

Recombination cloning e.coli experiment



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Nucleic acid manipulation is one of the most widely used techniques for the production of various therapeutic and commercial products. The technique mainly involves micro isolation plasmid DNA from cells by using lysozymes, restriction of isolated DNA by using restriction endonucleases, ligation of restricted DNA and desired gene using DNA ligases, transformation of recombinant DNA into host cell by using suitable vector and expression of gene by allowing cells to replicate. In the present experiment DNA (foreign DNA), plasmid pUC19 (vector), restriction endonuclease EcoRI, T4 DNA ligase and heat shock in calcium chloride were used. The experiment was carried out to produce ampicillin resistant recombinant clones. The main advantage of using the technique is its ease and cheap availability of raw materials that is microbes. The main disadvantage of the technique is only skilled professionals can perform the technique. The applications of the process involves production of antisense nucleic acids which are useful in therapy and research, production of therapeutically important compounds like hormones, vaccines and proteins, production of transgenic animals,

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production of agricultural compounds like bio-fertilizers, herbicides and pesticides and usage of technique in Gene therapy to treat defective genes.

Introduction:

Nucleic acid manipulation is otherwise called as genetic engineering, gene splicing, nucleic acid manipulation, recombinant DNA technology and gene cloning. It can be defined as changes in structure of nucleic acids. It mainly involves the following sequential steps: selection and isolation of desired gene (foreign gene), micro isolation of plasmid DNA from the bacterial cells, restriction of plasmid DNA, ligation of desired gene and restricted plasmid DNA, transformation of recombinant DNA and identifying host cells containing recombinant DNA. (James, S. & C. B., 2002).

Selection involves the identification of gene that is responsible for the production of required substance (like proteins, hormones and more).

Isolation of gene can be carried out by constructing a complimentary DNA using reverse transcriptase enzymes or by fishing out gene from genome using nucleic acid probes.

The plasmid DNA is isolated from the E. coli cells by using lysozymes. The lysozymes cause the breakdown of cell wall and release the cytoplasmic material. This method is called alkaline lysis method or Birnboim and Doly method.

The restriction of plasmid is carried out by using restriction endonucleases. These enzymes recognise a particular sequence of codes and cuts the plasmid DNA at the recognition sequence. The restriction endonuclease,

EcoRI cuts at recognition sequence G*GATCC/CCTAG*G, where * denotes cutting of endonuclease. (Larry, S & Wendy, C., 2007).

The ligation of desired gene and restricted plasmid DNA is carried out using T4- DNA ligases. The ligase produced by bacteriophage which is used to join restriction fragments is called DNA ligase. The ligation of plasmid DNA and desired gene lead to formation of recombinant DNA or chimeric DNA.

The recombinant DNA is transferred into suitable host cell (E. coli). The DNA can be transferred to host cell by using heat shock method or electroporation. In heat shock method, calcium chloride solution containing host cells and chimeric DNA are incubated at 0°C and are suddenly subjected to a temperature of 37-43°C. In electroporation method the host cells and chimeric DNA are suddenly subjected to high voltage pulse.

The growth of host cells is maintained at predetermined or specific conditions, which allows only the growth of host cells containing chimeric DNA. The efficiency of the procedure may be very low but a single cell can produce numerous cells. The cells were isolated and then allowed for replication for expression of gene. (David, L. N. & Michael, M. C., 2000).

Aim:

The aim of experiment is to obtain recombinant clones of E. coli with strains of genome inserted into plasmid pUC19.

Objectives:

The objectives of the experiment include:

- Micro isolation of plasmid from bacterial cells.

- Cutting of DNA using restriction enzymes.
- Analysis of DNA using Agarose gel electrophoresis.
- Making of recombinant DNA by using DNA ligase.
- Transformation of recombinant DNA into E. coli to obtain clones.

Materials and methods:

The materials and methods were followed as per the practical booklet of core molecular biology with the following amendment in the gel electrophoresis of using a voltage of 120V instead of 100V and the agar plates were incubated for 3 weeks instead of overnight.

Results:

Agarose gel electrophoresis result for samples.

In first Lane, a smear of DH5 α extract and also a scattered band was observed. In second lane, a light band of pUC19 and smear of DH5 α extract was observed. In the third lane, five distinct bands of restricted DNA can be observed. The fourth lane shows series of bands of pUC19. In the fifth lane, a restricted band of pUC19 along with series of bands were observed. In the sixth lane, a recombinant DNA was observed which has travelled the least distance along with series of other bands. In the seventh lane, no bands were observed.

Discussions and conclusions:

The method involved in separation of DNA fragments is gel electrophoresis. Electrophoresis is a process in which charged particles move towards opposite charge in presence of electricity. In the gel, the molecules with less

molecular weight moves faster and molecules with high molecular weight moves slower.

In the experiment, a continuous smear was observed in the lane 1 and lane 2. This may be due to excessive shaking of the extracts or vortexing of the extracts. Moreover the buffer containing bottle may not be enclosed properly after its use as a result interaction might have taken place between sodium hydroxide of buffer and carbon dioxide in air leading to salt formation. In the lane 3, no bands were observed. This may be due complete denaturation of restriction endonucleases EcoRI and vector pUC19. The possible cause for denaturation may be improper storage conditions that is usage of excess temperature. The temperature of the water bath may be excess than its reading or the sample might have been placed very nearer to the heat producing zone of the water bath. In the lane 4, no bands were observed this may be due to the denaturation of the pUC19 due the usage of excessive temperature in the water bath. In lane 5, no bands were observed. This may be also due the usage of excessive temperature that is more than 37°C or may be due excess incubation time for more than one hour. In the lane 6 and lane 7, no bands were observed. This may be due to failure of storage at very low temperatures that is around 4-15°C. More over before ligation, the restriction endonuclease are to be completely inactivated for proper ligation. Due improper inactivation of restriction endonucleases, the ligation has not been achieved resulting in no bands.

No colonies were formed in transformation. This may be due to improper ligation. The failure for transformation may be due lack of proper annealing of foreign DNA to plasmid which may result in destabilization. More over the <https://assignbuster.com/recombination-cloning-ecoli-experiment/>

crucial factors effecting transformation are optimum temperature and requirement of salts like calcium ions. To overcome this problem proper maintenance of temperature, usage of laminar-air-flow while transferring the solutions, proper dilutions has to be carried out.