PACBIO®

Technical Note

Sample Prep

PACBIO®

Preparing Samples for PacBio® Whole Genome Sequencing for *de novo* Assembly: Collection and Storage

Introduction

Single Molecule, Real-Time (SMRT®) Sequencing uses the natural process of DNA replication to sequence long fragments of native DNA. As such, starting with high-quality, high molecular weight (HMW) genomic DNA (gDNA) will result in better sequencing performance across difficult to sequence regions of the genome. To obtain the highest quality, long DNA it is important to start with sample types compatible with HMW DNA extraction methods.

This technical note is intended to give general guidance on sample collection, preparation, and storage across a range of commonly encountered sample types used for SMRT Sequencing whole genome projects. It is important to note that all samples and projects are unique and may not be comprehensively addressed in this document.

Topics Covered

- Sample and storage recommendations for:
  - Vertebrates - mammals, birds, fish, amphibians, reptiles
  - Invertebrates - marine, terrestrial
  - Arthropods - insects, crustaceans
  - Fungi - microorganisms, mushrooms, algae*
  - Plants - broad leaf plants, grasses

  *Algae is included with fungi due to similar growth and storage conditions
- Additional considerations for planning HMW DNA isolation

Sample and Storage Recommendations

Vertebrates

When sampling from vertebrates it is recommended to use a single individual.

	Sample Type	Sample Storage
1	Cell-dense tissue (brain, kidney, muscle, etc.)	Fresh (within ~24 hours of collection and kept at 4°C) or flash frozen with liquid nitrogen and stored at -80°C.
2	Whole blood (if red blood cells are nucleated)	Collected in tube with anticoagulant such as EDTA, blood sample will be viable at 4°C for 24-72 hours after collection. After this time, sample will be viable at -20°C for several months.
3	Cell-culture	Room temperature cell suspension or pellet is best entering the DNA isolation step. However, cryopreserved cells (slow frozen in cryoprotectant) should be viable at -80°C for several months.
4	Sperm	Fresh collected, room temperature sperm samples are best entering the DNA isolation step. However, customers have been successful with sperm frozen at -20°C.

Table 1 - Vertebrate sample types in order of preference

Note: It is NOT recommended to use liver tissue as input sample due to high abundance of enzymes that may degrade DNA.

PACBIO®

Invertebrates

When sampling from invertebrates, it is recommended to use a single individual. Some invertebrates have mucous membranes that inhibit the ability to obtain high-quality DNA. Please consider an extra cleanup step of the DNA if mucous-coated samples are used (see cleanup protocol here). It is also common to encounter invertebrate samples that are not easily separated from contaminants or do not have cell-dense tissues readily available. In these cases, sperm can be used as the input sample.

	Sample Type	Sample Storage
1	Cell-dense tissue (brain, kidney, muscle, etc.)	Fresh (within ~24 hours of collection and kept cold) or flash frozen with liquid nitrogen and stored at -80°C should be viable for several months.
2	Cell-culture	Room temperature cell-suspension or pellet is best entering the DNA isolation step. However, cryopreserved cells (slow frozen in cryoprotectant) should be viable at -80°C for several months.
3	Sperm	Fresh collected, room temperature sperm samples are best entering the DNA isolation step. However, customers have been successful with sperm frozen at -20°C.

Table 2 - Invertebrate sample types in order of preference

Note: It is NOT recommended to use liver tissue as input sample due to high abundance of enzymes that may degrade DNA.

Arthropods

When sampling from arthropods, it is recommended to use a single individual. However, it is often the case that arthropod individuals, such as insects, are too small to obtain the necessary quantity of DNA. In these cases, it is recommended to use as few directly related individuals as possible.

