protocols.io

Social network platforms have a “cold start” problem. For science efforts, it’s the North Pole.

Image credit:Ice near the North Pole, Christopher Michel, CC-BY 2.0

Lenny Teytelman

lenny@protocols.io

@lteytelman

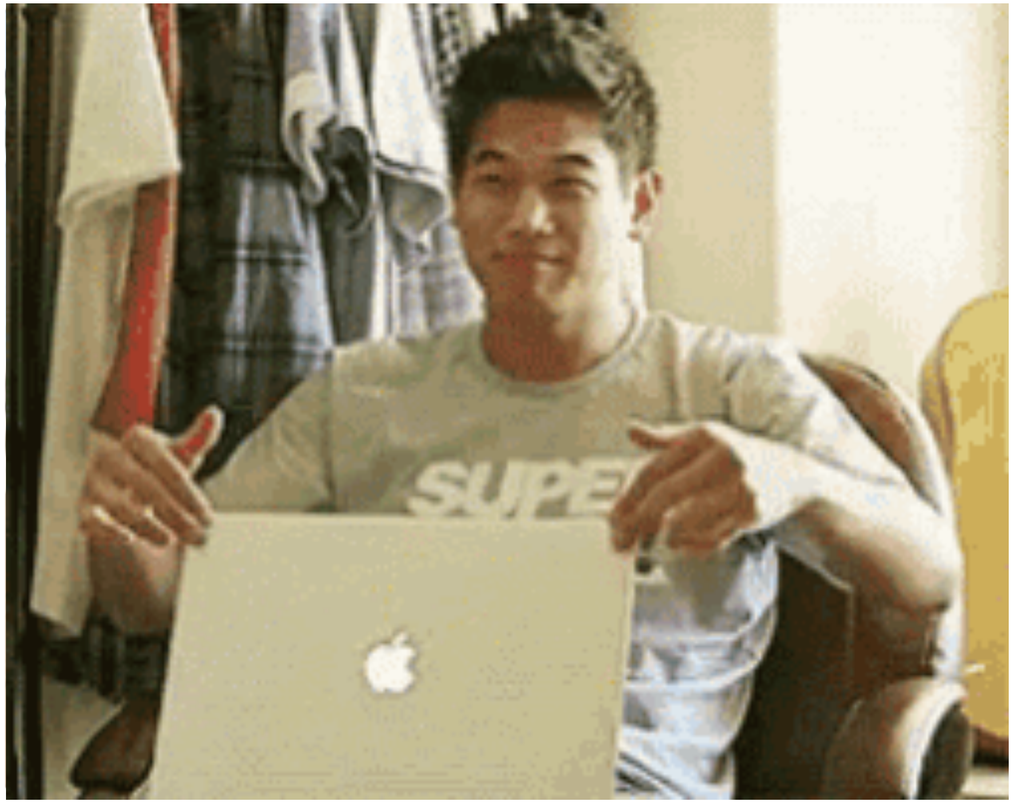
Talk outline

- Quick intro to protocols.io
- The “cold start” problem
- What we tried **before** launch
- The brutal adoption numbers at protocols.io
- What we tried **after** launch
- Lessons learned

Morgan Halane
@themorgantrail

Follow

Looking for protocol in 1997 paper: "as described in (x) et al '96". Finds '96 paper: "as described in (x) '87." Finds '87 paper: Paywall.



9:20 PM - 1 Nov 2017 from Pohang-si, Republic of Korea

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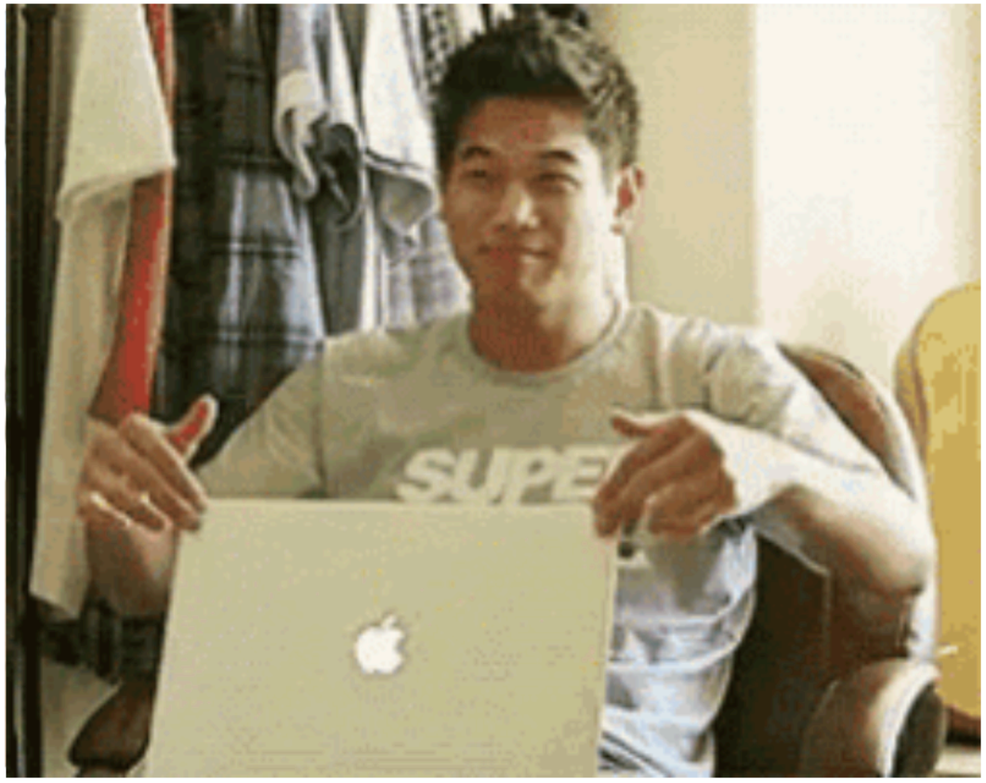
Morgan Halane
@themorgantrail

Follow

Looking for protocol in 1997 paper: "as described in (x) et al '96". Finds '96 paper: "as described in (x) '87." Finds '87 paper: Paywall.



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Daniel Gonzales
@dgonzales1990

Follow

2017: “Devices were fabricated as previously described [ref 8]”

[ref 8] 2015: “Devices were fabricated as previously described [ref 4]”

[ref 4] 2013: “Devices were fabricated as previously described [ref 2]”

[ref 2] 2009: “Devices were fabricated with conventional methods”

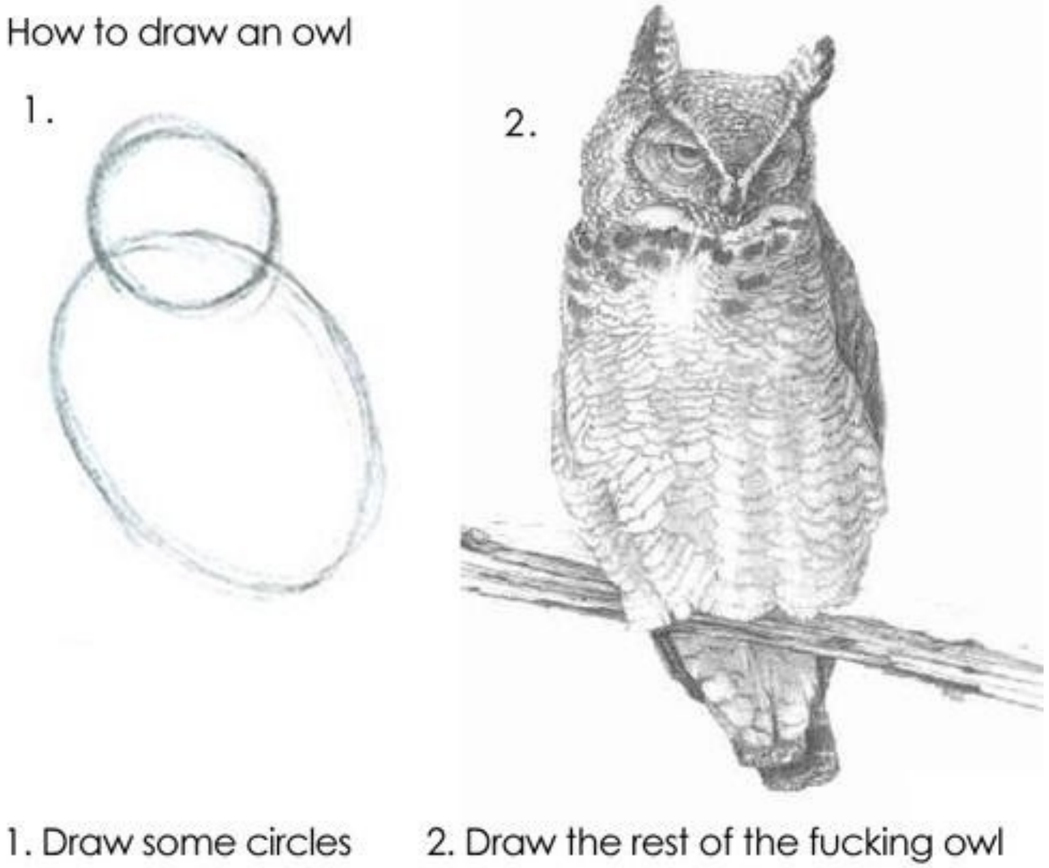
1:16 PM - 17 Jan 2018

231 Retweets **800** Likes

29 231 800

Timothée Poisot Follow
Ecologist. Not that kind of doctor.
Sep 8, 2015 · 2 min read

