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

Contents

1. General Considerations ... 2
2. Sample Quality and Quantity Requirements ... 2
3. Labelling and Packaging Instructions ... 5
4. Sample information submission to CNAG Submission Site ... 6
5. Shipping samples to CNAG from EU ... 11
6. Non EU shipments ... 11

STEPS:

1. Project/subproject Creation – by CNAG Project Management
2. Tubes/Plates and Barcodes shipment – by CNAG Biorepository
3. Samples shipment to CNAG - by the Collaborator
4. Samples Quality Control and Report - by CNAG Biorepository
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

- CNAG Biorepository will contact you to provide the materials and a URL link to the submission site for sample data collection.
- Use only the material provided by CNAG for 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.crg.eu or Ana González, ana.gonzalez@cnag.crg.eu) or to Oxford Nanopore Team (ONT_team@cnag.crg.eu)

2. Sample Quality and Quantity Requirements

- **Tables 1** and **2** show CNAG input quantity and quality general requirements for samples. Samples for Oxford Nanopore Sequencing platform have specific guidelines.
- CNAG 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:
  i) replace the samples
  ii) 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 the CNAG Project Manager according to the experiment and/or the genome size of the studied organism before shipping your samples.

INPUT QUANTITY:

Table 1. Protocols and sample amount requirements (*consult with Project Management or Oxford Nanopore Team, ONT)

Sequencing protocol	**MINIMAL quantity requested** (fluorescence based quantification method)	**Concentration** range (ng/ul) (fluorescence based quantification method)
Regular Whole Genome	2.5 ug	50-200
Low Input Whole Genome	250 ng *	10-50
Ultra Low Input	*	*
WGS for de Novo Assembly	8,5 ug *	50-200
Nanopore DNA 1D	2-17 µg depending on the MW, the genome size, the species and the experimental design. Please contact us before sending less material than 10 µg*	150-200
Nanopore DNA Low Input	*	*
Whole Genome Bisulphite	2 ug	10-50
EnzymaticMethyl-seq (EM seq)	1 ug	20-50
Regular Exome/Custom Capture (Agilent)	4,5 ug	50-100
Low Input Exome/Custom Capture (Agilent)	800 ng *	10-50
Regular Exome and Custom Capture (Kapa Roche)	500 ng	50-100
Amplicon Sequencing	Same protocols as Whole Genome Sequencing	
Clone Sequencing	Same protocols as Whole Genome Sequencing	
Nanopore RNA: PCR-cDNA	100 ng*	10-50
Nanopore RNA: direct cDNA	500 ng of mRNA*	10-50
Stranded total RNA or mRNA Sequencing	2 ug total RNA or 400 ng depleted RNA	50-200 for total RNA; 10-50 for depleted RNA
Non-stranded Low Input RNA Sequencing	*	*
Hi-C protocol	*	*
Genotyping by Sequencing	**Pilot Phase, 1 enzyme**: 1 sample 4 ug; 7 samples 1 ug	20-50
	Pilot Phase, 2 enzymes: 1 sample 7 ug; 3 samples 2 ug	20-50
	Large Scale: 400 ng	20-50

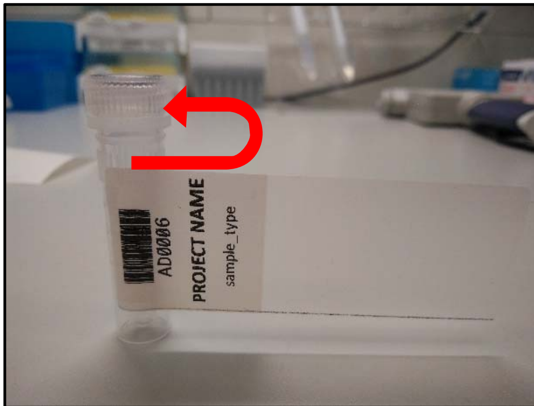
INPUT QUALITY:

Table 2. Sample quality requirements

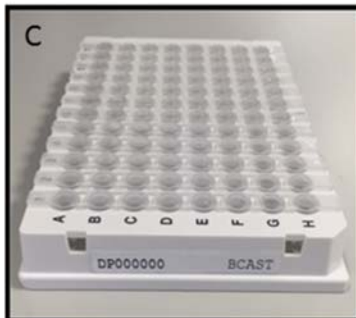
Sample type	Quality requirements
gDNA	• Pure DNA, free of RNA contamination. Optical Density measurements: OD 260/280 1.8-2.0 and OD 260/230 1.8-2.2. Depending on the extraction method employed, RNAse treatment is required. • High molecular weight DNA, no degradation smear • Free of other species DNA contamination • Free of PCR inhibitors • Sample buffer must be water or 10Mm Tris/1mM EDTA • Quantified by fluorescence-based method specific for dsDNA; Absorbance based quantification is inadequate (Nanodrop or equivalent) • See dedicated guidelines for Oxford Nanopore Sequencing
Whole Genome Amplified DNA	Contact Project Manager • Always refer to amplification method employed in data submission site
FFPE DNA	Contact Project Manager
PCR amplicons	Contact Project Manager • Always provide amplicon size in data submission site
Cloned DNA	Contact Project Manager • Always provide insert size in data submission site
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 • Quantified by fluorescence-based method specific for RNA; Absorbance based quantification is inadequate (Nanodrop or equivalent) • See dedicated guidelines for Oxford Nanopore Sequencing
FFPE RNA	Contact Project Manager

