

How to make your protocol more reproducible, discoverable, and user-friendly

protocols.io team

Abstract

Protocols.io offers extensive flexibility in entering and presenting detailed methods. However, simple suggestions in here can help to make your protocol more useful to you and others. Importantly, easy steps outlined here can greatly increase the visibility of your protocol and ensure that scientists who need can find it.

If you have any questions, please feel free to reach out to the protocols.io team by e-mailing us at [info \[at\] protocols.io](mailto:info@protocols.io).

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protocols.io

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Guidelines

The importance of detailed protocols for reproducibility.

With the recent publication in eLife of the first studies from the [Reproducibility Projects: Cancer Biology](#), there has been a lot of emphasis on the missing details in the Materials & Methods sections of published research papers.

[Monya Baker & Elie Dolgin in Nature News:](#)

Perhaps the clearest finding from the project is that many papers include too few details about their methods, says Errington. Replication teams spent many hours working with the original authors to chase down protocols and reagents, in many cases because they had been developed by students and postdocs who were no longer with the lab. Even so, the final reports include long lists of reasons why the replication studies might have turned out differently — from laboratory temperatures to tiny variations in how a drug was delivered. If the project helps to bring such confusing details to the surface, it will have performed a great service, Errington says.

[Ed Yong in The Atlantic:](#)

The hardest part, by far, was figuring out exactly what the original labs actually did. Scientific papers come with methods sections that theoretically ought to provide recipes for doing the same experiments. But often, those recipes are incomplete, missing out important steps, details, or ingredients. In some cases, the recipes aren't described at all; researchers simply cite an earlier study that used a similar technique. "I've done it myself: you reference a previous paper and that one references a paper and that one references a paper, and now you've gone years and the methodology doesn't exist," admit Errington. "Most people looking at these papers wouldn't even think of going through these steps. They'd just guess. If you asked 20 different labs to replicate a paper, you'd end up with 10 different methodologies that aren't really comparable."

So, in every case, he had to ask the scientists behind the original experiments for the details of their work. Oftentimes, the person who actually did the experiments had left the lab, so an existing team member had to rummage through old notebooks or data files. The project ended up being hugely time-consuming for everyone concerned. "We spent a boatload of time trying to get back to ground zero," says Errington.

[Julia Belluz, VOX:](#)

Scientists and journals need to get better at describing their methods and sharing data

It is not hard to make your protocol reproducible for the future you (when you try to repeat the experiment in 6 months) and for others (future members of your lab or company, other scientists after publication of your papers). Please see the simple suggestions in the 'Steps' section here and also read the excellent [How to Write an Easily Reproducible Protocol](#) from [Arsalan Daudi](#).

(Arsalan's guide is cross-posted below with his permission.)

How to Write an Easily Reproducible Protocol

SUMMARY

- Research articles seldom describe experimental protocols in sufficient detail to reproduce them in another lab
- Protocols should be thought of as stand-alone scientific articles that provide context and detail on the research methodology
- There are a growing number of resources that provide the research community with access to

Have you ever looked at the Materials and Methods section of a research paper to try and find details of a protocol you want to implement in the lab? Have you come across short paragraphs that concisely summarize a method but leave out critical details? Have you found yourself asking questions like:

- How long did they spin those samples for?
- Did they store the overnight suspension in a fridge or freezer?
- Exactly which ultra high metal affinity column did they purchase?

If you answered yes to any of these, don't be alarmed – you are not alone.

The art of writing a precise yet detailed and contextualized, relatable and reproducible protocol has far too often been confined to a lab notebook that will never again be seen by anybody other than the lab notebook owner and a handful of their colleagues.

This article will share some pointers that provide a framework for writing an easily reproducible protocol.

Structure as a whole

It is important to think of a protocol (may it be wet or dry lab-based, biology or chemistry-focused, simple or advanced) as a brief, modular and self-contained scientific publication. To this end, several factors can be considered when preparing a draft.

