Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen

Sample Requirements Nucleic Acids

General Information

By utilising our core facility services, you confirm that you have carefully read and understood the information provided in the "NCCT General-Guidelines" here.
This section covers important aspects of the quality and handling of isolated nucleic acid for sequencing at the NCCT.

In order to get the highest quality out of the samples, we recommend you to consult our recommendation on nucleic acid extraction here.

Nucleic acids sent for sequencing applications must meet specific quality parameters in order to be able to be processed. Those parameters are volume, concentration, integrity and purity. It is recommended to assess as many parameters as possible before submitting the samples. We highly recommend you to make every effort to eliminate DNA from RNA samples and to eliminate RNA from DNA samples. Please indicate the use of nucleases! Additional quality control will be performed before library preparation at the NCCT. Samples that do not meet the minimum requirements may be processed with a higher likelihood of failure. Please contact us in case of low input or low quality protocols. All requirements for offered applications are summarised in the two following tables. We recommend elution of the DNA in a low-TE buffer or a standard buffer (as provided in most kits).

Application	Required volume	Required total amount	Required concentration	Additional parameters
DNA library / WGS / Methyl-Sequencing	> 20 µl **FFPE:** >40µl	3 µg **FFPE:** >200 ng **Low input:** >100 ng	> 50 ng/µl **FFPE:** >5 ng/µl **Low input:** >10 ng/µl	
WES	> 15 µl	1 µg **Low input:** 200ng	> 50 ng/µl **Low input:** >5 nl/µl	
Low Input WES	> 15 µl	200 ng	>20 ng/µl	
Bacterial WGS Metagenomics	> 15 µl	1 µg	> 10 ng/µl	
16S / Amplicon	> 15 µl	250 ng	> 15 ng/µl	
LRS WGS Nanopore Eukaryotes	> 20 µl	10 µg	> 50 ng/µl	
LRS Nanopore Prokaryotes	> 20 µl	1.5 - 5 µg	> 30 ng/µl	
LRS PacBio	> 20 µl	15 µg	> 50 ng/µl	
RNA-Sequencing polyA	> 15 µl	500 ng **Paxgen:** 2µg	> 25 ng/µl **Paxgen:** > 30 ng/µl	RIN > 7 **Paxgen:** RIN > 6
RNA-Sequencing ribodepletion	>20 µl	1 µg **FFPE:** 100 ng	> 50 ng/µl **FFPE:** >10 ng/µl	RIN > 6 **FFPE:** 50% DV200
Low Input RNA-Sequencing	> 15 µl	100 ng	>5 ng/µl	RIN > 7
miRNA/small RNA (require consulting)	> 15 µl	20 ng	> 5 ng/µl	Isolation protocol that retains miRNA
cDNA-PCR (ONT)	> 20 µL	1 µg	> 50 ng/µl	RIN > 8.5
Iso-Seq (PacBio)	> 20 µL	1 µg	> 50 ng/µl	RIN > 8.5

Abreviations: Whole-Genome-Sequencing (WGS), Whole-Exome-Sequencing (WES), Long-Read-Sequencing (LRS)

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Nucleic acid quantification

NanoDrop nucleic acid quantification

The most common and abundant methods to analyse the quality of nucleic acids are NanoDrop spectrophotometry and agarose gels.

For high quality DNA the NanoDrop assessed 260/280 nm ratio indicates high-quality DNA at 1.8 and high-quality RNA at 2.0. The 260/230 nm ratio is considered pure between 1.8-2.2.

	260 nm/280 nm	260 nm230 nm
High quality DNA	1.8	2
High quality RNA	1.8-2.2	1.8-2.2

These values reflect the purity of the samples to a certain degree but do not provide information about the integrity. The concentrations provided by NanoDrop spectrophotometry are fairly reliable with high-quality nucleic acids, but generally tend to be overestimated.

We recommend column- or bead-based cleanup as any chemical contamination of the sample (e.g. with Guanidinium thiocyanate, phenol, etc.) may cause problems with downstream processing steps. We can do this for you at a small extra-cost.

Qubit Fluorometer quantification

Nucleic acid quantifications assessed with the Qubit fluorometer are far more specific, but do not provide purity information. Nevertheless, for NGS applications it is essential to determine the input quantity of nucleic acid as precisely as possible, which is why Qubit assessed concentrations are to be preferred over NanoDrop measurements.

Nucleic Acid Integrity

The integrity of nucleic acids is most important for FFPE, RNA-sequencing and long-read-sequencing.

