Abstract
SDS-PAGE and western blotting are two commonly taught protein detection techniques in biochemistry and molecular biology laboratory classrooms. A pitfall associated with incorporating these techniques into the laboratory is the significant wait times that do not allow students to obtain timely results. The waiting associated with SDS-PAGE comes from staining and destaining, whereas with western blotting it is the times required for antibody incubations and the numerous wash steps. This laboratory exercise incorporates 2,2,2-trichloroethanol (TCE) into the SDS-PAGE gel allowing for visualization of migrated proteins in a matter of minutes, saving both the time and chemical waste associated with traditional Coomassie staining. Additionally, TCE staining does not affect protein transfer eliminating the requirement for duplicated gels for total protein and western analyses. Protein transfer can be confirmed immediately without the use of Ponceau S staining. Lastly, this western blot procedure has been further shortened by using an HRP-conjugated primary antibody, which eliminates the secondary antibody incubation and washes, and uses a colorimetric detection to allow for visualization by students without the need for specialized equipment.

Keywords: SDS-PAGE; western blot; 2,2,2-trichloroethanol (TCE); laboratory exercises

Introduction
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting are two of the most common protein analyses used in biochemistry and molecular biology. These techniques are often incorporated into undergraduate laboratory courses, but the traditional protocols include significant wait times and often require steps to be performed outside of scheduled laboratory periods. Figure 1a illustrates the timeline needed to complete a traditional western blot protocol, also referred to as immunoblotting. The general procedure requires protein samples of interest to be run on an SDS-PAGE gel, transferred from the gel to nitrocellulose, and detected by immunoblotting. The steps involved in detection include blocking the membrane to prevent nonspecific protein binding, incubation with a primary antibody that binds the protein of interest, incubation with a conjugated secondary antibody that binds to the primary antibody, and exposure to an enzymatic substrate that reacts with the conjugated antibody to indirectly detect the protein of interest [1]. Additionally, there are numerous wash steps between the incubation periods. This entire procedure can take more than five hours over the course of two laboratory periods.

As in any multistep procedure, there are several points in which problems can arise, especially when inexperienced students are performing the assay. Therefore, it is beneficial in the teaching laboratory to include supplementary procedures to assess the success of intermediate steps (Fig. 1a). These additional steps also serve as valuable tools to illustrate important concepts in the overall procedure. For example, it is often useful to view the total protein composition of samples so that the specificity of western blotting detection can be directly compared with the total proteins in the stained gel. Coomassie Brilliant Blue staining is commonly used for this purpose because it is inexpensive and easy to perform. It has a sensitivity limit in the submicrogram range depending on the staining properties.
of the protein [2]. Unfortunately, this technique generates significant amounts of methanol and acetic acid waste and requires time-consuming staining and destaining steps (destaining is usually performed overnight) that prevent visualization of the results in a timely manner. Another significant drawback to Coomassie staining is that the proteins become fixed in the gel, requiring a duplicate gel be run for comparative analysis by western blotting [3].

Another beneficial checkpoint is visualization of transferred proteins on the nitrocellulose, since mistakes can occur in several steps when setting up the transfer apparatus. The blotting papers and membrane must be matched to the exact dimensions of the gel to prevent wicking of the transfer reaction. Additionally, the transfer materials must be assembled carefully in the proper order to ensure that protein transfer occurs evenly throughout the gel, in the correct direction (onto the membrane), and to completion. Ponceau S is a reversible stain that can be used to confirm protein transfer to nitrocellulose or polyvinylidene fluoride membranes [4]. Staining is performed by immersing the membrane in Ponceau S for 5 min and rinsing the membrane with two to three 5-min washes with deionized water on an orbital shaker. Ponceau S detection limits are approximately 100 ng protein per band and the stain can be removed by additional washing [1]. While this technique is easy to perform and generates little waste, it adds at least 30 min to the overall procedure.

