**Article:**

Harrison, Adrian, Evans, Gareth and Blanco, Gonzalo (2024) Expanding science skills: teaching tissue culture, data analysis, and reporting through imaging the actin cytoskeleton. Journal of microbiology & biology education. e0019023. ISSN: 1935-7877

https://doi.org/10.1128/jmbe.00190-23

AMERICAN SOCIETY FOR MICROBIOLOGY | JMBE Journal of Microbiology & Biology Education

Check for updates

| Tips and Tools

# Expanding science skills: teaching tissue culture, data analysis, and reporting through imaging the actin cytoskeleton

**Adrian Harrison,**[1] **Gareth Evans,**[1] **Gonzalo Blanco**[1]

**AUTHOR AFFILIATION** See affiliation list on p. 15.

**ABSTRACT** Within the eukaryotic cell, the actin cytoskeleton is a crucial structural framework that maintains cellular form, regulates cell movement and division, and facilitates the internal transportation of proteins and organelles. External cues induce alterations in the actin cytoskeleton primarily through the activation of Rho GTPases, which then bind to a diverse array of effector proteins to promote the local assembly or disassembly of actin. We have harnessed the extensively studied functions of RhoA in the dynamics of the actin cytoskeleton to craft a practical series for Stage 2 Biology students. This series not only imparts essential tissue culture laboratory skills but also reinforces them through repetition. These activities are presented in a scenario designed for students to explore the function of a hypothetical RhoA family member. Students produce slides from transfected cells, undertake fluorescence microscopy, process the images using ImageJ, and compile their findings in a comprehensive scientific report. The composition of the report requires independent acquisition of new knowledge and synoptic learning. According to student feedback, this early experience greatly aids in solidifying and honing the skills required to report on more extensive and intricate research projects, such as capstone projects.

**KEYWORDS** tissue culture, actin cytoskeleton, RhoA, transfection, cell shape

The aim of bioscience undergraduate research skills training is to prepare students to enter the workforce or to continue into postgraduate training. However, undergraduate research skills training often culminates in a capstone research project or senior thesis in the final year. Before reaching this, the second year (hereafter referred to as Stage 2) is a critical stage where core skills taught in the first year are applied to more authentic research scenarios, thus developing competence in more specialized laboratory techniques, experimental design, data acquisition, analysis, interpretation, and critical evaluation, communicated through a structured report. We designed a Stage 2 cell biology practical course that addresses these aims, through a series of laboratory practicals, supported by workshops and lectures. The course provides students with insight into a research process commonly used to assess protein function *in vivo*. It is based around a series of experiments that explore the cellular role of the small GTPase RhoA and the well-established paradigm of its regulation of the actin cytoskeleton in mammalian cells (1). The practicals evaluate RhoA-mutation/phenotype correlations at a cellular level, allowing students to answer questions related to the expression, localization, and function of RhoA variants and their effects on the actin cytoskeleton. Moreover, the signaling mechanisms downstream of RhoA are explored through the use of an inhibitor of the RhoA effector, Rho-associated coiled-coil-containing kinase (ROCK) (1–3).

In order to encourage students to think about how research is conducted in novel situations, we themed the course around the investigation of a novel fictitious protein called "Largando." This fictitious protein is presented to students as a recently discovered

**Editor** Laura J. MacDonald, Hendrix College, Conway, Arkansas, USA

Address correspondence to Gonzalo Blanco, gonzalo.blanco@york.ac.uk.

The authors declare no conflict of interest.

**Received** 31 October 2023
**Accepted** 13 April 2024
**Published** 9 May 2024

member of the Rho family GTPases and the aim of the experiments is to investigate whether it has a role in the regulation of actin polymerization.

The practicals encompass a range of high-level bioscience techniques, including tissue culture, transfection of cells, expression of proteins, cell staining, fluorescence microscopy, and image analysis using ImageJ (4). It also allows students to consolidate their core practical techniques, such as pipetting and centrifugation, introduced in earlier practicals. The series of practicals convey the rationale of using multiple techniques in a series of experiments combined to address a hypothesis or a wider research question. The practicals allow students to develop critical analysis skills through the evaluation of methodology, the application of statistics, and the requirement to tie findings to those in the scientific literature. Synoptic learning is encouraged and evaluated in the assessment, an extended write-up (hereafter referred to as a "scientific report") at a level between reports based on single-stage experiments and those required for a capstone project or senior thesis. The practicals are supported by workshops and lectures that explain the theory behind the use of different techniques and the understanding of the background science behind the work.

Laboratory activities are distributed over 3 weeks. In the first week, students practice the basics of tissue culture and learn how transfection of mammalian cells to overexpress proteins provides insights into protein function. By the end of week 1, students have produced a set of slides ready for fluorescence microscopy analysis in which the effect of Largando on cell size can be visualized and quantified. The second week covers identical techniques but introduces an inhibitor so that the morphological changes observed in week one can potentially be attributed to a downstream effector of Largando. In week three, slides collected from week one and two are analyzed by fluorescence microscopy, culminating with a set of images ready for quantification analyses. Supportive lectures and workshops running alongside the practicals provide guidance and resources to help students build figures toward the main outcome of this practical, the production of a scientific report.

### Learning objectives

Upon completing this series of practicals students will have the ability to:

- Use aseptic technique to successfully grow and passage mammalian cells *in vitro*.
- Transfect DNA into mammalian cells using the calcium phosphate method.
- Evaluate transfection efficiency.
- Set up and operate a fluorescence microscope to examine the subcellular distribution of proteins tagged with fluorescent markers in mammalian cells.
- Qualitatively and quantitatively analyze changes in cellular morphology and size using fluorescence microscopy images and image analysis using the open-source image analysis package ImageJ.
- Appraise scientific literature to further explore the scientific background of the experimental model.
- Identify the key components of a compelling scientific report based on a series of interrelated experiments.

