# Sample Preparation Guidelines

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**** Please do not send samples prior to being contacted by a Dovetail Scientific Project Manager, who will provide specific instructions and documents regarding sample submission** Projects require a purchase order to be initiated.**

## 1.0 PacBio Sample Preparation Guidelines

- De novo assemblies should be generated from the DNA of only one individual and free of contaminants such as parasites, endosymbionts, bacteria or heavy metals.

- If more than one individual is used, they should be true clones. If this is not possible, review the details below and contact your project manager prior to sending your sample.

- Possible options for effective inbreeding, when a single individual cannot yield enough DNA:

  - Minimum bottleneck of one individual, from which a larger sample is then obtained by solo reproduction: clonally, parthenogenetically, or by selfing.
  - Minimum bottleneck of two sexual individuals, with that bottleneck repeated multiple times (multiple generations as single pair of siblings), and only then, allow a generation with more than two individuals for DNA sampling. This process will generally achieve down to two haplotypes, but this cannot be guaranteed.

- Evaluating whether more than two haplotypes are an issue would require at least 30x shotgun sequencing for genotype validation and this evaluation may not provide sufficient insight to determine if de novo assembly will be successful.

### 1.1 PacBio Animal Tissue and Blood

**Amount and Type:** Recommended tissues are below, ordered from most to least preferable:

| Tissue Type | Minimum Amount |
|---|---|
| Nucleated Blood | 50-100 uL |
| Blood | 50-100 uL |
| Sperm Cells | 5x107-10x107 |
| Liver | 1 g |
| Spleen | 1 g |
| Brain | 1 g |
| Heart | 1 g |
| Muscle | 1 g |
| Lung | 1 g |

**Tissue Preparation:**

- Provide only fresh tissue, flash frozen in liquid nitrogen and stored at-80˚C until shipment.
- Please do not send tissue that has been fixed or preserved.
- The library and subsequent data quality will decrease for tissue samples stored at 2–8°C or –20°C.

## 1.1. PacBio Animal Tissue and Blood continued

**Blood Preparation:**

- The fresher the blood sample, the better it will perform.
- We ask that all blood samples are collected with an anticoagulant. EDTA is the anticoagulant of choice, as it inhibits DNase activity and does not introduce volume changes.
- Blood collection tubes pre- coated with EDTA can be purchased; samples should be inverted 8-10 times immediately after collection to ensure proper mixing of the anticoagulant.
- If a blood sample will be stored prior to shipment, it should be immediately flash frozen in liquid nitrogen after collection and stored at -80˚C until shipment. Frozen mammalian blood requires additional volume, as freeze/thaw negatively impacts yield.
- If a fresh blood sample will be shipped immediately (to arrive at Dovetail within 48 hours of collection), it should be stored at 4˚C prior to shipment.

**Shipping:**

- Ship frozen tissue or blood overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.
- Fresh blood should be shipped overnight in an insulated container **on wet ice** to ensure the sample remains cold throughout shipment.

## 1.2. PacBio Plant Tissue

**Amount:** Minimum 5g, 10+ g preferred.

**Type:** High molecular weight plant DNA is notoriously difficult to extract. We strongly recommend the following tissues, ordered from most preferred to least:

1. Whole plants or parts of plants at the one- or two-leaf seedling stage, preferably cotyledons for species that are abundant in polyphenolics and/or polysaccharides, such as cotton or rose.
2. Very young leaves or meristems from more mature plants.
3. Tissues collected from plants that are pretreated in the dark for 2–3 days.

**Preparation:** Flash freeze tissue in liquid nitrogen and store at -80 °C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.

### 1.3. PacBio Arthropods

**Amount:** The amount of arthropod sample that will generate 20+ µg of DNA is very difficult to estimate. Usually a few cell-rich individuals are sufficient.

**Type:** A major concern for making PacBio libraries from arthropods is the potential for contamination from their food. Therefore, the recommended developmental stages of arthropods in order of preference:

1. Embryos
2. Newly hatched larvae
3. Early pupae
4. Adults

**Preparation:** We recommend degutting adults prior to freezing. Alternatively, they may be starved for a few days prior to freezing. Flash freeze the individuals in liquid nitrogen, either in bulk for inbred species or individually for outbred species, and store at -80°C prior to shipping.

**Shipping:**

- Ship frozen sample overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs, or other ship delays.

### 1.4. PacBio Marine Animals/Invertebrates

**Amount:** 200+ mg.

**Type:** Assembling PacBio libraries for marine animals (including fish, oysters, scallops and shrimp) is often more difficult than for other animals. Their tissues may contain high concentrations of several metabolic compounds and enzymes, *e.g.*, mucopolysaccharides and alkaline phosphatases, which interfere with the PacBio library procedure. If such difficulties are expected, please communicate them to your project manager.

