Adhere to the following instructions for the successful completion of your project. This document will provide guidelines on DNA or RNA sample quality requirements and sample submission to CNAG.

Contents

STEPS:

1. Project/subproject Creation – by CNAG Project Management (experimental design, sequencing protocol, number of samples, analysis (if required), invoicing matters, prioritization, deadlines…)

2. Barcoded Tubes shipment – by CNAG Biorepository (shipment logistics, Support in selecting DNA/RNA extraction methods, …)

3. Samples shipment to CNAG - by the Collaborator

4. Samples Quality Control and Report - by CNAG Biorepository (QC results, replacement issues…)

5. Samples selection approval – by the Collaborator

6. Libraries preparation and Sequencing – by CNAG Sequencing Unit

7. Data QC and Transfer – by CNAG Data QC team

1. General Considerations

- After your project has been reviewed and approved by CNAG Project Management, the CNAG Biorepository will contact you to provide the necessary materials and a sample submission consisting of a URL link to the sample data collection site.
- Use only the materials provided by CNAG for the sample shipment.
- Questions related to DNA and RNA sample requirements and shipment details should be directed to the CNAG Biorepository (Lídia Agueda, Biorepository Laboratory Manager, lidia.agueda@cnag.eu or Ana González, ana.gonzalez@cnag.eu) or to Long Read Sequencing (laura.aguilera@cnag.eu). Other inquiries regarding experimental design, quotations, changes in the number of samples, or related matters should be directed to the CNAG Project Manager (projectmanager@cnag.eu).

2. Sample Quality and Quantity Requirements

- **Tables 1** and **2** summarize CNAG's general quantity and quality requirements for samples. Samples intended for Long Read Sequencing applications have specific guidelines that are not included in this document.
- CNAG will provide a quality control (QC) report for all samples received. Any suboptimal samples that do not meet the requirements will be classified as FAIL or UNDER REVIEW.
- For suboptimal samples the collaborator must decide whether to:
  i) Replace the samples, or
  ii) Proceed with the available samples, accepting the risk of failure and the billing regardless of final data quality.
  Please contact the Project Manager for further details.
- Input material concentration should be determined by fluorescence-based quantification methods such as Qubit or Quant-It. If only absorbance-based quantification is available, provide as much material as possible.
- Table 1 indicates the MINIMUM amount of material required. Whenever possible, send more material than the minimum. If this is not feasible and you have to adhere to the minimum requirement, provide the sample at the lowest concentration within the specified range to minimize the material amount used for quality control procedures.
- If your samples cannot meet our requirements, please consult the CNAG Project Manager before shipping your samples.

Table 1. Protocols and sample amount requirements (*check with Project Management or Long Read Sequencing Team, LRST)

Sequencing protocol	MINIMAL quantity requested (fluorescence-based quantification method)	Concentration range (ng/ul) (fluorescence-based quantification method)
Regular Whole Genome	1.7 ug	50-100
Low Input Whole Genome (with PCR)	50 ng *	5-10
Low Input Whole Genome (without PCR)	Discuss with PM	5-50
Ultra Low Input Whole Genome	100 ng *	5-10
Nanopore DNA 1D	6-16 ug depending on the MW, the genome size, the species and the experimental design. Please contact LRST*	150-300
Nanopore DNA 1D Barcoding	*	150-300
Nanopore DNA Low Input	1 ug*	80-120*
PacBio HiFi Whole Genome	5 ug* (depends on DNA integrity and multiplexing)	100-200
PacBio Ultra Low Input Whole Genome	125ng	5-10
Enzymatic Methyl-seq (EM seq)	225 ng	5-10
E5hmc-seq	225 ng	5-10
Low Input Exome/Custom/Clinical Capture Agilent	800 ng	10-50
Roche Kapa exome	225 ng	5-10
Roche Kapa exome for FFPE	500 ng	10-20
Roche Kapa exome for cfDNA	*	*
Nanopore Adaptive Sampling	*	*
Amplicon Sequencing	Same protocols as Whole Genome Sequencing	
Stranded total RNA	1.5 ug total RNA	50-100
Stranded total RNA for FFPE samples	2.5 ug total RNA	150-200
Stranded mRNA	1.5 ug total RNA	50-100 for total RNA
Stranded/non-Stranded Low input options	*	*
Nanopore RNA: PCR-cDNA	1.5 ug total RNA* 50 ng polyA RNA*	50-120 ng/ul (total RNA) 10-100 ng/ul (polyA-RNA)
Nanopore RNA: direct cDNA	2.5 ug total RNA* 850 ng polyA RNA*	150-200 ng/ul (total RNA) 50-100 ng/ul (polyA-RNA)
Hi-C (OmniC)	(tissue)*	(tissue)*