Putting the methodology in context

You never know who will refer to your protocol in the future. It may be a new graduate student joining the lab you're preparing to leave or a new postdoc joining several years later when you're a busy and established PI thousands of miles away possibly researching something entirely different.

Alternatively, it may be a researcher you have never met who is working on a different species but looking for a protocol they can adapt for their needs. Whoever it may be, they will likely benefit from reading a three- to four-sentence abstract at the onset that briefly explains the system you worked on, describes any major obstacles or challenges you faced, and highlights the applicability of your workflow.

Materials and tools that were used in the protocol

Lets face it: two different batches of agarose or of magnesium sulfate may not be the same. Commonly used biochemical reagents could vary in terms of purity, yield, pH, hydration state, grade, and possibly additional biochemical or biophysical features. For many researchers this may not matter; yet for others it may be a deal breaker in terms of implementing your protocol into their workflow.

Similarly, when you mention “centrifuge,” this may not indicate how fast it can spin or how cold it can keep your samples. When a researcher finds out half way through an experiment that the process they set up based on your protocol cannot be completed because a simple detail was missing, the outcome is frustrating for everyone concerned.

This can lead to a poor reflection of your protocol, wasted resources in the lab, and time and effort that could have been better utilized. Share as many details as you can, such as catalog numbers and manufacturer details.

A chronology of steps

Bullet points are easy to follow and help others track a particular workflow. Always think about exactly what you did when you were using your protocol in the lab, and that the person using your protocol may be a novice experimenter.

Think about the quantities you used, sample replication, storage conditions, spinning and mixing, and details on how to operate various instruments and equipment. Listing all steps in a chronological and modular fashion can be a very effective interface for researchers that are trying to implement your protocol within their own laboratory workflows.

Notes, recipes, tips, and tricks

I would recommend wrapping up your protocol draft with a brief section that highlights details you might take for granted. Sometimes it is the little things that make an experiment work, flicking a tube gently versus vortexing it, spraying water on a leaf surface before infiltrating it with a syringe, or using PBS and tweezers to remove glue from a piece of sliced gel.

These are exactly the types of simple tricks or “secret sauce” that can save researchers weeks and potentially months of time and effort in the successful implementation of a protocol.

Conclusion

A well-curated database of protocols can serve as the backbone of a high performing research laboratory. There are a growing number of resources such as [Bio-protocol](#) and [Protocols.io](#) that allow you to share your protocols effectively with the research community and build your own portfolio of research methodologies.

Resources such as these as well as other established protocol journals and organizations such as [JOVE](#), [Science Exchange](#), [COS](#), and [GBSI](#) are filling an important niche to ensure that the scientific community maintains its core mission of delivering reproducible results.

Before start

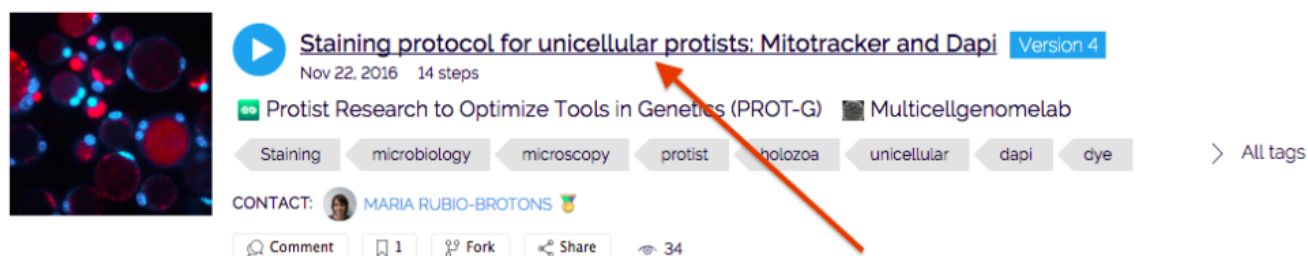
If you are just getting started, you might want to first see the [How to create a new protocol](#) guide.

