Sample Collection Recommendations for Long-Read Sequencing and Gene Annotations

We wrote these recommendations originally for the California Conservation Genomics Project (https://www.ioes.ucla.edu/project/california-conservation-genomics/). The first two pages describe the requirements for samples for HMW-DNA isolations. Page **3** has recommendations for samples for RNA isolations.

Specimen Requirements for HMW-DNA Isolation for Reference Genome Sequencing

General Notes:

- Submit a minimum of two samples per species from the same individual. Providing more aliquots of sample in pre-dissected amounts will increase our chances of success in extraction.
- The very highest quality DNA samples can be isolated from fresh blood and fresh or frozen cell culture samples. Suitable fresh frozen tissue samples can also yield excellent data.
- Frozen samples: In general samples should be flash-frozen in liquid nitrogen, stored at -80°C and shipped on dry ice. Freeze samples after removing excess liquid. Any thawing of the samples must be avoided.
- If there is no chance to arrange immediate flash-freezing please reach out to us to discuss storage options.
- Do not pool samples of different individuals.
- Submit higher sample amounts and volumes than requested below if possible. Additional samples are also highly beneficial for specimen-specific DNA isolation tests.
- We can NOT accept samples potentially infectious to humans.

If you plan on submitting cell culture samples:

Please submit a minimum of two vials with~10 million cells or more. Cells should be washed with 1x PBS, then pelleted again. The supernatant should be removed. The pellet should be flash frozen in liquid nitrogen (or between dry ice) and stored at -80°C. Please ship the cell pellets on dry ice.

If you plan on submitting vertebrate tissue samples:

- Soft tissue samples with a high content of nucleic acid (such as spleen, liver, heart) are preferable.
- Submitting sterile or very clean samples is of greatest importance: Guts, gills, skin, other connective tissues and hair should be removed before flash freezing, if possible. Rinse newly collected sample with cold saline to remove blood and other contaminants before freezing.
- Dissect fresh tissue samples on ice into pieces less than 0.5 cm in at least one dimension (or into pieces of 50 mg or less). Pack the samples into labeled cryo vials and flash freeze them in liquid nitrogen immediately. Store at -80°C and ship on dry ice. Please ensure the tissue sections are not jammed into the tubes and can be easily removed from the tubes without thawing.
- Submit at least 1 g of soft tissue per sample if possible. When working with small animals (smaller than a penny) provide the entire organism in a tube and flash freeze the specimen.

- Samples should be flash-frozen immediately. Please contact us to discuss storage options in the event that flash freezing in not possible.

If you plan on submitting blood samples:

- Please contact us beforehand to set up an appointment if you plan to ship fresh blood samples with overnight shipping.
- If blood samples will arrive within two days after the blood draw, it is generally better to not freeze the samples. Instead keep the samples cold at all times (refrigerator; 4°C) and transport them between a generous number of cold packs.
- EDTA-blood samples can also be flash frozen in liquid nitrogen and stored at –80°C. Frozen samples should be shipped on dry ice.
- Mammals: Minimum of 2 samples containing ~5 ml of mammalian blood sample are required each for HWM DNA isolation. For smaller animals submit samples with at least 2.5 ml of blood.
- Birds and reptiles: For blood samples with nucleated RBCs such as birds and reptiles, 500µl (0.5 ml) of unfrozen whole blood in EDTA anticoagulant is sufficient.
- Fish: Use acid citrate dextrose (ACD) as anticoagulant for unfrozen fish blood samples. 500 µl (0.5 ml) or more should suffice.
- All tubes should be sealed and individually double packed in packaging that protects the tubes from physical damage and vibrations. A secondary cyro-tube box within the shipping packaging is sufficient.

If you plan on submitting insects and other small animals:

- Animals should best NOT be fed for appropriate time spans before collection, to avoid isolating food DNA and to reduce microbial content.
- Pupa or larva tend to provide the best DNA samples and are preferable to mature animals.
- If possible, pupa and larva should be shipped live. In this case the shipment has to be coordinated with the receiving lab.
- Insects, pupae and larva can also be packed in cryo-vials and flash-frozen in liquid nitrogen, stored at -80°C and shipped on dry ice.

If you plan on submitting fish samples:

Fresh blood is the preferred sample type (see above). If this is not possible, then good tissue samples for fish are heart and gills (the latter only if microbial contamination can be avoided). Fin clips and tissue biopsies that include non-scaly skin pieces are also suitable options for specimens that cannot be sacrificed. For fish that live in very cold water please avoid muscle tissue.

If you plan on submitting plant samples:

Submit 5 to 10g of young leaf tissue. Wash the leaves and remove the midrib and any other hard tissue. Flash freeze the tissue distributed in several aluminum foil envelopes in liquid nitrogen and store at -80°C. Transport the samples on dry ice.

Tissue Sample Recommendations for RNA Samples for Gene Annotations

General Notes:

- The DNA Technologies Core does not offer RNA isolations as a service. We require high integrity isolated total RNA samples for RNA-Seq, PacBio Iso-Seq, or Nanopore RNA-sequencing (https://dnatech.genomecenter.ucdavis.edu/sample-requirements/)
- Samples for RNA-sequencing should be freshly dissected and flash frozen in liquid nitrogen. They should be stored at -80°C and shipped on dry ice. Any thawing of the samples must be avoided.
- The objective of the RNA-sequencing is the generation of data for as many different transcripts as possible. Since gene expression varies between tissues, between different life stages, and genders it is advantageous to collect a diverse set of samples. These should comprise several tissue types and samples from different life stages if possible.
- In contrast to the samples for the DNA isolations, the samples for the RNA isolations can be derived from several individuals.
- Submit a minimum of two samples per species from the same tissue type.
- Samples should be very clean. Rinse tissues in RNAse-free water before dissecting them.
- Samples are best frozen, stored and shipped in 1.5 ml or 2 ml cryo-vials with screw caps.
- Samples for RNA isolation will need to be shipped to a different address than the samples for DNA isolation.

If you plan on submitting plant samples:

- Tissues should be best include young leaves, root tips, flowers, buds, seeds.
- Remove any woody or hard parts. Quickly cut tissues into smaller pieces (500 mg or smaller) and deposit into pre-chilled cryo-vials and then flash-freeze them in liquid nitrogen.

If you plan on submitting insects and other small animals:

- Flash-freeze entire animals
- Submit different life stages if possible (pupae, larvae, mature animals).

If you plan on submitting animal samples:

- Dissect animals on ice.
- Quickly cut tissues into smaller pieces (~100 mg) on ice and flash freeze in liquid nitrogen.
- Collect multiple tissues: e.g. brain, liver, skin, testis, ovaries, spleen, lung. Brain and testes tend to express the highest number of genes.