For reproducibility, we need the methods behind the data

Lenny Teytelman, lenny@protocols.io

Morgan Halane
@themorgantrail

Folge ich

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.

Tweet übersetzen

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34 Retweets 96 „Gefällt mir"-Angaben

Morgan Halane
@themorgantrail

Folge ich

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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34 Retweets **96** „Gefällt mir"-Angaben

Daniel Gonzales
@dgonzales1990

Folge ich

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

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

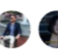
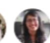
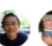
[ref 4] 2013: “Devices were fabricated as previously described [ref 2]”

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

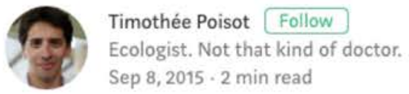
Tweet übersetzen

13:16 - 17. Jan. 2018

230 Retweets **798** „Gefällt mir“-Angaben

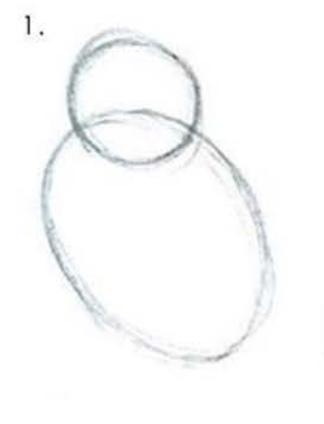
  

28 230 798



Step 2—do the rest of the fucking analysis

How to draw an owl



1. Draw some circles   2. Draw the rest of the fucking owl

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.

Mission:

Making it easy to share method details *before*, *during* and *after* publication.

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- Sharing
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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

dx.doi.org/10.17504/protocols.io.p9kdr4w

Human Cell Atlas Method Development Community

Aleksandar Janjic
Ludwig-Maximilians-Universität...

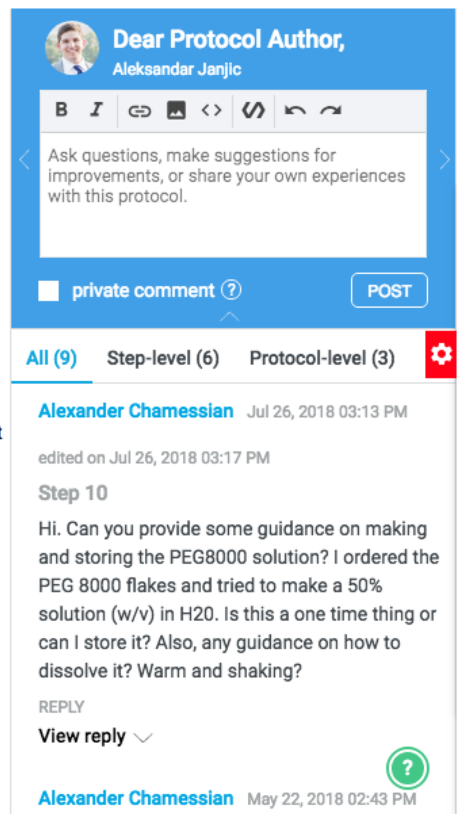
BEFORE STARTING

Wipe bench surfaces with RNAse Away and keep working environment clean.

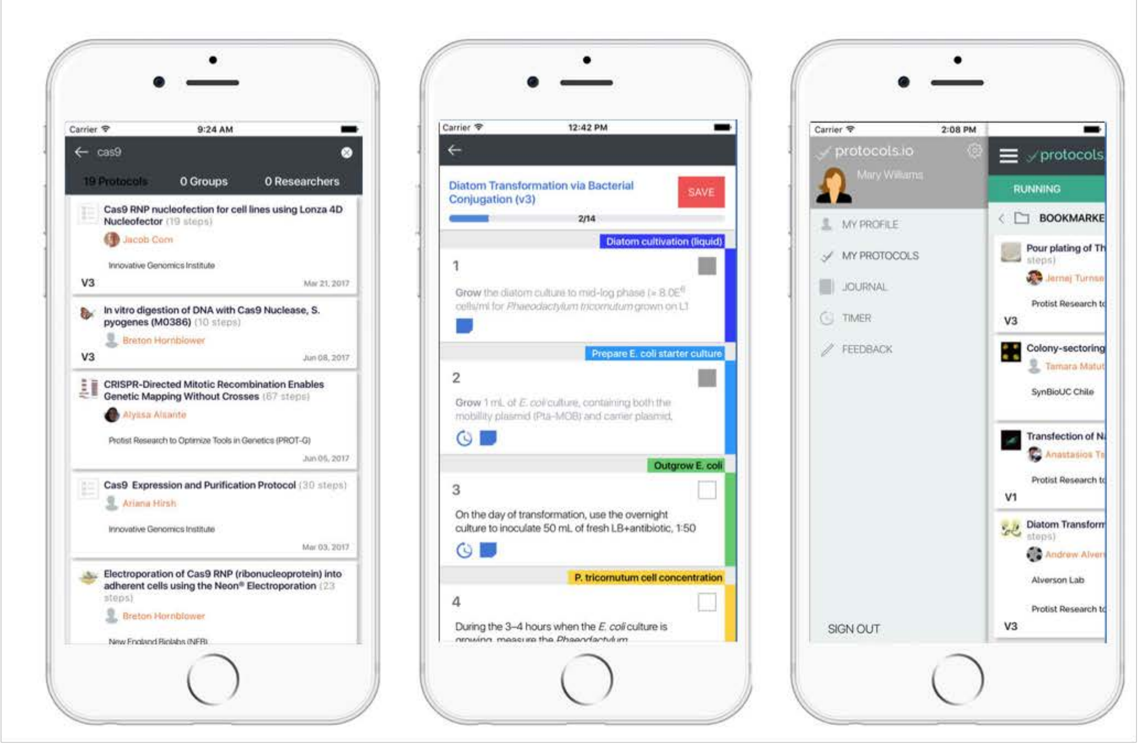
Preparation of lysis plates

1 Prepare **Lysis Buffer** according to the number of plates to be filled.

	A	B	C
1	**Reagent**	**96-well plate**	**384-well plate**
2	NEB HF Phusion buffer (5x)	1.1 µL	4.4 µL
3	Proteinase K (20 mg/mL)	27.5 µL	110 µL
4	UltraPure Water	411.4 µL	1645.6 µL
5	**Total**	**440 µL**	**1760 µL**