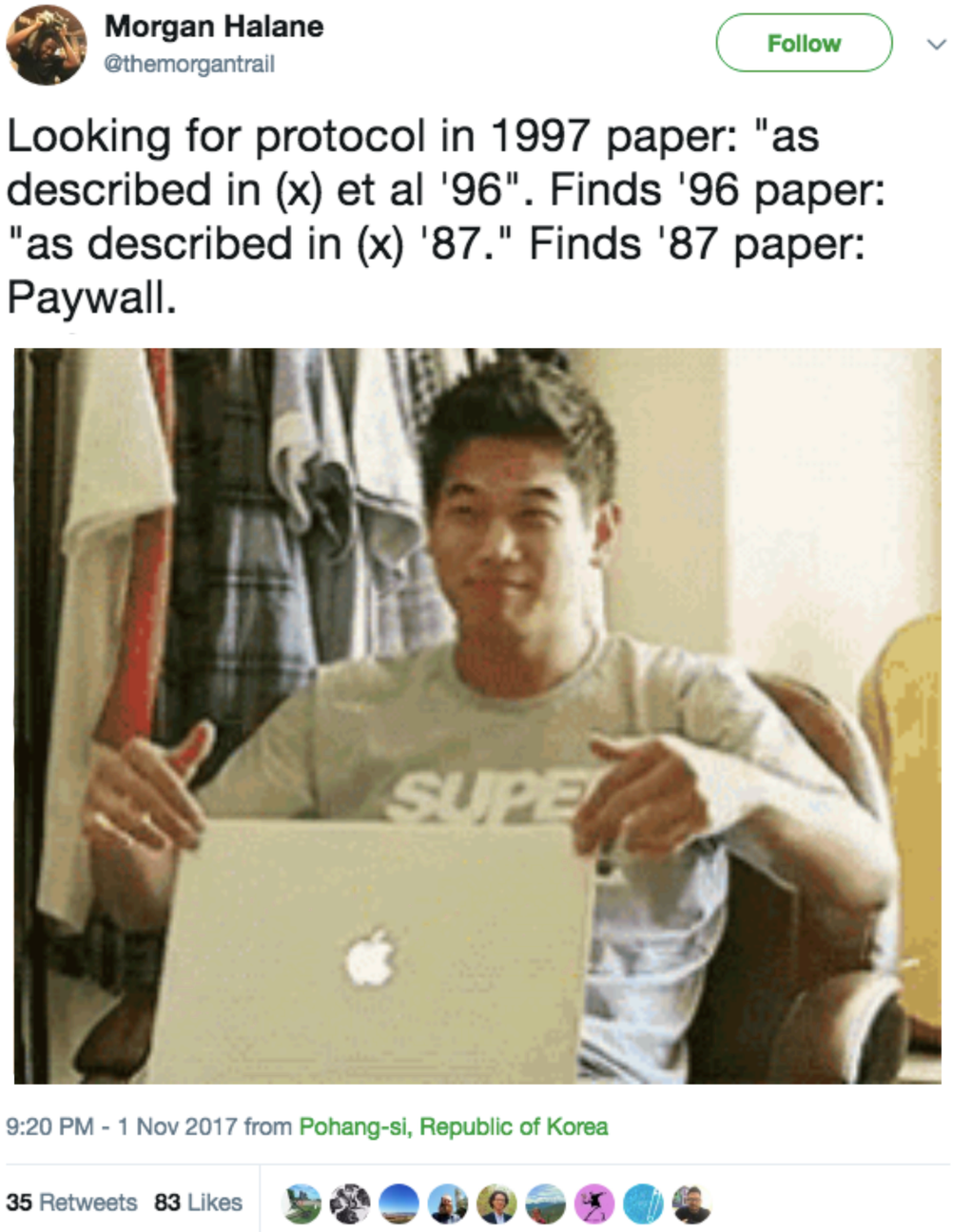
Step 2—do the rest of the fucking analysis



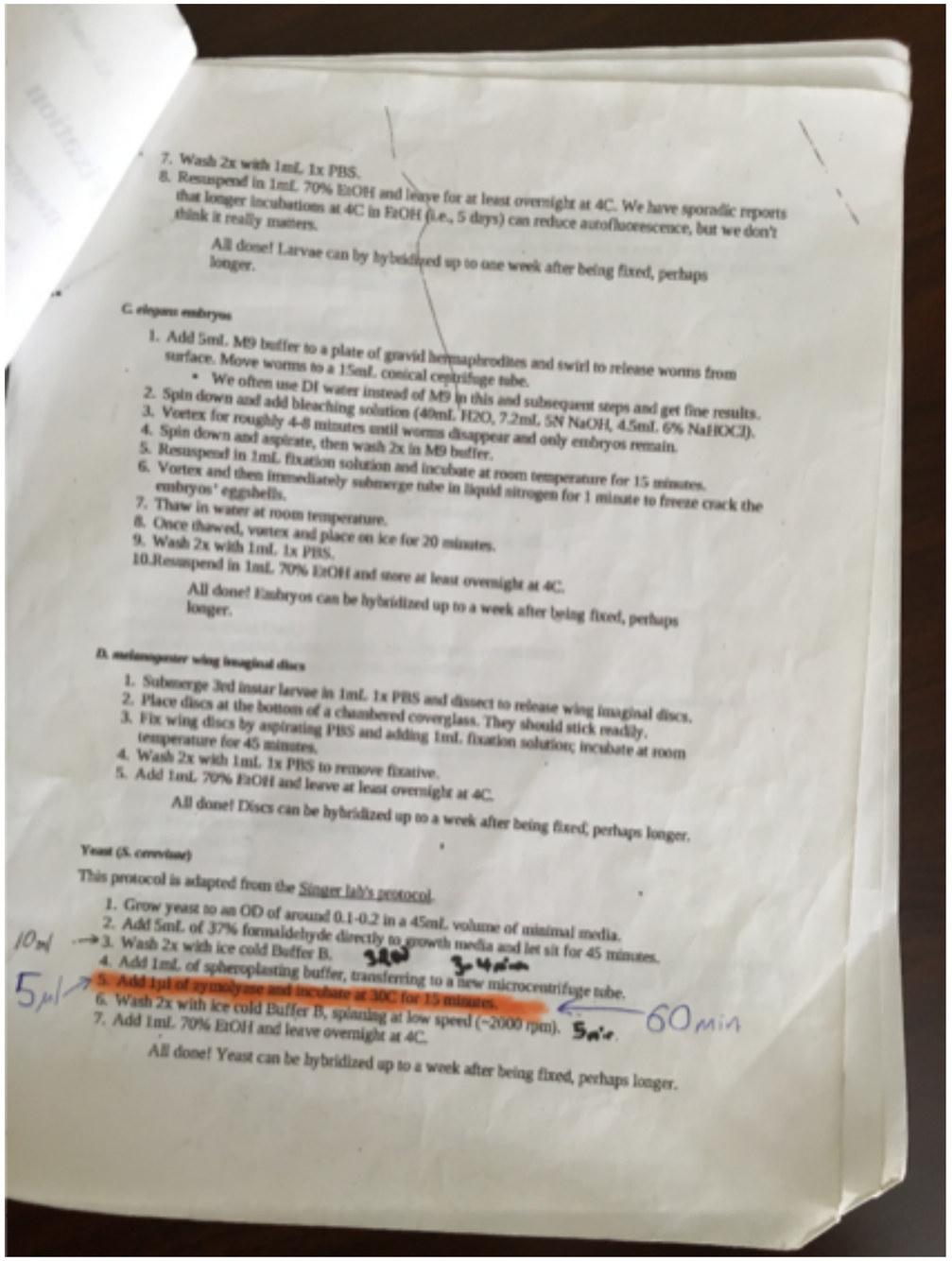
So when starting a new research project, one can feel like one is trying to draw an owl using the above tutorial. This is because we tend to learn about methods by reading papers, and the Methods section of any given paper is often, to put it mildly, *terse*. To pursue the *How to draw an owl* analogy, a Methods section could read

We draw the owl on 60.2 gsm white paper of the A4 dimension (210mm by 297mm), using 3H and 6B graphite pencils (Derwent, Cumbria, UK). We did so by looking at owls, and drawing what we saw on paper. This protocol yielded one drawn owl.

https://medium.com/@tpoi/do-the-rest-of-the-fucking-analysis-8fcef22fd991



Mission: to make it easy to share method details before, during, and after publication.



7. Wash 2x with 1mL 1x PBS.
8. Resuspend in 1mL 70% EtOH and leave for at least overnight at 4C. We have sporadic reports that longer incubations at 4C in EtOH (i.e., 5 days) can reduce autofluorescence, but we don't think it really matters.

All done! Larvae can by hybridized up to one week after being fixed, perhaps longer.

C. elegans embryos

1. Add 5mL M9 buffer to a plate of gravid hermaphrodites and swirl to release worms from surface. Move worms to a 15mL conical centrifuge tube.
   - We often use DI water instead of M9 in this and subsequent steps and get fine results.
2. Spin down and add bleaching solution (40mL H2O, 7.2mL 5N NaOH, 4.5mL 6% NaHOCl).
3. Vortex for roughly 4-8 minutes until worms disappear and only embryos remain.
4. Spin down and aspirate, then wash 2x in M9 buffer.
5. Resuspend in 1mL fixation solution and incubate at room temperature for 15 minutes.
6. Vortex and then immediately submerge tube in liquid nitrogen for 1 minute to freeze crack the embryos' eggshells.
7. Thaw in water at room temperature.
8. Once thawed, vortex and place on ice for 20 minutes.
9. Wash 2x with 1mL 1x PBS.
10. Resuspend in 1mL 70% EtOH and store at least overnight at 4C.

All done! Embryos can be hybridized up to a week after being fixed, perhaps longer.

D. melanogaster wing imaginal discs

1. Submerge 3rd instar larvae in 1mL 1x PBS and dissect to release wing imaginal discs.
2. Place discs at the bottom of a chambered coverglass. They should stick readily.
3. Fix wing discs by aspirating PBS and adding 1mL fixation solution; incubate at room temperature for 45 minutes.
4. Wash 2x with 1mL 1x PBS to remove fixative.
5. Add 1mL 70% EtOH and leave at least overnight at 4C.

All done! Discs can be hybridized up to a week after being fixed; perhaps longer.

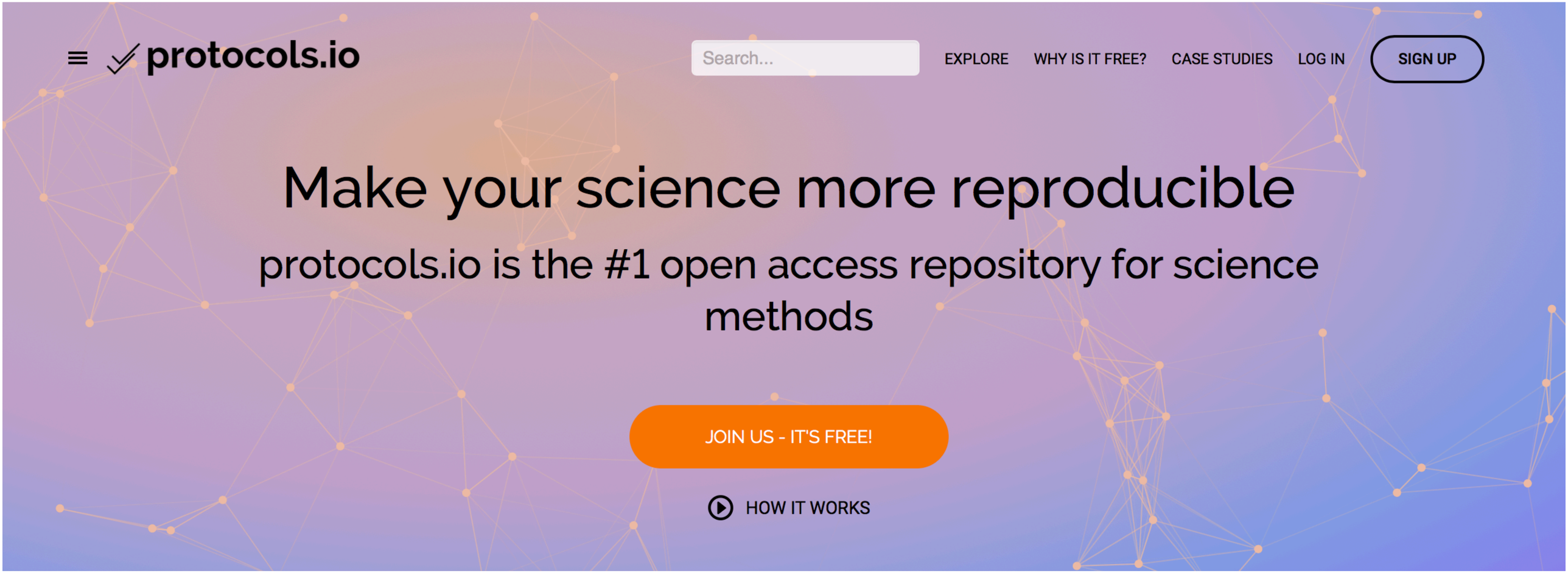
Yeast (*S. cerevisiae*)

This protocol is adapted from the Singer lab's protocol.

