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 librariesand 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: Plant subsampling

Part 1: Specimens for extraction

Quality/quantity requirements for specimens

Sample Category	Tissue type	Minimum mass (mg)	Maximum mass (mg)
Plant – DNA or RNA	Soft (ex: grasses, leaves)	10 mg (wet weight)	30 mg (wet weight)
Plant – DNA	Hard (ex: needles)	30 mg (wet weight)	60 mg (wet weight)
Plant – RNA	Hard (ex: needles)	10 mg	30 mg
Animal – DNA	Soft (ex: muscle, liver)	20 mg	50 mg
Animal – RNA	Soft (ex: muscle, liver)	10 mg	50 mg
Ancient – DNA	All	10 mg	100 mg
Don't see your sample type listed here? Email **genomics@arbor.daicel.com** for custom sample type specifications.			

Q: Can I submit a larger sample such as a leaf or bone for Arbor to take a smaller piece of?
A: Yes, please contact **sales@arbor.daicel.com** to add our subsampling service to your project.

Formatting requirements for specimens

Sample Category	Required submission format
All non-ancient sample types	**Plate type:** Cole-Parmer 1.4 mL, 96-well reinforced square well plate for homogenizers (PN 2205-50) **Seal type:** Cole-Parmer silicone sealing mat (PN 2206-50)
Plant – DNA	Lyophilized (shipped at room temperature)
Animal - DNA	Frozen (shipped on dry ice)
Plant & Animal – RNA	**Sample collection:** On ice (if possible), harvest into plates containing sufficient volume (~300-500 uL) 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 or dry ice).
Ancient – DNA	Any temperature, individual tubes accepted

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

Q: Can I use different plates or seals from what you have listed?
A: We tested a wide variety of plastics and seals before making this recommendation. This is the combination that we have identified as capable of standing up to homogenization without leaking, so we do require these specific plastics to be used. Having trouble sourcing them? Contact **genomics@arbor.daicel.com** for help.

Q: Can you lyophilize my samples for me?
A: Sure can! Contact our sales team at **sales@arbor.daicel.com** and let us know if you need subsampling and lyophilization or just lyophilization.

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
Degraded 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 2mM EDTA in buffer before drying*				

Formatting requirements for specimens

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 & Degraded) 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

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

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

Part 3: Libraries & sequencing tools

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 2mM 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 **genomics@arbor.daicel.com** for help)
- Email a completed spreadsheet version to **genomics@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 **genomics@arbor.daicel.com** for help)

Ship to

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

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

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

International shippers (outside of USA)

- 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.

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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:

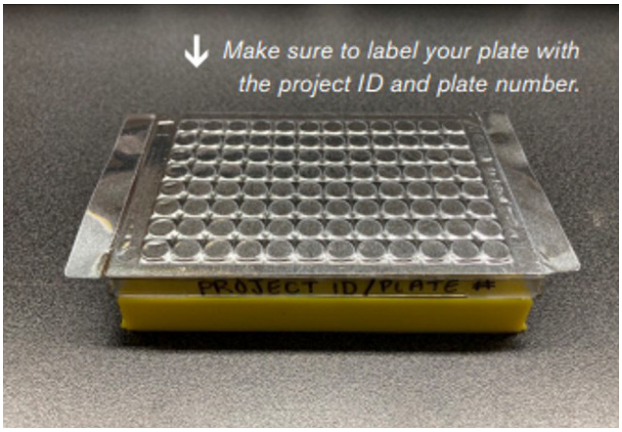
All DNA submissions are required to be fully dried and in plate format.

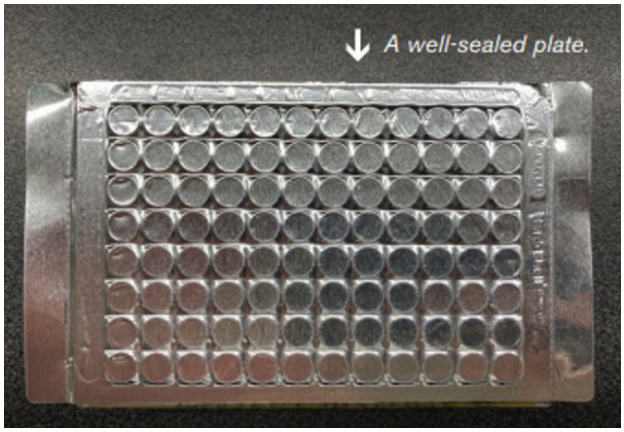
Why:

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: Plant subsampling guide

- **We get it – it can be tough to get small pieces of plant samples into plates.**
- **Here are our pro-tips for getting your plant samples into our required format**

Equipment/consumables:

- **Plate:** Cole-Parmer 1.4 mL, 96-well reinforced square well plate for homogenizers (PN 2205-50)
- **Seal #1:** Non-tacky adhesive film, such as Microseal® 'A' PCR Plate and PCR Tube Sealing Film
- **Seal #2:** Tacky plastic film, such as MicroAmp™ Clear Adhesive Film
- **Seal #3:** Silcone mat for shipping
- "Funnel" - conical paper cups work well
- Sterile tweezers
- Sterile scalpel
- Weigh boat(s)
- Anti-static equipment (e.g. ESD mat for benchtop, lab coat, ionizing air blower, static neutralizing gun, etc)

