guidelines v1.05

Submission guidelines for Oxford Nanopore (ONT) sequencing

Submission process

Users should fill in the excel submission form found on GECF website, and submit it by email to gecf@epfl.ch together with the profiles. Fields highlighted in red in the form are mandatory.

- DNA must have been cleaned up before submission with beads or columns. For elution, H2O, Tris or TE are acceptable.

- Fill in the gDNA **submission excel file** found on our website and email it to us. This must contain all relevant information; no information can be transmitted only within the email text or orally.

- In parallel to submitting the form, please bring DNAs in a clear bag (provided on top of our fridge), labeled with your name, lab PI, short description and date. We recommend storage of high molecular weight genomic DNAs at 4°C, in this case you can hand your samples over to us during regular office hours. For other samples such as amplicons you can directly place the bag in submission drawer of our -20°C. Once we have received both the emailed submission form and the samples, the project enters the queue.

- Bring your samples in **low-bind DNase-free PCR-grade 0.5ml-2ml microtubes**, but not in PCR tubes/strips/PCR plates. Leave some space on the side of tubes for GECF codes.

- Indicated tube **labels** must match exactly the ones on your physical tubes. If available, labels of profiles must also match perfectly either tube labels or sample names.

- The GECF is an open access facility, therefore we cannot guarantee the confidentiality of data. If this is an issue, the easiest solution is to code your samples names. Upon request we can create access-controlled folders for data delivery.

Recommendations for gDNA preparation

- If your project requires sequencing of ultra-high MW DNAs (N50 >50 kb), please contact us in advance so we can discuss upstream how to handle DNA extraction.

- Avoid **bacterial contamination**, for instance during dissection or FACS sorting (when using old contaminated buffers).

- Perform the **RNase treatment** that is often only optional in mainstream DNA extraction kits. RNAs can heavily contaminate gDNA prep and may affect gDNA quantification and other downstream procedures.

- We recommend the **Qubit** fluorometer to determine the mass of the sample. Nanodrop measurements of gDNA concentration may be very inaccurate, in particular at low concentrations (<20 ng/ul). Therefore, always report 260/280 and 260/230 ratios when nanodrop is used. Alternatively, a more accurate and sensitive method is Qubit DNA HS, which is recommended.

- Quantification of ultra-high MW DNAs can be challenging if the sample is viscous. You can first heat the sample 10’ minutes (or more) at 37°C for homogenization, before taking an aliquot for quantification. If

needed, heating at 56°C for 2' and harshly vortexing this aliquot (not the main sample) can help quantification by breaking up ultra-long DNA molecules.

DNA amounts to submit

The minimum amount of DNA that is required depends on the application, but is the same for MinION or PromethION.

The values below are minimal requirements, but larger volumes and higher concentrations are preferred. Indeed, to obtain expected yield, ONT flow cells may require the loading of additional library amount during the run, and we thus may need to start again the library prep. This may specially be the case for lower quality samples.

Genomic DNA (extracted with standard gDNA extraction procedure such as DNeasy)

- Without multiplexing: We need a minimum of **2'500 ng** of DNA, with a concentration in the range of 25–50 ng/µL, ideally **around 50 ng/µL**.

- With multiplexing:

- Minimum 2'500 ng gDNA per sample if using ≤4 barcodes (conc. >95 ng/ul).
- Minimum 1000 ng gDNA per sample if using >4 barcodes (conc. >45 ng/ul).

If the profile analysis indicates the presence of shorter DNA fragments, a size-selection step may be necessary. In that case, a higher DNA input may be required, typically 3–10 µg, depending on the fragment size distribution.

HMW Genomic DNA (extracted with HMW-specific method such as HMW Monarch)

Our typical workflow includes DNA extraction. In such case, we would discuss ahead but the range is around 10mio cells minimum.

If you perform the extraction on your side, a minimum of 30-40ug DNA is required.

cDNA from oligo-dT reverse transcription

Note that we recommend discussing ahead any cDNA sequencing projects.

We assume here a good quality of the cDNAs (large smear with a peak between 1.5 and 2kb).

- Without multiplexing: We need 200-400 fmoles, in maximum 100ul. This is typically 250-500ng cDNA.

- With multiplexing: We need a minimum of 400 fmoles/sample, in maximum 25ul. This is typically 450-500ng cDNA.

Amplicons

- Without multiplexing: We need 250-500 fmoles, in maximum 120ul.

- With multiplexing: We need >500 fmoles/sample, in maximum 30ul.

Direct RNA

- We offer the recently released direct RNA method from ONT. This is especially valuable to detect RNA modifications, or when a PCR-free method is absolutely required. Please inquire before submitting your RNAs.

- Multiplexing is available.

- We need a minimum of 20ug total RNA per sample.

Data handling

- By default we give fastq (and ubam files when relevant) to users but not the raw pod5 files. Let us know in advance if you need them too to generate your own basecalling.

- We store the whole dataset for at least 3 months in a fully backed-up fashion (*note: shorter than for short reads sequencing*), therefore please make sur to also have it secured on your side.

Versions log

- v1.01- v1.02: clarification regarding heating sample for quantification.
- v1.03 (01.09.2025): We don't give raw data by default. We store data for 3 months in a fully backed-up fashion. Mentioned that people can request a lab-specific access-controlled data delivery folder.
- v1.04 (29.01.2026): Removed refs to flongle (discontinued by ONT). Few minor changes. Direct RNA addition.
- v1.05 (08.07.2026): Clarified the DNA/RNA amounts requirements.