1. Grow yeast to an OD of around 0.1-0.2 in a 45mL volume of minimal media.
2. Add 5mL of 37% formaldehyde directly to growth media and let sit for 45 minutes.
3. Wash 2x with ice cold Buffer B.
4. Add 1mL of spheroplasting buffer, transferring to a new microcentrifuge tube.
5. Add 1µl of zymolyase and incubate at 30C for 15 minutes.
6. Wash 2x with ice cold Buffer B, spinning at low speed (~2000 rpm).
7. Add 1mL 70% EtOH and leave overnight at 4C.

All done! Yeast can be hybridized up to a week after being fixed, perhaps longer.

Mission: to make it easy to share method details before, during, and **after** publication.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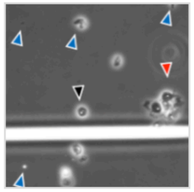


Nuclei isolation from human kidney for single-nucleus RNA-seq

Ben Humphreys [1], Yuhei Kirita [2]

[1] Washington University in St. Louis, [2] The Humphreys Lab

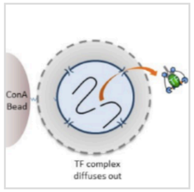
Oct 05, 2018

Working

Human Cell Atlas Method Development Community

CONTACT
Ben Humphreys

122 views 2 bookmarks 3 comments ...



CUT&RUN: Targeted in situ genome-wide profiling with high efficiency for low cell numbers

Peter J. Skene [1], Steven Henikoff [1]

[1] Howard Hughes Medical Institute, Basic Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, Washington, USA 98109

Version 1

Jan 16, 2018

Working

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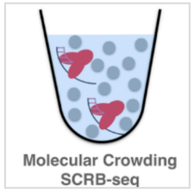
CONTACT
Steven Henikoff

2,569 views 24 bookmarks 17 comments 14 forks ...



mcSCRB-seq protocol

Nature Communications

Johannes Bagnoli [1], Christoph Ziegenhain [1], Aleksandar Janjic [1], Lucas Esteban Wange [1], Beate Vieth [1], Swati Parekh [1], Johanna Geuder [1], Ines Hellmann [1], Wolfgang Enard [1]

[1] Ludwig-Maximilians-Universität München

Version 2

May 22, 2018

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CONTACT
Aleksandar Janjic

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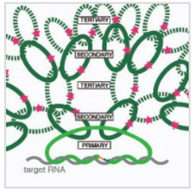


ClampFISH

Sara H Rouhanifard [1], Ian A Mellis [1], Margaret Dunagin [1], Sareh Bayatpour [1], Orsolya Symmons [1], Allison Cote [1], Arjun Raj [1]

[1] University of Pennsylvania

May 31, 2018

Working

Human Cell Atlas Method Development Community

CONTACT
Sara Rouhanifard

Version 2

May 24, 2017

Working

RNA (and optional DNA) extraction from environmental samples (filters)

Sarah Hu [1]

[1] University of Southern California

dx.doi.org/10.17504/protocols.io.hk3b4yn

Caron Lab - Protistan Ecology , SCOPE view 1 more group

Sarah Hu
University of Southern Califor...

BEFORE STARTING

- Please read through all of the warnings, steps, and considerations for Qiagen extraction kit
- Ensure that Buffer RLT+ (or RLT) has beta mercaptoethanol added, as per the Qiagen instructions
- Consider filter type (or however you are collecting cellular material) and compatibility to reagents (RLT+ buffer)

Lysis steps

1 Take frozen tubes out of the -80C freezer, keep on ice.

See other Caron lab protocol for collecting environmental samples!

2
1. While filters are thawing, add RNase/DNase-free* 0.5mm Silica beads to each sample tube
2. If RLT+ buffer (with beta mercaptoethanol) was not added previously, add it here.

*Clean your silica beads

Dear Protocol Author,

Sarah Hu

Ask questions, make suggestions for improvements, or share your own experiences with this protocol.

private comment POST

All (2) Step-level Protocol-level (2)

Evina Gontikaki Oct 3, 2018 07:44 AM

thank you for sharing your protocol! I just needed to ask if you've ever succeeded in getting DNA and RNA using this protocol with smaller sample sizes e.g. filtration of approx. 100 ml of seawater. Thank you.

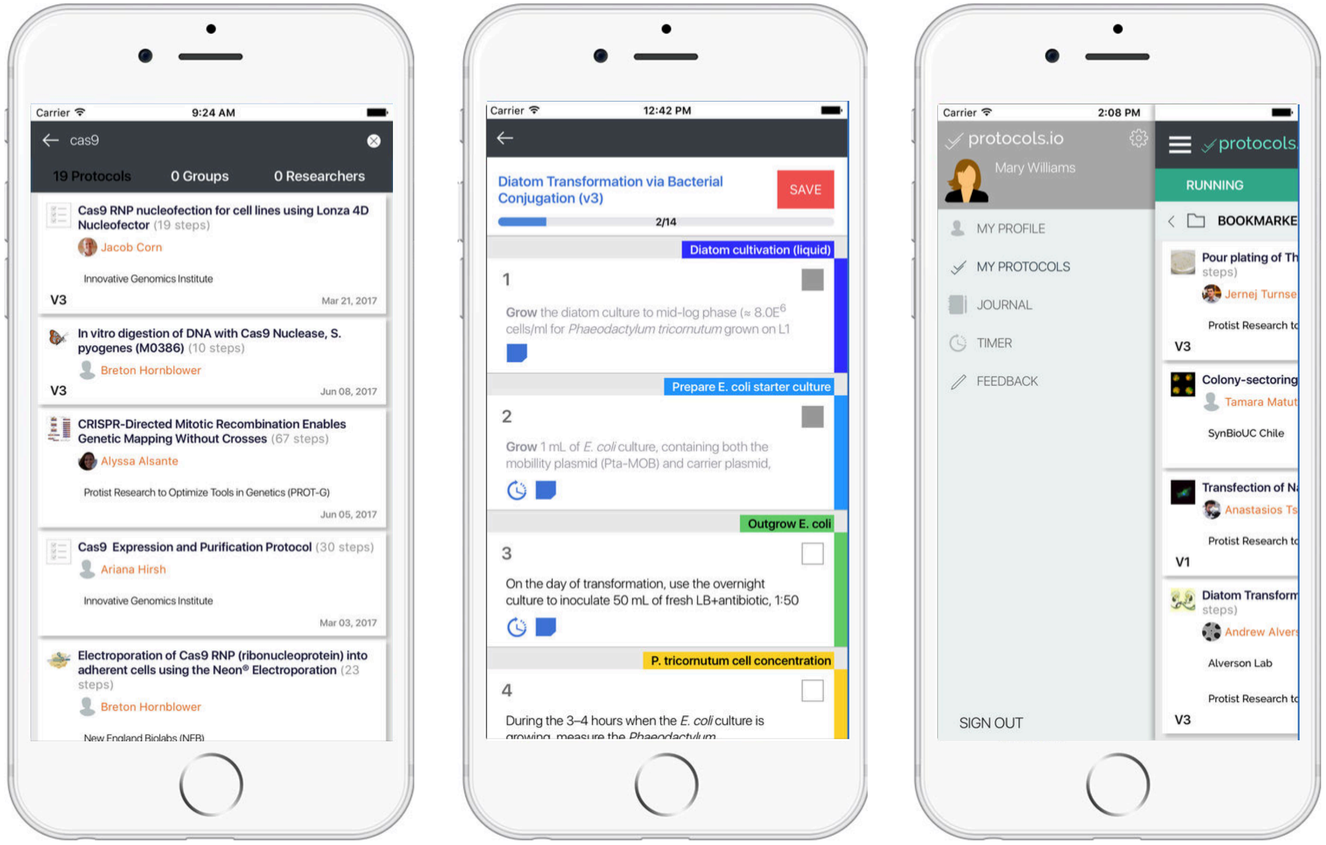
REPLY

Hide reply

Sarah Hu Oct 3, 2018 12:13 PM

University of Southern California

Hi Evina, It really depends on what organisms and what the concentration of those organisms is in that 100 mL of seawater. Often 100 mL isn't enough volume to get a good representative sample from seawater (my own experience).
That said, when I've worked with very concentrated samples o high biomass (i.e. the filter has a significant amount of color), 100



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The Cold Start Problem

How to solve the cold-start problem for social products

Social products need mass before scaling growth
I often write on the topic of how social products can scale growth, resulting in inbound emails to the effect of "how do I get my product to go viral?" The problem is, until you have a strong baseline of engagement, it's nearly impossible to have a metrics-oriented discussion on growth and virality. So you have to get that first, before you can talk about the next step.

