

Overview of lab

Short talk to introduce lab

In vivo labeling and neomycin treatment

~2 hr prep and then examination of larvae
groups of 2 (benchmates)

Testing rheotaxis behavior

~ 20 minute assay sometime during incubation intervals for neo treatment
groups of 4-5 (long benches)

As time permits:

Examine wildtype fish under the stereomicroscope.

Place embryo into ~7 ml of embryo media with 350 ul MESAB,

Examine transgenic embryos under the fluorescent dissecting scopes.

Place embryo into ~7 ml of embryo media with 350 ul MESAB.

Mount an embryo for confocal (transgenic or dye labeled):

1. Place embryo into ~7 ml of embryo media with 350 ul MESAB.
2. Using wide bore pipette, transfer fish in as small a volume as possible into 3 ml culture tube with low melting agarose.
3. Immediately pipette up embryos in agarose and onto bottom of Matek dish.
4. Using either Dumont forceps or poker/looper (fishing line in capillary glass), orient fish.
5. Allow agarose to harden (~5-10 min).
6. Add a few drops of embryo medium with MESAB.
7. Examine on confocal.

Later tonight we can set up a time-lapse with neomycin.

Transgenics available:

Brn3c: gfp

gfp under control of the hair cell specific brn3c promoter.

Labels HCs in lateral line and inner ear)

Tg(H2AZ::gfp); Tg(alpha-tubulin::tomato

Histone H2 Az variant fused to EGFP, labels nuclei of all cells

Alpha-tubulin fused to tomato fluorescent protein, labels hair cells

Tg(NeuroD::gfp); Tg(alpha-tubulin::tomato

NeuroD protein fused to EGFP, labels afferent nerves

Alpha-tubulin fused to tomato fluorescent protein, labels hair cells

In vivo labeling and neomycin treatment

Reagents:

YO-PRO1, 1 mM	dilute 63 ul in 21 ml embryo media (3 uM final)
FM1-43FX, 1mM	dilute 63 ul in 21 ml embryo media (3 uM final)
DASPEI, 0.05%	add 710 ul per well
Neomycin	dilute to 400 uM (614 ul in 25 ml) and serially to 50 μ M

One plate with 6 basket inserts
12 plates (without baskets)
one wide depression slide
poker/loop for manipulating embryos
One petri dish of ~ 60 wildtype fish

THROUGHOUT protocol,

Only transfer fish with wide-bore pasteur pipettes.
Keep fish in incubator between handling (28 degrees C)
Gently swirl entire plate after adding fish to new reagents to mix well.
Keep fish covered in foil once label with fluorescent dyes.

1. Label 6 baskets on the side:
FM/Y 0 =FM1-434FX, YO-PRO-1 labeled fish, mock treated
FM/Y 50, =FM1-434FX, YO-PRO-1 labeled fish, 50 μ M neomycin
FM/Y 400 =FM1-434FX, YO-PRO-1 labeled fish, 400 μ M neomycin
D0, =DASPEI labeled fish, mock treated
D50 =DASPEI labeled fish, 50 μ M neomycin
D400 =DASPEI labeled fish, 400 μ M neomycin
2. Plate fish in baskets containing embryo medium in a 6 well plate using wide-bore pipette (8-10 fish in each of 6 baskets). LEAVE a few fish in the original Petri dish to examine under stereomicroscopes.

FM1-43 labeling:

2. Dilute FM1-43FX in embryo medium to 3 μ M final (63 μ l in 21 ml embryo media).
3. Fill the wells of two plates with embryo medium for washes.
4. In a third plate, fill 3 wells with 7 ml diluted FM1-43FX.
5. Transfer the **3** baskets of fish (FM/Y labeled baskets) to FM1-43FX for 45 SECONDS. Keep fish covered with foil.
6. Rinse fish rapidly in wash plates, four washes per basket. DO NOT let fish dry out between rinses. DO not let medium drain.

YO-PRO1 labeling

7. Transfer 3 baskets of fish labeled with FM1-43FX into diluted YO-PRO1 (7 ml/well). Place fish in incubator for 30 min.

- Rinse 3 baskets of FM1-43FX/Yo_PRO1 labeled fish 4x in embryo medium (ie two 6 well plates).

Neomycin treatment:

- Dilute neomycin to 400 μM (614 μl in 25 ml embryo medium) and serially dilute to 50 μM (2 ml 400 μM neomycin in 14 ml embryo medium).
- Set up one plate with two wells of embryo medium only, two wells of 50 μM neomycin and two wells of 400 μM neomycin.
- Transfer baskets into the appropriate wells and incubate for 60 minutes.
- Rinse embryos four times in fresh embryo medium.

Six well plate for neo treatment:

	FM1-43 FX/YOPR O Labeled fish	Not labeled (yet)
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Embryo medium only	FM/Y 0	D0
50 μM neomycin	FM/Y 50	D50
400 μM neomycin	FM/Y 400	D400

DASPEI labeling:

- TO ONLY the three DASPEI baskets (D0, D50, D400),** add 710 μl 0.05% DASPEI (.005% final). Incubate 15 minutes.
- Rinse these three baskets twice in fresh embryo medium.

View embryos

- Anesthetize fish in all six baskets with MESAB (350 μl per basket).
- Use wide bore pipette to transfer embryo to depression slide and examine on one of the fluorescent dissecting scopes. Optionally, these embryos can be mounted in low melt agarose for confocal.