## PROCEDURE

### Methodology and execution of activities

Students should have the following knowledge and skills before completing this activity. These practicals are aimed at students who already have a previous experience in:

- pipetting and dilutions,
- practical understanding of aseptic conditions and manipulations,

- basic microscopy,
- laboratory recordkeeping, and
- basic statistical skills.

## Sessions

This course was run for 36 students split into groups of three students. The order and timings of the sessions described next is outlined in Appendix 2 (Technician Notes). Note that spacing between the sessions within week 1 or week 2 below is determined by the constraints of tissue culture. A period of 24 or 48 h can be accommodated between sessions. The period between week 1 and week 2 is flexible. Four tissue culture hoods and four fluorescence microscopes were available. Within groups, students are encouraged to switch roles. For example, in a 3-h microscopy session students rotate between focusing slides, taking images, and keeping records.

### *Week 1*

*Introductory lecture to week 1*

The introductory lecture may outline a scenario in which the students will be testing a hypothetical novel protein. This virtual scenario models a typical research pipeline, in which the results can then be treated and reported as original research. The recently discovered and uncharacterized protein is called Largando. The scenario sets out that the DNA coding sequence of Largando has been obtained and sequence similarities indicate that Largando belongs to the Rho GTPase family. The latter encompass a group of small signaling GTPases that play a crucial role in regulating various cellular processes, including cell migration, cell adhesion, cell shape, and intracellular transport. In reality, all constructs used in the practicals encode different variants of RhoA, but have consequently been renamed to Largando. The virtual scenario allows students to compare and contrast their results with those from well-characterized proteins of the Rho family in the literature. This facilitates the write-up of a critical discussion on potential roles for Largando as a novel protein. A second scenario that can also be explored using this practical series is to present students with a straightforward experimental outline to test the effect of RhoA variants on the actin cytoskeleton. In this case, the discussion in the scientific report will be based on contrasting their results with historical data available on RhoA in the literature.
The introductory lecture would include the following points:

- Research question: introduction of Largando as a new protein with sequence similarities with Rho GTPases. What is its function?
- Background: may include a simple schematic diagram of the regulation of Rho GTPases, mentioning the role of GTPase-activating proteins (GAPs), guanine nucleotide exchange factors (GEFs), and guanine nucleotide dissociation inhibitors (GDIs) to regulate the active/inactive states of the protein [e.g., reference (5)]. It may also include a generic description of the differential effects of the small GTPases Cdc42, Rac, and Rho on the regulation of actin polymerization (6, 7).
- A hypothesis regarding the function of Largando given sequence similarities with Rho GTPases: *Largando reorganizes the actin cytoskeleton*.
- An experimental design based on the transfection of COS7 fibroblasts with constructs containing different versions of Largando in frame with GFP: (i) a constitutively active (Addgene plasmid # 12968, hereafter referred to as GFP-Largando$^{Q63L}$); (ii) a dominant negative (Addgene plasmid # 12967, hereafter referred to as GFP-Largando$^{T19N}$); (iii) a wild type (Addgene plasmid # 12965, hereafter referred to as GFP-Largando); and (iv) a GFP only control (Addgene plasmid # 129020, hereafter referred to as GFP). These Largando constructs would have been

designed using amino acid substitutions described in the homologous protein RhoA to produce active and inactive protein variants (8).

- An explanation of how the constitutively active mutation (Q63L) located in the GTP/GDP binding domain of Largando renders the protein constitutively active by interfering with the hydrolysis of the γ-phosphate of GTP (5).
- An explanation of how the dominant negative mutation (T19N) located in the GTP/GDP binding domain of Largando increases binding affinity for GEFs, so that Largando may no longer bind to downstream targets. An explanation of how intracellular GEFs are known to interact with different GTPases. Overexpressed Largando$^{T19N}$ can therefore potentially sequester GEFs and prevent the activation of endogenous Largando or homologous proteins using the same GEFs. This mechanism then results in a dominant negative effect, not just a constitutively inactive protein (5).
- An introduction to GFP and rhodamine/fluorophore-conjugated Phalloidin (9) to visualise transfected cells and actin filaments, respectively, by fluorescence microscopy.
- An outline of the practical sessions 1–3, emphasizing that the overall objective is to produce slides for further analysis. These slides will be imaged in the microscopy session in week 3 to obtain quality images from transfected cells. The images will be analyzed in ImageJ to measure the cell area from at least 30 transfected cells for each of the constructs.
- An outline of the resources and the support that will be made available to the students for image processing and data analysis (e.g., interactive workshop on image analysis in ImageJ).

### *Practical session 1: cell trypsinization, counting and plating (2–3 h)*

A step-by-step protocol for all practical sessions is provided in Appendix 1 (Student Instructions). A summary of the main activities that will be undertaken in these practicals is outlined in Appendix 2 (Technician Notes). Appendix 2 also details the specific preparations ahead of each practical session, including equipment, reagents, and solutions. The main objective of this session is to develop an understanding of the critical factors involved in maintaining a healthy mammalian cell line. In this session, students are introduced to the basics of tissue culture, the materials required, the use of a hemocytometer, and the importance of using correct dilution calculations when passaging cells. For these practicals, COS-7 (African green monkey kidney fibroblast-like cell, ATCC CRL-1651) is suitable because of their high transfection efficiency, they grow well on glass and plastic surfaces and the effects of RhoA have been well documented in these cells (3, 8). Students are provided with an almost confluent culture of COS-7 cells in a T25 flask to illustrate a monolayer of cells continually dividing, highlighting that once fully confluent, the cells will enter $G_O$ and become quiescent. The use of trypsin and its role in passaging cells is briefly outlined. Students are demonstrated aseptic practice, including spraying of materials with 70% alcohol before placing them inside the flow hood and filter sterilization of phosphate-buffered saline. Once cells have been trypsinized, collected, and resuspended in media, students use a hemocytometer to count the total number of cells. Use of the hemocytometer can either be introduced as part of this practical, or the practical can be used to consolidate practice if introduced previously. Once the total number of cells has been estimated, cells would be diluted to give an 8-mL suspension of 40,000 cells/mL. This suspension would then be used to seed the coverslips in a 24-well plate at 5,000, 10,000, and 20,000 cells/well, by applying dilutions as appropriate (Fig. 1A). Appendix 1 (Student Instructions) contains detailed instructions to assist with dilution calculations.