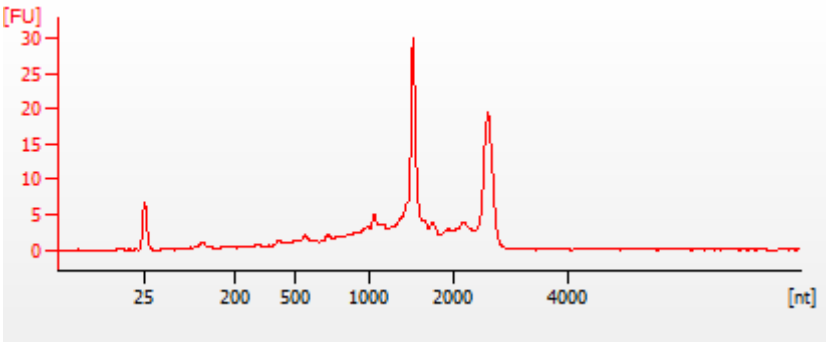
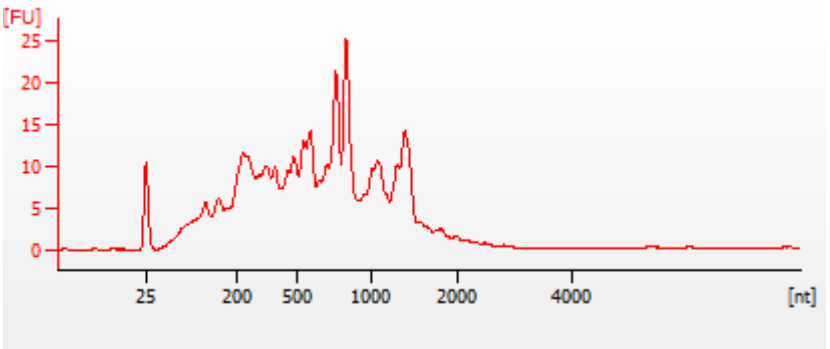
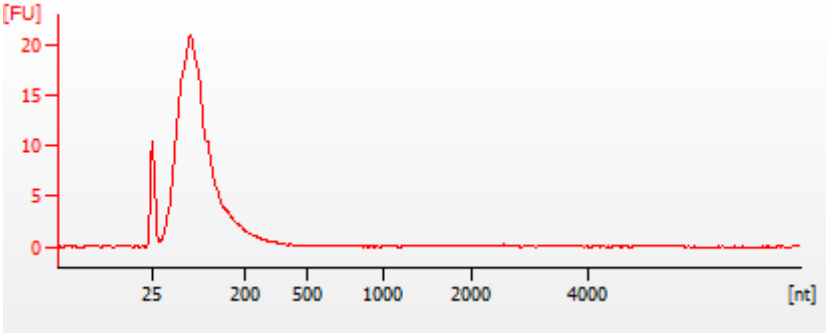
FFPE integrity

DNA samples isolated from **FFPE tissue exhibit significant variations** in quantity and fragment length, and it is common for all available fragments to be shorter than 200 bp. Assessing the level of crosslinks with DNA, RNA, or proteins, as well as chemical modifications to the bases or the DNA backbone, is not feasible. We recommend considering the grouping of samples with matching quality characteristics to mitigate the issue of inhomogeneous library fragment lengths.

RNA Integrity

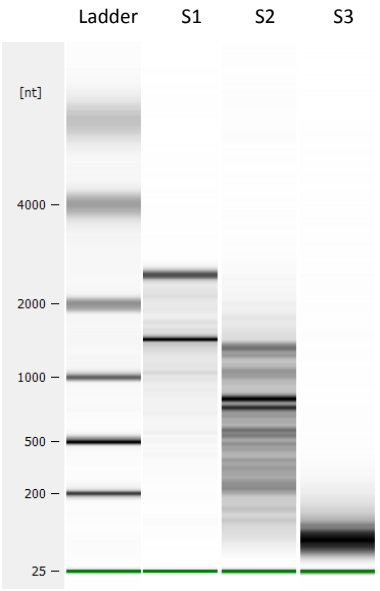
RNA integrity can be assessed with agarose gels or automated electrophoresis systems. Two clearly separated bands between 1000 – 3000 nt indicate high-quality RNA. The lack of two bands in this region indicates RNA degradation.

Automated electrophoresis systems can provide clear RNA quality parameters that reflect the degree of degradation like the RNA-integrity-number (RIN), RNA-Quality-Number (RQN) or DV200. RIN and RQN values are measured in a scale from 1 to 10, with 10 representing high quality, undegraded RNA. The DV200 value represents the percentage of fragments that are larger than 200 base pairs. Low DV200 values are an indication for low quality, degraded samples.

