Sample requirements

NCCT
NGS Competence Center Tübingen

Sample requirements

To ensure quick and easy processing, please make sure to always enclose the completed sample sheet with the quote ID. We cannot start processing your samples if we do not have all necessary information.

As we are trying to ensure the best possible way of processing each project, the following list of sample types and requirements may not cover your specific topic. If you do not find any specifications of your sample type, or if your sample does not meet the here mentioned requirements, please do not hesitate to contact us. We will find an individual solution.

In general, please send extracted RNA or DNA if possible. Some samples may be problematic to extract, or there may be already established manual methods for RNA/DNA extraction in your own lab. We have expertise in extraction using commercial kits, but we cannot warrant good results with other protocols or problematic starting material, which may also slow down the turnaround time of your samples.

General Information

All tubes should be labelled clearly with a sample ID, a date and the name of the sender. We recommend the use of nuclease-free, DNA-free, PCR-clean low-bind 1,5 ml tubes (no autoclaving). We do not handle tubes smaller than 1,5 ml, as storage and labelling of these tubes is problematic. For large sample batches, please use 96- or 384-well lo-bind-plates. Samples should be sent on dry ice if possible. Preserving reagents, such as RNAlater, should only be used if unavoidable and cannot replace appropriate handling.

Please do not send less than 15 µl, even if you have high concentrations. In this case, you can dilute the samples accordingly. We require 5µl of each sample for quality control (QC) measurements.

If you treat your samples with RNAse, please declare this visibly on the sample sheet! It is not a problem, but as an RNA-lab, we have to take special precautions with RNAse treated samples.

If you work with organisms of safety-level S2 or higher, please make sure to inactivate them completely or only send extracted nucleic acids. We do not handle S2 (or higher) organisms nor genetically modified organisms (GMO).

Tissue

For extraction of nucleic acids, we recommend using either a homogenizer or a bead-based lyzer, depending on the plasticity of the tissue and on the number of samples. Addition of mercaptoethanol or DTT to the lysis buffer is done only for specific tissues or cell types when protein contamination is detected in the quality control. If you prefer not to extract the nucleic acids yourself, we can do the isolation at small extra cost. Please refer to the following sample requirements:

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Fresh tissue

At least 5 mg, but not more than 25mg, fresh tissue, snap frozen in liquid nitrogen.
The sample should be sent in an 1,5 ml tube on dry ice.

Fixed material

Please send at least 10 slides (up to 10 µm thickness each with a surface area of up to 250 mm2). Please remove as much of the fixative as possible to ensure best results.

Plants

Please send one or several specimen of a > 50 mg wet weight. The sample should be sent in a 1,5 ml tube on dry ice.

Cells

Should be sent as a pellet in 1,5 ml tubes on **dry ice**. Please send at least $1x10^7$ cells (snap frozen) or refer to the kit specifications [e.g. QIAamp DNA Mini Kit]. In order to ensure easy processing, please provide an estimation of the number of cells on the sample sheet [Hyperlink]. For nuclein extraction, we recommend the homogenization and lysis using a commercial kit.

Please note that we do not handle revivable S2 or higher safety-level organisms (nor GMOs). Please make sure to **completely inactivate** them before shipping. To avoid any hazard of contaminations, we strongly recommend only sending extracted nucleic acids.

Blood

For DNA extraction: Whole blood, EDTA-Blood (human: 5 ml), PAXgene, blood cards.
For RNA extraction: PAXgene tubes (human: 2,5 ml)
For cell-free DNA: PAXgene tubes (human: 10 ml)

Please refer to the PAXgene Manual:
Whole blood and PAXgene tubes should be inverted **at least 10 times to mix well**, incubated at RT for at least 2 hours (for lysis) and 4°C overnight; after that, please freeze the samples at -80°C. Freeze-thaw-cycles should be avoided. Even if samples can be stored in PAXgene tubes for up to 7 days at room temperature, we do not recommend this. Especially for cfDNA, we recommend to process the samples as fast as possible within 2 days. To ensure best results, extraction of the cfDNA prior to shipping is advisable.

Note: **Please do not freeze EDTA-Blood!** For EDTA-Blood shipping at room temperature is recommended.

We do not process heparin-blood for NGS applications. Heparin-blood is useful for karyotyping but not for enzymatic processing. Heparin efficiently inhibits PCR reactions.

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Pure DNA

Please send us >20 µl or a total of 1 µg purified DNA provided in clearly labelled 1,5 ml tubes. If you cannot obtain this quantity, we can also obtain high-quality results with much less (as low as a total of 160 ng), but we only recommend this in urgent cases with a very low yield. Please note that we always need 5 µl of each DNA sample for quality control, independent of the DNA quantity.