Recommended tissue sources in the preferred order are:

1. Newly hatched larvae
2. Sperm
3. Blood
4. Muscle

**Preparation:** Flash freeze tissue in liquid nitrogen and store at -80°C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight **with sufficient dry ice to ensure that sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs, or other ship delays.

### 1.5. PacBio Purified DNA

**Amount:** 20+ µg

**Preparation:** The highest possible molecular weight DNA should be extracted. DNA should be pure (no contamination from nucleases or RNA) and at a concentration of at least 100 ng/µl. DNA may be eluted in TE or the elution buffer that comes with the extraction kit and stored at 4°C prior to shipping.

- DNA concentration should only be measured using fluorescence-based methods. We recommend using the Qubit Broad Range DNA quantification method.
- It is critical that the DNA be high molecular weight (50+ kbp). The quality of DNA should be assessed by Pulsed Field Gel Electrophoresis. The mean fragment size must be at least 50 kbp, though higher molecular weight DNA is always preferred.

**Shipping:**

- Ship purified DNA overnight on wet ice.
- In-House Quality Assessment: For every DNA sample received, Dovetail Genomics will use 1 µl to measure the DNA concentration and 200 ng to run a Pulsed Field Gel to measure fragment size distribution.
- We will contact you if the DNA received does not meet specifications.

### 1.6 PacBio Cell Pellets

**Amount:** Minimum $1x10^7$cells per aliquot. We advise sending multiple (2-3) cell aliquots to allow for repeated extraction, if needed.

**Preparation:** Wash cells in PBS and pellet; flash freeze in liquid nitrogen and store at -80°C prior to shipping.

**Shipping:**

- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs, or other ship delays

## 2.0 Omni-C Sample Preparation Guidelines

### 2.1. Omni-C Cells

**Amount:** Minimum of $1x10^7$ cells. Please indicate the total number of cells per sample on the sample submission form.

**Preparation:** Store cells fresh in their growth media or frozen pellets at -80°C.

**Shipping method**:

- Ship cells in culture 2-day or overnight in an insulated container (to avoid ambient temperature extremes) at room temperature in a T25 flask filled to the top with growth media.
- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment**. It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs, or other ship delays

### 2.2. Omni-C Animal Tissue

**Amount & Type:** Recommended tissues are below, ordered from most to least preferable:

| Tissue Type | Minimum Amount |
|---|---|
| Nucleated Blood | 50 uL |
| Fresh Blood | 3mL |
| Frozen Blood | 6mL |
| Sperm Cells | $2x10^6$-$1x10^7$ |
| Liver | 100 mg |
| Spleen | 100 mg |
| Heart | 100 mg |
| Muscle | 200 mg |

**Omni-C Tissue Preparation:**

- Provide only fresh tissue, flash frozen in liquid nitrogen and stored at-80˚C until shipment.
- Please do not send tissue that has been fixed or preserved.
- The library and subsequent data quality will decrease for tissue samples stored at 2–8°C or –20°C.

**2.3 Omni-C Blood Preparation**

- The fresher the blood sample, the better it will perform.
- We ask that all blood samples are collected with an anticoagulant. EDTA is the anticoagulant of choice, as it inhibits DNase activity and does not introduce volume changes.
- Blood collection tubes pre- coated with EDTA can be purchased; samples should be inverted 8-10 times immediately after collection to ensure proper mixing of the anticoagulant.
- If a blood sample will be stored prior to shipment, it should be immediately flash frozen in liquid nitrogen after collection and stored at -80˚C until shipment. Frozen mammalian blood requires additional volume, as freeze/thaw negatively impacts yield.
- If a fresh blood sample will be shipped immediately (to arrive at Dovetail within 48 hours of collection), it should be stored at 4˚C prior to shipment.

**Shipping:**

- Ship frozen tissue or blood overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs, or other ship delays.
- Fresh blood should be shipped overnight in an insulated container on wet ice to ensure the sample remains cold throughout shipment. Please pack considering potential customs or other ship delays.

**2.4. Omni-C Plant Tissue**

**Amount:** Minimum 1g, 3+ g preferred.

**Type:** We recommend the following tissues, ordered from most preferred to least:

1. Leaves of plants at the one- or two-leaf seedling stage, preferably cotyledons for species that are abundant in polyphenolics and/or polysaccharides, such as cotton or rose.
2. Very young leaves from more mature plants.
3. Leaves collected from plants that are pretreated in the dark for 2–3 days.
4. Tissue from young plant parts other than leaves.

