicals laboratory solvents and reagents An entirely new section on Chemical Biology and Drug Design gathers data on amino acid antagonists click chemistry plus glossaries for computational drug design and medicinal chemistry Each table is exhaustively referenced giving the user a quick entry point into the primary literature New tables for this edition Chromatographic methods and solvents Protein spectroscopy Partial volumes of amino acids Matrix Metalloproteinases Gene Editing Click Chemistry [Development of Circadian Clock Functions, volume II](#) Daisuke Ono, Takahiro J. Nakamura, Jeff Jones, Jihwan Myung, Rae Silver, 2024-07-31 The temporal order of physiological functions such as sleep wakefulness is regulated by the circadian clock This intrinsic clock starts ticking in the embryo and matures during development with attenuation of the clock function in the elderly illustrated by attenuation of synchrony entrainment and outputs of cellular circadian rhythms in the SCN This age related diminution can contribute to the emergence of diseases such as sleep disorders infertility diabetes mental disorders etc Over the course of our lives a variety of internal and external factors are under the influence of the circadian clock This inherent developmental plasticity of the circadian system is critical for the establishment of normal bodily functions to adapt to the changing environment on earth In mammals the central circadian clock is located in the suprachiasmatic nucleus SCN of the hypothalamus Circadian rhythms in the SCN are observed during the embryonic period and input and output pathways from the SCN are formed after birth During postnatal development functions of neurotransmitters in the SCN are changed which have an influence on special temporal patterns of circadian rhythms in the SCN and outputs signals to the outside of the SCN During postnatal development properties of circadian rhythms in the SCN and its inputs and outputs can be modulated by environmental light conditions Recent optical manipulation and imaging techniques have revealed that cell type specific functions for circadian rhythms However it remains unclear which cell types or networks in the brain are modulated during postnatal development and how these cells regulate circadian rhythms depending on postnatal period [IP Multimedia Subsystem \(IMS\) Handbook](#) Mohammad Ilyas, Syed A. Ahson, 2018-10-03 Take Part in the Future of Wireless Wireline Convergence The IP multimedia subsystem IMS established as the foundation for future wireless and wireline convergence is the bedrock that will facilitate easy deployment on new rich personalized multimedia communication services that mix telecom and data services Designers planners and researchers of communication systems will need to make full use of the

technology occurring with this convergence if they want to be the ones providing end users with new and efficient services that are as cost effective as they are innovative To provide researchers and technicians with the tools they need to optimize their role in this communication revolution the IP Multimedia Subsystem IMS Handbook presents all the technical aspects of the IMS needed to support the growth of digital traffic and the implementation of underlying networks This guide covers everything from basic concepts to research grade material including the future direction of the architecture Organized in three sections the book brings together the technical savvy of 50 pioneering experts from around the world providing complete coverage of relevant concepts technologies and services Learn How IMS Will Speed Innovation Filling the gap between existing traditional telecommunications and Internet technologies IMS has led to an environment in which new services and concepts are introduced more quickly than ever before such as reusable service components and real time integration The technology promises to be a cost effective evolutionary path to future wireless and wireline convergences that will meet next generation service requirements

**Integrated Genomics** Guy A. Caldwell, Shelli N. Williams, Kim A. Caldwell, 2006-08-04 Integrated Genomics A Discovery Based Laboratory Course introduces the excitement of discovery to the basic molecular biology laboratory Utilizing up to date molecular biology protocols and a basic experimental design this text offers experience with three different model systems Students will become familiar with the simplicity and power of single celled organisms Escherichia coli and Saccharomyces cerevisiae as they search for genes that interact and function within the nematode Caenorhabditis elegans Incorporated throughout the course are exercises designed to offer students familiarity with the wealth of bioinformatics data that can be accessed on the World Wide Web Following completion of interaction studies within the yeast the course is designed to allow students to examine the functional consequences of reducing a gene s function within the multicellular worm that is both simple and inexpensive to maintain within a laboratory The inclusion of alternative experiments allow for flexibility in determining the ending date or goal of the laboratory as well as working within the available budget and resources of most any classroom environment Further striking features of this title are An accompanying Web site providing PowerPoint slides plus links to the internet and regular updates as bioinformatics databases evolve and methods improve www.wiley.com/go/caldwell Inclusion of modern genomic proteomic technologies such as the yeast two hybrid system and RNAi Detailed experimental protocols and easy access to instructional materials This discovery based laboratory course provides excellent practical training for those pursuing career paths in biomedicine pharmacy and biotechnology

**RNAi** Martin Latterich, 2008-09-04 A key feature of RNAi is the highlighting of the pitfalls that can occur and how to minimize them The book also contains a complete list of abbreviations as there are many confusing terms associated with RNAi Jacket

**PeopleSoft PeopleTools Data Management and Upgrade Handbook** Paula Dean, Jim J. Marion, 2012-09-04 Master Oracle s PeopleSoft PeopleTools Data Administration and Upgrade Toolset Maximize data integrity maintain peak application performance and keep your PeopleSoft environment up to date PeopleSoft

