A Structured Inquiry-Based Module for the Undergraduate Cell Biology Laboratory That Teaches Fundamental Concepts of Cell Differentiation

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Abstract

Pedagogical research in science education has shown that students effectively learn science by doing science. As a result, there is increased interest in bringing research-like experiences into the classroom, particularly for laboratory courses. This lesson describes a structured inquiry laboratory module focused on the examination of muscle cell differentiation. Muscle differentiation is a complex process that provides a unique opportunity for undergraduate students to explore various aspects of cell biology in the laboratory. The students engage in a project spanning eight weeks in which they utilize three complementary techniques (including fluorescence microscopy, Western blotting, and reverse transcriptase-based polymerase chain reaction) to examine the morphological and genetic changes that occur during muscle cell differentiation in culture. The instructor assesses students on the quality of their laboratory notebooks, including how thoroughly they document each experiment, as well as on their participation in discussions regarding experimental design, techniques, and results. Ultimately, students compile their work into an individually written research report, the format of which parallels typical journal articles published in the field of cell biology. The design of this module allows students to explore fundamental cell biology concepts while learning key experimental techniques. In addition, the instructor teaches this module in a structured inquiry-based manner to engage students in learning through investigation and discovery.

Learning Goals

Students will:

◊ discuss principles of experimental design.
◊ evaluate the use of different lab techniques (fluorescence microscopy, Western blotting, and reverse transcriptase-based polymerase chain reaction) to address an experimental question in cell biology.
◊ formulate reasonable conclusions based on their results.

◊ From the Cell Biology Learning Framework:
  » Cell Communication: How do cells send, receive, and respond to signals from their environment, including other cells?
  » Cellular Specialization: How can and why do cells with the same genomes have different structures and functions?
  » Protein Targeting & Trafficking: How are cellular components targeted and distributed to different regions and compartments of a cell?
  » Methods & Tools of Cell Biology: How do the methods and tools of cell biology enable and limit our understanding of the cell?

Learning Objectives

Students will be able to:

◊ perform a variety of cell and molecular biology techniques that are used in typical research projects.
◊ relate the information provided by specific techniques to essential concepts of cell biology.
◊ evaluate and integrate their overall results.
◊ effectively communicate their results through written assignments.

◊ From the Cell Biology Learning Framework:
  » Cell Communication: explain how a cell's interaction with its environment can influence its differentiation ability.
  » Cellular Specialization: describe the process of muscle differentiation, including the role of specific gene regulation and muscle-specific protein expression.
  » Protein Targeting & Trafficking: examine and compare the differential localization and expression of two muscle-specific proteins and relate structure to function.
  » Methods & Tools of Cell Biology: compare the use of three complementary techniques to examine cell differentiation and describe strengths and limitations of each.

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INTRODUCTION

Cell differentiation is a critical concept for students to understand at the undergraduate level. Students must be able to explain how cells with the same genome can manifest different structures and functions. Laboratory experiences that focus on differentiation allow students to visualize and explore the process hands on. This lesson describes an eight-week laboratory module examining muscle differentiation in mammalian cell culture using three complementary techniques (fluorescence microscopy, Western blotting, and reverse transcriptase-based polymerase chain reaction). Students investigate muscle differentiation by examining the expression of two muscle-specific genes (myogenin and desmin) using the C2C12 cell line as a model system. The C2C12 cell line was originally isolated from mouse skeletal muscle (1). These cells can be maintained in a relatively undifferentiated state as proliferative myoblasts, and then induced to undergo differentiation into myotubes. Confluent mononucleated myoblasts express muscle-specific genes and undergo fusion, resulting in the formation of multi-nucleated tube-like cells that exhibit contractile properties (2). Myogenin is a well-characterized muscle-specific transcription factor that is required for differentiation. Myogenin expression is absent in myoblasts at lower confluency, however it is rapidly upregulated as cells reach higher confluency and begin to fuse together (3–6). Desmin, an intermediate filament protein, expresses at low levels in myoblasts. Desmin expression increases as myoblast confluency increases and continues to be upregulated throughout differentiation (7).

This module represents a cross between a traditional laboratory course and an inquiry-based one. In traditional laboratory courses, most aspects of the laboratory experience are instructor-defined or instructor-driven. The findings are previously established, and the instructor role is to teach students new skills (8). In this module, the study design and methodology are instructor-driven, and the purpose of the investigation is instructor-defined. The students examine the expression and localization of two muscle-specific proteins by following provided procedures for three techniques. Students form hypotheses and predict expected results based on information regarding these proteins in the literature. The main role for the instructor is to teach skills required to carry out the techniques, as well as to facilitate discussion and interpretation of results.

The literature describes a range of inquiry-based curricula, with different levels corresponding to the degree of autonomy that students have over the research questions and experimental procedures. While “inquiry-based” is an extensive term that encompasses a variety of instructional design methods, all inquiry methods provide an opportunity for students to engage in the process of discovery to some degree, with varying levels of guidance from the instructor. Inquiry-based pedagogy is associated with improved student outcomes, including increased understanding of course material (9). Windschitl describes four types of laboratory instruction methods including traditional, structured inquiry, guided inquiry, and open inquiry (10). In traditional laboratory experiences, also known as confirmation experiences, students corroborate known information by following a set of provided instructions. In structured inquiry laboratories, the instructor poses a question with an answer that is unknown to the students, and the students determine an answer by following a given set of procedures. In contrast to this, guided inquiry laboratory experiences allow for an instructor-defined problem or question to focus the investigation, but the students determine the methods to answer the question. In open inquiry laboratories, students determine both the research question and the procedures to develop their own independent study (10). The laboratory module described in this lesson aligns with the structured inquiry mode in which students follow a given set of procedures to determine an answer to an instructor-defined question.

In comparison to the classifications posed by Windschitl, Domin describes four types of laboratory instruction styles including expository, inquiry, discovery, and problem-based (11). According to these categories, the laboratory instruction style for this module can be classified as discovery (or guided inquiry), where the outcome is predetermined, the approach is inductive, and the procedure is provided for the students. In an inductive approach, students form an understanding of essential concepts and principles by observing a particular occurrence. Domin defines guided inquiry as a discovery-learning mode in which the instructor is aware of the outcome, but the students are not, and the role of the instructor is to guide students towards the outcome by providing specific steps (11).

