

# CCGP Reference Genome Specimen Requirements

This document describes the CCGP reference genome specimen requirements for samples collected for **DNA isolations** (pages 3-5) and for **RNA isolations** (page 6).

These requirements are for reference genome tissues only.

**CCGP Technical Board:** for technical questions, please email with “CCGP specimen submission inquiry” as your subject header. Lutz Froenicke ([lfroenicke@ucdavis.edu](mailto:lfroenicke@ucdavis.edu)) and Shana McDevitt, ([shana.mcdevitt@berkeley.edu](mailto:shana.mcdevitt@berkeley.edu)).

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## General Guidelines

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### Number and Target Species of Reference Genomes

- CCGP will provide a reference genome for **one** species in each project genus. For projects that include several closely related congeneric species, we suggest that you choose either the species with the widest California distribution, or one that will be most useful for your taxonomic group and community. Please contact Brad Shaffer ([brad.shaffer@ucla.edu](mailto:brad.shaffer@ucla.edu)), if you are unsure of which species you should choose.
- Estimated genome sizes of target species should be less than 5Gb. Please contact Brad or Erin ([etoff@ucla.edu](mailto:etoff@ucla.edu)) for special considerations.

### Overview of Sample Requirements

#### Sample Splitting Strategy

- Reference genome library components will be completed on separate UC campuses.
  - 1) UC Davis (PacBio HiFi, **required**)
  - 2) Dovetail Omni-C (UCSC, **required**)
  - 3) Transcriptome (UCLA, **optional but encouraged**)
  - 4) Archival samples of both DNA tissue and RNA tissue in case of shipping/library failures (UCLA, **required**).
- **Please prepare three separate sets of DNA tissue PLUS two sets of each RNA tissue type.**
  - When possible, DNA samples should come from the same individual.
  - RNA tissues should come from multiple tissues and when possible, multiple life stages.
  - For most taxa, this means that you will prepare at minimum 6 tubes of tissue for HMW DNA extractions (two each sent to UCSC, UC Davis, and UCLA) and at minimum 4 tubes per tissue type for RNA extractions (2 for RNA extraction, 2 for archive, sent to UCLA).
  - Providing more aliquots of sample in pre-dissected amounts will increase our chances of success in extraction.
- Please contact both UCDavis (Ruta Sahasrabudhe, [rmsaha@ucdavis.edu](mailto:rmsaha@ucdavis.edu) AND Mohan Marimuthu at [mpamarimuthu@ucdavis.edu](mailto:mpamarimuthu@ucdavis.edu)) and UCSC (Sam Sacco, [ssacco@ucsc.edu](mailto:ssacco@ucsc.edu)) **at least a week in advance** to set up an appointment if you plan to ship fresh samples with overnight shipping.

### Very small species

This includes most insects: If you feel a single individual does not contain enough tissue to split between at least UC Davis and UC Santa Cruz (see guidelines below for quantities), please submit multiple individuals to each shipping destination. It is better to send different whole animals to each lab (rather than splitting e.g. each of two specimens in half and sending half of each to two labs).

### Sample Preparation

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**Tissues should be split before flash freezing. Never allow samples to thaw.**

We strongly recommend using liquid nitrogen for flash freezing. If you don't have access to liquid nitrogen, a slurry of dry ice and isopropanol will work.

### **Specimen Requirements for HMW-DNA Isolation for Reference Genome Sequencing**

#### General Notes

- 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.
- If there is no chance to arrange immediate flash-freezing please reach out to the CCGP technical board 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 very useful for specimen-specific DNA isolation tests.
- Please use the heterogametic sex, when applicable.
  - If submitting multiple small individuals, please try to submit multiple of the same sex.
- The CCGP labs **CANNOT** accept samples potentially infectious to humans.

## Preferred Sample Types

Based on feedback from both the UC Davis and UC Santa Cruz labs, the following is a non-exhaustive list of preferred sample types. We realize these might not be possible for some species.

See sections for each sample type for specific sample preparation details.

### **Shipping to UC Davis (PacBio HiFi)**

- Vertebrates: blood (treated with anti-coagulants and shipped immediately or flash frozen).
- Plants: as much tissue as possible (>5g). Flash Frozen.

### **Shipping to UC Santa Cruz (Dovetail Omni-C)**

- Birds, Fish, Reptiles, and Amphibians: red blood cells, flash frozen; or whole blood treated with EDTA (1.5mg/ml of blood) and flash frozen or shipped fresh.
- Mammals: Blood, treated with EDTA (1.5mg/ml), flash frozen or shipped fresh.
- Plants: As much tissue as possible (> 1g is best)! Flash frozen.
- Invertebrates (marine or terrestrial): Whole flash frozen individuals, ideally starved

## Cell culture samples

Please submit a minimum of two vials each 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.

## Vertebrate tissue samples

- Soft tissue samples with a high content of nucleic acid (such as spleen, liver, heart) are preferable to those with more connective tissue (muscle, skin).
- 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. If you are unsure if your sample is appropriately sized for splitting, please contact the CCGP technical board to discuss how best to split small samples.
- Samples should be flash-frozen immediately. Please contact the CCGP technical board to discuss storage options in the event that flash freezing is not possible.

## Blood samples

- 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.
  - Please contact both UC Davis (Ruta Sahasrabudhe, [rmsaha@ucdavis.edu](mailto:rmsaha@ucdavis.edu) AND Mohan Marimuthu at [mpamarimuthu@ucdavis.edu](mailto:mpamarimuthu@ucdavis.edu)) and UCSC (Sam Sacco, [ssacco@ucsc.edu](mailto:ssacco@ucsc.edu)) **at least a week in advance** to set up an appointment if you plan to ship fresh blood samples with overnight shipping.
  - For the archival samples, please flash freeze prior to shipping to UCLA.