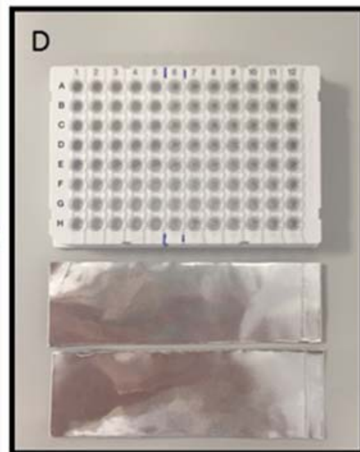
3. Labelling and Packaging Instructions

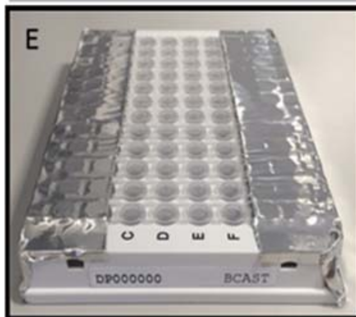
- If you receive screw cap tubes and labels with barcodes, stick first the white part and go around the tube with the transparent part covering the barcode and text with the transparent part for further protection. Keep the label orientation as shown in these pictures:



- If you receive 96-well plates, strip caps, sealing foil and labels for plates place the labels and seal the wells with the strip caps and the sealing foil as shown in these pictures:







- Do not alter the labels in anyway:
  - Tube labels show the project name, material type and a unique CNAG sample barcode.
  - Plate labels show the project name and a unique CNAG plate ID (one plate ID is linked to up to 96 individual CNAG sample barcodes already assigned to a unique plate position).
- Never apply Parafilm around the tubes cap, they have an anti-leakage system in the cap (rubber O-ring).
- For the shipment, use a container to keep the tubes or plates organized and safe from damage.
  - Tubes. Never ship tubes scattered directly in dry ice without any bag or box.
  - Plates: Tight stacking or hard hits during transport can brake plate wells.
- Additional material can be requested to CNAG Biorepository.
- RNA samples must be shipped in dry ice (frozen).
- DNA samples can be shipped refrigerated (with blue ice/cooling blocks) or at room temperature.

4. Sample information submission to CNAG Submission Site

- All the barcodes you receive from CNAG Biorepository will be accessible through a URL link to the submission site, that allows data collection. The SAME link is active until all provided barcodes have been used.
- For sample plates, each unique plate ID is automatically associated to a group sample barcodes, also accessible through the URL link to the external server.
- Submit sample data BEFORE sample shipment and notify by email to CNAG Biorepository (lidia.agueda@cnag.crg.eu or ana.gonzalez@cnag.crg.eu).
- CNAG barcodes that appear on the submission site can be used in different shipment batches. Select and submit the barcodes used for each shipment. Next time the URL is used it will only display the unused barcodes

- There are some submission site auto-fill tips:

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 after submitting if any of the parameters fail to comply with CNAG requirements.
- Contact CNAG Biorepository for any questions or doubts regarding the data submission (lidia.agueda@cnag.crg.eu or ana.gonzalez@cnag.crg.eu).

- **CNAG Submission Site contains the following fields:**

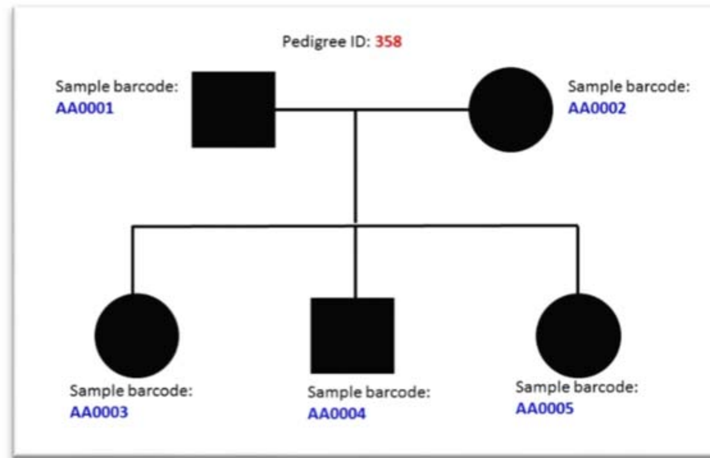
The fields with black font are mandatory, the ones with grey font are optional.

