## Innovations

# rDNA Technology and Genomic Library Construction Project in an Undergraduate Molecular Cell Biology Laboratory Class

**Subir Ghosh, Ph.D.**

**Division of Natural Sciences, College of Natural & Applied Sciences**

**University of Guam,**

**UOG Station, Mangilao, Guam 96923, USA.**

**E-Mail: sghosh@triton.uog.edu**

**Tel. No. (671) 735-2789**

**Abstract**

Undergraduate students of a molecular cell biology laboratory class learn basic recombinant DNA (rDNA) technology by engaging in a Genomic Library Construction Project. Significantly, in this lab project students learn experimental design strategy. The challenge for students is to determine the strategy that they can take to efficiently screen for the success of the genomic library construction. The collaborative rDNA technology project requires basic equipment and reagents available in the molecular biology teaching lab and yet provides an advanced molecular cell biology learning experience and sparks enthusiasm for the molecular life sciences and biotechnology.

Keywords: Undergraduate; Genomic Library Project; Experimental Design Strategy

Running Title: Undergraduate Genomic Library Project.

## Introduction

Engaging students in hands-on experimental work in a molecular cell biology laboratory class is an effective way to spark enthusiasm for the molecular life sciences and biotechnology. It is well documented that learning is more effective when students work together collaboratively in problem solving activities (Frey et al., 2022; Ghosh, 2019; Premo et al., 2018; Allchin, 2017; Bierema et al., 2017; Freeman et al., 2014; Jacobs, 2014; McLaughlin and Metz, 2016; Waldrop, 2015). The National Academies of Sciences, Engineering, and Medicine in their 2017 report has included course-based experiences as part of UREs (undergraduate research experiences) for STEM students.

A Genomic Library Construction Project has been designed as part of an upper-division level cellular and molecular biology laboratory coursework to introduce rDNA technology to undergraduate juniors and seniors who have completed the pre-requisite Genetics course. The 1 unit (three hours per week) Cellular and Molecular Biology laboratory class enrolls a maximum of 20 students. Two laboratory class sections are conducted every Spring semester for the 3 credit Cellular and Molecular Biology lecture course. In this cloning project performed collaboratively, students learn fundamental techniques used in rDNA technology. The students work in pairs and at every step of the multi-step project share their observation and results as well as difficulties encountered with other student groups and Instructor. This activity enables students to develop skills in troubleshooting experiments and the opportunity to review the science involved in the techniques. The multi-step project involves construction of a genomic library starting with genomic DNA fragments prepared from high molecular weight yeast genomic DNA that is recombined into a plasmid cloning vector. The recombinant plasmids are transformed into host E. coli bacteria and plated on selective bacterial media plates. The challenge the students face is to distinguish on the media plates between host bacterial colonies that have taken up the recombinant plasmid in contrast to bacteria that

have incorporated the plasmid cloning vector only without the genomic DNA inserts. This provides an opportunity for all students to design a strategy that can indicate the success of the Genomic Library Construction Project directly by observing the colonies growing on the media plates without having to perform any further experimental steps that would involve taking up more lab class sessions and reagents.

In this cloning project, students learn first-hand gene cloning techniques and analytical thinking skills critical for designing experimental strategies. In the prerequisite genetics course, students learn the mechanism of transformation, a genetic event that generates variation in prokaryotic bacteria. The Genomic Library Cloning Project, conducted in the cellular and molecular biology laboratory course, provides students the opportunity to apply their prior knowledge of transformation towards a major cloning endeavor. Students learn that the knowledge of science is critical for applications that directly benefit the further progress of science and contribute to solving practical problems. All other techniques in the multi-step cloning project are introduced to the students for the first time in their undergraduate biology curriculum. The multi-step Genomic Library Construction Project activity, and strategy taken by students to evaluate success of the cloning project is discussed in this paper.