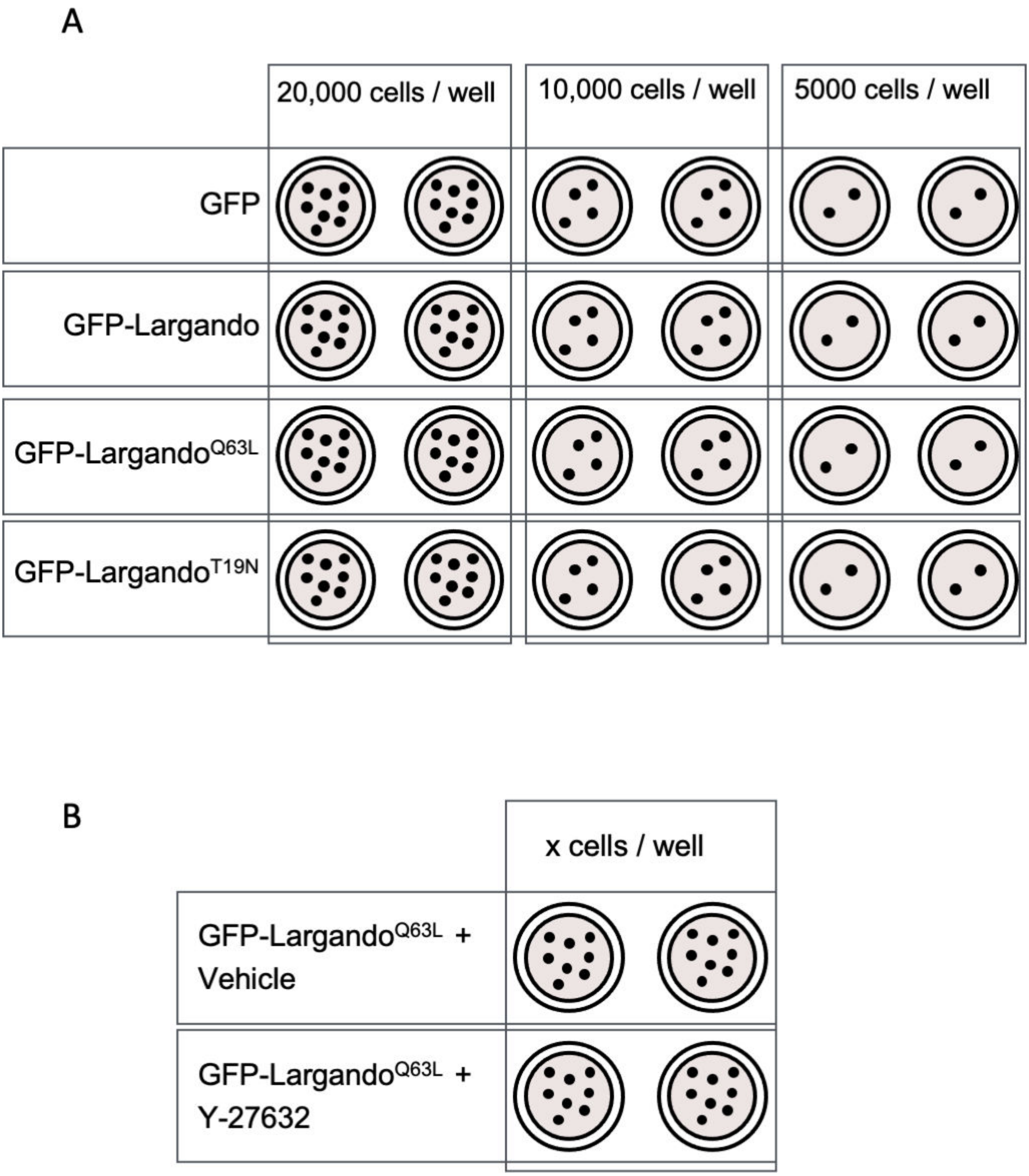


**FIG 1** (A) A schematic of a 24-well plate with coverslips seeded at different concentrations of cells and transfected with the indicated constructs in week 1. (B) A schematic of the layout of transfections performed in week 2, with construct and conditions as indicated. A single slide can accommodate a maximum of four coverslips of 12 mm in diameter.

*Practical session 2: cell transfection (2–3 h)*

This session can be placed 24 or 48 h after session 1. In this session, in order to assess the ability of Largando variants to affect the actin cytoskeleton and general morphology of COS-7 cells, cells that were plated in session 1 are now being transfected. For this, three variants of GFP-Largando (wild type, constitutively active, and dominant negative) and GFP alone as control are tested. The corresponding plasmids have been previously purified and are provided to the students in labeled tubes. The concentration of plasmids encoding for GFP-Largando$^{Q63L}$, GFP-Largando$^{T19N}$, GFP-Largando, and GFP alone can be provided or estimated by the students using a spectrophotometer. Calcium phosphate has been selected as the method of transfection as it is inexpensive and suitable for large groups. Moreover, this method allows for reinforcement of laboratory skills as it requires more manipulations than commercially available transfection kits. An outline of the calcium phosphate transfection method is introduced, placing particular emphasis on the importance of obtaining a fine precipitate of DNA-$CaCl_2$ for better transfection efficiency. Figure 1A shows a schematic of a transfected 24-well plate with the different constructs.

