Casting agarose arrays v2 SOP

Background

Agarose arrays of slots are used to ensure the zebrafish lie in a regular pattern. These agarose arrays need to be cast prior to fish sorting experiments.

There are two molds, one for sorting embryos and one for sorting larvae. The embryo mold contains a grid of 400 wells. The larvae mold contains a grid of 595 wells. The top left and bottom right wells for both molds are triangular shaped. These are used to locate the corners for calibration of the fish sorter instrument. The remaining wells are circular embryos and rectangular for larvae.

Agarose arrays can be reused several times if stored properly (ie. in a fridge, filled with E3 media and covered with a lid to prevent drying out). Agarose arrays should be replaced when any of the following is observed:

- Damage
- Shrinkage or change in shape (due to array drying out)
- Cloudiness (which hurts imaging quality)

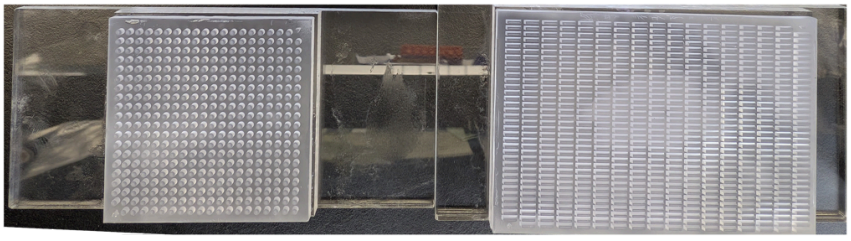
Materials

- Thermo Fisher Nunc OmniTray
  - Nunc™ OmniTray™ Single-Well Plate
- Male grating mold (see top of document for link/ID for latest version)
- Agarose powder (Bio-Rad Certified Molecular Biology Agarose, Cat # 1613102 is confirmed to produce successful molds for arraying and imaging of fish)
- E3 media
- 25 mL serological pipette
- **(Optional)** Razor blade

Molds:

Current male molds: `V53 - Zebrafish sorting`

- Larvae: Use configuration `Large - sorting - 2 corner`
- Embryo: Use configuration `Medium-sorting-2 Corner`



Version 53 Fish Sorter v2, Left: embryo mold, Right: larvae mold

Workflow

Cleaning mold and OmniTray

The OmniTrays are reusable. Both the mold and OmniTray should be cleaned before use:

1. Rinse the mold and OmniTrays with Di-water
2. Dry with house dry compressed air
    a. Be sure to dry the mold very well
3. Spray the OmniTray with 70% ethanol
4. Dry with house dry compressed air

Casting agarose array

1. Make a 2% agarose solution, dissolved in E3 media
    a. Typically prepare 100 mL of 2% agarose:
        i. weigh 2 g agarose
        ii. add 100 mL E3 media
        iii. heat in the microwave to dissolve
2. Aspirate 15 mL agarose solution with the serological pipette
    a. If you have extra agarose, store in a sealed jar in the incubator set to 68 deg C so the agarose stays liquid and can be used in future casts
3. Dispense the agarose solution in the OmniTray
4. Wet the mold in the agarose (to minimize bubbles on mold surface while casting)
5. Place the mold over the agarose solution
6. Check for bubbles by lifting the OmniTray and viewing the agarose/mold through the clear bottom surface
    a. If there are bubbles, lift the mold out of the agarose and place the mold down again
    b. Repeat as necessary
7. Place a heavy item (eg. E3 media jar) over the mold
8. Set agarose for 15 minutes

Embryo and larvae molds in

Removing the mold

1. Add E3 media into the plate, with the male mold still in place. (This make it easier to separate the mold from the agarose)
2. Attempt to slowly lift the mold, starting from one corner
    a. Once a single corner is lifted, E3 media should flow between the mold and the agarose
    b. The whole mold should then be easy to remove
3. **(Optional)** If it's difficult to perform Step 2 without tearing the agarose, try the following:
    a. Use a razor blade to separate the outer edges of the mold from the agarose
    b. Repeat Step 2

4. Cover with the OmniTray lid and store in the refrigerator until used

Storing agarose array

After experiments, fill the agarose array with E3 media and cover with the lid before storing in a fridge. This will prevent the agarose from drying out.

Label each agarose array with the following:

- Date
- Male mold version and slot size (eg. S/M/L)
- Agarose solution percentage (eg. 2%) and media (eg. E3)
- Unique identifier (eg. A, B, C, etc.) if multiple agarose arrays are cast on the same day