Table 2. 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 DNA contamination from other species • Free of PCR inhibitors • Sample elution buffer must be water or low-salt buffer. • Quantified by fluorescence-based method specific for dsDNA; Absorbance-based quantification is inadequate (e.g. Nanodrop or equivalent) • See dedicated guidelines for Long Read Sequencing applications
Whole Genome Amplified DNA	Contact Project Manager • Always refer to amplification method used in the data submission site
FFPE DNA	Contact Project Manager
PCR amplicons	Contact Project Manager • Always provide the amplicon size in the data submission site
Total RNA	• Pure RNA, free of DNA contamination • High integrity. Bioanalyzer profiles RIN/RQN>7 • mRNA samples must be free of rRNA. By means of TapeStation profiles, rRNA contamination <22% • Sample buffer must be water • Quantified by fluorescence-based method specific for RNA; Absorbance-based quantification is inadequate (é.g. Nanodrop or equivalent) • See dedicated guidelines for Long Read Sequencing applications
FFPE RNA	Contact Project Manager

3. Labelling and Packaging Instructions

- You will receive 500ul tubes labelled with a lateral 1D barcode and a 2D barcode on the bottom in a LVL 96-rack.

- The tubes can be handled individually, either manually or using compatible automated platforms.
- Do not overfill tubes with more than 500ul.
- Use Blue-capped tubes for DNA samples and red-capped tubes for RNA samples.
- Tubes are arranged numerically **by columns**, but numbering may not always be consecutive. We strongly recommend verifying each tube barcode during sample handling and again when uploading samples data to the external server.
- Use the provided LVL rack for shipment to CNAG, or an equivalent packaging alternative that ensures proper tube protection during transport.
- **Do not modify the tubes or labels in any way.**
- Each tube label contains a unique CNAG sample barcode (format: 3 letters followed by 5 numbers).
- The rack label shows the project name and sometimes plate order in case submission contains more than one rack (1/n, 2/n, … n/n).
- **Never wrap Parafilm** around the tube caps. The caps include an anti-leakage system.
- Never place any additional labels on tubes.
- For the shipment, ensure the LVL racks are securely closed to prevent tubes from scattering inside the transport box or directly into dry ice.

- Clearly indicate both the delivery address and sender/shipper/consignor address on the external package, even when using internal institution courier services.
- CNAG Material shipments and sample submission links typically include **extra tubes/barcodes.** These may be used for sending additional or replacement material, or also future agreed subprojects. Additional tubes can be requested to the CNAG Biorepository at any time. We strongly recommend keeping the extra tubes for future use.
- RNA samples must be shipped **frozen on dry ice.**
- DNA samples may be shipped refrigerated (with blue ice/cooling packs) or at room temperature.

4. Sample information uploading to CNAG Submission Site

- All the barcodes provided by the CNAG Biorepository will be available through a URL link to the submission site, that allows data collection. The **same link** will remain active until all the barcodes provided have been used.
- Submit the data **before** shipping the samples and notify the CNAG Biorepository by email (lidia.agueda@cnag.eu or ana.gonzalez@cnag.eu) once the data is submitted.
- The CNAG barcodes displayed on the submission site may be used in different shipment batches. For each shipment, select and submit the barcodes included in that shipment. Each time the submission URL is accessed, only the unused barcodes will be displayed.
- The list of submitted barcodes can be downloaded in Excel format directly from the submission site:

- There are some submission site auto-fill features to facilitate data entry:

> Submission site auto-fill tips:
> Hover over field names for more details
> - **To fill a whole column with same value**: fill in the 1st row and Ctrl + space or Ctrl + shift
> - **Copy/paste from excel**: copy and go to 1st row in the column and paste the whole column, ensure number or copied cells and rows match.
> - **No special characters are accepted (**¨; &; …)
> - **Species**: type species name in Latin and select from the displayed list

- **Warning messages will appear** if any submitted parameters do not comply with CNAG requirements. These warnings are intended to raise awareness of potential non-compliance in your samples; however, they do not prevent you from saving and submitting the data.

