Marker genes

Marker systems are tools for studying the transfer of genes into an experimental organism. In gene transfer studies, a foreign gene, called a transgene, is introduced into an organism, in a process called transformation. A common problem for researchers is to determine quickly and easily if the target cells of the organism have actually taken up the transgene. A marker allows the researcher to determine whether the transgene has been transferred, where it is located, and when it is expressed. Marker genes exist in two broad categories:

I. Selectable marker genes and II. Reporter genes.

Selectable Marker Genes:

The selectable marker genes are usually an integral part of plant transformation system. They are present in the vector along with the target gene. In a majority of cases, the selection is based on the survival of the transformed cells when grown on a medium containing a toxic substance (antibiotic, herbicide, antimetabolite). This is due to the fact that the selectable marker gene confers resistance to toxicity in the transformed cells, while the non- transformed cells get killed.

A large number of selectable marker genes are available and they are grouped into three categories—antibiotic resistance genes, antimetabolite marker genes and herbicide resistance genes.

(a) Antibiotic Resistance Genes

In many plant transformation systems, antibiotic resistance genes (particularly of *E. coli*) are used as selectable markers. Despite the plants being eukaryotic in nature, antibiotics can effectively inhibit the protein biosynthesis in the cellular organelles, particularly in chloroplasts.

Eg: Neomycin phosphotransferase II (npt II gene):

The most widely used selectable marker is npt II gene encoding the enzyme neomycin phospho-transferase II (NPT II). This marker gene confers resistance to the antibiotic kanamycin. The trans-formants and the plants derived from them can be checked by applying kanamycin solution and the resistant progeny can be selected.

(b) Antimetabolite Marker Genes

Eg: Dihydrofolate reductase (dhfr gene)

The enzyme dihydrofolate reductase, produced by dhfr gene is inhibited by the antimetabolite methotrexate. A mutant dhfr gene in mouse that codes for this enzyme which has a low affinity to

methotrexate has been identified. This dhfr gene fused with CaMV promoter results in a methotrexate resistant marker which can be used for the selection of transformed plants.

(c) Herbicide Resistance Markers

Eg: Enolpyruvylshikimate phosphate synthase (epsps/aroA genes):

The herbicide glyphosate inhibits photosynthesis. It blocks the activity of enolpyruvylshikimate phosphate (EPSP) synthase, a key enzyme involved in the biosynthesis of phenylalanine, tyrosine and tryptophan. Mutant strains of *Agrobacterium* and *Petunia hybrida* that are resistant to glyphosate have been identified. The genes epsps/aroA confer resistance to transgenic plants which can be selected.

Lists of selectable markers genes:

TABLE 49.3 A selected list of selectable marker genes used for gene transfer in plants, their source and substrates used for their selection

Selectable marker gene (encoded enzyme)	*Abbreviation*	*Source of gene*	*Substrate(s) used for selection*
Antibiotic resistance			
Neomycin phosphotransferase II	*nptII*	*E. coli*	Kanamycin, geneticin (G418)
Neomycin phosphotransferase III	*nptIII*	*Streptococcus faecalis*	Kanamycin, geneticin (G418)
Hygromycin phosphotransferase	*hpt/hyg*	*E. coli*	Hygromycin
Bleomycin resistance	*ble*	*E. coli*	Bleomycin
Aminoglycoside adenyltransferase	*aadA*	*Shigella flexneri*	Streptomycin, spectinomycin
Antimetabolite markers			
Dihydrofolate reductase	*dhfr*	Mouse	Methotrexate
Dihydropteroate synthase	*dhps/sul*	*E. coli*	Sulfonamides
Herbicide resistance			
Phosphinothricin acetyltransferase	*bar/pat*	*Streptomyces hygroscopicus/ S. viridochromogenes*	Glufosinate, L-phosphinothricin, Bialophos
Enolpyruvyl shikimate phosphate synthase	*epsps/aroA*	*Agrobacterium* sp/ *Petunia hybrida*	Glyphosate
Acetolactase synthase	*als*	*Arabidopsis* sp/maize/tobacco	Sulfonylureas
Glyphosate oxidoreductase	*gox*	*Achromobacter LBAA*	Glyphosate
Bromoxynil nitrilase	*bxn*	*Klebsiella pneumoniae*	Bromoxynil
Others			
β-Glucuronidase	*gus/uidA*	*E. coli*	Cytokinin glucuronide
Xylose isomerase	*xylA*	*Thermoanaerobcterium thermosulfurogenes*	Xylose
Mannose 6-phosphate isomerase	*pmi/manA*	*E. coli*	Mannose
Betaine aldehyde dehydrogenase	*badh*	Spinach	Betaine aldehyde