**Preparation:** Flash freeze tissue in liquid nitrogen and store in a freezer at -80 °C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight in an insulated **container with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.

### 2.5. Omni-C Arthropods

**Amount:** Minimum of 200 mg

**Type:** A major concern for making Dovetail Omni-C libraries from arthropods is the potential for contamination from their food. Therefore, the recommended developmental stages of arthropods in the preferred order are:

1. Embryos
2. Newly hatched larvae
3. Early pupae
4. Adults

**Preparation:** We recommend degutting adults prior to freezing. Alternatively, they may be starved for a few days prior to freezing. Flash freeze the individuals in liquid nitrogen, either in bulk for inbred species or individually for outbred species, and store in a freezer at -80 °C prior to shipping.

**Shipping:**

- Ship frozen sample overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.

### 2.6. Omni-C Marine Animals/Invertebrates

**Amount:** Minimum of 500 mg.

**Type:** Assembling Dovetail Omni-C libraries for marine invertebrates is often more difficult than for other animals. Their tissues may contain high concentrations of several metabolic compounds and enzymes, *e.g.*, mucopolysaccharides and alkaline phosphatases, which interfere with the library construction procedure. If such difficulties are expected, please communicate them to your project manager.

Recommended tissue sources in the preferred order are:

1. Internal organs (applicable for larger marine invertebrates)
2. Newly hatched larvae
3. Sperm
4. Blood
5. Muscle

**Note:** Mantle tissue does not perform well in the Dovetail Omni-C protocol. As such, usage of mantle tissue is not recommended.

**2.7. Omni-C Marine Invertebrates, continued**

**Preparation:** Flash freeze tissue in liquid nitrogen and store at -80°C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.

## 3.0. RNA-Seq Sample Preparation Guideline

Biological replicates are vital to confirm the accuracy of RNA-Seq data. Please send three replicates of two different samples to provide diversity and the ability to QC the data.

### 3.1 Animal Tissue

**Amount:** Minimum 2mg; 10mg preferred.

**Preparation:** Flash freeze tissue in liquid nitrogen and store at -80°C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight in an insulated **container with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays

### 3.2 Cell Pellet

**Amount:** Minimum $10^4$ cells, $10^6$ preferred

**Preparation:** Pellet cells and flash freeze in liquid nitrogen; store at -80°C prior to shipping.

**Shipping:**

- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays

### 3.3 Plant tissue

**Amount:** 200 mg of tissue

**Preparation:** Flash freeze tissue in liquid nitrogen and store in a freezer at -80°C prior to shipping.

**Shipping:**

- Ship frozen tissue overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays

## 4.0 Micro-C Proximity Ligation Sample Preparation Guidelines

### 4.1 . Micro-C (Mammalian cells)

**Amount:** For each sample, Dovetail requires a minimum of 5 million flash frozen human, mouse cells. Please send five aliquots of 1 million cells each and indicate the number of cells per aliquot on the sample submission form.

**Cell Preparation:**

- Harvest the cells, wash with 1X PBS and count.
- Aliquot 1 x $10^6$ cells into a 1.5 mL tube. Prepare five aliquots of 1 x $10^6$ cells each.
- Spin the 1 x $10^6$ cell aliquots at 3,000 x g for 5 minutes. Carefully remove and discard the supernatant
- Flash freeze the cell pellets in liquid nitrogen and store at -80°C until shipment.

**Shipping:**

- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the samples remain frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for 24-hour period of shipment. If you are shipping items for a longer duration, increase the amount of dry ice used. Please pack considering potential customs or other ship delays.

### 4.2 Micro-C Human/Mouse Tissue

**Amount & Type:** Recommended tissues are below, ordered from most to least preferable:

| Tissue Type | Minimum Amount |
|---|---|
| Fresh Blood | 3mL |
| Frozen Blood | 6mL |
| Sperm Cells | $2x10^6$-$1x10^7$ |
| Liver | 100 mg |
| Spleen | 100 mg |
| Heart | 100 mg |
| Muscle | 200 mg |

**Micro-C Tissue Preparation:**

- Provide only fresh tissue, flash frozen in liquid nitrogen and stored at-80˚C until shipment.
- Please do not send tissue that has been fixed or preserved.
- The library and subsequent data quality will decrease for tissue samples stored at 2–8°C or –20°C.