According to the National Research Council, inquiry-based pedagogy includes methods that involve students in thinking and working as scientists. This denotes a range of activities including recording observations, constructing questions, collecting reproducible evidence, determining conclusions by assimilating new evidence with previously established findings, conveying results through oral and written formats, and modifying conclusions or experimental design in response to critical feedback (12). There are several inquiry-based aspects in this laboratory module. Students engage in multiple scientific practices, including developing hypotheses, using the tools of science, gathering and analyzing data, identifying meaningful variation, developing and analyzing interpretations and arguments, and communicating findings. In addition, the outcome of the study is unknown to students, so they are participating in the discovery process as they progress through the lab sessions. As indicated above, the instructor role is to train students in new skills as well as to assist in the interpretation of results and promote meaningful discussion. This guided process is important since the results may be unclear, and the instructor acts as a facilitator to help students analyze the data that they have gathered. Through discussions, students troubleshoot issues and determine changes to the experimental design. Within the timeline that is available in the regular semester, it is not typically possible for students to repeat experiments. If desired, an instructor may incorporate iteration with some modifications to the timeline (for example by leaving an extra week or two with open lab sessions specifically designated for experimental repeats). Finally, the relevance of the work completed is limited to the course, which is a feature of both traditional and inquiry-based labs (8).

This laboratory module also requires students to use higher order thinking processes, as described by Bloom’s taxonomy. Through sessions that build upon one another, students are required to analyze their results, synthesize new information,
and evaluate the ability of complementary techniques to provide different information about the same cellular process (13). This laboratory module challenges students to assimilate the content on a deeper level, which is a characteristic of meaningful learning (14). The instructor introduces muscle differentiation and the context of each laboratory session but does not provide students with the expected outcome of each experiment. Instead, the instructor asks students to formulate hypotheses by integrating each new laboratory experience with their current knowledge. This promotes several higher order thinking processes that are components of inquiry, such as hypothesizing, explaining, analyzing, and evaluating results (15).

A structured inquiry laboratory design offers advantages for both the instructor and students. For the instructor, it is easier to plan for logistical aspects such as supply needs and semester timeline. For students, this type of experience is ideal to allow them to gain benefits of the discovery process while maintaining a consistent experience for the entire class. However, the success of this approach depends on appropriate execution. While the overall question is instructor-defined, the instructor must challenge students to consider their own hypotheses and gather their own background knowledge. Students should take an active role in discussions to determine the answers to questions and expected results for experiments. Likewise, the interpretation of results should fall to students first, with the instructor facilitating the analysis. This will ensure that the process remains inquiry-based for students, rather than devolving into a traditional confirmatory or expository laboratory experience.

A potential disadvantage of the structured inquiry approach is that students do not have the opportunity to develop their own research question or choose their own methods of analysis. Because the entire class is working on the same question and experimental techniques, it is possible that some students will not engage in the discovery process themselves but instead allow other students to formulate answers or analyze data for them. This possibility exists with any mode of instruction that involves groups working on the same topic. If desired, an instructor could make modifications to this module to align more closely with an open-inquiry mode of instruction, and I have outlined these further in the discussion.

**Intended Audience**

I have taught this laboratory module to junior and senior life sciences majors at a four-year university (typically including biology, biochemistry, and forensic science majors). The module is part of a semester-long cell biology laboratory course that is a co-requisite to a cell biology lecture course. Both the lecture and the laboratory are required for the majors listed above. The lecture typically consists of 16 students, and there are two accompanying lab sections, capped at eight students each. This laboratory module would also work for larger section sizes with adequate space, equipment, and instructor support.

**Required Learning Time**

This laboratory module spans eight weeks during the semester, with sections meeting once a week for three hours. I maintain the cell cultures outside of the lab sessions but if desired, an instructor could choose to modify this so that students are required to come in outside of lab time to split cells, change media, and perform other routine culture maintenance procedures.

**Prerequisite Student Knowledge**

Students have typically taken introductory biology, microbiology, general chemistry, and organic chemistry. A prior genetics course would be helpful but is not required. I remind students of basic skills such as micropipetting and teach all major techniques within the context of the lab sessions. It is important to note that I teach this module in the second half of the semester after students have already spent several weeks learning basic cell culture terminology and techniques.

**Prerequisite Teacher Knowledge**

Instructors should be well versed in eukaryotic animal cell biology. A basic background in skeletal muscle structure, function, and differentiation is also required. Instructors should have prior training in mammalian cell culture, including aseptic technique, media preparation, trypsinization, quantitation, and subculturing of cell lines. In addition to cell culture, instructors should have prior knowledge and experience with each of the three main techniques used, including fluorescence microscopy, Western blotting, and reverse transcriptase-based polymerase chain reaction (RT-PCR).

**SCIENTIFIC TEACHING THEMES**

**Active Learning**

Students work in pairs to carry out experimental techniques. While I maintain the cell cultures outside of the lab sessions, students are responsible for preparing and analyzing their own samples within each lab session. They also participate in discussions focused on experimental design and interpretation of results. This includes think-pair-share to engage students in discussion of specific topics. Students may also work collaboratively to write the lab report at the end of the module.

**Assessment**

This laboratory module includes three forms of assessment, outlined below.

**Individual laboratory notebooks:** Students must maintain a laboratory notebook that details each experiment performed, and includes introduction, methods, results, and discussion sections. I evaluate the notebooks based on completeness, effort, accurate interpretation of results, effective formulation of conclusions, and critical discussion. I have included the rubric that I used to grade the lab notebooks at the end of the module (Supporting File S16). Since this module occurs in the second half of a semester-long laboratory course in cell biology, I provide students with feedback on their notebook-keeping from the first half of the semester using a similar rubric prior to the start of this module. This allows students to receive feedback and make improvements. Instructors may choose to provide feedback more frequently if desired.

**Laboratory reports:** Students complete a final laboratory report outlining their results and interpreting them in a broader context. The report format mirrors typical journal articles in the
Students are assessed on their participation in class discussions. In addition, students wish to form groups or pairs based on how individual students approach challenges, as well as taking other considerations into account. Inclusion of cell biology. I grade the lab reports for specific elements using a rubric (Supporting File S15). Students may complete these reports individually or in pairs through collaborative writing. I have used both methods in different iterations of the course. I collect the reports at the end of the module without submission of earlier drafts. This works well in the semester-long course since students complete a similar lab report on a different topic prior to the start of this module, so they can take what they have learned from that experience and apply it to this report. In addition, the rubric is detailed and provides clear guidance on what to include in the report. Instructors could choose to have students submit drafts, or alternatively complete a peer review process to receive additional feedback. This is further outlined in the Teaching Discussion.

