DAICEL arbor biosciences

Sample Preparation Guide

myReads®

Solutions for a variety of sample types and project requirements

- Please follow the instructions below carefully when planning your project and preparing, packaging, and shipping samples.
- Shipments that do not follow these guidelines may result in sample loss/degradation during shipment, may be refused or destroyed at customs, and/or may not be eligible for any applicable service outcome commitment(s).

NEVER SHIP IRREPLACEABLE MATERIALS

How to use this guide

1. Find your sample submission type

- Part 1: Specimens for extraction
- Part 2: Nucleic acids
- Part 3: Pre-made libraries and sequencing pools

2. Review required quantity and quality of material

- Part 1: Specimens for extraction
- Part 2: Nucleic acids
- Part 3: Pre-made libraries and sequencing pools

3. Confirm required submission format

- Check the required submission format including plates, seals, and packaging for your submission type

4. Access pro-tips in the appendices

- Appendix 1: Sample drying guide
- Appendix 2: Frequently asked questions

Part 1: Specimens for extraction

Quantity requirements for specimens for standard extractions (short-read sequencing)

Sample category	Minimum mass (mg)	Maximum mass (mg)
Plant	30*	60*
Animal	20	50
Insect	25	50
Invertebrate	50	100
Ancient/Degraded	10	100

*Wet weight, pre-lyophilization
*Dried weight should be ~10mg

Quantity requirements for specimens for HMW extraction (long-read sequencing)

Sample category	Minimum mass (mg)
Gram negative bacteria	5.0 x 10^9 cells
Mammalian cell line	1.0 x 10^6 cells
Insect parts	1 thorax
Soft plant tissue	>= 100 mg (wet weight)

Part 1: Specimens for extraction *continued*

Formatting requirements for specimens for standard extractions (short-read sequencing)

Sample category	Extraction type	Required submission format
All non-ancient sample types	All	**Collection tubes and caps:** https://www.qiagen.com/us/products/discovery-and-translational-research/lab-essentials/plastics/collection-microtubes-and-caps?catno=19560 Secure caps to tubes with parafilm. Place lid on top and secure with rubber band. See Appendix 2 for pictures.
Plant	DNA	Lyophilized. Can be shipped at room temperature. Weigh before lyophilizing.
Animal Insect Invertebrate	DNA	Frozen and shipped on dry ice.
Plant Animal Insect Invertebrate	RNA	**Sample collection:** on ice (if possible), harvest into collection tubes (above) containing sufficient volume (~300-500 µL) of RNALater to completely cover sample. Incubate at 4°C overnight to permeate. Store at -20°C (> 1 month) or 4°C (< 1 month). **Shipping:** Frozen (ice packs).
Ancient/Degraded	Any	Any temperature. Individual tubes.

Formatting requirements for specimens for HMW extractions (long-read sequencing)

Sample category	Minimum mass (mg)
Non-cell cultures	Ship in Eppendorf 1.5-mL LoBind Tubes (e.g. Avantor Cat# 80077-230)
Cell cultures	Snap-frozen, shipped on dry ice. Shipping in culture medium is okay. Any standard cryotube is acceptable, e.g. Corning 2 mL vials (Sigma Cat# CLS431417)
Insect parts	If fresh frozen, ship on dry ice. Otherwise, ship on cold packs.
Soft plant tissue	Ship fresh on cold packs (do not freeze)

Questions on sampling procedures or submission requirements? See Appendix 2.

Part 2: Nucleic acids

Quality/quantity requirements for nucleic acids

Sample category	Total mass (ng)	Modal fragment length	UV 260:280	Bioanalyzer (or similar) trace
Standard DNA	> = 500 ng	> 10 kbp	1.7-1.9	Required
Flex DNA	> 10 ng (recommended)	Any	Any	Optional
Ancient DNA	Any	< 1 kbp	Any	Optional
Standard RNA	> 250 ng (> 2500 ng for depletion)	n/a	1.9-2.1	Required
Long Insert DNA	> 5000 ng	> 10 kbp	1.7-1.9	Required
***All sample types:** maximum concentration of 0.1 mM EDTA in buffer before drying*				

Formatting requirements for nucleic acids

Sample category	Required submission format
DNA	• Dried down fully • Placed in adhesive-sealed, colorless, skirted or half-skirted 96-well plate • Protected in a covered plate rack, if possible • Shipped at room temperature (no ice needed) • Do not include samples of differing quality (e.g. Standard & Flex) in the same plate
RNA	• Dried or liquid accepted • If liquid, all samples at same volume. No more than 100 uL. • Placed in adhesive-sealed, colorless, skirted or half-skirted 96-well plate • Protected in a covered plate rack, if possible • Use copious dry ice in styrofoam OR dried/in RNA Stabilizer at room temperature
Long Insert DNA for WGS	• Ship frozen on dry ice. Domestic shipments may use cold packs. • Shipments may be sent in plates as above or parafilm-sealed 1.5mL Eppendorf Lo Bind tubes (e.g. Avantor Cat# 80077-230) • Do not use Axygen brand tubes or tips while handling samples as they interfere with PacBio SMRTbell preparation

Can I submit my DNA samples in liquid form? Can I submit them in tubes?

