

Sample Submission Guide for RNA, DNA and RTR

Successful Sample Submission



Project Initiation

Your project will be initiated upon receipt of the completed and signed PO form.



Sample Submission Form

After your project is initiated, you will receive a confirmation email with a project number and a Sample Submission Form (SSF). It is essential to return the sample submission form fully completed by email and included as a copy in your sample shipment. Please, do not ship samples before the fully completed sample submission form has been shared digitally.



Sample Shipment/Delivery

If you are considering delivering your samples in person, please consult our lab team in advance. If you plan to ship your samples, please assign the package to your project manager and ship to the following address:

GenomeScan B.V.
Plesmanlaan 1d, 4th floor
2333 BZ LEIDEN
The Netherlands

- Samples shipped to GenomeScan need to be free of biological contaminants. Our laboratory operates in compliance with BSL-1 and BSL-2 requirements and cannot handle potential hazardous materials. In general RNA/DNA samples extracted from cells or tissue do not represent a biological threat.
- Ship your samples in (a) sealed 96-well skirted PCR plate(s). Additional costs will be charged when samples are provided in separate (Eppendorf) tubes.
- Each sample plate must be labelled with your GenomeScan project ID. Be sure that the samples associated with positions A1-H12 correspond to the sample ID as indicated on the SSF.

- Sample plates can be shipped in a sealed bag/box in a polystyrene container.
- To ensure optimal preservation of the sample, we recommend shipment of (g)DNA using icepacks.
- Use dry ice for sending RNA samples.
- Remember that international shipment may take longer than expected. Make sure that your package contains sufficient cooling materials to preserve the quality of your samples during transport.
- Avoid shipment of samples on days that will require transit on a weekend or over a holiday period. We are closed on the following generally recognized public holidays: New Year's Day, Easter Monday, Kings Day (27th April), Ascension Day, Whit Monday and Christmas (25th, 26th December).



Sample QC and Requirements

Starting material of good quality is of great importance to produce high quality NGS data. Therefore, when samples arrive at GenomeScan, all samples will go through a QC check for quality and quantity and a subset of your samples will be checked on sample purity and contamination (unless this is stated otherwise in the quotation). Depending on the library preparation type specified for your project, certain sample requirements apply. These can be found in the sample requirements tables on the next page.

- Provide samples free of (chaotropic) salts, phenol, ethanol, proteins etcetera as this affects downstream processing.
- Provide samples eluted or diluted with nuclease free water, 10mM Tris-HCl or Low TE.
- Samples should be delivered in a total volume > 20µl
- RNA samples should be free of DNA
- DNA samples should be free of RNA
- Please be aware that if samples don't meet the specified requirements our "Samples falling outside the requirements policy" applies, this can be found on the final page of this document.

Sample Requirements

Application	Input material	Required input amount (per sample)	Concentration range
All samples should be submitted in $\geq 20 \mu\text{l}$ with an OD260/280 $\sim 1.8-2.0$ and OD260/230 $\sim 2.0-2.2$			
DNA			
Whole Genome Sequencing (WGS)*	Purified gDNA	With PCR $> 1 \text{ ng}$ PCR free $> 25 \text{ ng}^{**}$	5 - 50 ng/ μl
ChIP-Seq/Targeted Resequencing	Enriched DNA	With PCR $> 1 \text{ ng}$ PCR free $> 25 \text{ ng}^{**}$	5 - 50 ng/ μl
Whole Exome Sequencing (WES)*	Purified gDNA	50 - 500 ng	25 - 200 ng/ μl
Whole Genome Bisulfite Sequencing (WGBS)	Purified gDNA	100 ng	5 - 50 ng/ μl
16S V4 Microbiome Sequencing**	Purified gDNA	$> 5 - 100 \text{ ng}$	5 - 50 ng/ μl
Whole Microbiome Sequencing (shotgun Seq)	Purified gDNA	50 - 500 ng	5 - 50 ng/ μl
Methylation EPIC BeadChip (935K)*	Purified gDNA	500 - 750 ng	$> 50 \text{ ng}/\mu\text{l}$
PacBio Sequencing	Purified gDNA/Amplicon	500 ng - 5 μg^{***}	$> 50 \text{ ng}/\mu\text{l}$
Prepared library			
Ready to run Sequencing	Indexed Library	$> 5 \text{ nM}$	3 - 10 ng/ μl