Direct incorporation of 2,2,2-trichloroethanol (TCE) into the SDS-PAGE gel eliminates the need for additional gel and membrane staining steps, saving a significant amount of time and reducing the chemical waste generated during total protein and transfer analyses [5–7]. After the gel is run, it is immediately exposed to a 300 nm transilluminator for up to 5 min. During this time, TCE covalently modifies tryptophan residues in the proteins facilitating their visualization by fluorescence [6]. The limit of detection for TCE staining is similar to Coomassie staining but is improved for proteins with a higher tryptophan composition [7]. Gel images can be captured immediately with a digital camera (or smartphone). Furthermore, TCE modified proteins efficiently transfer to nitrocellulose eliminating the requirement for a duplicate gel to observe total protein composition. Subsequent to the transfer, proteins bound to the membrane can once again be imaged by placing the membrane on the transilluminator, thus saving the time originally required for Ponceau S staining and destaining. When TCE is included in the gel and a semi-dry apparatus is used to transfer the proteins to nitrocellulose, the time required to complete the steps from the first day of the traditional SDS-PAGE/western protocol can be reduced by thirty minutes (Fig. 1b, Lab 1). Importantly, both quality check steps (total protein composition within the gel and transfer to the membrane) are retained. Additionally, the total protein composition is seen immediately following electrophoresis as opposed to during the second lab after Coomassie destaining is completed (Fig. 1a, Lab 2).

The most time-consuming components of immunoblotting are those required for antibody binding and washing (Fig. 1, Lab 2). The primary antibody is commonly added to the membrane for 1 hour; although this time may need to be extended to an overnight incubation depending on the amount of the protein on the membrane, the concentration of the antibody used (cost is often an issue), and the affinity of the antibody for the protein of interest [8]. Following primary binding, the membrane is washed three times and then a secondary antibody conjugated to an enzyme is generally used to bind to the primary antibody. After another hour of binding (for most secondary antibodies) and three more washes, a chromogenic or fluorescent substrate is added to the membrane to detect the enzyme conjugated to the bound secondary antibody and, therefore, indirectly detect the protein of interest. This laboratory exercise uses a glutathione S-transferase (GST) fusion protein which can be readily induced in Escherichia coli and detected in whole bacterial cell lysates or from fractions following column purification [9]. Since GST is a commonly used tag for fusion proteins, there are several commercially available antibodies that can be used for western blot analysis. We have chosen an HRP conjugated primary anti-GST antibody which eliminates the need for a secondary antibody and subsequent wash steps. Additionally, no specialized equipment is required for the analysis as this antibody is easily detected with a chromogenic substrate. Figure 1b illustrates the new timeline required for the series of experimental steps described in this paper. Ultimately, the entire SDS-PAGE and western analyses can be completed within two laboratory periods, each less than two hours, with additional time left for introduction by the instructor and/or discussion by the class.

Materials and Methods
Pre-preparation
The GST-tagged protein (66.2 kDa) in this exercise is a yeast reductase encoded by the YDL124w open reading frame [9]. The plasmid containing the YDL124w gene was chemically transformed into BL21 E. coli cells (New England Biolabs, Ipswich, MA). A single colony was used to inoculate 50 mL of LB medium containing 50 μg/mL kanamycin (Fisher Scientific, Waltham, MA) and grown at 37°C with shaking at 225 rpm overnight. The next morning, 10 mL of the overnight culture was used to inoculate 500 mL LB. The fresh culture was grown at 37°C with shaking at 225 rpm to an OD600 of 1.0. To prepare uninduced lysates, 1 mL aliquots of the culture were removed, subjected to centrifugation at 16,000 × g for 5 min, and pellets were stored at −20°C. Reductase expression was induced by adding IPTG (Gold Biotechnology, St. Louis, MO) (final concentration of 1 mM) to the remaining culture and
growing for an additional 18 hours. Before induced lysate preparation, 1 mL aliquots were harvested and cell pellets were stored as described above. Both uninduced and induced lysates were prepared using 50 μL Bugbuster® HT Protein Extraction Reagent (EMD Millipore, Billerica, MA), 2.5 μL Triton X-100, and 1.5 μL of 10 mg/mL lysozyme per cell pellet. Samples were vortexed for 5 min and centrifuged at 16,000 × g for 5 min, after which the supernatants were stored at −20°C until needed for SDS-PAGE. The GST-tagged protein was purified from IPTG-induced cultures using an AKTA Purifier FPLC (GE Healthcare, Piscataway, NJ) with a 5 mL glutathione column (Fisher Scientific). Cell pellets were lysed as previously described using the Bugbuster® mixture including a 2 mM final concentration of the proteinase inhibitor 4-(2-aminoethyl) benzensulfonyl fluoride hydrochloride (AEBSF) (Fisher Scientific). After equilibrating the column with start buffer (50 mM Tris, pH 7.5, 150 mM NaCl, and 1 mM EDTA), the cell lysate was loaded and nonspecific proteins were washed off the column with excess start buffer. The GST-tagged protein was eluted with elution buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10 mM Glutathione, and 1 mM DTT). Purified protein was aliquoted and stored in 50% glycerol at −20°C.