http://andrewchen.co/how-to-solve-the-cold-start-problem-for-social-products/

3 Ways To Solve Your Startup's Cold Start Problems

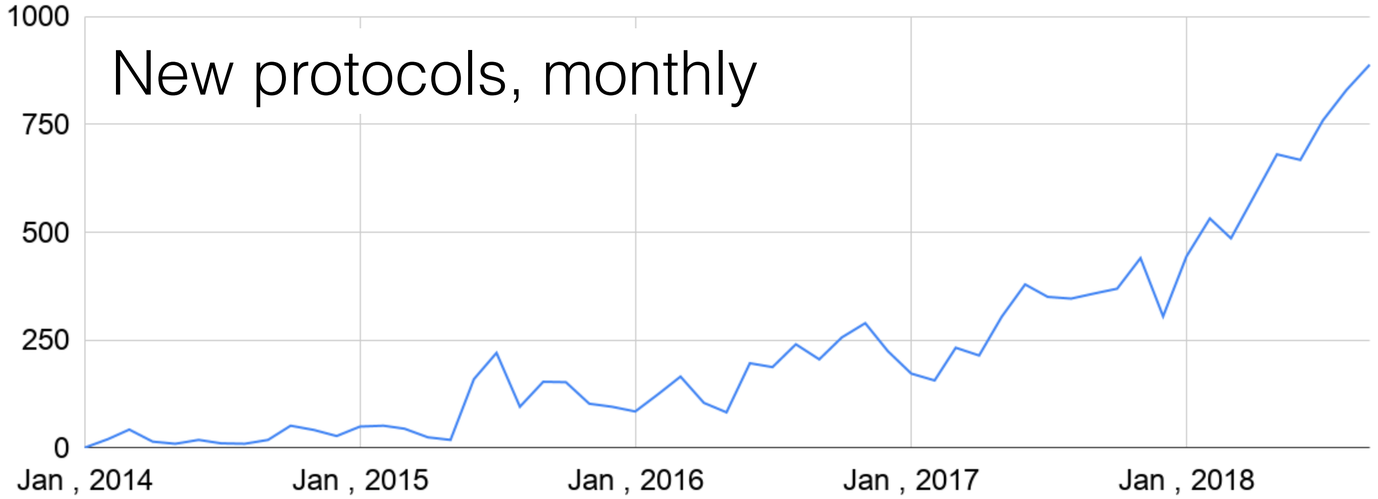
Doug Mallette

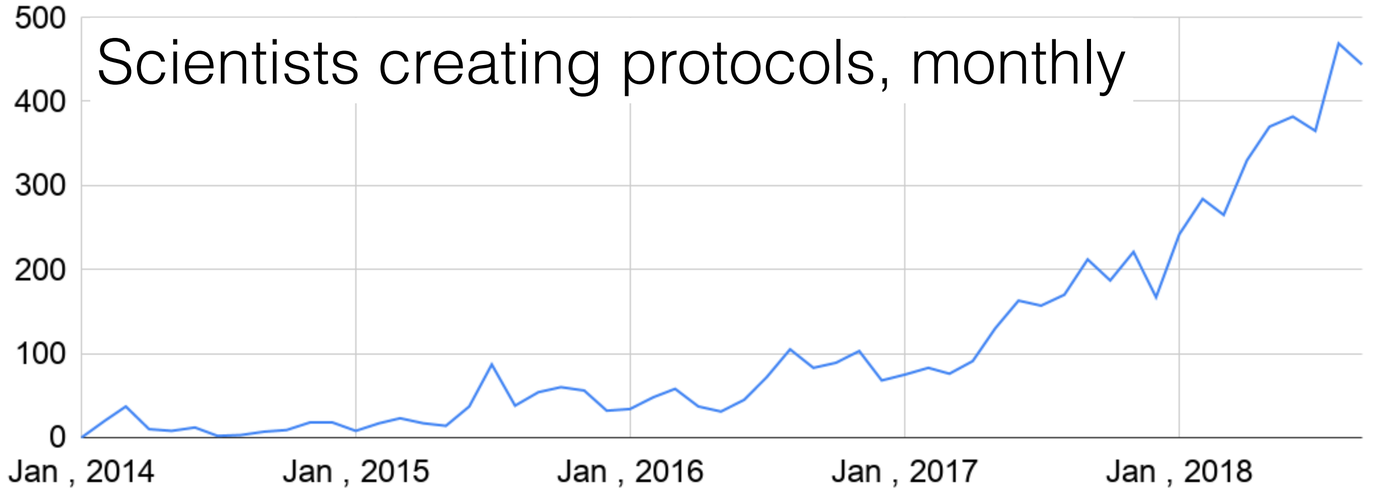
SEO Marketer / Copywriter for Neon Roots

Cold start problems affect all startups, big or small, but it's how these companies address these issues that ultimately determine if they sink or swim. Without brand recognition on your side, you're basically launching to an empty room. It's daunting, but there are ways to overcome cold start and actually come out with substantial heat behind your new business venture.

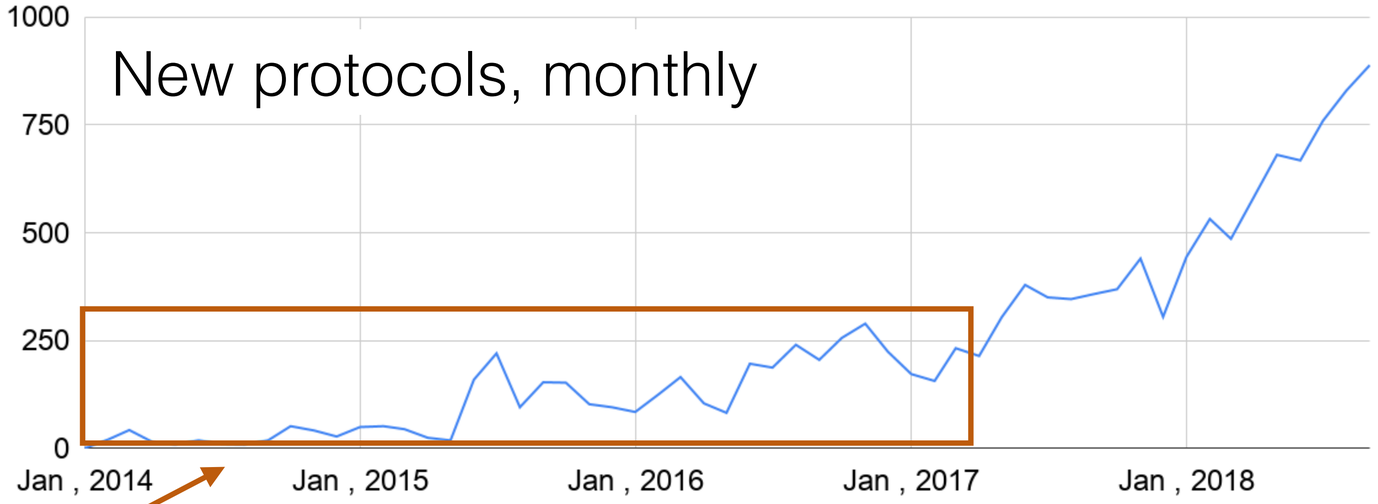
https://www.neonroots.com/blog/3-ways-to-solve-your-startups-cold-start-problems/

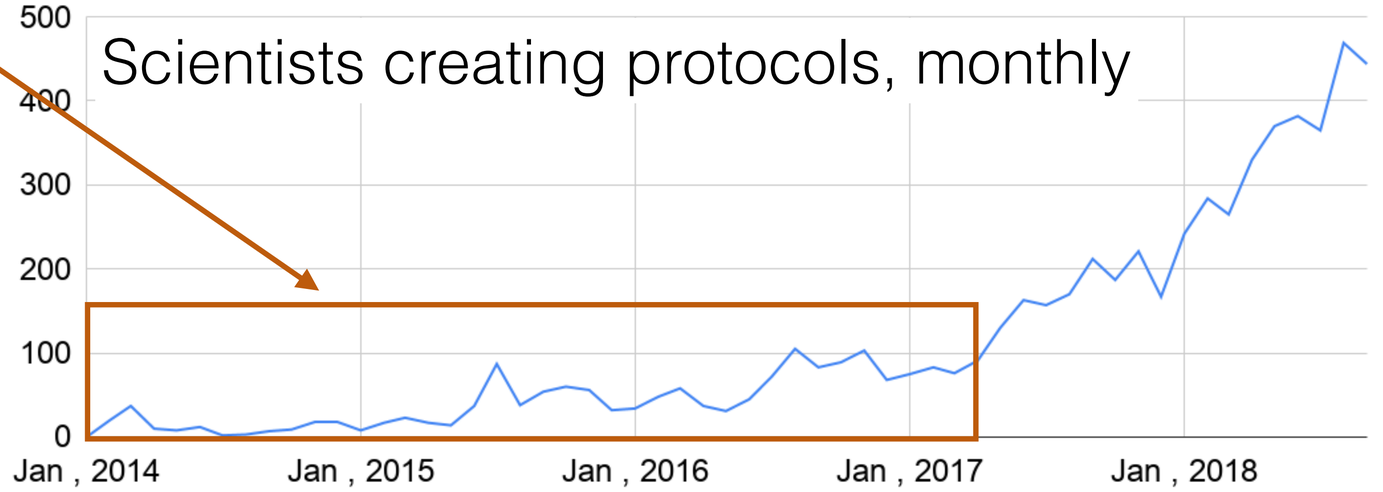
The long, freezing phase





The long, freezing phase





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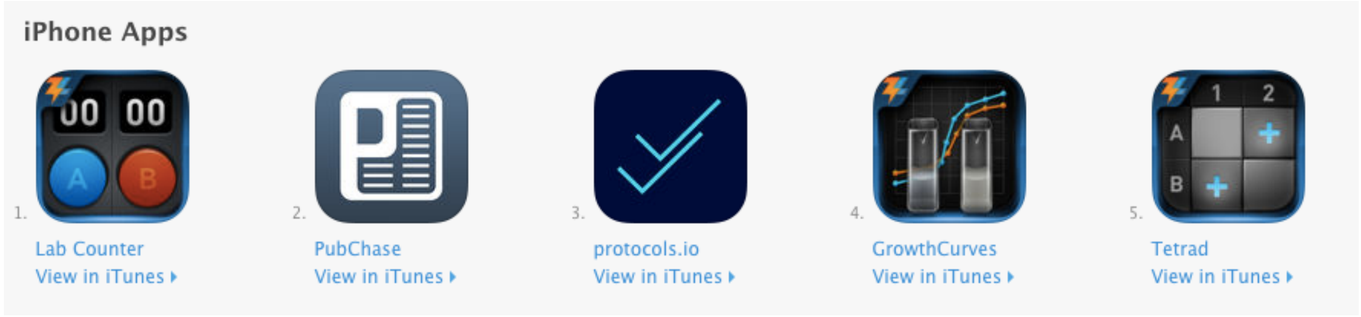
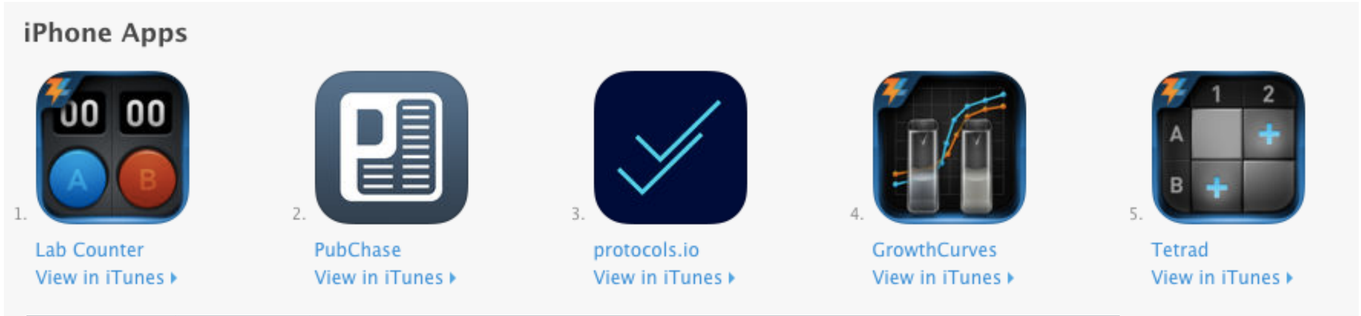
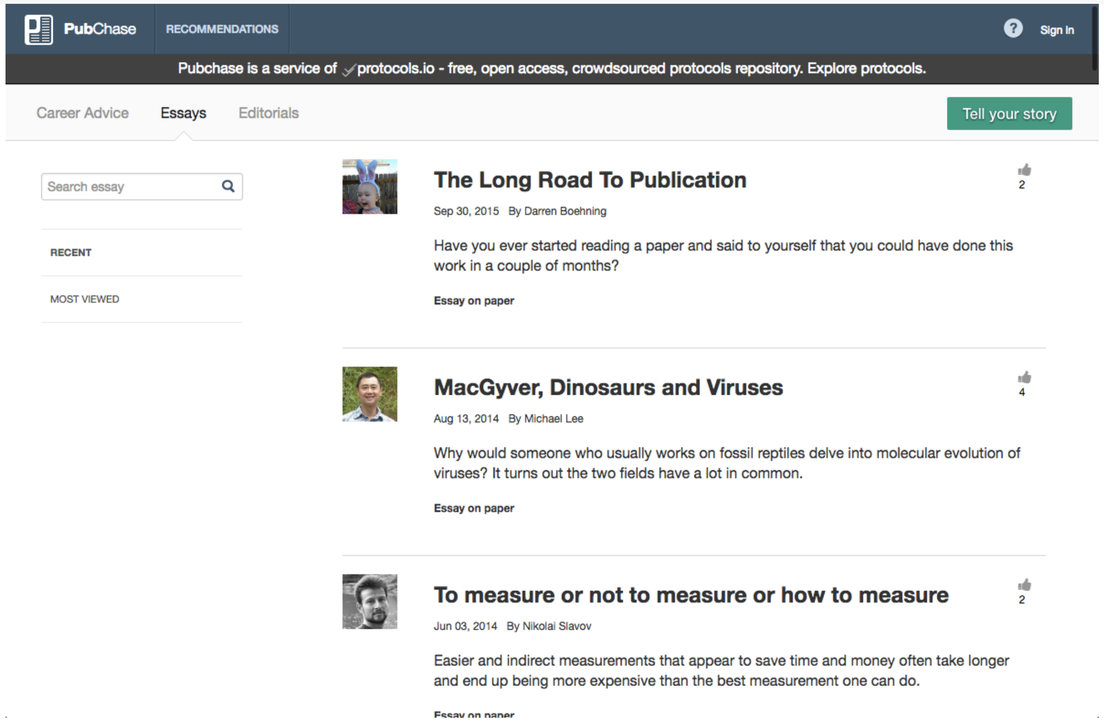
Building the crowd, pre-launch



