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 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                        |

### 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.

#### 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.

#### 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.

### 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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