Working with DNA: To our experience, best results can be obtained with column-based or bead-based isolation kits and DNA-free filtered tips. We recommend elution of the DNA in a standard buffer (as provided in most kits). If you dilute in water, please use **nuclease-free, not DEPC-treated water. Never use autoclaved water, tap water or Millipore water for NGS-applications!** It can contain DNA of any source (that will in the best case reduce your on-target sequencing results) and may contain components that inhibit downstream processing. In general, we strongly recommend **not using an autoclave** for NGS-applications. Autoclaves are usually used to autoclave all sorts of labware to inactivate microorganisms and enzymes. The autoclave does not entirely destroy or remove DNA. Pre-stacked filtered tips and PCR-clean tubes are strongly recommended.

Please provide the elution buffer on the sample sheet for correct calibration of our instruments. If it is not a standard buffer, please send us an aliquot. Please note that any chemical contamination of the sample (e.g. with Guanidinium thiocyanate, phenol, etc.) may cause problems with downstream processing steps that are sensitive to chemical contamination. If you isolate with protocols that contain these components (especially home-made ones), we recommend a column- or bead-based cleanup afterwards. We can do this for you at small extra-cost.

Please declare any RNAase treatment on the sample sheet! It is not a problem, but as an RNA-lab, we have to take special precautions for samples that have been treated with RNAse.

We recommend **shipping on dry ice if possible.**

NOTE: If you want to perform epigenetic analyses, make sure to indicate this on the sample sheet. Samples should be stored at -80°C and **shipped on dry ice** to avoid degradation.

A first rough assessment of the DNA quality and quantity can be obtained using a photometer. Please note our recommendations for the absorption ratios:

260/280: ≥ 1,7 → good ≥ 1,0 → medium < 1 → possibly problematic
Values <1,7 can indicate protein contamination and may lead to inhibition of downstream processing or degradation
260/230: ≥1,7 → good ≥ 1,0 → medium < 1 → possibly problematic
Values <1,7 can indicate solvent contamination, e.g. phenol, ethanol, and may lead to inhibition of downstream processing

If you notice any abnormal values, please reconsider re-extracting your samples or a column-based cleanup. Although in many cases processing and sequencing is possible despite medium or even bad

ratios, we cannot warrant good sequencing results and we exclude all liabilities for any results of the respective samples (for further information on sample quality control see QC Hyperlink).

Libraries

Of course, you can also send your ready-for-sequencing libraries. In this case, we require

- Dual indexes. If you are not familiar with dual indexing, please contact us. Dual indexing ensures correct sample assignment.
- Please only use Illumina standard sequencing primers. If you need to use custom sequencing primers, please specify them on the sequencing sample sheet (name, sequence and concentration). Please also send an aliquot of at least 20 µl these primers in a clearly labelled, nuclease-free 1,5 ml tube.
- Concerning the library:
    - for NovaSeq: fragment size 90% < 600 nt; concentration > 10 nM; volume > 15 µl in EB
    - for MiSeq and NextSeq: fragment size < 900 nt; concentration > 10 nM; volume > 15 µl in EB
    - Please provide the usual library quality parameters:
        - Concentration measured using a Qubit device
        - Average library size in base pairs (bp), measured using Bioanalyzer, Tapestation or Fragment Analyzer (please also provide the electropherogram)

Pure RNA

Our requirements are > 15 µl or a total of 1 µg purified RNA provided in clearly labelled RNase-free 1,5 ml tubes. If you cannot obtain this quantity, we can also obtain high-quality results with much less (as low as a total of 160 ng), but we only recommend this in urgent cases with a very low yield.

Working with RNA: All working areas should be treated with RNase decontamination solution. Optimally, the room temperature should be kept between 15°C to 25°C. We strongly recommend the usage of DNase digestion during the isolation. To our experience, best results can be obtained with column-based or bead-based isolation kits. We recommend elution in nuclease-free water (as provided in most kits). Please use **nuclease-free, not DEPC-treated water. Never use autoclaved water, tap water or Millipore water for NGS-applications!** It can contain DNA of any source (that will in the best case reduce your on-target sequencing results) and may contain components that inhibit downstream processing. In general, we recommend **not using an autoclave (e.g. for tubes or tips)** for NGS-applications. Autoclaves are usually used to autoclave all sorts of labware to inactivate microorganisms and enzymes. The autoclave does not entirely destroy or remove DNA. Pre-stacked filtered tips and PCR-clean tubes are strongly recommended.

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Shipping should be on dry ice.

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- Average library size in base pairs (bp), measured using Bioanalyzer, Tapestation or Fragment Analyzer (please also provide the electropherogram)