**Student performance in lab:** Students are assessed on their participation in the lab sessions, including execution of techniques and effective work with their lab partner. Students must also participate in discussions regarding experimental design and results. I evaluate students for laboratory performance using a rubric that includes separate scoring sections for Laboratory Work and Discussion Participation (Supporting File S17). I go over this rubric with students prior to the start of the module so that they understand the expectations. Since this module is part of an upper-level laboratory course, students are expected to use information provided in the prelab lectures and lab handouts to efficiently carry out procedures. I let students know that questions in lab are encouraged, but they should rely on the information provided in the prelab lectures and lab handouts. For participation in discussions, students have several options including answering questions posed to the class, asking follow up questions or other questions related to the topic, and offering other relevant comments or thoughts. Students raise their hands to offer questions or comments and I call on them in turn. By doing this, I can provide opportunity for all students to participate in the discussion. I also ask students to speak with me if they are concerned about participating in class. I encourage them to consider that oral communication is an important transferrable skill that will benefit them beyond the classroom and participating in discussions in a small class format is an excellent way to develop confidence. I also provide another option, such as writing down their contributions on a notecard, to promote fair evaluation for all students. However, since the sections are small, this is not typically an issue.

**Inclusive Teaching**

This laboratory module provides an ideal setting for group work. Students learn effectively in groups when completing difficult tasks and working on problems with more than one correct answer (16). Typically, I allow students choose partners. In some instances, however, student pairings may not be optimal and result in ineffective work (17). Instructors may wish to form groups or pairs based on how individual students approach challenges, as well as taking other considerations into account.

In-class discussions provide another mechanism of inclusive teaching. These discussions engage students in thinking about the science behind the experiments. In addition, students may develop a sense of capability and confidence through participation in class discussions.

Finally, I also use several types of assessment to provide students with diverse skill sets and backgrounds with the opportunity to excel in the lab. As noted in the previous section, this includes laboratory notebooks, laboratory reports, and performance in lab.

**LESSON PLAN**

The teaching timeline for this lesson can be found in Table 1.

**Week 1**

**Pre-Lab Preparation**

A few weeks prior to the start of the laboratory module, the instructor should obtain, expand, and freeze several stocks of C2C12 cells (available from ATCC® CRL-1772®). Culture cells according to the recommended protocol from ATCC and see Supporting File S1 for detailed instructions for weekly culture preparations.

Prepare 35 mm cell culture plates containing sterilized coverslips coated with 0.1% gelatin, as well as regular growth medium and differentiation medium (Supporting File S1). Prepare enough plates so that each student group has four total. Groups will immunostain two plates of C2C12 myoblasts and two plates of C2C12 myotubes so you must seed the plates at appropriate times prior to the first and second lab sessions. I would recommend seeding plates for myotubes approximately three to five days prior to the first lab session (depending on the density) to allow the cells to grow to 100% confluency. Students can add differentiation medium during the first lab session and allow the cells to differentiate over one week to lab session two (the instructor should change the medium approximately every two days on differentiation plates). Seed plates for myoblasts two to three days prior to the second lab session. Myoblasts should be approximately 60% confluent on the day of lab session two.

Review the handout for students (Supporting File S2) and the introductory PowerPoint (Supporting File S3).

**Lab Session**

**Introduction to Lab**

Review the handout and introductory PowerPoint with students. I teach this laboratory module during the second half of the semester, so students are already familiar with the basic techniques and terminology associated with cell culture. If students are not already familiar with these, you may wish to review additional background information (Supporting File S4).

**Student Work**

Students observe the confluent myoblast plates using the phase contrast microscope. Students will change the medium on these plates from regular growth medium to differentiation medium. The plates will differentiate over seven days until lab session two, with medium changes performed by the instructor approximately every two days. In lab session two, students will receive additional plates of undifferentiated myoblasts to immunostain and compare with the differentiated plates.

**Class Discussion**

Students discuss questions regarding fundamental concepts of cell differentiation. What makes a muscle cell different from
other cells, and how do these differences occur? What are some experimental techniques to examine such differences between cells? These questions provide a connection to the concept of Cell Specialization from the Cell Biology Learning Framework. Use think-pair-share to encourage further discussion among students. Ultimately, the discussion should connect back to the information provided to students in the handout and introductory PowerPoint.

**Follow-Up**

Students do not need to come into lab outside of the regular session since I prepare the cell plates for lab session two and perform the medium changes. To help students visualize what occurred during the intervening week between lab sessions one and two, I document the differentiation process using the phase contrast microscope and attached camera, so that students can see how the cells changed over the course of time. I also prepare plates of myoblasts a few days prior to lab session two, so that each group will have two plates of undifferentiated cells (approximately 60% confluent) to compare with the differentiated myotubes.

**Week 2**

**Pre-Lab Preparation**

Seed 35 mm plates containing gelatinized coverslips with myoblasts a few days prior to lab session two. The timing is somewhat flexible. On the day of the lab, the cells should be attached and growing, but should not be too confluent. You can seed them closer to the lab session if you add more cells per plate, or you can seed them several days prior if you add fewer cells per plate. In addition, prepare 60 mm plates for differentiation for lab session three during this week. See Supporting File S1 for instructor information regarding culture plate set up.

Review the introductory board notes (Supporting File S5) and the handout for lab session two for the students (Supporting File S6).

**Lab Session**

**Introduction to Lab**

Due to the length of time for this lab, students start with the procedure immediately, and then I go over the introduction during the one-hour primary antibody incubation (Supporting File S5).

**Student Work**

Groups observe their plates using a phase contrast microscope (two plates of myoblasts and two plates of myotubes), and then continue with the immunostaining steps. Groups should work around the same pace so that everyone gets to the one-hour primary antibody incubation within several minutes of each other. Once that is started, go over the board notes for immunostaining (Supporting File S5). You may also wish to review fluorescence microscopy if necessary. By the time I am finished with the introduction, students are usually ready to continue the procedure. If necessary, you can provide additional information for immunostaining and fluorescence microscopy during the 30-minute secondary antibody incubation. Once students finish, they will store their slides until viewing at the next session.

**Class Discussion**

Since the procedure takes the entire time, I do not have a discussion at the end of this lab, however I do encourage some discussion as we go over the immunostaining background. Specifically, I ask students to comment on where they think they will visualize the proteins based on their roles in the cell. Myogenin is a regulatory transcription factor and desmin is an intermediate filament protein, so their expected localizations are in the nucleus and cytoplasm, respectively. This provides a good opportunity to discuss localization as it relates to function and is connected to the concept of Protein Targeting & Trafficking from the Cell Biology Learning Framework.

