

## REPORTER GENES, GENE TRAPS, ENHANCER TRAPS

### Reporter genes:

- These genes aid in the determination of the function of the gene of interest by attaching its coding region to the gene's promoter region allowing the scientist to follow in on the gene's activity (i.e. synthesis of functional proteins), when the gene is turned on, it not only synthesizes proteins it synthesizes the reporter gene as well, thus a scientist can detect when and where the gene is active in the genome. Since reporter genes help explain the role of a particular gene by identifying when and where the proteins are produced, they are given the name reporter gene.
- The promoter region of the gene can be attached to the coding region of the reporter gene so that instead of protein production which is usually controlled by the promoter, now, the promoter region controls the production of the reporter proteins.

Steps involved in the attachment of reporter genes:

1. Attach the reporter gene DNA to a promoter region, and insert this into a vector
2. Insert vector into cell.
3. Select for transformant by screening for the selectable marker gene.

Advantages:

- Reporter genes allows the study of genes at various stages of development
- Reporter genes allows a way to visualize how any gene is regulated

Disadvantages:

- When reporter gene is inserted within the exon, it can interrupt the activity of the gene, and if the gene is part of a family, the other genes part of the family will take over the responsibility of the inactive gene, therefore, changes cannot be depicted in the genome.

### Selectable marker genes:

Scientists must be able to select cells that have been transformed.

Selecting cells is done by transforming the cells with the reporter gene plus an additional gene called a selectable marker gene. Selectable marker genes are genes that encode easily detectable traits making marked cells different from non-marked cells. The two most commonly used selectable marker genes encode the traits of herbicide and antibiotic resistance (ampicillin resistance - amp<sup>R</sup>).

### Gene trap:

- A technique used that **randomly** disrupts genes throughout the genome by inserting a DNA element, which contains a reporter gene and a selectable marker.
- These DNA elements are sometimes inserted into the endogenous gene so that the reporter will be expressed in a similar pattern as the endogenous gene. The disruption mutates the endogenous gene.
- Gene traps have splice acceptor sequences instead of having a promoter so that reporter gene's activity can occur only when that insertion is within a transcriptional unit and in the correct orientation.
- The gene's activity is monitored by the reporter gene when the reporter gene is inserted into an intron and or exon. If the reporter gene is inserted in between exons, the splice donating site of the exon and the splice accepting site of the reporter gene are spliced together and by this fusion the reporter gene is able to be transcribed because it is treated like an exon.
- If the reporter gene gets inserted into the correct frame of the exon, the reporter will function normally i.e it will produce a functioning tag. If however, the reporter gene gets inserted into the exon but not in the correct orientation or frame, the exon may produce proteins but the reporter tag will not be produced (DNA sequence will be read in a different frame - frame-shift mutation).

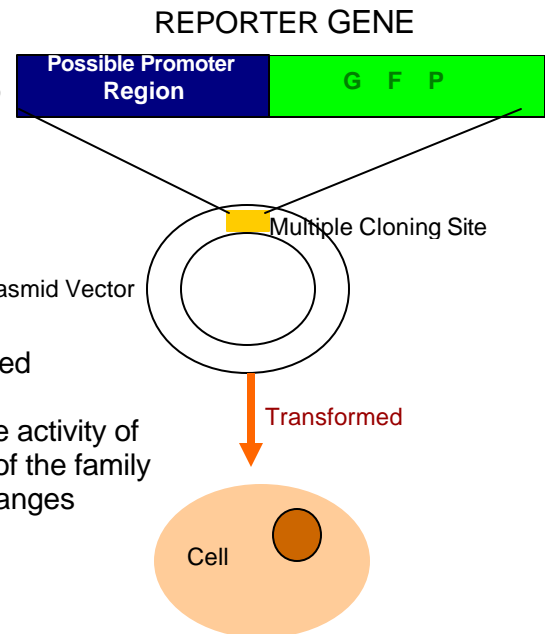
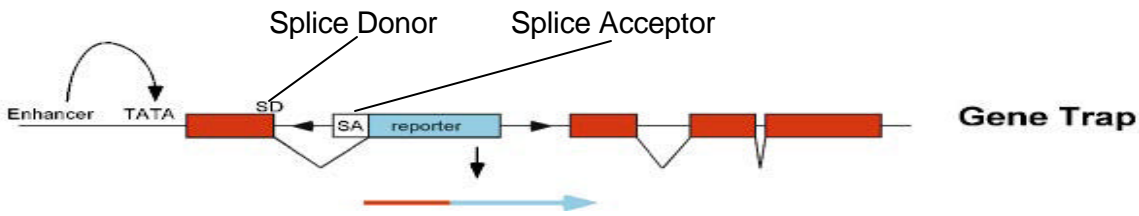


Figure 2: Structure of Gene trap with reporter inserted into an intron



(reference:<http://www.cepceb.ucr.edu/resources/pdf/springer/plantcell.pdf>)

**Advantages:**

- Any cell can be used for the technique of gene trap, this technique is not cell- specific
- Simultaneous identification and mutations of genes can be seen using the gene trap method.

**Disadvantages:**

- Due to random insertion, the DNA element or reporter gene can disrupt the gene activity (i.e produce inactive proteins
- Insertion could be lethal to the cell/organism

**Enhancer trap:**

- Enhancer traps allow the investigation of how and when enhancer DNA sequences can affect gene regulation, and also aid in the determination of their possible location.
- A technique that uses reporter genes fused to a minimal promoter, typically containing the TATA box and transcription start site. Minimal promoter is a promoter on the reporter gene that can only be activated by nearby enhancer sequences.
- This method uses reporter genes that can be inserted randomly within the genome. When a reporter gene is placed near the enhancer, the reporter gene product is detected within the cells.

**Steps involved in enhancer trap technique:**

1. Insertion of reporter gene (the reporter gene must have its own minimal promoter region).
2. Check for expression of reporter (expression indicates presence of a nearby enhancer).

**Advantages:**

- Due to the fact that a reporter gene is inserted into the DNA, it experiences the effect of the enhancer, and the effect the reporter gene experiences can be monitored and the role of the enhancer in that particular region can be determined.

**Disadvantages:**

- Since insertion of reporter gene is random, it could disrupt endogenous genes which may affect phenotype and gene expression.