Demo: www.protocols.io



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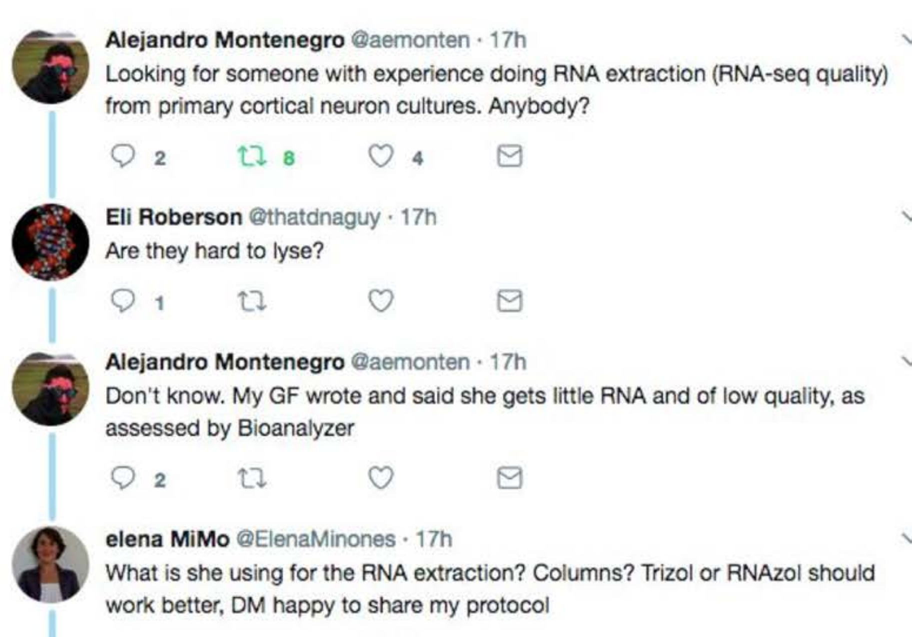
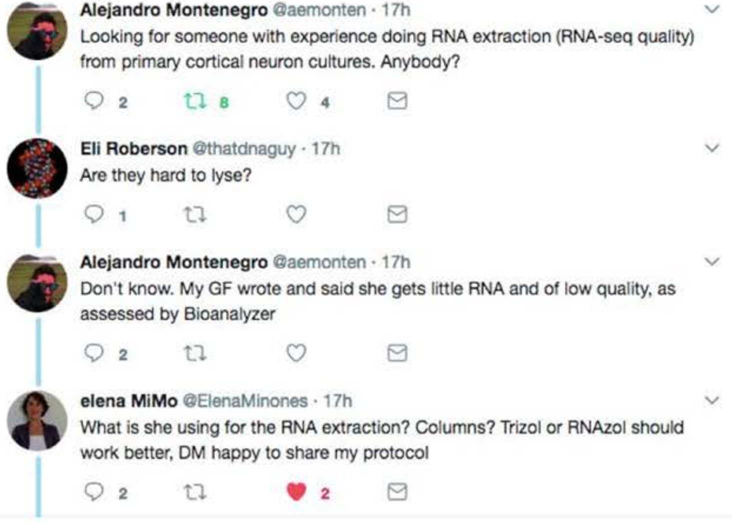

Alejandro Montenegro @aemonten · 17h
Looking for someone with experience doing RNA extraction (RNA-seq quality) from primary cortical neuron cultures. Anybody?
2
8
4
Eli Roberson @thatdnaguy · 17h
Are they hard to lyse?
1
Alejandro Montenegro @aemonten · 17h
Don't know. My GF wrote and said she gets 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

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 ...)

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*

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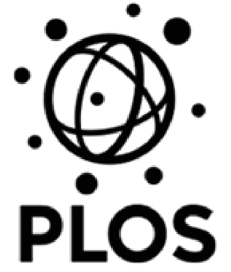

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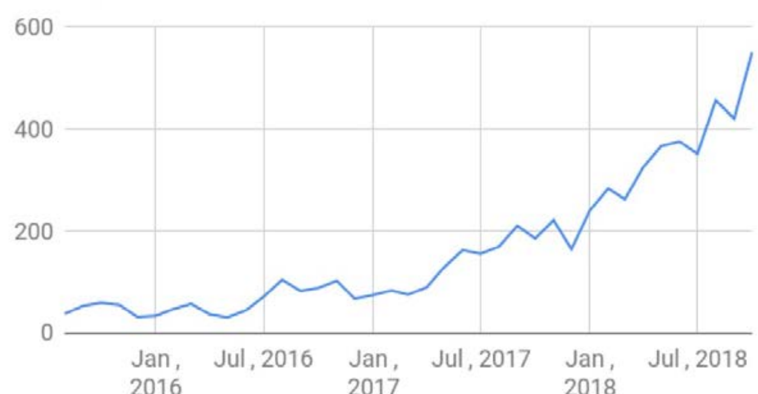

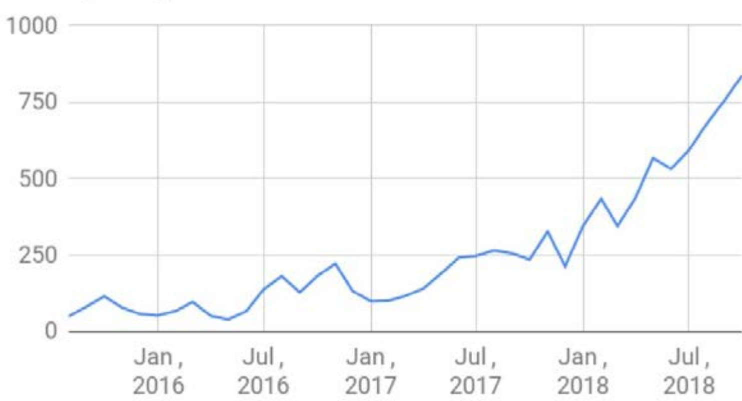

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Irina Makkaveeva
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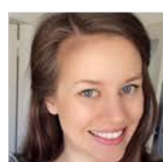

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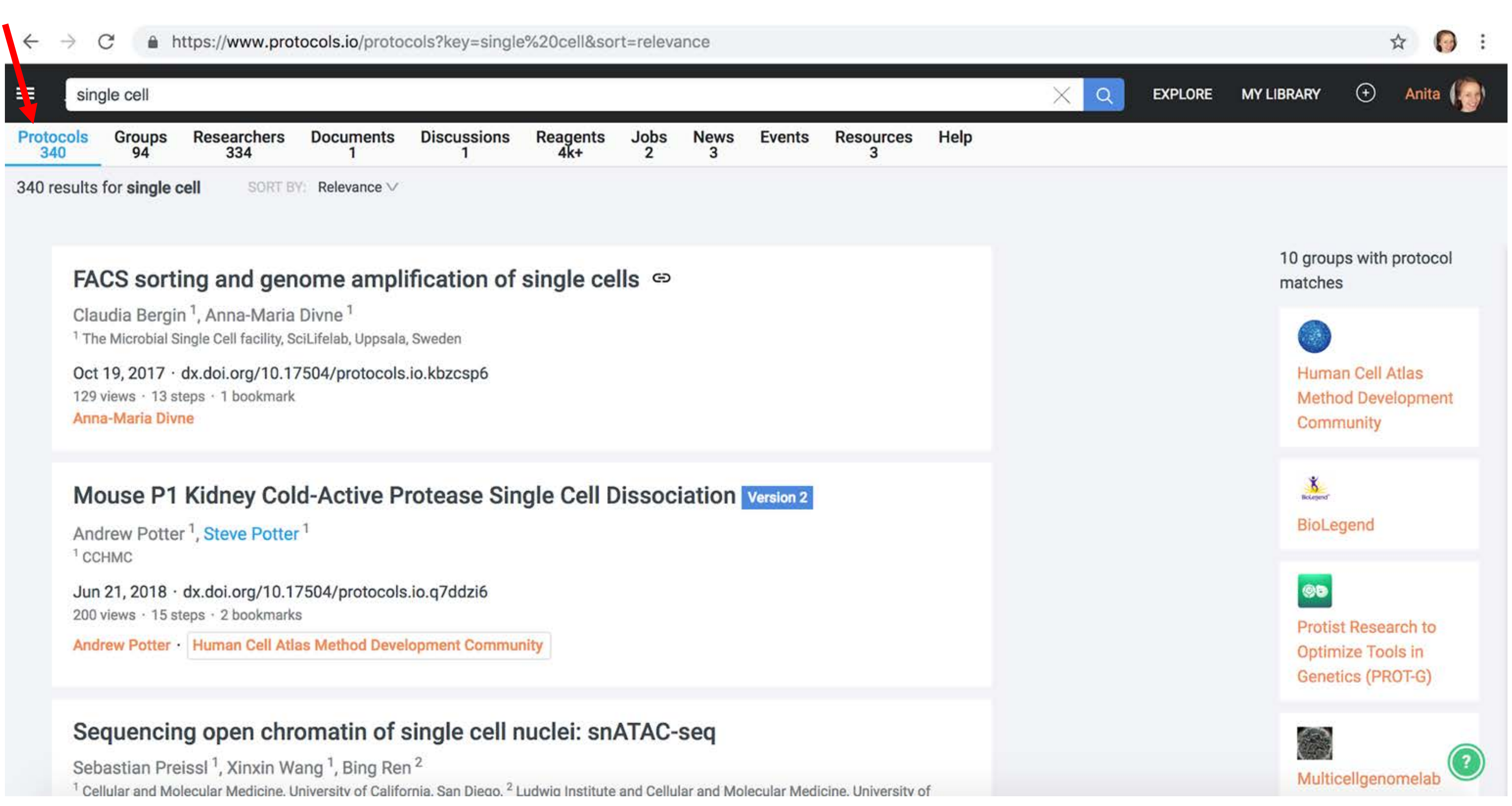

