VBCF SHORT READ SAMPLE SUBMISSION GUIDELINES

BEFORE SEQUENCING WITH US:

- ➔ Create an account in Forskalle (our request submission tool) **https://ngs.vbcf.ac.at/forskalle3/login**
- ➔ Make yourself familiar with the tool, pricing and general guidelines.
- ➔ Set up your sequencing request (specify cost assignment, select sequencing platform, generate samples with IDs, ...). For more detailed information please check out our Forskalle guide.

We are happy to help!
Contact us via
ngs-support@vbcf.ac.at

SAMPLE HANDLING AND INPUT REQUIREMENTS:

- ➔ Samples and libraries shall be delivered in **H_2O** or **low TE buffer**.
  (Alternative buffers might create problems in the following enzymatic steps.)
- ➔ Our offered preparation types require different amounts of input material. Please check out the detailed tables below to submit sufficient amounts! In case of doubt please refer to our NGS protocol guide or contact the facility.
- ➔ Please always provide more input material if available. The requirements given are minimum values.

User-prepared libraries:

- ➔ min. concentration: 2.5 nM
- ➔ min. volume: 30 µl

Vienna BioCenter Core Facilities GmbH, Dr. Bohr-Gasse 3, 1030 Vienna, Austria
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Raiffeisenlandesbank NÖ-Wien AG IBAN: AT87 3200 0000 1027 0429, BIC: RLNWATWW
Firmenbuchnummer FN350396 p, Firmenbuchgericht Handelsgericht Wien, UID ATU65928179

RNA samples:

- ➔ We request high-quality RNA: **RIN >7 and DV200>70%**.
- ➔ Always store RNA samples at -80°C.

Don't dilute RNA input unnecessarily!

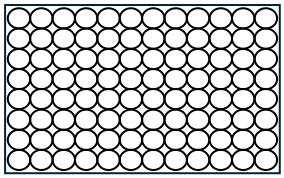
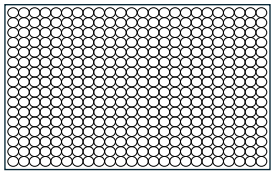
Application	Protocols offered	Sample requirements
mRNA sequencing	**mRNA enrichment**	min. 20µl of min. 10ng/µl total RNA
	with poly-A full transcript	
mRNA sequencing	**Lexogen, Quantseq**	min. 20µl of min. 40ng/µl total RNA
	QuantSeq 3' mRNA only + UMI	
mRNA, lncRNA, sn/snoRNA, non-poly(A) mRNA, cRNA	**rRNA depletion**	min. 20µl of min. 25ng/µl total RNA, DNase treated
	RiboVanish	
total RNA	**no enrichment** (including rRNA etc.)	min. 20µl of min. 20ng/µl total RNA
Low input RNA sequencing	**SMART-Seq v3**	min. 15µl of min. 0.05ng/µl total RNA
	RNA-seq full transcript + 5' UMI	
Low input RNA sequencing	**TM3'seq**	min. 15µl of min. 0.05ng/µl total RNA
	3' mRNA only + UMI	
miRNA	**miRNA Library preparation**	min. 20µl of min. 20ng/µl total RNA, DNase treated, column purified

DNA samples:

Application	Protocols offered	Sample requirements
DNA >800bp	**Enzymatic Shearing (PCR)**	min. 1 ng total DNA (min. conc. 0.05 ng/µl, min 20 ul)
	DNA Library Prep with Enzymatic Shearing	gDNA, 50%>800 bp
Genomic DNA (WGS)	**Enzymatic Shearing PCR-free**	min. 250 ng total DNA (min. conc. 10 ng/µl, min 25)
	DNA Library Prep with Enzymatic Shearing (PCR-free)	gDNA, 50%>1 kb
ChIP – Seq and similar assays	**Ligation**	min. 10 ng total DNA (min. conc. 1ng/µl, 20ul)
	sheared/short DNA input, ligation-based library prep	sheared DNA, 65% in the range 200-800 bp
Purified Amplicon Sequencing	**Barcode amplification NEB/Nextera**	min. 20µl of min. 0.5 ng/µl DNA (10ng)
	Second round of PCR on existing adaptors (NEB) or Nextera	purified amplicons
Metagenomics	**16S/ITS Metagenomics**	min. 20 µl of min. 1 ng/µl DNA (20 ng)
	16S/ITS rRNA Gene Amplicons	gDNA
Whole Genome Bisulfite Sequencing	**EM-seq**	100-500 ng in min. 20 µl
	NEBNext UltraShear with NEBNext Enzymatic Methyl-seq Kit (NEB)	gDNA

