Blue-White Screening for Positive Bacterial Colonies/Clones.

Prepared by Ms Alex Aitken

Background

The blue-white screen is a screening technique that allows for the detection of successful ligations in vector-based gene cloning.
DNA of interest is ligated into a vector. The vector is then transformed into competent bacterial cells. The competent cells are grown in the presence of X-gal. If the ligation was successful, the bacterial colony will be white; if not, the colony will be blue. This technique allows for the quick and easy detection of successful ligation.
X-gal (5-bromo-4-chloro-indolyl-β-D-galactopyranoside) is an organic compound consisting of galactose linked to a substituted indole. X-gal is much used in molecular biology to test for the presence of an enzyme, β-galactosidase. X-gal is one of many indoxyl glycosides and esters that yield insoluble blue compounds similar to indigo as a result of enzyme-catalyzed hydrolysis in this case by β-galactosidase.β-galactosidase is a protein encoded by the *lacZ* gene of the *lac* operon, and it exists as a homotetramer in its active state.

Theory

In Molecular Biology a mutant β-galactosidase with its N-terminal residues 11—41 deleted (termed the ω-peptide) is unable to form a tetramer and is inactive. This mutant form of protein however may return fully to its active tetrameric state in the presence of an N-terminal fragment of the protein, the α-peptide.

In this method of screening, the host *E. coli* strain carries the *lacZ* deletion mutant (*lacZΔM15*} which contains the ω-peptide, while the vectors used carry the *lacZα* sequence which encodes the first 59 residues of β-galactosidase, the α-peptide. Neither are functional by themselves. However, when the two peptides are expressed together, as when a vector containing the *lacZα* sequence is transformed into a *lacZΔM15* cells, they form a functional β-galactosidase enzyme.

The blue/white screening method works by disrupting this α-complementation process. The vector carries within the *lacZα* sequence an internal multiple cloning site (MCS). This MCS within the *lacZα* sequence can be cut by restriction enzymes so that the foreign DNA may be inserted within the *lacZ*α gene, thereby disrupting the gene and thus production of α-peptide. Consequently, in cells containing the vector with an insert, no functional β-galactosidase may be formed.

The presence of an active β-galactosidase can be detected by X-gal within the agar plate. X-gal is cleaved by β-galactosidase to form 5-bromo-4-chloro-indoxyl, which then spontaneously dimerizes and oxidizes to form a bright blue insoluble pigment 5,5'-dibromo-4,4'-dichloro-indigo. This results in a characteristic blue colour in cells containing a functional β-galactosidase. Blue colonies therefore show that they may contain a vector with an uninterrupted *lacZα* (therefore no insert), while white colonies, where X-gal is not hydrolyzed, indicate the presence of an insert.

Practical considerations

The correct type of vector and competent cells are important considerations when planning a blue white screen. The vector must contain the *lacZ*α, and examples of such vectors are pUC19 and pBluescript and their derivitatives. The *E. coli* cell should contain the mutant *lacZ* gene and some of the commonly-used cells with such genotype are JM109, DH5α, and XL1-Blue.

Drawbacks

Some white colonies may not contain the desired recombinant vector for a number of reasons. The ligated DNA may not be the correct one, and it is possible for some linearized vector to be transformed, its ends "repaired" and ligated together such that no LacZα is produced and no blue colonies may be formed.

A colony with no vector at all will also appear white, and may sometimes appear as satellite colonies after the antibiotic used has been depleted. It is also possible that blue colonies may contain the insert. This occurs when the insert is "in frame" with the LacZα gene and a STOP codon is absent in the insert. This can lead to the expression of a fusion protein that is still functional as LacZα. The correct recombinant construct can sometimes give lighter blue colonies which may complicate its identification.

However as this is a recombinant insert mutant and therefore undesirable the fact that it is blue and not considered is adventitious.