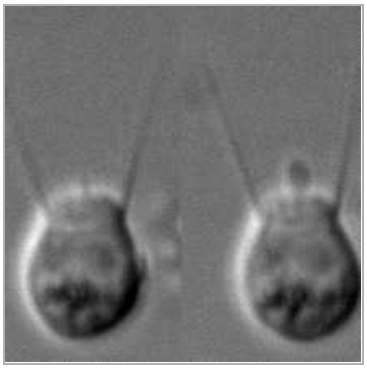

VERSION 2

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Choanoflagellate Ciliogenesis Live Imaging V.2

Maxwell C Coyle[1]

[1]University of California Berkeley

King Lab

Maxwell C Coyle

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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).

Protocol status: Working
This protocol works well, although de-ciliation efficiency is in the 80-90% range, not 100. Also you may need to adjust the timing of the -20C step depending on the exact temp and heat exchange of your freezer. The goal is to go as long as possible before the solution freezes.

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PROTOCOL integer ID: 78159

MATERIALS

High Nutrient Media: 4% AKCGM3 + 4% AKSWC in AKSW - Artificial Known Sea Water (See Booth 2018 Molecular Biology of the Cell for sea water details).

AKSW without addeed supplements

Incubator

Tabletop centrifuge

75cm^2 vented flasks

Haemocytomer or automated cell counter

16% Paraformaldehyde

0.1 mg/ml Poly-D-lysine

Forceps

Surface corona treater

Fluorodishes

Circular (22 mm diameter) coverslips

70% ethanol

50% glycerol

-20C freezer for incubation

Widefield microscope

Concentrate cells and remove cilia

1 Grow choanoflagellate cells (*Salpingoeca rosetta* fed with *Echinicola pacifica,* ATCC PRA-390) in High Nutrient Media to a density of 1-2 x 10^6 cells/ml. Grow at 22 ºC, 60% humidity

We grow 30 ml of culture in 75 cm^2 vented flask. Typically, inoculating this flask with a choanoflagellate cell density of 8,000 cells/ml 48 hours before ciliogenesis works well.

2 Count cells by haemacytomer or automated cell counter*. Shake culture flask vigorously to 5m

homogenize cell populatin and thene mix 99 µl of cell culture with 1 µl of 16% paraformaldehyde to fix cells for counting. Typically 10 µl of fixed cells can be loaded into a haemocytometer or automateed cell counting slide.

*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 1m

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. 2m

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. 1m

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. 1m

8 Add 200 µl of 50% glycerol (final concentration: 10% glycerol) and mix by pipetting 1m

9 Add cells to a second Fluorodish (i.e. one not treated with poly-D-lysine) and incubate 8m

-20 °C 7 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 13m

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 1m

12 Transfer cells to Fluorodish coated with poly-D-lysine and lay clean coverslip slowly on top using forceps 1m

13 Mount dish on microscope* and find focus. Let cells settle for 1 minute. 2m

*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. 1m

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.