guidelines v1.02

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 binding 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.

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.

	Flongle w/o multiplexing	**MinIon or P2 solo w/o multiplexing**	**If multiplexing**
high MW genomic DNA	1200 ng (conc. >25 ng/ul)	2500 ng (conc. >25 ng/ul)	2500 ng gDNA per sample if using ≤4 barcodes (conc. >95 ng/ul) 1000 ng gDNA per sample if using >4 barcodes (conc. >45 ng/ul)
amplicon DNA	125-250 fmol in max 60 ul	250-500 fmol in max 120 ul	500 fmol / sample to be barcoded in max 30 ul

Larger volumes and higher concentrations are preferred, in consideration of the fact that the yield of the library prep is dependent on the sample quality (in particular length of the fragments). Knowing that Nanopore flow cells may require loading of additional library, for instance after flushing, we may need to start again the library prep.

Versions log

- v1.01: initial release.
- v1.02: clarification regarding heating sample for quantification.