Electropherogram	Sample names and quality parameters
	Sample 1 (S1) RIN 7.2 DV200 95% Good quality
	Sample 2 (S2) RIN 4.7 DV200 85% Low quality
	Sample 3 (S3) RIN 2.6 DV200 5% Bad quality

RNA profiles generated by automated electrophoresis systems

Gel picture



Sample names and quality parameters

Sample 1 (S1)

RIN 7.2
DV200 95%
Good quality

Sample 2 (S2)

RIN 4.7
DV200 85%
Low quality

Sample 3 (S3)

RIN 2.6
DV200 5%
Bad quality

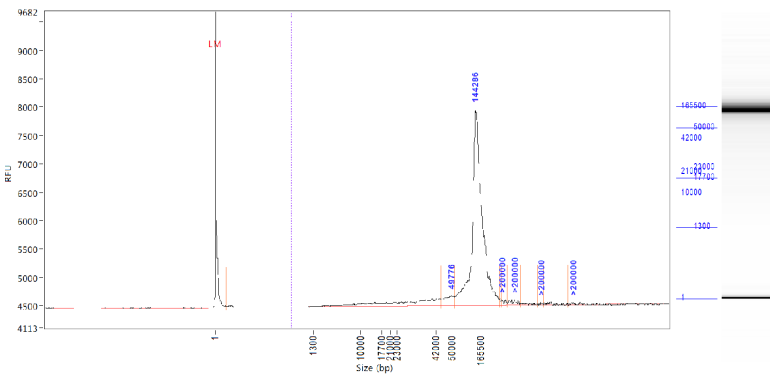
RNA gel picture generated by automated electrophoresis systems

High Molecular Weight DNA

In case only a lower amount of sample is available, PCR-based protocols starting from 100 ng or less can be applied. However, it should be kept in mind that the length distribution of the reads generated will be much shorter (<10kb).

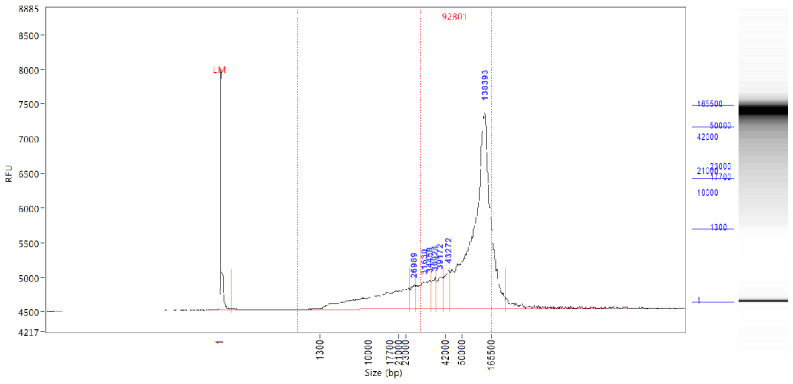
High molecular weight DNA QC: Fragment length distribution of isolated genomic DNA is assessed by pulse-field capillary electrophoresis. The measured DNA for long-read-sequencing should be >30 kb and ideally not show any signs of degradation (smear).

High molecular weight DNA

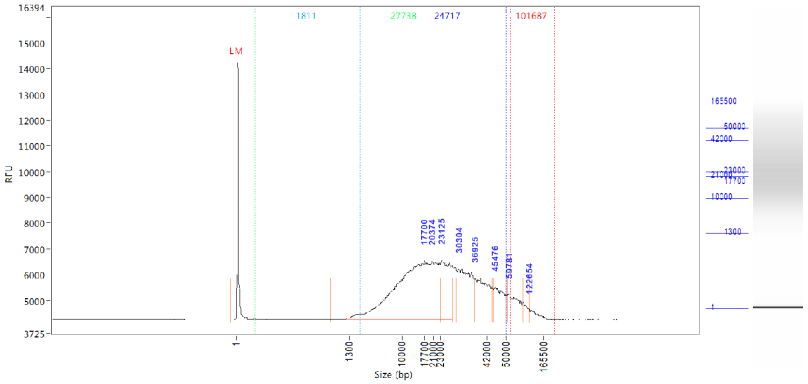


Example of HMW DNA isolated with Phenol-chloroform (toxic chemicals, not recommended)

Bead-based (commercial kit)



Not high-molecular weight DNA



Column-based (commercial kit)

We recommend using wide-bore pre-stacked filtered tips when handling already isolated high-molecular weight DNA. In order to preserve the long fragments of DNA for long-read sequencing please take the following precautions when handling samples: pipette slowly and do not mix by pipetting. **Do not vortex** samples and mix by inverting tubes. Tubes can be spin down.