Sample requirements & Guidelines for sample preparation and shipping

Eurofins Genomics

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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
- Receive UPS Labels for shipment free of charge within the European Union (no dry ice shipment)

1.2. Sample shipment

- Assure that all information and documents (e.g., offer confirmation in written form) are available at project start.
- If not specified differently, samples have to be sent in properly labelled 1.5 ml snap cap microcentrifuge tubes (e.g., Eppendorf Safe Lock Tubes™). Differently sized tubes, or tubes that have screw caps may not be used.
- 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

  Please note: in case you requested a quote with high sample numbers for extraction please check your quote. Sometimes samples should then be shipped to Ebersberg:

Eurofins Genomics Europe Applied Genomics GmbH
Extraction lab
c/o Jan Enke & Fabian Hauser
Anzinger Str. 7a
86650 Ebersberg
Germany

1.3. Project tracking & data access*

- Track all processing steps of your project via your online account in real time:
  - Initial QC
  - Library preparation
  - Sequencing
  - Bioinformatic analysis
- Invite colleagues to track your project to facilitate team working or represent you in your absence
- Receive all raw data as well as the analysed data online via your online account and via our secure FTP folder

1.4. 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. DNA sample preparation

INVIEW Genome, INVIEW Metagenome, INVIEW Exome,
INVIEW Oncoprofiling, INVIEW Virus (dsDNA), INVIEW Plasmid Verification
NGSelect DNA, NGSelect Amplicon on NovaSeq (150 – 270 bp), NGSelect Ready-2-Load

2.1. 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).

2.2. 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.

2.3. 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).

2.4. 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.

2.5. Shipping temperature

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

2.6. Starting material

	Product	Starting material
DNA sequencing	**INVIEW Resequencing** **INVIEW Metagenome** **NGSelect DNA**	100 ng gDNA Qubit quantified (up to 100µl / concentration > 1 ng/µl); (NanoDrop quantified > 200 ng recommended) 200 ng - 500 ng for DNA from FFPE (up to 100µl / concentration >2 ng/µl); (NanoDrop quantified > 200 ng recommended) cDNA and amplicons as starting material can only be processed on customer risk (no guarantees)
	Whole Genome Sequencing	200 ng gDNA Qubit quantified (up to 200µl / concentration > 1 ng/µl); (NanoDrop quantified > 400 ng recommended) 500 ng - 1.000 ng for DNA from FFPE (up to 200µl / concentration >2 ng/µl); (NanoDrop quantified > 1000 ng recommended)
	Premium Whole Genome Sequencing	200 ng DNA (up to 200 µl / concentration > 2.5 ng/µl) per sample (Qubit quantified); not available for FFPE samples
	INVIEW Plasmid Verification	*Projects up to 48 samples:* 100 ng gDNA Qubit quantified (up to 100µl / concentration > 1 ng/µl) *Projects > 48 samples:* 10-20 µl with 5-10 ng/µl

	INVIEW Virus (dsDNA)	100 ng - 500 ng gDNA Qubit quantified (up to 100µl / concentration > 1 ng/µl)(NanoDrop quantified > 200 ng recommended) cDNA cannot be accepted as starting material
Enrichment	**INVIEW Human Exome** **INVIEW Oncoprofiling**	100 ng for Qubit quantified gDNA (up to 100 µl / concentration > 1 ng/µl) 200 ng for Qubit quantified DNA from FFPE (up to 100µl / concentration >2 ng/µl) QC passed if DQN ≥ 4.0, Threshold = 10.000 (gDNA), if DQN ≥ 2.0, Threshold = 1.000 (FFPE DNA)
Amplicon sequencing	**NGSelect Amplicon – NovaSeq** **INVIEW CRISPR Check - NovaSeq**	100 -200 ng PCR products/amplicons Qubit quantified (up to 100µl / concentration > 1 ng/µl) (NanoDrop quantified > 200 ng recommended)
Ready-to-Load	**NGSelect Ready-to-Load**	300ng library (up to 30µl / concentration > 10 ng/µl), Qubit quantified

3. RNA sample preparation

INVIEW Transcriptome, NGSelect RNA, INVIEW Virus (RNA virus)

3.1. 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.

3.2. 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).

3.3. 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.

3.4. 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).

