

Review

Principles of Genetic Engineering

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Abstract: Genetic engineering is the use of molecular biology technology to modify DNA sequence(s) in genomes, using a variety of approaches. For example, homologous recombination can be used to target specific sequences in mouse embryonic stem (ES) cell genomes or other cultured cells, but it is cumbersome, poorly efficient, and relies on drug positive/negative selection in cell culture for success. Other routinely applied methods include random integration of DNA after direct transfection (microinjection), transposon-mediated DNA insertion, or DNA insertion mediated by viral vectors for the production of transgenic mice and rats. Random integration of DNA occurs more frequently than homologous recombination, but has numerous drawbacks, despite its efficiency. The most elegant and effective method is technology based on guided endonucleases, because these can target specific DNA sequences. Since the advent of clustered regularly interspaced short palindromic repeats or CRISPR/Cas9 technology, endonuclease-mediated gene targeting has become the most widely applied method to engineer genomes, supplanting the use of zinc finger nucleases, transcription activator-like effector nucleases, and meganucleases. Future improvements in CRISPR/Cas9 gene editing may be achieved by increasing the efficiency of homology-directed repair. Here, we describe principles of genetic engineering and detail: (1) how common elements of current technologies include the need for a chromosome break to occur, (2) the use of specific and sensitive genotyping assays to detect altered genomes, and (3) delivery modalities that impact characterization of gene modifications. In summary, while some principles of genetic engineering remain steadfast, others change as technologies are ever-evolving and continue to revolutionize research in many fields.

Keywords: CRISPR/Cas9; embryonic stem (ES) cells; genetic engineering; gene targeting; homologous recombination; microinjection; retroviruses; transgenic mice; transgenic rats; transposons; vectors

1. Introduction

Since the identification of DNA as the unit of heredity and the basis for the central dogma of molecular biology [1] that DNA makes RNA and RNA makes proteins, scientists have pursued experiments and methods to understand how DNA controls heredity. With the discovery of molecular biology tools such as restriction enzymes, DNA sequencing, and DNA cloning, scientists quickly turned to experiments to change chromosomal DNA in cells and animals. In that regard, initial experiments that involved the co-incubation of viral DNA with cultured cell lines progressed to

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Table of Contents Direct Reading Chapter 11 Section Genetic Engineering

1. Understanding the eBook Direct Reading Chapter 11 Section Genetic Engineering
 - The Rise of Digital Reading Direct Reading Chapter 11 Section Genetic Engineering
 - Advantages of eBooks Over Traditional Books
2. Identifying Direct Reading Chapter 11 Section Genetic Engineering
 - 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 Direct Reading Chapter 11 Section Genetic Engineering
 - User-Friendly Interface
4. Exploring eBook Recommendations from Direct Reading Chapter 11 Section Genetic Engineering
 - Personalized Recommendations
 - Direct Reading Chapter 11 Section Genetic Engineering User Reviews and Ratings
 - Direct Reading Chapter 11 Section Genetic Engineering and Bestseller Lists
5. Accessing Direct Reading Chapter 11 Section Genetic Engineering Free and Paid eBooks

- Direct Reading Chapter 11 Section Genetic Engineering Public Domain eBooks
 - Direct Reading Chapter 11 Section Genetic Engineering eBook Subscription Services
 - Direct Reading Chapter 11 Section Genetic Engineering Budget-Friendly Options
6. Navigating Direct Reading Chapter 11 Section Genetic Engineering eBook Formats
- ePub, PDF, MOBI, and More
 - Direct Reading Chapter 11 Section Genetic Engineering Compatibility with Devices
 - Direct Reading Chapter 11 Section Genetic Engineering Enhanced eBook Features
7. Enhancing Your Reading Experience
- Adjustable Fonts and Text Sizes of Direct Reading Chapter 11 Section Genetic Engineering
 - Highlighting and Note-Taking Direct Reading Chapter 11 Section Genetic Engineering
 - Interactive Elements Direct Reading Chapter 11 Section Genetic Engineering
8. Staying Engaged with Direct Reading Chapter 11 Section Genetic Engineering
- Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Direct Reading Chapter 11 Section Genetic Engineering
9. Balancing eBooks and Physical Books Direct Reading Chapter 11 Section Genetic Engineering
- Benefits of a Digital Library
 - Creating a Diverse Reading Collection Direct Reading Chapter 11 Section Genetic Engineering
10. Overcoming Reading Challenges
- Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Direct Reading Chapter 11 Section Genetic Engineering
- Setting Reading Goals Direct Reading Chapter 11 Section Genetic Engineering
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Direct Reading Chapter 11 Section Genetic Engineering
- Fact-Checking eBook Content of Direct Reading Chapter 11 Section Genetic Engineering
 - 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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