We require all DNA samples to be shipped fully dried and in plates. No exceptions will be made for Standard/Flex/Long Insert unless plate reformatting fee is paid (contact **salesteam@arbor.daicel.com** for help). If you have ancient DNA samples, an exception may be granted depending on project size. Contact **service@arbor.daicel.com** for help.

Part 3: Libraries & sequencing pools

Quality/quantity requirements for libraries & sequencing pools

Sample category	Total mass (ng)	Modal fragment length	UV 260:280	Bioanalyzer (or similar) trace
Individual short-insert libraries for capture	> = 1000 ng per library	< 1 kbp	1.7-1.9	Required
Pooled short-insert libraries for capture	> = 1000 ng per pool	< 1 kbp	1.7-1.9	Required
Short-insert sequencing pools	Minimum 10 nM in 30-50 uL	< 1 kbp	1.7-1.9	Required
***All sample types:** maximum concentration of 0.1 mM EDTA in buffer before drying*				

Formatting requirements for libraries & sequencing pools

Sample category	Required submission format
Individual or pooled libraries for capture	• Dried down fully • Placed in adhesive-sealed, colorless, skirted or half-skirted 96-well plate • Protected in a covered plate rack, if possible • Shipped at room temperature (no ice needed)
Sequencing pools	• Dried fully or liquid format accepted • If liquid, no more than 100 uL • Plates as above or clearly-labeled snap-cap tubes sealed with parafilm accepted • Protect during shipment with padding (e.g. falcon tube with paper towels for tubes, covered plate rack for plates) • Use copious dry ice in styrofoam if shipping liquid

Part 4: Packaging, documentation, & shipping

All projects require a submission form

- Complete the appropriate submission form for your sample type (contact **service@arbor.daicel.com** for help)
- Email a completed spreadsheet version to **service@arbor.daicel.com** for review & approval before shipping
- Include a signed physical copy of your submission form in your shipment

Packaging

- Follow "Required submission format" for your sample type
- Clearly label plates on two sides with black marker
- Package materials so that seals cannot be pierced and wells cannot be crushed in transit (e.g. wrap in bubble wrap or similar cushioning)
- Follow submission form instructions above
- Write your myReads Project ID on the outside of the box (contact **service@arbor.daicel.com** for help)

Ship to

Daicel Arbor Biosciences
myReads c/o Jennifer Klunk
5840 Interface Drive, Suite 101
Ann Arbor, MI, 48103
USA

+1-734-998-0571
service@arbor.daicel.com

⚠ **Samples not packaged in this fashion will be shipped back to the return address.**

International shippers (outside of USA)

- Do not ship samples until a member of our team has reviewed your commercial invoice, SSF, and institutional document
- Use the shipping address above. For the Notify Party, indicate "Heather Sayer, hsayer@arbor.daicel.com, telephone 734-998-0751"
- Once cleared to ship, provide courier and tracking number to **service@arbor.daicel.com**
- Identify any export and import permits necessary for your species and inform us before shipment
- Indicate "non-hazardous" as well as the type of ice used (if applicable) in both the item description and Proform Invoice
- On institutional letterheads, include a document containing the following pieces of information:
    - Species of origin (list all!)
    - Whether or not the specimen is being used to study any agriculture disease or pest
    - An accurate invoice/packing list accounting for all vials/plates that also includes the name of the responsible scientist sending the material
    - This paragraph, modified as appropriate:

" Non-hazardous, sterile DNA from [species], [common name] fully dried in plastic plates. For scientific analysis only. NON-INFECTIOUS, NON-HAZARDOUS, NOT PATHOGENIC, NOT AN ETIOLOGIC AGENT. Contain no known pathogens or viruses and are not hazardous to human health. Not a biohazard and in case of damage will not affect other organisms. Safe for air transport and all substances comply with applicable regulations. End us for laboratory research purposes only. No monetary value"

⚠ **Failure to include these descriptions with international shipments can result in unexpected and potentially harmful delays in customs.**

Appendix 1: Sample drying guide

- **Please follow the instructions below carefully when preparing your DNA samples for submission.**
- **Samples which do not meet these requirements will not be processed and may incur additional fees.**

What:

