STEPS OF CLONING

Exercise 1: to amplify a small fragment from the T70 gene in order to clone it into a cloning vector

Before amplification

After amplification

Exercise 2: to confirm the amplification of the target region in order to screen the amplified DNA before using the constructs in several experimental procedures

Before cloning

After cloning

Exercise 3: to transform the strain used to carry the amplified DNA before using the construct in several experimental procedures

Before transformation

After transformation

Propagation

Confirmation of transformation and propagation
What is cloning?
According to Paul Berg (Nobel Laureate):
"We use the word cloning in science as a term to describe the production of many copies of a starting material".

What is a clone?
Clone is a group of cells that stem from a single cell.

In order to clone a gene:
1. Isolate the DNA of interest.
2. Introduce the DNA into a cloning vector (such as a plasmid), thus creating a recombinant plasmid.

A plasmid is a double-stranded DNA that can gain admission to, and replicate in, the cytoplasm of many kinds of bacterial cells.

A cloning vector is a modified plasmid, capable of replication in a host organism (such as bacteria), and into which a piece of foreign DNA can be inserted.
In today's experiment you will be using a new type of vector called TOPO®.

TOPO has two advantages over the pUC19 vectors:

a) it is linear
b) it has single, overhanging 3' deoxythymidine (T) residue

Since the Taq polymerase, used in the PCR reaction, adds a single deoxyadenosine (A) to the 3' ends of the PCR product, this allows the product to ligate efficiently with the vector (A → T ligation).
2. Electrophorese the gel for 30 min at 90 volts.

3. Photograph the gel, view the bands, and save the tubes that show the 1.3 kb fragment.

The gel must be consistent with the figure shown below.

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**Evaluation of Your Performance**

From last week: 1.3 kb band visible on gel..............2

This week: At least 15 white & blue colonies (combined) visible on agar plate.........................5

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**Why Blue and White Colonies?**

The wild-type strain of E. coli has the gene lacZ which codes for the enzyme β-galactosidase (β-gal).

To indicate the expression of β-gal, an organic compound is used which is called X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside "BCIG").
1. Transform E. coli with non-recombinant TOPO vector (the vector does not carry an insert DNA)

- Functional β-gal
- Blue color

2. Transform E. coli with recombinant TOPO vector (the vector does carry an insert DNA)

The insert interrupts the reading frame of the gene, therefore:
- Defective β-gal
- No blue color (white)