All electrophoresis reagents were prepared in advance of the scheduled laboratories and stored at room temperature unless otherwise indicated. The SDS protein loading buffer (PLB) was prepared as a 6× stock solution (0.6 M Tris HCl, pH 6.8, 30% glycerol, 9.0% SDS, and 0.01% bromophenol blue). On the day of use, 6 μL fresh β-mercaptoethanol (reducing agent) was added for every 94 μL 6× PLB needed. The loading buffer was diluted by the students to 1× with each protein sample and water before loading. The electrophoresis buffer was prepared as a 5× stock solution (0.125 M Tris base, 0.96 M Glycine, 0.5% SDS, pH 8.3) and diluted to a 1× concentration before use. Depending on the time available in the laboratory class, polyacrylamide gels were either prepared in advance...
stored up to 1 week at 4°C) or during class. Gels were comprised of a standard 5% stacking gel on top of a 10% polyacrylamide resolving gel with 1% (v/v) TCE. Alternatively, stain free precast gels can be purchased directly from Bio-Rad (Criterion Stain Free TM Tris-HCl gels, Hercules, CA). The Precision Plus Protein™ All Blue Standards were purchased from Bio-Rad. For Coomassie staining, gels were fixed and stained (50% methanol, 10% glacial acetic acid, 0.25% Coomassie Brilliant Blue R-250) for 1 hour followed by destaining (18% methanol, 9.0% glacial acetic acid) overnight with rocking.

All western blotting reagents were prepared in advance of the scheduled laboratories and stored at room temperature unless otherwise indicated. Transfer buffer included 25 mM Tris, 0.192 M glycine, and 20% methanol (pH 8.3). For convenience, a 10× concentrated stock was prepared without the methanol. When preparing the working concentration, methanol was added with the water when a 1× working solution was prepared. NitroBind nitrocellulose (0.22 μm) was acquired from GE Healthcare Life Sciences (Piscataway, NJ) and extra thick blotting paper was purchased from Bio-Rad. The wash buffer (Tris buffered saline with Tween 20; TBS-T) was prepared as a 10× stock solution (0.1 M Tris-HCl, 1.5 M NaCl, pH 8, and 1% Tween 20) and diluted with water before use. The blocking buffer was prepared by adding 10% non-fat skim milk powder to 1X TBS-T and stored at 4°C for up to 1 week. THETM GST Antibody (mouse monoclonal conjugated to HRP) was purchased from GenScript (Piscataway, NJ), aliquoted, and stored at −20°C. The chromogenic substrate (1 Step TMB-Blotting Substrate, 3,3',5,5'-tetramethylbenzidine) was purchased from ThermoScientific (Rockford, IL) and stored at 4°C. Before use, an aliquot of the substrate was warmed to room temperature.

**Protein Electrophoresis**

Bacterial lysates (100 μg) and purified GST-tagged protein (0.1–2.5 μg) were mixed with 6× protein loading buffer. All samples were boiled for 5 min and cooled on ice before loading onto an SDS-10% polyacrylamide gel with 1% TCE. The Bio-Rad Mini Protean Tetra gel system was assembled and used according to manufacturer’s instructions. After proteins were run at 150 V for approximately 1 hour, the gel was carefully removed from between the casting plates and transferred to the pre-wetted surface of a 300 nm transilluminator. After 1 to 2 min of UV activation, proteins in the gel were visualized and the total protein image was captured using a smart phone with an 8-megapixel camera (Fig. 2a). The gel was immediately placed into a container of transfer buffer to prevent drying.

**Transfer to Nitrocellulose**

A Bio-Rad trans-blot SD semi-dry transfer cell was used to transfer the proteins in the gel to nitrocellulose. Manufacturer’s instructions were followed for assembly of the transfer sandwich and operation of the semi-dry blotter. Since there is no buffer chamber, it is especially important to pre-wet and keep all components of the sandwich (gel, store up to 1 week at 4°C) or during class. Gels were comprised of a standard 5% stacking gel on top of a 10% polyacrylamide resolving gel with 1% (v/v) TCE. Alternatively, stain free precast gels can be purchased directly from Bio-Rad (Criterion Stain Free TM Tris-HCl gels, Hercules, CA). The Precision Plus Protein™ All Blue Standards were purchased from Bio-Rad. For Coomassie staining, gels were fixed and stained (50% methanol, 10% glacial acetic acid, 0.25% Coomassie Brilliant Blue R-250) for 1 hour followed by destaining (18% methanol, 9.0% glacial acetic acid) overnight with rocking.

