



## Role of Genetic Transformation in Recombinant DNA Technology and Demonstration Using *Escherichia coli* strain DH5alpha

\*<sup>1</sup>Anushree Srivastava

Bio-Axis DNA Research Centre (P) Ltd, Lucknow

\*Phone: 7376900582, Email Id: anushree.srivastava02@gmail.com

Received: March 5, 2013; Accepted: March 19, 2013; Published: May 1, 2013

### Abstract:

In Molecular Biology, genetic transformation occurs when an organism's character is changed or modified by inserting one or more desired gene(s) in order to utilize them for different purposes, for instance, the betterment of plant species by insertion of genes coding for drought, pest, and frost resistance, transformation of common bacterium *E. coli* in order to generate mass quantities of the hormone insulin that can be used to treat diabetes, enabling bacteria by genetically transforming them to digest oil spills<sup>[1]</sup>, and in biological research organisms are genetically engineered to discover the phenotypic expressions and functions of specific genes. So, given the consequential role of genetic transformation in extension of recombinant DNA technology, the present study regarding the demonstration of successful transformation experiment establishes its relevance. In the study the common lab strain *Escherichia coli* DH5a has been used to demonstrate the transformation experiment and the parameter streptomycin resistance has been used as a marker. First, *Escherichia coli* strain DH5a were exposed to antibiotic sensitivity test and cells having streptomycin resistant plasmid were used for the plasmid DNA isolation. Agarose gel electrophoresis confirmed the presence of plasmid DNA in the sample. *E. coli* cells containing genes sensitive to streptomycin were used for the competent cell preparation through CaCl<sub>2</sub> treatment. Transformation was performed using heat shock method. Finally, the blue-white lacZ screening method was employed in the identification of successfully transformed white-color colonies of *E. coli*, which therefore concludes that genetic transformation may avail many commercial and research motives.

**Keywords:** *Escherichia coli*, Plasmid DNA, Recombinant DNA Technology, Transformation.

### Introduction:

The potential and promise of genetic engineering is enormous and the dissemination of the products of Recombinant DNA Technology is limitless and useful to the mankind<sup>[2]</sup> In recombinant DNA technology, genes from one kind of organism are introduced by lateral gene transfer into the genome of another kind of organism and genetic transformation is one of those few natural means of lateral gene transfer to be artificially manipulated and create recombinant DNA and organism<sup>[3]</sup> Transformation is an important tool in recombinant DNA technology as the addition of new genes to a recipient

cell introduces a heritable modification in the recipient cell's phenotype<sup>[4]</sup> Although recombinant DNA technology first emerged in the 1960s and 1970s, the basic principle of recombination had been discovered many years earlier. Indeed, in 1928, Frederick Griffith, an English medical officer studying the bacteria responsible for a pneumonia epidemic in London, first demonstrated what he termed "genetic transformation"; here, living cells took up genetic material released by other cells and became phenotypically "transformed" by the new genetic information. More than a decade later, Oswald Avery repeated Griffith's work and isolated the transforming molecule, which turned out to be DNA. These experiments showed that DNA can be transferred from one cell to another in the laboratory, thus changing the actual genetic phenotype of an organism<sup>[5]</sup>.

### Transformation Mechanism and Competence Factor:

The mechanism of transformation involves extraction of DNA from donor organisms by a complex biochemical process that yields hundreds of naked DNA fragments from each bacterial chromosome. (Naked DNA is DNA released from an organism, often after the cell is lysed) When the extracted, naked DNA is placed in a medium with organisms capable of incorporating it, most organisms can take up a maximum of about 10 fragments. Uptake of DNA occurs only at a certain stage in a cell's growth cycle, in response to high cell density and depletion of nutrients. In this stage, a protein called competence factor is released into the medium and apparently facilitates the entry of DNA. When competence factor from one culture is used to treat a culture that lacks it, cells in the treated culture become competent to receive DNA- they can now take up DNA fragments. Competence in most naturally transformable bacteria is regulated, and special proteins include a membrane-associated DNA-binding protein, a cell wall autolysin, and various nucleases play a role in the uptake and processing of DNA<sup>[6]</sup> DNA entry depends on factors such as modifications of the cell wall and the formation of specific receptor sites on the plasma membrane that can bind DNA. Once DNA reaches the entry sites, endonucleases cut double-stranded DNA. The strands separate and only one strand enters the cell. Splicing of a DNA strand involves breaking the strand, removing a segment, inserting a new segment, and attaching the ends. Enzymes in the recipient cell excise a portion of

the recipient's DNA and recombine it with the donor DNA, which now becomes a permanent part of the recipient's chromosome.