Protocol

How to make your protocol easily discoverable

Step 1.

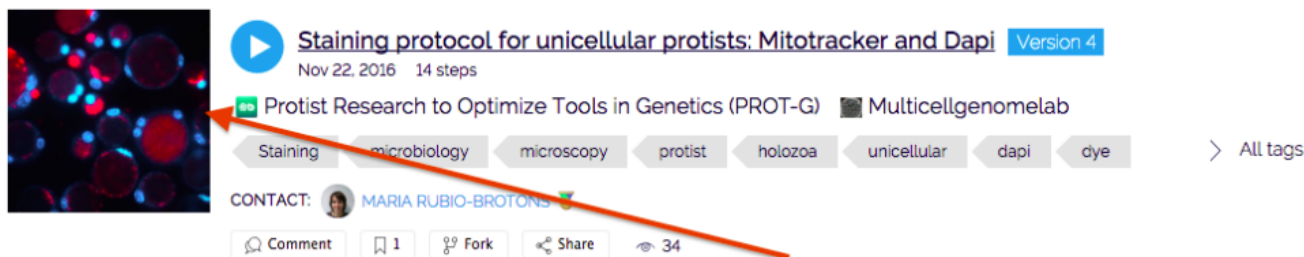
Title: Try to make your title descriptive, as you would for a manuscript. This helps for searches on Google & protocols.io.



How to make your protocol easily discoverable

Step 2.

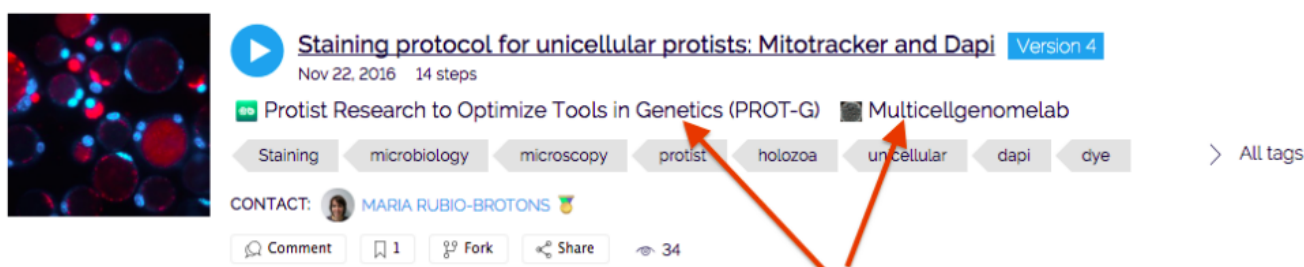
Thumbnail Picture: Having a picture to identify the protocol helps you and other users find it in the lists of protocols they use.



How to make your protocol easily discoverable

Step 3.

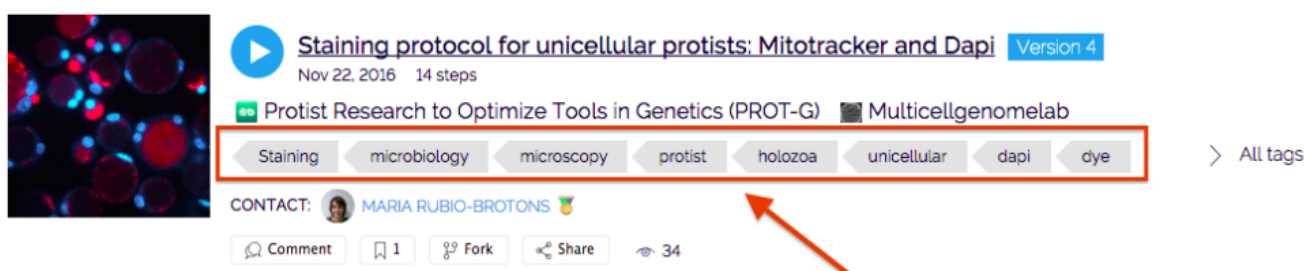
Groups: You can add your protocol to any relevant groups that you joined so that the members of these groups will receive a notification and be able to look at your protocol. It will enable you to show your protocol to the right audience.