How we tried to melt the ice





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How we tried to melt the ice

iPhone Apps

1. Lab Counter
View in iTune

PubChase

Career Advice

Search essay

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MOST VIEWED

The Spectroscope is a service of protocols.io - free, open access, crowdsourced protocols repository. Explore protocols.

THE SPECTROSCOPE

Search

RECENT STORIES

bioRxiv, the Chan-Zuckerberg Initiative, #ASAPbio, and scooping

If you've been accused of sexual harassment, maybe don't sing about how wonderful it is to be a woman in science?

A reproducible effect: failing to credit women scientists for their leadership.

The programming language does make a difference for bioinformatics. (From a Perl lover who knows no Python.)

Smile, you've got a genetic disorder!

ARJUN RAJ JUL 04, 2014 COMMENTS

Check out this paper in eLife, in which the authors use machine learning applied to facial images to determine whether people have genetic disorders. So cool! From what I can gather, they use a training set of just under 3000 images of faces (1300 or so of them have a genetic disorder) and then use facial recognition software to quantify those images. Using that quantification, they can cluster ~ **read more**

Carbon Sniffing from Space

CATHERINE KUHN JUL 04, 2014 COMMENTS

NASA successfully launched the new Orbiting Carbon Observatory 2 (OCO-2) satellite from Vandenburg Air Force Base in California today. After a 2009 foiled attempt ending with the original satellite, OCO-1, plunging into the Indian Ocean, this second iteration will be the sixth satellite joining the A-train, a constellation of satellites designed to monitor various aspects of earth's atmosphere ~ **read more**

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How to see, how to sample, how to shuffle, and how to see that

Why would someone who usually works on fossil reptiles delve into molecular evolution of viruses? It turns out the two fields have a lot in common.

Essay on paper

To measure or not to measure or how to measure

2

Jun 03, 2014 By Nikolai Slavov

Easier and indirect measurements that appear to save time and money often take longer and end up being more expensive than the best measurement one can do.

Essay on paper

How we tried to melt the ice

iPhone Apps

1.

The Spectroscope is a service of protocols.io - free, open access, crowdsourced protocols repository. Explore protocols.

THE SPECTROSCOPE

Smile, you've got a genetic disorder!

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protocols.io - Life Sciences Protocol Repository

A free, up-to-date, crowdsourced protocol repository for the life sciences.

Created by

ZappyLab

506 backers pledged $54,600 to help bring this project to life.

ed to facial images to
ather, they use a training
rder) and then use facial
can cluster ~ read more

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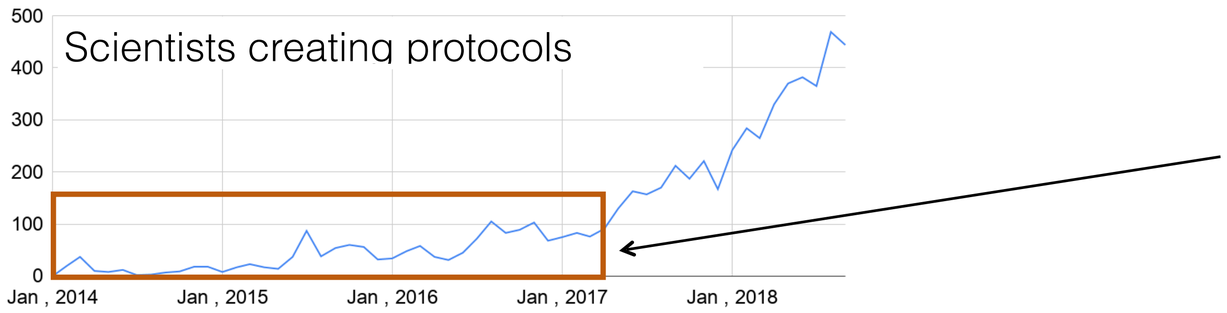
how to see that

Essay on paper

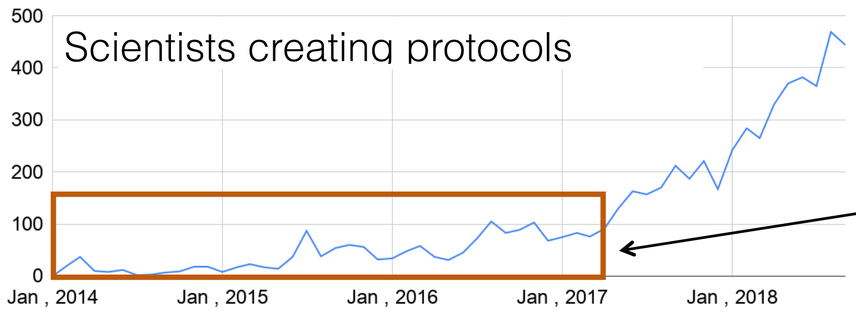
How we tried to melt the ice



Before protocols.io launch:

- Free mobile tools
- Blogging platform for scientists
- Literature-recommendation service
- Story-behind-the-paper
- Career Forum
- Kickstarter

How we tried to melt the ice



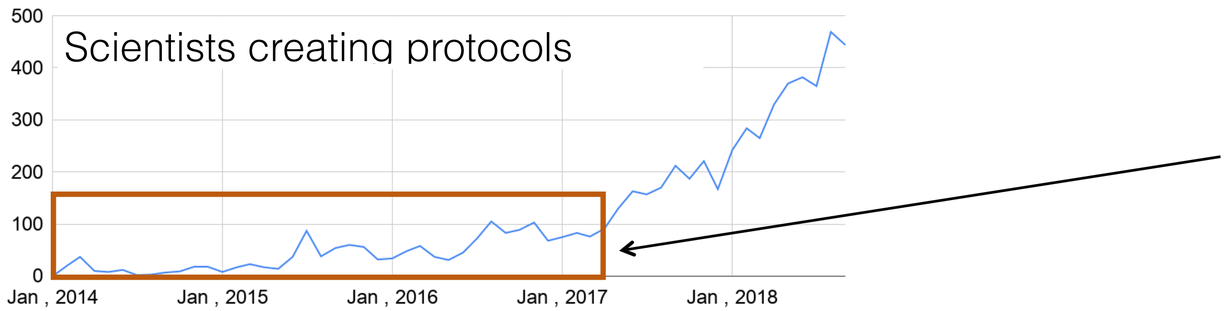
Before protocols.io launch:

- Free mobile tools
- Blogging platform for scientists
- Literature-recommendation service
- Story-behind-the-paper
- Career Forum
- Kickstarter

These efforts didn't work. Next slides will focus on ideas that were effective.

For fuller list of ideas that did not help much, see supplementary slide.

Beware of your own expectations

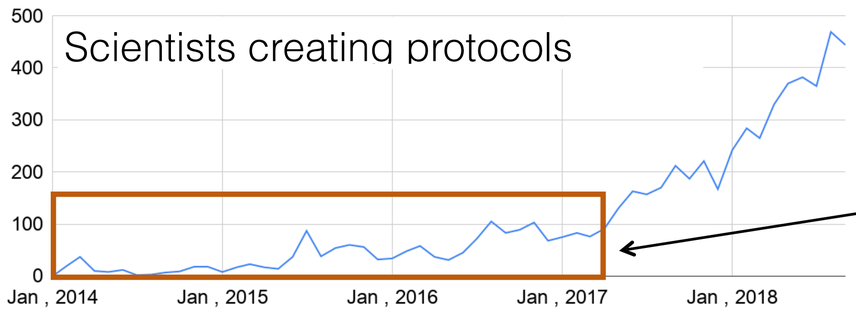


(my early conversation with Victor Henning, cofounder of Mendeley)

***Me**: Does no one need protocols.io? Are we terrible & failing?*

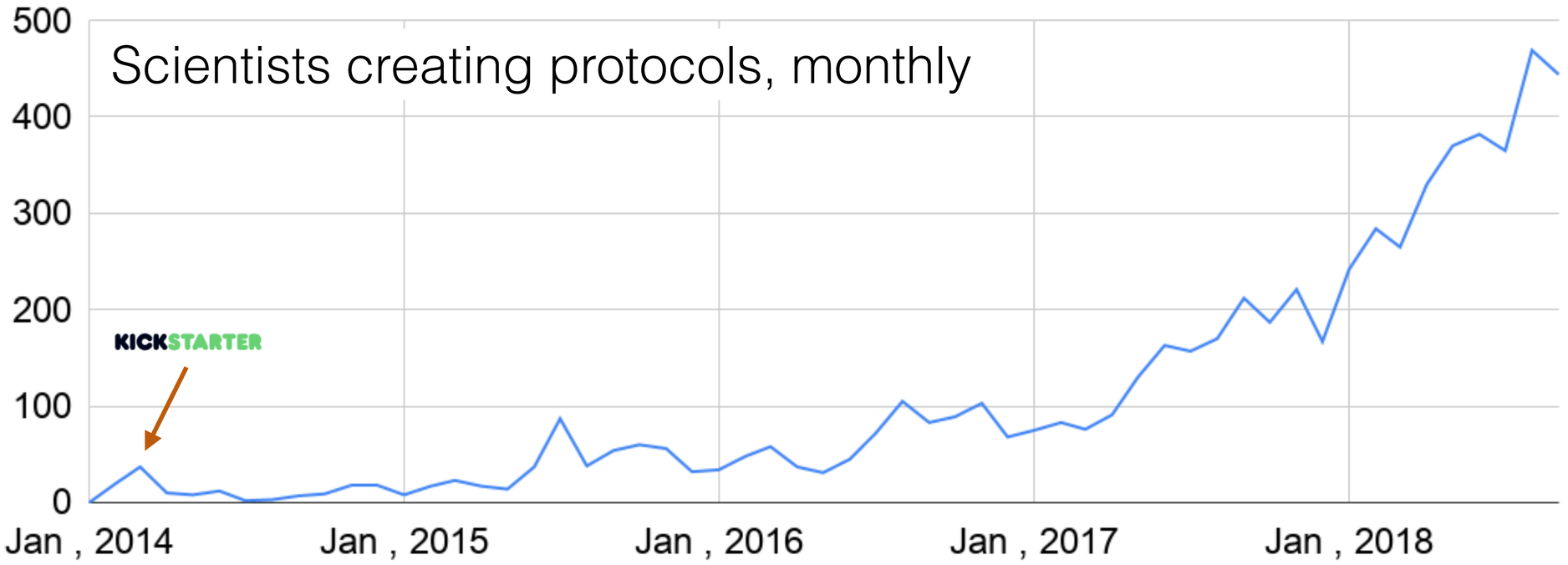
***Victor**: No, you're doing great. You just need to reset your expectations.*

Why so hard for science platforms?

