Cover page for Ottilie Schillig Special Teaching Projects

Title of Proposed Project:		
Project Director (PD) Name:	PD Phone Number:	PD Email:
PD Department:	PD College:	Estimated # of Students Impacted each Semester:
Requested Amount from Schillig Funds ($ 3,000.00 maximum):	Cost Share Amount (optional):	Total Amount for Project:
PD Signature:		Date:
Department Head Signature (if cost share included):		Date:
Co-PD Name:	Department:	Email Address:
Co-PD Name:	Department:	Email Address:
Co-PD Name:	Department:	Email Address:

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READS: Introducing a Realtime DNA Separation, Visualization and Documentation technique for the Undergraduate Students in Molecular Biology Methods

Dr. Aswathy Rai (anr424@msstate.edu), Dr. Seung-Joon Ahn (sa1838@msstate.edu) and Dr. Natraj Krishnan (nk260@msstate.edu)
Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

In molecular biology and biochemistry, the size of biomolecules (e.g.the molecular weight of protein, length of nucleic acids) is an important key information in any experimental analysis. One of the popular methods for size fractionation of such molecules is electrophoresis. Electrophoresis is thus arguably the most commonly used technique in molecular biology, making it an important component of any undergraduate molecular biology curriculum. Although polyacrylamide gel is also usable, agarose gel is the most commonly used compound for the electrophoresis of nucleic acids because of its ease of handling [1]. Hence, agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb [2]. After separation, the DNA molecules can be visualized under UV light after staining with a suitable dye –ethidium bromide (EtBr). The technique usually takes at least 60 minutes, often time-consuming when added with an introduction and training demonstration. In addition, there are safety concerns with using EtBr to stain the DNA (which is toxic) and UV light to visualize the DNA (which is harmful).

Figure 1: The Lonza FlashGel™ system

EtBr is a hazardous chemical as defined by the OSHA Hazard Communication Standard described in 29 CFR 1910.1200(d). It is a primary chemical safety concern when creating or working with gels in laboratories. Because of its ability to bind to DNA, EtBr is a potent mutagen capable of causing genetic damage. Exposure can occur via inhalation (if aerosolized), ingestion, and skin absorption Acute exposure can irritate the eyes, mouth, skin, and upper respiratory tract. Also, physical hazards are present during the heating portion of agarose gel preparation. Spilling and splashing may occur during the heating, mixing, and pouring of the hot liquefied gel, which poses a burning hazard to the body, including the face, hands, and upper body. Electrocution is also a potential hazard as typical voltages of 100 V can be applied across gels resulting in 25 mA of current. There are other stains and dyes which can substitute for EtBr [3], but what is required is a rapid yet sensitive and safe system that students can utilize for observing DNA migration through the gel in real-time. Here, we propose to employ the **Lonza FlashGel™ system** [4] (**Figure 1**) as a **Rea**ltime **D**NA **S**eparation, visualization, and documentation technique (**READS**) to engage undergraduate students of biochemistry in their Molecular Biology Methods course (BCH 4804). There are **several advantages of using this system**:

(a) It is fast, allowing for the separation of DNA bands in 5 – 7 minutes.

(b) It also has an integrated alternative transilluminator light (not UV) that allows students to watch DNA migrating in real-time (not usually possible with traditional methods).

(c) Furthermore, the DNA stain is not considered hazardous according to OSHA or EU hazard criteria and the gels come pre-made in a sealed cassette, meaning that students will have reduced exposure to any chemicals.

Approach: READS employs a 4-step approach (**Figure 2**):

1. **Loading DNA:** Load up to 32 DNA samples on a FlashGel™ cassette.
2. **Real time separation:** Observe real-time separation of DNA in 5 minutes without EtBr.
3. **Visualization:** Visualize the migrating DNA without UV at the bench.
4. **Documentation:** Document the gel digitally.

