How to make your protocol more reproducible, discoverable, and user-friendly V.3

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1 Works for me

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protocols.io news

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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.

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

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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 high quality protocols

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.

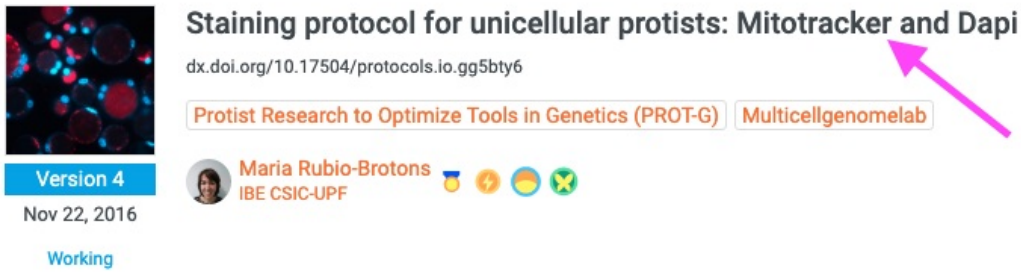
Resources such as these as well as other established protocol journals and organizations such as JOVE, Science Exchange, COS, and GBSI are filling an important niche to ensure that the scientific community maintains its core mission of delivering reproducible results.

BEFORE STARTING

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

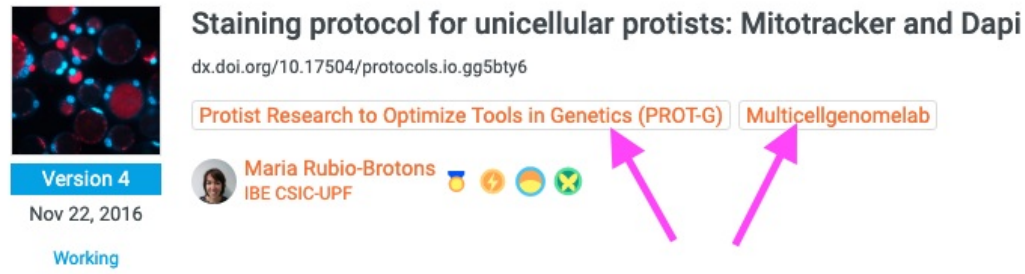
How to make your protocol easily discoverable

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



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

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.



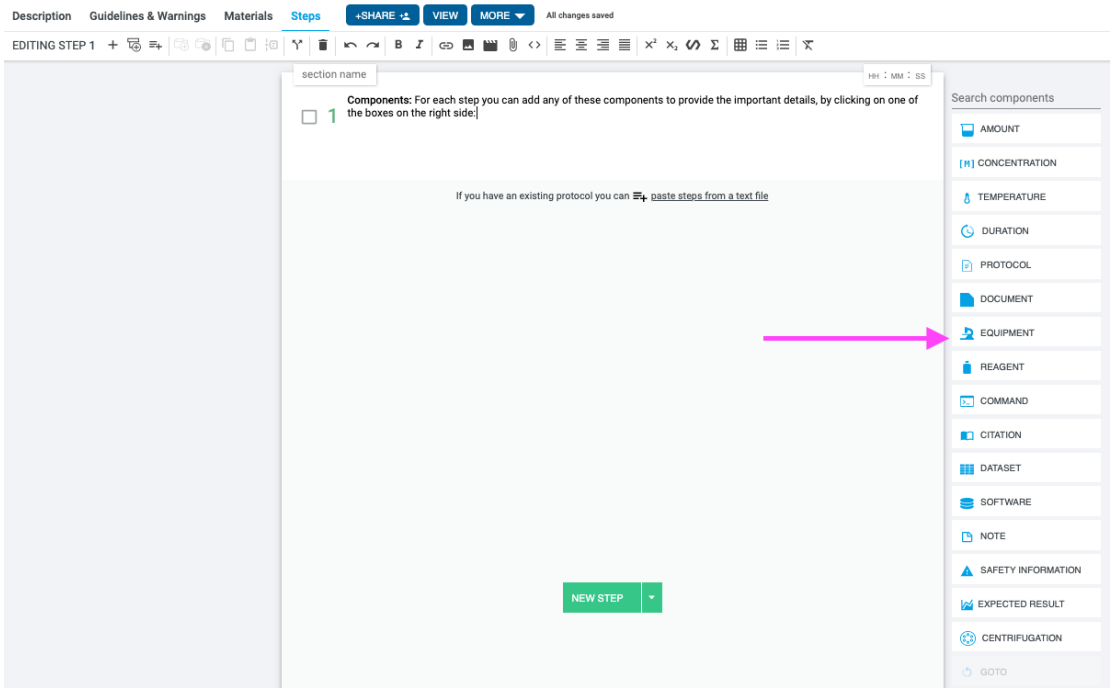
4 **Keywords:** When enter your protocol, you can add keywords on the 'description' tab. The keywords will be visible under the 'matadata' tab of you protocol and your protocol will appear in the search results for queries with these keywords.