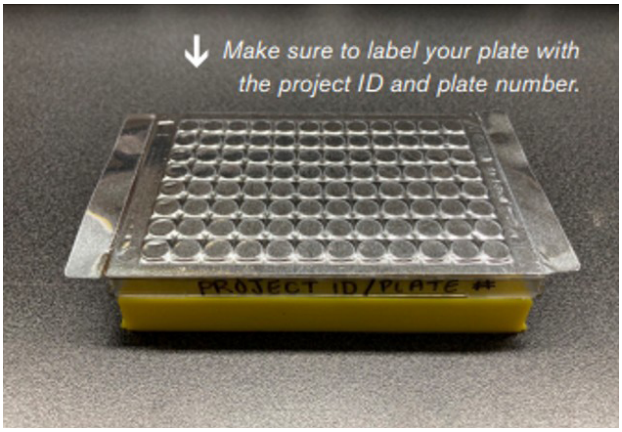
All DNA submissions are required to be fully dried and in plate format.

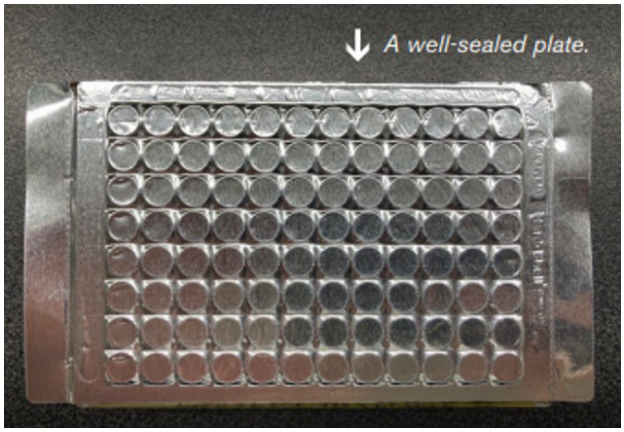
Why:

Samples that are in liquid format (even if frozen) are vulnerable to thawing, and potentially splashing, resulting in cross contamination. If samples are in liquid format and a well is crushed during the journey, the samples will leak out and be lost. With dried samples, there is a chance for recovery from a damaged tube/plate. Dried nucleic acids are typically stable at room temperature for several months and can survive an extended delay due to customs holds/shipment errors/etc.
The reason we ask for samples to be dried is because we want them to arrive safely!

How:

Below, we suggest three options for drying. Other methods are acceptable as long as your plate is full- or semi-skirted and your seal is an adhesive foil. **Pro-tip** – make sure you press thoroughly around and between the wells when you seal the plate!





Suggested plastics/consumables:

- 96-well clear semi-skirted plate: Bio-Rad, catalog number HSS9601 | **Purchase here**
- Adhesive foil seal: VWR, catalog number 490007-318 | **Purchase here**

Three options for drying

1. Vacuum centrifugation (vacuum concentration):

Suggested vacuum concentrator protocol:

Time for drying will vary based on starting volume and equipment. **For standard/fresh samples:** preheat a vacuum centrifuge to high heat (60°C). Set the plate into the vacuum centrifuge without a plate seal and start the protocol, seal the plate when the liquid has evaporated. Check every 15 minutes to monitor progress. **For heavily degraded/ancient samples:** set vacuum centrifuge to no heat, or lowest possible heat. Set the plate into the vacuum centrifuge without a plate seal and start the protocol, seal the plate when the liquid has evaporated. Check every 30 minutes to monitor progress.

Pros & cons:

Fast, but requires special equipment.

2. Passive drying:

Suggested passive drying protocol:

Leave the plate open in a gently heated (~37°C) thermal cycler until the liquid has evaporated. Seal plate.
Pro-tip – make sure no bugs or bits of fluff fall into the wells during drying!

Pros & cons:

No special equipment needed, but slow.

3. Bind to a silica membrane:

Suggested silica membrane binding protocol:

Follow the protocol of the kit through the binding, washing, and drying steps, but do not perform the final elution; just seal the plate instead. Send myReads the specifications for volume and type of elution buffer.

Pros & cons:

No special equipment required, but silica membrane plates are more expensive than regular plates and purification always results in some loss of gDNA mass.

Suggested kits:

- Standard: QIAquick 96 PCR Purification Kit, Qiagen, catalog number 28181 | **Purchase here**
- Highly Degraded: QIAquick Nucleotide Removal Kit, Qiagen, catalog number 28306 | **Purchase here**

What if I don't comply?

You have two options:

- We re-seal your shipping box and ship it right back to you, at your costs; or
- You pay a $500/plate reformatting fee, which must be paid before we touch your samples.

Questions? Email us at service@arbor.daicel.com.