- Mammals: Minimum of 2 samples containing ~5 ml of mammalian blood in EDTA (1.5mg/ml) 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 (1.5mg/ml) 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.
- EDTA-blood samples can also be flash frozen in liquid nitrogen and stored at –80°C. Frozen samples should be shipped on dry ice.
- Purple top Vacutainer tubes are unsuitable for flash freezing, so please transfer EDTA-blood to cryovials before flash freezing.

### 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. Probably two days is sufficient.
- Pupae or larvae tend to provide the best DNA samples and are preferable to mature animals.
- If possible, pupae and larvae should be shipped live. **In this case the shipment has to be coordinated with the receiving lab.**
  - Please contact both UCDavis (Ruta Sahasrabudhe, [rmsaha@ucdavis.edu](mailto:rmsaha@ucdavis.edu) AND Mohan Marimuthu at [mpamarimuthu@ucdavis.edu](mailto:mpamarimuthu@ucdavis.edu)) and UCSC (Sam Sacco, [ssacco@ucsc.edu](mailto:ssacco@ucsc.edu)) **at least a week in advance** to set up an appointment if you plan to ship fresh live animals.
  - For the archival samples, please flash freeze prior to shipping to UCLA.
- Insects, pupae and larvae can also be packed in cryo-vials and flash-frozen in liquid nitrogen, stored at -80°C and shipped on dry ice.
- If you feel a single individual does not contain enough tissue to split between at least UC Davis and UC Santa Cruz (see guidelines below for quantities), please submit multiple individuals to each shipping destination

### Fish samples

- Fresh blood is the preferred sample type (see section on blood samples).
- 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.
- Use acid citrate dextrose (ACD) as anticoagulant for unfrozen fish blood samples. 500  $\mu$ l (0.5 ml) or more should suffice.
- For fish that live in very cold water please avoid muscle tissue.

### Plant samples

- Submit 5 to 10g of **young leaf tissue for each shipping address**. If this is not possible for your species, please contact the core labs for advice.
- Wash the leaves and remove the midrib and any other hard tissue.
- Flash freeze the tissue distributed in several aluminum foil envelopes or cryotubes in liquid nitrogen and store at  $-80^{\circ}\text{C}$ .
- Transport the samples on dry ice.
- Please contact the CCGP technical board if your plant does not have leaves.

## Specimen Requirements for RNA Isolation for Gene Annotations

### General Notes

- 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 to generate data for as many different transcripts as possible. Since gene expression varies between tissues, between different life stages, and between 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**, and they need not be the DNA-sample individual.
- Submit a minimum of two samples per species from the same tissue type (for multiple tissue types).
- 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 shipped to UCLA.**

### 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 (~100 mg) and deposit into pre-chilled cryo-vials and then flash-freeze them in liquid nitrogen. Please provide two samples, each of at least 100 mg, for each tissue in separate tubes.

### Insects and Other Small Animals:

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

### Larger Animal Samples

- Dissect animals on ice.
- Quickly cut tissues into smaller pieces (~50 - 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 and therefore are very useful tissues.



## Shipment of Reference Genome DNA and RNA Samples

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In each package, please include a **paper copy** of the sample submission form **AND** email a copy of the submission form to each shipping destination. This submission form can be downloaded [here](#).

### Packaging Guidelines

- Packing should be leak-proof and meet carrier packaging requirements
- Samples should be sent on dry ice, taking care that tubes are protected from crushing. Secondary containers are recommended.
- Packages should be labeled externally with a Dry Ice label (UN 1845), and the shipping label should reflect that dry ice is inside the package.
- Senders should use next-day service.
- Shipments should only arrive Monday through Friday, and avoid weekends and holidays. We recommend shipping on Mondays or Tuesdays.
- Due to the ongoing fluidity of the Covid-19 situation, senders should check with each destination to ensure that packages can be received *prior* to sending samples.
- Please conform to all shipping and permit regulations regarding the shipment of biological specimens.
- **The CCGP labs CANNOT accept samples potentially infectious to humans**

### Shipping Destinations

If you plan on shipping fresh blood or live animals, please contact each destination at least a week in advance to ensure that the samples can be processed.

Aliquots of your tissues should be sent to each of three destinations:

#### 1) UC Davis (PacBio long-reads)

Attn: Ruta Sahasrabudhe & Mohan Marimuthu / **CCGP** Sample Submission  
DNA Technologies Core  
Genome and Biomedical Sciences Facility (GBSF), Room 1410  
University of California-Davis  
451 Health Sciences Drive  
Davis, CA 95616  
530-754-9143

Please send the tracking number and details of what is included in your package to Ruta at [rmsaha@ucdavis.edu](mailto:rmsaha@ucdavis.edu) AND Mohan Marimuthu at [mpamarimuthu@ucdavis.edu](mailto:mpamarimuthu@ucdavis.edu)

**2) UC Santa Cruz (Omni-c):**

Attn: Sam Sacco / **CCGP** Sample Submission  
Thimann Receiving/Biomed Rm. 155  
UC Santa Cruz  
1156 High St  
Santa Cruz, CA 95064  
907-231-0696

Please send the tracking number and details of what is included in your package to Sam Sacco at [ssacco@ucsc.edu](mailto:ssacco@ucsc.edu).

**3) UCLA (transcriptomes and archiving)**

Attn: Tara Luckau / **CCGP** Sample Submission  
Shaffer Lab, TLSB 4140  
UC Los Angeles  
610 Charles E Young Drive East  
Los Angeles, CA 90095  
310-825-3598

Please send the tracking number and details of what is included in your package to Tara at [tkluckau@ucla.edu](mailto:tkluckau@ucla.edu), so that someone can be on site when the sample arrives.