Title: Recombineering and Gene Targeting

Abstract: One of the most widely used tools in modern biology is molecular cloning with restriction enzymes, which create compatible ends between DNA fragments that allow them to be joined together. However, this technique has certain restrictions that limit its applicability for large or complex DNA construct generation. A newer technique that addresses some of these shortcomings is recombineering, which modifies DNA using homologous recombination (HR), the exchange between different DNA molecules based on stretches of similar or identical sequences. Together with gene targeting, which takes advantage of endogenous HR to alter an organism’s genome at a specific loci, HR-based cloning techniques have greatly improved the speed and efficacy of high-throughput genetic engineering.

In this video, we introduce the principles of HR, as well as the basic components required to perform a recombineering experiment, including recombination-competent organisms and genomic libraries such as bacterial artificial chromosomes (BAC). We then walk through a protocol that uses recombineering to generate a gene-targeting vector that can ultimately be transfected into embryonic stem cells to generate a transgenic animal. Finally, several applications that highlight the utility and variety of recombineering techniques will be presented.

Application videos:

1. Identification of Protein Complexes in *Escherichia coli* using Sequential Peptide Affinity Purification in Combination with Tandem Mass Spectrometry **(4057 Thumbnail @1:56 – recombineering schematic)**

Description: Recombineering, often carried out in bacteria engineered to express the components of the λ phage Red recombination system, can be used to directly and efficiently modify the bacterial genome, as researchers show in this protocol. A cassette containing an affinity tag for biochemical purification, as well as an antibiotic-resistance gene as a selection marker, were amplified and transformed into a Red-expressing bacterial strain. Induction of recombination led to integration of the cassette into a target gene, which leads to the production of a modified protein with the affinity tag at its carboxy terminus. The modified protein was then purified using affinity chromatography, and protein interaction partners of the target protein were identified via mass spectrometry.

2. Genetic Manipulation in Δku80 Strains for Functional Genomic Analysis of *Toxoplasma gondii* **(50598 Thumbnail @0:33 – gene-targeting schematic)**

Description: The development of recombination-based engineering techniques has greatly facilitated the ability to genetically manipulate “non-model” organisms – those that are not widely used in standard genetic studies but yet often have important medical implications. In this video, a recombineered vector was used for gene targeting in the obligate intracellular parasite *Toxoplasma gondii*, which causes the disease toxoplasmosis. The vector in this case was generated in yeast, a naturally recombination competent organism. This targeting vector was then introduced into strains of the parasite that favor homologous, rather than nonhomologous, recombination of DNA fragments into its genome, which enhanced the ability of scientists to target modifications to specific genomic locations. Together, these experimental innovations made it easier to study gene deletions in *Toxoplasma*, facilitating the study of gene functions and the development of therapeutics.

3. Subcloning Plus Insertion (SPI) - A Novel Recombineering Method for the Rapid Construction of Gene Targeting Vectors **(52155 Thumbnail @1:26 – Recombineering strategy schematic)**

Description: Here, a novel recombineering strategy designed to increase the speed and efficiency with which gene-targeting vectors can be generated is presented. Retrieval of genomic sequence from BAC DNA, as well as insertion of cassettes containing selection markers, occur simultaneously in the subcloning plus insertion (SPI) protocol. This concurrent reaction replaces the iterative steps of transformation and induction of recombination in traditional recombineering, greatly reducing construct generation time.

4. Site-specific Bacterial Chromosome Engineering: ΦC31 Integrase Mediated Cassette Exchange (IMCE) **(3698 Thumbnail @1:14 – IMCE schematic)**

Description: Site-specific recombination (SSR) is another form of genetic recombination, where genetic exchange is facilitated by short sequences that are recognized by enzymes known as recombinases. This mechanism has also been harnessed for use in genetic engineering. In this article, researchers demonstrate a technique known as integrase mediated cassette exchange, which uses a bacteriophage recombinase enzyme – ΦC31 integrase – to quickly and efficiently make targeted modifications to DNA. By engineering into the DNA of a “recipient” bacterial strain a “landing pad” flanked by two recombinase recognition sites (RRS), and then mating it with “donor” strains with RRS-flanked DNA cassettes of interest, the integrase facilitates the “swapping” of the target sequence with the desired insert DNA.

5. The MultiBac Protein Complex Production Platform at the EMBL **(50159 Thumbnail @0:20 – MultiBac schematic)**

Description: Here, we see the use of various recombination-based cloning techniques to generate a BAC designed to simultaneously express multiple protein subunits of a protein complex of interest for functional and structural characterization. First, researchers designed and generated individual plasmids containing the coding region for each protein. These are distributed amongst various “donor” and “acceptor” plasmids containing sites for Cre-mediated SSR, and addition of Cre recombinase to this mixture concatenates these individual plasmids into one multi-expression construct. This construct is then integrated into the BAC via Tn7 integration, which takes advantage of the ability of a particular transposon (a mobile genetic element) to insert into bacterial DNA at a specific site. This BAC is finally packaged into baculovirus particles and used to infect insect cells, where the protein complexes are expressed and can be purified and characterized.

Related Videos

5060 – Bacterial Transformation: Electroporation

5062 – Plasmid Purification

5327 – Genetic Engineering of Model Organisms

5552 – Introduction to Genetic Engineering