3.5. 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)

3.6. Starting material

	Product	Starting material
RNA-Seq	**INVIEW Transcriptome Bacteria**	Prokaryotes 150 ng total RNA (up to 15 µl / optimal concentration 6 - 50 ng/µl; absolute maximum concentration 200 ng/µl); rRNA depleted / mRNA enriched RNA: 20ng (up to 15 µl / concentration > 1.4 ng/µl)
	NGSelect RNA **INVIEW Transcriptome Discover**	* Eukaryotes (polyA): 150 ng total RNA (up to 25 µl / optimal concentration 6 - 50 ng/µl (absolute maximum concentration 200 ng/µl) * Eukaryotes & prokaryotes (rRNA depleted / mRNA enriched RNA): 20ng (up to 15 µl / concentration > 1.4 ng/µl)
	INVIEW Transcriptome Ultra-Low	Only for Eukaryotes with Poly-A: Only for Eukaryotes with Poly-A: at least 10µL of total RNA, at least 0.15 ng/µL Quality:

		• RIN > 6.5: QC passed • RIN < 6.5: Customer override required -> increased 3´bias to be expected For RNA isolation: no restrictions for input material for RNA isolation but also no guarantee for success
	INVIEW Virus (RNA)	Type: rRNA depleted or total RNA Quantity: 100 ng per sample Concentration: min 5 ng/µl Dissolved in: RNase-, DNase- and protease-free molecular grade water

4. Samples for DNA or RNA isolation – General

All INVIEW and NGSelect products except INVIEW Microbiome Profiling 3.0

4.1. General – Risk group classification sheet

- Please enclose a hard copy of the completed “risk group classification” of genetically modified organism for all projects, where DNA or RNA isolation is required.
- You can find the risk group classification sheet here: https://eurofinsgenomics.eu/ngs-guide

4.2. FFPE tissue

- To keep the rate of cross-linking and fragmentation of RNA / DNA at a minimum the tissue should be fixated as quickly as possible.
- Make sure that samples are completely dehydrated prior to embedding.
- FFPE samples have to be delivered as slices (unstained and uncovered) and should be as freshly cut as possible.
- Slices should not be thicker than 10 µm (surface approx. 250mm2) as the RNA / DNA yield decreases with increasing thickness.
- FFPE samples for DNA isolation can be sent at room temperature.
- Not suited for INVIEW Metagenome and INVIEW Microbiome Profiling 3.0

4.3. Tissue

- Tissue should be immediately snap-frozen in liquid nitrogen after harvesting.
- To facilitate fast freezing the tissue should be split into several pieces.
- Frozen material should be stored at -80°C and repeated freeze-thaw cycles should be avoided or kept to a minimum as they enhance nucleic acid degradation.
- Tissue has to be shipped on dry ice.
- RNA samples could be stored in RNAlater™ or similar stabilization reagents. Regarding the amount of RNALater usually 1x sample to 5x RNAlater™ is recommended. Please follow the manufacturer’s instructions for more details.

4.4. Cell culture

  - No lysis buffer is necessary.
  - Please determine the cell count and harvest the cells by centrifugation.
  - . Then snap-freeze the cells using liquid nitrogen.
- As we perform a washing step as the first step for extraction, cells do not necessarily need to be washed. Cultured cell lines have to be shipped on dry ice.
- RNA samples could be stored in RNAlater™ or similar stabilization reagents. Regarding the amount of RNALater usually 1x sample to 5x RNAlater™ is recommended. Please follow the manufacturer’s instructions for more details.

4.5. Whole blood, plasma and serum

- Freshly drawn blood should be directly collected in appropriate tubes that already contain anticoagulant-preservative agents (like e.g. EDTA, citrate). Heparin may not be used as anticoagulant as it inhibits downstream processes such as PCR. The maximum storage time (i.e. from sampling to nucleic acid extraction) at 4°C may not exceed 4 days.
- Plasma and serum samples should be immediately snap-frozen (if possible in liquid nitrogen) after collection.
- Frozen material should be stored at -80 °C and repeated freeze-thaw cycles should be avoided or kept to a minimum as they enhance nucleic acid degradation.
- Serum and plasma have to be shipped on dry ice.

4.6. BACs

- Stab culture or agar plate can be shipped at room temperature (no incubation needed), glycerol stocks have to be shipped on dry ice.
- Please provide us with information about the corresponding antibiotic resistances and enclose a hard copy of the completed “Risk group classification” of genetically modified organism

5. Samples for DNA isolation – for Microbiome Profiling 3.0

INVIEW Microbiome Profiling 3.0

Please ship the raw material samples in appropriate safe lock tubes. If not stated otherwise, you may ship your samples at ambient temperature.

