Creating an order in B-Fabric for Illumina / Short Read Sequencing:
Ready-made Libraries

Important:	Please precisely follow these instructions when submitting **ready-made libraries**. If you are submitting **RNA or DNA samples for library preparation,** please select the Service Type "**High Throughput Sequencing (NGS)**" instead. If you are submitting **Single-Cell Suspensions for library preparation,** please select the Service Type "**Single Cell Sequencing**" instead. If you are submitting **10x Ready-made libraries,** please also have a look at the additional instructions found **here**. For **10x Visium Ready-made libraries** more information can be found **here**.

1. Minimum order is 1 physically separated lane. *(1 lane of the NovaSeq for SP, S1, S2 and half an S4 flowcell).*
2. Pool samples together that will be sequenced on the same lane. Please use 1.5 ml Eppendorf tubes.
3. Dilute your samples to 10nM or an agreed upon concentration. We still need to QC and dilute your pooled libraries properly. We have to start with 10nM for the best results.

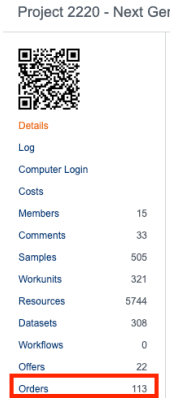
   At the end we will need 3nM of your pooled libraries and use the following volumes:

SP/S1	150ul
S2	200ul
S4	400ul

1.1) Order submission: step-by-step guide: Ready-made libraries

1. Please login to your project main page on B-Fabric (https://fgcz-bfabric.uzh.ch/bfabric/). If you have more than one project at the FGCZ, please make sure you are on the correct project page.