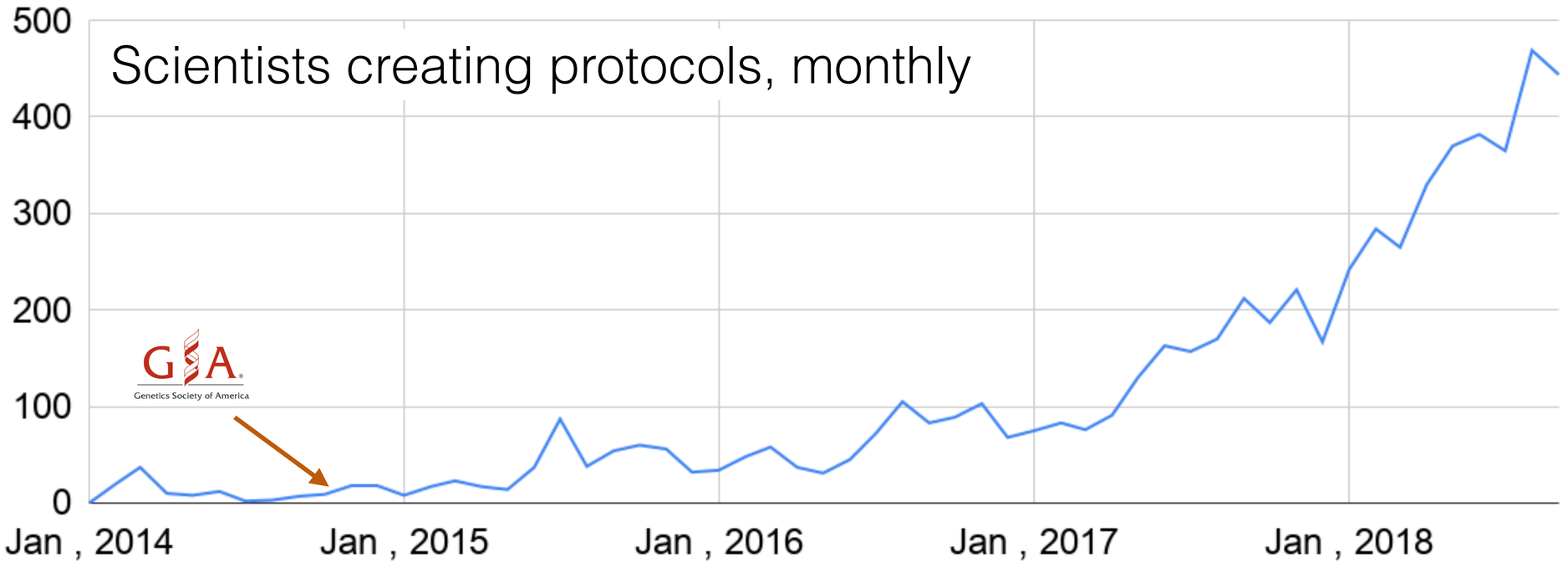


- Scientists are busy & overwhelmed
- Librarians are busy
- Corporations (publishers/vendors) & everyone afraid you will fail
- Need popular content/protocols

Effective efforts



Effective efforts



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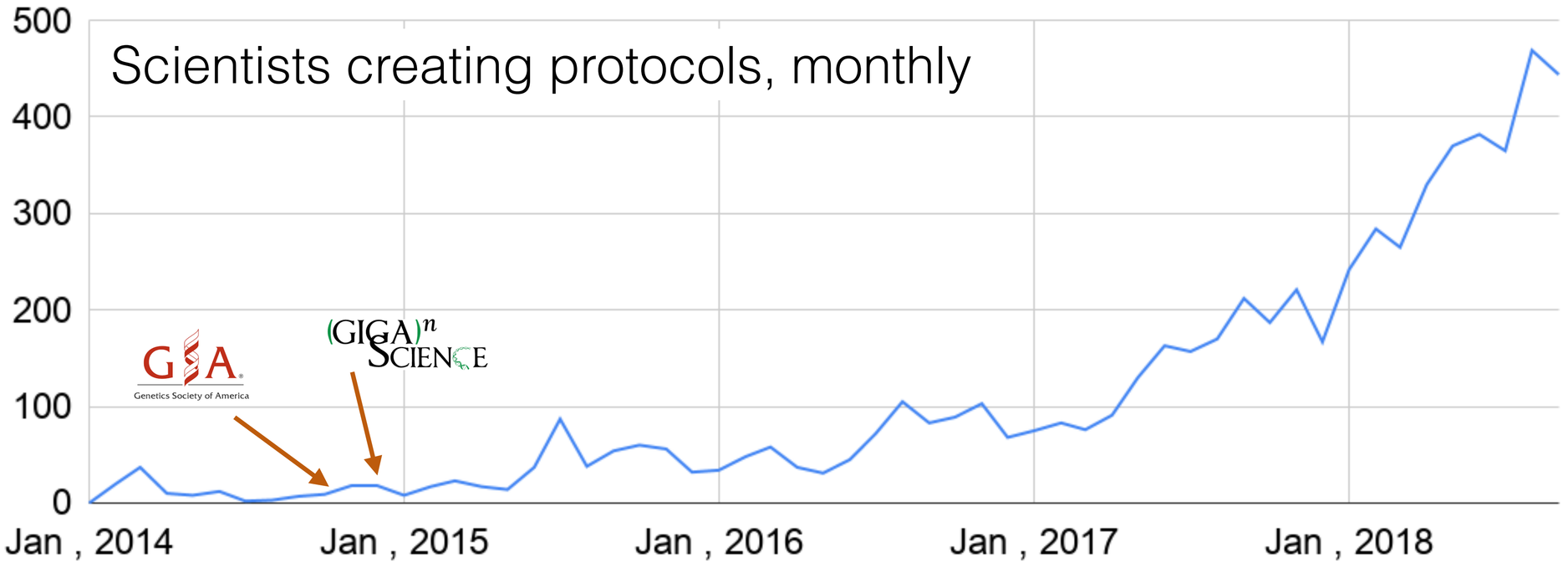
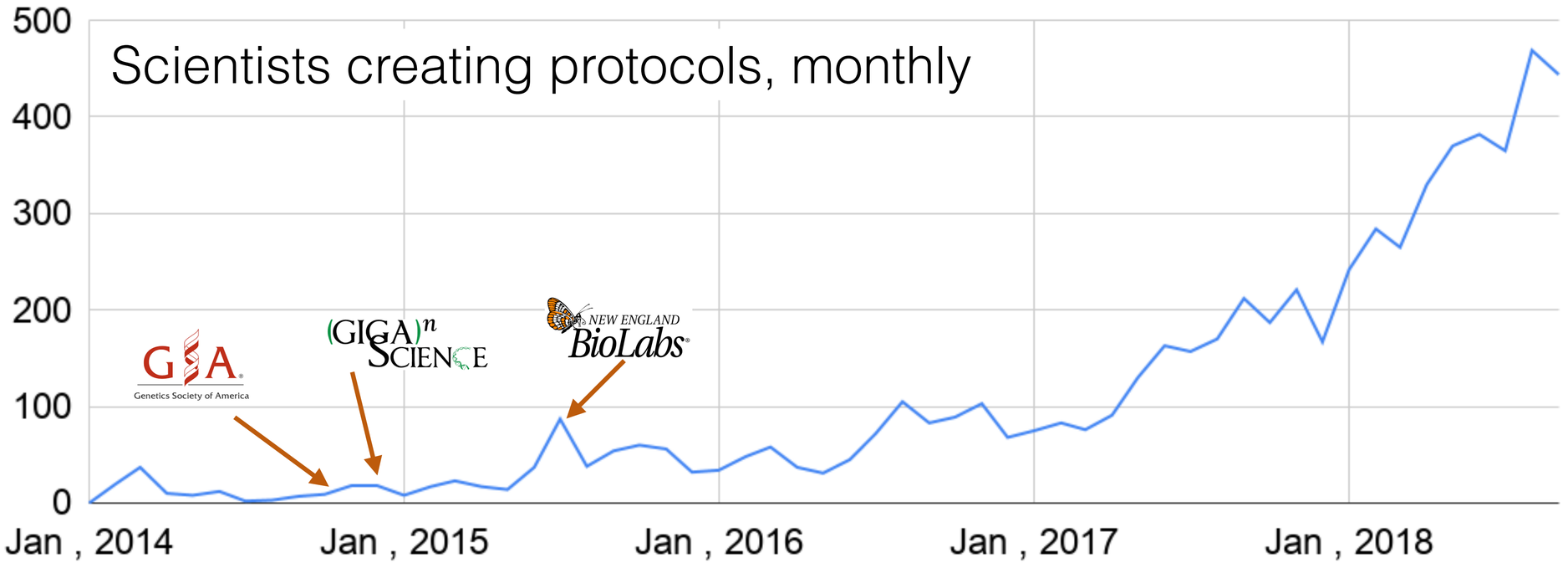
Effective efforts