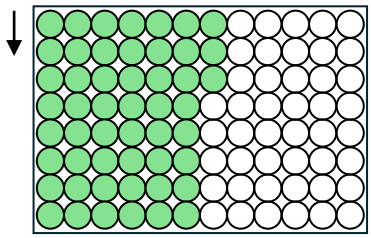
HOW TO SUBMIT THE SAMPLES:

➔ Create the request as the first step, before pipetting the samples!
➔ Depending on the number of samples:

# of samples	1-16 samples	17-94 samples	> 94 samples
container	**1.5ml tubes** safe-lock Eppendorf tube 	**96-well plates** BioRad HSP-9601 	**384-well plates** BioRad HSP3831 
labeling	**6-digit sample ID** (e.g. 200001) or **5-digit Multiplex ID** (e.g. M19123)	**sample IDs (first-last), requestID, plate #, preparation type**	

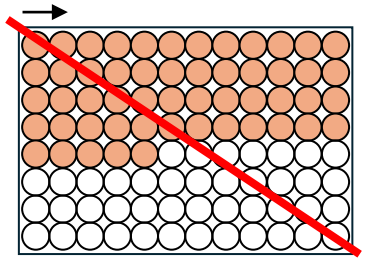
➔ Forskalle will generate a plate layout for sample loading. Please exactly follow these instructions!
➔ For RNA samples: To cover the plate always use alu-seal e.g. adhesive pierceable aluminum foil, **which are compatible with – 80^0 C storage**.

CORRECT



Start from A1, B1, C1, …

WRONG!



don't fill from A1, A2, A3, …

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GENERAL CONSIDERATIONS:

- If you bring your samples/libraries to the lab, please put them in the respective drawer of the designated freezer (libraries, DNA). There is a fridge accessible close to the reception anytime during the day. Or you can hand the samples over personally to facility staff (RNA, to be stored at -80). Our opening hours are Monday to Friday from 9.00-16.00.
- shipping address:
  VBCF/NGS, Vienna Biocenter 6, Dr.-Bohr-Gasse 7, 1030 Vienna
- To avoid RNA degradation, RNA samples must be shipped on dry ice.
- Always provide the signed request sheet together with the correctly labeled samples. Unlabeled or mislabeled samples might be rejected or even trashed, if the origin is not traceable.
- Check the cost assignment, Users are responsible for providing the correct invoicing information. University users must provide a valid cost center or funding assignment.
- If you want to re-sequence libraries, you can add them to your request via the "Pick Old Samples/Multis" button in Forskalle.
- Contact the facility upfront if you already know that your samples won't fulfill any of the here listed criteria.
- Be aware that mixing different types of libraries can impact quality.
  - For example, 10X libraries will propagate low qualities after cycle 28 in read 1 to other libraries in the same lane due to low complexity. We do not recommend to sequence more than 25% 10X libraries in a shared lane with non-10X libraries.
  - Pooling libraries with different fragment sizes might lead to imbalanced read output ratios since shorter fragments cluster more efficiently (especially on patterned flowcells). You can account for the differential clustering efficiencies by adjusting your pooling ratios or submitting the individual libraries and letting the facility pool.
- We recommend unique dual indices for pooling libraries. **For shared lanes or spike-ins the use of UDI and declaring them in Forskalle is mandatory!**
- If you plan to use two or more platforms (e.g. Illumina and PacBio) with the same samples, you are requested to deliver individual aliquots for each of them and register them as individual samples/requests in Forskalle. Take the different requirements and recommendations for each platform into account (e.g. storage conditions).

Important: We **only accept BSL-1** samples because our facility does not have the certified containment, validated SOPs, or licensed personnel required to handle samples of higher biosafety levels safely. Any samples above BSL-1, including BSL-1+, material will be refused on arrival — this policy is final and non-negotiable.

MAXIMAL TURNOVER TIMES FOR ILLUMINA SERVICES*:

User prepared samples: **3 weeks**

Facility prepared samples: **5 weeks**

*** Turnover time might be prolonged for shared lanes due to required barcode compatibility**

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