

DNA Cloning

How bacterial plasmids are used to clone genes for biotechnology (see fig. 20.1)

Isolation of plasmid DNA and DNA containing the gene of interest

Insertion of gene of interest into plasmid to form recombinant DNA

Introduction of recombinant plasmid into a bacterial cell to form recombinant bacterium

Cloning of recombinant bacterium containing the gene of interest

Identification of desired clone

Various applications such as the production of recombinant organisms or the production of useful proteins

Using a restriction enzyme and DNA ligase to make recombinant DNA (see fig. 20.2)

Restriction enzymes cut DNA asymmetrically at restriction sites (palindromes) giving rise to DNA with single-stranded sticky ends

If DNA fragments from different sources cut by the same restriction enzyme are mixed together, various combinations will be formed as fragments base pair via the nucleotides in their sticky ends

DNA ligase catalyzes the formation of covalent bonds to seal the strands

A closer look at cloning a human gene in a bacterial plasmid (see fig. 20.3)

Isolation of vector and gene-source DNA

The plasmid to be used, from the bacterium *E. coli*, has three important characteristics

The *amp^R* gene, which confers resistance to the antibiotic ampicillin on its *E. coli* host cell

The *lacZ* gene, encoding the enzyme β -galactosidase, which hydrolyzes the sugar lactose and also hydrolyzes X-gal to a blue-colored product

A single restriction site for the restriction enzyme being used, the site located within the *lacZ* gene

The DNA containing the gene of interest is isolated from cultured human cells

Insertion of human DNA into the vector

The plasmids and human DNA are cut with the same restriction enzyme

Each plasmid is cut at the single restriction site, giving rise to an open circle with two sticky ends and a disrupted *lacZ* gene

Each complement of human DNA is cut at numerous restriction sites giving rise to thousands of fragments, almost all of which have two sticky ends and one of which carries the gene of interest

The human DNA fragments are mixed with the clipped plasmids giving rise to many combinations of fragments and plasmids including the desired combination of a plasmid and the human fragment containing the gene of interest

DNA ligase is used to join the DNA molecules by covalent bonds

Many recombinant molecules are formed, including the desired recombinant plasmid containing the gene of interest and a disrupted *lacZ* gene

Introduction of the cloning vector into bacterial cells

Recombinant DNA, including the desired recombinant plasmid, is mixed with *lacZ*⁻ (cannot hydrolyze lactose or X-gal) *E. coli* cells, which take up DNA from the surrounding solution in a process called transformation

Some bacteria acquire the desired recombinant plasmid DNA

Cloning of cells along with foreign genes

Bacterial cells are plated out on solid nutrient medium containing ampicillin and the sugar X-gal

Bacterial cells capable of reproducing form cell clones that appear as colonies on the nutrient medium, and any human genes carried by recombinant plasmids are cloned at the same time

Only plasmid-containing cells can grow, because they are the only cells that have the *amp*^R gene conferring ampicillin resistance

Plasmids that carry foreign DNA have a disrupted, nonfunctional *lacZ* gene, and the bacteria themselves are *lacZ*⁻; therefore, bacteria containing recombinant plasmids do not produce β-galactosidase and cannot hydrolyze X-gal

When X-gal is hydrolyzed by β -galactosidase, it yields a blue product; therefore, colonies of cells containing non-recombinant plasmids (with an intact *lacZ* gene) will be blue, while colonies containing recombinant plasmids (with a nonfunctional *lacZ* gene) will be clear

Identification of cell clones carrying the gene of interest

If at least part of the nucleotide sequence of the gene of interest is known, an RNA or DNA probe complementary to it can be synthesized and used to identify cells carrying the gene (see fig. 20.4)

Bacterial colonies containing cloned segments of foreign DNA are grown on agar, and some cells from each colony are transferred to special filter paper by pressing the filter against the colonies

The filter is treated to break open the cells and denature the DNA, and single-stranded DNA sticks to the filter paper

A solution of probe molecules labeled with a radioactive isotope is incubated with the filter, the probe DNA hybridizes (base pairs) with any complementary DNA on the filter, and excess DNA is rinsed off

The filter is placed on photographic film, and any radioactive areas on the filter expose the adjacent film (autoradiography)

The developed film (autoradiograph) is compared to the master culture plate to locate colonies carrying the gene of interest

If the gene of interest can be translated into protein, it is possible to screen the colonies for production of the protein

If the objective was to fully sequence the gene of interest, large numbers of cells containing the gene can be grown in liquid culture in a large tank, and then large amounts of the gene can be isolated from the cells and analyzed

If the objective was to have bacterial cells make large quantities of a eukaryotic protein, it is necessary to produce a modified DNA and insert it into an expression vector

Eukaryotic genes contain long noncoding regions (introns), and bacteria do not contain the RNA-splicing machinery necessary for processing the primary transcript into mature mRNA

This problem can be overcome by producing complementary DNA (see fig. 20.5)

From eukaryotic cells that produce the desired protein, mature mRNA molecules are isolated after primary transcripts have been modified by splicing out the introns

The mRNA molecules are incubated with deoxyribonucleotides and reverse transcriptase to produce a complementary DNA strand

The RNA is then degraded, and DNA polymerase is used to synthesize a second DNA strand complementary to the first

The result is a double stranded complementary DNA (cDNA) molecule, which contains only exons and no introns

Molecules of cDNA can be cloned and translated into protein by inserting them into an expression vector, a plasmid with a prokaryotic promoter just upstream of the restriction site where the cDNA gene is inserted, and inserting the vector into bacterial cells that can be cultured in large quantity

It is also possible to use eukaryotic rather than prokaryotic cells for cloning and expressing eukaryotic genes

Yeasts are single-celled fungi that are easy to grow in culture and have plasmids

If the objective is to obtain a large quantity of DNA from a small sample so that various types of testing and(or) analysis can be done, it is possible to amplify the desired DNA fragment(s) without using cells

Polymerase Chain Reaction (PCR) is a method for making many copies of a specific segment of DNA (see fig. 20.7)

PCR requires a heat-resistant DNA polymerase, a supply of all four deoxyribonucleotides, and primers, which are synthetic molecules of single-stranded DNA complementary to the ends of the targeted DNA

The DNA is heated to separate the strands

It is then cooled to allow the primers to bind to the ends of the target sequence, one primer on each strand

DNA polymerase adds nucleotides to the 3' ends of the primers and new strands are synthesized along the original strands in the mandatory 5' → 3' direction

Each time the cycle is repeated, the number of molecules doubles

The cycle is run again and again until the targeted sequence has been duplicated enough times

DNA Libraries

Cloning of genes in plasmid vectors begins with a mixture of fragments from the entire genome of the organism being studied; therefore, thousands of different recombinant plasmids are produced and a clone of each ends up in a clear colony (see fig. 20.3)

These recombinant plasmid-containing bacterial clones can be maintained in culture as a genomic library, which can be used as a source of other genes of interest or for genome mapping (see fig. 20.6)

It is also possible to splice fragments of foreign DNA into a bacteriophage genome, package recombinant phage DNA into capsids, infect bacterial cells with them, and produce new phage particles containing the foreign DNA

These phage clones can be maintained in culture as a genomic library (see fig. 20.6)

cDNA libraries can be made as well, but these represent only the genes being transcribed in the starting cells; this is an advantage for studying genes responsible for specialized functions of particular kinds of cells or for studying changes in patterns of gene expression in a particular type of cell at different times in the life of an organism

This lecture outline was prepared mainly from *Biology*, by Campbell and Reece, 2002 (6th edition), and may contain phrases or entire sentences taken verbatim from that source.