*Practical session 3: cell fixation, staining, and mounting of slide 1 (2 h)*

This session can be placed 24 or 48 h after session 2. In this session, the cells will be fixed and stained with Rhodamine conjugated phalloidin. The session will hence introduce: (i) the role of formaldehyde as a cross-linker. Crosslinking of proteins and other macromolecules is commonly used to preserve tissue and cells for further analysis. The precautions to be taken when handling this hazardous substance as described in the protocol handout are emphasized; (ii) the use of detergents for membrane permeabilization to facilitate the access by fluorescently labeled compounds to intracellular targets; and (iii) the use of DAPI to identify all cells on the preparation. DAPI allows the calculation of proportion of transfected cells as well as facilitating a side-by-side morphological comparison between transfected and non-transfected cells. Once the fixation steps have been completed, the coverslips sitting at the bottom of the wells with the cells on top are lifted from the wells and mounted on a slide. For this, MOWIOL mountant containing DAPI will be used. A set of slides are now ready for visualization under a fluorescence microscope after completion of all practical sessions.

*Workshop 1: scientific report writing (2 h)*

The workshop outlines in detail the different sections of a scientific publication. This workshop can be built using the resources included in Appendix 7 (Report Writing Guidelines), adapting the content to the level of the course. For each of the sections mentioned next, groups composed of four to six students are presented with examples of contrasting quality. Students are then prompted to identify their preferred example and justiy their choice. The following points are considered: (i) the importance of having an informative title that aligns to the main finding(s) and serves to attract the reader's attention; (ii) making a distinction in the Introduction between background and the specific purpose of the work. The Introduction summarizes the established facts of the main topic and identifies knowledge gaps. The latter are then used to link to and justify the specific questions that will be addressed in the report; (iii) a discussion of common mistakes in chart presentations and figures, including accessibility. Examples of poorly constructed figures and figure legends can be used here to identify key issues and discuss possible improvements; (iv) likewise, examples of accurate reporting from a given chart can be provided to illustrate the importance of using calibrated language; (v) the elements of a good figure legend can be summarized following a brief analysis by the students of good and poorly constructed examples; (vi) the components of a Results section paragraph (e.g., brief justification of experimental approach to the question, work done, what it shows and what it means, while leaving wider conclusions and implications for the Discussion); (vii) the notion of maintaining a consistent narrative from

Introduction to Discussion; and (viii) the basic elements of a critical Discussion: summary of findings, detail comparing and contrasting of results with published data that can support or contradict own conclusions, the limitations of the actual experimental design, possible improvements and what else could be done next. The use of an appropriate citation technique. This workshop can either run as part of the structure of the series of practicals or it could be covered elsewhere in the course.

### ***Week 2***

#### *Introductory lecture to week 2*

This lecture begins with a recap of the work the students have done in week 1. The microscopy slides prepared in week 1 have been stored and will not be analyzed until week 3. In preparation for this analysis, prompts are offered to facilitate the interpretation of the results. For example: (i) If the results for GFP-Largando and GFP-Largando$^{Q63L}$ are the same/different, what does this mean?; (ii) If the results for GFP-Largando and GFP-Largando$^{T19N}$ are the same/different, what does this mean? The lecture now introduces the effectors of RhoA, which has been previously presented as a well-characterized closest homologue to Largando. Various downstream targets of active GTP-bound RhoA and their specific functions can be mentioned. Since ROCK is the primary mediator of RhoA activity [for a review, see reference (10)], we focus on ROCK and its inhibitor Y-27632 to test the hypothesis that Largando regulates the actin cytoskeleton through ROCK. To test this hypothesis, the construct encoding the constitutively active GFP-Largando$^{Q63L}$ will be transfected in the presence or absence of the inhibitor Y-27632. The notion of testing the solvent used to dilute Y-27632 as a necessary control is introduced. The experimental design that will be followed may be sufficient to detect an inhibitory effect of Y-27632, but is far from a complete set of experiments. For example, it assumes that Y-27632 is effective at the selected concentration in the protocol, but there is no margin of error, that is, no titration is performed or additional concentrations are used above and below those recommended in the literature. This offers the opportunity for the students to troubleshoot and suggest improvements to the experimental design in the Discussion section of the assessment. On the other hand, the protocols are identical to week 1, which will increase confidence and skills in those techniques.

#### *Workshop 2: ImageJ cell sizing software training (2 h)*

Students are guided through the options in ImageJ for selecting and measuring the area of cells using images obtained from the same preparations and microscope. The focus is on practicing: i) Setting the scale; (ii) Measuring cell areas using the region of interest (ROI) submenu and exporting the data to Excel; (iii) Merging channels and (iv) Building a final figure. Detailed instructions for instructors and students can be found in Appendix 5 (Cell Area in ImageJ) and Appendix 6 (Image Overlay in ImageJ).

#### *Practical session 4: cell trypsinization, counting, and plating 2*

This week addresses whether ROCK1 mediates the effect of active Largando. For this, only the constitutively active version of Largando will be transfected. A simpler plating plan is followed using a layout to account for the addition of Y-27632 in session 5 (Fig. 1B). In this session, the steps for trypsinization, cell counting, and plating onto coverslips in 24-well plates are performed exactly as in Practical session 1. Cells are allowed to grow for 24/48 h to get them ready for use in Practical session 5.

#### *Practical session 5: cell transfection 2*

In this session, only two sets of COS-7 cells with cDNA encoding GFP-Largando$^{Q63L}$ will be transfected. In one set, cells will be treated with Y-27632 and in the other set with vehicle to separate the effects of both components, even if the vehicle is just $H_2O$ in this case (Fig. 1B).