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Claudia Bergin 1, Anna-Maria Divne 1
1 The Microbial Single Cell facility, SciLifelab, Uppsala, Sweden
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Andrew Potter 1, Steve Potter 1
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Andrew Potter · Human Cell Atlas Method Development Community
Sequencing open chromatin of single cell nuclei: snATAC-seq
Sebastian Preissl 1, Xinxin Wang 1, Bing Ren 2
1 Cellular and Molecular Medicine, University of California, San Diego, 2 Ludwig Institute and Cellular and Molecular Medicine, University of
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Protist Research to Optimize Tools in Genetics (PROT-G)
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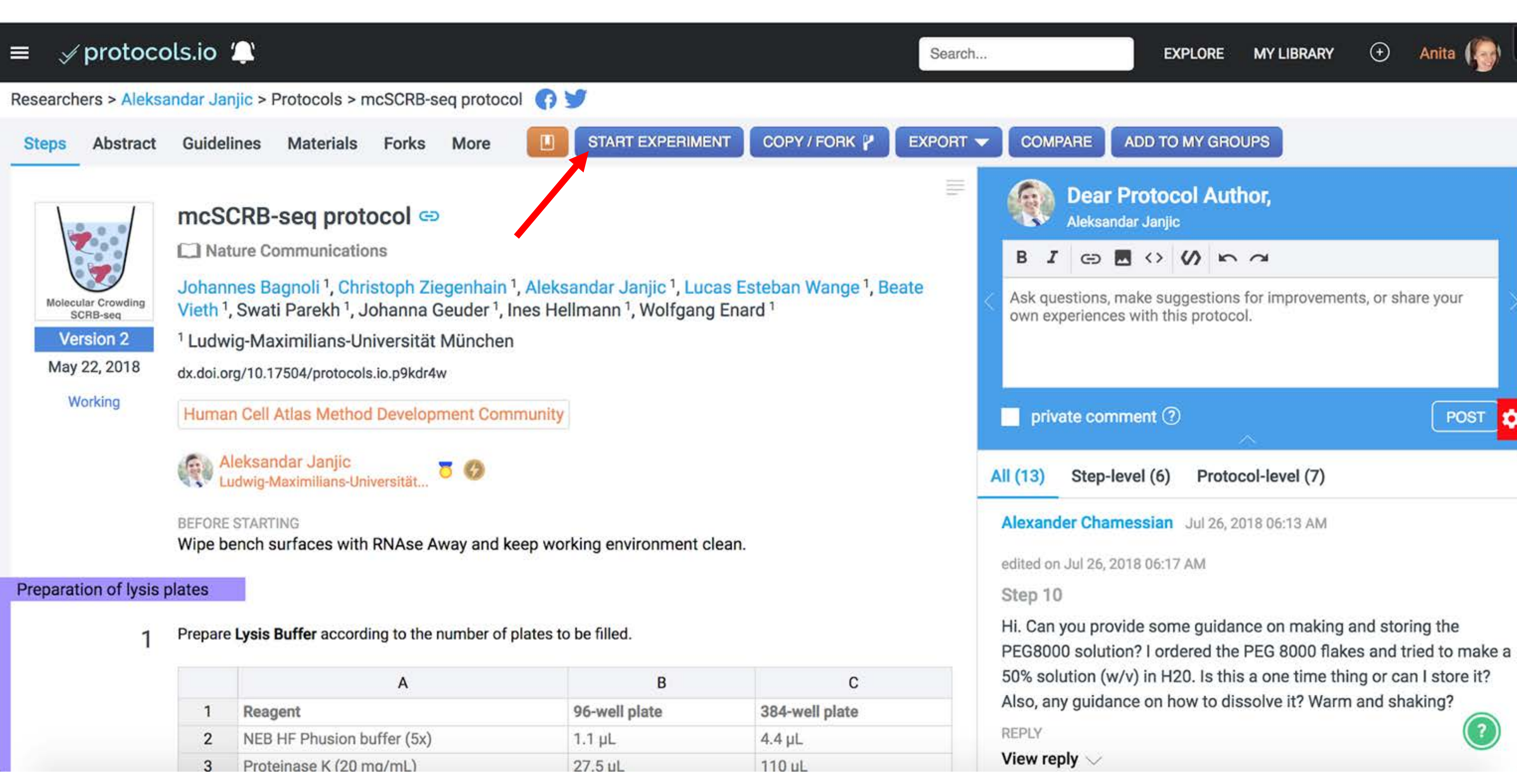