PeopleTools Data Management and Upgrade Handbook explains the latest techniques and provides best practices case studies and programming examples Find out how to develop and deploy data mover scripts audit database health apply patches and generate project comparison reports This Oracle Press guide offers thorough coverage of Oracle s PeopleSoft life cycle management tools Understand PeopleSoft PeopleTools architecture Manage metadata using PeopleSoft Application Designer Create and execute PeopleSoft Data Mover scripts Learn how to use PeopleSoft Data Archive Manager Read best practices for applying updates patches and fixes Implement PeopleSoft change control features Build automated functional tests Plan and execute application and platform upgrades

**Student Handbook** Southwestern,2005 The Student Handbook is designed to provide students with ready access to information with problem solving techniques and study skill guides that enable them to utilize the information in the most efficient manner Amazon com

**Hoover's Handbook of Emerging Companies 2004** Hoover's, Incorporated,2004-04 [Hoover's Handbook of American Business](#) Hoovers Inc,2006-12

**The Internet Searcher's Handbook** Peter Morville,Louis Rosenfeld,Joseph Janes,1996 Here is a practical step by step guide to resource discovery designed to be both a tutorial for how and a resource for now The handbook helps novice or advanced Internet users conduct comprehensive research investigations and find the answers to quick reference queries It shows how to find information on the Internet by providing in depth coverage on all useful and usable resources including virtual libraries Internet directories communities of people and Internet search tools Examples of real searches are given for each resource Methods to streamline locating information and addresses and instructions for a broad but selective list of Internet subject guides directories and search engines are also offered Additional sections including a review of other types of information resources such as electronic discussion lists and conferencing tools round out this useful manual that is ideal for reference librarians students and researchers of any kind

*Gene Function Analysis* Michael F. Ochs,2007-08-23

With the advent of high throughput technologies following completion of the human genome project and similar projects the number of genes of interest has expanded and the traditional methods for gene function analysis cannot achieve the throughput necessary for large scale exploration This book brings together a number of recently developed techniques for looking at gene function including computational biochemical and biological methods and protocols

**The New Walford Guide to Reference Resources** Ray Lester,2005 The New Walford highlights the best resources to use when undertaking a search for accurate and relevant information saving you precious time and effort For those looking for a selective and evaluative reference resource that really delivers on its promise look no further In addition to print sources The New Walford naturally covers an extensive range of e reference sources such as digital databanks digital reference services electronic journal collections meta search engines networked information services open archives resource discovery services and websites of premier organizations in both the public and private sectors But rather than supplying a list of all available known resources as a web search engine might The New Walford subject specialists have carefully selected and evaluated

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**Reference & User Services Quarterly**, 2002. **Molecular and Genetic Characterization of Acp29AB and Identification of Acp Interactors in Drosophila Melanogaster** Shannon Noelle Albright, 2004. [Science](#) John Michels (Journalist), 2002. [Medical and Health Care Books and Serials in Print](#), 1986. [Short Protocols in Protein Science](#) John E. Coligan, 2003-10-24. Short Protocols in Protein Science provides condensed descriptions of more than 500 protocols compiled from Current Protocols in Protein Science. Drawing from both the original core manual as well as the quarterly update service, this compendium includes all step-by-step descriptions of the principal methods covered in Current Protocols in Protein Science.

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## **Table of Contents Gateway Cloning Handbook**

1. Understanding the eBook Gateway Cloning Handbook
  - The Rise of Digital Reading Gateway Cloning Handbook
  - Advantages of eBooks Over Traditional Books
2. Identifying Gateway Cloning Handbook
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Gateway Cloning Handbook
  - User-Friendly Interface
4. Exploring eBook Recommendations from Gateway Cloning Handbook
  - Personalized Recommendations
  - Gateway Cloning Handbook User Reviews and Ratings
  - Gateway Cloning Handbook and Bestseller Lists
5. Accessing Gateway Cloning Handbook Free and Paid eBooks
  - Gateway Cloning Handbook Public Domain eBooks
  - Gateway Cloning Handbook eBook Subscription Services
  - Gateway Cloning Handbook Budget-Friendly Options

6. Navigating Gateway Cloning Handbook eBook Formats
  - ePub, PDF, MOBI, and More
  - Gateway Cloning Handbook Compatibility with Devices
  - Gateway Cloning Handbook Enhanced eBook Features
7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Gateway Cloning Handbook
  - Highlighting and Note-Taking Gateway Cloning Handbook
  - Interactive Elements Gateway Cloning Handbook
8. Staying Engaged with Gateway Cloning Handbook
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Gateway Cloning Handbook
9. Balancing eBooks and Physical Books Gateway Cloning Handbook
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Gateway Cloning Handbook
10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
11. Cultivating a Reading Routine Gateway Cloning Handbook
  - Setting Reading Goals Gateway Cloning Handbook
  - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Gateway Cloning Handbook
  - Fact-Checking eBook Content of Gateway Cloning Handbook
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
  - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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