*Practical session 6: cell fixation, staining, and mounting of slide 2*

Cells will be processed in this session following the same protocol as in Practical session 3.

### *Week 3*

*Practical session 7: imaging cells using a fluorescent microscope (3 h)*

Working in groups of three per microscope, students collect images from their slides. Students receive an introduction to using the fluorescent microscope and the associated imaging software. This guidance will be specific to the make and model of microscope in the institution. A brief outline of the basics of fluorescence microscopy and the use of fluorescent tags or proteins for the identification of cell structures can be provided. Examples from the student's slides can be used to illustrate what they should be focusing on. The number of cells that should be collected per construct as well as the advantages and disadvantages of using different magnifications is discussed at this time. For example, a distinction can be made between selecting a 20× objective for illustration purposes and 10× objective for quantification purposes. Students are encouraged to change roles as they progress through the preparations of their transfected cells.

The anticipated effects of transfections with GFP-Largando or GFP-Largando$^{Q63L}$ would be a reduction of cell size, as active RhoA causes cell shrinkage by promoting stress fiber formation through the ROCK1 effector (11). The latter effect would be attenuated in the presence of the ROCK1 inhibitor Y-27632 (12). GFP-Largando$^{T19N}$ would cause an increase in cell area, as the dominant-negative mutation titrates out the GEFs, preventing the formation of stress fibers by inhibiting downstream interactions with effector proteins (13), resulting in larger rounded cells. Figure 2 shows an example of what can be generated from the images obtained in these practicals. Tables 1 and 2 show examples of measurements obtained from COS7 cell transfections and the mean obtained from each independent treatment, respectively. The *P* value corresponding to the *F*-statistic of one-way ANOVA is lower than 0.01, indicating that one or more pairs of treatments are significantly different. Application of Tukey's HSD to test each of the six pairs shows that all pairs except GFP-Largando/GFP exhibit a statistically significant difference (Table 3).

## CONCLUSION

Every eukaryotic cell contains an actin cytoskeleton that plays essential roles in maintaining cell shape, regulating cell migration and division, and providing a mechanism for intracellular transport of proteins and organelles. Extracellular stimuli induce changes in the actin cytoskeleton primarily through Rho GTPases, which once activated bind to a variety of effector proteins that directly or indirectly, affect the local assembly or disassembly of actin (3). We have taken advantage of the well-characterized role of RhoA in actin cytoskeletal dynamics in COS7 cells to design a practical series suitable for Stage 2 Biology students. The practical series introduces tissue culture lab skills and reinforces them through repetition. It contextualizes each experiment by introducing an overarching narrative that provides sufficient detail to facilitate the interpretation of results. For example, by analyzing in the introductory lecture the RhoA mutations that have been selected for the transfection experiments. This background establishes the scenario for students to explore the function of a new hypothetical RhoA family member in this practical series and report on their own findings. For the practical sessions, the inexpensive $CaCl_2$ method generally produces high transfection efficiencies in COS7 fibroblast cells, but other liposome-based methods may be considered. As expected, the transfection efficiency and quality of the final slides vary greatly among groups. In our experience, from a cohort of 32 students, enough slides of good quality are produced as a whole to provide measurements for statistical analyses for each student. The slides can be shared and re-imaged so that every group of three students has a chance to collect good-quality images during the microscopy session. Data collection and analyses