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Version 2
May 22, 2018
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mcSCRB-seq protocol
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dx.doi.org/10.17504/protocols.io.p9kdr4w
Human Cell Atlas Method Development Community
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1
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A
B
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3 Proteinase K (20 mg/mL) 27.5 µL 110 µL
Dear Protocol Author,
Aleksandar Janjic
Ask questions, make suggestions for improvements, or share your own experiences with this protocol.
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Step-level (6)
Protocol-level (7)
Alexander Chamessian Jul 26, 2018 06:13 AM
edited on Jul 26, 2018 06:17 AM
Step 10
Hi. Can you provide some guidance on making and storing the PEG8000 solution? I ordered the PEG 8000 flakes and tried to make a 50% solution (w/v) in H20. Is this a one time thing or can I store it? Also, any guidance on how to dissolve it? Warm and shaking?
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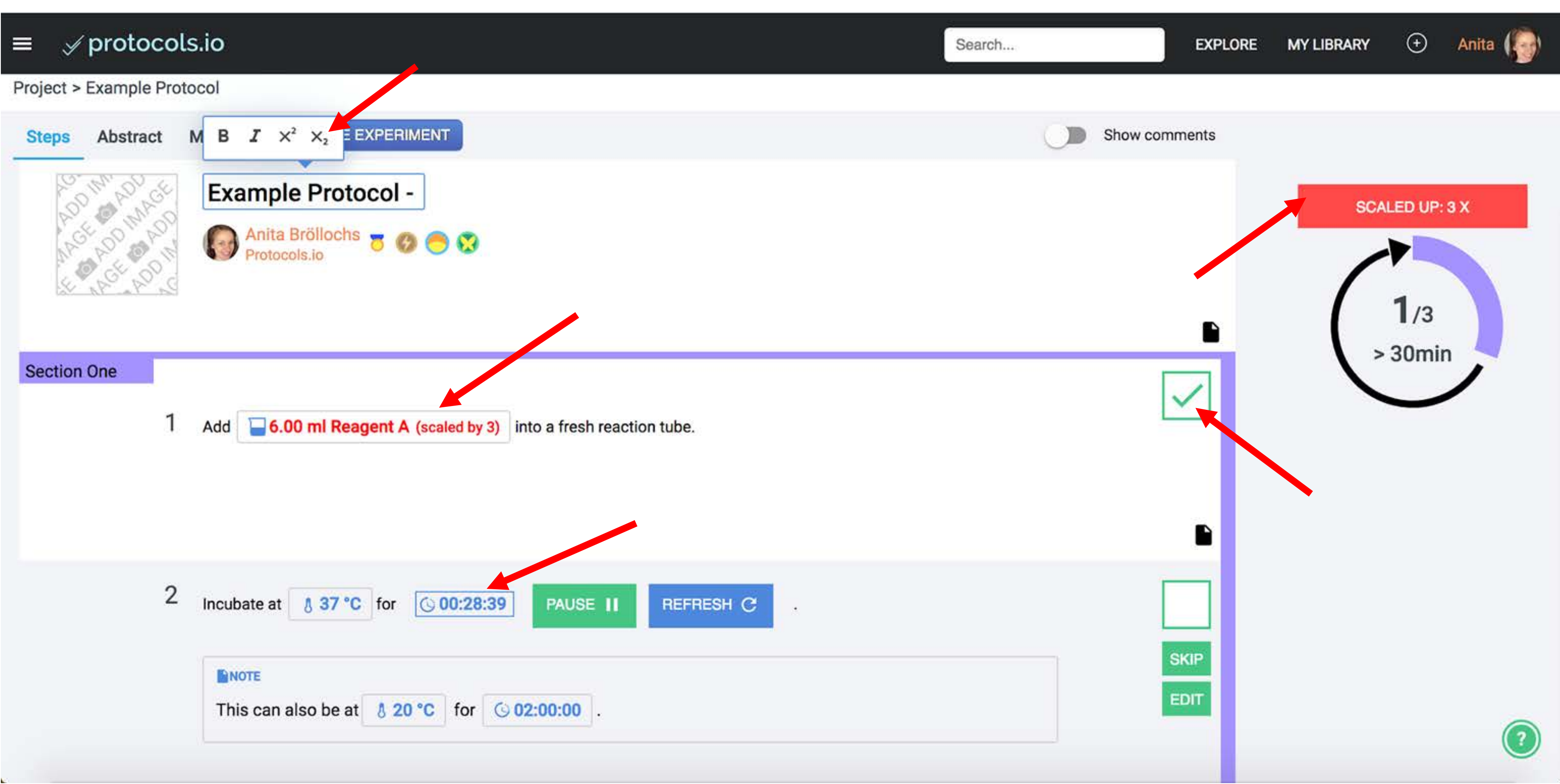
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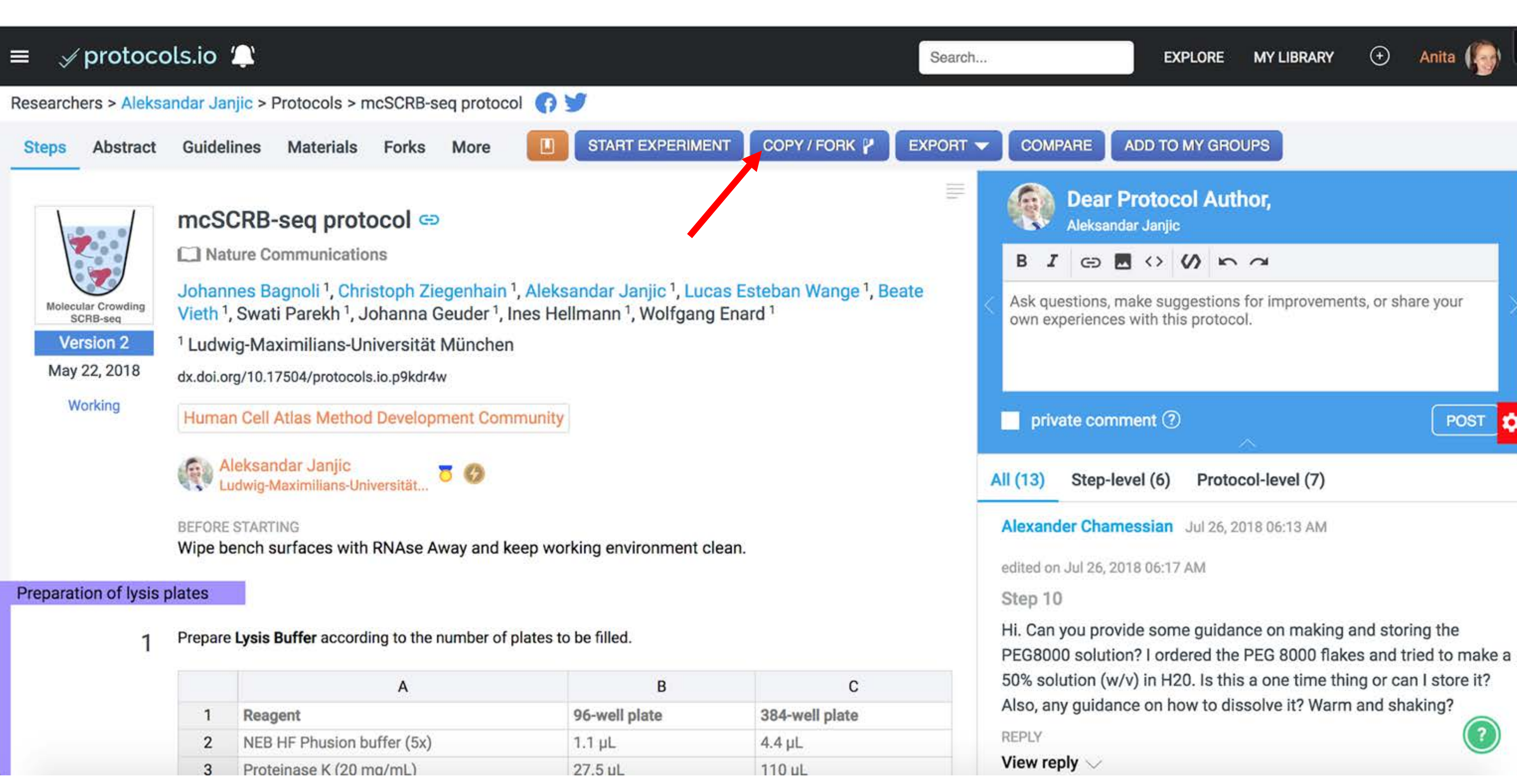
SCALED UP: 3 X
1/3
> 30min
Section One
1 Add 6.00 ml Reagent A (scaled by 3) into a fresh reaction tube.
2 Incubate at 37 °C for 00:28:39 PAUSE REFRESH .
NOTE
This can also be at 20 °C for 02:00:00 .
SKIP
EDIT



