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Guidelines for sample preparation and shipping for DNA / RNA and Amplicon samples

Eurofins Genomics

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1. Service workflow and general guidelines for preparation and shipping 2
2. Starting material 4
3. DNA sample preparation 5
4. RNA sample preparation 7
5. Amplicons on MiSeq 9
6. DNA for INVIEW CRISPR Check 10
7. Sample Preparation for GridION projects 11

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1. Service workflow and general guidelines for preparation and shipping

1.1. Sample assignment

- The sample assignment has to be done in your online account.
- The sample submission form can be downloaded during the acceptance process of your online quote or order
- Assign your samples to the corresponding barcodes used in your online account
- Provide information about all quality and quantity measurements performed at your side to assure fast and optimised sample processing
- You can buy UPS Labels for shipment within the European Union (no dry ice shipment, no shipment of S2 organisms)

1.2. Sample shipment

- Assure that all information and documents are available at project start.
- Sample shipment
    - Tubes: properly labelled 1.5 ml snap cap microcentrifuge tubes (e.g., Eppendorf Safe Lock Tubes™)
    - Plates: Eppendorf twin.tec PCR Plate 96, full-skirted
    - For sample shipment in any other container we may add a handling fee to your project
- We recommend shipping the samples in a padded envelope, box, or other protective shipping package designed for mailing fragile items.
- Packages should be shipped overnight Monday to Thursday, especially if samples have to be cooled.
- Customers outside the European Union should enclose a “proforma invoice” to declare sample value and provide general descriptions.
- Please note, that the DropBoxes / Collection Points cannot be used for the shipment of NGS samples.
- Raw Material with potential S2 organisms: Samples need to be packed in sealed & thick plastic bags before placing in the transport box. For more information please consult the respective guidelines, e.g. UN3373 (“Shipping Dangerous Goods UN3373 Biological Substance, Category B).
- Recommended shipping temperature can be found in the sections below.
- Send labeled samples to

  Eurofins Genomics
  Jakob-Stadler-Platz 7
  78467 Konstanz
  Germany

1.3. Contact

In case of any questions, please do not hesitate to contact Customer Care by email (ngs-support@genomics.eurofinseu.com) or by phone.

All phone numbers are available at: www.eurofinsgenomics.eu/phone

Operating hours: Mondays to Fridays from 8 am to 6 pm CET.

2. Starting material

Sample type	Customer Requested Input / Concentration range / RIN	Definition Plates	Further Comments
RNA (Poly A selection)	150 ng, 6-200 ng/µL, RIN ≥ 8	semi-skirted* leave position H12 empty	Min. 25 µl
RNA (depletion) for bacteria rRNA, HMR rRNA and globin	375 ng, 37,5-200 ng/µL, RIN ≥ 8		Min. 10 µl
rRNA depleted / mRNA enriched by customer	min 20 ng, > 1.4 ng/µL, RIN ≥ 8		up to 15µl
RNA FFPE	200 ng, 8-200 ng/µl, DV200 > 35%		min. 25 µl
RNA ultra low	2 ng, ≥ 15 µL with 0.45-10 ng/µL, RIN > 8		min. 15 µl
RNA Exome	**FFPE:** 150 ng, 18-200 ng/µl, DV200 > 35% **Intact RNA**: 20 ng, 2,5-200 ng/µl, RIN > 8		min. 15 µl
TruSeq RNA	300 ng, 6-500 ng/µl, RIN > 8		min. 50 µl
miRNA (QiaSeq)	150 ng, 30-200 ng/µl, RIN > 8		min. 10 µl
DNA	600 ng / > 3 ng/µl	semi-skirted*	min. 20 µl
Enrichment	100 ng / 1-50 ng/µl	full-skirted*	min. 25 µl
Enrichment FFPE	200 ng / 2-50 ng/µl		min. 25 µl
Amplicon /Microbiome	200 ng / 10-50 ng/µl	semi-skirted* or Deepwell Plate*	min. 25 µl
Microbiome own target	10 ng / 0.5 ng/µl	full-skirted* leave position G12 & H12 empty	min. 25 µl
Adapter Ligation	100 ng-200 ng		
Enrichment cfDNA	10 ng-100ng	full-skirted*	
RNA for RNA fusion detection	**FFPE:** 400 ng, min. 40 - 80 ng/µl **Intact RNA** (fresh frozen): 100 ng, min. 20 ng/µl		

3. DNA sample preparation

3.1. Requirements

- Double-stranded high molecular weight DNA with an OD 260/280 ≥ 1.8-2,0 and an OD 260/230 ≥ 1,8-2,2
- Preferably dissolved in RNAse-, DNAse- and protease-free Tris-HCl buffer (pH 8.0 – 8.5)
- “Ready to load” genomic libraries, ready to load PCR products or PCR products without sequencing adapters must be column purified from low molecular weight impurities (like e.g., primers, and nucleotides) and reaction buffer and should appear as single band on an agarose gel. Please note that a “smear” besides the specific band will interfere with following preparation steps. Upon consultation Eurofins Genomics can perform an additional bead purification step (at extra charge) in order to optimize the sample quality prior to further processing.
- The solution must not contain any impurities e.g., biological macromolecules (e.g., protein, polysaccharides, lipids), chelating agents (e.g., EDTA), divalent metal cations (e.g., Mg2+), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., SDS, Triton-X100).

