CCGP Mini-Core Sample Submission Guidelines

The Mini-Core can assist CCGP projects with lab work leading to sequence generation, including DNA extraction from tissue and library preparation.

Please note that we are only able to process samples that are part of CCGP and we are not able to accept payment from non-CCGP funding sources.

For general inquiries about CCGP WGS, please contact Erin Toffelmier (etoff@ucla.edu). For inquiries regarding the CCGP Mini-Core, please contact Andrew Tully (andrewtully@g.ucla.edu).

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Submission of tissues for DNA extraction followed by library preparation

- The Mini-Core can extract DNA from high-quality tissues.
- We will not work with highly degraded samples, herbarium specimens, old leaf tissues and other low-quality samples.
- In all cases, recently collected samples are preferred
    - we are unable to process formalin-fixed samples
- For challenging to extract species (i.e. many plants, marine invertebrates, small insects), or very small species or samples:
    - If possible, please provide additional or backup samples for extraction troubleshooting.
    - If there are known extraction protocols that work for your species, please share them with us!
        - Attach them during sample submission or contact Andrew (andrewtully@g.ucla.edu)
- We cannot accept samples that are potentially infectious to humans

Plants

- Submit at least 1g of leaf tissue per sample.
    - Fresh, young leaf tissue is very much preferred.
    - We will not work with herbarium specimens
- If possible, flash freeze the tissue in individually labeled cryotubes or aluminum foil envelopes in liquid nitrogen and then store at -20°C or -80°C. Transport flash-frozen samples on dry ice.
- Fresh samples may also be desiccated with silica-gel beads and placed individually in a manila paper envelope. Ensure that all samples have been thoroughly desiccated prior to shipping.
- Please contact Andrew (andrewtully@g.ucla.edu) if your plant does not have leaves.

Insects and Other Small Animals

- Submit entire individuals or at least 0.2g of material per sample.
- Avoid feeding samples prior to collection, to avoid isolating food DNA and microbial content. This will lower your on-target sequences and your final coverage.
- If providing partial animals, please attempt to exclude the gut
- If possible, flash-freeze individuals or samples in cryotubes in liquid nitrogen, store at -20°C, and ship on dry ice.
- Alternatively, store individuals or samples in ethanol (75%-99%) at -20°C and ship with frozen ice packs or dry ice.
- Please do not submit live animals.

- If you cannot submit an entire individual, and feel as if the individual does not contain enough tissue for extraction, please submit additional individuals if possible. Contact Andrew (andrewtully@g.ucla.edu) with questions.

Vertebrate Soft Tissues

- Soft tissues (such as spleen, liver, heart, and muscle) **are strongly recommended.** They will give you better data.
- Submit at least 0.2g of material per sample.
- Soft tissues should be divided into roughly 0.5cm^3 pieces (multiple pieces per tube is fine).
- Newly collected samples should be rinsed with cold saline to remove blood and other contaminants prior to freezing.
- If possible, flash-freeze individuals or samples in cryotubes in liquid nitrogen, store at -20°C, and ship on dry ice.
- Alternatively, store individuals or samples in ethanol (75%-99%) at -20°C and ship with frozen ice packs or dry ice.

Vertebrate Hard Tissues

! Hard tissues may require additional extraction attempts and special handling. Please contact Andrew (andrewtully@g.ucla.edu) to discuss your options before creating a submission with hard tissues.

- Hard tissues include skin, scales, feathers, fin clips, claws, and other tissue types are difficult to work with and often produce poor results.
- For small samples, if possible submit additional material (i.e. multiple claws, feathers, or fin clips) to maximize extraction success.
- Submit at least 0.2g of **extractable material** (not just keratin) per sample.
- Store samples in ethanol (75%-99%) at -20°C and ship with frozen ice packs or dry ice.