**rDNA Technology Learning Objectives from the Genomic Library Construction Project**

rDNA technology has been the foundation for the rapid advances in investigating and understanding life at the molecular level. In the Cellular and Molecular Biology lecture class, students learn of molecular cloning techniques that enable scientists to identify genes and investigate their function in health and disease. The Genomic Library Construction Project laboratory class activity, performed collaboratively, provides undergraduate students with first-hand experience identifying, isolating, and manipulating biomolecules. The students learn to isolate genomic DNA from a species; isolate plasmid DNA from bacteria; restriction analysis of DNA by gel electrophoresis; purification of DNA bands from the agarose gel, post electrophoresis; perform ligation reaction to clone genomic DNA fragments into plasmid cloning vector DNA; transform host cloning E. coli bacteria with the recombinant DNA molecules and plate onto selective media. An important task the students perform is to examine the plasmid cloning vector map and learn of the important components that constitute a cloning vector. Students learn of the multiple cloning sites that enable insertion of DNA sequences into the plasmid vector; of the origin of replication that enables the recombinant plasmid to autonomously replicate in the transformed cloning host bacteria; and the presence of the selectable marker gene that is critical for growth of only transformed bacteria on selective media. Students also learn that the optimum size of a library of completely random fragments of genomic DNA necessary to ensure representation of a particular sequence of interest is dictated by the size of the cloned fragments and the size of the genome. They further learn to use a statistical equation to determine the number of independent clones, N, that must be screened to isolate a particular sequence with probability P (provided in Materials & Methods). Significantly students learn experimental design and molecular strategies. Students are given the challenge of determining the strategy that can be taken to determine successful construction of genomic library by differentiating directly on the media plate, bacterial transformant colonies that have the genomic DNA fragments cloned as opposed to bacteria that have taken the plasmid vector DNA that did not ligate successfully with the genomic DNA inserts.

The Genomic Library Construction Project requires standard molecular biology equipment and reagents available in most institutions. Student fees for the laboratory course are available for the purchase of lab supplies and kits used for the molecular biology project. The multi-step project can be completed in four

three-hour long laboratory class sessions with active supervision and assistance from the instructor.

**Genomic Library Construction Project Implementation Timeline:**

**Laboratory class schedule for the cloning project**

Each lab class is 3 hours long; Students work in pairs; Handout explaining cloning project and protocols for all procedures provided to students.

Lab Class 1: Isolation of Genomic DNA & Plasmid DNA; Restriction Endonuclease digestion of Genomic and Plasmid DNA.

Lab Class 2: DNA Agarose Gel Electrophoresis of Restriction Endonuclease digested Genomic and Plasmid DNA; Purification of DNA from Gel.

Lab Class 3: Ligation Reaction; Transformation of E. coli with Ligation Mix; Plating of E. coli on Selective Media plates.

Lab Class 4: Strategy to evaluate success of Genomic Library Construction Project; Examination of Selective Media plates to detect bacterial colonies transformed with the recombinant plasmid containing a genomic DNA insert fragment.

Genomic Library Construction Project Experimental Design

**Yeast Genomic DNA preparation to generate inserts for cloning into vector**

Standard molecular biology protocols for construction of the genomic DNA library are followed as provided in Molecular Cloning: A Laboratory Manual, 3rd Edition; and as per manufacturers protocols for the molecular biology kits and reagents used in this cloning project. Students isolate high molecular weight genomic DNA from yeast (Saccharomyces cerevisiae) using the Monarch Genomic DNA Purification Kit protocol (New England Biolabs, NEB). The enzyme Lyticase (Sigma-Aldrich) is used for enzymatic lysis of yeast cells to prepare genomic DNA. [In the event that students are not successful in isolating genomic DNA, the instructor can provide prepared yeast genomic DNA, Sigma-Aldrich]. Next, they restrict the yeast genomic DNA with a restriction endonuclease enzyme (NEB) to generate fragments of varying length. The specific restriction endonuclease enzyme used is revealed to the students during the last stage of the project where the strategy to evaluate success of the Genomic Library Construction Project is formulated. The restricted genomic DNA is electrophoresed in a 1% DNA agarose gel in Tris-Acetate-EDTA (TAE) electrophoresis buffer to obtain a range of DNA fragments from high to low molecular weight and visualized as a smear by staining the gel with DNA specific dye SYBR™ Green (Thermo Fisher Scientific) and illuminated with a Syngene™ blue light transilluminator (Fisher Scientific). For safety reasons, the instructor (not the students) excises different parts of the restricted genomic DNA smear in the gel representing a specific molecular size range using a razor blade. Collectively all the student groups in the class approximately cover the entire molecular size range of the restricted yeast genomic DNA. The genomic DNA fragments are purified from the agarose gel by the students using Freeze 'N Squeeze™ DNA gel extraction spin columns (Bio-Rad) to generate a large population of yeast genomic DNA fragments of varying length for insertion into a plasmid cloning vector to prepare a yeast genomic library.