- For any questions or concerns regarding data submission, please contact the CNAG Biorepository (lidia.agueda@cnag.eu or ana.gonzalez@cnag.eu).

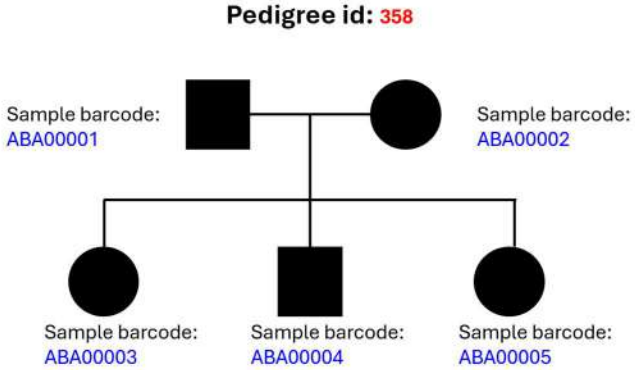
CNAG Submission Site contains the following fields:

Field name	Field description:
LAB_CENTER (opt)	*Laboratory identifier.* Optional, useful for projects involving multiple participant laboratory or centers.
COHORT_NAME (opt)	*Cohort identifier.* Optional, useful if samples belong to different study groups.
SAMPLE_BARCODE	*Sample unique identifier.* **Assigned by CNAG**
REPLACEMENT_OF (opt)	*Sample barcode of the original sample that is being replaced by this new sample.* **Mandatory** when sending additional material. When a sample is additional material for a previous one, the SAMPLE_NAME must be identical (see further details below).
SAMPLE_NAME	*Sample unique identifier.* DO NOT include patient names or surnames Use alphanumerical characters only (no spaces, dashes, or dots). ⚠ Two aliquots from the same sample must have the same SAMPLE_NAME but different SAMPLE_BARCODE. ⚠ Additional material from the same sample must have the same SAMPLE_NAME but different SAMPLE_BARCODE. ⚠ Different samples from the same individual (e.g. normal/tumor; treated/untreated…) must have different SAMPLE_NAME and different SAMPLE_BARCODE. ⚠ Experimental replicates must have different SAMPLE_NAME and different SAMPLE_BARCODE. (see section 4.1 for further details)
SAMPLE_ TYPE	*Type of material* e.g. gDNA, total RNA, small RNA. **Assigned by CNAG.**
FIXATIVE (opt)	*Fixative employed for sample conservation if any.* **Mandatory** for FFPE samples.
SPECIES	*Species from which the DNA/RNA has been obtained.* Use species names compatible with NCBI Taxonomy Browser. For non-human samples, include the known or approximate genome size in the COMMENTS column.
MATERIAL_SOURCE	*Specify the source (e.g. whole blood, buccal swabs, **FFPE tissue**, liver, whole organism, etc.) from which the DNA/RNA was obtained.*
EXTRACTION_METHOD	*Nucleic Acid extraction method employed.* Specify kit and manufacturer, when known. For whole-genome amplified samples, specify the amplification protocol performed.
RESUSPENSION_BUFFER	*Buffer used in final resuspension for the material extraction.*
INITIAL_VOLUME (ul)	*Sample volume provided in µl.* **Exact sample volume do not provide approximate values.**
STOCK_CONCENTRATION (ng/ul)	*Sample concentration in ng/µl.* The accepted concentration may vary according to the project characteristics. Please confirm with CNAG staff if needed.