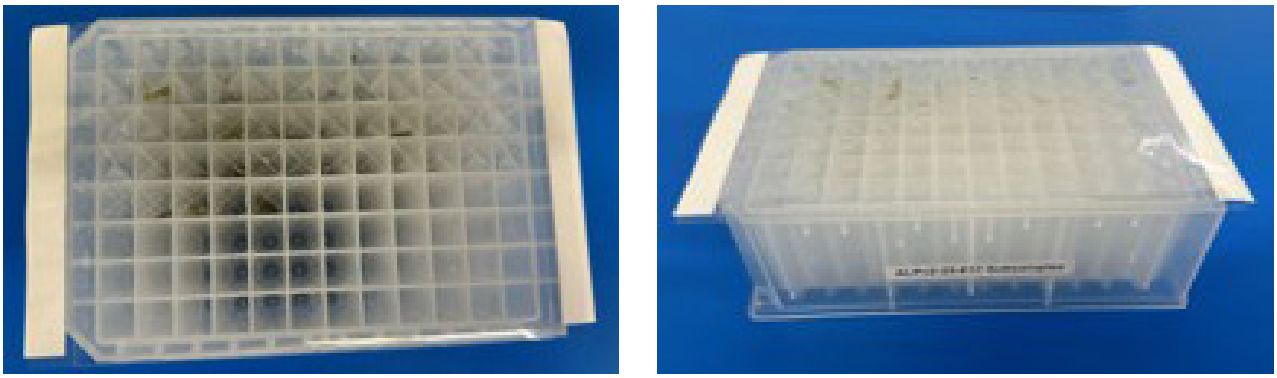


Figure 1: Double-sealed plate with "X" cuts

Figure 2: Preparing the funnel – just a small cut!

Step-by-step instructions

1. Place the non-tacky adhesive film on your empty plate. Seal it.
2. Place the tacky plastic film over top of the adhesive film. Seal it.
3. Using a sterile scalpel, cut an “X” shape in the seals at the well you will place your sample in.
4. Cut off a VERY small piece of the tip of the conical paper cup funnel, if using.
5. Prepare your small sample in the weigh boat with scalpel and tweezers. Cut into smaller pieces if desired, but static may make it harder to get small pieces into the well.
6. Place the funnel into the appropriate well.
7. Use the tweezers to place the sample into the funnel and guide it into the well
8. Well will self-seal once paper funnel is removed.
9. Once all the samples are in the plate, spin it down.
10. Carefully remove plastic seals and replace with silicone mat for shipment.
11. Wrap plate in bubble wrap to protect during shipment.

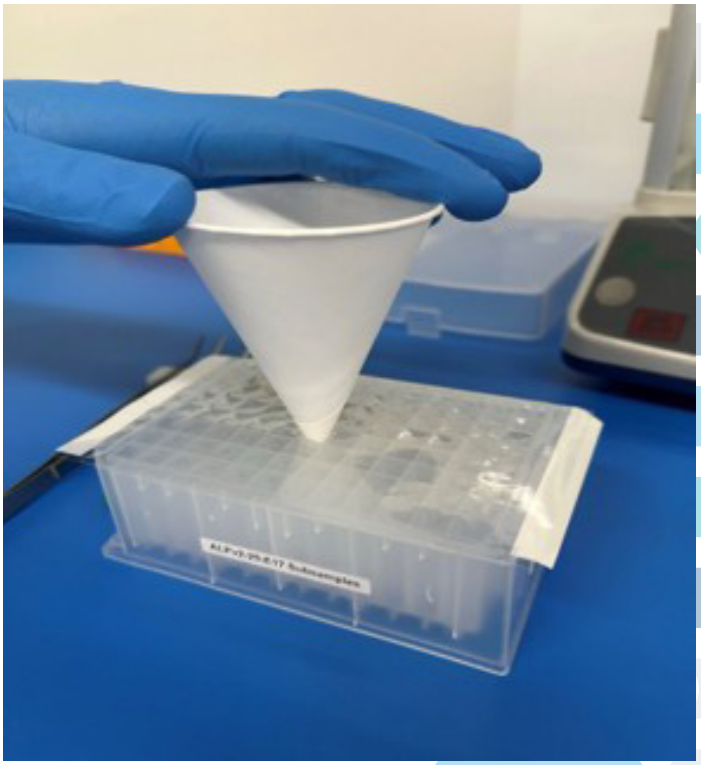
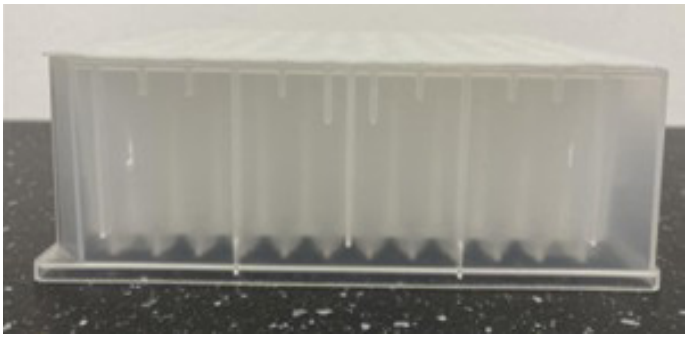


Figure 3: The final set up



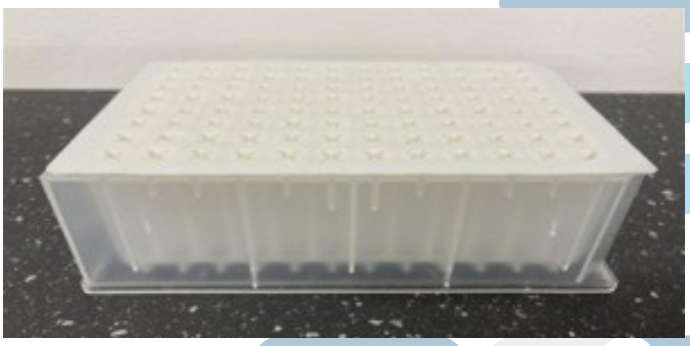


Figure 4: Plate fully sealed with silicone mat

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

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