KEYWORDS

staining, microbiology, microscopy, protist, hoiozoa, unicellular, dapi, dye

How to make your protocol more reproducible

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:



6 **Wetlab Components**: Specifying the reagents and equipment with vendor names, catalog numbers, links, RRID, etc. 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 'materials' section.

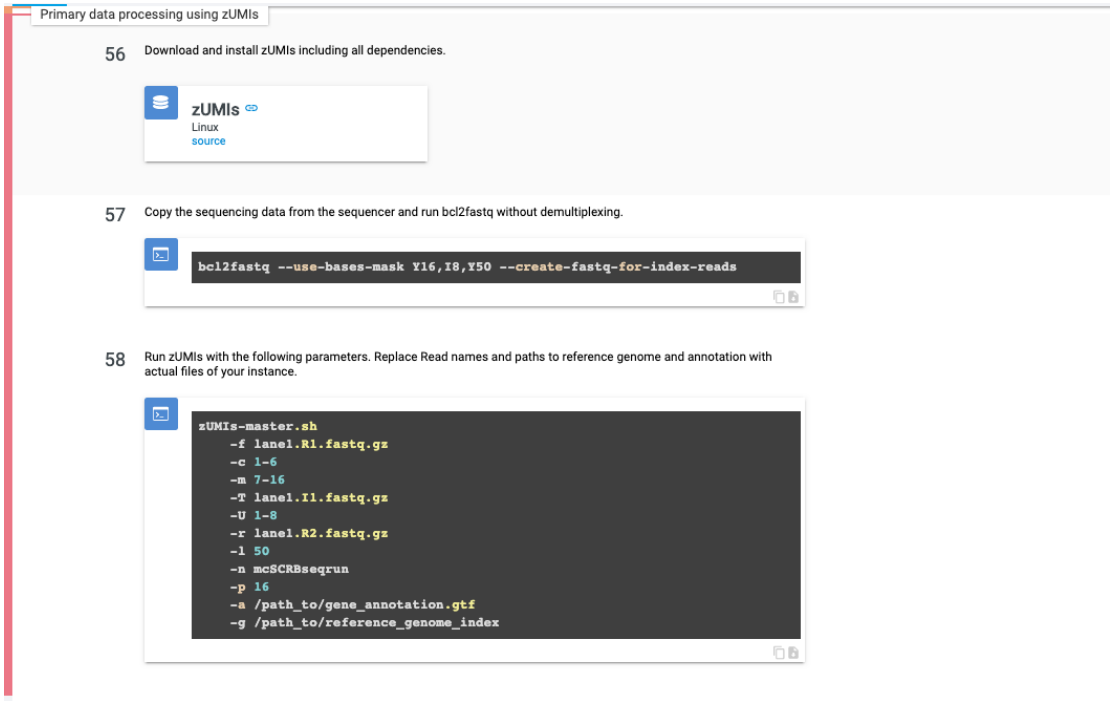
MATERIALS

NAME	CATALOG #	VENDOR
DNAse I, RNAse-free	79254	Qiagen
PBS without Ca2 or Mg2	10010-031	Gibco, ThermoFisher
Dispase	354235	Corning
Collagenase CLS I	C1-28	Biochrom AG
Elastase	20931	Serva, Germany
FBS	S 0615	Biochrom AG