**Plasmid Cloning Vector preparation**

Students isolate using the Aurum™ Plasmid Mini Kit (Bio-Rad), the covalently closed circular plasmid cloning vector DNA (pGlo) from E. coli bacteria that contains the plasmid (Source: Instructor; prepared using the Bacterial Transformation Kit, Bio-Rad). The isolated plasmid DNA is restricted with the same restriction endonuclease used to generate yeast genomic DNA fragments. This results in linearization of the plasmid with compatible cohesive ends that would allow insertion of the genomic DNA fragments into the plasmid cloning vector. The restricted plasmid is electrophoresed on a 1% DNA agarose gel to separate completely

restricted plasmid from unrestricted plasmid. The linearized plasmid forms a sharp 5.3 kilobase pair DNA band and is purified from the gel, using Freeze 'N Squeeze™ DNA gel extraction spin columns (Bio-Rad).

**Ligation Reaction to insert Yeast Genomic DNA fragments into Plasmid Cloning Vector**

The purified population of linear double-stranded yeast genomic DNA fragments are inserted into the linearized double-stranded plasmid cloning vector DNA in a ligation reaction using the T4 DNA Ligase enzyme and protocol provided by NEB. The ligation reaction is performed at room temperature for an hour. During this incubation time, the mechanism of the ligation reaction is discussed, and students are quizzed on the expected final products of the joining reaction. In the quiz students are asked to draw the starting substrates in the ligation reaction and then predict all the possible outcomes at the end of the joining reaction. Typically, most of the students provide diagrams of the recombinant plasmid cloning vector containing genomic DNA inserts of varying length. The students are quizzed again and asked to formulate a strategy to determine the success of their ligation reaction. Students find it challenging and are unable to answer this question. The students are informed that after completion of the ligation reaction, an aliquot of the ligation reaction can be electrophoresed in an agarose gel and after DNA staining the products of the joining reaction can be examined to determine the success of the ligation reaction. Students are provided a hint by discussing the science of DNA staining in an agarose gel and the fact that the intensity of the stain indicates the amount of DNA present in the DNA band observed in the gel. The students are asked to generate a hypothesis for the expected result of a successful ligation when the completed ligation reaction is examined in a DNA gel after electrophoresis. The students find this question challenging and are unable to provide the correct answer. The students learn that the staining intensity of the plasmid vector DNA band would decrease if successfully ligated to the large population of genomic fragments of varying sizes. The entire class benefits from the discussions since they learn analytical thinking that leads to developing scientific hypothesis.

**Transformation of Cloning Host E. coli with the Ligation Reaction Mix**

Students introduce the ligated recombinant cloning vector plasmid containing the yeast genomic DNA fragments into cloning host HB101 strain of E. Coli bacteria using the Calcium Chloride Transformation procedure (Hanahan, 1983; Bacterial Transformation Kit, Bio-Rad). Next, the E. coli bacteria are plated on antibiotic selection media plates (LB agar nutrient media plates containing the ampicillin antibiotic, Bio-Rad) and incubated overnight at 37 $^{0}$C to select for transformed bacteria that potentially contain the recombinant plasmids into which the yeast DNA genomic fragments have been cloned. In the final step of the cloning project, the students address the challenging task of evaluating the success of the Genomic DNA Library Construction Project and this exercise is the highlight focus of this paper and is discussed in the next section.

**Strategy to Evaluate Success of the Genomic Library Construction Project**

A highlight of the Genomic DNA Library Construction Project is for students to learn molecular strategies taken in designing experimental approaches. Cloning of the genomic fragments into a standard plasmid cloning vector results in recombinant plasmids bearing genomic DNA inserts. However, during the ligation reaction a population of plasmids do not take up the genomic inserts and self-seal to recreate the original plasmid. When plated onto antibiotic selection media plates, both host E. coli containing the recombinants as well as bacteria bearing only the plasmid vector are observed to grow on the media plates. The bacterial colonies are indistinguishable, and the students cannot determine if they have been successful in construction of the genomic library.

Students are given the challenging task of determining a strategy that can be taken to

evaluate the success of constructing the genomic library. Rather than use a standard plasmid cloning vector, students are provided the pGlo recombinant plasmid (Bio-Rad) to be used as a cloning vector for yeast genomic DNA library construction. The pGlo plasmid contains a jellyfish Green Fluorescent Protein coding gene (Prasher et al., 1992). The plasmid has a cloning site bearing multiple restriction endonuclease recognition sites and has restriction sites at other regions of the plasmid including in the antibiotic selection gene, the origin of replication, and within the GFP gene sequence. Students are provided a map of the pGlo plasmid (Bio-Rad) and given the task of determining a suitable strategy that can be taken to differentiate E. coli host bacteria that are transformed with recombinant plasmids containing yeast genomic fragments in contrast to E. coli transformed with the pGlo plasmid alone that self-sealed during ligation and failed to insert the genomic fragments. This task is given as homework to the students followed by a lab class meeting where the strategy is discussed and the final step of evaluation of the success of the cloning project is completed.

