Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen
Core Facility Genomics

Sample Isolation Specifications for Eukaryotic Samples

Unless stated otherwise, it is crucial to strictly adhere to the specifications provided below when handling the samples. The isolation kits are not designed to accommodate lower or higher input quantities. Any deviation from these specifications can lead to a complete failure of the RNA/DNA extraction process. If the samples need to be cut, divided, aliquoted, or transferred, there is a risk of material loss or thawing, which can ultimately result in distorted results.

Tissue

To extract nucleic acids from tissue samples, we suggest using either a homogenizer or a bead-based lyzer, depending on the tissue's plasticity and the number of samples being processed. The addition of mercaptoethanol or DTT to the lysis buffer is necessary only for specific tissues or cell types when protein contamination is detected during quality control. Please review the following sample requirements:

Fresh tissue

It is ideal to send approximately 10mg of fresh tissue with a deviation of 10%. We recommend a minimum of 5mg and a maximum of 25mg. Ensure that the fresh tissue samples are snap-frozen in liquid nitrogen and sent in 2 ml tubes on dry ice.

Fixed material

For fixed material, we strongly recommend contacting the molecular pathology department in Tübingen for their isolation service of DNA or RNA from FFPE (Formalin-Fixed Paraffin-Embedded) fixed material.

Plants

For plant samples, please send one or several specimens with a wet weight of more than 50mg. These samples should be placed in 2 ml tubes on dry ice for shipment.

Cells

For short read sequencing

To facilitate the sequencing process, please send an ideal quantity of 5 million cells with a deviation of 10%. The samples should be prepared as a dry pellet and placed in 2 ml tubes on dry ice. To ensure smooth processing, kindly provide the counted number of cells on the sample sheet.

For long read sequencing

For each sample intended for long read sequencing, please send four aliquots of dry cell pellets. Each pellet should contain 1 to 2 million cells and be snap frozen in 2 ml tubes. If a different number of cells is pelleted, please indicate an approximate count on the sample sheet. To ensure quality, we recommend including a positive control (cell culture prepared in our facility) alongside the samples. This control will help ensure that the samples were not degraded during sample preparation, transportation, or DNA extraction.

Blood

Handling blood samples requires specific considerations, and it is essential to carefully review the recommendations provided by the product supplier before the first usage. Some critical steps typically involved are as follows:

- Mixing blood with the tube solution
- Incubation of the tube at room temperature
- Performing DNA or RNA isolation as quickly as possible
- Isolation of DNA or RNA should be performed as fast as possible

If isolation is not feasible on-site:

- Freezing samples often leads to a reduction of DNA and RNA quality (particularly for cell free DNA)
- Freezing samples can complicate the automation of isolation procedures

For DNA extraction from whole blood

We recommend using EDTA-Blood BD Vacutainer (5 ml)

When possible, do not freeze EDTA-Blood and isolate DNA within 2 days. For large projects requiring biobanking without the option of DNA isolation, solutions for isolation should be discussed prior to the start of the project.

Whenever possible, avoid freezing EDTA-blood and perform DNA isolation within 2 days. For large projects that require biobanking without the option of immediate DNA isolation, it is advisable to discuss a suitable isolation solution before initiating the project.

Please note that we do not process heparin-blood for NGS (Next-Generation Sequencing) applications. While heparin-blood is useful for karyotyping, it is not suitable for enzymatic processing as it efficiently inhibits PCR reactions.

DNA extraction methods

To prioritize read length (at expense of total yield), high-molecular weight DNA can be obtained with salting-out, bead-based isolation or phenol-chloroform. To optimize the total yield (at the expense of read length), ensure your DNA preparation is free of any contaminants.

DNA extraction tips

Avoid phenol-chloroform extractions which can result in residual phenol. For highest purity opt for column-based isolation of genomic tips. By using columns, the DNA will in turn not produce very long reads since the DNA is fragmented during isolation. However, reads >10kb can still be achieved at very high yields. If sufficient DNA is obtained, size selection can be performed to remove short fragments present in the sample.

For RNA extraction from whole blood

For blood RNA collection, we recommend using PAXgene Blood RNA tubes (human: > 2.5 ml), catalog number of 762165 (100 tubes). To ensure proper handling and usage instructions, please consult the PAXgene tube manual provided by the manufacturer.