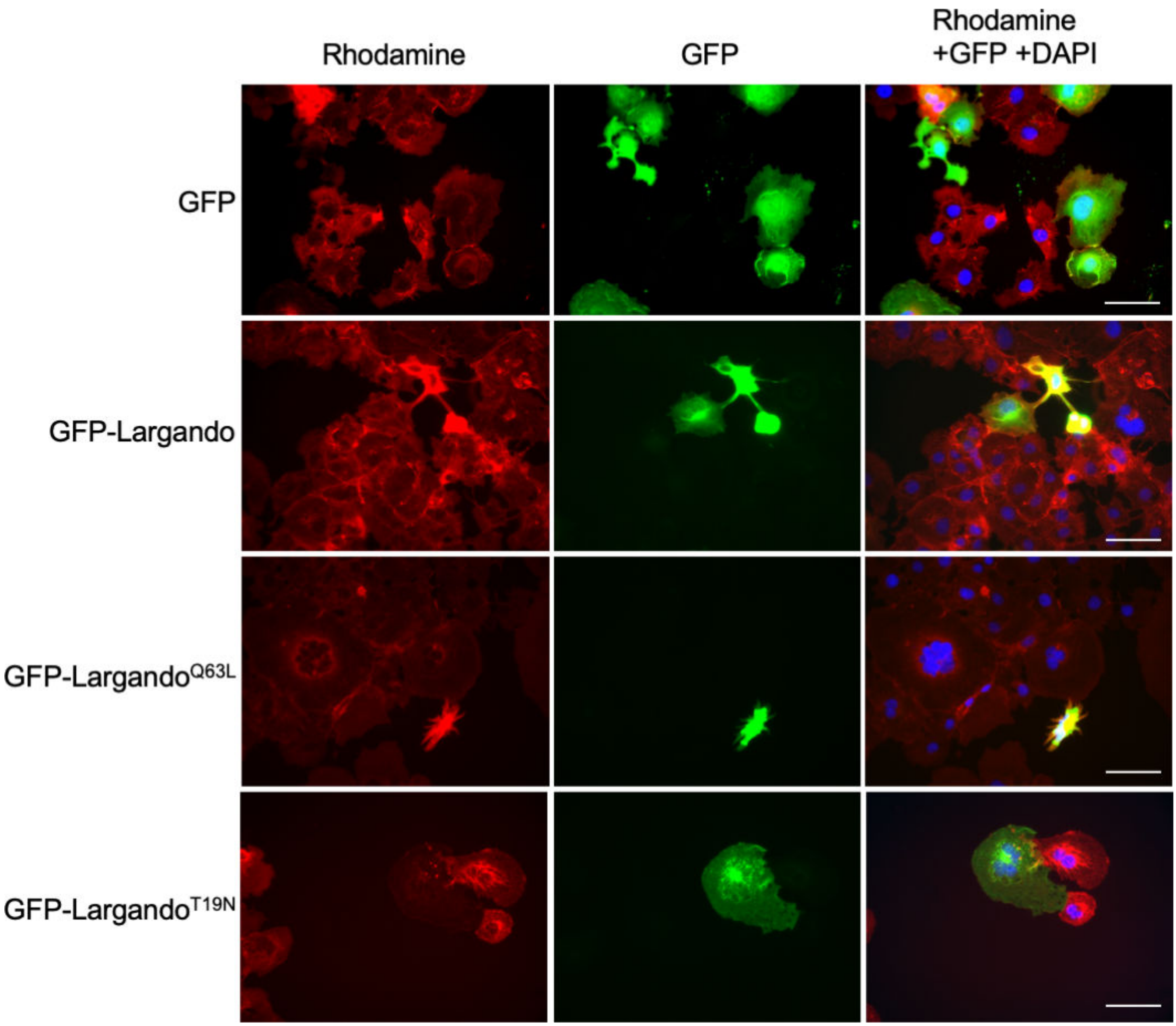


**FIG 2** Illustrative examples of transfected COS7 cells with GFP, GFP-Largando, GFP-Largando$^{Q63L}$, and GFP-Largando$^{T19N}$ as indicated. Note that constitutively active Largando (GFP-Largando$^{Q63L}$) and wild-type Largando (GFP-Largando) cause cell shrinkage and stronger GFP and rhodamine-phalloidin signals. Expression of GFP-Largando$^{T19N}$ results in larger cells with reduced rhodamine-phalloidin signal. Scale bar: 50 µm.

are undertaken independently by the students. The "Report writing" and "ImageJ" workshops are designed to provide the toolkits to deal with a scientific report and the processing of data, respectively. Students can then reach their own conclusions, while remaining aware that the results have been obtained from a limited set of experiments. Students are encouraged to explore primary scientific literature in order to appraise other experimental designs and independent evidence and use those references to calibrate their own conclusions. It should be expected that building an evidence-based discussion through exploring the wider context is still a challenging task at this stage of the curriculum. Therefore, ample guidance should be provided in the report writing workshop. We also recommend providing access to an online discussion board where students can ask questions until the submission deadline. This discussion board is extensively used by the students. In addition to questions related to background and techniques, questions often refer to the structure and expectations of the assessment.

**TABLE 1** Cell area measurements

| ID | Area (μm²) |
|---|---|
| GFP | |
| 1 | 1,421 |
| 2 | 1,479 |
| 3 | 1,038 |
| 4 | 1,076 |
| 5 | 1,185 |
| 6 | 928 |
| 7 | 1,015 |
| 8 | 1,354 |
| 9 | 1,300 |
| 10 | 966 |
| 11 | 1,535 |
| 12 | 1,366 |
| 13 | 1,010 |
| 14 | 1,018 |
| 15 | 1,495 |
| 16 | 928 |
| 17 | 1,237 |
| 18 | 1,485 |
| 19 | 1,298 |
| 20 | 1,516 |
| 21 | 927 |
| 22 | 1,396 |
| 23 | 1,307 |
| 24 | 908 |
| 25 | 1,114 |
| 26 | 1,445 |
| 27 | 1,391 |
| 28 | 1,308 |
| 29 | 1,131 |
| 30 | 1,486 |
| 31 | 1,148 |
| 32 | 952 |
| 33 | 1,317 |
| 34 | 1,554 |
| 35 | 1,559 |
| 36 | 1,359 |
| 37 | 1,000 |
| 38 | 1,222 |
| 39 | 1,133 |
| 40 | 1,272 |
| 41 | 1,364 |
| 42 | 1,160 |
| 43 | 1,265 |
| 44 | 1,045 |
| 45 | 1,054 |
| 46 | 1,578 |
| 47 | 1,110 |
| 48 | 1,081 |
| WT | |
| 1 | 953 |
| 2 | 919 |

(*Continued on next page*)

**TABLE 1** Cell area measurements (*Continued*)

| ID | Area (μm²) |
|---|---|
| 3 | 1,037 |
| 4 | 1,174 |
| 5 | 931 |
| 6 | 1,468 |
| 7 | 1,229 |
| 8 | 1,017 |
| 9 | 1,200 |
| 10 | 975 |
| 11 | 1,379 |
| 12 | 1,051 |
| 13 | 1,341 |
| 14 | 1,222 |
| 15 | 1,398 |
| 16 | 1488 |
| 17 | 1,048 |
| 18 | 1,137 |
| 19 | 1,099 |
| 20 | 977 |
| 21 | 1,264 |
| 22 | 1,224 |
| 23 | 948 |
| 24 | 1,199 |
| 25 | 1,208 |
| 26 | 1,040 |
| 27 | 1,024 |
| 28 | 1,512 |
| 29 | 969 |
| 30 | 1,443 |
| 31 | 1,000 |
| 32 | 945 |
| 33 | 1,371 |
| 34 | 1,535 |
| 35 | 1,326 |
| 36 | 1,464 |
| 37 | 945 |
| 38 | 1,124 |
| 39 | 1,468 |
| 40 | 1,214 |
| 41 | 1,472 |
| 42 | 1,002 |
| 43 | 992 |
| 44 | 1,080 |
| 45 | 1,291 |
| 46 | 1,156 |
| 47 | 1,443 |
| 48 | 1,048 |
| Q63L | |
| 1 | 504 |
| 2 | 627 |
| 3 | 421 |
| 4 | 679 |
| 5 | 326 |

