



Guidelines for Sample Collection: Flash Freezing Fresh Tissue

Overview: The VGL strongly prefers the collection of at least 100mg of tissue *per technology used* (i.e. if analyzed with Bionano, PacBio, 10X, and Hi-C a minimum of 400mg is needed plus an additional 100mg to account for additional extractions and archival).

Flash freezing fresh tissue is considered the gold standard for high molecular weight (HMW) DNA extraction. These requirements are based on previous experiences with flash frozen tissue samples and guidelines by Bionano Genomics. Tissue must be collected fresh and **never thawed**. After flash freezing, immediately store samples at -80C and ship later to VGL on dry ice overnight.

Materials: 1-2mL cryovials; liquid nitrogen dewar, tube rack, and bowl; sterile scalpel; sterile forceps; sterile surface for tissue dissection

Tissue Preferences: muscle, liver, spleen, kidney, brain, gonads

Collection Instructions:

1. Label cryovials with the species name, sex of individual, and date of collection.
2. Place cryovials on dry ice to prechill. This step is critical for ensuring that your tissue maintains a constant temperature after flash freezing.
3. Prepare a container of liquid nitrogen, preferably bowl-shaped or wide mouthed. A mortar will work as well.
4. Using your preferred dissection protocol, section off 50mg chunks of tissue (about the size of a pea; do not weigh).
5. Using forceps, dunk a chunk of tissue into the liquid nitrogen bowl until it is fully frozen. Place the chunk in the prechilled cryovial. You may choose to include multiple 50mg-chunks into one cryotube.
6. Store at -80C immediately. Complete the VGL Shipping and Specimen forms, and ship samples to VGL on dry ice (at least 10kg) using overnight shipping.

Ship to:

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