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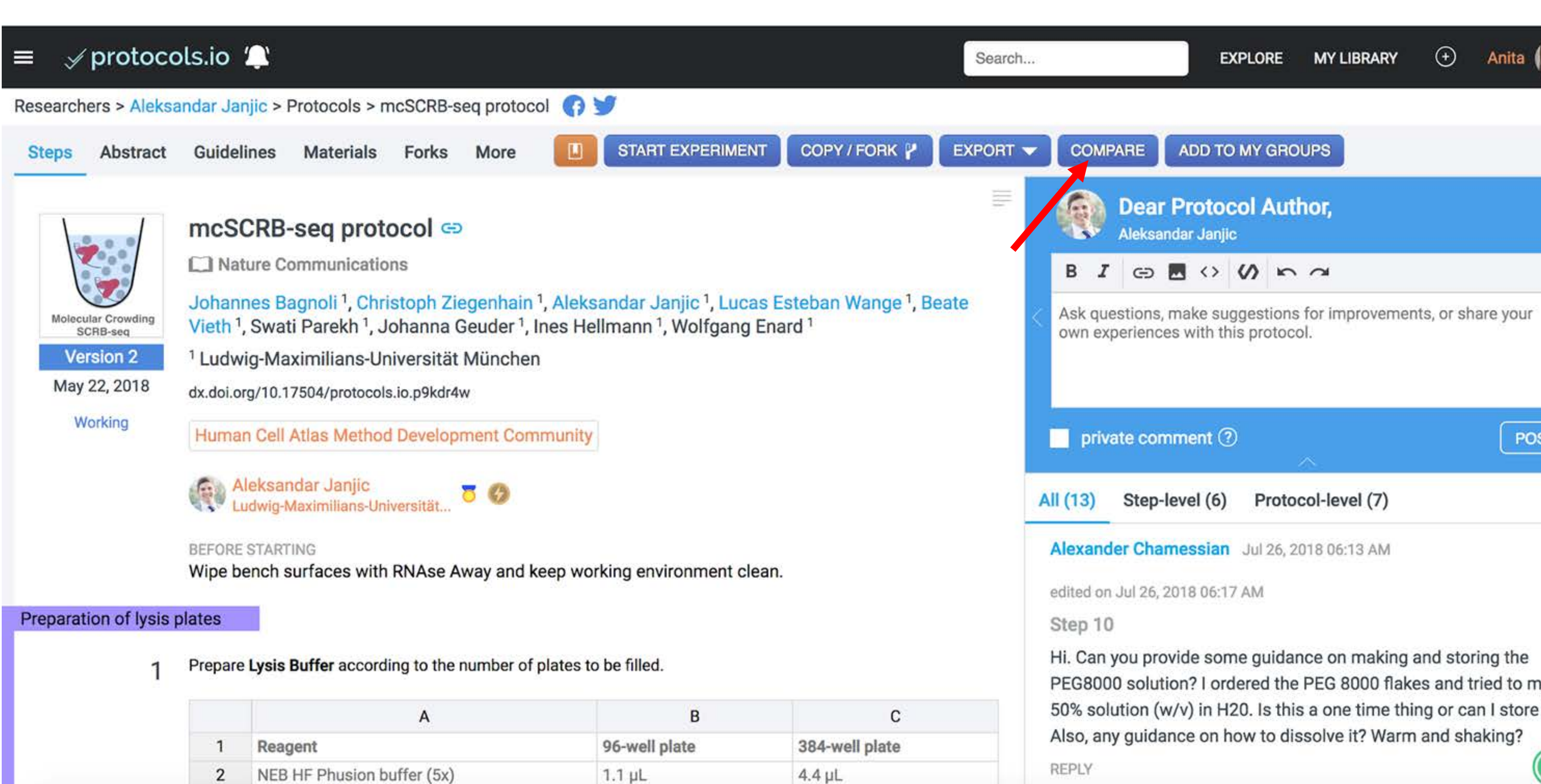
