# myReads® Sample Preparation Guide

## 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

- Check the required quantity and quality of materials for your submission type

### 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 DNA extraction

**Table 1:** Quality/quantity requirements for specimens

| Sample category | Tissue type | Minimum mass (mg) | Maximum mass (mg) |
|---|---|---|---|
| Plant - DNA | Soft (E.g., grasses, leaves) | 10 (wet weight) | 30 (wet weight) |
| Plant - DNA | Hard (e.g., needles) | 30 (wet weight) | 60 (wet weight) |
| Animal - DNA | Soft (E.g., grasses, leaves) | 20 | 50 |
| Animal - DNA | All | 10 | 100 |

Don't see your sample type listed here? Email genomics@arbor.daicel.com for custom sample type specifications

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

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

**Table 2:** Quality/quantity requirements for specimens

| Sample category | Tissue type |
|---|---|
| All non-ancient sample types | Plate type: Cole-Parmer 1.4 mL, 96-well reinforced square well plate for homogenizers (PN 2205-50)<br>Seal type: Cole-Parmer silicone sealing mat (PN 2206-50) |
| Plant - DNA | Lyophilized (room temperature) |
| Animal - DNA | Frozen (dry ice) |
| Ancient - DNA | Any temperature, individual tubes accepted |

**Can I submit frozen plant samples?**

*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.*

**Can I use different plates or seals from what you have listed?**

*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.*

**Need subsampling + lyophilization or just lyophilization? Contact us at sales@arbor.daicel.com**

# Part 2
# Nucleic acids

**Table 3:** Quality/quantity requirements for nucleic acids

| Sample category | Total mass (ng) | Model 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 (>2,500 ng for depletion) | n/a | 1.9-2.1 | Required |
| Long insert DNA | >5,000 ng | >10 kbp | 1.7-1.9 | Required |

**All sample types: maximum concentration of 2mM EDTA in buffer before drying**

**Table 4:** Formatting requirements for nucleic acids

| Sample category | Required submission format |
|---|---|
| DNA | • Dried down fully<br>• Placed in adhesive-sealed, colorless, skirted or half-skirted 96-well plate<br>• Protected in a covered plate rack, if possible<br>• Shipped at room temperature (no ice needed)<br>• Do not include samples of differing quality (e.g. Standard & Degraded) in the same plate |
| RNA | • Dried or liquid accepted<br>• If liquid, all samples at same volume. No more than 100 uL.<br>• Placed in adhesive-sealed, colorless, skirted or half-skirted 96-well plate<br>• Protected in a covered plate rack, if possible<br>• Use copious dry ice in styrofoam or dried/in RNA Stabilizer at room temperature |

### Can I submit my DNA samples in liquid form or in tubes?

*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 and sequencing pools

**Table 5:** Quality/quantity requirements for libraries and sequencing pools

| Sample category | Total mass (ng) | Model fragment length | UV 260:280 | Bioanalyzer (or similar) trace |
|---|---|---|---|---|
| Individual short-insert libraries for capture | ≥1,000 ng per library | <1 kbp | 1.7-1.9 | Required |
| Pooled short-insert libraries for capture | ≥1,000 ng per pool | <1 kbp | 1.7-1.9 | Required |
| Short-insert sequencing pools | Minimum 10nM in 30-50 uL | <1 kbp | 1.7-1.9 | Required |

**All sample types: maximum concentration of 2mM EDTA in buffer before drying**

**Table 6:** Formatting requirements for libraries & sequencing pools

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

# Part 4
# Packaging, documentation, and shipping

## All projects require a submission form:

- Complete the appropriate submission form for your sample type
- 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

## Ship to

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

+1 734 223 5014
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 Daicel Arbor Biosciences before shipment
- Indicate "non-hazardous" as well as the type of ice used (if applicable) in both the item description and Proforma 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. Contains 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 use 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**

# myReads® Sample Drying Guide

## Reliable techniques for drying samples in plate format

- Please follow the instructions below carefully when preparing your DNA samples for submission
- Samples that 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

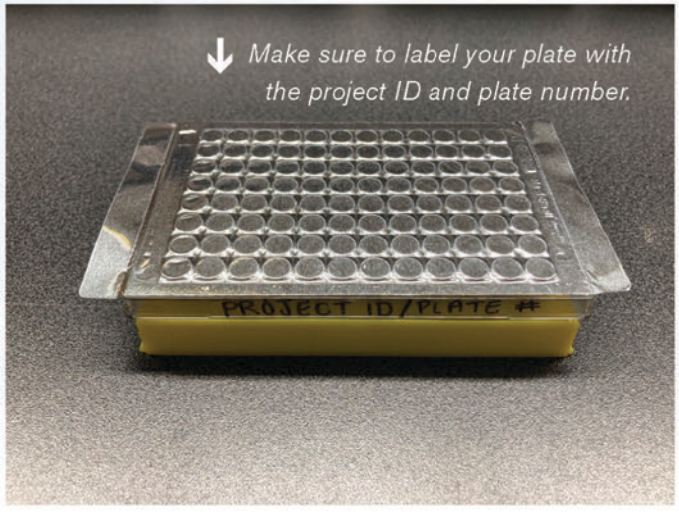
Liquid samples, even when frozen, are susceptible to thawing and possible splashing, which can lead to cross-contamination. If a liquid sample well becomes crushed during transportation, there's a significant risk of leakage and loss of the sample. In contrast, dried samples offer a better chance of recovery, even if a tube or plate is damaged. Dried nucleic acids generally maintain stability at room temperature for several months, making them resilient to delays caused by customs holds or shipping issues. We request that samples be dried to ensure they arrive safely and intact!

