VBCF LONG 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 shall be delivered in **low TE buffer**. If the samples are in a different buffer, enter the used buffer in Forskalle and provide an aliquot.
- ➔ Please **do not** subject the DNA to **multiple freezing/thawing cycles**. Keep it at 4°C!! **Don't store high molecular weight DNA at -20°C!**
- ➔ Sample purity: pure DNA has an A260/280 ratio of 1.8 and an A260/230 ratio of 2-2.2 or higher.
- ➔ For DNA extraction we can recommend the Monarch HMW DNA Extraction kit for tissue or for cells & blood (NEB).

DNA samples:

Application	Protocol/Preparation kit	Minimal input
PacBio- De novo genome assembly	Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0	**5-8µg**
PacBio gDNA Sequencing	Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0	**1µg**
PacBio- De novo genome assembly w/ ultralow input (Ampli-Fi)	Amplifying genomic DNA for SMRTbell library preparation and HiFi sequencing	**1-30ng**
PacBio Microbial Multilexing	Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0	**1µg** for de novo assembly; **15µg** for variant detection: **3µg** for structural variant detection
PacBio Amplicon Sequencing	Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0	**300ng-1µg** per sample
ONT Whole Genome Sequencing using Ligation (single sample)	Ligation Sequencing gDNA V14	**1-2µg**
ONT Whole Genome Sequencing using Ligation & Multiplexing (multiple samples)	Ligation Sequencing gDNA - Native Barcoding Kit V14	**400ng-1µg** depending on the number of multiplexed samples
ONT Rapid DNA Sequencing	Rapid Sequencing DNA V14 with Barcoding	**200ng** per sample
ONT Rapid DNA Sequencing - 16S	Rapid sequencing DNA - 16S Barcoding Kit V14	**10ng** HMW DNA

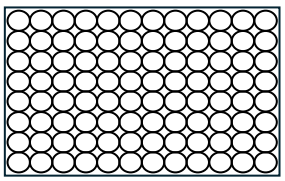
RNA samples:

- ➔ We request high quality total RNA with RIN >7.
- ➔ DNase treatment is strongly recommended.

Application	Protocol/Preparation kit	Minimal input
PacBio RNA Iso-seq sequencing	Preparing Iso-Seq libraries using SMRTbell prep kit 3.0	**>300 ng** per library in max. 7µl ; RIN >7
PacBio RNA sequencing using Kinnex kit	Preparing Kinnex libraries using the Kinnex full-length RNA kit	**300 ng** per library (min. conc. 43ng/µl per library); RIN >7
ONT direct RNA Sequencing	Direct RNA sequencing kit	**300ng** of poly(A)RNA or **1-2µg** of total RNA in 8µl
ONT RNA sequencing using cDNA (PCR barcoding)	cDNA-PCR Sequencing V14 - Barcoding	**10ng** poly(A) RNA or **500ng** of total RNA per sample

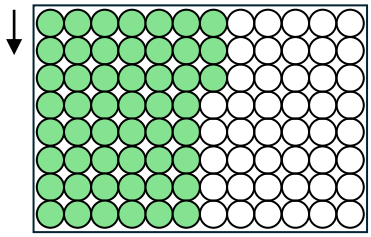
HOW TO SUBMIT THE SAMPLES:

- ➔ Depending on the number of samples:

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

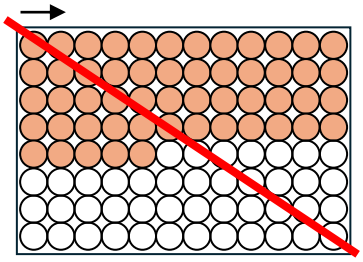
- ➔ Forskalle will generate a plate layout with sample loading. Please exactly follow these instructions!

CORRECT



Start from A1, B1, C1, ...

WRONG!



don't fill from A1, A2, A3, ...

INSTRUMENTS AND TECHNICAL INFORMATION:

Pacific Biosciences:

	REVIO
SMRT cell type	25M Cell
Pol. Read Number	12-18M
gDNA library	
av. Pol read length HiFi mode	70+ Kb
expected output HiFi mode	60-100Gb
Amplicons/Iso-Seq libraries	
av. Pol read length	14-17Kb
Number HiFi reads	7.5M

Oxford Nanopore:

	MINION	**PROMETHION**
gDNA library		
av. read length	10-30Kb	10-30Kb
expected output	up to 20-30Gb	30-120Gb
cDNA/ amplicons		
av. read length	1-10Kb	1-10Kb
number of reads	1-10M	40-70M
direct RNA		
av. read length	1-2Kb	1-2Kb
number of reads	up to 3M	up to 3M

Depending on the input final output and read length may vary from sample to sample.

GENERAL CONSIDERATIONS:

- If you bring your samples/libraries to the lab, please put them in the respective box of the designated fridge (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 from 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 fulfil any of the here listed criteria.
- 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.