AQUATIC ZEBRAFISH CORE

Mounting Zebrafish Larvae for Microscopy

There are various techniques to mount larvae for microscopy. They can be put on a drop of methyl cellulose, a good technique for an upright microscope, or they can be mounted in low melting point agarose or under thin glass fibers in a glass bottom dish, which is adequate for inverted microscopes and confocal microscopies. Agarose is also used for time-lapse imaging. For longer experiments the agarose has to be removed around the tail and/ or head to give the larvae room to grow.

- Anesthetize larvae with 0.1-0.3mg/ml Tricaine. For longer experiments 0.1mg/ml should be used (250ul 4mg/ml in 10ml E3).

Mounting on Methyl Cellulose

Mounting on cellulose is a good technique to get a quick picture. However, fish do not stay steady for an extended time.

- Use a probe to scoop some methyl cellulose on a depression slide.
- Create a line that is about 3mm thick and wide.
- Transfer larvae to slide and mount on Cellulose with probe by gently pressing them into Cellulose.

Mounting in Agarose

- Make a 1% agarose solution by adding 100mg low melting agarose (SeaPlaque #50101) to 10 ml of E3 in a 50 ml tube, mix well.
- Boil up in microwave: put open tube in small beaker to upright, put beaker off center to avoid boil over. Wear heat resistant gloves safety glasses. Watch while heating and stop microwave as soon as agarose starts to boil, mix. Repeat 1-2 times till all agarose is dissolved.
- Cool in 37°C water bath for at least 10 minutes, add 250 ul Tricaine.
- Float glass bottom dish in water bath and warm probe to 37°C in bath.
- Use a pipette to transfer larvae to agar in as little liquid as possible.
- Pipette larvae from agar on glass bottom dish, use pre-warmed probe to arrange.

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- Transfer dish under dissecting scope and hold in air. Arrange larvae to desired position with heated probe.
- To cool agarose rest dish on glass plate of microscope with some water under plate for better contact. Check positioning while agarose hardens.
- Larvae can stay in agar for about 15 minutes before it starts to dry out. If transport is necessary transport them without additional liquid. Add liquid once arrived at destination, otherwise the agar plug might float off the dish. Use E3 with Tricaine to cover agar.