How to make your protocol easily discoverable

Step 4.

Tags: Click on the 'More' tab and enter a tags to supplement the keywords in your title. Your protocol will appear in the search results for queries with these keywords.



■ ANNOTATIONS

Michael Strack 08 Sep 2017

Do you have any best-practise recommendations for differential use of visible tags vs. invisible keywords? I'm guessing the search algorithms aren't weighted towards one or the other.

How to make your protocol more reproducible

Step 5.

Components: For each step you can add any of these components to provide the important details, by clicking on one of the boxes on the right side:

How to make your protocol more reproducible

Step 6.

Wetlab Components: Specifying the reagents and equipment with vendor names, catalog numbers, links is crucial. Just naming the antibody you used is not enough, as vendor-to-vendor and even lot-to-lot variability can make or break the experiment. Use the 'reagent' component inside steps or in the 'more' section.

STEPSDESCRIPTIONGUIDELINESWARNINGSCOMMENTSRÉSULTSMETRICSFORKSMORE

MATERIALS

| Name | Vendor name | Catalog # | Link |
|---|----------------------|---------------|----------------------|
| 10g/L Fe Stock Solution | Contributed by users | | View |
| 0.1M EDTA-0.2M MgCl2-0.2M Ascorbate Buffer | Contributed by users | | View |
| ✓ Millipore Express PLUS filter 0.22 µm, 142 mm | Contributed by users | GPWP14250 | View |
| ✓ Pall Supor-800 filter | Contributed by users | 60114 | View |
| ✓ GE Polycarbonate Membrane filter | Contributed by users | MAINE 1216611 | View |

CITATION

Seth John, Bonnie Poulos, Christine Schirmer (2015). Iron Chloride Precipitation of Viruses from Seawater. [protocols.io](https://protocols.io/view/Iron-Chloride-Precipitation-of-Viruses-from-Seawat-c2wyfd)
<https://protocols.io/view/Iron-Chloride-Precipitation-of-Viruses-from-Seawat-c2wyfd>

How to make your protocol more reproducible

Step 7.

Bioinformatics Components: Use the "command", "software package", "dataset", and "external link"

OTU Summary

Step 11 To compute some summary statistics of the OTU table we can run the following command.

Run Diversity Analysis

Step 12 Run diversity analyses. Here we're running the core_diversity_analyses.py script which applies many of the 'first-pass' ... [read more](#)

Step 13 The results above treat all samples independently, but sometimes (for example, in the taxonomic summaries) it's useful t... [read more](#)

Step 11

To compute some summary statistics of the OTU table we can run the following command.

COMMAND

```
biom summarize-table -i
otus/otu_table_mc2_w_tax_no_pynast_failures.biom
```

ANNOTATIONS [Add new](#)

 **BONNIE HURWITZ** Nov 6, 2015 12:47

The key piece of information you need to pull from this output is the depth of sequencing that should be used in diversity

components to specify the code, version of software, and location/version of the datasets used in the analysis.

How to make your protocol more reproducible

Step 8.

Pictures/Videos: You can add pictures or videos for any step of your protocol (or in the guidelines). This often makes all the difference when someone is trying your method for the first time.

protocols.io > researchers > Benjamin Schwessinger > protocols > High quality DNA from Fungi for long read sequencing e.g. PacBio

STEPS DESCRIPTION GUIDELINES COMMENTS RESULTS METRICS FORKS MORE

Step 36 Use AMPure beads for secondary clean up at beads 0.45 (Vol/Vol) following the PacBio protocol.

