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Eggs injections

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

Protocol for microinjections of 0-dpf eggs @FishFloorUCL.



Set-up breeding pairs

- 1 Set up the breeding pairs the night before or order them from the fish facility. Arrive early in the morning, 9AM for single cell, 9:30 for fish facility injections.

Make sure to book the injector in advance.

Calibrate the needle

- 2 Open the red valve for your injector, you should see the dial turn to ~40
- 3 Power on switch
- 4 Set the time to 14-15ms
- 5 Take a needle from the large Petri dish on the side and stick in the blue tack above the microscope
- 6 Add what you want to inject (less is better, eg: 1-3 nL)
- 7 Place the black measuring slide under the microscope, add a drop or two of mineral oil and focus on the little ruler.
- 8 When tilting the whole needle base use both hands. When not using make sure it is tilted up
- 9 Tilt the needle into the oil
- 10 Break the needle with forceps and press the pedal until the correct amount is coming out of your needle (using the table on the wall to calibrate)

Collect eggs

- 11 Take the dividers out of the fish boxes



12 wait 6-7 minutes

13 Transfer the fish to new boxes and replace the dividers for another round of eggs

14 Collect the eggs by pouring the water through a small sieve and transferring this to a Petri dish, clearly labelled

Inject the eggs

15 Use a Pasteur pipette to transfer eggs to a new pipette

16 Use a thin ended pipette to remove all the water and then line them up along the edge of a glass slide placed in the Petri dish

17 Go down the line of cells and inject one at a time

18 Collect a new clutch and repeat.

Turn everything off/clean after yourself

19 Turn off the red tap.

20 Turn time to minutes, press pedal until the dial has run completely down, flick it back to ms

21 Turn off the power switch

22 Wipe down everything with ethanol.