**Follow-Up**

Unless the lab session is longer than three hours, students will visualize their slides outside of class or in the following session. Students take fluorescent images of each slide and save them for making a figure. The control fluorescence slides are expected to show only non-specific background staining. Using a mounting medium with DAPI is preferable so that students can visualize the nuclei when viewing control slides. The experimental fluorescence slides should show specific staining patterns for myogenin in the nucleus and desmin in the cytoplasm. Students image a representative area for each slide (400X magnification recommended).

**Week 3**

**Pre-Lab Preparation**

This lab requires 60 mm plates of C2C12 cells for protein extraction. Each student group receives one plate each of myoblasts and myotubes. Start the myotube plates at least one week prior, similar to the preparation of the 35 mm plates. In addition, prepare buffers and bovine serum albumin (BSA) standards. See Supporting File S1 for detailed instructor protocols. Finally, review the handout for lab session three for students (Supporting File S7).

**Lab Session**

**Introduction to Lab**

I briefly go over major points from the introduction and procedural steps in the handout. Students should move through the procedure in a timely manner, since they will also visualize their cells on the fluorescent microscope during this session.

**Student Work**

Students complete each step of the procedure for protein extraction. Once again, it is useful for groups to work around the same pace so that everyone can centrifuge their tubes together, unless you have multiple centrifuges available. Once students have obtained concentrations for their protein extracts, we go over graphing the standard curve and calculating the protein concentrations in Excel. As groups are ready, they visualize their slides from last week and take images.

**Class Discussion**

There are two main areas of discussion for this lab session, including the purpose of protein extraction and quantification and comparison to the immunostaining technique used in the last lab session. Students should be able to explain the importance of accurately quantifying protein concentration in preparation of the Western blot analysis that they will perform over the next two sessions. In addition, lab sessions three, four, and five introduce a biochemical approach to studying protein expression, in contrast to the microscopy approach used in lab session two. Students participate in the discussion with the instructor providing necessary points for guidance.
Follow-Up

Students store their protein extracts for performing Western blotting at the next lab session. They also print their standard curve graphs and protein concentration quantifications from Excel, as well as their fluorescent images, for the results section of their notebooks.

Week 4
Pre-Lab Preparation

Prepare solutions for Western blotting and obtain gel and transfer rigs. I use premade 10% SDS-PAGE gels (from Bio-Rad), due to time constraints and a lack of materials for students to make the gels on their own. See Supporting File S1 for instructor protocols. Review the board notes (Supporting File S8) and handout for students (Supporting File S9).

Lab Session
Introduction to Lab

Students start with the procedure and then go over the introduction once the gels are running for the most efficient use of time. This includes information from the introduction in the handout and the board notes (both referenced above). Students obtain their protein extracts from last week and proceed through each step as outlined in the handout. Groups can share a gel and electrophoresis station (either two groups sharing one set up or different variations depending on the class size and amount of equipment). Loading the vertical gel and setting up the membrane transfer will likely require a demonstration and close guidance unless the students have performed these techniques previously.

Class Discussion

Students will not see the results of the Western until the next session, so this provides an excellent opportunity for them to discuss potential expected results.

Follow-Up

Once the transfer is complete, membranes are stored until the next session.

Week 5
Pre-Lab Preparation

In this week’s session, students finish the Western blots and extract RNA from myoblasts and myotubes. For Western blotting, I use the WesternBreeze Chromogenic Western Blot Immunodetection Kit (Invitrogen), with solutions prepared according to manufacturer’s protocol. The entire Western blotting protocol takes about four hours from start to finish, so I start the first few steps prior to the lab session and have students add the primary antibodies to the membranes at the start of the lab session. During the primary antibody incubation, students can complete the RNA extraction. Each student pair should have one 60 mm plate each of myoblasts and myotubes. These are prepared in the same way that the 60 mm plates were for protein extraction. See Supporting File S1 for detailed instructor preparation and materials for this session. Review the handout for students (Supporting File S10).

Lab Session
Introduction to Lab

Briefly introduce the set-up for the second part of the Western blotting and explain what steps have been completed (for a three-hour lab, this is usually up to the point of the primary antibody incubation). Students may note that there is a lot of similarity between the Western and immunostaining procedures as far as requiring a blocking step, antibody incubations, and washes, however they also represent different techniques as a biochemical and microscopy approach, respectively. After you bring students up to speed on the Western, introduce the reagents and protocol steps for RNA extraction.

Student Work

Students proceed with RNA extraction while the Western blots incubate with the primary antibody for the first hour of lab. Each student group extracts RNA from their own set of samples (one plate each of myoblasts and myotubes). Once students complete the RNA extraction, they place their samples on ice and store them in the -80 °C freezer until the next session. Students then proceed with the remainder of the Western blotting protocol. Groups can share a station (either two groups sharing one set up or different variations depending on the class size and amount of equipment). In a three-hour lab session, we usually add the chromogenic substrate around 15 minutes from the end of the session, and students are able to see the bands beginning to form on the membrane.

Class Discussion

Students will observe the initial results of the Western blots, even if the incubation with the chromogenic substrate proceeds beyond the end of the session. Students discuss their potential expected results in comparison to the initial results that they observe on the membranes. This is also an excellent opportunity to discuss caveats of the Western blot, including antibody specificity, control protein detection, and sources of error.

Follow-Up

If bands are slow to develop, the incubation with the substrate can go up to one hour. I complete the final few steps by rinsing the membranes, drying them, and then posting images of the results on the course website so that students can review them after lab and print a copy to include in their notebooks. You could also have students come in outside of lab time to image the membranes.

Week 6
Pre-Lab Preparation

Prepare reagents and materials for agarose gel electrophoresis. See Supporting File S1 for detailed instructor preparation and review the handout for students (Supporting File S11).

In this session, students will run the extracted RNA from last week out on an agarose gel to check for integrity and estimate concentration.

Lab Session
Introduction to Lab

Briefly introduce the set up for the gels and electrophoresis stations. Emphasize proper technique for loading the gels. It is
important that students load samples fully to allow for the most accurate interpretation.

**Student Work**

Students pour the agarose gels as soon as they come into lab. Once solidified, each student group loads their own samples. Once the electrophoresis is complete, students can visualize the results and capture electronic images.

