RESEARCH IN THE CLASSROOM: CLONING AND SEQUENCING THE GAPC GENE FROM MULTIPLE PLANT SPECIES IN THE UNDERGRADUATE LEARNING OF MOLECULAR BIOLOGY

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ABSTRACT

Students participated in hands-on research, in addition to lecture, to improve their understanding of molecular biology. Hands-on research included cloning and sequencing a gene that codes for Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in multiple species of plants. The impact of hands-on learning in students’ understanding of molecular biology was qualitatively assessed. Results confirm that this approach can be effective in increasing students’ confidence with basic laboratory techniques and knowledge of bioinformatics.

INTRODUCTION

The purpose of this research was to provide undergraduate students with the opportunity to learn molecular biology through hands-on experimentation, in addition to traditional lecture and to qualitatively assess how hands-on learning improved their understanding of molecular biology. During the semester, approximately half of the time in the classroom was spent cloning the GAPC gene in multiple plants. The following semester was spent performing bioinformatics analysis on sequences of GAPC clones. During their hands-on research students accomplished the following:

- Extraction and purification of genomic DNA from plants
- Amplification of target DNA using polymerase chain reaction (PCR)
- Assessment of PCR amplification using gel electrophoresis
- Purification of PCR products using size exclusion chromatography
- Ligation of PCR products into a plasmid vector
- Cloning target DNA by transformation via bacteria
- Identification and isolation of transformed bacteria using a selection marker
- Extraction and purification of cloned DNA plasmid from bacteria
- Sequencing and bioinformatics analysis of cloned genes
- Troubleshooting for unexpected results and subsequent reassessment

METHODS

The Cloning and Sequencing Explorer Series kit from Bio-Rad (#166-5000EDU) was used for extraction, cloning, and amplification of the GAPC gene in all plants used. Cloned GAPC fragments were analyzed using NCBI’s basic local alignment search tool, CAP3 Sequence Assembly Program, and the European Bioinformatics Institute’s sequence translation tool. The plants shown below were used in addition to the pGAP control plasmid containing the GAPC target region from Arabidopsis.

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REFERENCES

- Bio-Rad. Biotechnology Explorer: Cloning and Sequencing Explorer Series #166-5000EDU
- European Bioinformatics Institute: http://www.ebi.ac.uk/
- MultiAlin: http://malign.bio.toulouse.inra.fr/

CLONING AND SEQUENCING

![Image of cloning and sequencing process]

Figure 1. Chromosomal locations of GAPDH genes in Arabidopsis. The GAPDH gene that was cloned is indicated in the red box (GAPDH).

![Image of sequence homology]

Figure 2. Sequence homology between GAPC in successfully cloned plants compared to chromosomal locations of GAPC in Arabidopsis. Exons are shown in red. The portion of GAPC including the active site of GAPDH was cloned in all five of these plant species.

![Image of multiple alignment]

Figure 3. Multiple alignment of amino acid residues of GAPDH in successfully cloned plant species compared to coding sequences for cytosolic GAPDH in Arabidopsis (MalAlin).

STUDENT LEARNING

![Table of student learning outcomes]

Table 1. Students ranked the concepts above on a 1 through 5 scale: Ratings prior to and after cloning exercises are shown, with the overall percent increase:
1 = poor, 2 = fair, 3 = good, 4 = very good, 5 = exceptional

CONCLUSIONS

- GAPC genes were successfully cloned in five of the six plant species (B. glabella, B. pilosa, C. annum, M. pudica, P. aristata) and coding sequences will be published in NCBI’s GenBank.
- GAPC clones showed high sequence homology when compared to GAPC in Arabidopsis.
- Introduction of cloning and sequencing exercises into the teaching of molecular biology was an effective way to increase student learning and retention, as shown in Table 1 and formal classroom assessments.
- Students reported at least a two-fold increase, on a 1-5 scale, in their understanding of concepts and techniques of molecular biology after completing hands-on exercises.
- An overall increase of 30.36% was reported in students’ understanding of molecular biology concepts and techniques following hands-on exercises.