FFPE tissue: this starting material is not accepted for Microbiome Profiling 3.0

5.1 Fermented products (cheese, yoghurt, etc.)

- Ideally, please send 5 -10 g of sample or a whole packing in a sealed container.

5.2. Enrichment cultures / starter cultures

- Please send a representative sample or an overnight culture freshly frozen or cooled on ice packs.

5.3. Buccal swabs

- Please send 1 swab per sample frozen or chilled. If available, please send 1 additional swab per sample.

5.4. Surface swabs

- Please send only dry swabs and if available 1 additional swab per sample.
- Please send the swabs chilled or frozen.

Please note that DNA extraction is only feasible if enough material is on the swab. This sample type will be processed on customer risk only.

5.5. Human or animal faeces / sewage sludge

- Please send minimal 800 mg frozen. Alternatively you may send faeces samples using the OMNIgene®•GUT collection and stabilization system from DNA Genotek.

5.6. Water and waste water

- Please filter 500 ml water sample through a 0.22 µm pore size filter. Filter should be filled to full capacity to harvest 10^9 bacterial cells. Filter should be free and removed from the holder (i.e. not attached to a column). Before sending the filter should be dried and wrapped in sterile aluminium foil.
- If you do not have the possibility to filter, please contact us.

5.7. Earthy Soil

- Please send minimal 800 mg of frozen samples ideally in tubes. Earthy soil only, no sand, sandy soil or sediment, Loam only on customer risk.

5.8. Plant leaves (for surface analysis)

- Please send plant leaves in 50 ml tubes cooled or at room temperature. Depending on the size of the leaves you might want to roll the leaves. Please take extreme care to keep the leaves intact.
- For extraction of bacterial or fungal DNA we will initially detach microorganisms from the plant leaves by swirling them in a small amount of buffer in order to concentrate the microorganisms. Intact leaves therefore will minimise the portion of reads derived from homologous plant sequences (most relevant for 16S sequences).

6. DNA sample preparation for Microbiome

INVIEW Microbiome Profiling 3.0

6.1 Requirements

- Please provide your DNA samples in Tris-buffer / buffer from commercial DNA extraction kits

Sample type	Purified microbial DNA	DNA from host and bacteria / fungi
Volume	**1 target: 20 µl 2 targets: 30 µl 3 targets: 40 µl 4 targets: 50 µl**	**1 target: 20 µl 2 targets: 30 µl 3 targets: 40 µl 4 targets: 50 µl**
Concentration	**10 - 50 ng/µl**	**50 ng/µl**

6.2 Quantitative and Qualitative Assessment:

- See sections 2.3 and 2.4.

7. Amplicons for Microbiome & NGSelect Amplicon MiSeq

INVIEW Microbiome Profiling 3.0 – your targets,
NGSelect Amplicon – MiSeq (280 – 570 bp)

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).

8. DNA for INVIEW CRISPR Check

INVIEW CRISPR Check

8.1 General Requirements

- See section 2 for amplicon preparation for NovaSeq
- see section 7 for amplicon preparation for MiSeq

8.2 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.

9. Plasma Preparation for cell-free DNA extraction

INVIEW Liquid Biopsy products

9.6 Blood collection with EDTA or citrate (do not use heparin)

Blood should be kept at room temperature and plasma should be prepared within 1 h after blood draw to avoid cell lysis and release of contaminating gDNA.

9.7 Blood collection with Cell-Free DNA BCT (Streck)

Blood should be kept at room temperature and plasma should be prepared within 36 h.

In order to separate plasma from blood samples, Eurofins recommends the following protocol:

- Place primary blood collection tubes in a centrifuge with a swing-out rotor.
- Centrifuge the blood samples for 10 min at 1900 x g at +20 °C.
- Carefully aspirate the plasma supernatant without disturbing the buffy coat layer. About 4–5 ml of plasma can be obtained from one 10 mL primary blood collection tube.
- Transfer the aspirated plasma into fresh 15 ml centrifuge tubes with conical bottoms.
- Centrifuge plasma samples for 10 min at 16,000 x g (in fixed-angle rotor) at +4 °C.
- Carefully transfer the supernatant (plasma) into a new tube with a pipette without disturbing the pellet.
- Plasma can be kept frozen at –80 °C.

10. Sample Preparation for GridION projects

INVIEW GridION WGS & INVIEW GridION Full-Length 16S & INVIEW GridION Amplicon

10.1 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

10.2 Full-length 16S sequencing

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

10.3 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%)