Next generation sequencing: Sample preparation and shipping guidelines

Eurofins GATC Biotech GmbH

May, 2018

1. Next generation sequencing service workflow

1.1. Contact

- Contact us to discuss your experimental requirements and to design your study
- Receive an offer for your experimental design
- Receive free NGS barcodes for unambiguous sample identification and tracking through your entire sequencing project

1.2. Sample preparation

- Assess sample quality and quantity
- Label each individual sample by affixing one barcode in front of the tube

For further information about sample preparation please refer to the additional information provided below.

1.3. Sample assignment

- Assign your samples during ordering in your NGS project
- 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

1.4. Sample shipment

- Send labeled samples to Eurofins GATC Biotech
- Assure that all information and documents (e.g., order confirmation page) are available at project start

For further information about sample shipment please refer to the additional information provided below.

1.5. Project tracking

- Track all processing steps of your project via your myGATC customer account in real time:
- initial QC
- library preparation
- sequencing
- bioinformatics analysis
- Invite colleagues to track your project to facilitate team working or represent you in your absence

1.6. Receive regular updates via email Data download

- Receive all raw data as well as the analysed data online via your online account

2. Sample requirements

Please refer to your offer for the specific sample requirements. If your samples do not fulfil the requirements, the processing might be delayed.
The quality and quantity of the starting material is crucial for the success of your project. The use of too little, damaged (e.g. apurinic/apyrimidinic sites, nicks, interstrand crosslinks etc.), degraded or contaminated starting material can result in low yield or failure of the library preparation.
To help assuring a fast and optimised processing please send an electronic copy of all available quality and quantity measurement results to nextgen@gatc-biotech.com prior to sending your samples. If you have any further questions regarding sample requirements, please do not hesitate to contact our experienced Project Management Team by telephone under +49 (0) 7531 81 60 50 60. The completed "GATC Sample Description Sheet" should be sent along with your sample.
The quality and quantity of each incoming sample will be determined by appropriate methods (e.g. agarose gel analysis / Qubit® Fluorometer / NanoDrop). If the amount, concentration, and / or quality of the starting material do not meet the requirements for the library preparation (as specified in your quote), we will contact you to discuss how to proceed. If possible, Eurofins GATC Biotech will recommend additional pre-processing steps in order to optimise the sample quality. If additional services are requested, the following prices per sample apply:

1. RNA / DNA concentrating: € 60
2. RNA / DNA purification: € 60
3. RNase treatment: € 60
4. Sample pooling after quantification by agarose gel: € 25
5. Sample pooling after quantification : € 40
6. Additional quality check: € 10

2.1. Sample submission checklist

☐ Concentration has been determined for each sample
☐ Samples are labelled and prepared for shipment
☐ Samples are assigned to the corresponding barcodes in your myGATC account
☐ All necessary information and documents (e.g., offer acceptance in written form) are included

2.2. Contact

In case of any questions, please do not hesitate to contact Customer Care by email (customercare@gatc-biotech.com) or by phone

Germany: +49 (0) 7531 81 60 68
United Kingdom: +44 (0) 207 69 14 92 1
France: +33 (0) 97 04 46 74 3
Sweden: +46 (0) 8 65 53 60 9
Operating hours: Mondays to Fridays from 8 am to 6 pm CET.

3. DNA sample preparation

3.1. 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 GATC Biotech 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., Mg^{2+}), 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.
- 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.

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 a 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.

4. RNA sample preparation

4.1. 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.

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 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.

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).

5. Samples for DNA or RNA isolation

5.1. 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.

5.2. 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.

5.3. Cell culture

- ☐ Cell lines should be lysed in lysis buffer, centrifuged (after determination of the cell count) and snap-frozen in liquid nitrogen.

5.4. 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.

6. Sample shipment

6.1. General

- ☐ 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.
- ☐ A hard copy of the completed "GATC Sample Description Sheet" should be enclosed, indicating the corresponding offer number, sample name and shipment date.
- ☐ Customers outside the European Union should enclose a "proforma invoice" to declare sample value and provide general descriptions.

Samples have to be sent to the following address:
Eurofins GATC Biotech GmbH
European Genome and Diagnostics Centre
Jakob-Stadler-Platz 7
78467 Konstanz
Germany

Please note, that the GATC Collection Points cannot be used for the shipment of NGS samples.
DNA

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

6.2. 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 that not all the couriers accept dry-ice shipments.

6.3. FFPE samples

- ☐ FFPE samples for DNA isolation can be sent at room temperature.
- ☐ FFPE samples for RNA isolation have to be shipped on dry ice.

6.4. Cultured cell lines, tissue, whole blood, plasma and serum

- ☐ Cultured cell lines, tissue, whole blood, serum and plasma have to be shipped on dry ice.

6.5. Environmental samples

- ☐ Stool samples should be shipped at -20°C.
- ☐ Soil, food and animal feed should be dried and shipped at room temperature or refrigerated (4°C)

6.6. 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 genetic