Appendix 2: Frequently asked questions

Samples for extraction

Can I submit a larger sample such as a leaf or bone for Arbor to take a smaller piece of?

Yes, please contact **salesteam@arbor.daicel.com** to add our subsampling service to your project.

Can I submit frozen plant samples for DNA extraction?

We strongly discourage sending frozen plant samples for DNA extraction because freeze-thaw cycles are terrible for DNA integrity. Also, we have found that lyophilization has large positive effects on DNA yield and purity for plants. If you do submit frozen material, ensure that once it is frozen, it is not allowed to thaw.

Which part of the plant should I send for extraction?

If possible, take a sample from young, healthy-looking leaves. If you need to send another tissue type, please contact **salesteam@arbor.daicel.com**.

Can you lyophilize my samples for me?

Sure can! Contact our sales team at **salesteam@arbor.daicel.com** and let us know if you need subsampling and lyophilization or just lyophilization.

The sample submission requirements for plants give masses for wet weight. I've already lyophilized/dried my samples and can only get the dry weight. How much should I send?

Please send ~10 mg of dry weight.

Can I use different tubes and caps from what you have listed?

We tested a wide variety of plastics before making this recommendation. This is the combination that we have identified as capable of standing up to homogenization without leaking, as we do require these specific plastics to be used. Having trouble sourcing them? Contact **salesteam@arbor.daicel.com** for help.

For insect DNA/RNA extraction, which part(s) should I send?

If shipping less than a whole insect, we suggest sending the thorax. Heads or legs (where legs have substantial muscle) may also work. Unless you are interested specifically in microbial content, we would not recommend using just the abdomen.

How should I sample my insects for DNA extraction?

For freshly collected whole insects, euthanize immediately prior to sampling (e.g., placing in a -80°C freezer overnight to induce hibernation and death). Working on dry ice, chop insect into sections of ~3 mm in width and place sections into collection tubes. If working on dry ice is not possible, follow the recommendations for shipping samples for RNA extraction (e.g. work on regular ice, ship in RNALater), but this may result in lower quality and or quantity of DNA.

Can I send dried insects/insect parts for DNA or RNA extraction?

We don't recommend it, but if that's the only option, we can work with dried insects. They can be shipped at room temperature. However, the quantity and quality of DNA/RNA from these samples may be low. Please send ~10 mg.

Can you perform a non-destructive extraction?

Unfortunately no, our extraction methods do consume the sample. Please prepare for the fact that any physical material you send us will not be returned, though if there is any remaining DNA/RNA or libraries, we can typically return them.

Can I do a pilot set of extractions?

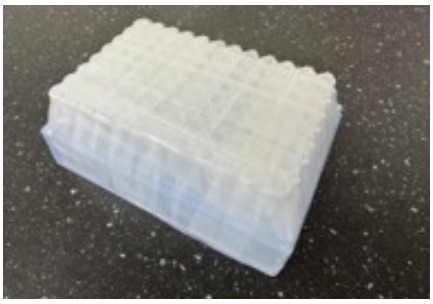
Yes, we offer an option for doing a pilot of 1-12 samples. Doing a pilot first is our strong recommendation – while we've handled a lot of different sample types, each species and tissue is unique and can behave just a little bit differently. Even if you prefer not to perform a separate pilot, we encourage you to send extra tissue for ~8-12 samples so that we can test to ensure there are no chemistry issues with your unique sample type.

What should I do if I cannot sample into RNALater?

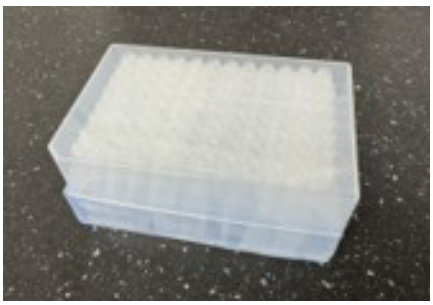
We strongly recommend using RNALater. If it is totally impossible, please work on dry ice and ship on dry ice. Keeping the sample very cold is your best chance of keeping the RNA from being digested or degraded.

How to package samples for nucleic acid extraction:

1. After placing the samples in the collection tubes, firmly seal the whole plate with the strip caps.



2. Ensure that the caps will stay on by wrapping the plate and base in parafilm.



3. Place the lid over the parafilm-sealed plate.

4. Secure the lid tightly with rubber bands or another similar method.

Make sure the plate is protected from crushing or excessive force during its journey with cushioning inside the shipping box!

Questions? Contact us via the methods listed below. Our team is happy to assist you!

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Web: www.arborbiosci.com
Email: info@arbor.daicel.com
Phone: 1-734-998-0751
X: @ArborBio
Bluesky: @arborbio.bsky.social

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