2. Click on the "Orders" button listed on the left part of the screen:

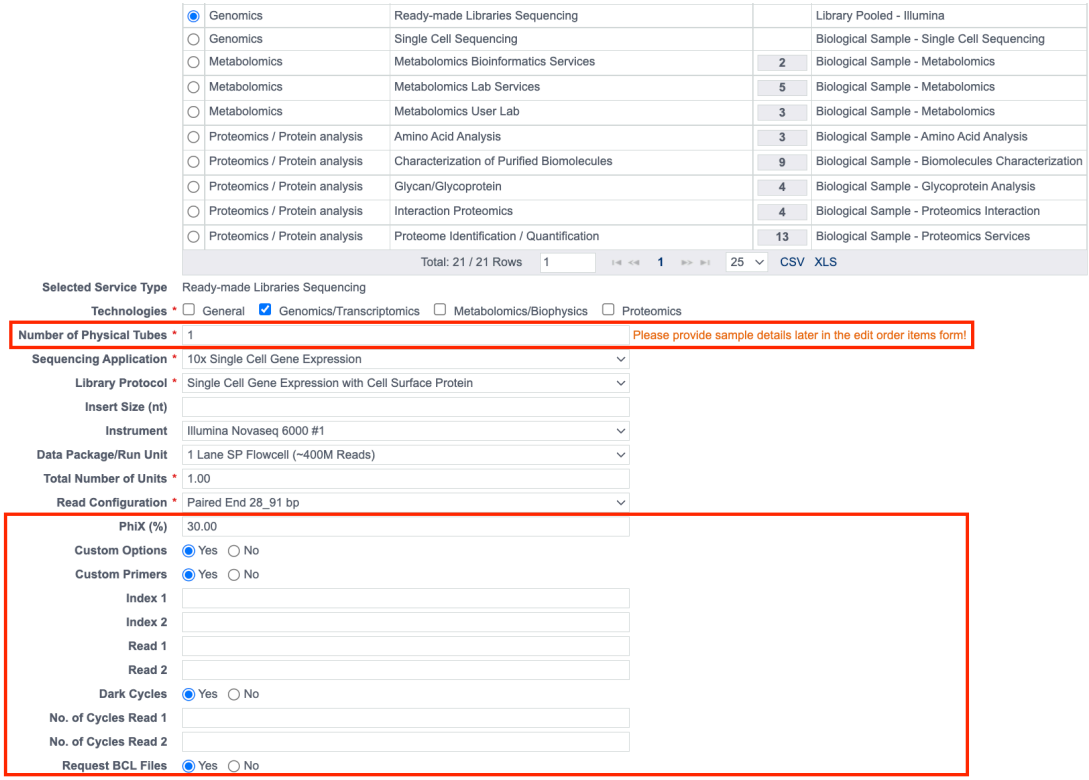


3. Click on the "Create Order" button.

4. Under “Service Area”, click on “**Ready-made Libraries Sequencing”** and fill up the remaining form (Sequencing Application, Library Protocol, Instrument, Data Package/Run Unit, Read Configuration etc.) as best you can. If you indicate “*I don’t know*”, your coach will get in touch with you to clarify your order.

Important: When creating an order please select the **number of physical tubes** that you will deliver. This corresponds to the **number of pools** you have. If you have one pool select 1, if you have two pools select 2 etc. (see figure below).

Each pool will get a **separate Tube ID** that you will have to use for the labelling later on.



◉	Genomics	Ready-made Libraries Sequencing		Library Pooled - Illumina
○	Genomics	Single Cell Sequencing		Biological Sample - Single Cell Sequencing
○	Metabolomics	Metabolomics Bioinformatics Services	2	Biological Sample - Metabolomics
○	Metabolomics	Metabolomics Lab Services	5	Biological Sample - Metabolomics
○	Metabolomics	Metabolomics User Lab	3	Biological Sample - Metabolomics
○	Proteomics / Protein analysis	Amino Acid Analysis	3	Biological Sample - Amino Acid Analysis
○	Proteomics / Protein analysis	Characterization of Purified Biomolecules	9	Biological Sample - Biomolecules Characterization
○	Proteomics / Protein analysis	Glycan/Glycoprotein	4	Biological Sample - Glycoprotein Analysis
○	Proteomics / Protein analysis	Interaction Proteomics	4	Biological Sample - Proteomics Interaction
○	Proteomics / Protein analysis	Proteome Identification / Quantification	13	Biological Sample - Proteomics Services

Total: 21 / 21 Rows 1 1 25 CSV XLS

Selected Service Type	Ready-made Libraries Sequencing
Technologies *	☐ General ☑ Genomics/Transcriptomics ☐ Metabolomics/Biophysics ☐ Proteomics
Number of Physical Tubes *	1 — Please provide sample details later in the edit order items form!
Sequencing Application *	10x Single Cell Gene Expression
Library Protocol *	Single Cell Gene Expression with Cell Surface Protein
Insert Size (nt)	
Instrument	Illumina Novaseq 6000 #1
Data Package/Run Unit	1 Lane SP Flowcell (~400M Reads)
Total Number of Units *	1.00
Read Configuration *	Paired End 28_91 bp
PhiX (%)	30.00
Custom Options	◉ Yes ○ No
Custom Primers	◉ Yes ○ No
Index 1	
Index 2	
Read 1	
Read 2	
Dark Cycles	◉ Yes ○ No
No. of Cycles Read 1	
No. of Cycles Read 2	
Request BCL Files	◉ Yes ○ No

You have the option to define the percentage of **PhiX (%)** that should be spiked in for the sequencing. This field is non-mandatory and can be left blank.

Furthermore, you have the possibility to define custom options for the sequencing of your ready-made libraries (see figure above: **Custom Primers, Dark Cycles, Request BCL Files**). Please only select custom options if you know what these parameters imply. If in doubt, please contact your coach.