(*Continued on next page*)

TABLE 1 Cell area measurements (*Continued*)

| ID | Area (µm$^2$) |
|---|---|
| 6 | 445 |
| 7 | 415 |
| 8 | 430 |
| 9 | 345 |
| 10 | 372 |
| 11 | 545 |
| 12 | 452 |
| 13 | 679 |
| 14 | 443 |
| 15 | 615 |
| 16 | 498 |
| 17 | 553 |
| 18 | 472 |
| 19 | 519 |
| 20 | 430 |
| 21 | 434 |
| 22 | 586 |
| 23 | 643 |
| 24 | 317 |
| 25 | 623 |
| 26 | 645 |
| 27 | 466 |
| 28 | 469 |
| 29 | 632 |
| 30 | 625 |
| 31 | 530 |
| 32 | 399 |
| 33 | 566 |
| 34 | 336 |
| 35 | 375 |
| 36 | 338 |
| 37 | 594 |
| 38 | 322 |
| 39 | 512 |
| 40 | 347 |
| 41 | 439 |
| 42 | 395 |
| 43 | 607 |
| 44 | 469 |
| 45 | 684 |
| 46 | 536 |
| 47 | 409 |
| 48 | 300 |
| T19N | |
| 1 | 1,366 |
| 2 | 1,208 |
| 3 | 1,902 |
| 4 | 1,772 |
| 5 | 1,425 |
| 6 | 1,836 |
| 7 | 1,951 |
| 8 | 1,154 |

(*Continued on next page*)

**TABLE 1** Cell area measurements (*Continued*)

| ID | Area ($\mu m^2$) |
|---|---|
| 9 | 1,335 |
| 10 | 1,824 |
| 11 | 1,835 |
| 12 | 1,509 |
| 13 | 1,593 |
| 14 | 1,021 |
| 15 | 1,135 |
| 16 | 1,263 |
| 17 | 929 |
| 18 | 1,946 |
| 19 | 1,033 |
| 20 | 1,090 |
| 21 | 1,685 |
| 22 | 1,499 |
| 23 | 1,815 |
| 24 | 1,525 |
| 25 | 1,301 |
| 26 | 1,836 |
| 27 | 1,246 |
| 28 | 1,712 |
| 29 | 1,248 |
| 30 | 1,311 |
| 31 | 1,398 |
| 32 | 1,719 |
| 33 | 969 |
| 34 | 1,609 |
| 35 | 1,791 |
| 36 | 1,873 |
| 37 | 1,483 |
| 38 | 976 |
| 39 | 1,586 |
| 40 | 1,227 |
| 41 | 1,841 |
| 42 | 965 |
| 43 | 1,257 |
| 44 | 962 |
| 45 | 1,749 |
| 46 | 1,022 |
| 47 | 1,911 |
| 48 | 1,104 |

At this stage of their studies, students have not been thoroughly tested in the criteria that apply to scientific publications in most of the assessments they have undertaken. Although referencing and brief single practical reports may have been practiced, producing a full scientific report in the style of a scientific publication is a challenging task at this stage of the degree course. This practical series and assessment can be considered an introduction to producing and reporting original research. Crucially, this learning outcome aligns with the larger capstone research project or senior thesis report in the final year. Here, in the supportive workshop, students are taught the basic compositional elements of a professional scientific publication and the various templates that journals use and that students can follow to produce their own reports. All of it helps to build toward the QAA Bioscience benchmark outcomes (14). These outcomes include hypothesis testing and interpretations of results in context within the research area,

**TABLE 2** Summary of measurements from Table 1

| Treatment | Mean | SEM | *N* |
|---|---|---|---|
| GFP | 1,234.1 | 29.3 | 48 |
| GFP-Largando | 1,182.3 | 27.8 | 48 |
| GFP-Largando$^{Q63L}$ | 486.8 | 16.2 | 48 |
| GFP-Largando$^{T19N}$ | 1,453.1 | 47.3 | 48 |

amongst others. The report contains title, introduction, a single section combining results and discussion, and references. The weighting is 25% for introduction, 60% for results and discussion, and 15% for presentation. Presentation includes title, overall quality of the layout, and references. The marking criteria include excellence indicators for first-class reports (see Appendix 3, Report Marking Criteria). A first-class report should demonstrate at least one of those indicators, for example, by showing an impressive ability to recognize the significance of the results obtained in relation to previous work, and providing thoughtful explanations for unexpected findings or discrepancies. The presence of other indicators is used to award marks in the range of 70–100. These marking criteria are available to the students. Students receive a feedback form that contains scores for each key component of the different sections of the report and a final mark (Appendix 4, Report Feedback Template). In addition, a summary of "what was done well" and "what can be improved" is also provided. Students are encouraged to take reflections on this feedback forward for preparing their capstone project report in the final year.

We ran this practical for three consecutive years, making adjustments in response to feedback from students. The introduction of the report writing workshop, for example, was included in response to how challenging many students found undertaking this new form of assessment. The feedback from students highlights improving both their practical and report writing skills, including constructing scientific figures, and thinking like a scientist. Students like the opportunity to acquire skills in specialized techniques they had not encountered before, particularly cell culture and fluorescence microscopy. The opportunity to learn and practice in small groups and to practice the techniques with the repeating structure of Weeks 1 and 2 is often mentioned as a positive experience. Students found some technical aspects challenging, such as tracing cell shapes when analyzing the fluorescence cell images. It is therefore advisable to have a complete bank of images as a backup for students who have been unable to acquire appropriate images. Students may choose to use the provided backup images instead of their own, noting that images will still need to be independently processed for quantification. Some students mentioned in their feedback that they found it challenging to cope with a fictitious protein. Interestingly, the fictitious protein scenario was introduced in response to earlier feedback comments. In an earlier run of the practical series, students found it difficult to compose a report using their own results as the information using similar experiments was readily available in the literature. We suggest that if delivery is built around a fictitious protein, consistency is critical and leaking of information to the students that the fictitious protein is actually RhoA should be avoided.