3.2. Isolation

- There are numerous commercial kits and a wide variety of suitable protocols available for the isolation of DNA (e.g., QIAGEN DNeasy kit). Researchers should select a protocol that meets their particular needs. Organic extraction methods (such as phenol or trizol) should not be used for the purification of total DNA as they can inhibit enzymes used during the library preparation and therefore increase the risk of failure of library preparation. If the use of phenol- or trizol-based methods is unavoidable (e.g. to obtain high molecular DNA), the total removal of these compounds should be guaranteed (that means, an extra clean-up step after extraction should be necessary).
- For best results use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C for the DNA isolation in order to minimize degradation through endogenous nucleases.
- DNA should have undergone a minimum of freeze-thaw cycles, no exposure to high temperatures, no exposure to pH extremes (< 6 or > 9) and no exposure to intercalating fluorescent dyes or ultraviolet radiation.
- DNA should be treated with RNase (e.g., from QIAGEN) to minimize contamination through RNA, which otherwise might be reflected in the sequencing results.

3.3. Quantitative assessment

- Preferred measurement method: fluorescence-based methods like e.g., Qubit® assay (Invitrogen, Life Technologies) or Quant-iT™ PicoGreen ds DNA kit (Invitrogen).
- When using UV-spectrometer-based methods, please be aware that they tend to overestimate the DNA concentration as they are not double-stranded DNA specific but also measure contaminants like e.g., RNA. It is therefore recommended to additionally check the DNA quantity on an agarose gel (see below).

3.4. Qualitative assessment

- Preferred method: UV electrophoresis on an agarose gel (low-percentage for gDNA or high percentage for amplicons) and / or capillary electrophoresis-based methods. Using visualisation techniques such as an agarose gel is a simple way to assess the quality of the DNA. High molecular weight DNA is greater than 50 kb in size and shows minimal smearing. Contamination, damage and degradation are revealed through a low molecular weight smear and should be removed using alternative cleanup strategies.

3.5. Shipping temperature

- DNA can be sent at room temperature, we recommend shipping samples refrigerated (4°C).

4. RNA sample preparation

4.1. Requirements

- High quality RNA with an OD 260/280 ratio ~2.0 and an OD 260/230 ratio between 2.0-2.2.
- RNA Integrity Number (RIN; resp. RNA quality indicator (RQI; Bio-Rad’s Experion) value ≥ 8.
- Preferably dissolved in RNase-, DNase- and protease-free molecular grade water (do not use DEPC-treated H2O).
- The solution must not contain any impurities e.g., biological macromolecules (e.g., protein, polysaccharides, lipids), chelating agents (e.g., EDTA), divalent metal cations (e.g., Mg2+), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., SDS, Triton-X100).
- If any preprocessing steps have been applied (rRNA depletion, mRNA enrichment), please provide us the results of quality and quantity measurements (e.g., charts) performed at your side prior and after the processing steps.

4.2. Isolation

- There are numerous commercial kits and a wide variety of suitable protocols available for the isolation of RNA (e.g., mirVana™ miRNA Isolation Kit from Ambion or QIAGEN RNeasy kit). Researchers should select a protocol that meets their particular needs.
- Extract and stabilize RNA as quickly as possible after obtaining samples and wear gloves at all times to minimize degradation of crude RNA by limiting the activity of endogenous RNases.
- All reagents should be prepared from RNAse-free components.
- We strongly recommend performing a final clean-up of the RNA using a DNAse treatment (e.g., from QIAGEN)
- Organic extraction methods (such as phenol or trizol) should not be used for the purification of total RNA as they can inhibit enzymes used during the library preparation protocol and therefore increase the risk of failure of library construction. If the use of phenol- or trizol-based methods is unavoidable, the total removal of these compounds should be guaranteed (i.e. an extra clean-up step after extraction should be necessary, using e.g. RNeasy kit from QIAGEN).

4.3. Quantitative assessment

- Preferred measurement method: capillary electrophoresis-based methods like e.g to determine the sample concentration.
- Alternatively, we recommend running a 1% formaldehyde agarose gel to provide information on sample concentration.

- Please note that DNA contamination will result in an underestimation of the amount of RNA.

4.4. Qualitative assessment

- Preferred measurement method: capillary electrophoresis-based methods like e.g., to determine the RNA Integrity Number (RIN) or UV spectrophotometry.
- Alternatively, we recommend running a 1% formaldehyde agarose gel to check the RNA integrity. The mRNA should appear as a smear between 500 bp and 8 kb (most of the mRNA should be between 1.5 kb and 2 kb).