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nitrocellulose, and blotting papers) wet during assembly and to remove bubbles between layers. Proteins were transferred for 30 min at 25 V (never exceeding the current limit of 5.5 mA/cm²). Transfer of the prestained marker to the nitrocellulose was confirmed before disassembly. The nitrocellulose was immediately placed onto the pre-wetted surface of a 300 nm transilluminator where transferred proteins were visualized and the image was captured by smartphone (Fig. 3). The membrane was placed into blocking buffer for a minimum of 1 hour with rocking. Due to time restraints of our laboratory classes, the membrane was stored in blocking buffer at 4°C until the next lab period. If this was more than 1 week, blocking buffer was removed, the membrane was rinsed with TBS-T, and freshly prepared blocking buffer was added at a frequency of once per week.

Immunodetection of GST-Tagged Proteins

The blocking buffer was removed and the membrane was rinsed well in TBS-T (especially if stored for an extended period of time). The anti-GST antibody was prepared at a 1:1,000 dilution (0.5 μg/μL) in blocking buffer and added to the membrane. The membrane was incubated, with rocking, for 1 hour at room temperature, ensuring there was sufficient volume to keep the membrane wet. After incubation, the antibody solution was removed and the membrane was washed three times in TBS-T with rocking for 5 min each. After the last wash was removed, room temperature 1-step TMB blotting substrate was carefully added to the surface of the membrane. Protein bands began to develop almost immediately. Once bands had fully developed, the reaction was stopped by rinsing the membrane in distilled water and the image was captured by smartphone (Fig. 4).

Precautions and Safety Considerations

Since unpolymerized acrylamide is a known neurotoxin, gloves were worn during gel casting and any time that gels were loaded or handled. When transilluminators were utilized, the plexiglass safety shield was in place whenever the UV bulb was on. All UV images taken by smartphone were captured through the safety shield. Gloves were also worn when nitrocellulose and blotting paper was handled since oils from skin can prevent proper wetting of transfer materials and skin proteins can contribute to background signals.

Timing and Student Participation

Depending on the available time in lab, most of the buffers and reagents were prepared in advance by the instructor or student lab assistants. The laboratory courses in which we have implemented SDS-PAGE met for 3 to 4 hours each week. Two laboratory periods were dedicated to the SDS-PAGE and western analyses. Before the first meeting, students were provided with a handout describing the background principles of the procedure and a detailed protocol. Due to time constraints, gels were pre-poured for the 3-
hour labs, whereas students in a 4-hour lab class prepared their own gels within class. In the first hour (if gels were provided), the instructor led a brief introductory discussion and students prepared and loaded their samples onto their gels. Within the second hour, students recorded their total protein results and began transferring their proteins to nitrocellulose. The remaining hour was used to record the transfer results, set up the blocking reactions, and for in-class discussion. Immunodetection was completed within the second laboratory period. In the first 10 to 20 min, the instructor led a brief discussion of the procedure, after which students worked in their groups to complete the immunodetection. In the second half of the lab, students documented the results of their western blots and determined the molecular weight of the GST-tagged protein using their results from the prestained protein standards.

Results
An SDS-PAGE and western blot laboratory series has been performed in our Immunology laboratory course for a total of five semesters. Several adaptations were made to the traditional western blotting laboratory until it evolved into the current method portrayed here. The laboratory sequence described in this document has been performed once in its final form in an Introduction to Biochemical Techniques laboratory class. Both of these courses typically have an enrollment ranging between 4 and 20 students. Immunology is an elective course that primarily targets upper-level biology majors while the biochemistry course is a requirement for all biochemistry majors. The prerequisites for Immunology include cell biology and microbiology, whereas the biochemistry course requires two semesters of organic chemistry and quantitative chemical analysis. Both courses meet for 3 (Immunology) to 4 hours (Biochemistry) per week during a 15-week semester.

The described procedure can easily be accommodated into two periods of a laboratory course that meets for a minimum of 2 hours during each session; however, 3-hour sessions would provide additional time for valuable in-class discussions. The first lab day introduces students to SDS-PAGE, total protein analysis, and transfer of proteins from the gel to nitrocellulose, while the second lab session focuses on probing the nitrocellulose for the specific detection of the GST-fusion protein using a chromogenic substrate (Fig. 1b).