**Class Discussion**

Students should discuss the appearance of the RNA on the gels and relate this to both RNA quality and quantity. They will use an estimated RNA concentration to set up the reverse transcription reactions the following week. This is also a good opportunity to discuss the limitations of this method and compare with other, more precise methods of determining nucleic acid concentration, such as spectrophotometry. You might consider incorporating another method such as this, if available.

**Follow-Up**

The RNA samples should be stored in a -80 °C freezer until the next lab session. Students can print images of the gels to include in their notebooks.

**Week 7**

**Pre-Lab Preparation**

Prepare reagents and materials for reverse transcription and polymerase chain reaction (PCR). See Supporting File S1 for detailed instructor preparation and review the board notes (Supporting File 12) and handout for students (Supporting File 13).

**Lab Session**

**Introduction to Lab**

In this session, students complete the third and final technique to examine gene expression in C2C12 differentiation. Students perform reverse transcription to make cDNA and then set up PCR reactions to examine the expression of myogenin, desmin, and GAPDH as a control. Briefly review the schematic for RT-PCR on the board and then have students proceed with setting up the reverse transcription reactions. You can spend more time on the introduction during the reaction incubation.

**Student Work**

Students will set up their reactions for reverse transcription using the RNA that they analyzed by electrophoresis last week. They should estimate the concentration of the samples by comparing them to a set of standards with known concentration and then use appropriate equal amounts for each sample. Once the reverse transcription is complete, students proceed with setting up the PCR reactions. Three reactions should be set up for each sample (one with myogenin primers, one with desmin primers, and one with GAPDH primers). See Supporting File S1 for information on PCR components and student set up.

**Class Discussion**

Students engage in two major points of discussion during this session. The first is what results they expect for the RT-PCR. They can relate this back to the results for Western blotting. The second is a comparison of the three complementary techniques (fluorescence microscopy, Western blotting, and RT-PCR) used to examine the expression of myogenin and desmin in C2C12 myoblasts and myotubes. Students consider the advantages and disadvantages of each technique, and why it is important to use more than one technique to address an experimental question.

**Follow-Up**

At the end of this lab session, students will put their PCR reactions in the machine to run for a few hours to overnight. I take the PCR reactions out once they are finished and store them in the -20 °C freezer until the final lab session.

**Week 8**

**Pre-Lab Preparation**

In this session, students will analyze the PCR reactions from the previous week by electrophoresis. Prepare reagents and materials for electrophoresis. See Supporting File S1 for detailed instructor preparation. Review handout for students (Supporting File S14).

**Lab Session**

**Introduction to Lab**

Briefly introduce the set up for the gels and electrophoresis stations. Students will be familiar with this from running out RNA on agarose gels in lab session six. Review proper pipetting and gel loading technique, as this is essential for accurate interpretation of results.

**Student Work**

Students pour the agarose gels as soon as they come into lab. Once solidified, each student group loads their samples. It is important that students do this accurately, as loss of sample will prevent accurate interpretation of results. Once the electrophoresis is complete, students can visualize the results and capture electronic images.

**Class Discussion**

Students should compare their actual results to their expected results, as well as to the Western results that they previously generated. This is also an excellent opportunity for students to discuss potential sources of error. Even though PCR is a relatively simple technique, there are multiple factors that may affect the outcome, including primer specificity and various components of the reaction conditions. Students should discuss these in relation to the observed results. They should identify the most likely sources of error and suggest potential modifications.

**Follow-Up**

Students include these results in their notebook, and if desired, in the laboratory report. See Supporting File S15 for a rubric that I use for the laboratory report. I do not have students include the RT-PCR results, but they should include both the immunostaining and Western results from previous sessions.

**TEACHING DISCUSSION**

**Effectiveness in Achieving Learning Goals and Objectives**

This laboratory module allows students to engage in key aspects of experimental design, such as considering a research question and developing a hypothesis, which they then test using different experimental techniques (fluorescence...
microscopy, Western blotting, and reverse-transcriptase based polymerase chain reaction or RT-PCR). By using fluorescence microscopy, students can visualize changes that occur in cell structure during differentiation, as shown through the specific staining patterns for two muscle proteins. Students are also able to study changes in gene expression using Western blotting and reverse transcription combined with polymerase chain reaction. These fundamental techniques are useful across a wide variety of research projects. Through discussion, students gain an appreciation for the value of each technique and their individual contributions to an overall understanding of a particular question in cell biology. They also recognize that these techniques are applicable in other contexts. Additional aspects of experimental design are emphasized as students engage in discussions regarding experimental controls, troubleshooting, and appropriate protocol modifications based on results obtained, although the timeline outlined here does not allow students to repeat modified experimental procedures.

This module requires students to state their conclusions in their laboratory notebooks as well as in a written report. Based on the results obtained, students develop reasonable conclusions through their consultations with other classmates. We take time to discuss various influences on the results and how these might affect the interpretation. Students also compare the actual results to the predictions and hypotheses that they generated previously. This is a critical step, since students will often get results that do not match what they expected (either because their predictions were inaccurate or because the results are flawed).

Students evaluate and integrate their results through laboratory discussions and laboratory notebooks. They also write a formal laboratory report that requires them to communicate their results in a cohesive manner. The report mirrors primary research papers typically published in the field of cell and molecular biology. I often remind students that they should consider the results that they obtained with previous techniques when predicting expected outcomes for a new one. For example, students might have some prediction of how expression will change in the RT-PCR experiment based on the previous Western results. Students learn to consider their results collectively to determine if they reveal consistent findings regarding the changes that occur during muscle cell differentiation. The laboratory notebook and laboratory report also provide an opportunity to assess the ability of students to connect their results to cell biology concepts, further outlined below.

Relevant concepts from the Cell Biology Learning Framework include Cell Communication, Cellular Specialization, Protein Targeting & Trafficking, and Methods & Tools of Cell Biology. Students consider how cells communicate with one another and how culture conditions affect cell structure and function. Muscle cells provide an ideal model system since students can easily observe morphological changes in living cultures. I introduce the concept of cell communication at the beginning of the module when students are learning about the C2C12 cell line and the ability of the cells to undergo either proliferation or differentiation. I ask students to explain why it is important for cells to grow to confluency to undergo differentiation and how the different medium conditions facilitate this process. I assess students’ responses to this in the laboratory notebooks.

