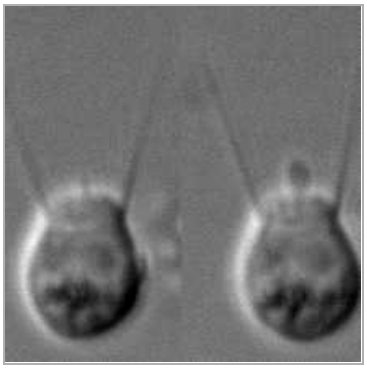

VERSION 1

MAR 05, 2023

OPEN ACCESS

Protocol Citation: Maxwell C Coyle 2023. Choanoflagellate Ciliogenesis Live Imaging. **protocols.io** https://protocols.io/view/choanoflagellate-ciliogenesis-live-imaging-cqjjvukn

MANUSCRIPT CITATION: Coyle, M. C. *et al.* An RFX transcription factor regulated ciliogenesis in the progenitors of choanoflagellates and animals. *bioRxiv* 2022.11.11.515474 (2022) doi:10.1101/2022.11.11.515474

Protocol status: Working
This works well although the efficiency of ciliary removal is not 100%, often closer to 80-90%.

Created: Mar 05, 2023

Last Modified: Mar 05, 2023

PROTOCOL integer ID: 78155

Choanoflagellate Ciliogenesis Live Imaging V.1

Maxwell C Coyle[1]

[1]University of California Berkeley

Maxwell C Coyle

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

This protocol removes the cilia/flagella from choanoflagellate cells and sets up the cells for live imaging of ciliogenesis. It has been developed for the species *Salpingoeca rosetta*, but may be portable into other choanoflagellate species. Cells begin to re-generate their cilia/flagella right after removal. The idea of using a cold shock with glycerol for ciliary removal came from Brokaw et al 1960 (doi: 10.1016/0014-4827(60)90027-6).

Concentrate cells and remove cilia

18m

1 Grow choanoflagellate cells in High Nutrient Media to a density of 1-2 x 10^6 cells/ml

2 Count cells by hemacytomer or automated cell counter*

*We use LUNA-FL Automated Fluorescence Cell Counter (Logos Biosystems L20001)

3 Aliquot and pellet 6 x 10^6 cells 2000 x g, 00:10:00

10m

4 Corona treat a fluorodish 5-10 seconds

Fluorodish **World Precision Instruments Catalog #FD35-100**

Equipment

BD-20AC Laboratory Corona Treater	NAME
Corona Treateer	TYPE
Electro-Technic Products	BRAND
12051A	SKU
https://www.electrotechnicproducts.com/bd-20ac-laboratory-corona-treater/	LINK

5 Rinse fluorodish for 5 seconds with 1 ml of 0.1 mg/ml poly-D-lysine, followed by 3x washes with 1 ml water. Dry by air or by capillary action of kimwipe, being careful to minimize contact with surface of imaging dish.

Poly-D-lysine hydrobromide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6407-5MG**

6 Rinse a coverslip (circular - 22mm diameter) in 70% EtOH followed by water and then lay on kimwipe to dry. Easiest to hold coverslip by forceps and dunk into 50 ml conical tubes with the ethanol or water.

7 When cells are done pelleting, remove supernatant and resuspend cell pellet in 800 µl of AKSW and transfer to 1.5 ml eppendorf tube.

8 Add 200 µl of 50% glycerol (final concentration: 10% glycerol) and mix by pipetting

9 Add cells to a second Fluorodish (i.e. one not treated with poly-D-lysine) and incubate

-20 °C 8 mins

Standard laboratory freezer is fine, but depending on exact temperature of your freezer or where in the freezer you place the cells, you may need to adjust the timing.

Set up cells for live imaging of ciliogenesis

18m

10 Transfer cells to 1.5 ml eppendorf tube and pellet 4200 x g, 00:08:00

8m

11 Remove supernatant and resuspend cells in 25 µl of AKSW

12 Transfer cells to Fluorodish coated with poly-D-lysine and lay clean coverslip slowly on top using forceps

13 Mount dish on microscope* and find focus. Let cells settle for 1 minute.

*We use a Zeiss Axio Observer.Z1/7 widefield with a 100x NA 1.40 Plan-Apochromatic oil immersion objective and a Hamamatsu Orca-Flash 4.0 LT CMOS digital camera

14 Float coverslip off of cells by adding AKSW around the side of the coverslip drop by drop with a plastic transfer pipette. If you don't do this, the cells will eventually suffocate.

15 Image!

On our system we use a short (5 ms exposure) with high light intensity (12.2 V) and a DIC condenser to get the best imaging of ciliogenesis. We use Zeiss Definite Focus and take a 10 µm z-stack with 1 µm between slices every 30 seconds for one hour.