**Plasmid DNA Vector and Escherichia coli strain**

**DH5a Host cell:** Vector is an agent that can carry a DNA fragment into a host cell in which it is capable of replication. Plasmids are autonomously replicating circular, double stranded DNA molecules found in bacteria, where they behave as additional genetic units inherited and replicated independently of the bacterial chromosome [7] The size of plasmids ranges from a few kb to 200 kb [8] Plasmids often contain genes that code for enzymes that can be advantageous to the host cell and may be involved in resistance to, or production of, antibiotics, resistance to toxins found in the environment, or the production of toxins by the bacteria itself. *E.coli* is a rod shaped Gram negative bacterium [8]. It is the host of choice for cloning experiments due to several reasons. It has a short doubling time (20 min.) and its genetics is well understood and can be easily transformed. The *E.coli* strain DH5a is used for most common cloning applications. These cells are compatible with *lacZ* blue/white selection procedure. The *lacZAM15* is a mutation of DH5a that deactivates *lacZ* activity in the bacteria producing an inactive form of  $\beta$ -galactosidase [9]. Strains with this mutation cannot cleave X-gal and remain colorless on X-gal plates, thus determining the success of transformation experiment [10] Hence, by deliberately introducing the foreign DNA into this bacterium, it is possible to make it produce useful medical products such as insulin or interferon [11]

**Materials and Methods:**

**Bacteria:** *Escherichia coli* DH5a, (genotype: F- $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$  (*lacZYAargF*) U169 *recA1 endA1 hsdR17* (rk-, mk+) *phoA supE44 thi-1 gyrA96 relA1 tonA*), was used in this study which was obtained from Bio-Axis DNA Research Center (P) Limited, Lucknow.

**Culture Media, Solutions and Buffers:** Luria-Bertani (LB) medium was routinely used to culture *E. coli*. For making plates medium was solidified with 1.6% agar. For selection *E. coli* of transformants, Streptomycin was added to LB agar plates at concentration 100 $\mu$ g/ml. For plasmid DNA isolation, Solution 1 [50mM Tris HCL, 20mM EDTA, 15% Glucose, Distilled water] Solution 2 [0.2 M NaOH, 1.5% SDS, Distilled water], Solution 3 [3M Sodium Acetate, Distilled water] and TE Buffer [1 mM EDTA, 10 mM Tris-HCl, pH 8.0] have been used.

**Antibiotic Sensitivity Test:** To detect the antibiotic resistant and sensitive strains of *E.coli* DH5a, this test has been performed. After sterilization and cooling the LB agar at 45°C, streptomycin was added into the media and poured into sterile Petri dishes under sterilized condition. After solidification, 1.5 ml of

sterilized distilled water was taken in an eppendorf tube and microbial suspension was prepared by taking 2-3 loops of *E. coli* colonies taken from EMB agar. After shaking the microbial suspension, 100 $\mu$ l of the suspension was taken into Petri dishes by spread plate method. After inoculation the plates were incubated at 37°C for 24 hours.

**Plasmid DNA Isolation:** Donor DNA was isolated from streptomycin resistant *E. coli* DH5a as described by Sambrook & Russel [12]. Agarose gel electrophoresis was performed to confirm the presence of DNA in the sample.

**Competent Cell Preparation:** 1.5 ml of overnight culture of streptomycin sensitive *E. coli* DH5a was diluted into 40ml of LB broth preheated to 37°C until the OD<sub>600</sub> reaches 0.4-0.6 (about 2.5-3h). The culture was chilled on ice for 20 min. The cell suspension was centrifuged at 3000 rpm at 4°C for 15 min. Supernatant was discarded and resuspended the pellet by gentle pipetting 20ml of sterile ice-cold 100mM CaCl<sub>2</sub>. Then it was chilled on ice for 20 min. and again centrifuged the for 15 minutes at 3000 rpm at 4 °C. Supernatant was discarded and resuspended the pellet by 4ml of sterile ice-cold 100mM CaCl<sub>2</sub> and kept on ice and in refrigerator for transformation procedure next day [13]

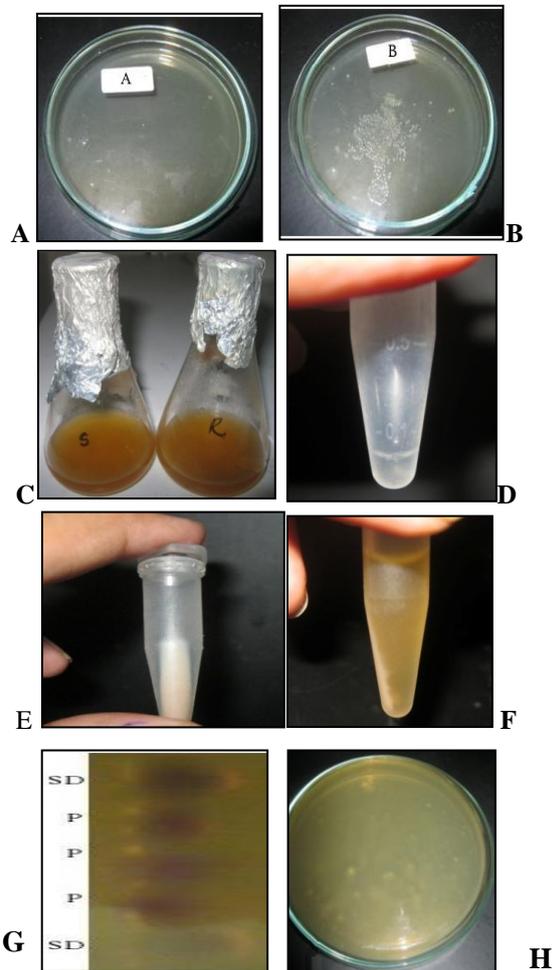
**Transformation:** 200 $\mu$ l of competent cells were transferred into a sterile eppendorf tube and 5  $\mu$ l of isolated plasmid DNA was added into it. Then transformation mixture was placed on ice for 30 min and then transformation mix was transferred to 42°C water bath and incubated for exactly 30 s. Transformation mixture was again placed on ice for 2 min. After that 1 ml of pre-warmed, at 37°C, LB broth was added into the tube and then incubated at 37°C for 1 h., shaking at approx 150 rpm to ensure good aeration [14]

**Screening:** 50ml of LB agar was prepared 50  $\mu$ l of streptomycin was added into it. Then it was poured into a Petri dish and left to solidify for 20 min. After solidification 40  $\mu$ l of X-gal added and spread evenly on the Petri dish. Then 10 $\mu$ l of IPTG was added, spread properly and left for 20 min. 100  $\mu$ l of transformation mixture prepared was spread properly on NAM-streptomycin plate and incubated for overnight at 37°C. White color colonies were identified next day.

**Results and Discussion:**

The *E. coli* strain DH5a, containing the streptomycin sensitive plasmid is not able to grow on LB agar sample plate A. On the other hand, the same strain with streptomycin resistant plasmid showed visible growth on the LB agar sample plate B after overnight incubation at 37°C. After identification of streptomycin sensitive and resistant *E. coli* strain DH5a, both

sensitive and resistant strains have been further used for plasmid culture inoculation. Plasmid DNA was successfully isolated from the culture which was inoculated with *E. coli* strain DH5 $\alpha$  containing streptomycin resistant plasmid. In the present study Agarose gel electrophoresis was performed to confirm the presence of plasmid DNA in the sample. Ethidium bromide–DNA complexes display increased fluorescence compared to the dye in solution. This means that illumination of a stained gel under UV light (254–366 nm) allows bands of DNA to be visualized against a background of unbound dye. The culture which was inoculated with streptomycin sensitive *E. coli* strain has been further used for competent cell preparation by calcium chloride treatment. After that, by heat shock method, transformation mixture was successfully prepared.  $\beta$ -galactosidase assay facilitated the visualization of white color colonies of *E. coli* and confirmed that transformation has occurred in the host cell *E. coli* strain DH5 $\alpha$ .



**Figure:** A, Streptomycin sensitive *E. coli* strain DH5  $\alpha$ ; B, Streptomycin resistant of *E. coli* strain DH5  $\alpha$ . C, [S]: culture inoculated with Streptomycin sensitive strains of *E. coli* and [R]: culture inoculated with Streptomycin resistant strains of

*E. coli*; D, plasmid DNA obtained, dissolved in 50  $\mu$ l of TE Buffer. E, Orange color bands of plasmid DNA are visible. (SD: standard DNA, P: Plasmid) F, successfully prepared competent cells. G, prepared transformation mixture. H, Transformed white color colonies of *E. coli* strain DH5  $\alpha$ .

The result of successful transformation experiment using *E. coli* strain DH5 $\alpha$  in the present study designates that artificially induced competence makes *E. coli* feasible host for the manipulation of DNA as well as expressing proteins. Transformation is the only technique available for gene mapping in some species. Mapping by transformation experiment is based on the principle that two markers transform together if they are near enough to be carried on the same DNA fragment.<sup>[15]</sup> Thus, transformation can be used to assess linkage<sup>[16]</sup>. The detailed genetic maps help locate the risk genes for a host of genetic diseases. The ability to investigate the root cause of diseases may allow medical researchers to develop strategies to avoid the environmental conditions that serve as triggers to disease, formulate customized drugs, and techniques for gene therapy<sup>[17]</sup> Bacterial transformation is crucial in producing mass quantities of human hormone like insulin by transforming common bacterium *E. coli*, an inhabitant of the human digestive tract and the 'factory' used in the genetic engineering of insulin<sup>[18, 19]</sup> Over the last two decades numerous transformation techniques have been developed for plants as well. Recent advancements in genetic engineering techniques have led to the identification of genes and proteins involved in the DNA transfer mechanism accomplished by *Agrobacterium* transformation<sup>[20]</sup> Chloroplast transformation technology is gaining importance due to its unique advantage of gene stacking without concern about gene silencing and of creating an opportunity to produce multivalent vaccines in a single transformation step. The recently developed zinc finger nuclease technology holds much promise in both basic and applied agricultural biotechnology. Hence, it is hoped that in the near future, GM plants with minimal genomic modifications can be developed<sup>[21]</sup>

*Froger & Hall (2007)*<sup>[22]</sup> support heat shock method, which has been used in the present study, as basic technique of molecular biology and can be used successfully to transform most commercially available competent bacteria such as *E.coli*. The study of *Roychoudhury et al (2009)*<sup>[23]</sup> also supports CaCl<sub>2</sub> method, used in the present study, as the “best standardized transformation method with very minor modifications”. Moreover, *Cosloy & Oishi (1973)*<sup>[24]</sup> state, “The possibilities of this transformation system in *E. coli* with its wealth and diversity of genetic and biochemical background material is for the most part self evident. Among the more intriguing possibilities is the potential for using this extremely well-characterized

organism as a model to study the introduction and integration of genetic information from various sources." Transformation efficiency is the efficiency by which cells can take-up foreign DNA and get transformed, which has not been computed in the present study, however, the significant occurrence of the observable transformed colonies of *E. coli* is satisfactory to exhibit the success of transformation experiment.

#### Conclusion:

The transforming principal - DNA is the material of heredity - led the birth of modern genetics<sup>[25,26]</sup> Transformation is the main route by which plasmid cloning vectors containing DNA inserted *in vitro* are introduced into bacteria. It is therefore a vital technique in both cloning and reversed genetics/allele replacements<sup>[27]</sup> procedures. The development of plasmid transformation regimes is thus an important prerequisite for the extension of recombinant DNA technology<sup>[28]</sup> Conclusively, the observation in the present study that DNA obtained from streptomycin resistant *E. coli* strain *DH5a*, coding for streptomycin resistance, is able to transform the sensitive *E. coli*, which indicates the utility of genetic transformation technique in genetic engineering. By putting a gene of interest into a plasmid, the transformed bacteria will express that gene and that expression can be exploited for the production of a needed protein or for selection purposes. Bacterial transformation is essential to the field of molecular biology in that it allows for the propagation, genetic expression, and isolation of recombinant DNA molecules<sup>[29]</sup>

#### Acknowledgement:

I wish to thank Mrs. Amita Sinha, Head of Academic Operations, Bio-Axis DNA Research Centre, for providing me the opportunity of training and project in the prestigious institute.

#### References:

- [1] Biotechnology Explorer, pGLO, Bacterial Transformation Kit, catalog# 166-0003EDU, p.1
- [2] O. S. Reddi, Recombinant DNA Technology: A Laboratory Manual, 2000, Allied Publishers, p. 8.
- [3] J. G. Black, Microbiology: Principals and Exploration, 2012, Edition: 8
- [4] Moss R., 1991. Genetic transformation of bacteria, The American Biology Teacher, 53(3), 179-180.
- [5] Pray, L. (2008) Recombinant DNA technology and transgenic animals. Nature Education 1(1)
- [6] M. T. Madigan, J. M. Martinko, D. A. Stahl, D. P. Clark, Brock Biology of Microorganisms, Pearson Education, 2011
- [7] Benchguide Qiagen, Ch.1.
- [8] S. Dhillon, A.K. Chhabra, P. Kharb; Recombinant DNA Technology and Biotechnology: Cloning Strategies and Screening of Recombinant DNA Clones.
- [9] Yanisch-Perron C., Vieira, J., Messing, J., 1985. Improved M13 phage cloning vectors and host strains: nucleotide

sequences of the M13mp18 and pUC19 vectors. *Gene* 33:103-19.

[10] M. Griffith, Genetic Modification of the *Escherichia coli* Strain DH5  $\alpha$  to Allow the Selection of Plasmids Carrying Complementary Yeast Genes, Projects in Biology 05.4111/6, 2001.

[11] J. Burgess, M. Marten, R. Taylor, Under the Microscope: A Hidden World Revealed, Archive: 1990.

[12] J. Sambrook, D. W. Russel, Condensed protocol from molecular cloning: A laboratory manual, 2006. p.

[13] T. Brown, Gene Cloning and DNA Analysis: An Introduction, 2010 Ed: 6

[14] N. Casali, A. Preston, E. Coli Plasmid Vectors: Methods and Applications, Springer 2003

[15] Microbial Genetics, Stanley Maloy, Jones and Bartlett Publishers, Inc.; 2nd edition, 1994

[16] Modern Genetic Analysis, Griffiths AJF, Gelbart WM, Miller JH, et al. New York: W. H. Freeman; New York, 1999.

[17] Benefits of Genetic Mapping, Copyright © 2006-2012 geneticmap.net.

[18] Genetic Engineering, Compton's Interactive Encyclopedia.

[19] Recombinant DNA Technology in the Synthesis of Human Insulin, translated in Romanian by Alexander Ovsov Copyright © 1994 [Ilanit ToF](#).

[20] Veena (2008) Engineering plants for future: tools and options *Physiol. Mol. Biol. Plants* 14, 131–135.

[21] Chapotin, S.M., and Wolt, J.D. (2007) genetically modified crops for the bioeconomy: meeting public and regulatory expectations. *Transgenic Res.* 16, 675–688.

[22] Alexandrine Froger, James E. Hall, Transformation of Plasmid DNA into *E. coli* Using the Heat Shock Method. *J Vis Exp.* 2007; (6): 253. 2007. doi: 10.3791/253.

[23] Roychoudhury A., Basu S. , Sengupta D.N., 2009, Analysis of Comparative Efficiencies of Different Transformation Methods of *E. coli* Using Two Common Plasmid Vectors, *Journal of Biochemistry & Biophysics*. Vol. 46, pp. 395-400.

[24] Cosloy S. D., Oishi M. 1973, Genetic Transformation in *E. coli* K 12, *Proc. Nat. Acad. Sci., USA*, Vol 70, pp. 84-87.

[25] DNA: The Transforming Principle and the Birth of Modern Genetics, The Rockefeller University Hospital.

[26] Recent Advances in Plant Transformation, S. Barampuram, Z. J. Zhang, J. A. Birchler, *Plant Chromosome Engineering: Methods and Protocols*, vol. 701, DOI 10.1007/978-1-61737-957-4\_1, © Springer.

[27] Weissmann C., Nagata S., Tanagucci T., Weber H. Meyer F., 1979, In Genetic Engineering, Principals and Methods, Vol.1 pp-133-150, Plenum Press, New York.

[28] Grinsted J., 1988, *Methods in Microbiology*, Vol.21, Academic Press, p.106.

[29] The Transformation Lab, Experiment Using *E. coli* and pFluoro Green, Maryland Loaner Lab Teacher Packet, EDVOTEK®.

\*\*\*\*\*