1. Objective

This document will provide guidelines on DNA or RNA samples or libraries quality requirements and sample submission to Genomics Unit CRG.

2. General considerations

a) Before starting a project at the Genomics Unit of the CRG, we strongly recommend to discuss it with project management to receive advice on the experimental design, services offered and prices, sending an e-mail to ngs@crg.eu and explaining briefly your project objectives and what do you want to analyze. If you already know the number of samples and the application needed, please, specify it on the mail.

b) After discussion, please fill the "**Sequencing Request Form**" and **send it** to ngs@crg.eu with the following information on the body of the mail:

- Number of samples
- Type of service (application)
- How to sequence (Single Read or Paired End, length of the read)
- Number of lanes needed or number of million reads per sample
- Information needed for the quotation of the service
    - Name of the PI (always)
    - E-mail of the PI (always)
    - Name of the Institute (always)
    - Address (for new users)
    - VAT number of the institute (for new users)

All services request forms are on CRG Genomics Unit web page:

- Sequencing Request Form: library prep and sequencing
  https://www.crg.eu/sites/default/files/crg/sequencing_request_samples-crg_v7.xlsm
- Sequencing Request form: Ready libraries to sequence
  https://www.crg.eu/sites/default/files/crg/sequencing_request_libraries-crg_v6.xlsm
- Capillary electrophoresis request form
  https://www.crg.eu/sites/default/files/crg/capillary_electrophoresis_request_form_v2.xlsx
- Covaris system request from
  https://www.crg.eu/sites/default/files/crg/covaris_request_form_20210219.xlsx

Genomics Unit will open a new project, send a quotation and provide you a slot to bring/send the samples. **Do not bring/send** any samples without the approval from Genomics Unit.

If you send the samples via courier, please send the parcel to:

Genomics Unit
Centre de Regulació Genòmica (CRG)
PRBB Building
C/ Dr. Aiguader 88, 5th floor, lab 520
08003 Barcelona
Phone: 93 3160126

Please include inside the package the service request forms in paper.
The CRG Genomics Unit is not responsible for parcels sent without prior notification and agreement with the Unit staff.

3. Sample Quality and Quantity Requirements

Tables 1 and 2 show CRG Genomics Unit quality and input quantity requirements for samples.
Table 3 shows input quantity requirements for libraries.

CRG will report the quality control results. Any suboptimal samples which do not meet the requirements will be referred as FAIL or UNDER REVIEW.

For suboptimal samples the collaborator must decide:

- replace the samples
- proceed with the samples accepting the risk of failure and the billing regardless of data quality. Please contact the Project Manager for further details.

Input material quantity should be determined by fluorescence-based quantification methods such as Qubit or Quant-It. When only an absorbance-based quantification is available, always provide as much material as possible.

If your samples cannot meet our requirements, discuss them with Project Management according to the experiment and/or the genome size of the studied organism before shipping your samples.

Table 1. Sample quality requirements

Sample type	Quality requirements
gDNA	Pure DNA, free of RNA contamination. Optical Density measurements: OD 260/280 1.8-2.0 and OD 260/230 1.8-2.2. Depending on the extraction method employed, RNAse treatment is required.
	High molecular weight DNA, no degradation smear
	Free of other species DNA contamination
	Free of PCR inhibitors
	Sample buffer must be EB buffer (Tris 10 mM Tris-Cl, pH 8.5) or similar
	Quantified by fluorescence-based method specific for dsDNA
Total RNA	Pure RNA, free of DNA contamination
	Good integrity. Bioanalyzer profiles RIN>8
	mRNA samples must be free of rRNA. By means of Bioanalyzer profiles, rRNA contamination <22%
	Sample buffer must be water

Table 2. Protocols and sample amount requirements (*consult with Project Management, ngs@crg.eu)