	Sample Type	Sample Storage
1	Single pupa or larva	Fresh (within ~24 hours of collection and kept cold) or flash frozen with liquid nitrogen and stored at -80°C should be viable for several months.
2	Single adult individual	Fresh (within 24 hours of collection) or flash frozen with liquid nitrogen and stored at -80°C should be viable for several months.
3	Multiple pupae or larvae	Fresh (within 24 hours of collection) or flash frozen with liquid nitrogen and stored at -80°C should be viable for several months.
4	Cell-culture	Room temperature cell-suspension or pellet is best entering the DNA isolation step. However, cryopreserved cells (slow frozen in cryoprotectant) should be viable at -80°C for several months.

Table 3 - Insect sample types in order of preference

	Sample Type	Sample Storage
1	Cell-dense tissue (brain, kidney, muscle, etc.)	Fresh (within ~24 hours of collection and kept cold) or flash frozen with liquid nitrogen and stored at -80°C should be viable for several months.
2	Cell-culture	Room temperature cell suspension or pellet is best entering the DNA isolation step. However, cryopreserved cells (slow frozen in cryoprotectant) should be viable at -80°C for several months.
3	Sperm	Fresh collected, room temperature sperm samples are best entering the DNA isolation step. However, customers have been successful with sperm frozen at -20°C.

Table 4 - Crustacean sample types in order of preference

Note: To reduce contaminants from food and gut microbiota, it is recommended to starve insects when using whole individuals and to dissect out the gut as best as possible.

PACBIO®

Fungi

When sampling from fungi or algae, it is recommended to culture the sample in order to acquire a single isolate/individual in the case of macroscopic organisms or an isogenic population in the case of microorganisms. These guidelines are not for metagenomic applications and should only be used for single genome analysis workflows.

	Sample Type	Sample Storage
1	Cultured single isolate/individual	Fresh culture is best entering the DNA isolation step. However, customers have been successful with fungal cultures that are lyophilized or spun down and stored at -80°C.
2	Cultured isogenic population	Fresh culture is best entering the DNA isolation step. However, customers have been successful with fungal cultures that are lyophilized or spun down and stored at -80°C.
3	Field sample	Customers have been successful with fungal samples that are lyophilized and stored blocked from light at 4°C or spun down and stored at -80°C.

Table 5 - Fungi sample types in order of preference

Note: If using a field sample, please consider an additional cleanup step for the isolated DNA to remove potential contaminants (see cleanup protocol here).

Plants

When sampling from plants, it is recommended to obtain leaves/new shoots from a single individual. The leaves/shoots should be the youngest possible and dark treated for 24-72 hours before collection. This reduces the abundance of secondary metabolites present in the tissue that may inhibit the ability to obtain high-quality DNA.

	Sample Type	Sample Storage
1	Young, dark treated leaf/shoot tissue	Flash frozen in liquid nitrogen and stored at -80° or lyophilized samples, blocked from light stored at 4°C should be viable for several months.
2	Mature, dark treated leaf tissue	Flash frozen in liquid nitrogen and stored at -80° or lyophilized samples, blocked from light stored at 4°C should be viable for several months.
3	Mature, non-dark treated leaf tissue	Flash frozen in liquid nitrogen and stored at -80° or lyophilized samples, blocked from light stored at 4°C should be viable for several months.

Table 6 - Plant samples in order of preference

Note: Plants have an abundance of secondary metabolites that may inhibit the ability to obtain high-quality, HMW DNA. Please consider an additional cleanup step for the isolated DNA to remove potential contaminants (see cleanup protocol here).

Additional Considerations for HMW DNA Isolation and Handling

- Minimize or eliminate the number of freeze/thaw cycles with your sample to reduce DNA damage
- Only use wide bore pipette tips when handling DNA and pipette very slowly to reduce shearing
- Minimize or eliminate any high-heating steps during isolation or preparation of DNA
- Minimize or eliminate high-speed vortexing; use gentle mixing techniques such as slow inversion
- Allow sufficient thawing time for aliquots of DNA, as partially frozen DNA is prone to shearing
- Shipping DNA: Overnight shipping at 4°C in is preferred. However, if shipping overnight is not an option, flash freeze the DNA sample with liquid nitrogen and ship frozen. Alternatively, DNAstable Plus allows for shipping of DNA at room temperature.

PN: TN100-040518