Effective efforts



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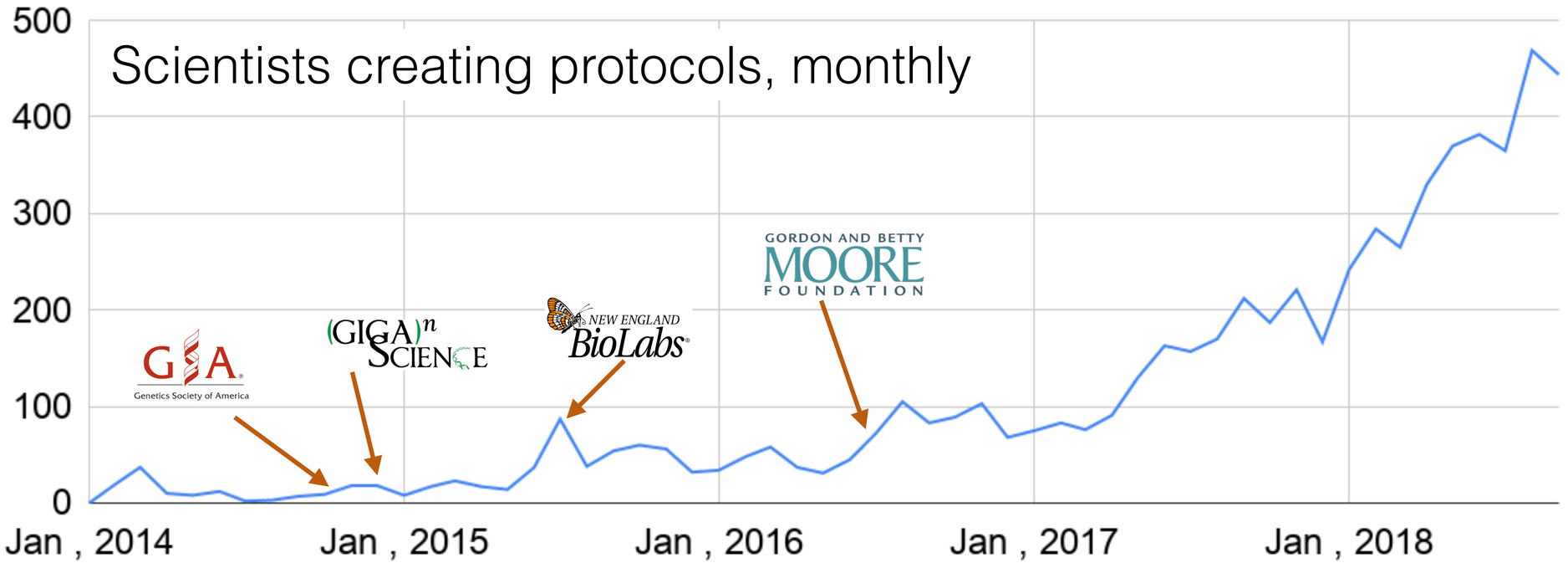

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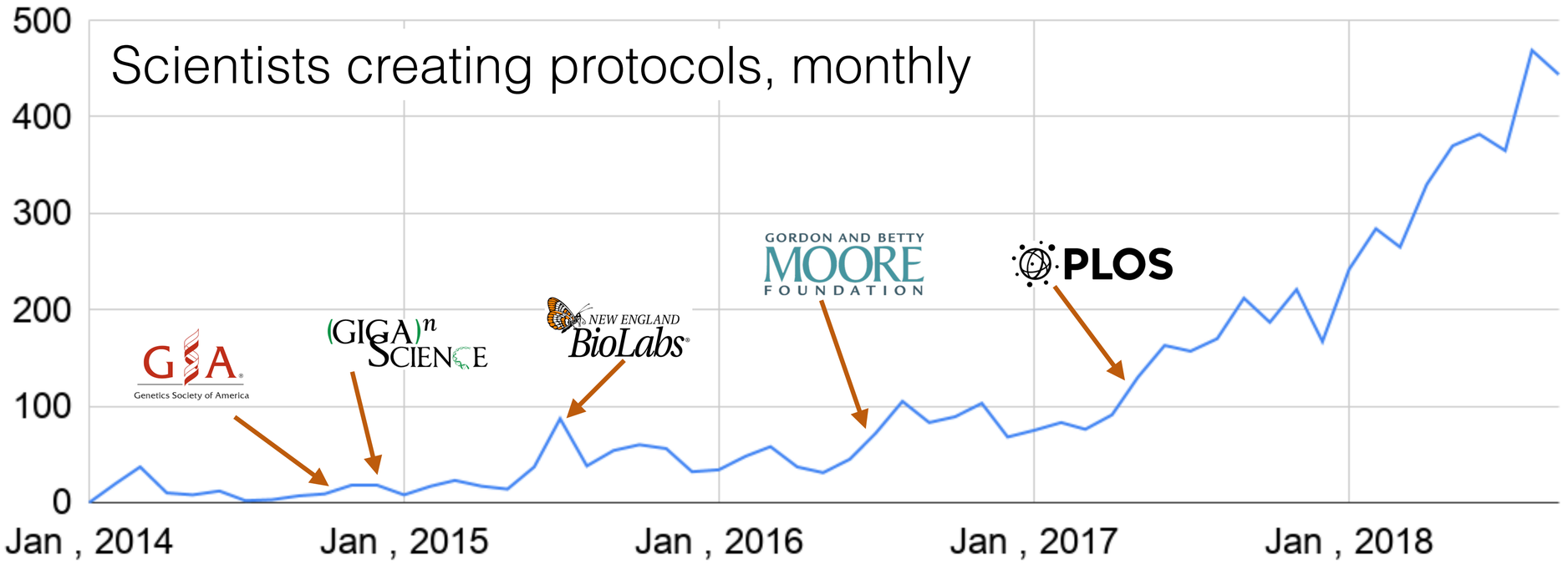

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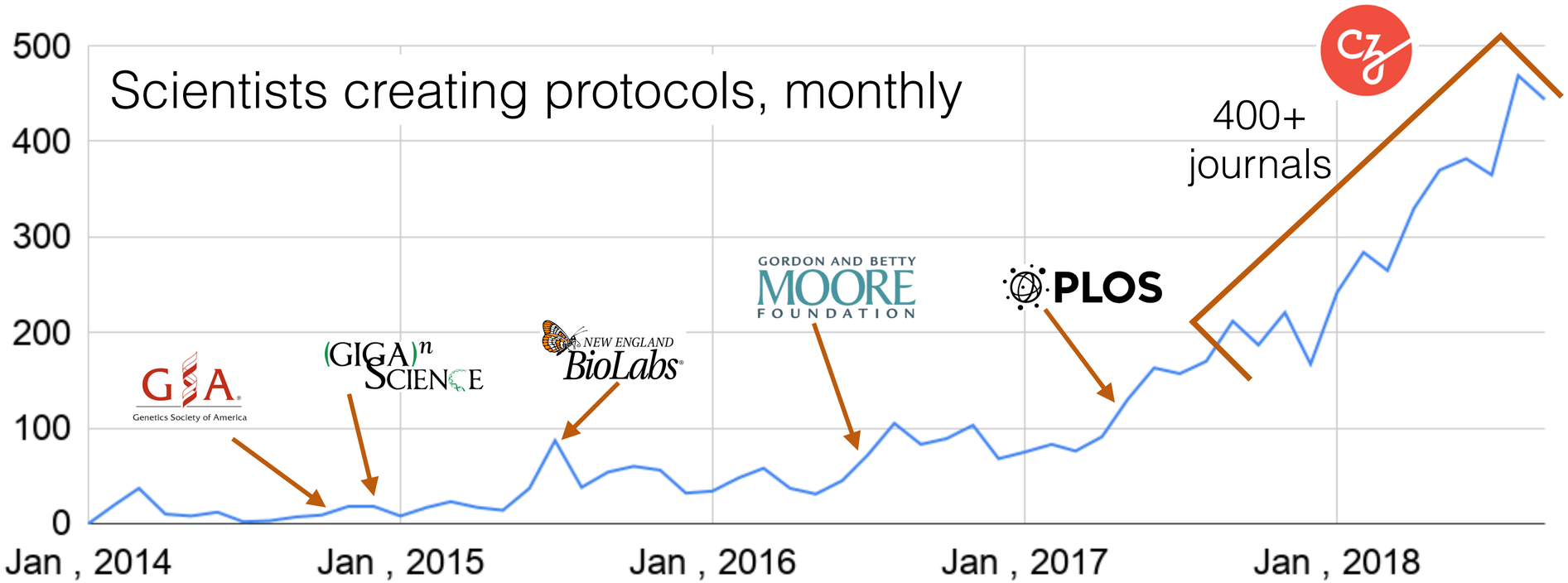
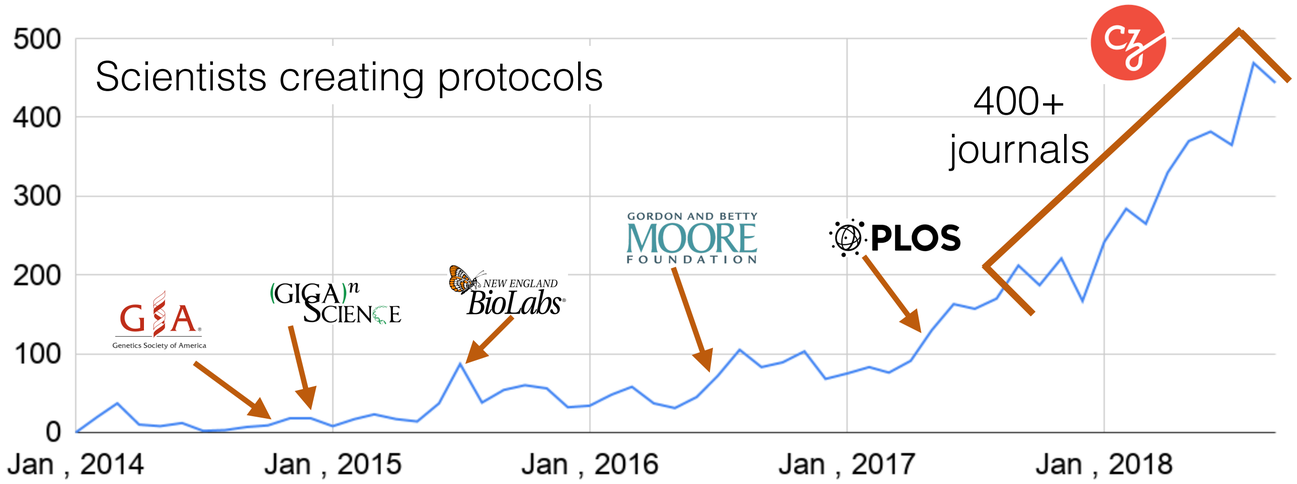


Effective efforts

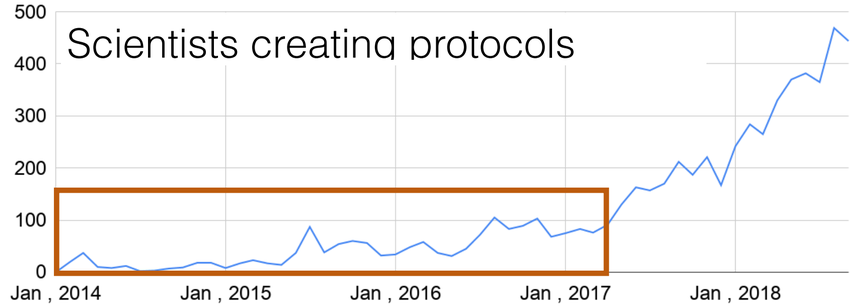


After launch:

- Partnerships w/ publishers
- Partnerships w/ vendors
- Social media
- Seeding popular content
- User interviews and feedback/rating prompts
- Funders
- Librarians
- Ambassador program
- Attention to the user interface & experience to encourage engagement
- Create micro-communities

Lessons Learned

- Set proper expectations (beginning is slow & painful)
- Make it simple - focus on the key product
- Look for partners who already have the community you need
- Ignore Silicon Valley's "Fail Fast" mantra. Failing fast is easy. It's succeeding that is hard and takes time.