This module challenges students to think about the fundamental concepts of cell specialization and protein targeting within the cell. They consider how cells with the same genomes take on different structures, directly relating to an understanding of cell-type specific gene expression and regulatory transcription mechanisms. By studying the expression and localization of a muscle-specific transcription factor, students can apply the concept of transcriptional regulation as a key step in cell differentiation. By studying the expression and localization of a muscle-specific structural protein, students can apply the concept that different structures exist to support different functions. They must be able to explain how cells that are proliferative and mononucleated are able to transform into muscle-like cells that are quiescent and multinucleated. Students can visualize the differentiation process through phase contrast and fluorescence microscopy. In the fluorescence microscopy experiment, students form a hypothesis for the locations of the two muscle proteins, based on their roles in the cell. Desmin localizes to the cytoplasm, where it functions in maintenance of the structural integrity of the muscle cell. As a transcription factor, translation of myogenin occurs in the cytoplasm but it functions the nucleus. This connects to the concept of compartmentalization and specific localization of proteins involved in different cellular processes. Based on this knowledge and the observation of the fluorescence microscopy results, students form the conclusion that myogenin must possess a nuclear localization signal, providing an opportunity for them to apply information that they learn in the textbook and lecture setting to the laboratory.

Students also hypothesize how protein expression levels change during differentiation as cells progress from mononucleated myoblasts to multinucleated myotubes. They then test their hypothesis using both Western blotting and RT-PCR. In the Western blotting experiment, students confirm the expected band size for each protein, compare the levels of protein expression for myogenin and desmin in myoblasts and myotubes, and discuss the importance of reliable controls. They also compare the two techniques that they used to examine protein expression (fluorescence microscopy for localization and Western blotting for levels of expression). This comparison encourages students to think about how changes in gene expression result in corresponding changes in cell structure and function. They can also appreciate that this is not specific to this experimental situation with muscle cells, but rather is a fundamental concept that underlies the structure and function relationship that is apparent throughout multicellular organisms.

In the RT-PCR experiment, students compare band intensities for myogenin, desmin, and GAPDH in myoblasts and myotubes. If band appearances are not clear or are absent (due to issues with the PCR or with gel loading), students may conclude that they cannot accurately interpret the results. However, even if the results are not as expected, students can still learn valuable information, since this allows for discussion of possible sources of experimental error and modifications for future experiments. The use of RT-PCR also allows students to distinguish techniques that analyze expression at the RNA level versus the protein level, providing a connection to the central dogma and the stages of gene expression.
It is important to note that students do not have pre-determined answers or outcomes for these questions and experiments. They use their collective knowledge and resources to formulate their own predictions. Following discussion in class, students often correctly identify the locations of each protein (the nucleus for myogenin and the cytoplasm for desmin). They frequently come up with different answers for how expression might change upon differentiation from myoblasts to myotubes, and they explain their choices based on the references that they have consulted.

Through this laboratory module, students develop an awareness of what information they can gain, and what they cannot gain, within the context of cell culture as a model system. They understand that cultured cells have been adapted from a three-dimensional tissue to a two-dimensional culture plate. By performing three separate techniques looking at the same proteins, they determine the information revealed by each technique, as well as the advantages and disadvantages of each one. Overall, this laboratory module helps students to recognize the value of using multiple approaches to yield a more complete understanding of a given cellular process.

**Student Reactions**

**Experimental Techniques**

In my experience, most students enjoy learning the techniques in this module. For most students, this is their first time working with cultured cells, and they have not typically performed fluorescence microscopy or Western blotting. In the previous course (RT-PCR and agarose gel electrophoresis) students became acquainted with doing DNA analysis, which many students take prior to this. Over the course of the module, they develop an awareness of how researchers use these methods to answer questions in cell biology. Student comments indicate that they liked using different techniques over several lab sessions to explore aspects of one scientific question, and that this helped them understand how research is performed. Some students specifically commented on fluorescence microscopy and immunostaining as favorite techniques.

**Experimental Design**

Given the timeline of this module, students do not have the opportunity to design their own experiments. I choose the techniques ahead of time for the purpose of planning the lab sessions. Students approach these methods from the standpoint of a novice and consider important aspects of experimental design throughout the lab sessions. Motivated students may benefit from greater autonomy. For example, one student indicated a desire to check on the cell cultures more often to allow for improved results and expressed interest in performing additional experiments. On the other hand, some students have commented that this would require too much time and effort outside of class.

**Cell Biology Concepts**

This module provides a connection to key concepts from the Cell Biology Learning Framework (including Cell Communication, Cellular Specialization, Protein Targeting & Trafficking, and Methods & Tools of Cell Biology). I frequently have a wide range of interest level and academic ability in my classroom. Students are usually in their junior or senior year, and have completed general biology, microbiology, and potentially some additional other upper-level biology courses. They have also completed general and organic chemistry. This module is part of a semester long laboratory course that students take in conjunction with a lecture course in cell biology. I often hear from students that this is among the most difficult biology courses, and they frequently struggle with concepts in the lecture. However, it seems that some students gain a better understanding of the concepts once they have applied them in an experimental setting. For example, one student commented on how the techniques from lab reflected the content covered in lecture, and specifically pointed out an example with performing immunostaining and observing the results, as opposed to just seeing fluorescent images in the textbook. Another student commented that the lab techniques were helpful for the comprehension of cell biology.

**Suggestions for Possible Improvements or Adaptations**

This module could be adapted to provide students with greater autonomy over the research question and analysis. Given the nature of the techniques, you may want to consider how feasible this would be for your own course and institution. I need to know the materials that I will order for the lab well ahead of time, which limits the ability of students to design their own research questions. For example, if different groups decided to look at different proteins, we would need to order multiple antibodies and reagents depending on what students chose to examine, which would not be feasible in terms of budget or timeline. It would also be necessary for students to maintain their own cultures outside of lab (for example if different groups are examining different time points in differentiation or some other variation). These limitations are the main reason that I have developed the laboratory module as a structured inquiry-based approach. To increase the autonomy, you could acquire materials for a few additional avenues of investigation and have student groups choose one to follow up on.

For the lab report, I provide three references that students are required to use, and they can refer to laboratory handouts as well. Other sources are encouraged but not required. You could modify this so that students perform their own literature search to develop the background and introduction for their lab reports. This would provide an opportunity for students to practice additional skills, such as finding relevant sources, reading the primary literature, and incorporating it appropriately into the report. As mentioned earlier under Assessment, I collect the report at the end and do not have students submit a draft. You could modify this to have students hand in a draft for instructor evaluation, or alternatively complete a peer review of each other's reports. With appropriate guidance and direction, students would gain meaningful feedback on their work from peers and would have the opportunity to scaffold their writing and make revisions (19).