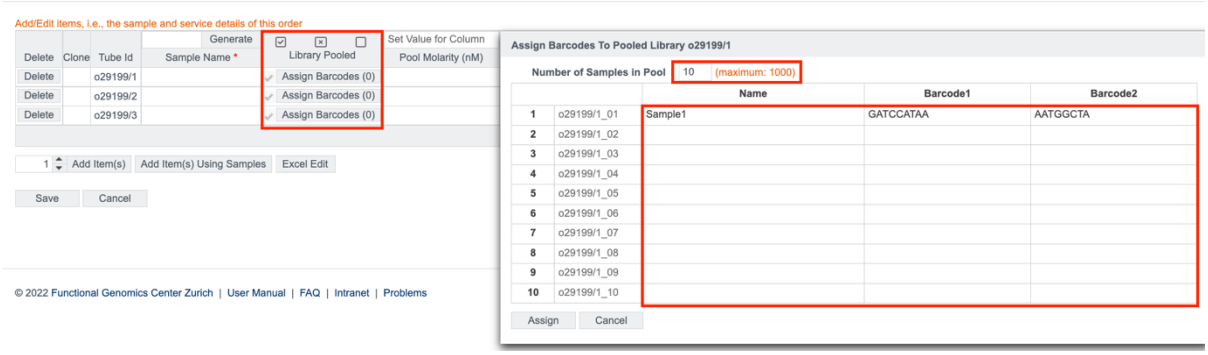
Any further special instruction which cannot be defined under custom options should be put in the “Remarks” field.

Important: Please fill up the form as best as possible by following the instructions in the section above. **We will rely on the information you provide in sequencing your libraries.** Please do not assume that we know how to sequence your libraries. We really prefer for you to be as specific as possible.

5. Check if the **billing address** is correct. Please note that ETH/UZH users will be required to enter their fund number for invoicing. If not sure about the fund number please ask your group leader. Click on **Agree** and **Save**.

6. A table will open with fields for the **number of physical tubes / pools** you have chosen previously.

7. Click on "**Assign Barcodes**" and define the number of samples in each pool (see figure below). Please carefully read the section on the next page about barcode assignment.



8. **Important: For each sample in the pool the barcode sequences have to be assigned** (Barcode1 = i7. Barcode2 = i5). Please be aware that only unique barcodes / barcode combinations are accepted in a given pool. You can also define the sample names in the column on the left. It is possible to copy and paste the barcodes and sample names directly from a table.

   Only the actual barcode sequences are accepted! Do not use any barcode names as the demultiplexing will not work. An exception are 10x ready-made libraries (e.g. SI-TT-A9). Please contact your coach if in doubt.

   Some Illumina sequencers read Barcode2 differently. We automatically correct the barcodes for the different mode. **ALWAYS** provide Barcode2 which is specified for **NovaSeq,MiSeq,HiSeq 2000/2500** (even in the case that your libraries are running on a different instrument). Please have a look at the figure below.

   For a more detailed guide about indexing refer to the following file:
   Which barcode sequences should I write?

IDT for Illumina–TruSeq DNA and RNA UD Indexes

These unique dual (UD) index adapters are arranged in the plate to enforce the recommended pairing strategy.

▶ Adapter Trimming

▼ Index Adapters

Index 1 (i7) Adapters

GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[i7]ATCTCGTATGCCGTCTTCTGCTTG

Index 2 (i5) Adapters

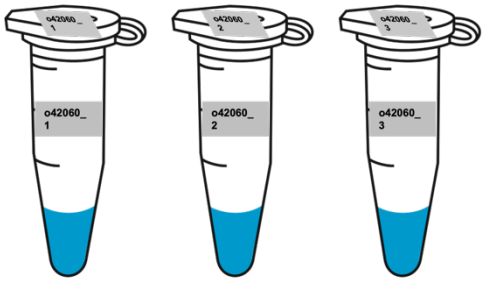
AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT

Barcode1 Barcode2

Index Name	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases in Adapter	i5 Bases for Sample Sheet NovaSeq 6000 with v1.0 reagent kits, MiSeq, HiSeq 2000/2500, NextSeq 2000 (Sample Sheet v2)	i5 Bases for Sample Sheet iSeq, NovaSeq 6000 with v1.5 reagent kits, MiniSeq, NextSeq 500/550, HiSeq 3000/4000/X, NextSeq 2000 (Sample Sheet v1)
UDI0001	CCGCGGTT	CCGCGGTT	AGCGCTAG	AGCGCTAG	CTAGCGCT
UDI0002	TTATAACC	TTATAACC	GATATCGA	GATATCGA	TCGATATC
UDI0003	GGACTTGG	GGACTTGG	CGCAGACG	CGCAGACG	CGTCTGCG
UDI0004	AAGTCCAA	AAGTCCAA	TATGAGTA	TATGAGTA	TACTCATA
UDI0005	ATCCACTG	ATCCACTG	AGGTGCGT	AGGTGCGT	ACGCACCT
UDI0006	GCTTGTCA	GCTTGTCA	GAACATAC	GAACATAC	GTATGTTC
UDI0007	CAAGCTAG	CAAGCTAG	ACATAGCG	ACATAGCG	CGCTATGT
UDI0008	TGGATCGA	TGGATCGA	GTGCGATA	GTGCGATA	TATCGCAC

