Willsey Lab Neural Crest Cell Dissection Protocol

Helen Willsey Lab
Created and Updated: April 10th, 2024 by Micaela Lasser

WILLSEY LAB

This protocol is for dissecting primary embryonic neural crest cells (NCCs) from *Xenopus laevis* to visualize cell migration and cytoskeletal dynamics. The cytoskeleton is a dynamic network, composed of microtubules and actin filaments, that create the architecture of a cell's shape and control critical events like cell division, cell migration, and intracellular trafficking. Here, we provide a protocol to obtain and analyze cell migration and cytoskeletal dynamics in both live and fixed embryonic neural crest cells of *X. laevis* embryos.

Xenopus laevis neural crest cell dissection for live or fixed imaging

Adapted from: Laura Anne Lowery (see CSHL protocol here)

General Reagents:

- 1/3 MR (for *X. laevis*)
- 1X sterile filtered PBS
- 35mm glass bottom MatTek dishes (product # P35G-1.5-14-C)
- mRNA fluorophore-tagged protein of interest (see Table 1)
- Stage 18 *X. laevis* embryos (~18hrs post fertilization before neural tube has closed. I recommend keeping at 20°C overnight)

Reagents for neural crest cell culture:

- Fibronectin (20ug/mL; Sigma F1141)
- Gelatin (Sigma G1890)
- Plating culture media for NCCs (see recipes)

Equipment for neural tube dissections:

- Clay-coated petri dish
- Eyelash knife

PROTOCOL

1.) Culture Dish Coating

- ☐ Heat 2mg/mL gelatin dissolved in distilled water in the microwave until boiling; allow to boil 5-10sec until dissolved, then allow to cool for a minute.
- ☐ Add 300uL of cooled gelatin to the center of a 35mm MatTek glass bottom culture dish and leave for 10 minutes at RT.
- ☐ Discard the gelatin and rinse the dish with 1X sterile PBS three times.
- ☐ Add 300uL of 20ug/mL fibronectin in 1X PBS and incubate the dish overnight at 4°C.

**You can make dishes the day of dissection by incubating a dish with fibronectin at 37°C for 1 hour, but best results will be overnight incubation.*

- ☐ On the next day, remove the fibronectin. Rinse plate 3 times with 1X PBS and add 2mL of DFA media (see recipes) to culture cells.

2.) Neural Crest Culture

**A very helpful guide to neural crest cell isolation is given by Milet and Monsoro-Burq (2014). It is advised to refer to this for tissue identification and dissection technique. You will not be good at identifying NCCs without PRACTICE!*

- ☐ Prior to performing the dissections, prepare the culture plates as described above. Fill in the culture dishes with DFA media (see recipes)
- ☐ Sort embryos based on brightest fluorescence along neural folds and cranial neural crest region at stage 18.
- ☐ Transfer embryos to a clay coated petri dish containing DFA media and strip the vitelline membrane with forceps. Embed them gently in the clay dish with the anterior dorsal regions exposed.
    **neural crest cells emerge from the tissue just along the anterior neural fold, which is raised slightly from the surrounding tissue.*
- ☐ Remove the skin above the neural crest using an eyelash knife. Apply gentle pressure along the edge of the neural fold to allow the neural crest to separate. Lift the explant with a lateral/ventral flicking motion.
- ☐ Transfer the explants with a P20 pipette into rows along the fibronectin-coated coverslip culture dish.
- ☐ Allow the explants to adhere to the coverslip for at least 45 minutes at RT before imaging for live cell imaging of migration and cytoskeletal dynamics.
    **tissue will begin collective cell migration within an hour of plating, and will subsequently delaminate to begin single-cell movement after 8 hours or more.*
- ☐ If fixing cells, let explants adhere and migrate for at least 4 hours before fixation.

3.) Imaging and Analysis of Cytoskeletal Dynamics

- ☐ If imaging live explants for **<u>microtubule dynamics</u>**, follow imaging instructions below:
    - ☐ Take plates to confocal microscope and put on 63x oil objective lens
    - ☐ Use airyscan SR 4 color frame switch flexible program
    - ☐ Select time lapse imaging and set for **2 minute video length with 1 second intervals**
        ** It will image each channel separately, but I find that this gives the best resolution without photobleaching*
        ** You can also use airyscan multiplex imaging or airyscan line switch - each has its own pluses and minuses - faster imaging and will image both channels simultaneously but not as crisp resolution*

- ☐ Image 15 cells per condition
- ☐ Analyze videos in ImageJ using MTrackJ plugin
- ☐ If imaging live explants for **cell migration**, follow imaging instructions below:
  - ☐ Use 20x phase contrast objective

    ** This is the best objective to use for very long time lapse imaging of NCC explants. NCCs will not migrate well with long exposure to lasers. Using phase contrast exposes NCCs to minimal amounts of light so that their migration is not obstructed.*
    - ☐ Set up time lapse imaging for **6 hours with 6 minute intervals**
    - ☐ Set up tiles that capture the entire explant PLUS surrounding area (cells will migrate VERY far away from initial explant; typically 3x3 is sufficient)
    - ☐ Go through and set your Z for each explant
    - ☐ Let time lapse run and wait one full cycle before leaving to confirm every explant is in frame and is in focus
    - ☐ If using guidance cues, flood in cues during imaging by using two P1000 pipettes - simultaneously pipette out media in dish and pipette in media containing guidance cue

RECIPES

Plating Culture Media (DFA)

Reagent	Amount	Final Concentration
NaCl	1.55g	53mM
Na_2CO_3	0.265g	5mM
Potassium gluconate	0.527g	4.5mM
Sodium gluconate	3.82g	35mM
$MgSO_4$	0.060g	1mM
$CaCl_2$	0.055g	1mM

Add reagents to 400mL deionized water. Adjust the pH to 8.0 with bicine and bring to a final volume of 500mL with deionized water. Sterilize by filtration. Store frozen at -20°C in small aliquots.

Table 1. Useful cytoskeletal markers to visualize microtubules, actin filaments, and focal adhesions

Marker	Labels	Purpose	Concentration per embryo	Imaging Technique
EB3-GFP, EB3-mCherry	MT plus-end	MT growth dynamics	60pg mRNA	Spinning disk confocal microscopy (SDCM)

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LifeAct-GFP, LifeAct-mCherry, LifeAct-mGarnet	F-actin	Actin dynamics	60pg mRNA	SDCM, TIRF
α-tubulin-mCherry	MTs	Uniform labeling to visualize MTs	60pg mRNA	SDCM
Paxillin-mCherry	Focal adhesion	Focal adhesion dynamics	20pg DNA	SDCM, TIRF