Area	Application	Protocol	Concentration requested	Input requested
Whole and Targeted Genome	WG-seq	WGS (PCR-free)	25-100ng/ul	1,5µg
		WGS (PCR-based)	25-100ng/ul	600ng
		WGS (Tn5)	10ng/ul	30ng
		Ultra Low Input WGS (Tn5)	*	*
		WGS long reads (Ligation)	150-200ng/ul	3ug
		Low Input WGS long reads (Transposase based)	10-50ng/ul	500ng
	ExomeCapture-seq and CustomCapture-seq	Regular Exome/Custom Capture Agilent	25-100ng/ul	1,5µg
	Amplicon-seq	Addition of barcodes and sequencing adaptors	5-25ng/ul	150ng
	Amplicon-seq (Metagenome)	Metagenome	*	25-50 ul (volume requested, not amount)
Transcriptome	mRNA-seq	mRNA-seq	50-100ng/ul	1,5ug
		Low Input mRNA-seq	10-25ng/ul	200ng
		Ultra Low Input mRNA-seq	*	pg to 10ng total RNA
	Ribodepleted RNA-seq	Ribodepleted RNA-seq	50-100ng/ul	1,5ug
		Low Input Ribodepleted RNA-seq	10-25ng/ul	200ng
		Ultra Low Input & Low Quality Ribodepleted RNA-seq	*	pg to 10ng total RNA
	Small RNA-seq	Small RNA-seq	200-250ng/ul	1,5ug
		Low Input Small RNA-seq	10-20ng/ul	200ng
	Ribo-seq	Ribosome profiling	*	All available material after footprint fragment purification
Epigenome	ChIP-seq	ChIP-seq protocol	*	All available material after ChIP. Ideally 5-10ng.
	ATAC-seq	ATAC-seq protocol	NA	10ul of transposed and purified DNA
	HiC-seq	Hi-C protocol (from Biotin pull-down on)	NA	200ul from Biotin pull-down on
	Methyl-seq	Enzymatic Methyl-seq	10-100ng/ul	1ug
	Cut and Run-seq	CUT&RUN-seq	*	10ng in 50ul
Single Cell genomics and transcriptomics	Single cell RNA-seq	Chromium Next GEM Single Cell 3' mRNA	800-1200 cells/ul	Ideally between 8300-13300 total cells in <19ul (for cell recoveries of 5000 to 8000 cells)
	Single cell ATAC-seq	Chromium Next GEM Single Cell ATAC	*Depends on Targeted Nuclei Recovery	Ideally between 7700-12300 total nuclei in max. 5ul (for nuclei recoveries of 5000 to 8000 cells)
	Single cell Multiome-seq	Chromium Next GEM Single Cell Multiome ATAC + Gene Expression	*Depends on Targeted Nuclei Recovery	Ideally between 8000-12900 total nuclei in max. 5ul (for nuclei recoveries of 5000 to 8000 cells)

Table 3. Libraries amount requirements depending on the sequencer to be used:

Instrument		Libraries or pools	Molarity	Volume
iSeq 100		Single libraries	2 nM	20 ul
		Already prepared pools	2 nM	35 ul
MiSeq		Single libraries	2 nM	15 ul
		Already prepared pools	2 nM	35 ul
NextSeq 500 (MID/HIGH)		Single libraries	2 nM	15 ul
		Already prepared pools	2 nM	35 ul
NextSeq 2000 (P1/P2/P3/P4)		Single libraries	2 nM	20 ul
		Already prepared pools	2 nM	35 ul
NovaSeq 6000	**SP/S1**	Single libraries	2 nM	15 ul
		Already prepared pools	3 nM	60 ul
	S2	Single libraries	2 nM	15 ul
		Already prepared pools	3 nM	85 ul
	S4	Single libraries	2 nM	15 ul
		Already prepared pools	3 nM	160 ul

4. Labelling and Packaging Instructions

It is mandatory that the samples are brought to the Unit in **1.5 ml tubes or plates.**

- The name of the samples and plates must be 6 characters long maximum and no special characters should be used (only underscore is accepted).
- The name written on the tube must be the same as the one entered in the request form.
- The tube wall and tap must be free of any sticker or tape.
- We recommend using "safe-lock" eppendorfs. Do not use parafilm.
- For samples in plates (sending samples in plates **must** be agreed with project management):
  - use one of the 2 options:
    - Eppendorf twin-tec PCR 96 semi-skirted (ref. 30128575)
    - NZYTECH 96 well PCR plates half skirt (ref. LW00801)
  - MANDATORY: always fill plates by columns, not by rows. Do not leave empty wells between samples (only G12 and H12 for microbiome projects).
  - If sending plates with dry ice, we recommend using the Bio-Rad Microseal "B" seals (ref.MSB1001) to prevent detachment of the foil.

5. How to recover samples from CRG genomics unit

Note: bear in mind that your samples will be stored at the Genomics Unit for 6 months after data delivery. After that time, samples will be discarded unless you have taken them back.

- Send an e-mail to ngs@crg.eu, indicating the name of the subproject and the name of the samples/libraries that you want to get back.
- Once CRG have collected them, a confirmation e-mail will be sent.
- If you come in-person, the time schedule for recovering samples is Monday to Wednesday, from 9 to 13h. Remember to bring dry ice or ice if needed (depending on sample nature).
- For samples that need to be sent via courier, **the user** is responsible of arranging the shipment. The day and time frame to send the parcel has to be agreed with the Genomics Unit staff. In case of RNA samples, a package with dry ice has to be sent by the user.

6. Shipping samples to Genomics Unit from non-EU shipments

For non-EU shipments: additional documentation will be requested by the custom authorities. CRG has to gather several documents and handle it to Spanish Customs, once the import is authorized, CRG contacts the collaborator to define shipment date.

- Contact CRG before any sample shipment.
- Any parcel missing customs complete documentations, unpaid customs duties, or containing restricted items, will be returned to the sender.
- Samples integrity is not granted if held at customs premise.