The strategy taken to screen transformed bacterial colonies on selective media plates for determining success of the construction of the genomic DNA library is a highlight of the project and presents a challenge to all students. Almost all students state that the yeast genomic DNA fragments should be cloned in the multiple cloning site of the pGlo plasmid leaving the GFP coding sequence intact. Thus, their strategy to evaluate the success of the Genomic Library Construction Project was that all bacterial colonies that were transformed with the recombinant pGlo plasmids containing genomic inserts would result in bacterial colonies glowing green. The strategy was reviewed, and students learn that their approach would not be able to evaluate the success of the cloning project since even the pGlo plasmids that did not contain the genomic inserts would contain the intact GFP coding sequence and would also result in glowing green transformed bacterial colonies. The students also learn that the yeast genomic DNA fragments should not be inserted into the "ori" site (origin of replication) of the pGlo plasmid since the origin of replication site enables the recombinant plasmid to autonomously replicate and amplify in the transformed cloning host bacteria. The antibiotic selectable marker gene present in the pGlo plasmid should also be intact since it is critical for growth of only the transformed bacteria on selective media.

Thus, the strategy that should be taken is to clone the yeast genomic DNA fragments directly into the coding sequence of the GFP gene via a unique restriction site, Xho1, present in the gene and not anywhere else in the pGlo plasmid. This would result in inactivation of the GFP gene and expression of the GFP protein would be lacking in bacteria transformed with recombinant pGlo plasmids containing the genomic DNA inserts. Thus, all transformed bacteria glowing green on the media plates would reflect bacteria containing plasmid lacking genomic inserts. Only the bacterial colonies not glowing green would reflect the success of the Genomic Library Construction Project. The cloning project provided the opportunity to all students to learn experimental molecular strategy.

**Discussion**

The Genomic Library Construction Project provides undergraduate students the opportunity to learn first-hand gene cloning and analytical thinking required for developing experimental strategies. Students collaborate and complete the multi-step cloning project to construct a genomic DNA library, learning fundamental molecular biology concepts and rDNA technology techniques as they perform each step of the project. Students performed a wide range of molecular techniques – DNA isolation; restriction endonuclease mapping of DNA; DNA analysis by DNA gel electrophoresis; purification of specific DNA fragments from the agarose gel; cloning vectors; ligation reaction to create recombinant DNA; transformation of cloning host bacteria; and screening of bacterial transformants on selective media plates.

Students further learnt of library screening procedures using labeled molecular probes to discover novel genes involved in a cellular process under investigation by scientists. The Genomic Library Construction Project requires standard molecular biology equipment and reagents available in most institutions. The project involves multiple steps taking four three-hour long laboratory class sessions with active supervision and assistance from the instructor. Standard safety procedures and rDNA technology guidelines as established by the NIH are followed in the laboratory class (The National Institutes of Health Guidelines for Research involving recombinant or synthetic Nucleic acid molecules, NIH, April 2019). Students collaboratively ensure success of each step thereby learning responsibility in undertaking projects. It is anticipated that active engagement in projects such as the genomic DNA library cloning exercise will go a long way in motivating students and raising enthusiasm for the molecular life sciences and future careers in research and the health field.

**Methods**

a. The NIH guidelines (The National Institutes of Health Guidelines for Research involving recombinant or synthetic Nucleic acid molecules) are followed for construction and handling of recombinant DNA molecules and host bacteria containing these molecules (https://osp.od.nih.gov/biotechnology/nih-guidelines/).

b. Standard molecular biology protocols for construction of the genomic DNA library are followed as provided in Molecular Cloning: A Laboratory Manual, 3rd Edition; and as per manufacturers protocols for the molecular biology kits and reagents.

c. Students learn that the size of a library of completely random fragments of genomic DNA that is necessary to ensure representation of a particular sequence of interest is dictated by the size of the cloned fragments and the size of the genome. The number of independent clones, N, that must be screened to isolate a particular sequence with probability P is given by

$$N = \ln(1-P)/\ln(1-(I/G)),$$

where, I, is the size of the average cloned fragment, in base pairs, and G is the size of the target genome, in base pairs. In order to have a 99% chance of isolating a desired sequence, the number of clones screened should be such that the total number of base pairs present in the clones screened (I x N) represents a 4.6-fold excess over the total number of base pairs in the genome (Molecular Cloning: A Laboratory Manual, 3rd Edition).