During the first laboratory activity, the instructor led a discussion focused on the principles of recombinant protein expression, protein purification, and SDS-PAGE. Students worked in groups of two to four to pour their own gels (for 4-hour lab courses only) and/or to prepare their samples for loading. When students prepared the gels, multiple problems were encountered (e.g., leaking gels and polymerization issues) that students found frustrating and required a third, unplanned lab session to complete the exercise. When gels were prepared in advance, students missed out on the hands-on experience of gel preparation but the lab went more smoothly and proceeded according to schedule. The instructor demonstrated how to assemble the gel apparatus and load samples into the wells of the gel. Students were encouraged to use the extra wells of their gel to practice loading 1× PLB before adding their samples.

Electrophoresis was monitored by following the migration of the tracking dye and protein separation was directly visualized by observing the separation of the standards in the prestained marker. While the gels were running, the instructor explained the traditional process of Coomassie blue staining and led a discussion of the pros and cons of Coomassie versus TCE staining of total proteins. The instructor also reviewed the concept of transfer of proteins from a gel to nitrocellulose and demonstrated how to set up the transfer assembly. When electrophoresis was complete, the gels were placed on a 300 nm transilluminator for photoactivation of the TCE. With the protective plexiglass shield in place, students were able to visualize the protein bands within 1 to 2 min and capture clear images of total protein staining with a smart phone or digital camera (Fig. 2a). TCE is unable to react with prestained proteins, explaining the absence of the protein marker in the TCE images. The instructor provided a sample Coomassie-stained gel for comparison (Fig. 2b). The sensitivity of both staining procedures was similar (the GST-tagged protein contains 1.5% tryptophan residues).

Students worked together to set up their transfer reactions. Four mini-gel transfers were assembled per semi-dry transfer apparatus. While the transfer reaction was run, students were expected to begin work on a series of discussion questions that directly related to core principles of SDS-PAGE and transfer or required preliminary analysis and interpretation of their total protein results (Supporting Information Laboratory Instructions). Additionally, students were asked to draw a figure of the results that they would predict to see upon completion of the western blot analysis in the following lab period. Once the transfer was complete, students did a preliminary visual check to confirm that the bands from their prestained protein standard had transferred to the membrane. Next, they documented the transfer of their sample proteins using the transilluminator and their camera phones (Fig. 3). Students began blocking their membranes and finished their questions (either in class or as an out-of-class assignment). Membranes were stored in blocking buffer until the following lab period, 1 week later.

During the second laboratory day, the instructor led a brief discussion contrasting immunodetection with a conjugated primary antibody and a traditional two-antibody detection protocol. Students continued to work in groups of two to four to probe their blots with the anti-GST HRP conjugated antibody. After washing, the chromogenic substrate was added and bands developed almost immediately.
GST-tagged proteins were clearly observed in the induced lysates and in all of the lanes containing purified protein (Fig. 4). Low levels were also seen in the uninduced sample, most likely due to leaky induction of the plasmid’s promoter [10]. Using the protein standards, students determined the molecular weight of the GST-tagged protein. Students completed a set of questions that required them to compare the results between their TCE-stained gels and their western blots and to troubleshoot theoretical problems in the western blotting procedure (Supporting Information Laboratory Instructions). The students were able to quickly discern that the antibody was specific for the GST fusion protein as none of the endogenous E. coli proteins were detected. Also, they were able to observe a dose-dependent detection in the lanes containing increasing amounts of the purified protein. This allowed students to semi-quantitatively determine the expression levels of the GST-fusion protein in the induced lysates.

Student Feedback
After an in-class discussion of Coomassie and Ponceau S staining, the students appreciated the almost instantaneous feedback obtained by visualization of their TCE-stained proteins following electrophoresis and transfer to nitrocellulose. For students with aberrant results, they were able to quickly pinpoint areas where their technique might require improvement based on whether the problems were observed in the gel or membrane staining. Most students were reassured that their gels and transfer reactions had proceeded according to plan and were more confident about beginning the detection of GST-tagged proteins. The students enjoyed being able to see the immunodetection develop in real-time as the HRP activated the substrate resulting in the rapid appearance of blue bands. Importantly, when the immunodetection was complete, students fully understood the specificity of the antibody for the target protein and saw increased amounts of the GST-tagged protein in the induced lysate compared with the uninduced lysate. This eventually led to a discussion about how the expression system works.