Step 37 Elute in 10mM Tris pH8

Step 38 Measure dsDNA concentration using BR Qubit and measure absorbance with Nanodrop

Step 39 At this stage Qubit to Nanodrop ratios were 0.64, 260/280 1.87 and 260/230 1.37

Results

Step 40 Samples were submitted to Ramaciotti (<http://www.ramaciotti.unsw.edu.au/>) sequencing centre in Sydney. Excellent persone... [read more](#)

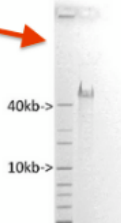
Step 40 Samples were submitted to Ramaciotti (<http://www.ramaciotti.unsw.edu.au/>) sequencing centre in Sydney. Excellent personel performed quality control, prepared 15-20kb libraries and we ran 13 SMRT cells with P6 chemistry. Some summary statistics are shown below.

EXPECTED RESULTS

Summary statistics for all 12 SMRT cells combined

| | |
|------------------------|-------------|
| Total sequence amount: | 1263691e+10 |
| Number of reads: | 1181543e+06 |
| Median read length: | 9632 |
| Mean read length: | 10695 |

Pippin Pulse Gel: 9hr 10-48kb 0.75% KBB gel



40kb->

10kb->

Making your protocol "runnable"

Step 9.

When doing an experiment, the protocols on this platform can be followed step-by-step on the web or on our [iOS](#) and [Android](#) apps.

[My protocols](#)
[Menu](#)

[save in journal](#)

Staining protocol for unicellular protists: Mitotracker and Dapi

Version 4

Nov 22, 2016 14 steps

CONTACT: MARIA RUBIO-BROTONS

Step 1

2% PFA Fixation: Add PFA drop by drop and mixing carefully into 1mL cells for a final concentration of 2%PFA

Step 2

Incubate cells for 4 min at room temperature

Step 3

Centrifuge at 1000g for 5 minutes

Step 4

Wash cells with 1XPBS (1/2)

Step 5

Wash cells with 1XPBS (2/2)

Wash

Step 4

Wash cells with 1XPBS (1/2)

Step 5

Wash cells with 1XPBS (2/2)

3/14

STEPS COMPLETED

[Help](#)

Making your protocol "runnable"

Step 10.

Single Step Instructions: Break up the method details into recipe-like checklist format. Instead of "incubate for 4min, then centrifuge at RT for 5min" separate as follows:

Step 1 2% PFA Fixation: Add PFA drop by drop and mixing carefully into 1mL cells for a final concentration of 2%PFA

Step 2 Incubate cells for 4 min at room temperature

Step 3 Centrifuge at 1000g for 5 minutes

Wash

Step 4 Wash cells with 1XPBS (1/2)

Making your protocol "runnable"

Step 11.

Repeated Steps: Instead of "wash 3 times" or "repeat steps 8-13", spell out the individual steps.

[protocols.io](#) > [researchers](#) > [Maria Rubio-Brotons](#) > [protocols](#) > [Staining protocol for unicellular protists: Mitotracker and Dapi](#)

[STEPS](#) [COMMENTS](#) [RESULTS](#) [METRICS](#) [MORE](#)

Wash

Step 4 Wash cells with 1XPBS (1/2)

Step 4

Wash cells with 1XPBS (1/2)

ANNOTATIONS [Add new](#)

Step 5 Wash cells with 1XPBS (2/2)

📌 NOTES

Lenny Teytelman 24 Feb 2017

This helps when "running" the protocol. Very frequently, we lost track when repeating the same thing of how many times we've done it. Am I on wash step 2/4 or 3/4?

Wash too much, and you lose the sample; wash too little, and there's contamination...

Making your protocol "runnable"

Step 12.

Be concise: Too much text in the instruction step makes it hard to follow. Move comments and tips to the "annotation" component.