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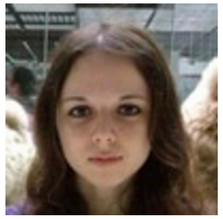
CFO, cofounder

Anita Bröllochs
Outreach

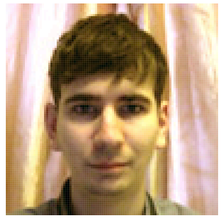
Vladimir Frolov
Development

Monika Khassan
Proj Manager



Yulia Kurnosova
Development

Nick Gulev
Development



Sergey Alekseev
Development

Ashley Humphrey
Editor

GORDON AND BETTY MOORE FOUNDATION

CHAN ZUCKERBERG INITIATIVE

Open Philanthropy Project

Genetics Society of America

(GIGA)n SCIENCE

PLOS

Supplementary Slides

Ineffective outreach efforts

Before protocols.io launch:

- Free mobile tools
- Posters on university campuses
- Blogging platform for scientists (The Spectroscope)
- Literature-recommendation service (PubChase)
- Story-behind-the-paper (on PubChase)
- Career Forum (on PubChase)
- Kickstarter

After launch

- Professional video (most won't watch it)
- Testimonials (most won't see them)
- Tech Media coverage (Forbes, Tech Crunch, etc.— scientist don't read them)
- Startup competitions
- Conference booths (conferences are great for partnerships, but not user bumps)
- Hiring a "growth hacker"

August, 2018 at protocols.io

August 2018

Pageviews:	126,000
Visitors:	27,000
Registrations:	1,600
Active scientists:	600 (out of 20,000 total registered)

Micro-communities: let people talk to colleagues

Protocols | Groups | Researchers | Documents | Discussions | Reagents | Jobs | News | Events | Resources | Help

384 results

MinION user group for high molecular weight DNA extraction from all kingdoms

DNA, sequencing, MinION, long reads, sequence EVERYTHING

250 members 32 protocols Follow

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

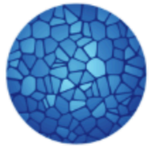
marine protists, genetics, transformation, experimental model systems, oceans, microbe

186 members 165 protocols

Sullivan Lab

viral ecology and evolution, virus-host interaction biology, viral discovery and metagenomics

40 members 153 protocols Join Follow



Human Cell Atlas Method Development Community

single-cell genomics, reference maps, molecules, cells, tissues, organs, systems

163 members 52 protocols

Aiptasia-Symbiodinium Model System

symbiosis, Aiptasia, Symbiodinium, ecology, genetics, onset of symbiosis, regulation of symbiosis, breakdown of symbiosis

57 members 24 protocols Join Follow

VERVE Net

viral ecology

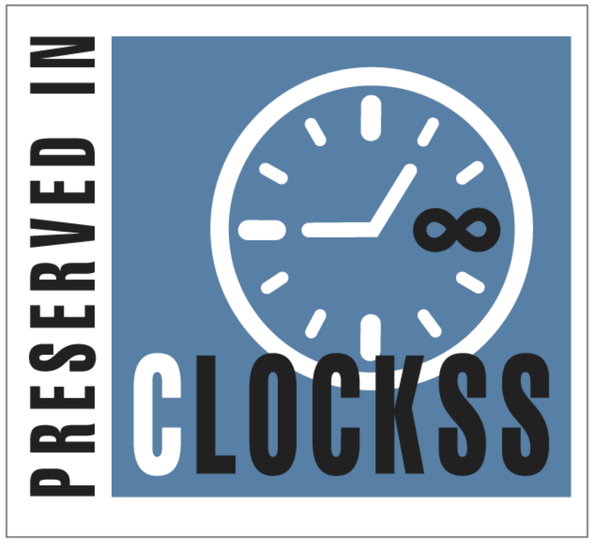
289 members 377 protocols Follow

Preservation and backups

Daily backups

Public APIs

Export in PDF and JSON
(download or Dropbox/GoogleDrive)



How is protocols.io free to read & publish?
(Business Model)

Private groups
Monthly dues to keep protocols visible only to group members

Vendor analytics
Subscription fee to access aggregated usage statistics

protocols.io

Business Model - Private Groups

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

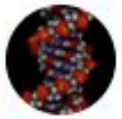
Contact us

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

Alejandro Montenegro @aemonten · 17h

Looking for someone with experience doing RNA extraction (RNA-seq quality) from primary cortical neuron cultures. Anybody?

2 8 4



Eli Roberson @thatdnaguy · 17h

Are they hard to lyse?

1

Alejandro Montenegro @aemonten · 17h

Don't know. My GF wrote and said she gets little RNA and of low quality, as assessed by Bioanalyzer

2

elena MiMo @ElenaMinones · 17h

What is she using for the RNA extraction? Columns? Trizol or RNAzol should work better, DM happy to share my protocol

2 2

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2 2

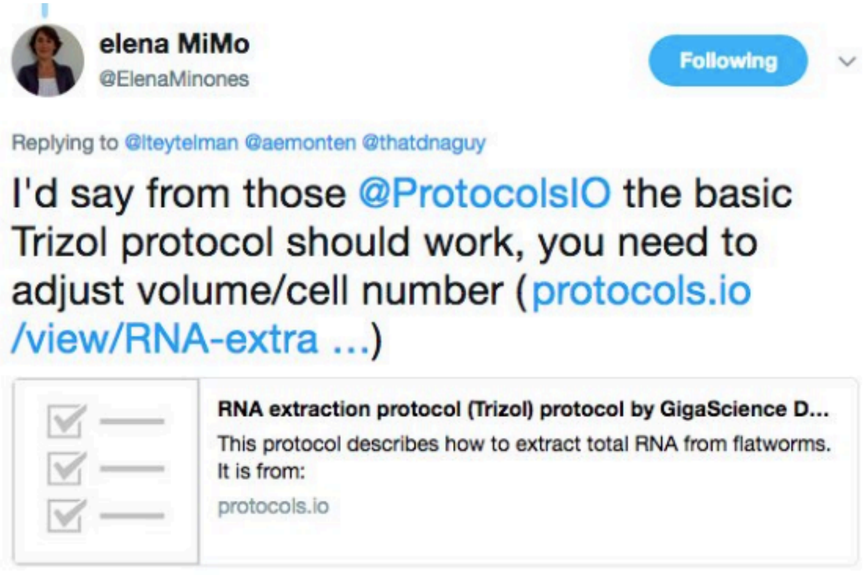
elena MiMo
@ElenaMinones

Following

Replying to @lteytelman @aemonten @thatdnaguy

I'd say from those @ProtocolsIO the basic Trizol protocol should work, you need to adjust volume/cell number (protocols.io /view/RNA-extra ...)

RNA extraction protocol (Trizol) protocol by GigaScience D...
This protocol describes how to extract total RNA from flatworms. It is from:
protocols.io



Hébert *et al. GigaScience* (2016) 5:24
DOI 10.1186/s13742-016-0128-3

GigaScience

DATA NOTE Open Access

Transcriptome sequences spanning key developmental states as a resource for the study of the cestode *Schistocephalus solidus*, a threespine stickleback parasite

François Olivier Hébert[1*], Stephan Grambauer[2], Iain Barber[2], Christian R. Landry[1] and Nadia Aubin-Horth[1]

Abstract

Background: *Schistocephalus solidus* is a well-established model organism for studying the complex life cycle of cestodes and the mechanisms underlying host-parasite interactions. However, very few large-scale genetic resources for this species are available. We have sequenced and *de novo*-assembled the transcriptome of *S. solidus* using tissues from whole worms at three key developmental states - non-infective plerocercoid, infective plerocercoid and adult plerocercoid - to provide a resource for studying the evolution of complex life cycles and, more specifically, how parasites modulate their interactions with their hosts during development.

Findings: The *de novo* transcriptome assembly reconstructed the coding sequence of 10,285 high-confidence unigenes from which 24,765 non-redundant transcripts were derived. 7,920 (77 %) of these unigenes were annotated with a protein name and 7,323 (71 %) were assigned at least one Gene Ontology term. Our raw transcriptome assembly (unfiltered transcripts) covers 92 % of the predicted transcriptome derived from the *S. solidus* draft genome assembly currently available on WormBase. It also provides new ecological information and orthology relationships to further annotate the current WormBase transcriptome and genome.

Conclusion: This large-scale transcriptomic dataset provides a foundation for studies on how parasitic species with complex life cycles modulate their response to changes in biotic and abiotic conditions experienced inside their various hosts, which is a fundamental objective of parasitology. Furthermore, this resource will help in the validation of the *S solidus* gene features that have been predicted based on genomic sequence.

Keywords: Transcriptome, RNA-seq, de novo assembly, *Schistocephalus solidus*, Parasite, Cestode, Flatworm, Threespine stickleback, *Gasterosteus aculeatus*

RNA extraction for plant samples using CTAB-pBIOZOL

Feb 28, 2017 14 steps
dx.doi.org/10.17504/protocols.io.gsnbwde

Bauhinia Genome | RNA extraction | plant science | transcriptomics

CONTACT: SCOTT EDMUNDS

Run

2x CTAB buffer production

Step 1 To make the 2x CTAB buffer used in the pre-lysis step make up the following b and then autoclave and aliquot. 2% CTAB ... read more

Pre lysis buffer

Step 2 Add 750µl 2x CTAB buffer to 60µl of beta-mercaptomethanol and 750µl pBIOZOL reagent in 2ml eppendorf tubes. Mix well.

Step 3 Warm up the lysis buffer to 65°C in a heat block

Grind plant tissues

Step 4 Cut 1-2 cm^2 sections of plant or leaf tissues and grind up in a pestle and mortar with liquid nitrogen. These roughly 80... read more

Incubate lysis reaction

Step 5 Incubate the samples in a thermo mixer with gentle mixing (700rpm) for 15 minutes at 65°C to permit the commplete dissoc... read more

Step 1

To make the 2x CTAB buffer used in the pre-lysis step make up the following b and then autoclave and aliquot.

2% CTAB (w/v)	20g
100mM Tris(PH 8.0 , 1M)	100ml
20mM EDTA(PH 8.0, 0.5 M)	40ml
1.4 M NaCl	81.8g

Then add distilled water to make it up to 1000ml

REAGENTS

CTAB (Hexadecyltrimethylammonium bromide)
by BBI Biotech
Catalog #: CB0108-100g

ANNOTATIONS Add new

Help

Journal partnerships

400+ journals with protocols.io in author guidelines (increased from 3 in 2017)