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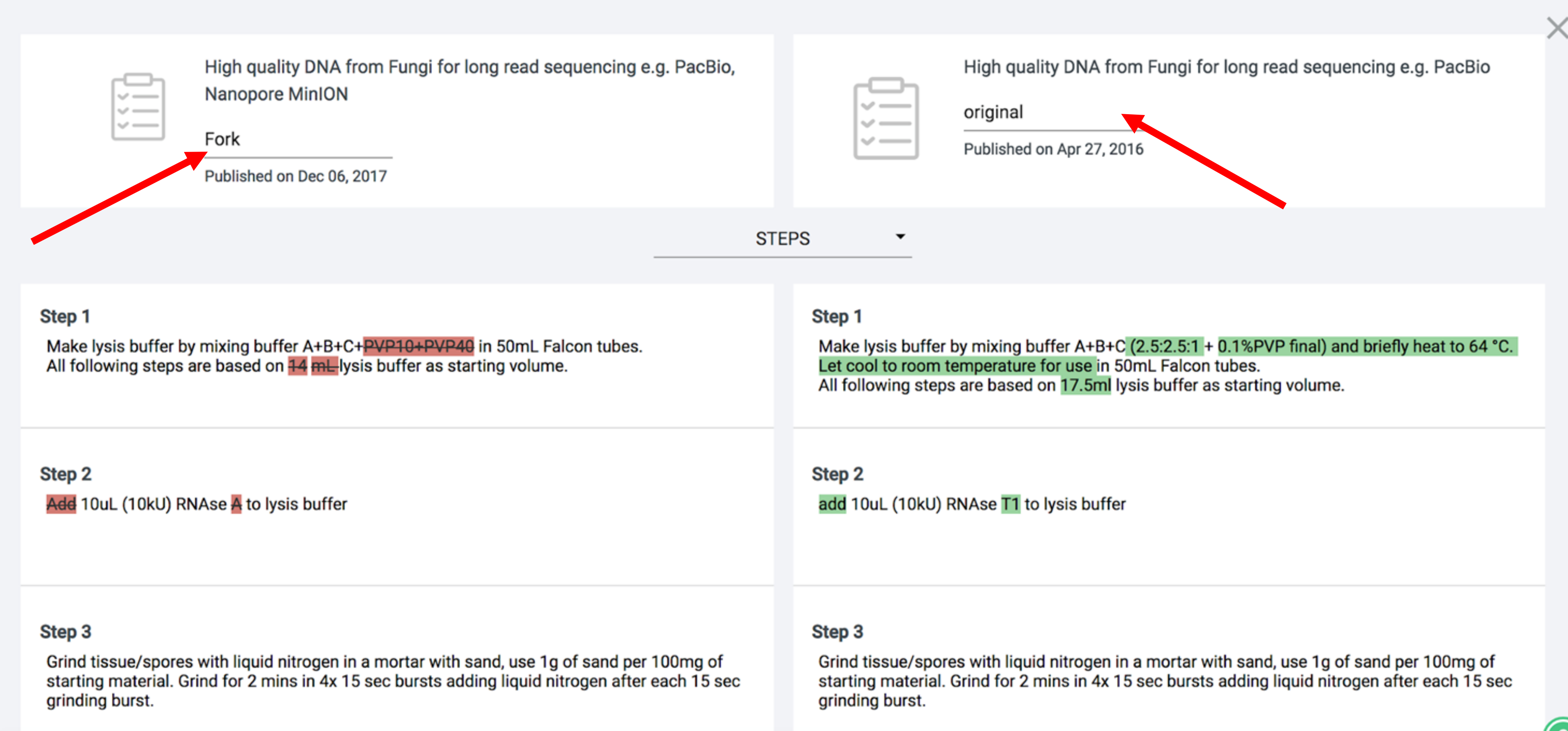
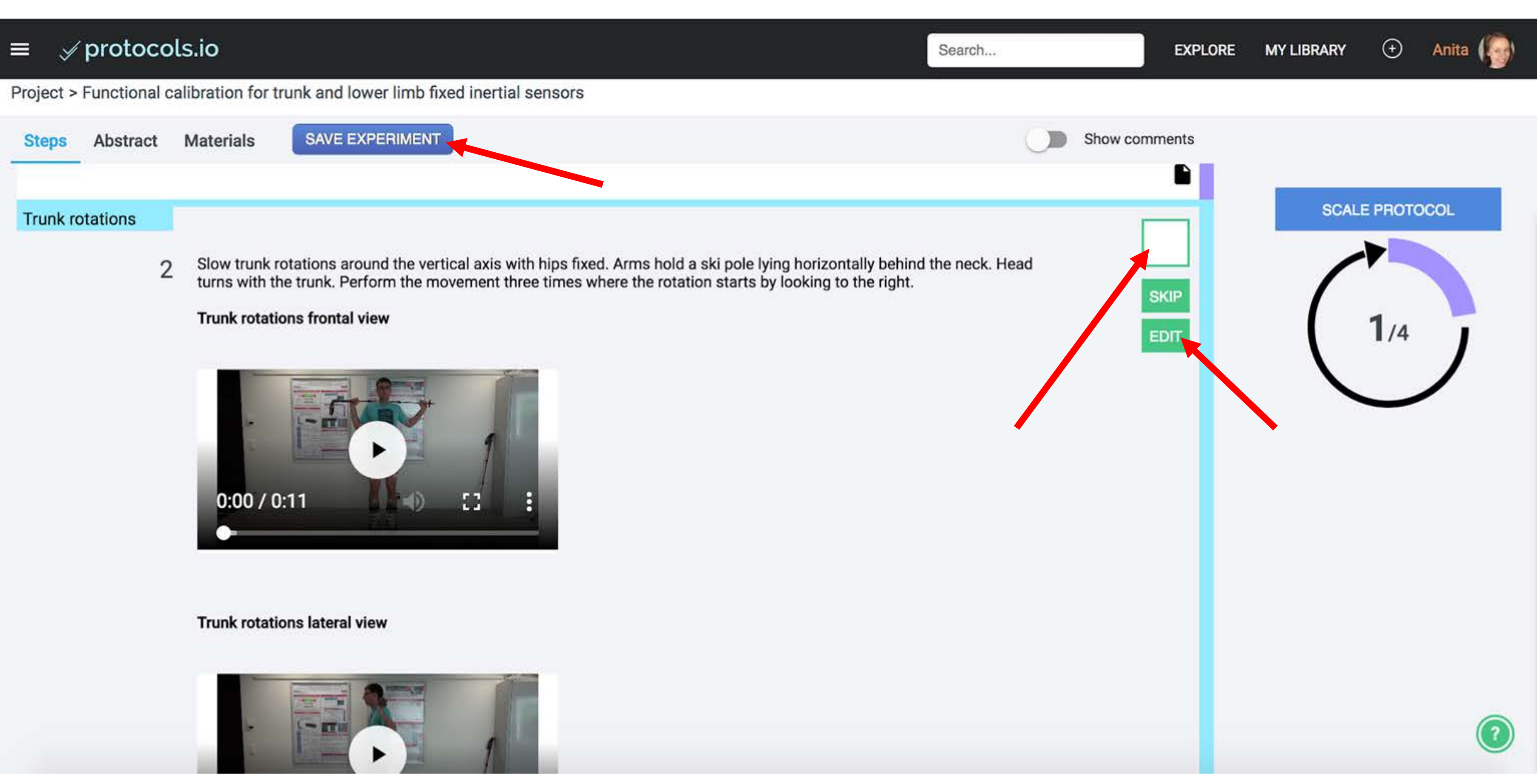

