DNA Sampling Protocol

DNA tissue samples should be taken using the protocols below and submitted to the Molecular Genetics Lab, Pacific Biological Station, Nanaimo. Samples should be submitted with proper vial labelling and documentation.

1) Sampling instructions fresh tissue –individual or bulk sample

- Use paper punch to take a tissue sample from the preferred location on fish (see below).
- **Bulk sample** - where tissue from greater than one fish is placed in a bottle or vial, but only one tissue per fish. **Individual sample** - where tissue from one fish is placed in bottle or vial, but there can be more than one tissue per fish.
- Tissue sample in order of preference:
    - 1. **Adipose**- Pink, chum, sockeye - easiest for lab to process, good for non-lethal sampling. **Do not use for chinook and coho salmon, reserved mark for coded wire tags.**
    - 2. **Operculum** – can delaminate in bulk sample causing multiple amplification of same fish, works best for individual sample, can damage gills if live sampling, harder to process in the lab.
    - 3. **Tail or other fin** – rays can fall apart in bulk sample can cause multiple amplification of same fish – works best for individual sample, good for non-lethal sampling.
    - 4. **Scale**s - harder to process in the lab requires removing scales from scale cards, good for non-lethal sampling, can get matching DNA and age.
- Place tissue in sample bottle containing 95% non-denatured ethanol solution. Do not dilute the ethanol. Do not use methanol or Reagent Alcohol solutions (i.e rubbing alcohol or denatured alcohol) because these chemicals disrupt DNA extraction.
- DNA will degrade if ratio of tissue to ethanol is too high. Should contain no more than ¼ tissue to ¾ ethanol.
- Label each bottle with geographic location, statistical area, species, date and sampler.
- If labels placed inside vials-- *******Do not use paper-based waterproof paper (ie Rite in the Rain©) because chemicals interfere with DNA extraction ******* Plastic paper is ok.
- Always label in pencil or solvent resistant markers to ensure the writing does not get dissolved by the ethanol.

2) Alternative tissues

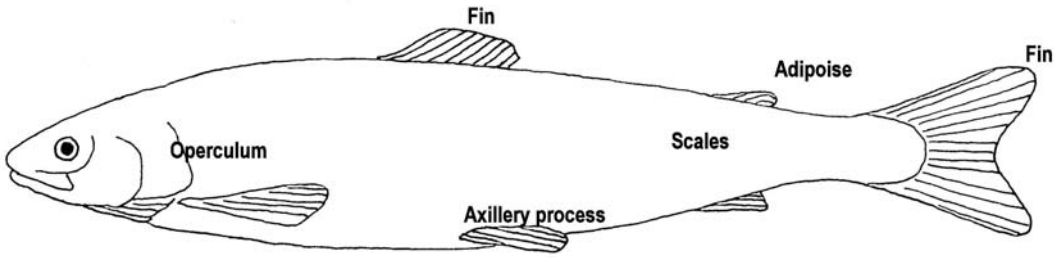
- Sometimes samples are previously frozen. Use the same sampling procedure as above except take small piece of muscle, heart, or liver instead. These tissues have more DNA so will amplify better after freezing. Do not add more the ¼ tissue to ¾ ethanol per vial as per above.
- *Axillary process* this tissue is used by ADFG see website: http://www.genetics.cf.adfg.state.ak.us/ Non-lethal tissue sampling (PDF)

3) Vial numbering

- Bulk or individual vials vial should be given a unique alphanumeric code by collection agencies/organizations. For example, West coast of Vancouver Island Troll fisheries i.e. 01WCTDFO56
- Format = Year + Fishery code + collection agency + vial number
- Length = (2digits)+(3digits)+(3digits)+(>=1digit)

4) Sample Sheets

- A standard reporting sheet (Excel spreadsheet) should be filled out that accompanies the samples sent to the Molecular Genetics Lab, Pacific Biological Station, Nanaimo.
- Minimum requirements on DNA sampling sheets:
  DATE GEAR AREA SPECIES VIAL # #TISSUES SAMPLER
- This is thought to be the minimum information required on a sample sheet. Additional information such as sub-area, location, vessel name, statistical week, and comments may be needed in some circumstances



If you have any further questions or comments please contact:
John Candy 250-756-7224
john.candy@dfo-mpo.gc.ca
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