Tissue and Tumor Collection, Storage, and Shipping Instructions

This document is intended to give guidance as to the preferred method for isolating, packaging, and shipping **tissues and tumors** to preserve gDNA quality when transporting. Following these instructions will increase the likelihood that the resulting gDNA will be suitable for Bionano processes.

Materials and Equipment

The following materials are used to prepare fresh tissue for storage, or to break off portions of tissues which have already been frozen.

Item	Description
Tissue Samples	≥ 10 mg (most tissues) ≥ 60 mg (tissues with low nuclei content relative to tissue mass)
Scalpel	e.g. ThermoFisher Scientific Catalog # 3120032
Forceps	e.g. ThermoFisher Scientific Catalog # 3120032
Cryovials	e.g. ThermoFisher Scientific Catalog # 5011-0012
Disinfecting spray or wipes	10% bleach or equivalent
Liquid Nitrogen	≥ 1 L
-80°C freezer	

The following materials are required for shipping frozen tissue samples to Bionano Genomics.

Item	Description
Dry Ice	≥5 lbs (domestic shipping) ≥15 lbs (international shipping)
Polystyrene box	At least 1.5 inches thick (2 inches preferred for international shipping)
Hard sided container	e.g. 50 mL conical vials or cryovial storage box
Plastic bag	e.g Ziplock bag
Shipping Form	Provided by Bionano Genomics representative
Soft packing material	e.g. packing peanuts or bubble wrap
Absorbent material	Paper towels, Kimwipes, etc.
Customs/Shipping documents	May vary according to country of origin
Dry Ice Label	e.g. UN1845 label

Freezing and Storage Instructions

Freezing Fresh Tissue

Tissue must be collected fresh, and never thawed. Necrotic tissue is not accepted. Divide, weigh, and freeze the tissue as soon as possible.

1. Before freezing, divide tissue into 10 - 30 mg portions for most tissues (e.g. spleen, liver, kidney, brain), or 60 mg portions for tissues with low nuclei content relative to tissue mass. Note: yield from tumor tissue may vary.
    - Tissues with higher nuclei content relative to tissue mass: spleen, brain, liver, lung, kidney, thyroid, colon, bladder, ovary, testes, colon, prostate, and most breast tissue.
    - Tissues with lower nuclei content relative to tissue mass: some fatty tissues, including fatty breast tissue, and most skeletal muscle.
2. Transfer the tissue portions into individual cryovials (one portion per tube).
3. Decontaminate surface of vials using disinfecting wipes. Ensure that sample identifiers are still legible after decontamination.
4. Flash freeze the cryovials in liquid nitrogen.
5. Transfer the cryovials containing the frozen tissue to -80°C for storage until gDNA isolation.

Removing a Portion of Frozen Tissue

Warning: Once frozen, the entire sample must remain frozen until immediately before gDNA extraction with a Bionano-developed gDNA Isolation Protocol. Follow the instructions below to break a frozen tissue sample into smaller portions without thawing it. If this cannot be accomplished, a smaller portion can also be removed at the time of gDNA isolation.

1. Pre-chill forceps, cryovials, and scalpel using liquid nitrogen. It is important to keep these materials cold so that the tissue doesn't stick to them, or thaw upon contact.
2. Submerge the sample in liquid nitrogen and use a scalpel to break off a 10-40 mg portion.
3. Use forceps to transfer tissue portions into cryovials.
4. Transfer the frozen tissue to -80°C for storage.

Packaging and Shipping Instructions

Packaging materials must be leak-proof and meet the general requirements of UN3373 Category B Biological Substances as described by the US Postal Service Packing Instruction 6F (346.321) and International Air Transportation Association Packing Instruction 650. Packaging should also comply with UN1845 Dry Ice requirements, as described by the US Postal Service Packing Instruction 9A and International Air Transportation Association Packing Instruction 954. Requirements of other carriers and customs authorities may apply.

1. Prepare a polystyrene box which is filled at least halfway with dry ice. Select a box that is large and thick enough to hold the samples, plus enough dry ice to keep the samples frozen during transit.
    - For domestic shipping, samples should be shipped in a polystyrene box with ≥ 5 pounds of dry ice. The walls of the box should be ≥ 1.5” thick.
    - For international shipping, samples should be shipped in a polystyrene box with ≥ 15 pounds of dry ice. The walls of the box should be ≥ 2” thick,
2. Label hard-sided secondary container(s) (e.g. 50 mL conical vials or cryotube box) and sealable plastic bag(s) “Biohazard.” Pre-chill both inside the polystyrene box with dry ice.
3. Inspect each cryovial containing frozen sample for leakage. Do not to allow sample to thaw.
4. Remove cryovials (primary container) from -80°C storage and immediately place them inside the pre-chilled hard-sided secondary container(s).
    - If flip-top microcentrifuge tubes were used instead of cryovials, seal the tubes with parafilm.
5. Securely close the secondary container(s). If using a cryotube box, use tape to prevent the lid from opening during transit.
6. Place secondary container(s) inside a sealable plastic bag, along with enough absorbent material (e.g. paper towels) to absorb any liquids that may leak from the samples.
7. Immediately return the sealed bag containing packaged samples to the polystyrene box containing dry ice.
8. Cover the samples with dry ice. Any remaining empty space within the polystyrene box should be filled with additional dry ice or soft packing material.
9. Place the polystyrene box inside a final cardboard box. The polystyrene box should not be able to move inside the outer cardboard box. If necessary, add cushioning material to fill excess space.
10. Ensure that the polystyrene box and outer cardboard box are secured shut, but not airtight.
    - **Note:** The dry ice package must be able vent CO_2 to maintain structural integrity.
11. Apply class 9 dry ice label (Dry Ice, UN1845) to the exterior of the box and specify the quantity of dry ice in kilograms.
12. Apply Category B Biological Substances UN3373 Diamond and add the mark “Biological Substance Category B, UN3373” to exterior of the box, along with the name and phone number of a responsible person.

13. Print a shipping form containing sample information. Place form in a sealed plastic bag and include inside the shipment container (affix to outside lid of polystyrene box).
14. For US domestic shipments, send the package by next-day delivery service (e.g. FedEx Priority Overnight or UPS Next Day Air). For International shipping, choose priority service.
15. Email recipient with the tracking number and the shipment delivery date.
    - If shipping internationally, ensure that samples are admissible and that proper declarations are made with customs authorities. Accommodate for customs inspection accordingly. We recommend couriers that will replenish dry ice during transit and while waiting in customs, such as World Courier or FedEx International Priority with the Priority Alert Plus option selected.
    - If shipping to US Bionano Genomics headquarters, please include shipping@bionanogenomics.com in your recipient list.

Plan shipments so that they will be delivered Monday through Thursday. Shipments scheduled to arrive on Fridays are discouraged because we are unlikely to receive them over the weekend in the event of delays. Avoid shipments arriving on Saturdays, Sundays, or national holidays.

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