Finally, you could modify this module to look at other muscle-specific proteins of interest, using the same techniques presented here, or you could adapt the module to look at differentiation in other contexts (for example, by using another commercially available cell line, such as PC12 neuronal cells). Several additional avenues of investigation are provided in Supporting File S18. These adaptations provide an opportunity for instructors to incorporate additional inquiry and broaden the applicability of this lesson.
SUPPORTING MATERIALS

- S1. Muscle Differentiation Inquiry Lab – Weekly Solution and Cell Preparations
- S2. Muscle Differentiation Inquiry Lab – Session 1 Handout for Students
- S3. Muscle Differentiation Inquiry Lab – Introduction to C2C12 Cell Line Slides
- S4. Muscle Differentiation Inquiry Lab – Additional Background Information
- S5. Muscle Differentiation Inquiry Lab – Immunostaining Board Notes
- S6. Muscle Differentiation Inquiry Lab – Session 2 Handout for Students
- S7. Muscle Differentiation Inquiry Lab – Session 3 Handout for Students
- S8. Muscle Differentiation Inquiry Lab – Western Blotting Board Notes
- S9. Muscle Differentiation Inquiry Lab – Session 4 Handout for Students
- S10. Muscle Differentiation Inquiry Lab – Session 5 Handout for Students
- S11. Muscle Differentiation Inquiry Lab – Session 6 Handout for Students
- S12. Muscle Differentiation Inquiry Lab – RT-PCR Board Notes
- S13. Muscle Differentiation Inquiry Lab – Session 7 Handout for Students
- S14. Muscle Differentiation Inquiry Lab – Session 8 Handout for Students
- S15. Muscle Differentiation Inquiry Lab – Laboratory Report Rubric
- S16. Muscle Differentiation Inquiry Lab – Laboratory Notebook Rubric
- S17. Muscle Differentiation Inquiry Lab – Laboratory Performance Rubric
- S18. Muscle Differentiation Inquiry Lab – Experimental Variations