9. Enter the **Sample Names** of your pools and optionally also the **Pool Molarity [nM]**.

10. Please leave the **Tube Id** unchanged and use these numbers (Order ID, e.g. o26381/1) when labeling your tubes. Please label your tubes on **top and on the side** with the Tube ID.



11. Click on "Save"

12. Click on "Submit Order" button on the bottom of the page.

13. Your project coach will check the order, accept the order and will add an offer based on your order. Please check the offer and if everything is in order, please bring the samples together with the signed offer and confirmation form.

    Once you are at the door of the FGCZ you can call the **Genomics Sample Delivery number (reachable from 09:00-17:00)**.

    Do not bring the samples if your order has not been accepted by your coach!

    IMPORTANT: Once you bring or sent the samples, we will process the samples according to the order details accepted by your coach in Bfabric. We will only refer to the order form on the offer and NOT look at any off-Bfabric communication you had with any staff member.

14. The signed confirmation form means that you agree to our terms and conditions.

15. You will receive status updates every time a milestone is reached in the processing of your order. The current state can always be checked on the order page.

Sequencing Output [Disclaimer]: Please note that we cannot guarantee the exact read numbers per sample but do our best to provide the requested amount of data.

1.2) Order submission: additional instructions for 10x Ready-made libraries

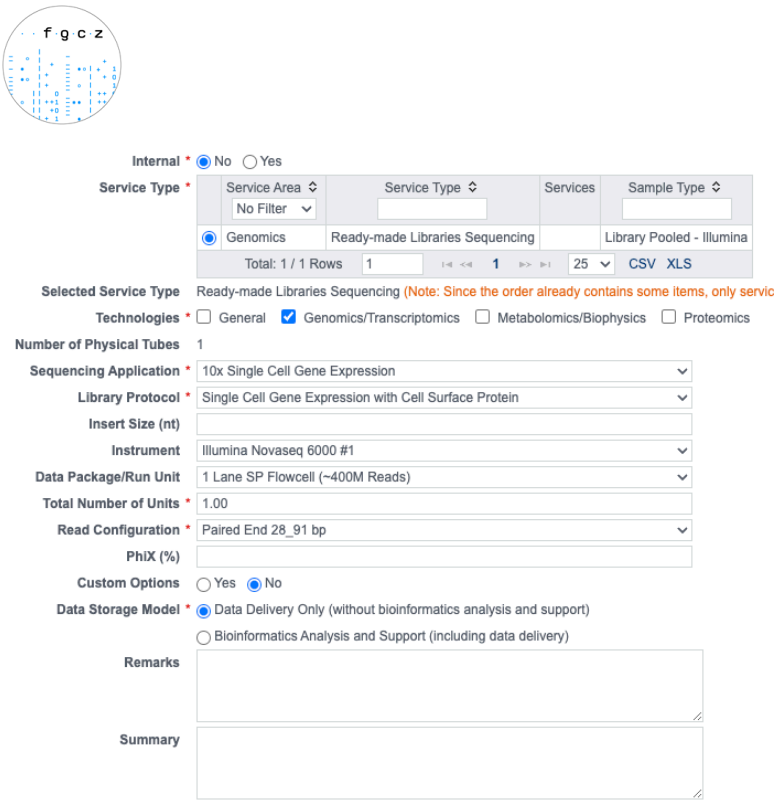
Only pre-pooled libraries can be submitted for sequencing at FGCZ. In B-fabric create an order as described in the previous section. Choose the corresponding **Sequencing Application** (e.g. 10x Single Cell Gene Expression) and **Library Protocol** (e.g. Single Cell Gene Expression with Cell Surface Protein) in the drop-down menu.