STEPS MATERIALS

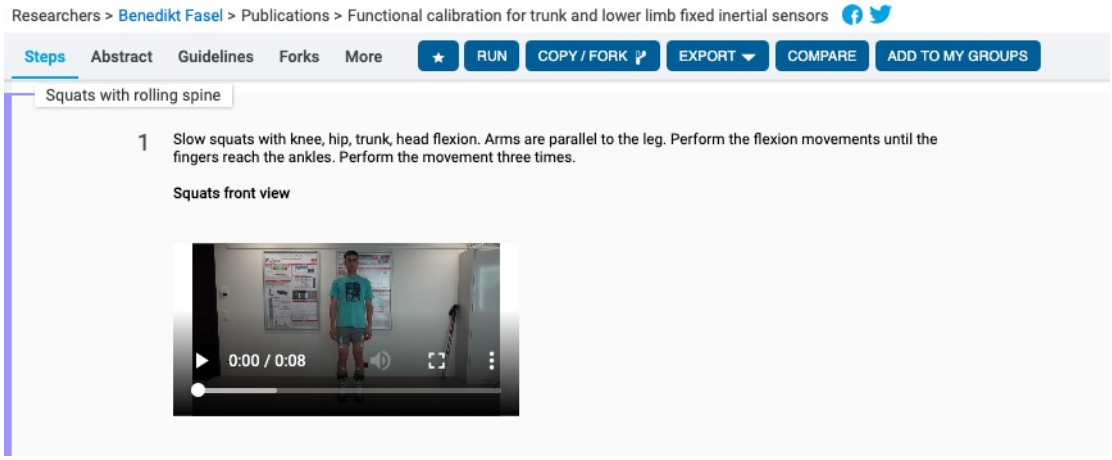
NAME	CATALOG #	VENDOR
RBC Lysis Buffer	00-4333-57	Invitrogen - Thermo Fisher

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



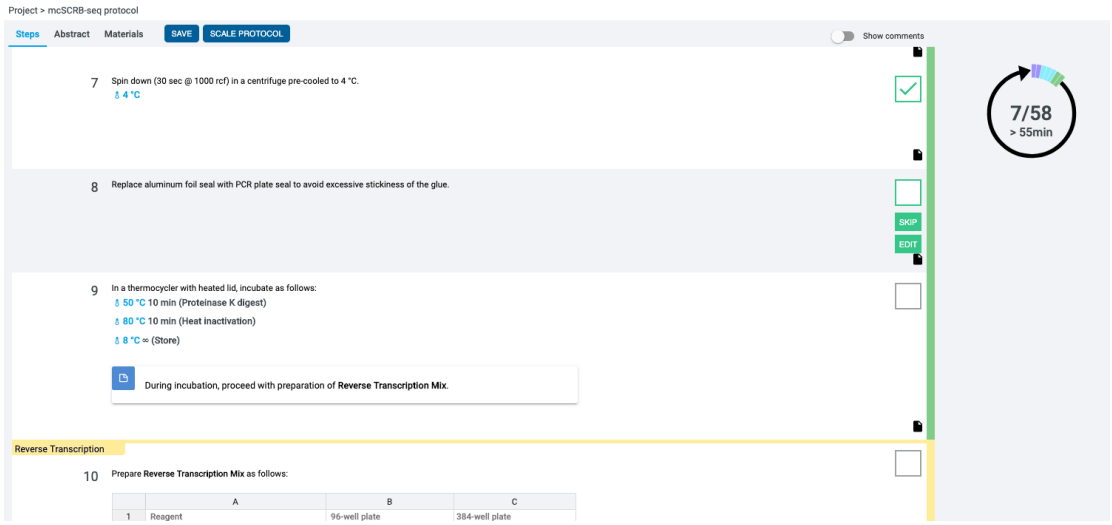
components to specify the code, version of software, and location/version of the datasets used in the analysis.

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.



Making your protocol "runnable"

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.



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:

1 Add PFA drop by drop and mix carefully into 1 ml cells for final concentration of [M]2 Mass Percent PFA .

2 Incubate cells for 00:04:00 at Room temperature .

3 Centrifuge at 1000 x g for 00:05:00 .

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

Wash

4 Wash cells with 1XPBS (1/3)

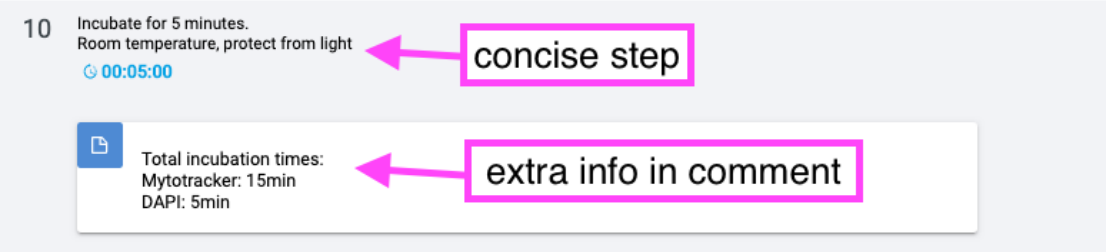
5 Wash cells with 1XPBS (2/3)

6 Wash cells with 1XPBS (3/3)

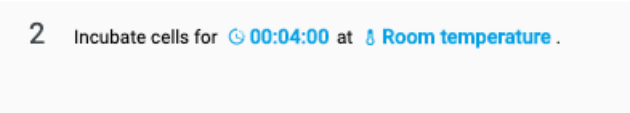
This helps when "running" the protocol. Very frequently, we lost track when repeating the same thing of how many times we've done it. Am I on wash step 2/4 or 3/4?

