

NGS sample submission guide

DNA & RNA samples

- Follow the instructions below for your sample type and application.
- BaseClear can supply sample collection kits, tubes, barcode stickers, barcoded plates or racks and DNA/RNA Shield solution upon request.
- Before sending samples please visit the Order Portal and submit the order form. Provide a PRINTED version of your order form with the shipped samples. **Samples received without an order form may cause project delay and extra fees may apply.**

Biosafety regulations:

BaseClear can only process micro-organisms of biosafety Class I and II according to European regulations. The customer has the responsibility not to ship samples that might contain class III or IV

organisms according to EU directive 2000/54/EU. For Class III and IV organisms, only deactivated cells (in Shield solution) or DNA and RNA can be provided. RNA from RNA viruses cannot be send intact but can be send in Shield solution. Provide samples in clean tubes. Extra fees may apply for additional decontamination work. Possibly contaminated samples will be destroyed and additional fees will apply.

Postal address:

BaseClear B.V.
Attn. Expedition
Galileiweg 4
2333 BE Leiden
The Netherlands.

✉ info@baseclear.com

☎ +31 (0)71 523 3917

DNA SAMPLES

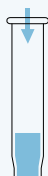
ILLUMINA SEQUENCING



At least 500 ng DNA
with a concentration
of > 25 ng/ µl

Final volume:
Min. 30 µl

NANOPORE SEQUENCING (ONT)



At least 2 µg of DNA with
a concentration
of > 20 ng/ µl

Final volume:
Min. 30 µl

Notes

- Provide material in excess whenever possible.
- DNA concentrations must be measured with an assay specific for double-stranded DNA, i.e. Qubit or Broad Range kit. Spectrophotometric measurements (i.e. Nanodrop) are not specific and can result in overestimated concentrations.
- gDNA must show a single high-molecular weight band; we recommend that customers check integrity and concentration by agarose gel electrophoresis and provide gel photos (or TapeStation or equivalent).
- gDNA must be pure (OD₂₆₀:280 ~1.8; OD₂₆₀:230 ~2.0, DIN>7.0 measured with TapeStation (or equivalent) in molecular-grade water.
- Do not provide DNA in TE buffer!
- gDNA must not contain RNA or inhibitors.
- Samples need to be sent in a microfuge tube (centrifugable).
- Samples can be provided in 96 well plates (see requirements page 2).
- We recommend shipping DNA frozen on dry ice by express courier.
- Provide any relevant sample-related information.

ONT:

- Provide material in nuclease-free water, elution buffer in safe-lock Eppendorf or Micronic tubes.
- DNA size should be >25 kb in a single major band (agarose gel electrophoresis, TapeStation or equivalent).
- Ship samples frozen on dry ice.

qPCR assay evaluation - required control strains

- Viable strain with growth conditions (recommended)
or
- At least 100 µl DNA with a concentration of >10ng/ µl
- Final amount: 1 µg DNA per assay

Note: If the client cannot provide control strains, the specificity with regard to a positive genomic target control and off-target control cannot be determined

qPCR quantification

- >15 µl DNA per sample per assay for TaqMan
- >35 µl DNA per sample per assay for SYBR Green

RNA SAMPLES

Eukaryotic mRNA-SEQ (Poly-A purification)
Bacterial mRNA-SEQ (rRNA depletion)
Total RNA library prep



At least 2 µg total RNA
with a concentration
of >50 ng/µl

Final volume:
Min. 30 µl

Notes

- Provide material in excess whenever possible.
- RNA samples must be of high quality, with a RIN value of >8.0.
- RNA concentrations must be measured with a non-UV method, i.e. Qubit or Broad Range kit, because UV methods (i.e. Nanodrop) often overestimate concentrations. Moreover, we recommend checking integrity and concentration with an Agilent BioAnalyzer (or similar) and providing the BioAnalyzer traces.
- Total RNA must be pure in RNase-free molecular-grade water.
- Total RNA must be free of contaminating gDNA. We recommend including a DNase treatment in the RNA isolation procedure, especially for bacterial RNA samples. BaseClear does not check for the presence of contaminating DNA in the samples during the standard QC and therefore does not take responsibility for genomic DNA contamination in the samples and/or data generated.
- We recommend shipping RNA frozen on dry ice by express courier.
- RNA can be provide in 96 well plates (see below requirements).

DELIVERY OF SAMPLES IN 96-sample FORMAT



BaseClear recommends the following racks:

1. Micronic 96-4 Rack with high cover for 1.4 ml tubes (MP51211) and TPE cluster caps (**do not use screw caps**) (MP53001) (NBS Scientific).
2. Micronic 96-4 Rack with high cover for 1.4 ml tubes including TPE cluster caps (MPW42047LBC3S-XS) (NBS Scientific), **available via BaseClear only!**
3. ZR-96 BashingBead Lysis Rack, 0.5 mm & 0.1 mm (S6002-96-3) (Zymo Research).

Notes

- For delivery of samples for high throughput analysis.
- For 22 or more samples, use a Micronic tube robo rack format. BaseClear can supply racks on request. See below for recommendations.
- Micronic racks can be shipped on dry-ice. Bashing racks can be shipped on ice.
- Use high quality caps and make sure the caps are properly closed to avoid cross contamination during shipment.
- Samples must be contiguous in a plate in column format. Do not leave empty wells between wells with samples.
- The maximum number of samples per rack is 94. Leave 2 spaces (G12 and H12) empty for our internal controls (PTC (Positive Template Control) and NTC (No Template Control)).
- For multiple racks, completely fill the first rack (up to 94 samples) before filling the next rack.
- Fill out the F089-NG-06-2 form with sample names and upload it together with the weborder in the Order Portal. The maximum number per form is 94. Leave 2 spaces (G12 and H12) empty for our internal controls (PTC (Positive Template Control) and NTC (No Template Control)). If you need multiple forms, fill each form with 94 samples before you use an additional form. If your boxes do not fit 94 samples, you still have to fill up the full form with 94 samples before you use an additional form.
- Request the F089-NG-06-2 form at info@bascclear.com in case you do not have this document.
- For samples intended for qPCR analysis it is mandatory to provide an excel table that clearly describes each file and its group, species, strain or gene target for the qPCR assay using the F089-qPCR-01 form.
- Request the F089-qPCR-01 form at info@bascclear.com in case you do not have this document.
- In our online order portal we create 1 sample entry line per rack. The name should match the name on the rack and of the F089-NG-06-2 form.