### How

Below, we suggest three options for drying. Other methods are acceptable as long as your plate in full- or semi-skirted and your seal is an adhesive foil.

**Pro tip**

Make sure you press thoroughly around and between the wells when sealing 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)*

- **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.
- **Heavily degraded/ancient samples:** set vacuum centrifuge to no heat or lowest possible heat. Place 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**

- *Fast*

**Cons**

- *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 - ensure no bugs or bits of fluff fall into the wells during drying.

**Pros**

- *No special equipment needed*

**Cons**

- *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. Seal the plate instead. Send myReads the specifications for volume and type of elution buffer.

### Suggested kits

- **Standard/fresh samples:** QIAquick 96 PCR Purification Kit, Qiagen, catalog number 28181 | Purchase here
- **Heavily degraded/ancient samples:** QIAquick Nucleotide Removal Kit, Qiagen, catalog number 28306| Purchase here

**Pros**

- *No special equipment needed*

**Cons**

- *Silica membrane plates are more expensive than standard plates*
- *Purification often leads to some loss of gDNA mass*

## What if I don't comply?

1. We reseal your shipping box and ship it back to you at your cost; or,
2. You pay a $500/plate reformatting fee, which must be paid before your samples will be processed

Appendix 2
Plant subsampling guide

# Simplifying the process of transferring small plant samples into plates

## Expert tips for effectively preparing your plant samples in 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 MicroAmp™ Optical Adhesive Film
- Seal #2: Tacky plastic film, such as MicroAmp™ Clear Adhesive Film
- Seal #3: Silicone mat for shipping
- "Funnel" - conical paper cups work well
- Sterile tweezers
- Sterile scalpel
- Weigh boat(s)
- Anti-static equipment (e.g. ESD mat for bench top, lab coat, ionizing air blower, static neutralizing gun, etc)

**Figure 1:** double-sealed plate with "X" cuts

**Figure 2:** preparing the funnel - make 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.

## Questions?

Talk to our dedicated team of scientists about your project at genomics@arbor.daicel.com.

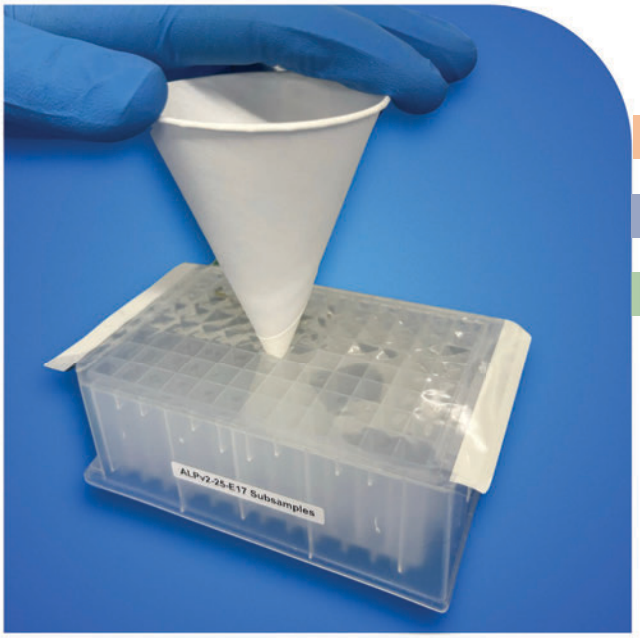



**Figure 3:** the final set-up

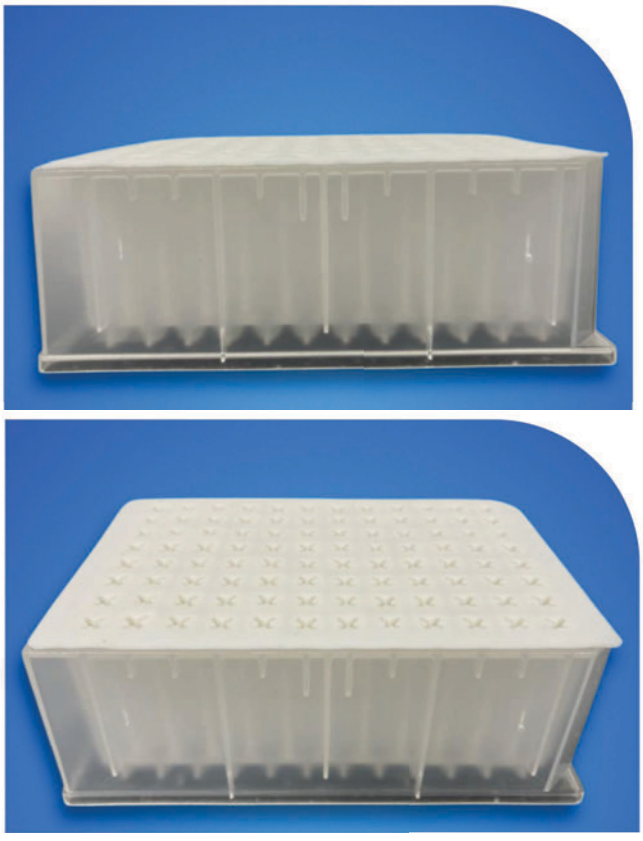


**Figure 4:** plate fully sealed with silicone mat

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