	If quantification was not permofed using a fluorescence-based method (e.g. Qubit or Quant-it), specify the method in the COMMENTS column.
ABSORBANCE_RATIO_ 260/280 (opt)	*ABSORBANCE RATIO 260/280* If samples are quantified by absorbance methods.
ABSORBANCE_RATIO_ 260/230 (opt)	*ABSORBANCE RATIO 260/230* If samples are quantified by absorbance methods.
SEX	*Sex of the individual* Accepted values: **Unknown/Male/Female/Othe**r
STATUS	*Status of the individual* Accepted values: **unknown** or not applicable / **unaffected** or normal or control or wild type / **affected** or tumor or index case.
PEDIGREE_NUMBER (opt)	*Pedigree identifier.* **Mandatory** only for **family studies**. Members of same family will have the same PEDIGREE identifier. (See an example below).
FATHER (opt)	*Sample_name or CNAG barcode of the father of this individual.* Optional, **mandatory** for family studies.
MOTHER (opt)	*Sample_name or CNAG barcode of the mother of this individual.* Optional, **mandatory** for family studies.
GEOGRAPHIC_ORIGIN	*Geographic origin of the sample.*
COMMENTS (opt)	*Any comments that the collaborator wishes to add, and/or any of the previously mentioned:* ✓ For non-human samples, add known/approximate genome size. ✓ Quantification method (if not fluorescence-based).

- **Provide the EXACT INITIAL VOLUME** to CNAG, not approximations. It will be used for total material availability calculation. Any library failure due to limited material is at your own risk.

- Example of data entry for pedigrees:



Sample_barcode	sample_name	pedigree_number	father	mother
ABA00001	father	358		
ABA00002	mother	358		
ABA00003	daughter1	358	ABA00001	ABA00002
ABA00004	son1	358	ABA00001	ABA00002
ABA00005	daughter2	358	ABA00001	ABA00002

4.1 Sending replacements or additional material to CNAG for FAIL/UNDER REVIEW samples:

- Additional material from the SAME SAMPLE (same individual, same extraction):
  a) use a NEW BARCODE for sample identification.
  b) use EXACTLY THE SAME sample_name. Merging can be considered.
  c) fill in the "replacement_of" column with the barcode of the original sample.

- Replacement of a suboptimal sample with a NEW EXTRACTION (from the same individual):
  d) use a NEW BARCODE for sample identification.
  e) use a SIMILAR sample_name (e.g. "xxx_2extr" or "xxx_b") to the original sample being replaced.
  f) fill in comments column with "to sequence instead of sample_barcode", referring to the replaced sample.

- Replacement of a suboptimal sample with a NEW INDIVIDUAL:
  g) use a NEW BARCODE for sample identification.
  h) use a NEW sample_name , different from the sample being replaced.
  i) fill in comments column with "to sequence instead of sample_barcode", referring to the replaced sample.

- Submit the sample data in the submission site and notify by email to CNAG Biorepository (lidia.agueda@cnag.eu/ana.gonzalez@cnag.eu) BEFORE shipping the samples.

5. Shipping samples to CNAG from EU

- Check that all the samples comply with CNAG requirements and that all administrative aspects are in place with the Project Management team.
- Prepare and pack samples according to the guidelines provided above.
- Data submission **MUST** be completed BEFORE sample shipment.
- Notify the CNAG Biorepository for every sample batch before shipping.
- Confirm the date of delivery.
- Provide the shipment tracking information whenever possible.
- Parcel reception times: preferably, send the parcels at the beginning of the week

  Monday to Friday 8-12h
  No reception on Saturday, Sunday and local bank holidays

- CNAG will not be responsible for parcels delivered outside these time frames or without prior notification.
- During bank holidays, summer breaks or Christmas period, confirm the reception schedules with the CNAG Biorepository

Shipment address:

ATT. Lídia Agueda, PhD / Ana González, PhD
Centre Nacional de Anàlisi Genòmica (CNAG)
Parc Científic de Barcelona – Torre I
C/Baldiri i Reixac, 4
Barcelona 08028 – Spain
☎ **+34 934020569**

6. Non-EU shipments

- For non-EU shipments, additional documentation will be required by the custom authorities.
- CNAG will prepare and submit the necessary documents to Spanish Customs
- The shipment date will be confirmed with the collaborator once the import authorization is granted.
- Contact CNAG Biorepository staff before sample shipping.
- Any parcel missing customs complete documentation, unpaid customs duties, or containing restricted items, will be returned to the sender
- Sample integrity is not granted if held at customs premises.

Shipment address:

ATT. Lídia Agueda, PhD / Ana González, PhD
Centre Nacional de Anàlisi Genòmica (CNAG)
Parc Científic de Barcelona – Torre I
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