Blood

- For species with nucleated red blood cell (birds, reptiles, fish, amphibians), please send at least 30µl per sample.
    - For smaller species, provide a minimum of 15µl of nucleated blood
- For species with non-nucleated red blood cells (mammals), please provide at least 1200µl.
    - For smaller species: provide a minimum of 600µl of blood.
- Treat fresh blood with anticoagulants and then freeze
    - mammals, birds, reptiles, and amphibians: EDTA
    - fish: citrate dextrose (ACD)

- Store all samples at -20°C and ship with frozen ice packs or dry ice.
- If samples were stored using an alternative method or at a different temperature, please contact Andrew (andrewtully@g.ucla.edu) before sending.

Submission of gDNA for Library Preparation

The Mini-Core will accept high quality gDNA extracts for library preparation and sequencing.

- Input DNA quantity and volume should meet or exceed the following requirements. Submitting sample amounts higher than the above requirements will improve the success of generating high quality libraries.
    - We cannot accept degraded DNA samples. DNA should be of high molecular weight (>15kb) and show no significant fragmentation (e.g. no DNA smear on an agarose gel). See below for characterization requirements.
    - For species with a genome size smaller than 0.5Gb:
        - Quantity: > 125ng
        - Volume: > 25µl
        - Concentration: > 5ng/µl
    - For species with a genome size greater than 0.5Gb:
        - Quantity: > 500ng
        - Volume: > 25µl
        - Concentration: > 20ng/µl

- EDTA concentration should be less than 1mM
    - An EDTA cleanup of extracted DNA can be performed at an additional cost, if necessary.
    - Please indicate the elution buffer used if you are unsure of the EDTA concentration.

- All submitted gDNA samples should be accompanied by two quality checks done by the submitting lab. An estimate of both quantity and quality for HMW gDNA for each sample must be submitted **prior to samples being shipped to the CCGP Mini-Core**, or additional QC costs will be incurred.
  1. Concentration:
      - A fluorometric quantitation (e.g.. Qubit, PicoGreen)

  2. Evidence of HMW gDNA:
      - A visible, clear, and labeled band should be present for each sample submitted via a gel image, **OR**

- Results from a Bioanalyzer should be submitted for each sample, demonstrating a clear peak for HMW gDNA with clearly labeled sample names

Mini-Core Details for Users

To submit either tissue or gDNA to the UCLA Mini-Core, please complete this submission form: https://airtable.com/shrwTLw4Z49ukKTmU

All submissions must include a completed Sample Data Sheet
If submitting samples in a plate, submissions must include a Plate Map

Shipping address:
Attn: Andrew Tully - CCGP/Shaffer, TLSB 4140
610 Charles E Young Dr East
Los Angeles, CA 90095
310-825-5063

- **Shipping directions:**
  Email Andrew (andrewtully@g.ucla.edu) before shipping to make sure someone is available to receive a package.
- Email the package tracking number and digital copy of the Sample Data Sheet to Andrew (andrewtully@g.ucla.edu)
- Include a hard copy of the Sample Data Sheet in the shipment.

Packaging guidelines:
- Packing should be leak-proof and meet carrier packaging requirements.
- For samples on dry ice, label package externally with a Dry Ice label (UN 1945).
- For all samples, use secondary containers to prevent tubes, foils, or bags from being crushed.
- Submitters should use next-day service.
- Shipments should only arrive Monday through Friday, and avoid weekends and holidays if possible. **We recommend shipping on Mondays or Tuesdays**.
- Do not ship over University of California holidays or closures.

Batch processing and workflow:
- If possible, please submit a complete batch of samples (i.e. if you plan on submitting 150 samples, submit them all at one time). Ideally, we will do extractions and library preps in complete batches. However, if this is not feasible, submit samples in as few batches as possible.

- If 5 or more samples in the batch fall below our quality standards, we will contact projects about how they would like to proceed.
- If fewer than 5 samples in the batch are below our quality standards, we will proceed with library prep. CCGP will attempt to include lower quality samples, but some samples might be dropped entirely. We will let you know if that is the case.

Data retrieval:

- Sequence data will be hosted by the sequencing Core for download. It is the project PI's responsibility to retrieve their own data.

Sample storage:

- All tissues or DNA submitted to the CCGP Mini-Core will be stored at -20°C.
- Excess samples (tissue, DNA, or library) will be stored for 6 months after use.
- After 6 months, all tissues, DNAs and libraries will be discarded.