Reporter Genes:

A reporter gene may be regarded as the test gene whose expression can be quantified. The plant transformation can be assessed by the expression of reporter genes. In general, an assay for the reporter gene is carried out by estimating the quantity of the protein it produces or the final products formed. A selected list of the reporter genes along with the detection assays is given in Table and some of the important ones are discussed below.

Table 49.4 A selected list of reporter genes used for gene transfer in plants, their sources, and detection assays

Reporter gene (enzyme/protein encoded)	*Abbreviation*	*Source of gene*	*Detection assay*
Octopine synthase	*ocs*	*Agrobacterium tumefaciens*	Electrophoresis, chromatography
Nopaline synthase	*nos*	*Agrobacterium tumefaciens*	Electrophoresis, chromatography
β-Glucuronidase	*gus/uidA*	*E. coli*	Fluorometric or histochemical or colorimetric
Green fluorescent protein	*gfp*	*Aequorea victoria* (jelly fish)	Fluorescence
Luciferase (bacterial)	*luxA/luxB*	*Vibrio harveyi*	Bioluminescence
Luciferase (firefly)	*luc*	*Photonus pyralis*	Bioluminescence
Chloramphenicol acetyltransferase	*cat*	*E. coli*	Autoradiography

β-Glucuronidase (GUS gene)

β-Glucuronidase producing gene (gus/uidA) is the most commonly used reporter gene in assessing plant transformation for the following reasons:

i. β-Glucuronidase assays are very sensitive.

ii. Quantitative estimation of the enzyme can be done by fluorometric method (using substrate 4-methylumbelliferryl P-D-glucuronide which is hydrolysed to 4-methylumbelliferone).

iii. Qualitative data on the enzyme can be obtained by histochemical means (enzyme localization can be detected by chromogenic substance such as substrate X-gluc).

iv. No need to extract and identify DNA.

Green fluorescent protein (GFP gene)

Green fluorescent protein (GFP), coded by gfp gene, is being widely used in recent years. In fact, in many instances, GFP has replaced GUS since assays of GFP are easier and non-destructive. Thus, screening of even the primary transplants can be done by GFP which is not possible with other reporter genes.

Gene for GFP has been isolated from jelly fish *Aequorea victoria* which is a luminescent organism. The original gfp gene has been significantly modified to make it more useful as a reporter gene. GFP emits fluorescence which can be detected under a fluorescent microscope.

Luciferase

1) Bacterial luciferase (luxA/luxB genes)

The bacterial luciferase genes (luxA and luxB) have originated from *Vibrio harveyi*. They can be detected in some plant transformation vectors. The detection assay of the enzyme is based on the principle of bioluminescence. Bacterial luciferase catalyses the oxidation of long-chain fatty aldehydes that results in the emission of light which can be measured.

Firefly luciferase (luc gene)

The enzyme firefly luciferase, encoded by the gene luc, catalyses the oxidation of D-luciferin (ATP dependent) which results in the emission of light that can be detected by sensitive luminometers. The firefly luciferase gene, however, is not widely used as a marker gene since the assay of the enzyme is rather cumbersome.