Step 10 Incubate for 5 minutes. Room temperature, protect from light

Wash

Step 11 Wash with 1xPBS (1/2)

Step 12 Wash with 1xPBS (2/2)

concise step

extra info in comment

Step 10
Incubate for 5 minutes.
Room temperature, protect from light

DURATION
00:05:00

ANNOTATIONS [Add new](#)

MARIA RUBIO-BROTOS Nov 22, 2016 07:18

Total incubation times:
Mytotracker: 15min
DAPI: 5min

Making your protocol "runnable"

Step 13.

Timed steps: Use the "Duration/Timer" component in the individual steps as appropriate. This makes it possible to use the timer when "running" your protocol.

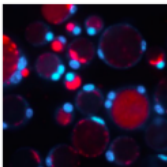
Step 3

Centrifuge at 1000g for 5 minutes

DURATION
00:05:00

ANNOTATIONS [Add new](#)


Running the protocol:



Staining protocol for unicellular protists: Mitotracker and Dapi

Version 4

Nov 22, 2016 14 steps

CONTACT:  MARIA RUBIO-BROTONS

1/14 STEPS COMPLETED

Step 1

2% PFA Fixation: Add PFA drop by drop and mixing carefully into 1mL cells for a final concentration of 2%PFA

✓

Step 2

Incubate cells for 4 min at room temperature

DURATION 00:03:47

Other tips

Step 14.

Sections: Groups related steps together into sections by giving sequential steps the same "section title".

protocols.io > researchers > Margaret Dentlinger > protocols > Near-Infrared (NIR) Western Blot Detection (PVDF membrane)

| STEPS | DESCRIPTION | GUIDELINES | COMMENTS | RESULTS | METRICS | FORKS | MORE |
|------------------------------------|---|------------|----------|---------|---------|-------|------|
| Prepare the membrane | | | | | | | |
| Step 2 | After you have air dried the membrane, pre-wet it for 1 minute in 100% methanol. | | | | | | |
| Step 3 | Rinse with ultra pure water. | | | | | | |
| Step 4 | Wet in 1X PBS or 1X TBS for 2 minutes (using the appropriate buffer system). | | | | | | |
| Block the membrane | | | | | | | |
| Step 5 | Place membrane in incubation box and block the membrane in Odyssey Blocking Buffer (PBS or TBS) for 1 hour with gentle... read more | | | | | | |
| Primary antibody incubation | | | | | | | |
| Step 6 | Primary antibody diluent: Odyssey Blocking Buffer (PBS or TBS) + 0.2% Tween® 20 (final concentration). | | | | | | |
| Step 7 | Dilute primary antibody in Odyssey Blocking Buffer + 0.2% Tween 20, using the vendor's recommended dilution for Weste... read more | | | | | | |

Other tips

Step 15.

Guidelines, warnings, before start: Use the "more" tab for adding "before start" instructions, warnings. Also in "more" is the "Guidelines" field for information about the method, references, tips, etc.

Other tips

Step 16.

Safety: In the "more" tab there is a "warnings tab". You can also add step-level safety information for specific reagents and equipment using the "Safety information" component. It's good to be redundant with these warnings, so consider putting them into relevant steps, "warnings", and Guidelines.

Other tips

Step 17.

See the [Guidelines](#) for more on making your protocols reproducible.

Also, see some of the detailed protocols from our users, used in the examples above:

[Staining protocol for unicellular protists: Mitotracker and Dapi](#), by [Maria Rubio](#)

[Iron Chloride Precipitation of Viruses from Seawater](#), [Matthew Sullivan lab](#)

[QIIME:Moving Pictures of the human microbiome](#), [Bonnie Hurwitz](#)

[High quality DNA from Fungi for long read sequencing e.g. PacBio](#), [Benjamin Schwessinger](#)

[Near-Infrared \(NIR\) Western Blot Detection \(PVDF membrane\)](#), [LI-COR Biosciences](#)