Field name	Field description:
LAB_CENTER (opt)	*Laboratory identifier.* Optional, useful for projects with several participant centers.
COHORT_NAME (opt)	*Cohort identifier.* Optional, useful if samples belong to different study groups.
SAMPLE_BARCODE	*Sample unique identifier.* Provided by CNAG in the same order as printed labels. Check that barcodes in the file correspond to the shipped barcode labels. For PLATES, sample barcodes are already assigned to a unique plate position.
REPLACEMENT_OF	*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, SAMPLE_NAME must be the exact same (see further details below).
SAMPLE_NAME	*Sample unique identifier.* No patient name or surname should appear in the field. ⚠ Two aliquots from same sample must have same SAMPLE_NAME but different SAMPLE_BARCODE. ⚠ When additional material from the same sample is required, both samples must have same SAMPLE_NAME but different SAMPLE_BARCODE. ⚠ If there are different samples from the same individual (i.e. normal/tumor; treated/untreated…) those must have different SAMPLE_NAME. ⚠ When two or more experimental replicates need to be sequenced, they must have different SAMPLE_NAME and different SAMPLE_BARCODE. Use only alphanumerical characters (no spaces, dashes or dots). (See further details below).
SAMPLE_ TYPE	*Type of material* (gDNA, total RNA, small RNA…). Provided by CNAG.
FIXATIVE	*Fixative employed for sample conservation if any.* Mandatory for FFPE samples.
SPECIES	*Species from which the DNA/RNA has been obtained.* Use NCBI Taxonomy Browser compatible species names. For non-human samples, add known/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.* Please specify kit and manufacturer, if known. If samples are whole genome amplified, specify amplification protocol employed.
RESUSPENSION_BUFFER	*Buffer used in final resuspension for the material extraction.*

INITIAL_VOLUME (ul)	*Sample volume provided in µl.* ⚠ **Exact volume provided to CNAG, no approximations.**
STOCK_CONCENTRATION (ng/ul)	*Sample concentration in ng/µl.* ⚠ Accepted concentration may vary according to the project characteristics, please, discuss with CNAG staff. ⚠ If the quantification method employed is not fluorescence-based (Qubit/Picogreen); please specify it in COMMENTS column.
ABSORBANCE_RATIO_ 260/280	*ABSORBANCE RATIO 260/280* If samples are quantified by absorbance methods.
ABSORBANCE_RATIO_ 260/230	*ABSORBANCE RATIO 260/230* If samples are quantified by absorbance methods.
SEX	*Sex of the individual* Unknown/Male/Female/Other
STATUS	*Status of the individual* **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 same PEDIGREE identifier. See 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.*
PLATE_BARCODE	*Plate unique identifier.* Provided by CNAG. Check that barcodes in the file correspond to the shipped plate labels. For PLATES, sample barcodes are already assigned to a unique plate position.
PLATE_POSITION	*Plate position.* Provided by CNAG. For PLATES, sample barcodes are already assigned to a unique plate position.
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 different than Qubit/Picogreen). Optional.

- 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	Pedigree_number	Father	Mother
AA0001	358		
AA0002	358		
AA0003	358	AA0001	AA0002
AA0004	358	AA0001	AA0002
AA0005	358	AA0001	AA0002

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

- If additional material from the SAME SAMPLE is sent (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 column “replacement_of” with the barcode of the sample.

- If any suboptimal sample is replaced by a NEW EXTRACTION (from the same individual):
  d) use a NEW BARCODE for sample identification.
  e) use a SIMILAR sample_name (“xxx_2extr” o be “xxx_b”) to the sample being replaced).
  f) fill in comments column with “to sequence instead of sample_barcode” of the replaced sample.

- If any suboptimal sample is replaced by 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” of the replaced sample.

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

5. Shipping samples to CNAG from EU

- Check that all the samples conform to our requirements (or have been otherwise discussed with the Project Manager) and that they are prepared and packed according to the guidelines given above.
- Data submission MUST be completed BEFORE any sample is sent
- Please make sure to notify CNAG Biorepository staff for every sample batch before shipment.
- The date of delivery needs to be confirmed.
- Provide the shipment tracking information whenever possible.
- Parcel reception times: send parcels preferably 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 of these time frames or without prior notification of parcel shipment.
- Please, confirm with CNAG Biorepository staff the reception timetables during bank or summer holidays or Christmas period.

6. Non EU shipments

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

a. Contact CNAG Biorepository staff before any sample shipment.
b. Any parcel missing customs complete documentations, unpaid customs duties, or containing restricted items, will be returned to the sender
c. Samples integrity is not granted if held at customs premises.

Shipment address:

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