Reporter Genes - Indicators of Transformation

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Article Summary: "A reporter or marker gene is a gene, which produces a specific phenotype, in turn enables the differentiation of the cells possessing this particular gene from those without this gene. Hence, the transformed cells can be selected easily among the thousands of non-transformed cells. Reporter genes are an invaluable tool to track .."

A reporter or marker gene is a gene, which produces a specific phenotype, in turn enables the differentiation of the cells possessing this particular gene from those without this gene. Hence, the transformed cells can be selected easily among the thousands of non-transformed cells. Reporter genes form specific protein products, which are easily detectable and quantifiable, sometimes even without destroying the tissue. Reporter genes are an invaluable tool to track and study another associated gene in bacterial and mammalian cell culture, animals and plants. One can easily find out the expression patterns of a gene within the cell by fusing its promoter with one of the several reporter genes and transfecting inside the living cells. So, it is very useful in for the monitoring and detection of plant transformation, for studying the activities of regulatory elements such as promoter and enhancer.

Features of an ideal reporter gene:-
- Easily quantifiable
- Relatively rapid degradation of the enzyme
- High signal-to-noise ratio (Low endogenous background)
- Should not be toxic to cells
- Products of the reporter gene should be resistant to the chemicals used in the processing
- Assay should be sensitive and reliable.

Types of reporter genes
Reporter genes are mainly of two types:

1) Scorable marker
2) Selectable marker

1) Scorable marker: - Expression of this kind of marker gene results in a quantifiable phenotype i.e., it will make the cells containing it to look different. The main principle behind the use of these reporter genes for the study of molecular processes in living cells means that in natural genes, synthetic modification have introduced in order to either simplify the detection of the product or to distinguish it from similar genes in the genome. These reporter genes were assayed at the level of protein.

Important examples are:
• **Chloramphenicol acetyl transferase (CAT)**
This gene was isolated from the transposon Tn9 of *E.coli* and codes for CAT enzyme, which catalyses the transfer of the acetyl groups from acetyl coenzyme A to chloramphenicol.

• **GUS (I2-glucuronidase)**
Predominant reporter used to study gene expression in plants, based on *E.coli* gene *uidA* encoding I2-glucuronidase enzyme, which catalyze hydrolysis of glucuronides. Transformed cells turn blue in the presence of substrate, X-gluc. The disadvantage associated with the use of GUS assay is the destruction of plant material.

• **Fire fly luciferase (LUC)**
Luc gene is isolated from *Photinus pyralis*. Luciferase enzyme confers the organism the ability to glow in the dark. The firefly luciferase catalyzes the bioluminescent oxidation of the luciferin in the presence of ATP, magnesium and oxygen:
Luciferin + ATP ? luciferyl adenylate +PPi
Luciferyl adenylate + oxygen ��' oxyluciferin + AMP + yellow-green light
This gene is not destructive to the plant and reflects real-time gene expression status of the transgenic tissue under investigation owing to its *in-vivo* short half life.

• **Green fluorescent protein (GFP)**
It is a small protein of 238 amino acids (26.9 KDa), first isolated from the jellyfish *Aequorea victori*. This reporter gene is used for the study of dynamic process (sub-cellular localization of proteins, etc.) inside the cell and to determine the zygosity of transgenic plants. It exhibits bright green fluorescence when irradiated with blue light. In 2008, Osamu Shimomura, Martin Chalfie, Roger Y. Tsien got Noble Prize for the discovery of GFP.

Other scorable markers include LacZ (I2-galactosidase).

2) Selectable marker: - The cells that contain this type of marker gene show the ability to survive under selective conditions. These selective conditions would otherwise result in the death of the cells lacking that specific gene.
Most commonly used selective agents are antibiotics. Out of the millions and billions of cells, only few of them get transformed by the foreign DNA. It is practically impossible to check every individual cell, so a selective agent is required to eliminate the non-transformed cells, leaving only the desired ones.

Usually, selectable markers are of two types
a) Antibiotic resistance marker (*nptII*, *hptII*, etc.)
• *nptII*: - Most commonly used neomycin phosphotransferaseII (*nptII*) gene is isolated from transposon Tn 5 (*E.coli* K12 strain). It encodes for aminoglycoside 3` phosphotransferase enzyme which inactivates a range of antibiotics such as kanamycin, neomycin, puromycin, etc.
• *hptII*: - Hygromycin phosphotransferase gene was isolated from *E.coli*, which codes for enzyme that inactivates the antibiotics, Hygromycin B; the latter is more toxic than kanamycin and kill sensitive cells more quickly.

b) Herbicide resistance marker (*bar* gene, *als* gene etc.)

• *Bar* gene
It was originally isolated from *Streptomyces hygroscopicus*, and confers resistance to the herbicide bialaphos (bar). This gene encodes phosphinothricin acetyl transferase (PAT) enzyme which acetylates phosphinothricin (PPT), a component of bialaphos. In normal cells, glutamine synthetase (GS) incorporates ammonia into protein. Thus maintain the level of ammonia in cells. PPT is a competitive inhibitor of GS, so its presence blocks the activity of latter. Consequently, PPT cells accumulate ammonia, which is toxic to cell. So, transfer of *bar* gene makes the plant resistance to PPT.
• Acetolactate Synthase gene (*als*)
This gene was isolated from *Arabidopsis thaliana* and encodes for acetolactate synthase enzyme that provides resistance against sulfonylurea. When *als* gene is transferred to crop of interest , it will become resistant to sulfonylurea.

Measurement of expression of reporter gene
• Enzyme activity assay of the expressed enzyme encoded by the reporter gene using chromo, fluoro, luminogenic substrate
• Immunological assay of the expressed protein encoded by the reporter gene
• Histochemical staining of cells or tissue typically to localize enzymatic activity expressed from reporter gene construct cells.

In conclusion, the marker genes can be used to identify a promoter, to study the expression pattern and strength of the promoter. They are used for the study of regulation of different cellular processes, in particular to study gene expression, protein localization and intracellular protein trafficking without the need of destroying the plants.

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