Discussion
SDS-PAGE and western blotting are cornerstone methods illustrating several important fundamental principles in undergraduate biochemistry and molecular biology courses. However, in many of these laboratory classes, time constraints and costs of equipment and reagents place limitations on the ability of instructors to implement these techniques effectively in the classroom setting. This exercise illustrates a greener, more time- and cost-effective approach for introducing students to these techniques without the need for expensive imaging equipment.

The use of TCE staining to visualize total proteins in SDS-PAGE is not a novel technique [5–7]; however, its adaptation and application to the teaching environment has yet to be considered. Incorporation of TCE into SDS-PAGE gels allows for visualization of total protein following electrophoresis and transfer in a matter of minutes, eliminating the need for accessory procedures such as Coomassie and Ponceau S staining. It is important to demonstrate the basic principles of visualizing gels and to provide students with data to interpret, but both of these staining approaches add significant time requirements to the overall western blotting process, leaving the instructor with the dilemma of sacrificing time or instruction of valuable concepts. While the Coomassie staining and destaining process is traditionally performed using methanol and acetic acid, protocols have been developed to accelerate this process using water and elevated temperatures [11], but this approach is still not as rapid as using TCE. Additionally, a duplicate gel is still required if western blot analysis is planned. TCE staining saves time, eliminates the need for a second gel, reduces the chemical waste generated, and provides nearly instantaneous results at a sensitivity equivalent to conventional Coomassie R-250 staining, depending on the number of tryptophan residues present in the proteins of interest [7].

This laboratory exercise utilizes a semi-dry transfer system and relies on the use of a conjugated primary antibody instead of the traditional two-antibody system employed in most western blotting procedures. A wet transfer system can easily be substituted without adding significant time to the transfer procedure, but will generate additional methanol waste. One must consider, while semi-dry transfer systems are more expensive, their reduced buffer requirement saves the instructor preparatory time, the cost of extra reagents, and the additional mess produced by wet transfer. Additionally, while the initial cost of a conjugated primary antibody is higher, ultimately the amount of money invested in antibodies, extra reagents, and student and instructor time is similar, if not less, than when a two-antibody system is employed. If sensitivity is a concern, a chemiluminescent substrate can be substituted; however, as this will require access to an appropriate detection system, a significant cost would be added to the course. Moreover, the instructor would need to explain how to use the software associated with this system resulting in a greater time commitment for all of the students in the class to document and analyze their individual results.

If time constraints are more restrictive than the overall budget for the course, modifications can further be made to shorten the time required to run this protocol. All of the required buffers for electrophoresis, transfer, and immunoblotting are commercially available. Companies sell precast, stain-free, SDS-PAGE gels (Bio-Rad TGX Stain-Free™) that run in 15 to 20 min. Additionally, Bio-Rad sells a V3 Western Workflow™ system that combines quick-running, stain-free gels, a rapid protein transfer system (proteins transfer in as little as 3 min), and an imaging system that
allows users to quantitate western blotting data using total protein normalization. With this type of workflow system, the entire procedure could be completed in approximately 2.5 hours.

This protocol can be easily adapted to suit the needs of the instructor and the course in which it will be implemented. Many instructors will find the TCE staining alone to be a significant time-saver for SDS-PAGE labs without incorporating western blotting into their curriculum. Others may be interested in expanding the preparatory steps to this exercise into a multi-week module encompassing bacterial cloning and transformation, verification of plasmid integrity by restriction digest or PCR, induction of plasmid expression, bacterial lysate preparation, protein quantification, and/or column purification/chromatography.

While this exercise was originally developed for biochemistry and immunology laboratory courses at a 4-year undergraduate institution, it can be applied to a variety of undergraduate or graduate courses in which molecular biology and/or biotechnology concepts are addressed. As there is little requirement for technical skill or specialized equipment, this procedure could be adapted and introduced to high school students as a method of screening patient samples for the presence of proteins of interest (e.g., HIV proteins). The instructor would simply need to rename the protein samples as mock samples that match the purpose of the exercise. Any protein with commercially available antibodies can be used; however, if a conjugated primary antibody is not available, extra time would be required in the classroom to implement a two-antibody detection protocol.

Conclusion
This laboratory exercise demonstrates an efficient procedure for teaching western blotting in the classroom setting. The protocol eliminates the generation of methanol and acetic acid waste while decreasing the time needed to visualize protein samples either following SDS-PAGE separation or their transfer to nitrocellulose. Additionally, the time-exhaustive steps of adding a secondary antibody and subsequent wash steps are eliminated with the use of a conjugated primary antibody.

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References