4.5. Shipping temperature

- RNA should ideally be shipped on dry ice
- If it is not possible to ship on dry ice, then you can precipitate your RNA in ethanol. Recommended precipitation protocol:
  - add 1/10 volume 3 M sodium acetate to 1 volume RNA (pH 5.2)
  - add 1/50 volume glycogen (5 mg /ml), final concentration will be 100 µg/ml
  - vortex to mix
  - add 3 volumes ice cold 100 % ethanol
  - vortex to mix thoroughly
  - Please do not decant and discard the supernatant, but send the sample diluted in ethanol.
- Tissues / cell cultures must be flash frozen in liquid nitrogen and have to be shipped on dry ice. Alternatively, fresh material can be stabilised in RNAlater or similar reagents (e.g. Ambion, Sigma or QIAGEN) and can be sent at room temperature.
- Please note: If you send your RNA samples in EtOH it is important that you inform us during ordering (sample questionnaire)

5. Amplicons on MiSeq

Please provide amplicons generated with NGS primers (consisting of the target specific sequences defined by the customer and the adapter sequences defined below).

Illumina adapter sequence that has to be added on the 5'- end of the forward primer:
5'- ACACTCTTTCCCTACACGACGCTCTTCCGATCT -forward primer target sequence-3'

Illumina adapter sequence that has to be added on the 5'- end of the reverse primer:
5'- GACTGGAGTTCAGACGTGTGCTCTTCCGATCT -reverse primer target sequence-3'

- Please note that the complete sequence of your primers is necessary for sorting of reads and the optional bioinformatics analysis. Sequences are to be given in our sample submission form.
- Amplicons size (not including the adaptors you introduced with your target specific PCR) needs to be between 280 – 540 bp in order to be accepted. Merging is usually possible.
- Quality check needs to be performed by agarose gel electrophoresis (= clear visible band(s) of expected size using 5 µl PCR product). The gel picture needs to be handed out to Eurofins Genomics. Please purify the samples (dilution preferred in EB buffer). The total required volume/amplicon is 25 µl. Please provide min. 20 ng in min. 20 µL (concentration min. 1 ng/µL). For Microbiome – your target, no concentration can be defined because bacterial content is still unknown. Per sample and PCR> 10 ng DNA with at least 10^3 bacterial / fungal / archaeal genomes are needed". Please specify the agarose gel including volume loaded, the size of the amplicons and the type of marker (incl. manufacturer).

6. DNA for INVIEW CRISPR Check

6.1 Amplicon Length Requirements

- Amplicon size needs to be adapted to the length of InDels you want to be able to analyse, the sequencing mode you chose and must allow merging of read pairs. Please find below the wildtype amplicon size requirements for two experimental cases.

INVIEW CRISPR Check Product type	Detection of deletions up to 100 bp and detection of insertions of up to 30 bp	Detection of deletions up to 50 bp and detection of insertions of up to 30 bp
MiSeq	450 – 500 bp	400 – 500 bp
NovaSeq	200 – 225 bp	150 – 225 bp

Table: Wildtype amplicon size requirement for two experimental cases.

- Wildtype amplicon size for INVIEW CRISPR Check MiSeq does not include the universal adaptors that are included in your lab
- Wildtype amplicon size for INVIEW CRISPR Check NovaSeq does not include the 10 nucleotide tags on fwd primers that you optionally include in your lab
- Target regions can be mutated in your unmodified cells and might differ from the available reference sequence/expected amplicon sequence. To evaluate this, we strongly recommend including at least one wildtype control sample for your experiment.
- The INVIEW CRISPR Check Gene Editing Bioinformatics Analysis requires a minimum of 10 000 read pairs per sample and target site. In case you are using the INVIEW CRISPR Check Adaptor Ligation approach with sample tagging, please plan accordingly.

7. Sample Preparation for GridION projects

7.1 Whole genome sequencing of small genomes (e.g. bacteria)

- OD 260/280: 1.8
- OD 260/230: 2.0-2.2
- For 1 sample: 4000 ng, conc. 40 ng/µl (Nanodrop 100 ng/µl), min vol 50 µl
- For 2-5 samples: 2000 ng, conc. 40 ng/µl (Nanodrop 100 ng/µl), min vol 25 µl
- For 16-8samples: 800 ng, conc. 40 ng/µl (Nanodrop 100 ng/µl), min vol 15 µl

7.2 Full-length 16S sequencing

- OD 260/280: 1.8
- OD 260/230: 2.0-2.2
- > 2.5 ng/µl in 20 µl

7.3 Amplicon sequencing

- OD 260/280: 1.8
- OD 260/230: 2.0-2.2
- For 1 sample: optimal > 25 ng/µl in 60 µl
- For 2-8 samples: optimal > 25 ng/µl in 30 µl, size range of different amplicons should be similar (+/- 10%)