**Reagents**

New England Biolabs (NEB): Monarch Genomic DNA Purification Kit, Catalog # T3010S; Xho1 Restriction endonuclease, Catalog # R0146SVIAL; DNA Molecular Weight Marker, Catalog # N3012S; and Quick Ligation Kit, Catalog # M2200S.

Sigma: Lyticase, Catalog # L2524 (for enzymatic lysis of yeast cells to prepare genomic DNA); High Molecular Weight Yeast Genomic DNA, Catalog # 69240-M.

Bio-Rad: Aurum plasmid Mini Kit, Catalog # 7326400EDU; Freeze 'N Squeeze™ DNA gel extraction spin columns, Catalog # 7326165EDU; Bacterial Transformation Kit (includes pGlo plasmid & restriction endonuclease map; cloning host HB101 strain of E. coli; Calcium Chloride Transformation solution; LB liquid and solid nutrient agar media; selective LB media agar plates prepared by adding ampicillin antibiotic, and arabinose sugar to induce GFP gene expression – provided in kit), Catalog # 1660003; DNA grade agarose, Catalog # 1613100EDU; nucleic acid electrophoresis buffer, Catalog # 1610743EDU; DNA Electrophoresis Sample Loading Dye, Catalog # 1660401EDU. [EDU – educational discount provided by Bio-Rad].

Thermo Fisher Scientific: SYBR™ Safe DNA Gel Stain, Catalog # S33111

Fisher Scientific: Syngene™ Slimline LED Blue Light Transilluminator, Catalog # 01-257- 274.

## Acknowledgements

The author acknowledges the students of the cellular and molecular biology laboratory course at the University of Guam for their active participation and enthusiasm.

## Conflict of Interest

The author declares no conflict of interest.

## References

Allchin, D. (2017). Problem- and Case-Based Learning in Science: An Introduction to Distinctions, Values, and Outcomes. CBE—Life Sciences Education Vol. 12, No. 3.

Bierema, A. M.-K., Schwarz, C.V., and Stoltzfus, J.R. (2017). Engaging undergraduate biology students in scientific modeling: analysis of group interactions, sense making, and justification. CBE-Life Science Education, Vol 16, No. 4.

Freeman, S., Eddy, S. L., McDonough, M., Smith, M. K., Okoroafor, N., Jordt, H., and Wenderoth, M. P. (2014). Active learning increases student performance in science, engineering, and mathematics. PNAS 111(23): 8410–8415.

Frey, R.F., Brame, C. J., Fink, A., and Lemons, P. P. (2022). Teaching discipline-based problem solving. CBE- Life Sciences Education, Vol 21 No. 2.

Ghosh, S. (2019). Learning strategies to initiate and motivate students of an introductory microbiology laboratory class to perform cooperatively an inquiry-based project. Bioscene, Vol 45 (1).

Hanahan, D. (1983). Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. 166(4), 557-580.

Jacobs, G. (2014). Introverts Can Succeed with Cooperative Learning. Parole 4(1): 83–93

McLaughlin, J., & Metz, A. (2016). Vision and change: Why it matter's. American Biology Teacher, 78(6), 456-462.

Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, 2001.

The NIH guidelines (The National Institutes of Health Guidelines for Research involving recombinant or synthetic Nucleic acid molecules), NIH, April 2019. https://osp.od.nih.gov/biotechnology/nih-guidelines/.

National Academies of Sciences, Engineering, and Medicine. (2017). Undergraduate research experiences for STEM students: Successes, challenges, and opportunities. Washington, DC: National Academies Press.

Prasher, D. C., Eckenrode, V.K., Warde, W. W., Prendergast, F.G., and Cormier, M.J.(1992). Primary structure of the Aequorea victoria green-fluorescent protein. Gene 111: 229-233.

Premo, J, Cavagnetto, A, and Davis W. B. (2018). Promoting collaborative classrooms: The impacts of interdependent cooperative learning on undergraduate interactions and achievement. CBE—Life Sciences Education, 17:ar32, 1–1.

Waldrop, M. M. (2015). Why we are teaching science wrong, and how to make it right. Nature, 523, 272-274.