Sample requirements & Guidelines for sample preparation and shipping

Eurofins Genomics

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.

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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)

Please note: *most of our products require the sample shipment* ***in tubes*** *using the NGS barcodes. But some products can also be shipped* ***in plates****. This is also mentioned during the online sample assignment. More details can also be found in the sections below.*

1.2. Sample shipment

- Send labeled samples to
  Eurofins Genomics Europe Sequencing GmbH
  Jakob-Stadler-Platz 7
  78467 Konstanz
  Germany
- 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.
- Recommended shipping temperature can be found in the sections below.

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

* *Please note that for some products the level of detail might vary*

1.4. Contact

In case of any questions, please do not hesitate to contact Customer Care by email (ngs-support@eurofins.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.

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2. DNA sample preparation

INVIEW Genome, INVIEW Metagenome, INVIEW Exome,
INVIEW Panel, INVIEW Microbiome Profiling High Specificity,
NGSelect DNA, NGSelect Amplicon Adaptor Ligation, NGSelect Ready-2-Load

2.1. Requirements

- Double-stranded high molecular weight DNA with an OD 260/280 ≥ 1.8 and an OD 260/230 ≥ 1.9
- 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 gel-purification step (at extra charge) in order to optimise 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.
- 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 minimise 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 minimise 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).

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3. RNA sample preparation

INVIEW Transcriptome, NGSelect RNA

3.1. Requirements

- High quality RNA with an OD 260/280 ratio ≥ 1.8 and an OD 260/230 ratio ≥ 1.7.
- 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 stabilise RNA as quickly as possible after obtaining samples and wear gloves at all times to minimise degradation of crude RNA by limiting the activity of endogenous RNases.
- All reagents should be prepared from RNAse-free components and be kept on ice.
- We strongly recommend performing a final clean-up of the RNA using commercial available RNA purification kits (e.g., RNeasy spin columns from QIAGEN) and 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.

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 has to be shipped on dry ice unless RNA is precipitated 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 or dry ice and have to be shipped on dry ice. Alternatively, fresh material can be stabilised in RNAlater (e.g. Ambion, Sigma or QIAGEN) and 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)

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4. Samples for DNA or RNA isolation – General

All INVIEW and NGSelect products except INVIEW Microbiome Profiling 3.0

4.1. 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.
- FFPE samples for RNA isolation have to be shipped on dry ice.

4.2. Tissue

- Tissue should be immediately snap-frozen (after weighing) 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.

4.3. Cell culture

- Cell lines should be lysed in lysis buffer, centrifuged (after determination of the cell count) and snap-frozen in liquid nitrogen.
- Cultured cell lines have to be shipped on dry ice.

4.4. 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. After collection the tubes should be thoroughly inverted and immediately be stored at -80 °C. The maximum storage time 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.

4.5. BACs

- Stab culture 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.

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. 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.

5.5. Water, waste water & other liquids

- 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.

5.6. Soil & other sediment samples

- Please send minimal 800 mg of frozen samples.

5.7. 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).

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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
- Samples need to be shipped in 96-well PCR plates with 200 µl well volume to ensure compatibility with our robotics platform (like e.g. # N8010560). To avoid leakage or cross-contamination, please seal the plates using cap stripes (like e.g. # N4323032). Many adhesive seals can come unsealed, or can hardly be removed (e.g. aluminum seal) and thus can cause damage for our devices. Shipping of plates can be done at ambient temperature.

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.

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7. Amplicons for Microbiome & NGSelect Amplicon 2nd PCR

INVIEW Microbiome Profiling 3.0 2nd PCR, NGSelect Amplicon 2nd PCR

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'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-forward primer target sequence-3'

Illumina adapter sequence that has to be added on the 5'- end of the reverse primer:
5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-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 340 – 760 bp in order to be accepted. Depending on the size of the amplicon you may expect an overlapping of reads. Merging is usually possible for amplicons smaller than 570 bp (without adaptor). For the longer regions the amplicon size is too long and read overlapping will not occur or only for some of the read pairs.
- 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. No purification step should be performed at customer's lab. The total required volume/amplicon is 25 µl. Please specify the agarose gel including volume loaded, the size of the amplicons and the type of marker (incl. manufacturer).
- Samples need to be shipped in 96-well PCR plates with 200 µl well volume to ensure compatibility with our robotics platform (like e.g. # N8010560). To avoid leakage or cross-contamination, please seal the plates using cap stripes (like e.g. # N4323032). Many adhesive seals can come unsealed, or can hardly be removed (e.g. aluminum seal) and thus can cause damage for our devices. Shipping of plates can be done at ambient temperature.

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8. DNA for INVIEW CRISPR Check

INVIEW CRISPR Check

8.1 General Requirements

- See section 2 for amplicon preparation for the Adaptor Ligation approach
- see section 7 for amplicon preparation for the 2nd PCR approach

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
2nd PCR	450 – 500 bp	400 – 500 bp
Adaptor Ligation 2x 150 bp	200 – 225 bp	150 – 225 bp
Adaptor Ligation 2x 250 bp	375 – 400 bp	325 – 400 bp
Adaptor Ligation 2x 300 bp	375 – 500 bp	325 – 500 bp

Table: Wildtype amplicon size requirement for two experimental cases.

- Wildtype amplicon size for INVIEW CRISPR Check 2nd PCR does not include the universal adaptors that are included in your lab
- Wildtype amplicon size for INVIEW CRISPR Check Adaptor Ligation 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.