Quick Short-Read Submission Guide Version 8.0 (July 2020)

Samples submitted to the facility need to fulfil certain criteria, otherwise they cannot be accepted. Please go through the checklist before you deliver your samples:

General considerations for libraries AND samples:

- Requests with less than 16 samples have to be delivered in 1.5 ml Eppendorf Safe-lock tubes; larger requests (for >16 samples Forskalle will generate pipetting scheme) in 96-well plates. As the plates must be compatible with our automation, we request Users to use BioRad HSP-9601 PCR plates. In case of shortage or time pressure, the facility can provide them.
- Library concentrations shall not exceed 2.5 ng/µl in case of amplicons and small RNAs and 5 ng/µl for samples/libraries with a broader size distribution.
- Sample tubes have to be labelled with the 6-digit sample number or the 4-digit multiplex number for pooled samples generated by Forskalle (Examples: M1234 or 543210). Plates must be labelled exactly as displayed in Forskalle after request creation (sample ID x-sampleID y l requestID plate #, preparation type) For labelling, please use a permanent marker, NO stickers.
- For user prepared libraries: Minimal molarity is 2.5 nM
- Delivery volume is at least 30µl, exception: NovaSeq, see table below.
- Check the cost assignment, Users are responsible for providing the correct invoicing information.
- If you bring your samples/libraries by the lab then put them please in the respective drawer of the designated freezer (libraries, DNA) or hand the samples over to facility personnel (RNA, to be stored at -80).
- If you want to resequence libraries you can add them to your request via the “Pick Old Samples/Multis” button in Forskalle.

Criteria for Facility Preparations:

DNA Samples (genomic DNA or cDNA):

- The minimal acceptable amount of DNA is 1ng. (applies for sheared and unfragmented DNA).
- The minimum concentration is 0.06ng/µl.
- When submitting sheared DNA, make sure that the majority of DNA fragments is already in the approximate size range that your library inserts are supposed to be.

NEB EM-seq: this is now our standard kit for or identification of 5-mC and 5-hmC; minimal requirement is 10ng of unfragmented DNA.

Bisultite Seq: The Zymo pico methyl kit is our low input alternative for sequencing methylated cytosines. We only apply it below 10 ng input amount (unfragmented DNA).

RNA Samples:

We request high quality total RNA (RIN >7 and DV200 >70%, higher quality leads to better results!). Due to discrepancies of quantifications methods for all facility RNA preparations we recommend to target **20ul with concentration of 300 ng/ul**. Absolute minimum amounts and concentration depending on the preparation method are:

Protocol / Kit	**Min. Amount**	**Min. Conc.**
NEB poly-A	400ng	8ng/µl
NEB total RNA	100ng	20ng/µl
rRNA depletion (Ribovanish)	500ng	60ng/µl
Lexogen Quant-Seq	300ng	60ng/µl
Qiagen SmallRNA	200ng	20ng/µl

- DNAse treatment by the user is mandatory for rRNA depletion protocols!
- For first time submissions of ATAC-Seq and Single Cell scRNASeq (smartseq2 and 10X Genomics), please contact Alexander Vogt in order to discuss details: alexander.vogt@vbcf.ac.at

10X Samples:

- Make an appointment with NGS lab to assure Chromium and labstaff is avaiable to procede with your cells. Important: Not just date, but also timeslot in which you want to bring samples.
- Bring your signed request with you to the appointment.
- Minimal concentration 1000 cells/ul and volume of 20ul (so 20000 cells). Bringing more cells is better, but please try a concentration of 1000-2000 cells/ul.
- Cells must have a viability of 90% or higher. In case of cell-suspensions (tissue, organoids etc.) viability >85% is acceptable. cellaggregates should not be higher than 5% (counted on NC 250).
- 10X solutions provided by VBCF NGS (status April 2020):
  - Single Cell Expression

- Single Cell ATAC
- Single Cell Immune Profiling (VDJ)

Criteria for NovaSeq Submissions:

User prepared libraries:

The required amount of library fragments is higher than for HiSeq/NextSeq. You likely need to use a couple of additional cycles when preparing your libraries. Minimal molarity is 2.5nM. Higher concentrated libraries require accordingly less volume.

Minimal volume of 2.5nM Library (µl)			
Flowcell	**SP/S1**	**S2**	**S4**
Standard workflow	50	70	130
Xp workflow (per lane/multiplex)	20	20	30

- We strongly recommend the use of unique dual barcodes in order to avoid index misalignment

- Current Flowcell pricing assumes that we load one multiplex per flowcell. If lanes are loaded individually we need to charge additionally for an XP kit (950 EUR for S4 or 300EUR for SP/S1/S2) which allows us to load each lane individually with a different multiplex.

- Pricing for S2 and S4 lanes include a library test on iSeq 100 instrument which delivers ~4M reads and allows for evaluation of libraries as its flowcells are very similar to the Novaseq ones. For individual loading of lanes, additional iseq tests might be advisable. We strongly recommend such a test for S1 lanes as well but leave it up to users to decide on it (additional fee: 690EUR).

Maximal turnover times for all Illumina services:

- User prepared samples: 3 weeks
- Facility prepared samples: 5 weeks