**Micro-C Blood Preparation:**

- The fresher the blood sample, the better it will perform.
- We ask that all blood samples are collected with an anticoagulant. EDTA is the anticoagulant of choice, as it inhibits DNase activity and does not introduce volume changes.
- Blood collection tubes pre- coated with EDTA can be purchased; samples should be inverted 8-10 times immediately after collection to ensure proper mixing of the anticoagulant.
- If a blood sample will be shipped immediately after collection (to arrive at Dovetail within 48 hours of collection), it should be stored at 4˚C prior to shipment.
- If a blood sample will be stored prior to shipment, it should be immediately flash frozen in liquid nitrogen after collection and stored at -80˚C until shipment. Frozen mammalian blood requires additional volume, as freeze/thaw negatively impacts yield.

**Shipping:**

- Ship frozen tissue or blood overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays.
- Fresh blood should be shipped overnight in an insulated container on wet ice to ensure the sample remains cold throughout shipment.

## 5.0 HiChIP Proximity Ligation Sample Preparation Guidelines

### 5.1. HiChIP (Human/Mouse Cells)

**Amount:** For each HiChIP Sample Dovetail requires a minimum of 20 million flash frozen human cells. We recommend sending four aliquots of 5 million cells each. Please indicate the number of cells per aliquot on the sample submission form.

**Preparation:**

- Harvest the cells, wash with 1X PBS and count.
- Aliquot 5 x $10^6$ cells into a 1.5 mL tube. Prepare four aliquots of 5 x $10^6$ cells each.
- Spin the 5 x $10^6$ cell aliquots at 3,000 x g for 5 minutes. Carefully remove and discard the supernatant.
- Flash freeze the cell pellets in liquid nitrogen and store at -80°C until shipment.

**Shipping:**

- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the samples remain frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for 24-hour period of shipment. If you are shipping items for a longer duration, increase the amount of dry ice used. Additionally, gel packs can also extend the life of dry ice.
- For international shipments, please increase the amount of dry ice used to account for custom clearance or potential shipment delays.

### 5.2 HiChIP (Human/Mouse Tissue)

**Amount & Type:** Recommended tissues are below, ordered from most to least preferable:

| Tissue Type | Minimum Amount |
|---|---|
| Liver | 500 mg |
| Spleen | 500 mg |
| Heart | 500 mg |
| Muscle | 500 mg |

**Tissue Preparation:**

- Provide only fresh tissue, flash frozen in liquid nitrogen and stored at-80˚C until shipment.
- Please do not send tissue that has been fixed or preserved.
- The library and subsequent data quality will decrease for tissue samples stored at 2–8°C or –20°C.

**Shipping:**

- Ship frozen tissue overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays

## 6.0 ChIP-Seq Sample Preparation Guidelines

### 6.1. ChIP-Seq (Human/Mouse Cells)

**Amount:** For each ChIP-Seq Sample Dovetail requires a minimum of 20 million flash frozen human cells. We recommend sending four aliquots of 5 million cells each. Please indicate the number of cells per aliquot on the sample submission form.

**Preparation:**

- Harvest the cells, wash with 1X PBS and count.
- Aliquot 5 x $10^6$ cells into a 1.5 mL tube. Prepare four aliquots of 5 x $10^6$ cells each.
- Spin the 5 x $10^6$ cell aliquots at 3,000 x g for 5 minutes. Carefully remove and discard the supernatant.
- Flash freeze the cell pellets in liquid nitrogen and store at -80°C until shipment.

**Shipping:**

- Ship frozen cell pellets overnight in an insulated container **with sufficient dry ice to ensure the samples remain frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for 24-hour period of shipment. If you are shipping items for a longer duration, increase the amount of dry ice used. Additionally, gel packs can also extend the life of dry ice.
- For international shipments, please increase the amount of dry ice used to account for custom clearance or potential shipment delays.

### 6.2. ChIP-Seq (Human/Mouse Tissues)

**Amount & Type:** Recommended tissues are below, ordered from most to least preferable:

| Tissue Type | Minimum Amount |
|---|---|
| Liver | 500 mg |
| Spleen | 500 mg |
| Heart | 500 mg |
| Muscle | 500 mg |

**Tissue Preparation:**

- Provide only fresh tissue, flash frozen in liquid nitrogen and stored at-80˚C until shipment.
- Please do not send tissue that has been fixed or preserved.
- The library and subsequent data quality will decrease for tissue samples stored at 2–8°C or –20°C.

**Shipping:**

- Ship frozen tissue overnight in an insulated container **with sufficient dry ice to ensure the sample remains frozen throughout shipment.**
- It is recommended to include 5-10 pounds of dry ice for each 24-hour period of the shipment. Please pack considering potential customs or other ship delays