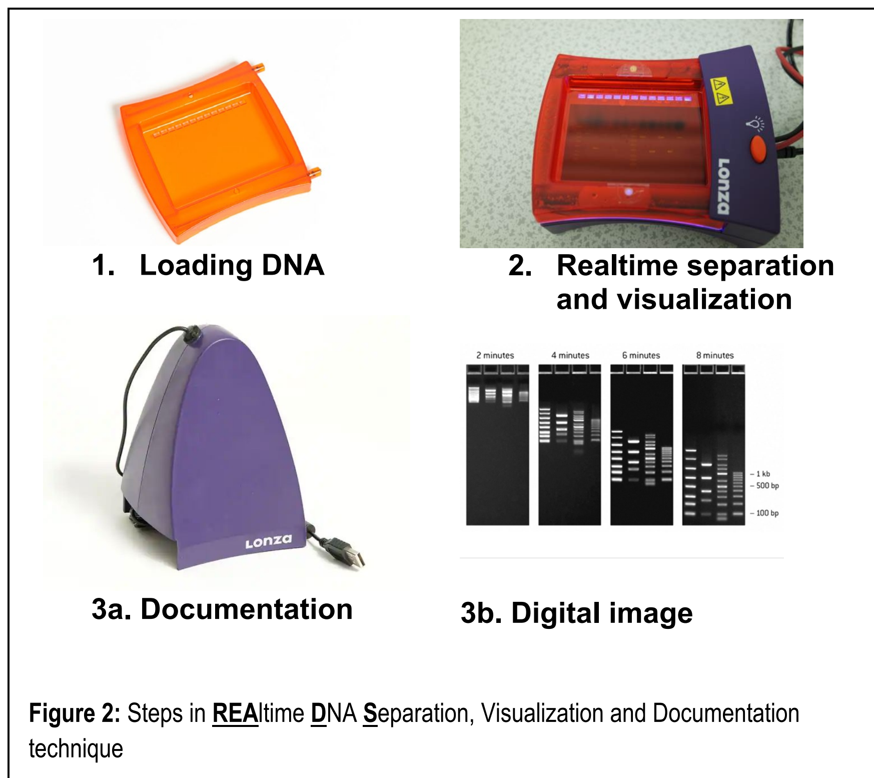

Figure 2: Steps in **REA**ltime **D**NA **S**eparation, Visualization and Documentation technique

Improvement of undergraduate teaching using READS:
DNA analytical techniques using **READS** can be effectively used in undergraduate teaching for fulfilling the following goals:

1) Reinforce theoretical concepts learned in the classroom in accordance with the curriculum in Molecular Biology Methods (BCH 4804).

2) Effectively train the students to conduct a series of experiments, thus exposing them to research concepts and achievement of objectives.

3) Prepare them for scientific careers as graduate or medical students.

4) Be feasible in terms of available funding.

The use of **READS** can fulfill all these goals as follows:

A) Theoretical concepts taught in the **Molecular Biology Methods** class **(BCH 4804)** meant for undergraduates will be reinforced with practical experience in laboratory demonstrations. **Dr. Rai** currently conducts the lectures and Labs in the course of Molecular Biology Methods and **Dr. Ahn** also participates in running the lab sections of the course. They are key for incorporating the **READS** technique in their curriculum.

B) Encouraging students to design their own experiments that may reveal mutations in genes. The most common approach in this regard will be to leverage the vast number of mutant and transgenic flies (*Drosophila melanogaster*) that Dr. Krishnan maintains. Students can easily understand how to spot genetic changes with specific genetic backgrounds or even perform crosses of their own design to generate transgenics. They can then check what has occurred in the DNA. The same approach can also be used in **Introduction to Forensic Science (BCH 2013) and Advanced Forensic Sciences (BCH 4333),** where students can easily analyze DNA fingerprints. Additional courses that will benefit include **Essential Biochemical Concepts and Analysis (BCH 3102), and Insect Biotechnology**

(EPP 8363). Students can subsequently create projects that utilize **READS** to determine changes at the nucleic acid level (DNA and RNA).

C) The undergraduate students are afforded the opportunity to participate in authentic scientific inquiry, by designing the project, maintaining the fly stocks (including crossing different strains) and gaining essential technical skills valued for a graduate student working in a *Drosophila* laboratory or even in the biomedical field. Students will also learn how to analyze data by taking part in running statistical analyses on the results which could lead to student publications.

D) Thus, in using **READS** technique, simple experiments can be conducted, providing an excellent means to teach undergraduates a scientifically sound method currently used in genetics. This would allow the students also to develop a tremendous amount of creativity and flexibility in designing experiments for their courses or collaborating with their research advisor.

Amount of funding requested: $3,000:

ITEM	COST
FlashGel™ System, includes dock, camera, cassettes, and accessories needed to use FlashGel™ Cassettes (Catalog #: 57067)	$ 1,763.00
FlashGel™ DNA Starter Kit, including a FlashGel™ Dock, a box of 1.2% FlashGel™ DNA Cassettes, FlashGel™ Loading Dye (5X) and FlashGel™ DNA Marker (Catalog #: 57026)	$ 815.00
FlashGel™ DNA Cassettes, 1.2% 16+1 well double-tier, 9 packs Easily screen 32 samples; Separation: 50 bp - 4 kb (Catalog #: 57029)	$ 422.00
Total	**$ 3,000.00**

Justification: The FlashGel™ System will be part of the teaching lab, once procured. The FlashGel™ Cassettes will be used initially as proof-of-concept test during the first two years of the program will eventually be factored into the lab fee after 24 months. Dr. Rai, Dr. Ahn, and Dr. Krishnan have extensive experience training undergraduate and graduate students through structured courses and Directed Individual Study programs. The proposed technique **will be invaluable for the BCH 4804 (Molecular Biology Methods) course and can be used to develop Course Based Undergraduate Research Experience for undergraduate students**.

References cited:

1. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments. 2012; 62:e3923. DOI: 10.3791/3923
2. Sambrook J, Russell DW. Molecular Cloning. 3rd 2001.
3. Hall, A.C. A comparison of DNA stains and staining methods for Agarose Gel Electrophoresis. bioRxiv. 2020; 568253; DOI: 10.1101/568253
4. https://bioscience.lonza.com/lonza_bs/US/en/Electrophoresis/p/000000000000190252/FlashGel%E2%84%A2-System