SCREENING AND SELECTION FOR RECOMBINANTS

The art of cloning is to find the one particular transformed cell that contains the cloning vector with the gene of interest (referred to as a recombinant cell). It is thus most important to be able to select recombinant cells from transformed cells (containing the vector without insert DNA) (Brown, 1990). If this goal has been achieved the gene in question is said to have been cloned.

Although there are many different procedures by which the desired clone can be obtained, all are variations of two basic concepts (Brown, 1990):

- **Direct selection**, which means that the cloning experiment is designed in such a way that the clones obtained are the clones containing the required gene. Almost invariably, selection occurs at the plating-out stage. Direct selection is the method of choice, as it is quick and usually unambiguous. However, it is not applicable to all genes.

- **Identification of the clone from a gene library**, which entails an initial “shotgun” cloning experiment, to produce a clone library representing all or most of the genes present in the cell, followed by analysis of the individual clones to identify the correct one.

**Direct selection:**

To be able to select for a cloned gene, it is necessary to plate the transformants on to an agar medium on which only the desired recombinants, and no others, can grow. The only colonies that will appear will, therefore, comprise cells that contain the desired recombinant DNA molecule. Most cloning vectors are designed so that the insertion of a DNA fragment into the vector destroys the integrity of one of the genes present on the molecule. This is referred to as insertional inactivation (Brown, 1990). Two examples will be discussed: (i) direct antibiotic resistance screening, and (ii) blue-white colour screening.

**Direct antibiotic resistance screening**

Most cloning vectors are designed so that the insertion of a DNA fragment into the vector destroys the integrity of one of the genes present on the molecule (usually an antibiotic resistance gene). For instance, if the vector carries an ampicillin resistance gene (amp

This would mean that when the clones are plated out on ampicillin-containing medium, only cells containing a plasmid would be resistant to the antibiotic and, therefore, be able to grow. However, this does not show whether the cell contains the recombinant plasmid (with the
inserted gene of interest) or only the re-ligated vector (without the inserted gene of interest). Figure 7 shows a diagram of screening for recombinants by using direct antibiotic resistance.

**Figure 7: Screening for recombinants using direct antibiotic resistance screening.**

**Blue-white colour screening**

A more sophisticated procedure for screening for the presence of recombinant plasmids, which can be carried out on a single transformation plate, is called blue-white screening. This method also involves the insertional inactivation of a gene and uses the production of a blue compound as an indicator (Brown, 1990). The gene is \( \text{lacZ} \), which encodes the enzyme \( \beta \)-galactosidase, and is under the control of the \( \text{lac} \) promoter. If the host \( E. coli \) strain is expressing the \( \text{lac} \) repressor, expression of a \( \text{lacZ} \) gene on the vector may be induced using IPTG (isopropyl- \( \beta \)-D-thiogalactopyranoside), and the expressed enzyme can utilize the synthetic substrate X-gal (5-bromo-4-chlore-3-indolyl- \( \beta \)-D-galactopyranoside) to yield a blue product. Insertion of a DNA fragment into the \( \text{lacZ} \) gene (insertional inactivation of \( \text{lacZ} \)) in the production of a recombinant plasmid would prevent the development of the blue colour. In this method, the transformed cells are spread onto a plate containing an antibiotic (to select for transformants in the usual way), IPTG and X-gal, to yield a mixture of blue and white colonies. The white colonies have no expressed \( \beta \)-galactosidase and are hence likely to contain the inserted target fragment. The blue colonies probably contain religated vector.
Identification of the clone from a gene library:

Many different techniques are available for screening a library. The most important approaches involve screening by nucleic acid hybridization and screening by functional analysis. Nucleic acid hybridization requires some prior knowledge of the DNA sequence either of the gene to be cloned or of stretches of DNA in the vicinity of the gene to be cloned. Functional screening involves the use of expression vectors that allow cells containing the vector with the desired gene to express the corresponding protein. Under these circumstances, cells containing a vector with the desired gene can be identified by means of antibodies directed against the protein. Cells producing the desired gene product can also be identified in bioassays detecting protein activities, if these are available (http://www.copewithcytokines.de/).

Nucleic acid hybridization

Detection of an individual clone in a library can be achieved by employing strategies of nucleic acid hybridization in which short chemically synthesized labeled oligonucleotides (probes) are used to detect complementary sequences in individual cells or phages containing an insert. The success of colony or plaque hybridization will depend on the availability of a DNA molecule that can be used as probe. This probe must share at least a part of the sequence of the cloned gene. If the gene itself is not available, it can be derived, for example, from known protein sequences, or so-called degenerate oligonucleotides (mixtures of oligonucleotides that differ from each other by base substitutions at identical and/or different positions) can be used.

The first step of a hybridization screening experiment involves the transfer of the DNA in the plaque or colony to a nylon or nitrocellulose membrane. The bacteria on the membrane are lysed to release their DNA, and the DNA is denatured with alkali to produce single strands that are bounded to the membrane by heat treatment or UV irradiation. The membrane is then immersed in a solution containing a nucleic acid probe (usually radioactively labeled) and incubated to allow the probe to hybridize to its complementary sequence. After hybridization, the membrane is washed to remove unhybridized probe, and regions where the probe has hybridized are visualized with autoradiography (Turner et al., 1997). Figure 8 shows a schematic sketch of a hybridization screening experiment.
Figure 8: An example of hybridization probing (Hybridization screening of a plasmid cDNA library using a radioactive probe) (Adapted from: http://www.genetics.biol.ttu.edu/genetics/Lecture/red28.html).

Functional screening

The desired gene can also be identified by the activities of the encoded gene product. In this case, one uses a so-called expression library that has been established by cloning DNA (cDNA in the case of eukaryotes) fragments into special cloning vectors allowing the functional expression of cloned DNA fragments. Functional gene products and hence the desired clones can then be detected either by antibodies (immunological screening) or other ligands that specifically recognize the encoded proteins or by exploiting a bioactivity of the gene product, if known (Brown, 1990).

The procedure of immunological screening has similarities to hybridization screening, discussed before, though in this instance it is the protein encoded by the DNA (or cDNA) rather than the DNA itself that is detected on the membrane. The membrane is treated to covalently attach the protein, and immersed in a solution containing the antibodies. When the antibody has bound to its epitope, it is detected by other antibodies and/or chemicals that recognize it.

Chromosome walking

Often, a genomic clone may not include all of the sequence for a particular gene so it is necessary to isolate overlapping clones that cover the genomic region of interest. Such clones can be isolated using probes derived from the outlying ends of the clones in hand. This process is known as chromosome walking.