High quality DNA from Fungi for long read sequencing e.g. PacBio, Nanopore MinION
Fork
Published on Dec 06, 2017
High quality DNA from Fungi for long read sequencing e.g. PacBio
original
Published on Apr 27, 2016
STEPS
Step 1
Make lysis buffer by mixing buffer A+B+C+PVP10+PVP40 in 50mL Falcon tubes.
All following steps are based on 14 mL lysis buffer as starting volume.
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 50mL Falcon tubes.
All following steps are based on 17.5ml lysis buffer as starting volume.
Step 2
Add 10uL (10kU) RNAse A to lysis buffer
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 2 mins in 4x 15 sec bursts adding liquid nitrogen after each 15 sec grinding burst.
Step 3
Grind tissue/spores with liquid nitrogen in a mortar with sand, use 1g of sand per 100mg of starting material. Grind for 2 mins in 4x 15 sec bursts adding liquid nitrogen after each 15 sec grinding burst.



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Slow trunk rotations around the vertical axis with hips fixed. Arms hold a ski pole lying horizontally behind the neck. Head turns with the trunk. Perform the movement three times where the rotation starts by looking to the right.
Trunk rotations frontal view
0:00 / 0:11
Trunk rotations lateral view
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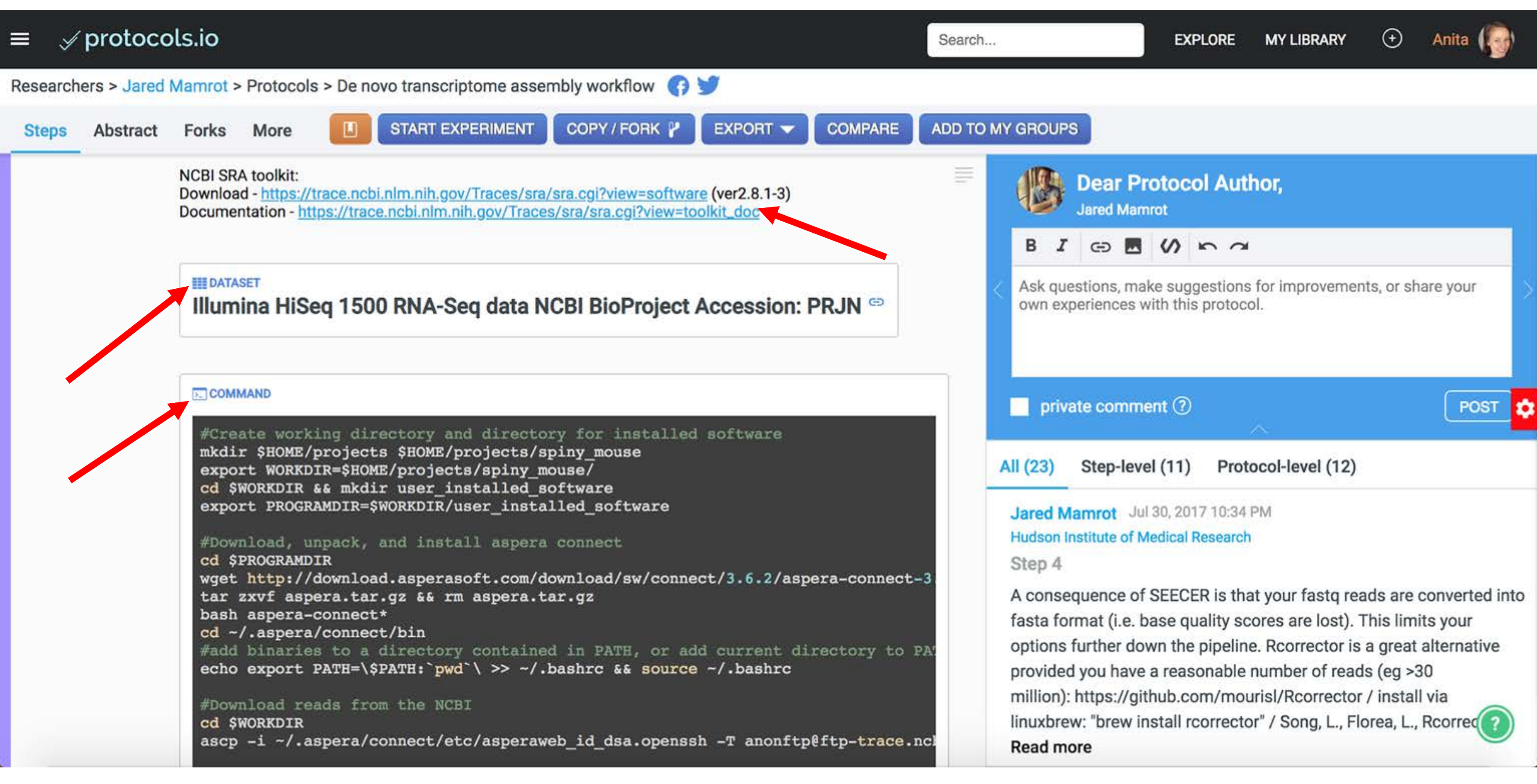
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DATASET
Illumina HiSeq 1500 RNA-Seq data NCBI BioProject Accession: PRJN
COMMAND
#Create working directory and directory for installed software
mkdir $HOME/projects $HOME/projects/spiny_mouse
export WORKDIR=$HOME/projects/spiny_mouse/
cd $WORKDIR && mkdir user_installed_software
export PROGRAMDIR=$WORKDIR/user_installed_software
#Download, unpack, and install aspera connect
cd $PROGRAMDIR
wget http://download.asperasoft.com/download/sw/connect/3.6.2/aspera-connect-3
tar zxvf aspera.tar.gz && rm aspera.tar.gz
bash aspera-connect*
cd ~/.aspera/connect/bin
#add binaries to a directory contained in PATH, or add current directory to PA
echo export PATH=\$PATH:`pwd`\ >> ~/.bashrc && source ~/.bashrc
#Download reads from the NCBI
cd $WORKDIR
ascp -i ~/.aspera/connect/etc/asperaweb_id_dsa.openssh -T anonftp@ftp-trace.nc
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Jared Mamrot
Ask questions, make suggestions for improvements, or share your own experiences with this protocol.
private comment
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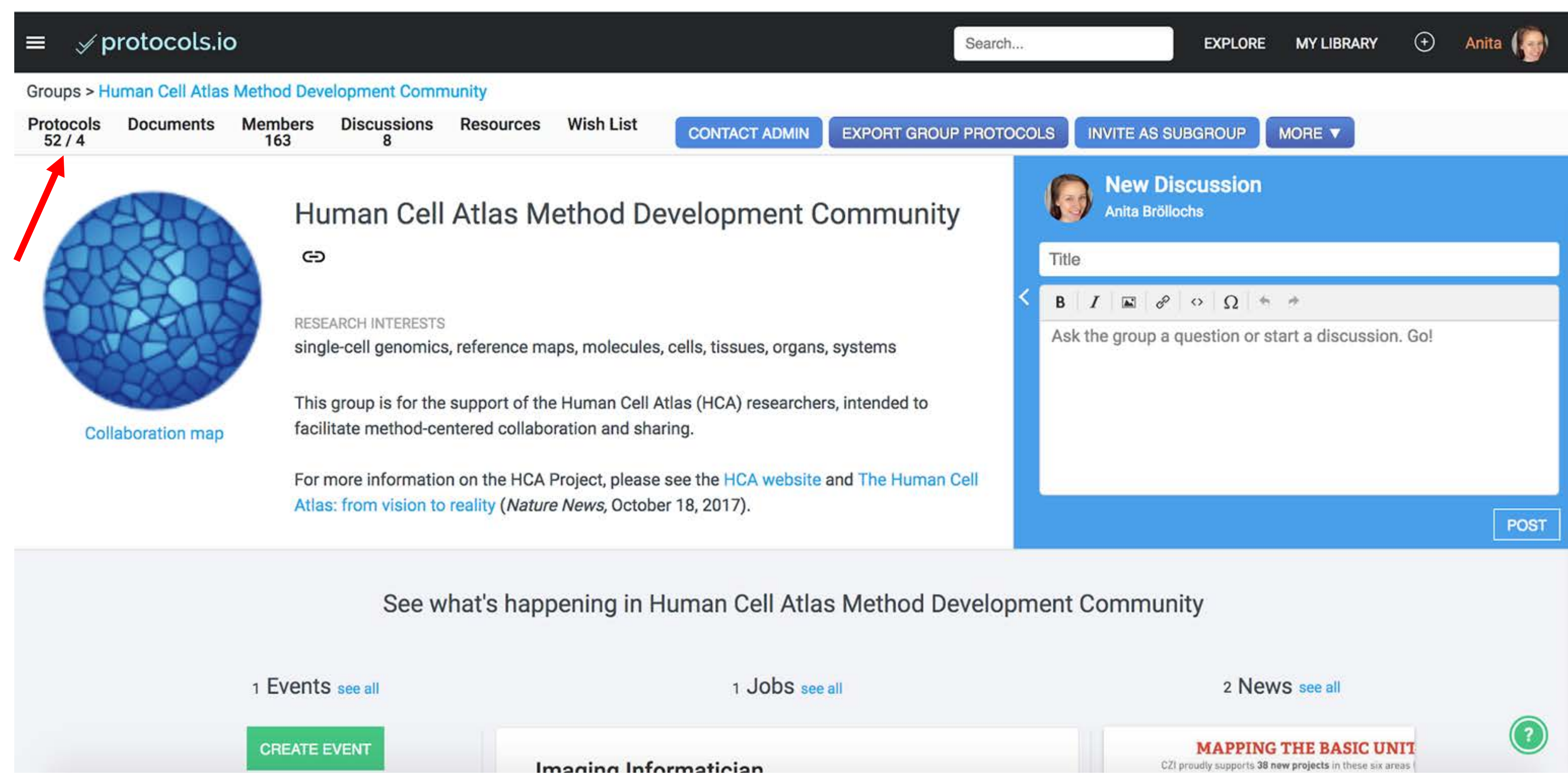
All (23)
Step-level (11)
Protocol-level (12)
Jared Mamrot Jul 30, 2017 10:34 PM
Hudson Institute of Medical Research
Step 4
A consequence of SEECER is that your fastq reads are converted into fasta format (i.e. base quality scores are lost). This limits your options further down the pipeline. Rcorrector is a great alternative provided you have a reasonable number of reads (eg >30 million): https://github.com/mourisl/Rcorrector / install via linuxbrew: "brew install rcorrector" / Song, L., Florea, L., Rcorrec
Read more