ACKNOWLEDGMENTS

An internal Innovation Grant from Alvernia University supported the development of this laboratory module.
Table 1. Lesson plan timeline. This table outlines instructor preparation and activities for each weekly lab session, with approximate time lengths. The notes column provides references to the associated supporting materials.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Estimated Time</th>
<th>Notes</th>
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<tbody>
<tr>
<td><strong>Preparation for Lab Session 1</strong></td>
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</table>
| Prepare handout, research article, and Introductory PowerPoint | 1. Make copies of Handout 1 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)  
2. Make copies of Primary Research Article for each student or pair (or post electronically)  
3. Review Introductory PowerPoint  
4. If desired, review additional background information on cell culture and include in PowerPoint | ~30–60 minutes, depending on time needed to review Introductory PowerPoint and additional background information | • Primary Research Article is cited in Handout 1 and is available online (open access)  
• Introductory PowerPoint is provided in Supporting File S3  
• Additional background information on cell culture is included in Supporting File S4 |                |
| Prepare lab materials                          | 5. Materials include 35 mm cell culture plates, sterilized coverslips and forceps, regular growth medium, and differentiation medium                                                                                                                                 | ~3–4 hours, including incubation time for plates | | • Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1 |
| **Lab Session 1**                             |                                                                                                                                                                                                             |                |                                                                                                                                                                                                           |
| Introduce lab                                 | 1. Review Introductory PowerPoint  
2. Review Handout 1 with students                                                                                                                                  | ~45 minutes   | • Handout 1 is provided in Supporting File S2  
• Introductory PowerPoint is provided in Supporting File S3 |
| Students perform lab work                     | 3. Students change medium on cultures for next week’s session (immunostaining)                                                                                                                                  | ~30 minutes   | | |
| **Preparation for Lab Session 2**             |                                                                                                                                                                                                             |                |                                                                                                                                                                                                           |
| Prepare handout and Board Schematic           | 1. Make copies of Handout 2 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)  
2. Review the first several procedural steps of Handout 2 with students briefly  
3. Students start immunostaining procedure at start of lab session | ~15–45 minutes, depending on time needed to review the Board Schematic | • Board Schematic for Immunostaining is provided in Supporting File S5  
• Handout 2 is provided in Supporting File S6 |
| Prepare lab materials                          | 2. Lab materials include cell cultures and immunostaining reagents for students                                                                                                                                 | ~2–3 hours    | • Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1 |
| **Lab Session 2**                             |                                                                                                                                                                                                             |                |                                                                                                                                                                                                           |
| Students perform lab work                     | 1. Review the first several procedural steps of Handout 2 with students briefly  
2. Students start immunostaining procedure at start of lab session                                                                                                                                 | ~3 hours, including 45 minutes to review Board Schematic with students during incubation | | • Handout 2 is provided in Supporting File S6 |
<p>| Introduce immunostaining                      | 3. Write out the Board Schematic on a white board or chalk board in lab and review during incubations                                                                                                        | ~30–45 minutes | • Board Schematic for Immunostaining is provided in Supporting File S5 |
| <strong>Preparation for Lab Session 3</strong>             |                                                                                                                                                                                                             |                |                                                                                                                                                                                                           |
| Prepare handout                               | 1. Make copies of Handout 3 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)                                                                 | ~15–30 minutes | • Handout 3 is provided in Supporting File S7 |
| Prepare lab materials                          | 2. Lab materials include cell cultures and protein extraction reagents for students                                                                                                                                              | ~2–3 hours    | • Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1 |</p>
<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Estimated Time</th>
<th>Notes</th>
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<tbody>
<tr>
<td><strong>Lab Session 3</strong></td>
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</tr>
<tr>
<td>Introduce lab</td>
<td>1. Review Handout 3 with students</td>
<td>~15 minutes</td>
<td>• Handout 3 is provided in Supporting File S7</td>
</tr>
<tr>
<td></td>
<td>2. Students complete the procedure for protein extraction and quantification</td>
<td>~1.5 to 2 hours</td>
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</tr>
<tr>
<td>Students perform lab work</td>
<td>3. Student groups also visualize slides from last week on the fluorescent microscope</td>
<td>~15 minutes per student group</td>
<td>• Student groups may also visualize slides at an agreed upon time outside of lab if there is not enough time in the lab session</td>
</tr>
<tr>
<td><strong>Preparation for Lab Session 4</strong></td>
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<tr>
<td>Review the Board Schematic for Western blotting and handout</td>
<td>1. Make copies of Handout 4 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)</td>
<td>~15–30 minutes, depending on time needed to review the Board Schematic</td>
<td>• Board Schematic for Western blotting is provided in Supporting File S8</td>
</tr>
<tr>
<td></td>
<td>2. Lab materials include reagents and equipment for Western blotting (electrophoresis and transfer)</td>
<td>~2 hours</td>
<td>• Handout 4 is provided in Supporting File S9</td>
</tr>
<tr>
<td><strong>Lab Session 4</strong></td>
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</tr>
<tr>
<td>Introduce lab</td>
<td>1. Review Handout 4 with students</td>
<td>~15 minutes</td>
<td>• Board Schematic for Western blotting is provided in Supporting File S8</td>
</tr>
<tr>
<td></td>
<td>2. Go over the Board Schematic once the gels are running</td>
<td></td>
<td>• Handout 4 is provided in Supporting File S9</td>
</tr>
<tr>
<td>Students perform lab work</td>
<td>3. Students complete the steps for protein electrophoresis and membrane transfer</td>
<td>~2.5 hours</td>
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<tr>
<td><strong>Preparation for Lab Session 5</strong></td>
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<tr>
<td>Prepare handout</td>
<td>1. Make copies of Handout 5 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)</td>
<td>~15–30 minutes</td>
<td>• Handout 5 is provided in Supporting File S10</td>
</tr>
<tr>
<td></td>
<td>2. Lab materials include reagents and equipment for Western blotting (antibody incubations and chromogenic detection)</td>
<td>~1–2 hours</td>
<td>• Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1</td>
</tr>
<tr>
<td><strong>Lab Session 5</strong></td>
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<tr>
<td>Start the Western blotting procedure prior to lab</td>
<td>3. Complete the first several steps of the Western blotting procedure prior to lab (up to the primary antibody incubation)</td>
<td>~1 hour</td>
<td></td>
</tr>
<tr>
<td>Introduce lab</td>
<td>4. Review Handout 5 with students</td>
<td>~10 minutes</td>
<td>• Handout 5 is provided in Supporting File S10</td>
</tr>
<tr>
<td>Students perform lab work</td>
<td>5. Students complete RNA extraction towards the beginning of lab, while Western incubation is going</td>
<td>~1 hour</td>
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<td></td>
<td>6. Students perform the rest of the Western blotting procedure</td>
<td>~2 hours</td>
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<tr>
<td>Complete the Western blotting procedure after lab ends</td>
<td>7. Lab time may end prior to the end of chromogenic substrate incubation—if needed, complete the last few steps and take an image of the membranes to post for students</td>
<td>~30–60 minutes</td>
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<tr>
<td>Activity</td>
<td>Description</td>
<td>Estimated Time</td>
<td>Notes</td>
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<tr>
<td><strong>Preparation for Lab Session 6</strong></td>
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<tr>
<td>Prepare handout</td>
<td>1. Make copies of Handout 6 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)</td>
<td>~15–30 minutes</td>
<td>• Handout 6 is provided in Supporting File S11</td>
</tr>
<tr>
<td>Prepare lab materials</td>
<td>2. Lab materials include equipment and reagents for electrophoresis</td>
<td>~1 hour</td>
<td>• Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1</td>
</tr>
<tr>
<td>Review lab report</td>
<td>3. Go over the lab report rubric with students</td>
<td>~15–30 minutes</td>
<td>• Lab report rubric is provided in Supporting File S15</td>
</tr>
<tr>
<td><strong>Lab Session 6</strong></td>
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<tr>
<td>Introduce lab</td>
<td>1. Review Handout 6 with students</td>
<td>~15 minutes</td>
<td>• Handout 6 is provided in Supporting File S11</td>
</tr>
<tr>
<td>Students perform lab work</td>
<td>2. Students complete the steps for RNA electrophoresis</td>
<td>~2 hours</td>
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<tr>
<td><strong>Preparation for Lab Session 7</strong></td>
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<tr>
<td>Review the Board Schematic for RT-PCR and prepare handout</td>
<td>1. Make copies of Handout 7 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)</td>
<td>~15–30 minutes, depending on time needed to review the Board Schematic</td>
<td>• Board Schematic for RT-PCR is provided in Supporting File S12</td>
</tr>
<tr>
<td>Prepare lab materials</td>
<td>2. Lab materials include equipment and reagents for reverse transcription and PCR</td>
<td>~1 hour</td>
<td>• Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1</td>
</tr>
<tr>
<td><strong>Lab Session 7</strong></td>
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</tr>
<tr>
<td>Introduce lab</td>
<td>1. Review Handout 7 with students</td>
<td>~15 minutes</td>
<td>• Board Schematic for RT-PCR is provided in Supporting File S12</td>
</tr>
<tr>
<td>Students perform lab work</td>
<td>2. Go over the Board Schematic once the reverse transcription reactions are incubating</td>
<td>~30 minutes</td>
<td>• Handout 7 is provided in Supporting File S13</td>
</tr>
<tr>
<td>3. Students complete the steps for protein electrophoresis and membrane transfer</td>
<td>~2.5 hours</td>
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<tr>
<td><strong>Preparation for Lab Session 8</strong></td>
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<tr>
<td>Prepare handout</td>
<td>1. Make copies of Handout 8 for each student or pair; alternatively, provide an electronic version on course management system (Blackboard, Moodle, etc.)</td>
<td>~15–30 minutes</td>
<td>• Handout 8 is provided in Supporting File S14</td>
</tr>
<tr>
<td>Prepare lab materials</td>
<td>2. Lab materials include equipment and reagents for agarose electrophoresis of PCR products</td>
<td>~1 hour</td>
<td>• Detailed Instructor information for weekly solution and cell preparations is included in Supporting File S1</td>
</tr>
<tr>
<td><strong>Lab Session 8</strong></td>
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</tr>
<tr>
<td>Introduce lab</td>
<td>1. Review Handout 8 with students</td>
<td>~15 minutes</td>
<td>• Handout 8 is provided in Supporting File S14</td>
</tr>
<tr>
<td>Students perform lab work</td>
<td>2. Students complete the steps for electrophoresis</td>
<td>~1.5 hours</td>
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</tbody>
</table>
A Structured Inquiry-Based Module for the Undergraduate Cell Biology Laboratory That Teaches Fundamental Concepts of Cell Differentiation

REFERENCES