Discover & share science methods
The Problem - Communication of methods

Lessons from the Cancer Reproducibility project (January 2017):

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

Perhaps the clearest finding from the project is that many papers include too few details about their methods. 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.

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…
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1. Missing method details (“contact authors” & “as reported elsewhere”)
2. Once published, impossible to correct and keep up-to-date
The Solution

✓ protocols.io: Discover & share science protocol knowledge

Discover & share science protocol knowledge
Increase the speed and reproducibility of your research
The Solution

### Staining protocol for unicellular protists: Mitotracker and Dapi

**Version 4**

**Nov 22, 2018** 14 steps

**Protist Research to Optimize Tools in Genetics (PROT-GI) | Multicel1genomelab**

**CONTACT:** MARIA RUBIO-BRITONS

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<td><strong>Step 1</strong></td>
<td></td>
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<tr>
<td>2% PFA Fixation: Add PFA drop by drop and mixing carefully into 1mL cells for a final concentration of 2% PFA.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Step 2** |  |  |  |  |
| Incubate for 5 minutes. |
| Room temperature, protect from light. |
| DURATION: 00:05:00 |

| **Step 3** |  |  |  |  |
| Centrifuge at 1000g for 5 minutes |

**Wash**

| **Step 4** |  |  |  |  |
| Wash cells with 1xPBS (1/2) |

| **Step 5** |  |  |  |  |
| Wash cells with 1xPBS (2/2) |

(Live demo if possible)
Who Needs It?

And who will pay?

protocols.io

Pharma & Biotech

Academic Community

Publishers

Vendors

$55K

$1.5m

$
GigaScience has made it its mission to move scientific publishing beyond static unreproducible journal articles. Working with Protocols.io has fit perfectly into these goals, allowing integration of our data publishing workflows with their expert handling and presentation of the crucial methodological details supporting the production and use of this data. Helping our papers become living, interactive research objects, protocols.io empowers our readers to much better understand, reproduce and build upon our published research.

Scott Edmunds - Ph.D., Executive Editor, GigaScience
Public groups on protocols.io are always free. Organize, discuss, and collaborate privately with one of the plans below.

**Academic/Non-Profit/Early-Stage Startup**
- Free
  - 15 GB of storage per user
  - User level permissions control
  - Security and privacy
  - Basic Support

**Organization**
- $10 per user/month
  - Starting at $35/month (includes the first 5 users).
  - 25 GB of storage per user
  - User level permissions control
  - Premium Customer support
  - Security and privacy
  - Support for initial set-up

**Enterprise**
- Contact us
  - On demand custom collaborative features
  - Unlimited storage
  - User level permissions control
  - Private cloud hosting
  - Security and privacy
  - Premium Customer support
  - Support for the implementation
Researchers

Free

Business Model - Analytics

$50b (annual reagent purchases for experimental research)

protocols.io

Researchers

Free

Vendors

Subscription fee to:

- connect to & engage with customers
- access analytics
- feature new products
- improve product manuals
Traction (adoption)

- Public protocols: 1,385
- Registered scientists: 16,000
- Private protocols: 2,500
- Groups/communities: 245

Public protocols on protocols.io versus Nature Protocol Exchange

![Bar chart showing adoption over time](chart.png)
Traction (partnerships)

Total reagent vendor partners

- 7/1/2014
- 10/1/2014
- 1/1/2015
- 4/1/2015
- 7/1/2015
- 10/1/2015
- 1/1/2016
- 4/2016
- 7/1/2016
- 10/1/2016
Management

Alexei Stoliartchouk, CTO
CNET, Yahoo, thisMoment

Irina Makkaveeva, CFO
MBA from UCLA

Lenny Teytelman, CEO
Ph.D. UC Berkeley, postdoc MIT

Team

Engineers:
Nick Gulev
Julia Kurnosova
Sergey Alekseev
Vladimir Frolov

UI Designer:
Denise Ting

Partnerships:
Anjuli Manche
protocols.io institutional round

$3MM Series A: Use of proceeds/goals
• Grow public protocol repository (to over 3000 protocols)
• Scale revenue (10 more analytic subscriptions; 10 more groups)
• User acquisition: 30,000 user target
• Hire director of sales
• Hire director of publisher partnerships

Funding Sources to Date:
Seed, angels & VC (convertible notes): $825K
Kickstarter: $55K
Grants (nondilutive): $1.5m

Contact information
site: protocols.io
e-mail: lenny@protocols.io
twitter: @protocolsio
Below are backup slides, in case live demo fails
ECOGE Omics Training: 1.0 Unix and Bioinformatics - Version 4

Aug 18, 2015   53 steps

BENJAMIN TULLY AND KEN YOUGS CLARK, EARTH CUBE OCEANOGRAPHY AND GEOLOGY ENVIRONMENTAL OMICS

CONTACT: ELISHA WOOD-CHARLSON

The Start

Step 1
Open terminal window

Step 2
Use ls to list items in the current directory.

Step 3
Many commands have additional options that can be set by a `-`

Directory System

Step 4
cd - change directory

Step 5
pwd (present working directory) can be used to show the current directory.
Super simple In vivo Hoechst staining of unicellular protists

Nov 22, 2019 | 5 steps

CONTACT: MARIA RUBIO-BRONTOS

Step 1
Add 1/1000 Hoechst dye (1 µL into 1 mL volume of cells). Keep cells with dye protected from light.

Step 1
Add 1/1000 Hoechst dye (1 µL into 1 mL volume of cells). Keep cells with dye protected from light.

