Do Buccal swabs from Zebrafish give enough of a sample of DNA to be used as a viable non-invasive method of genotyping?

Sarah Lawton, Manchester University

Abstract

The Zebrafish has become a valuable research model within the laboratory. Due to the increased numbers used of transgenic Zebrafish, genotyping of the fish has become an everyday necessity to facilitate their use in order to identify the fish with the correct or necessary genes. Genotyping of the fish is usually performed at 6 months of age. By this time, the fish are classed as adults in size and are ready for breeding.

Currently the proven method of genotyping involves taking a tail fin clip from the fish to gain the sample. This involves surgically removing a piece of the tail fin under anaesthetic.

In previous studies buccal swabs have been taken from different species such as humans, mammals and larger species of fish. This involves taking a swab from the inside of the mouth, gathering cells which are used to extract the DNA. This study investigates if taking buccal swabs from zebrafish gives enough of a sample of DNA to be a viable non-invasive method for genotyping.

This project was conducted within the principles of the 3Rs (Reduction, Refinement, and Replacement).

All fish were euthanised prior to sample taking to prevent risk of suffering.

Sample taking.

The sample taking method for each fish needs to be repeatable. The method will change for each experimental group depending on the number of rotations of the swab but everything else will remain the same. For the purposes of this study I tested for the gene IPIP27A_F.

All equipment needs be set out beforehand ready for the sample taking.

Materials and Procedure.

Remove one fish at a time from the MS222, selecting each fish noting if it is male or female. Start the timer. Gently dab the fish on paper towel (TORK Hygiene Roll) to remove excess water, and place the fish on sampling area, facing forwards towards you.

Using tweezers (Pake Dissecting Forceps Curved Fine Serrated Jaw 115m), gently open the mouth of the fish, just wide enough to be able to insert the swab (TAMIYA Craft Cotton Swab, Triangular, Extra small, 50PCS. Insert the swab. Remove tweezers from the mouth, leaving the swab in place, allowing the mouth to close around the tip of the swab. Rotate the swab for the necessary amount of times depending upon the experimental group. Remove swab from mouth, ensuring not to touch anything with the tip. Keeping hold, cut off the tip with the sample on, off into the correct epindorph tube with scissors. Close the lid on the epindorph tube and stop the timer.

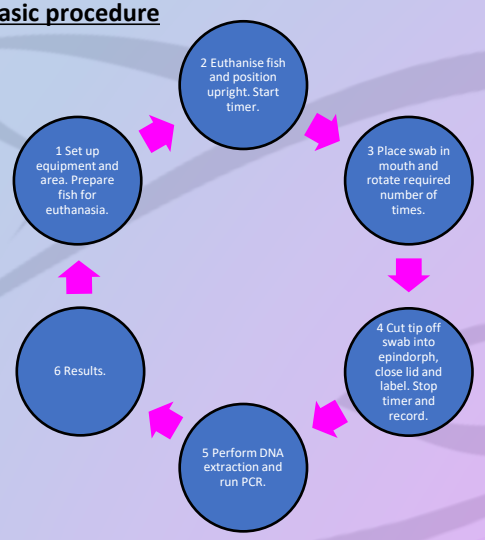
Dispose of the holding end of swab into the bin.

Now an inspection of the fish is carried out, looking for any signs of damage to the mouth.

Record all results on the recording sheets and take any pictures.

The fish can now be disposed of in the yellow disposable bag. Reset the timer and wipe down the tweezers ready for the next fish.

Basic procedure



DNA Extraction and PCR.

First add 50 milimolar (mM) of Sodium Hydroxide to the sample inside the epindorph tube, repeating for all samples. Vortex each sample for 5 seconds, then heat all samples to 95oc for 20 mins in a thermal cylinder. After this add 5 mM solution Tris buffer (PH8 1 molar (M) solution) to each sample. The samples can now be stored at 4oc ready for PCR.

For PCR begin with using 0.2ml PCR tubes, adding 2ul of the DNA sample mixed with 8.5 ul H2O, 1 ul of primer IPIP27A_F, 1ul of primer IPIP27A_R, and 12.5 ul GoTag® green master mix (Promega). Run this on the following PCR cycle using a Techne 3Prime Thermal Cycler for:

Run Order	Cycles	Temperature (Degrees Celsius)	Time (seconds)
1	1	95	120
2	30	95	30
		60	30
		72	30
3	1	72	120
4	1	4	Hold

These samples are then loaded onto a 2.5% Agarose gel containing SafeView and run at 100V for 40 minutes. They are then imaged using an UV transilluminator.

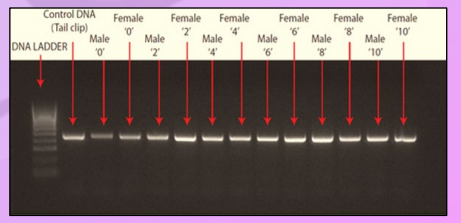
Results

The results gained from set 1 were very promising. Each sample gave a positive result when compared to the control sample. In total 12 samples were taken, 2 samples for a range of rotations (1 male and 1 female for each) including 0, 2, 4, 6, 8,and 10. This helps to indicate firstly if a DNA positive result can be given and secondly if there are any differences to be found between the number of rotations.

The first male sample of 0 rotations is not as clear as the rest of the samples. Due to this being the first sample, which had been taken it may be the technique that was the cause. It is not possible to rule out that this may also be a result of a lack of DNA being taken due to a low number of rotations. The signal from the results becomes very strong on the female sample of 2 rotations and remains strong throughout the rest of the samples.

Pictures of the fish taken post sampling will to help evaluate if there were any visible signs of damage being caused. From the pictures, no signs of mouth damage are visible.

UV transilluminator image of results for Set 1



Record sheet for set 1

TRIAL RUN	STRAIN	D.O.B	SEX	NUMBER OF ROTATIONS	TIME TAKEN (seconds)	SIGNS OF DAMAGE
1	WT	29.1.2015	M	0	56.4	NO
2	WT	29.1.2015	F	0	19.7	NO
1	WT	29.1.2015	M	2	24.4	NO
2	WT	29.1.2015	F	2	15.3	NO
1	WT	29.1.2015	M	4	27.2	NO
2	WT	29.1.2015	F	4	16.5	NO
1	WT	29.1.2015	M	6	35.2	NO
2	WT	29.1.2015	F	6	20.9	NO
1	WT	29.1.2015	M	8	27.8	NO
2	WT	29.1.2015	F	8	22.9	NO
1	WT	29.1.2015	M	10	29.3	NO
2	WT	29.1.2015	F	10	22.1	NO

Further investigations

This method of genotyping for Zebrafish needs to be further investigated. The results gained from this project show that it is possible to gain a positive result using this method, but it has been limited to using only euthanised animals, being tested using a single gene. For the future, investigations into other genes will be needed to ensure it is functional across the Zebrafish genome. Investigations using live animals will be necessary to ensure that there is no damage to the mouth area, or any other lasting harm has occurred, and that the fish are able to function fully after the procedure.

The length of time (seconds), taken to take each sample is something to look at reducing. A possible cause may be due to using tweezers to open the mouth of the fish first which is no longer necessary, as the mouth will open when touched by the swab.

.

For full paper e-mail
Sarah.Lawton@Manchester.ac.uk

Special thanks to Graham Morrissey (Director, BSF), Adam Hurlstone, and Anthony Jackson.