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, a packing slip, shipment instructions and a Sample Submission Form (SSF) or Batch Confirmation Form (BCF). It is essential to return the fully completed SSF/BCF via email in .xlsx-format before proceeding with the sample shipment. Additionally, please include a printed copy of the SSF/BCF with your sample shipment.

Sample Shipment/ Delivery Requirements

For sample shipments, please address the package to your project manager by attaching a printed packing list on the outside of the container (please use the version from the project initiation email) and ship to the following address:

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

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 sensitive and/or unique samples to use cold chain shipments.

Sample shipments should be delivered by courier or in person between 9:00 and 16:00 on regular workdays. Avoid scheduling sample shipments on days that would result in transit over weekends or during national holidays. Packages delivered outside opening hours cannot be accepted or may remain unopened overnight. Delivery in person needs to be announced with a message to laboratory@genomescan.nl. You are required to either leave the cooling materials on site or remain present until the samples can be personally handed over to a laboratory staff member. Please also attach the printed SSF/BCF to the package for quick identification.

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).

- 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 potentially 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 (semi) skirted PCR plate(s), transparent wells, (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, with the lid taped shut. Ensure each tube has a proper and clear label referencing the customer ID or GenomeScan ID matching the SSF/BCF. 'Strip tubes' with connected lids are not permitted.
- Each sample plate must be labelled with your GenomeScan project ID. Ensure that sample positions in the plate correspond accurately to the sample IDs 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. To prevent damage of the sample plate during shipment, it is recommended to put the plate in a protective box with the lid taped shut.
- For RNA samples, please use dry ice during shipment to prevent degradation.

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/librarypreparation/general/library-preparation-generalreference_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 concentration and for quality via a profile assessment. In case the profiles are not showing optimal distribution, we can discuss alternative options for low quality or the "Samples falling outside the requirements policy" applies, which can be found below.

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 of at least 25 µl
- RNA samples should be free of DNA
- DNA samples should be free of RNA
- Please be aware that if samples do not 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 do not 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 standard sample QC procedure.
  - The affected samples proceed "own risk", as outlined below.
  - 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 standard sample QC procedure.
  - We can proceed only with the PASSED samples, excluding the FAILED samples.
  - If feasible, an alternative library preparation kit may be used, additional costs may apply.
  - The affected samples proceed "own risk", as outlined below.

Own Risk

When samples are categorized as "own risk" after our sample QC procedure, we will proceed with processing them after written confirmation to maximize customer's desired results. However, successful outcomes cannot be guaranteed. This means that if the library preparation results in aberrant size profiles or low library yields, we will not repeat the library preparation. In this case the customer will be informed and all samples will continue to sequencing. In this scenario, sequencing will only be tried once, even if the desired yield is not reached. 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 quality of data, including the results of secondary data analysis.

Sample Requirements

Application	Input material	Required input amount (per sample)	Concentration range
DNA	All samples should be submitted in ≥ 25 µl • DNA samples should be free of RNA • DNA samples should be free of (chaotropic) salts, phenol, ethanol, proteins, EDTA, heparin, etc. • Elution buffer: nuclease free water		
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	Purified gDNA	50 - 500 ng	25 - 200 ng/µl
Target Sequencing by oligo panels	TNA*** or DNA	> 50 ng	> 5 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
Long Read Sequencing (PacBio)	Purified gDNA/Amplicon	500 ng - 5 µg	> 50 ng/µl
Long Read Sequencing (Nanopore)	Purified gDNA	Minimal 1-5 µg (per flowcel) ****	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
*** TNA = total nucleic acid
**** 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 be free of DNA • RNA samples should be free of (chaotropic) salts, phenol, ethanol, proteins, EDTA, heparin, etc. • Elution buffer: nuclease free water			
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
Minimal quality	Good quality: RNA should be intact, with minimal signs of degradation in the fragment profile. Two distinct ribosomal peaks should be visible.	Moderate degradation is acceptable (based on the fragment profile) if sufficient RNA is available for library preparation.	Good quality, not degraded	Degrated: DV200 25%

Determined by fluorescent measurement
** Library preparation performed only once

Long-Read RNA Sequencing

Long-Read RNA Sequencing			
ONT	Amount	Concentration	Quality
Direct RNA seq	> 3 µg total RNA or > 300 ng enriched mRNA	> 43 ng/µl	Good, not degraded
cDNA seq (PCR based)	> 300 ng total RNA		
PacBio	Amount	Concentration	Quality
Iso-seq	> 300 ng total RNA	> 43 ng/µl	Good, not degraded
Kinnex	> 300 ng total RNA		