Calculate how many reads you need according to the recommendations of sequencing depth from 10x, e.g.:

Sequencing Parameters	
Library	**Sequencing Depth**
Gene Expression	Minimum 20,000 read pairs per cell
Cell Multiplexing	Minimum 5,000 read pairs per cell (Minimum required Read 2 length is 15 bp)
CRISPR Screening	Minimum 5,000 read pairs per cell (Minimum required Read 2 length is 70 bp)
Cell Surface Protein	Minimum 5,000 read pairs per cell (Minimum required Read 2 length is 25 bp)
Sequencing Type	
Paired-end, dual indexing	
Sequencing Read	Recommended Number of Cycles
Read 1	28 cycles
i7 Index	10 cycles
i5 Index	10 cycles
Read 2	90 cycles

Afterwards choose the corresponding **Sequencing Instrument** and **Flow Cell** (e.g. NovaSeq 6000, 1 Lane SP Flowcell).

Choose "*10x Universal Paired End (28_91 bp)*" as **Read Configuration**, which is suitable for both single and dual index libraries.



If you want to pool libraries together for sequencing, make sure each library has unique indexes and pool them according to 10x recommendations, e.g:

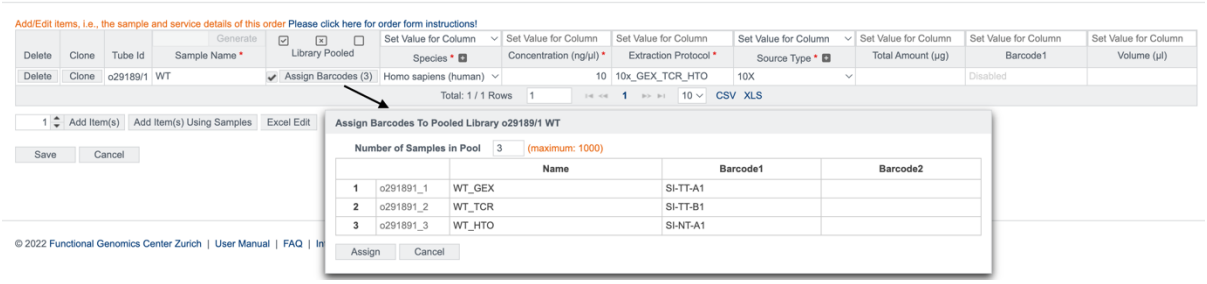
Libraries	Sequencing Depth (read pairs per cell)	Library Pooling Ratio
3' Gene Expression library	20,000	4
Cell Surface Protein	5,000	1
Cell Multiplexing library	5,000	1

10x Genomics does not support sequencing single index and dual index libraries together in the same lane.

Please add the library type after your sample name and follow the terminology below:

Library Type	Abbreviations
Gene expression	GEX
Antibody-derived tag	ADT
Hashtag oligonucleotide	HTO
Cell multiplexing oligo	CMO
B cell receptor	BCR
T cell recepter	TCR

Put the **10x index name** (e.g., SI-TT-A1) for each library clearly in the "*Barcode 1*" column (see figure below):



If your libraries are **stained with surface antibodies or multiplexed**, please also provide us the information of the oligos conjugated with surface proteins, hashtags or lipid tags via excel document attached in the comments section. We need this information to run the Cellranger multi app.

Antibody staining:

Antibody Description	Sequence
anti-human CD86	GTCTTTGTCAGTGCA
anti-human CD274 (B7-H1, PD-L1)	GTTGTCCGACAATAC

Hashtag:

Sample	Hashtag Name	Sequence
o29189_WT	TotalSeq™-C0251 anti-human Hashtag 1 Antibody	GTCAACTCTTTAGCG
o29189_KO	TotalSeq™-C0252 anti-human Hashtag 2 Antibody	TGATGGCCTATTGGG

Lipid tag:

Sample	CMO Name	Sequence
o29189_WT	CMO301	ATGAGGAATTCCTGC
o29189_KO	CMO302	CATGCCAATAGAGCG

1.2) Order submission: additional instructions for 10x Visium Ready-made libraries

Only pre-pooled libraries can be submitted for sequencing at FGCZ. