Activity-based Gene Cloning through PCR Module as a part of Undergraduate Minor Project in Biotechnology

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ABSTRACT:
Identification and characterization of native isolates from environmental sources assumes immense significance from research, and commercial perspective. Microbial identification using a molecular biology approach based on 16S rDNA has been the popular and well-established method. Experiments of Molecular Biology and rDNA Technology have always been a challenge for effective teaching owing to the cost, time, inconsistent results and expertise. The study was undertaken with a broad objective of giving a hands-on experience of selected molecular biology and rDNA technology experiments and related activities to the undergraduate students of Biotechnology Engineering, as a module of Minor project identified as flagship course. The genomic DNA of selected environmental soil isolates was isolated by the conventional lysis method, followed by amplification of its 16S rDNA by PCR, using universal primers. The resulting amplicons were purified, cloned into the TA vector, and analyzed for insert and the recombinant vector was transformed into host cells. The ligated cloned vector showed a size of 4500 bp as against the control vector of 3000 bp. The growth of transformants was confirmed by selective growth against ampicillin antibiotic and by blue-white screening. The exercise helped in addressing eight graduate attributes related to (investigation of technical issues, modern engineering tools, discipline-specific tools, team-work and produce technical report). Formal feedback from the students evidenced that the students had good experiential learning from the exercise. Thus, the study related to cloning exercise was instrumental in providing hands-on experience and enhanced the skill sets of the students related to fundamental molecular biology.

Key Words: PCR, rDNA, TA Cloning. recombinant screening.

1. INTRODUCTION

Molecular biology has evolved as an indispensable part of biology discipline. It has profound applications in various branches of studies, relevant applications and commercial significance. Despite some of its associated and perceived controversies in terms of application, it has emerged as a powerful subject of knowledge. This in-turn has snowballed into increased demand for skilled personnel in the field which demands expertise and experience. This comes at a cost concerning molecular biology owing to its need for costly chemicals, reagents, infrastructure and long training periods due to inconsistent and delayed results. The graduates with exposure to molecular biology are most sought after in industries as it saves on their time and efforts needed to train the graduates. In this regard, effective teaching of biotechnology in general and molecular biology in particular in the academic institutions assume significance (Zeller, 1994). Several pedagogical methods have been practiced to enhance the teaching-learning of molecular biology. Virtual molecular biology labs have been successful to a great extent in this direction (Zumbach et al, 2006). An in silico cloning module was developed as part of biochemistry by Elkins (2011). McClean et al (2017) developed animations related to molecular biology to compensate for the two-dimensional teaching of conventional methods and demonstrated their long-lasting effects on students' learning. Postner and Markstein (1994). employed cooperative mode of pedagogy to teach molecular biology and enhance the academic performance of the students. 16S rDNA cloning and sequencing leading to molecular characterization and biodiversity analysis have been extremely needed for any study of academic and research interest. A ten-week research-based curriculum was developed for teaching molecular biology by Boomer et al (2002). An investigative approach of teaching modern DNA techniques to graduate laboratory course was practiced by Lencastre et al (2017).

In light of the above background, the present study focuses on teaching the gene cloning module of molecular biology as experiential learning for undergraduate students of Biotechnology engineering.

2. METHODOLOGY

A. Genesis and Objective:

A theme-based minor project titled 'Theme-Based Minor Project Implementation for Basic Skill-Set Development in Biotechnology' of six credits is being implemented as a flagship project for VI semester undergraduate students of the Department of Biotechnology, KLE Technological University, Hubballi. The present study is a part of the flagship project focussing on skill-sets related to molecular biology.

The key objectives of the present exercise were:

1. to give an insight into the basic steps involved in gene cloning of TA vector and screening of recombinants and
2. Provide hands-on experience of working on molecular biology techniques.

B. Delivery of the module.

The gene cloning module was a team exercise with 4 students members in each group. It was executed in two stages: a training stage followed by a practical implementation stage in the lab.

1). Training Phase: The training phase included theory sessions wherein the objectives of the exercise, basic concepts needed for understanding and the expectations are dealt with.

2). Implementation phase:

The students performed the unit exercises sequentially in the laboratories giving them a hands-on feel of the module.

C. Sequential steps involved in the cloning of gene and screening of recombinants

*1).*Selection of isolate:

Moist soil samples were collected from the surrounding environment of Hubballi-Dharwad for isolation. The samples were serially diluted and spread plated onto pre-poured nutrient agar plates (NA), incubated at 37 °C for 24-48 h. The isolated colonies were subjected to pure culture techniques, subcultured on NA slants and preserved at 4 °C for further use.

2). Genomic isolation of the isolate:

The bacterial cells of the isolate were grown overnight in nutrient broth at 37 °C and pelleted by centrifugated at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed and genomic DNA was extracted by lysis method using SDS and NaOH. The quality of the genomic DNA thus extracted was analyzed qualitatively (agarose gel assay) and quantitatively (spectrophotometric assay).

3). 16S rDNA amplification and purification:

The 16S rDNA was amplified using a pair of universal primer set (8F and 1492 R). The reaction was carried out in 25 µL reaction volume. PCR mixtures contained extracted genomic DNA as a template, the four deoxynucleoside triphosphates (dNTPs) and Taq polymerase.
PCR assays were carried out in a Bio Era thermocycler programmed for the following PCR conditions:
Initial denaturation at 94 °C for 5 minutes
Denaturation at 94 °C for 30 seconds
Annealing at 50 °C for 30 seconds
Extension at 72 °C for 90 seconds and
Final extension at 72 °C for 5 minutes
Total number of cycles- 30
The amplified product was purified by a silica column-based PCR purification kit (Chromous Biotech) according to the manufacturer's instructions.

4). TA cloning and ligation:

The purified amplicons were cloned into the TA vector (Chromous T vector)and ligated at 16 °C for 2 h.

5). Transformation:

The vector was transformed with a heat-shock (42 °C for a minute) method into DH5α cells.

6).Screening of recombinants:

The transformed cells with heat-shock treatment were screened for recombinants by blue-white screening method using media containing x-gal and IPTG as substrate and inducer respectively.

3. Assessment

The formal assessment of the module was performed in two phases:
In-semester assessment (ISA) and End-semester assessment (ESA) each comprise 50 marks. The assessment was performed with appropriate rubrics. A formal written technical report was obtained from each group followed by an oral presentation.

4. Results

A. Genomic DNA

The genomic DNA extracted was a single band (Fig.1) with A_{260}/A_{280} ratio of 1.9 indicating a good quality of DNA free of protein contamination.

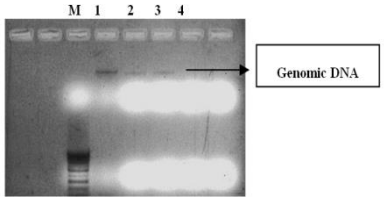

Fig.1 Genomic DNA on agar gel electrophoresis

B. 16S rDNA amplification and purification.

The amplification of extracted genomic DNA with a set of universal primers resulted in a band with an expected size of 1.5 kb as shown in fig.2

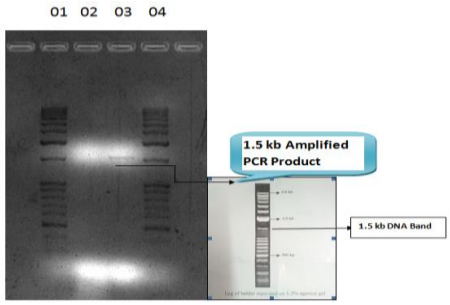

Fig.2. PCR amplification of 16S rDNA with a 1.5 kb band.

C. Cloning and transformation of recombinants.

The purified amplicons cloned onto the TA vector and the blue-white screening of recombinants revealed some colonies colorless in nature indicating the successful transformation and presence of recombinant strains.

D. Program Outcomes addressed.

The exercise was instrumental in addressing some of the program outcomes as listed in Table 1.

Table 1. Program Outcomes addressed by the cloning exercise.

Sl. No.	PO/PSO No.	Program Outcomes/Program Specific Outcomes
1	PO 2	Problem analysis
2	PO 4	Conduct investigations of complex problems
3	PO 5	Modern tool usage
4	PO 9	Individual and teamwork
5	PO 10	Communication
6	PSO 13	Good Lab Practices

E. Student feedback.

A formal feedback from the student participants revealed that the module gave an insight into the basics of gene cloning and its significance leading to sequence analysis for identification of the organism at the molecular level. The content of the module enhanced their understanding of gene cloning. The unit exercises of the module complemented the theory aspects they had learned. They could have the first-hand feel of handling the molecular biology reagents, pipettes and related instruments like a centrifuge, thermocycler, gel electrophoresis, and gel documentation unit. They honed their skills in term usage of pipettes, handling solutions and buffer stock preparations and its related calculations for working stocks.

5. Conclusions

The module of gene cloning was instrumental in giving an insight into the nuances of molecular biology and the associated techniques. The exercise was an experiential learning for the students. Further scope for

getting the cloned gene sequenced, using the sequence data for identification of the organism involved using bioinformatics tools exists.

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