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For more information on the HCA Project, please see the HCA website and The Human Cell Atlas: from vision to reality (Nature News, October 18, 2017).
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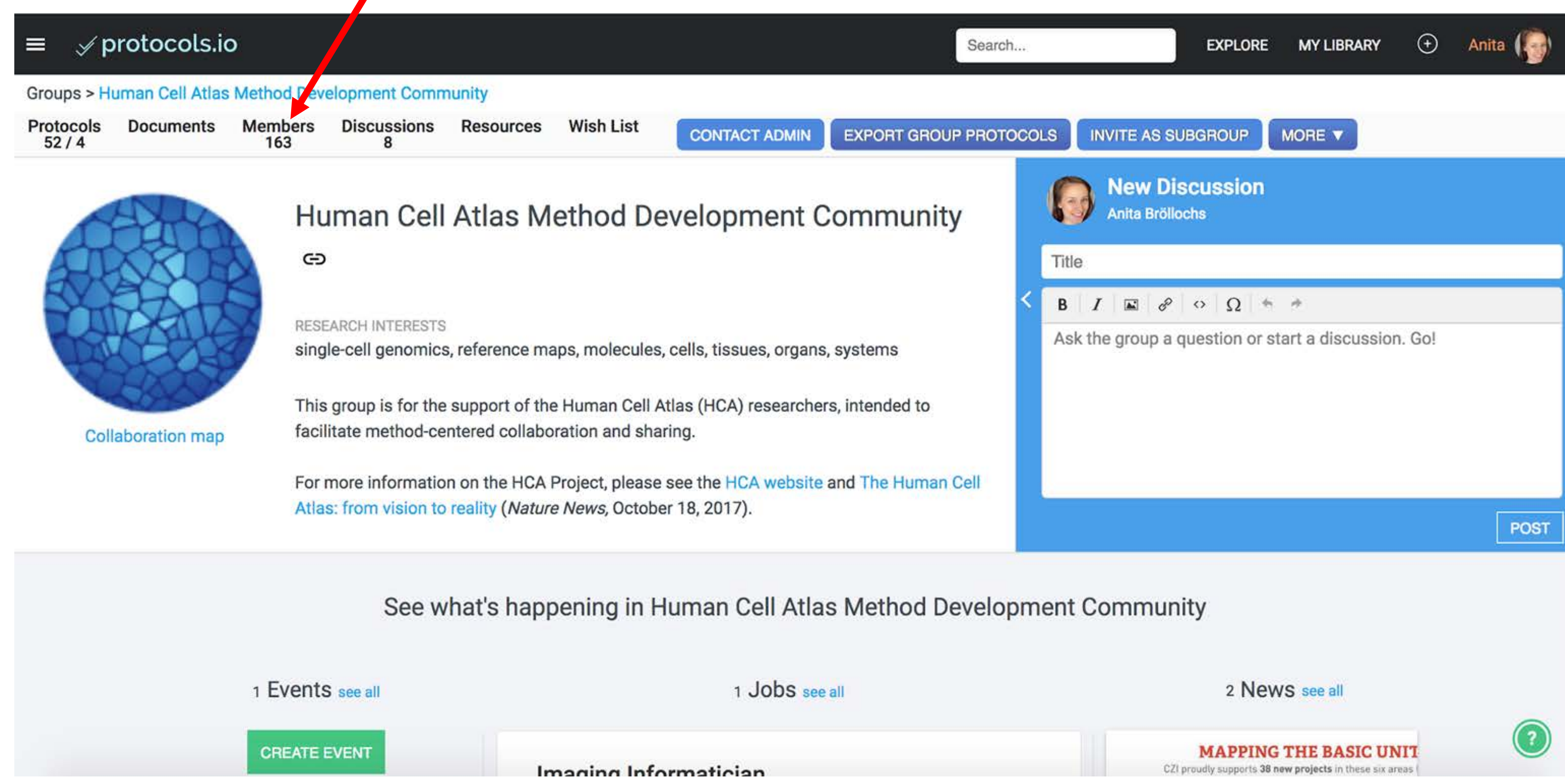

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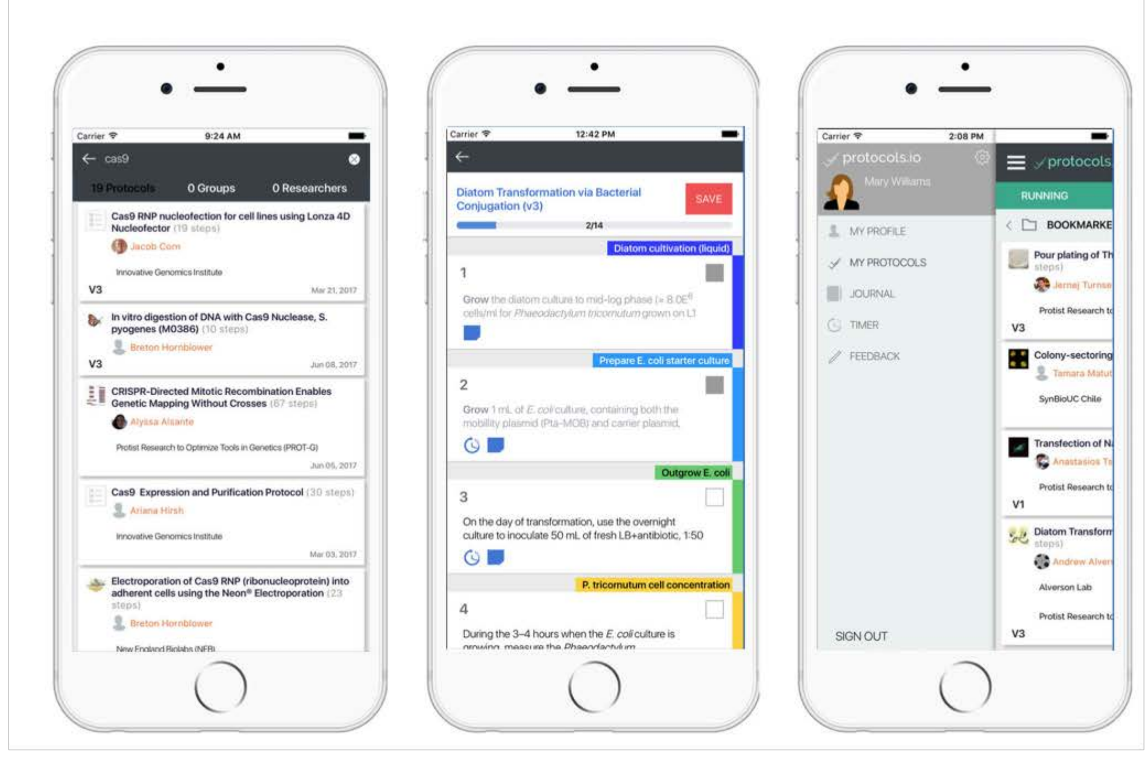

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