



Version 2

May 18, 2021

How to make your protocol more reproducible, discoverable, and user-friendly V.2

Version 1 is forked from [How to make your protocol more reproducible, discoverable, and user-friendly](#)

Lenny Teytelman¹, protocols.io team¹, Anita Broellochs¹, Ayu Nagane Uchikawa²

¹protocols.io; ²EBSCO Information Services Japan

1 Works for me

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Ayu Nagane Uchikawa

ABSTRACT

protocols.io is a platform for sharing and documenting protocols. It is designed to be reproducible, discoverable, and user-friendly. This protocol describes how to use protocols.io to share your protocols.

protocols.io info [at] protocols.io

protocols.io

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Version created by [Lenny Teytelman](#)

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FORK NOTE

FORK FROM

Forked from [How to make your protocol more reproducible, discoverable, and user-friendly](#), Lenny Teytelman

KEYWORDS

protocols, help, FAQ, optimization

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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 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.

Conclusion

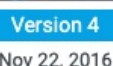
Resources such as these as well as other established protocol journals and organizations such as [JOVE](#), [Science Exchange](#), [COS](#), and [GBSI](#) are filling an important niche to ensure that the scientific community maintains its core mission of delivering reproducible results.

BEFORE STARTING

How to create a new protocol

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

1 www.google.com/protocols

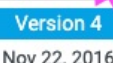


Working

Staining protocol for unicellular protists: Mitotracker and Dapi

[dx.doi.org/10.17504/protocols.io.gq5bty6](https://doi.org/10.17504/protocols.io.gq5bty6)

Protist Research to Optimize Tools in Genetics (PROT-G) Multicellgenomelab

[illegible]

Nov 22, 2016

Working

Staining protocol for unicellular protists: Mitotracker and Dapi

[dx.doi.org/10.17504/protocols.io.qg5bty6](https://doi.org/10.17504/protocols.io.qg5bty6)

Protist Research to Optimize Tools in Genetics (PROT-G) Multicellgenomelab

[illegible]

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. **Primary data processing using zUMIs**: **command** **software package** **dataset** **external link**

Primary data processing using zUMIs


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Download and install zUMIs including all dependencies.

 **zUMIs**
Linux
source

57


Copy the sequencing data from the sequencer and run bcl2fastq without demultiplexing.



```
bcl2fastq --use-bases-mask Y16,I8,Y50 --create-fastq-for-index-reads
```

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Run zUMIs with the following parameters. Replace Read names and paths to reference genome and annotation with actual files of your instance.



```
zUMIs-master.sh
-f lane1.R1.fastq.gz
-c 1-6
-m 7-16
-T lane1.I1.fastq.gz
-U 1-8
-r lane1.R2.fastq.gz
-l 50
-n mcSCRBSegrun
-p 16
-a /path_to/gene_annotation.gtf
-g /path_to/reference_genome_index
```

8. **8/8**: **8/8**

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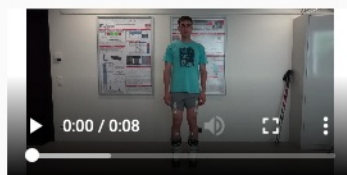
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Squats with rolling spine

- 1 Slow squats with knee, hip, trunk, head flexion. Arms are parallel to the leg. Perform the flexion movements until the fingers reach the ankles. Perform the movement three times.

Squats front view



runnable

9

Project > mcSCRB-seq protocol

Steps Abstract Materials [SAVE](#) [SCALE PROTOCOL](#) [Show comments](#)

- 7 Spin down (30 sec @ 1000 rcf) in a centrifuge pre-cooled to 4 °C.
- 8 Replace aluminum foil seal with PCR plate seal to avoid excessive stickiness of the glue.
- 9 In a thermocycler with heated lid, incubate as follows:
 - 50 °C 10 min (Proteinase K digest)
 - 80 °C 10 min (Heat inactivation)
 - 8 °C ∞ (Store)

During incubation, proceed with preparation of Reverse Transcription Mix.

Reverse Transcription

- 10 Prepare Reverse Transcription Mix as follows:

	A	B	C
1	Reagent	96-well plate	384-well plate

7/58 > 55min

10

- 1 Add PFA drop by drop and mix carefully into 1 ml cells for final concentration of 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

Wash

- 4 Wash cells with 1XPBS (1/3)
- 5 Wash cells with 1XPBS (2/3)
- 6 Wash cells with 1XPBS (3/3)

running 2/4 3/4

...

12: annotation

- 10 Incubate for 5 minutes.
Room temperature, protect from light
00:05:00

concise step

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

extra info in comment

13: Duration/Timer

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




2 Incubate cells for 00:03:54 PAUSE REFRESH at Room temperature .

14:


Steps
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Prepare the membrane

- After membrane transfer and you have removed the membrane from the transfer stack, allow the membrane to air dry. This takes about 1 hour at room temperature, depending on lab conditions.
 01:00:00
- After you have air dried the membrane, pre-wet it for 1 minute in 100% methanol.
 00:01:00
- Rinse with ultra pure water.
- Wet in 1X PBS or 1X TBS for 2 minutes (using the appropriate buffer system).
 00:01:00

Block the membrane

- Place membrane in incubation box and block the membrane in Odyssey Blocking Buffer (PBS or TBS) for 1 hour with gentle shaking. Be sure to use sufficient blocking buffer to cover the membrane (a minimum of 0.4 mL/cm² is suggested).
 01:00:00

 For a detailed Western blot blocker optimization protocol, see Odyssey Western Blot Blocker Optimization (www.licor.com/optimize)

Primary antibody incubation

- Primary antibody diluent: Odyssey Blocking Buffer (PBS or TBS) + 0.2% Tween® 20 (final concentration).

15  Guidelines & Warnings  Guidelines & Warnings  Guideline

16  Guidelines & Warnings  Safety Warings  Safety information  warnings  Guideline

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 Protocols.io 

[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](#)