Sample Submission Guide
DNA, RNA 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 containing a project number and a Sample Submission Form (SSF). It is essential to return the fully completed SSF via email before proceeding with the sample shipment. Additionally, please include a copy of the SSF with your sample shipment.

Sample Shipment/Delivery Requirements

If you are considering delivering your samples in person, please consult our lab team in advance.
For sample shipments,, please address the package to your project manager (mentioned in the SSF) 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) (preferably Eppendorf Twin.tec PCR Plate 96). If this is not feasible, inform us before proceeding to sample shipment. Please be aware that additional charges will be applied if samples are provided in individual (Eppendorf) tubes.
- If samples need to be provided in Eppendorf tubes, the tubes should be placed inside a box in the correct order. Ensure each tube has a proper and clear label referencing the customer ID or GenomeScan ID.
- Each sample plate must be labelled with your GenomeScan project ID. Ensure that samples in positions A1-H12 correspond accurately to the sample IDs as indicated on the SSF.
- Sample plates should be shipped in a sealed bag or box within a polystyrene container.
- To ensure the optimal preservation of genomic DNA (gDNA), we recommend shipping it with ice packs.
- For RNA samples, please use dry ice during shipment to maintain its integrity.
- Remember that international shipment may experience longer than anticipated transit times. Ensure that your package includes adequate cooling materials to preserve the quality of your samples during transport. We highly recommend for sensible and/or unique samples to use cold chain shipments.
- Avoid scheduling sample shipments on days that would result in transit over weekends or during national holidays. As a company based in the Netherlands, 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 and 26th December).

Sample Quality Control (QC) and Requirements

Sample Purity

The purity of the sample is of high importance when determining the input and quality required to proceed with the library preparation. When measuring concentrations with nanodrop, the presence of compounds like guanidium isothiocyanate and phenol can greatly inflate the concentrations, giving a much higher concentration than the actual value.
These compounds as well as others, like EDTA and isopropanol, also greatly influence the effectiveness of the sample preparation. All these compounds can be found in the samples due to the isolation process and the reagents involved, especially during the final elution step.

Refer to the following articles for more details on the influence of these contaminants.

- *https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001249*
- *https://community.nanoporetech.com/contaminants (the Ligation Seq Kit library prep efficiency graphs are comparable to our prep method)*

Quality Control (QC)

Good quality starting material is critical 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 (10% of the samples, throughout the plate, with a minimum of 2 samples) will be checked on sample purity and contamination (unless this is stated otherwise in the quotation). An OD 260/230 ratio between 1.8 and 2.2 is optimal. A ratio lower than 1.6 will be marked as failed and we recommend a purification step before starting the library preparation. Otherwise, the "Samples falling outside the requirements policy" applies, which can be found below. Be aware that the addition of extra purification steps implies additional costs.

Sample Requirements all projects

Depending on the library preparation type specified for your project, certain specific sample requirements apply. These can be found in the sample requirements tables on the next page.
The following requirements apply for all sequencing projects:

- Provide samples free of (chaotropic) salts, phenol, ethanol, proteins etcetera
- Provide samples eluted or diluted with nuclease free water, 10mM Tris-HCl or Low TE.
- Samples should be delivered in a total volume > 25 µl
- 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.

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 specified in the table above, the customer will be informed via e-mail, and the project is paused until a plan for continuation can be established. Several options are available:

- When samples fail on Purity and/or Contamination the following options apply:
  - The project can proceed after an additional purification step, subject to customer approval, additional costs apply.
  - Replacement samples can be provided, additional costs apply. These replacement samples will undergo normal sample QC procedures again.
  - The project is cancelled.

- When samples fail to meet Concentration and/or Quality the following options apply:
  - Spare samples, if already supplied, can replace the failed samples.
  - Replacement samples can be provided, additional costs apply. These replacement samples will undergo normal sample QC procedures again.
  - We can proceed only with the PASS samples, excluding the FAIL samples.
  - If feasible, an alternative library preparation kit may be used, additional costs may apply.
  - We can continue with samples that did not meet our quality requirements under "own risk" conditions, as outlined below.

Own Risk

When samples are categorized as "own risk", we will proceed with processing them after written confirmation in an effort to maximize customer's desired results. However, successful outcomes cannot be guaranteed. This means that if the library preparation results in aberrant profiles or low library yields, we will not repeat the library preparation. In this case the customer will be contacted, to discuss available options.
The turnaround-time is paused when continuation with the project is uncertain. In this scenario, the turnaround time exposed in the quotation is no longer valid and a new timing will be discussed. If the library preparation is successful, sequencing will start. However, it is important to note that even if sequencing is successful, it may not yield the desired amount or quality of data, including the results of secondary data analysis.

Sample Requirements

Application	Input material	Required input amount (per sample)	Concentration range
All samples should be submitted in ≥ 25 µl with an OD260/280 ~1.8-2.0 and OD260/230 ~2.0-2.2			
DNA			
Whole Genome Sequencing (WGS)*	Purified gDNA	With PCR >1 ng PCR free > 25 ng**	5 - 50 ng/µl
DNA Sequencing	Small fragments or ampliconcs	1– 500 ng	5 - 50 ng/µl
Whole Exome Sequencing (WES)*	Purified gDNA	50 - 500 ng	25 - 200 ng/µl
16S V4 Microbiome Sequencing	Purified gDNA	> 5 - 100 ng	5 - 50 ng/µl
Whole Metagenome Sequencing (shotgun Seq)	Purified gDNA	50 - 500 ng	5 - 50 ng/µl
Methylation EPIC BeadChip*	Purified gDNA	500 - 750 ng	> 50 ng/µl
Long read Sequencing (PacBio)	Purified gDNA/Amplicon	500 ng - 5 µg***	>50 ng/µl
Long read Sequencing (Nanopore)	Purified gDNA	On request	On request
Prepared library			
Ready to run (RTR) Sequencing	Indexed Library	> 5 nM	3 - 10 ng/µl

* Different input criteria apply to FFPE material, recommended DV200 should be >20%
** Higher input is required (> 100 ng) for deep sequencing
*** Application dependent

	Gene-expression profiling	Total transcriptome	Low input RNA seq	Low quality RNA seq
Sample requirements	**Poly-A selection**	**rRNA reduction**	**Low input**	**Low input, low quality (FFPE)**
RNA	**All samples should be submitted in ≥ 25 µl.**		**RNA samples should free of DNA**	
Input material	Purified RNA	Purified RNA	Purified RNA	Purified RNA
Optimal RNA quantity#	≥1000 ng	≥500 ng	30 ng	30 ng
Optimal RNA concentration#	40 ng/µL	20 ng/µL	1.2 ng/µL	1.2 ng/µL
Minimal RNA quantity**	200 ng	100 ng	≥0.25 ng	≥0.25 ng
Minimal RNA concentration	8 ng/µL	4 ng/µL	0.01 ng/µL	0.01 ng/µL
Optimal quality	RQN: ≥6 RIN: ≥7	RQN: ≥6 RIN: ≥7	RQN: ≥6 RIN: ≥7	DV200 ≥60%
Minimal quality	N/A	RQN: 3 RIN: 3	RQN: 3 RIN: 3	DV200: 25%
Purity	OD260/280: 1.8-2.2 OD260/230: 1.8-2.2	OD260/280: 1.8-2.2 OD260/230: 1.8-2.2	N/A	N/A
Elution buffer	Salt free (free from EDTA, heparin, etc.)			

Determined by fluorescent measurement.
** Library preparation performed only once

genomescan.nl
+31 71 5681050
info@genomescan.nl

GUID007, version 13, Authorized by QA