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

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



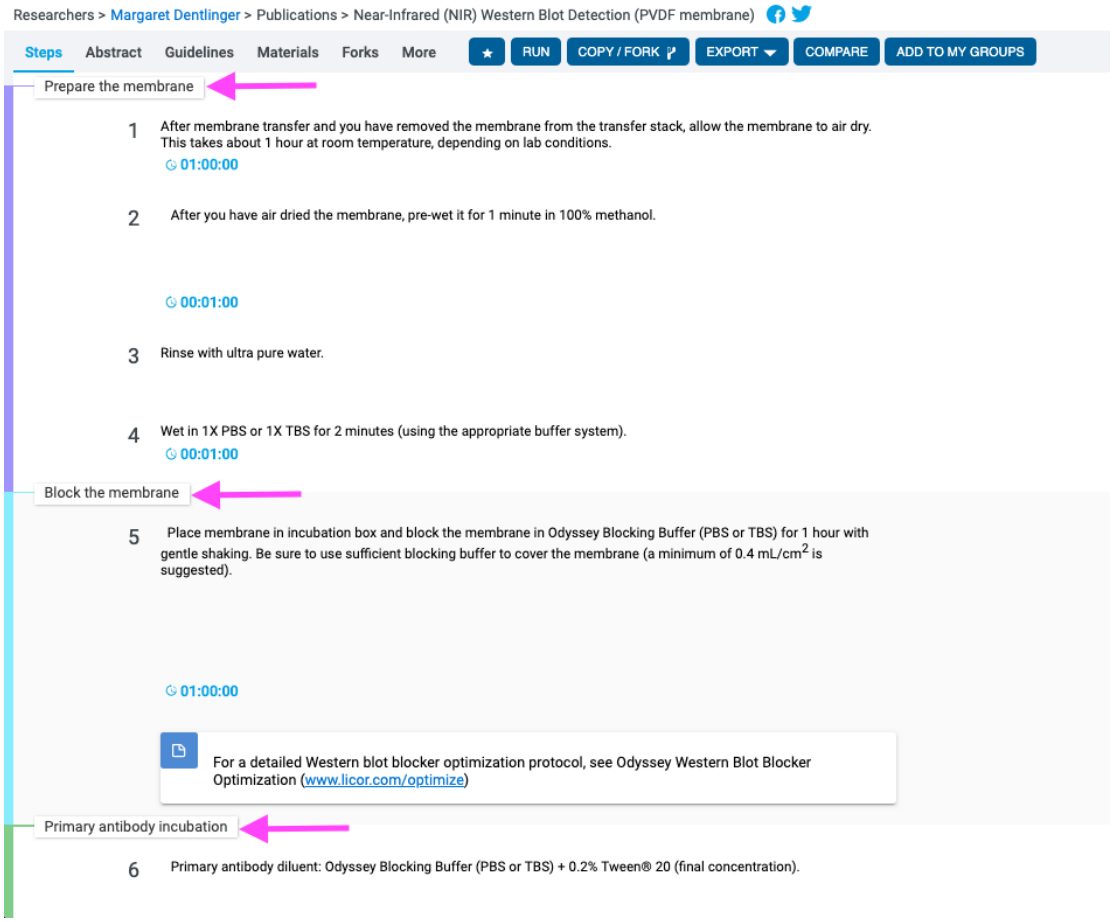
13 **Timed steps:** Use the "Duration/Timer" component in the individual steps as appropriate. This makes it possible to use the timer when "running" your protocol.



Running the protocol:

Other tips

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



15 **Guidelines, warnings, before start:** Use the "Guidelines & Warnings" tab for adding "before start" instructions, warnings. Also in "Guidelines & Warnings" tab is the "Guidelines" field for information about the method, references, tips, etc as well as an option to label the protocol as confidential or containing sensitive content.

16 **Safety:** In the "Guidelines & Warnings" tab there is a "Safety Warings" field. 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 relevants steps, "warnings", and Guidelines.

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
Functional calibration for trunk and lower limb fixed inertial sensors, Benedikt Fasel