Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen

Sample Requirements Nucleic Acids v.1

This section covers important aspects of the quality and handling of isolated nucleic acid for sequencing at the NCCT.

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. All requirements for offered applications are summarised in the two following tables.

Application	Required volume	Required total amount	Required concentration	Additional parameters
WGS/ MethylSeq	> 20 µl	3 µg	> 50 ng/µl	
Whole Exome Sequencing	> 15 µl	5 µg	> 50 ng/µl	
Bacterial WGS Metagenomics	> 15 µl	1 µg	> 10 ng/µl	
16S / Amplicon	> 15 µl	250 ng	> 15 ng/µl	
Long-Read-Sequencing Nanopore Eukaryotes	> 20 µl	10 µg	> 50 ng/µl	
Long-Read-Sequencing Nanopore Prokaryotes	> 20 µl	1.5 - 5 µg	> 50 ng/µl	
Long-Read-Sequencing PacBio	> 20 µl	3 µg	> 50 ng/µl	
RNA-Seq	> 15 µl	1 µg	> 30 ng/µl	RIN > 7

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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 nm and high-quality RNA at 2.0 nm. The 260/230 nm ratio is considered pure between 1.8-2.2 nm.

	260/280 nm	260/230 nm
High quality DNA	1.8 nm	2 nm
High quality RNA	1.8-2.2 nm	1.8-2.2 nm

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.

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 RNA-sequencing and long-read-sequencing.

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.

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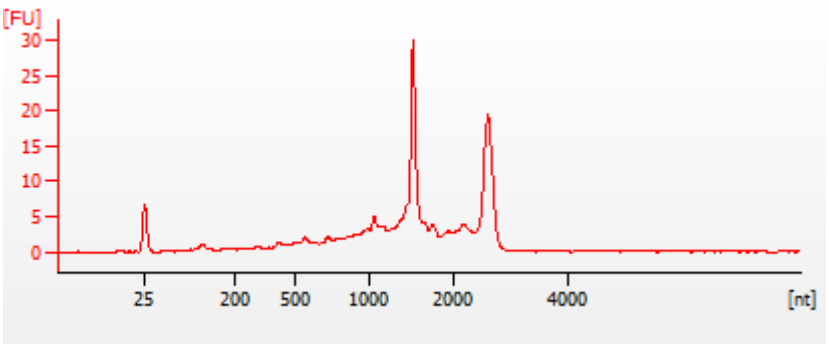
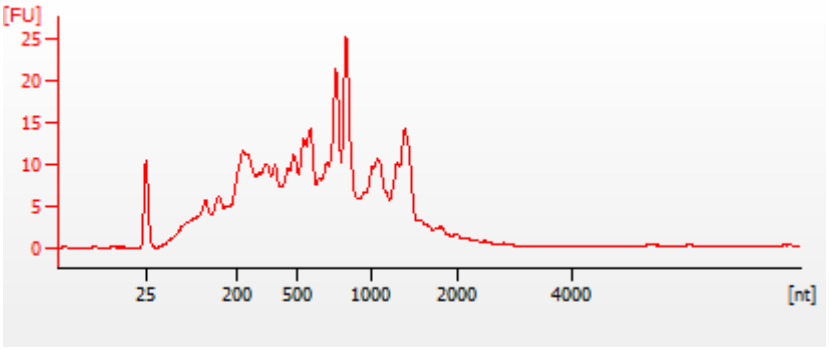
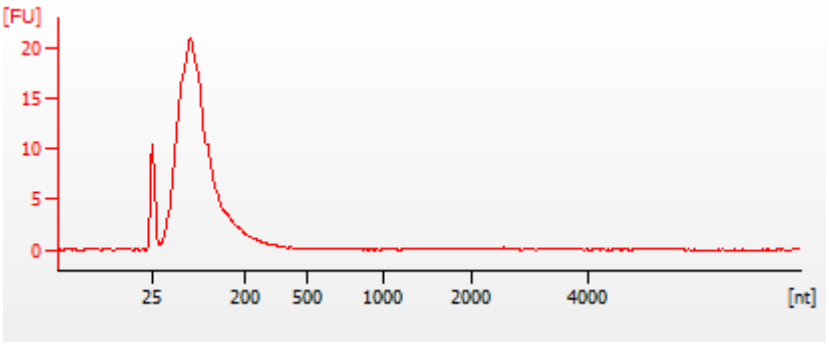
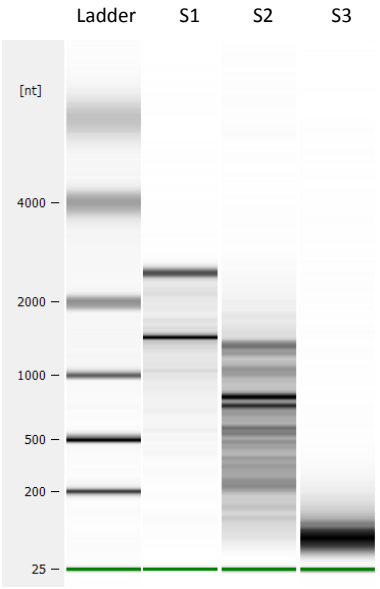
Electropherogram	Sample names and quality parameters
	Sample 1 (S1) RIN 7.2 DV200 95% Good quality
	Sample 2 (S2) RIN 4.7 DV200 85% Low quality
	Sample 3 (S3) RIN 2.6 DV200 5% Bad quality

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

Integrity of high molecular weight DNA can be assessed with a low percentage gel or the automated electrophoresis systems. The measured DNA for long-read-sequencing should be >30 kb and ideally not show any signs of degradation (smear). DNA for long-read-sequencing is best to be eluted in low-EDTA TE buffer.