Genetic Engineering laboratory protocol

Cloning of Green Fluorescent Protein into *E.Coli* DH5α

Aim: To perform the basic concept of cloning and transformation using the GFP gene (Green Fluorescent Protein)

Principle: Molecular cloning refers to the process by which recombinant DNA molecules are produced and transformed into a host organism, where they are replicated. A molecular cloning reaction is usually comprised of two components:

1. The DNA fragment of interest to be replicated/expressed .

2. A vector/plasmid backbone that contains all the components for replication/expression in the host.

Cloning involves the following steps:

1. Isolation of vector and insert DNA.
2. Restriction digestion of vector and insert DNA.
3. Purification of digested of vector and insert by gel elution method.
4. Determination of the quantity of purified vector and insert.
5. Ligation of vector and insert in 3:1 molar ratio.
6. Transformation of ligated mixture in *E.Coli* DH5α competent cells.
7. Screening of recombinant clones.

In present experiment, digested and purified vector and insert DNA (GFP gene isolated from a bioluminescent jelly fish *Aequorea Victoria*) are provided to you. You have to perform ligation, transformation and screening.

Procedure:

Ligation of Vector to Insert

1. Thaw the Ligase Assay Buffer, vector and Insert DNA.

Note: Thaw the Ligase Assay Buffer vial on ice, store at -20^{0}C immediately after use.

2. Set up the ligation reaction as indicated in table 1:

Reagent	**Amount**
Water	11 µL
Vector DNA	2 µL
Insert DNA	4 µL
Ligase assay buffer	2 µL
T4 DNA ligase	1 µL
Total volume	20 µL

Table: 1

Mix the contents by tapping gently and incubate at 16˚C water bath, overnight.

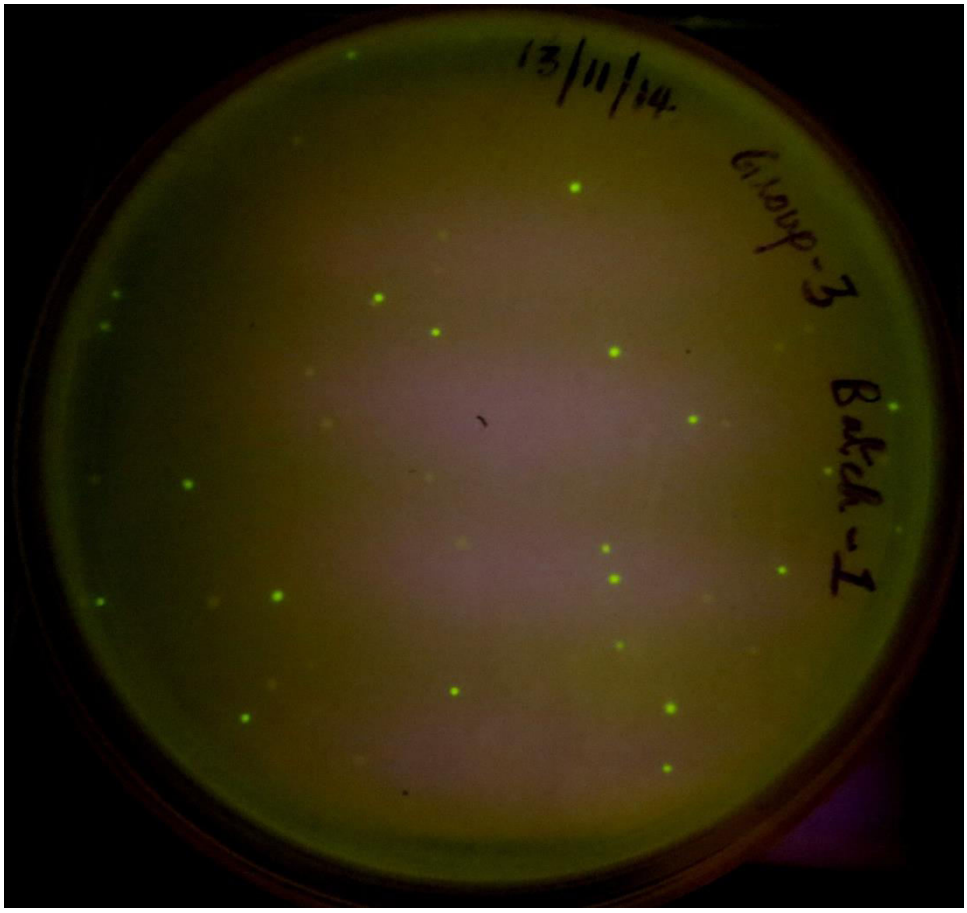
Preparation of competent cells: (As per the previous protocol and use the competent cells which are kept in -80^{0}C).

Transformation of ligated product in *E. coli* DH5α competent cells: (Follow the previous protocol). After transformation incubate the plate at 37^{o}C for overnight.

Next day: If ligation reaction is successfully done , you may observe bacterial colony in plate.

Screening: The plates will then be visualized under UV light to screen for clones. This is because, the unique 3 dimensional conformation of the GFP releases energy in the form of visible green light on exposure to UV light. Hence, only cells having the ligated vector and insert in the right orientation will glow. However, there will be other clones where in the insert is ligated in the reverse orientation resulting in no expression of the protein. These clones will not glow on exposure to UV light.

Observation:



GFP expression in *E.Coli* DH5α (The experiment was performed by B.Tech Biotechnology 2016 Batch, NIT Warangal)