In summary, composing a scientific report requires new knowledge that is mainly acquired through independent study and synoptic learning. The report is a significant deviation in style and content from other assessments students may be accustomed to,

**TABLE 3** Tukey HSD results after one-way ANOVA was applied to Table 1

| Pair | *Q* | *P* value |
|---|---|---|
| GFP/GFP-Largando | 1.6121 | 0.6653 |
| GFP/GFP-Largando$^{Q63L}$ | 23.2588 | 1.00E−10 |
| GFP/GFP-Largando$^{T19N}$ | 6.8159 | 0.0000175 |
| GFP-Largando/GFP-Largando$^{Q63L}$ | 21.6467 | 1.00E−10 |
| GFP-Largando/GFP-Largando$^{T19L}$ | 8.428 | 7.34E−08 |
| GFP-Largando$^{Q63L}$/GFP-Largando$^{T19N}$ | 30.0747 | 1.00E−10 |

and we noted that some students found it hard to manage the amount of time they should be spent exploring a topic for which they have not received a defined amount of content to review. It is likely that this early experience helps students to cement and develop skills to report on longer and more complex research activities, such as capstone projects.

## AUTHOR AFFILIATION

[1]Department of Biology, University of York, York, United Kingdom

## AUTHOR ORCIDs

Gonzalo Blanco http://orcid.org/0000-0003-3827-3111

## ADDITIONAL FILES

The following material is available online.

### Supplemental Material

**Appendix S1 (jmbe00190-23-s0001.docx).** Student Instructions (Protocol that students follow in each practical session).
**Appendix S2 (jmbe00190-23-s0002.docx).** Technicians Instructions (Instructions for supporting staff to prepare all materials and reagents required).
**Appendix S3 (jmbe00190-23-s0003.docx).** Scientific Report Marking Criteria (Marking criteria to evaluate the scientific report submitted by students).
**Appendix S4 (jmbe00190-23-s0004.docx).** Feedback template (Feedback information for students following evaluation of the report).
**Appendix S5 (jmbe00190-23-s0005.docx).** Measuring Cell Area in ImageJ (Step by step instructions to measure cell area using ImageJ).
**Appendix S6 (jmbe00190-23-s0006.docx).** Image Overlay in ImageJ (Step by step instructions to overlay fluorescence images in ImageJ).
**Appendix S7 (jmbe00190-23-s0007.docx).** Report Writing Guidelines (Generic guidelines to write a scientific report).

## REFERENCES

1. Paterson HF, Self AJ, Garrett MD, Just I, Aktories K, Hall A. 1990. Microinjection of recombinant p21rho induces rapid changes in cell morphology. J Cell Biol 111:1001–1007. https://doi.org/10.1083/jcb.111.3.1001
2. Freitas MR, Eto M, Kirkbride JA, Schott C, Sassard J, Stoclet J-C. 2009. Y27632, a Rho-activated kinase inhibitor, normalizes dysregulation in alpha1-adrenergic receptor-induced contraction of Lyon hypertensive rat artery smooth muscle. Fundam Clin Pharmacol 23:169–178. https://doi.org/10.1111/j.1472-8206.2008.00658.x
3. Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, Narumiya S. 1999. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 285:895–898. https://doi.org/10.1126/science.285.5429.895
4. Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089
5. Heasman SJ, Ridley AJ. 2008. Mammalian Rho GTPases: new insights into their functions from *in vivo* studies. Nat Rev Mol Cell Biol 9:690–701. https://doi.org/10.1038/nrm2476
6. Narumiya S. 1996. The small GTPase Rho: cellular functions and signal transduction. J Biochem 120:215–228. https://doi.org/10.1093/oxfordjournals.jbchem.a021401
7. Hall A. 1998. Rho GTPases and the actin cytoskeleton. Science 279:509–514. https://doi.org/10.1126/science.279.5350.509
8. Subauste MC, Von Herrath M, Benard V, Chamberlain CE, Chuang TH, Chu K, Bokoch GM, Hahn KM. 2000. Rho family proteins modulate rapid apoptosis induced by cytotoxic T lymphocytes and Fas. J Biol Chem 275:9725–9733. https://doi.org/10.1074/jbc.275.13.9725
9. Glass JR, DeWitt RG, Cress AE. 1985. Rapid loss of stress fibers in Chinese hamster ovary cells after hyperthermia. Cancer Res 45:258–262.
10. Schmandke A, Schmandke A, Strittmatter SM. 2007. ROCK and Rho: biochemistry and neuronal functions of Rho-associated protein kinases. Neuroscientist 13:454–469. https://doi.org/10.1177/1073858407303611
11. Wheeler AP, Ridley AJ. 2004. Why three Rho proteins? RhoA, RhoB, RhoC, and cell motility. Exp Cell Res 301:43–49. https://doi.org/10.1016/j.yexcr.2004.08.012
12. Rao JN, Guo X, Liu L, Zou T, Murthy KS, Yuan JX-J, Wang J-Y. 2003. Polyamines regulate Rho-kinase and myosin phosphorylation during intestinal epithelial restitution. Am J Physiol Cell Physiol 284:C848–C859. https://doi.org/10.1152/ajpcell.00371.2002
13. Feig LA. 1999. Tools of the trade: use of dominant-inhibitory mutants of Ras-family GTPases. Nat Cell Biol 1:E25–E27. https://doi.org/10.1038/10018
14. Quality assurance agency. 2023. " BioSciences" 2023