Tail Clipping Larval Zebrafish

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COMMENTS 0

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IN DEVELOPMENT

ABSTRACT

Tail clipping of ~ 2–4 dpf zebrafish larvae for genotyping.

PROTOCOL CITATION

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Introduction

1 On FishFloor UCL, Gareth Powell would be the best person to ask for a live demonstration.

Technique originally published by Kosuta et al (2018) – video protocol:

https://www.jove.com/v/58024/high-throughput-dna-extraction-genotyping-3dpf-zebrafish-larvae-fin

Kosuta, C., et al. (2018). “High-throughput DNA Extraction and Genotyping of 3dpf Zebrafish Larvae by Fin Clipping.” Journal of Visualized Experiments(136).

This method can be used to genotype larvae before they are 5 dpf. This way, only growing up larvae with the desired genotype (e.g., when generating a new line). This reduces the number of fish used and fulfils the 3R aims of the Home Office.

Cons

- It is a time-consuming process. Therefore, it is advised that you first ensure that your parent fish does have the mutation in their germ line. To do this, first take a cohort of embryos, extract their DNA and sequence it, or do a headloop PCR, to check. This adds time to an already laborious technique.
- The Fish Facility does not currently give you enough time to screen the animals before they are put into the nursery.
- The first lab to use this technique have grown these fish to adulthood and bred them with no issues - that has not been achieved at UCL though so it is likely there will be a drop off in survival

It would be a good idea to try this first with raising a new generation of a line that we know doesn't have any problems with viability in the nursery, just to check that the tail clipping doesn't introduce any additional mortality in our system. Other labs might not have experienced problems, but our systems and our regulations are different.

It is worth verifying that it is included in your project license too, before you go ahead and use the procedure.

Materials

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- 1X Tricane in fish water (to anaesthetise the fish)
- Forceps
- Microfeather 30o microblade
- a Petri dish with autoclave tape stuck to the bottom in a strip – this will be the hard surface on which to cut

Procedure

3 Fish need to be at least 48 hours post fertilisation

1. Use a Pasteur pipette to add the fish to the 1X tricane solution and wait for it to stop moving. It is best to do the fish one at a time to avoid over anesthetising.
2. Use the pipette to transfer the fish on to the tape on the bottom of a Petri dish. You may want to use a piece of tissue to soak up some of the excess water.
3. You want to cut the tail without clipping the blood vessels (indicated by red arrows below). Just after the blood vessel turns back on itself, there is a small pigment gap. You can use this as a guide of where to cut the tail (indicated by the black dotted line below).
4. Use the forceps to pick up the tail end (or a piece of tissue – you can then put the whole tissue fragment in to the well).
5. Put the fish in to fresh fish water and check 5 minutes later that they have revived by gently poking them with a pipette and seeing if they move.

If you are doing this procedure to genotype, you are going to put the tail ends and fish in to separate 96 well plates. Make sure the tail well corresponds to the fish well so you can keep track of which fish is which.

This video (YouTube) shows the blood vessels very clearly.