gDNA PCR GENOTYPING PROTOCOL FOR ZEBRAFISH

1. Supplies needed for tissue isolation:
    a. Plastic beakers - labeled by number (one beaker per fish to be genotyped)
    b. Thermocycler tubes (typically 8- or 12-tube strips) numbered so that each plastic beaker has a corresponding tube
    c. Fresh razor blade
    d. Forceps
    e. Plastic spoon
    f. Beaker with Tricaine solution for anesthetizing fish
    g. Spray bottle with 70% ethanol
    h. Kimwipes
    i. Place a clean strip of tape along a bench top for caudal fin clipping

2. Prepare 25 μl of lysis buffer per gDNA isolation according to the recipe below. Distribute to thermocycler tubes. Solution may be kept at room temperature during tissue isolation.

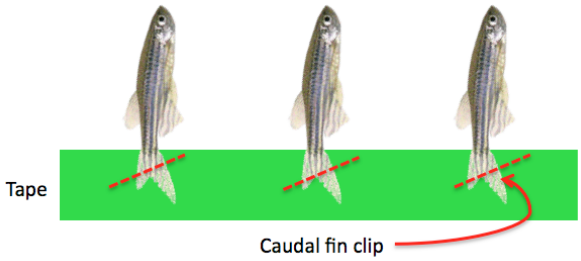
3. Place 2-3 fish at a time in a beaker with Tricaine. When the fish are anesthetized, remove them with the spoon and array on the bench so that their caudal fins are lying on the tape (see figure). With a razor blade, amputate a small fragment of the caudal fin. Transfer the tissue with forceps into the lysis buffer. Make certain that the entire tissue is submerged in the buffer.

4. CLEAN THE RAZOR BLADE AND FORCEPS WITH 70% ETHANOL AFTER EACH AMPUTATION TO AVOID CROSS-CONTAMINATION.

5. Cap the tubes and incubate the reactions at 65˚C in the thermocycler for 1 hour. Remove the tubes and vortex briefly to dissolve the tissue. BE SURE THAT THE CAPS ARE FULLY CLOSED PRIOR TO VORTEXING. Quickspin the samples and return to thermocycler. Incubate at 65˚C for another 30 minutes.

6. Inactivate the proteinase K in the reaction by heating to 95˚C for 15 minutes. Remove reactions and carefully vortex to mix. Quickspin the samples to ensure that the contents are at the bottom of the tube. The gDNA is now ready for subsequent PCR analysis. Samples can be stored at -20˚C for future use.

7. Set up the PCR reaction according to the conditions below.



Lysis buffer (per sample):

Water	21.25 µl
10X Taq buffer (with or without Mg2+)	2.5 µl
10 mg/ml proteinase K	1.25 µl
Total volume	**25 µl**

PCR conditions (per sample):

Water	17.85 µl
DNA	2 µl
Taq buffer	3 µl
Primer #1	1 µl
Primer #2	1 µl
dNTP (2.5 mM)	5 µl
Taq Polymerase	0.15 µl
Total volume	**30 µl**

PCR reaction profile: 55C60s

95˚C	2 min	
95˚C	30s	30x
55˚C	45s	
72˚C	60s	
72˚C	10 min	
10˚C	Forever	