Step 2
Incubate at room temperature for 10 minutes. Keep cells with dye protected from light.

Step 3
Centrifuge cells at 1000g for 5 minutes

Step 4
Resuspend with fresh growing medium. Use minimum volume of medium in order to plate in to the slide immediately

Step 5
Mount your slide and ready for observation under microscope
Super simple In vivo Hoechst staining of unicellular protists

Nov 22, 2016  5 steps

CONTACT: MARIA RUBIO-BROTOS

Step 1 Add 1/1000 Hoechst dye (1 μL into 1 mL volume of cells). Keep cells with dye protected from light.

Step 2 Incubate at room temperature for 10 minutes. Keep cells with dye protected from light.

Step 3 Centrifuge cells at 1000g for 6 minutes

Step 4 Resuspend with fresh growing medium. Use minimum volume of medium in order to plate in to the slide immediately

Step 5 Mount your slide and ready for observation under microscope
Super simple In vivo Hoechst staining of unicellular protists

Nov 22, 2016  5 steps

CONTACT: MARIA RUBIO-BROTOS

---

**Step 1**
Add 1/1000 Hoechst dye (1 UL into 1 mL volume of cells). Keep cells with dye protected from light.

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Resuspend with fresh growing medium. Use minimum volume of medium in order to plate in to the slide immediately

**Step 5**
Mount your slide and ready for observation under microscope
Resuspension Buffer P1

Oct. 12, 2015  5 steps

Qiagen, Matthew Sullivan Lab, University of Arizona, The Ohio State University

CONTACT: Bonnie Poulos

Steps

Step 1  Dissolve 6.06g Tris Base in 800 mL MilliQ water

Step 2  Add 3.72g EDTA disodium salt, dihydrate to the 800 mL Tris base and stir to dissolve

Step 3  Adjust the pH to 8.0 with HCl

Step 4  Adjust the volume to 1 liter with MilliQ water

Reagents

Tris Base
by Fisher Scientific
Catalog #: BP152-1

Annotations

Add new

Chris Upton  Oct. 12, 2015 12:40
Isn't this usually made from stock Tris and EDTA solutions?

Bonnie Poulos  Oct. 12, 2015 13:42
It can be made either way depending on what you have available in the lab. Some find it easier to use stock solutions, but it is not necessary as long as final pH is adjusted.
High quality DNA from Fungi for long read sequencing e.g. PacBio

There is a newer version of this protocol available.

Apr 19, 2016 40 steps

BENJAMIN SCHWESSINGER, ANU

CONTACT: BENJAMIN SCHWESSINGER

View 4 comments on prior versions of this protocol

BENJAMIN SCHWESSINGER Apr 20, 2016 22:40

The sequencing part of the project was performed at the Ramaciotti Centre in Sydney (http://www.ramaciotti.unsw.edu.au/) and supported financially by Bioplatforms Australia (http://www.bioplatforms.com/).

reply

THANH LAM Apr 21, 2016 08:13

Hi Benjamin,

Thank you for sharing your protocol here. May I ask what PVP 1% solution is? Is it Polyvinylpyrrolidinone (PVP)?

reply

BENJAMIN SCHWESSINGER Apr 24, 2016 00:52

Apologies for being unspecific, I will fix this in the final version.

PVP is Polyvinylpyrrolidinone (PVP-20K) and you can get it from Sigma http://www.sigmaaldrich.com/catalog/product/sig/104791
Extraction I

Step 1
Mix lysis buffer by vortexing and briefly head to 64 °C. Let cool to room temperature for use in 50mL Falcon tubes.

Step 2
Add 10uL (100uL) RNase T1 to lysis buffer.

Step 3
Grind tissue/spores with liquid nitrogen in a mortar with sand, use 1g of sand per 100mg of starting material, grind for...

Step 4
Transfer powder to 50mL Falcon containing lysis buffer and RNase, mix well by vortexing.

Step 5
Incubate at RT for 30 mins mixing by inversion every 5 mins.
**High quality DNA from Fungi for long read sequencing e.g. PacBio**

April 28, 2018 - 40 steps

doi.org/10.137504/protocols.io.xv6bfm

**BENJAMIN SCHWEISSINGER, ANU**

MinION user group with fungi and plants on their mind

**CONTACT:** BENJAMIN SCHWEISSINGER

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**Extraction I**

**Step 1**
Make lysis buffer by mixing buffer A+B+C (1.25:2.5:1 + 0.1%PVP final) and briefly heat to 64 °C. Let cool to room temperature... read more

**Step 2**
Add 10uL (10KU) RNase T1 to lysis buffer

**Step 3**
Grind tissue/spores with liquid nitrogen in a mortar with sand, use 1g of sand per 100mg of starting material. Grind for... read more

**Step 4**
Transfer powder to 50mL Falcon containing lysis buffer and RNase, mix well by vortexing

**Step 5**
Incubate at RT for 30mins mixing by inversion every 5 mins
High quality DNA from Fungi for long read sequencing e.g. PacBio

**Step 1**

**Step 1**

Make lysis buffer by mixing buffer A+B+C (2.5:2.5:1 + 0.1% PVP final) and briefly heat to 64 °C. Let cool to room temperature for use in 80mL Falcon tubes.

Annex all following steps are based on 17.5ml lysis buffer as starting volume.

**Step 2**

Add 10uL (10kU) RNase T1 to lysis buffer
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
| 2    | DESCRIPTION (changed)  
add 10uL (10kU) RNase A to lysis buffer |
| 3    |             |
| 4    |             |
| 5    |             |
| 6    |             |
| 7    |             |
| 8    |             |