* Different input criteria apply to FFPE material, recommended DV200 should be $> 20\%$

** Higher input is required ($> 100 \text{ ng}$) for deep sequencing

*** Application dependent

Application	Input material	Required input range (Validated)	Preferred input amount (per sample)	Concentration range	Quality
All samples should be submitted in $\geq 20 \mu\text{l}$ with an OD260/280 $\sim 1.9-2.1$ and OD260/230 $\sim 2.0-2.2$					
RNA					
RNA-Seq using Poly-A selection (Gene-expression profiling)	Purified RNA	25-1000 ng	$> 250 \text{ ng}$	10-250 ng/ μl	RIN ≥ 7 RQN ≥ 6
RNA-Seq using rRNA reduction (Total transcriptome)	Purified RNA	10-1000 ng	$> 250 \text{ ng}$	10-250 ng/ μl	RIN ≥ 3 (optimal > 7) RQN ≥ 3 (optimal > 6)
Small RNA (Purified RNA)	Purified RNA	10-1000 ng	$> 250 \text{ ng}$	10-250 ng/ μl	RIN ≥ 7 RQN ≥ 6
Small RNA (Enriched small RNA)	Enriched RNA	—	$> 2 \text{ ng}$	$> 0.1 \text{ ng}/\mu\text{l}$	N/A
Low Input RNA-Seq (Poly-A selection)	Purified RNA	0.01-10 ng	$> 1 \text{ ng}$	$> 0.1 \text{ ng}/\mu\text{l}$	RIN ≥ 8 RQN ≥ 8
Low Input RNA-Seq (rRNA reduction)	Purified RNA	0.25-10 ng	$> 1 \text{ ng}$	$> 0.1 \text{ ng}/\mu\text{l}$	DV200 $> 50\%$

* Different input criteria apply to FFPE material, recommended DV200 should be $> 20\%$

Samples falling outside the requirements policy

Multiple metrics play a crucial role in ensuring high-quality data generation. Key factors include the origin, integrity, preparation input, and purity of samples. Insufficient purity has the potential to significantly interfere with or disrupt the library preparation process. Additionally, decreased integrity and input levels can adversely impact the resulting data.

Samples not meeting the requirements

When samples don't meet the requirements indicated in the table above this will be communicated via e-mail and the project is halted until a plan is made for continuation of the project. There are now several possibilities:

- When samples fail on Purity and/or Contamination the following options apply:
 - The project can be carried out only after an additional clean-up if approved by the customer, additional costs apply.
 - Replacement samples can be provided, additional costs apply. For these samples the normal sample QC will again be performed.
 - The project is stopped.
- When samples fail to meet Concentration and/or Quality the following options apply:
 - We can use spare samples, if already supplied, to replace the failed samples.
 - Replacement samples can be provided, additional costs apply. For these samples the normal sample QC will again be performed.
 - We can continue with only the PASS samples and leave out the FAIL samples.
 - If possible, we could make use of an alternative library preparation kit, additional costs may apply.
 - We can continue with the samples that did not meet our quality requirements under the conditions of "own risk", see below.

Own Risk

When samples are deemed "own risk", we can continue processing the samples to try and maximize the customers desired results, however successful sample processing cannot be guaranteed. This means that library preparation will not be repeated if the library preparation results in aberrant profiles or low library yields. In this case the customer will be contacted, and options will be discussed. The turn-around-time is paused when continuation with the project is unclear. If the library preparation is successful, the sequencing will start, however this might still not yield the desired data amount or quality, also including the results of secondary data analysis.