SOP-P049

Preparation of Grass Carp Fry for Triploid Screening

Objective: To prepare fish fry samples for DNA ploidy analysis, for J.M Malone & Sons.

NOTE: Samples come in large bags of about 1 liter of water with very small fish (fry) in them. Each bag will be labeled as either a diploid control, or with a batch number. Must do in duplicate, one bag = one batch. So will have samp1es 1A and 1B for batch #1. **MUST ONLY SAMPLE THE LIVE FRY.**

Procedure:

1. Check all bags for leakages. (One bag = one batch)
2. Estimate viability and record for all batches.
3. Carefully cut open one corner of the bag, and carefully empty the contents into a 2 liter beaker properly labeled with the batch number.
4. Using a serological pipet, transfer some of the fry to a large (14cm) petry dish.
5. Using a long (9inch) Pasteur pipet, aspirate **ONLY live fry** from this petry dish, into the pipet.
6. Count them as you expel them from the Pasteur pipet onto a mesh screen, which is over a standard size petry dish. You need to count 50 fry for each duplicate (100 fry total for each batch). So sample 1A will contain 50 fry from batch 1, and sample 1B will contain 50 fry from batch 1.
7. Before smashing the fry, pour off the liquid from the petry dish, and add back 5ml of PBS to the fry.
8. Smash the fry through the screen using a rubber policeman.
9. Transfer the smashed fry sample to a clean, labeled 15cc conical tube.
10. Filter approximately 2.5-3ml thru a 60µn nylon mesh filter into a clean, labeled 12 x 75mm test tube.
11. Filter again thru a 30µn nylon mesh filter into a clean, labeled 12x75mm test tube.
12. Transfer 1 ml of the filtered fry sample into a clean, labeled 12x75mm test tube.
13. Add 1 ml of modified Vindelov's Propidium Iodide stain.
14. Vortex 1 second.
15. Cap the tube.
16. Incubate 1 hour on ice (or 4°), protected from the light.
17. Run on FC500, using fry protocol.
18. Run the diploid control samples first, centering the peak in the gate on the left labeled diploid.
19. Collect 5,000 cells per sample.
20. Print the data as the tubes are run.
21. Create the report to fax to J.M. Malone & Sons in EXCEL go to
    a. n:\flow\outside projects\fry\report
    b. open one of the previous reports and save with a new name (use Malone, then the date of the samples)
    c. delete the old values
    d. enter the %diploid and %triploid from the cytometer printout
    e. edit the title of the report to reflect the corrected date of the sample.
    f. Save and print the report

22. Fax a cover letter and the excel data sheet to Beverly Jones at J.M. Malone & Sons.
    a. In the fax cover letter be sure to indicate the status of the batches, i.e. which bags, if any leaked out, and if there were not enough fry to get 50 per sample for any of the batches.
    b. Sample cover letters can be found in n:\flow\outside projects\fry\fax
23. Dispose of remaining fry. **DO NOT PUT LIVE FRY DOWN THE SINK.**
    a. Pour some bleach into the beaker containing the unsampled fry.

b. Stir it around a bit with the serological pipet left after aspirating the sampled fry.
c. Let sit till all fish are dead.
d. Discard down the sink flushing with lots of water.

Contact information:

Beverly Jones, or Robert Glennon

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Verified by:_____Date:_____ _____

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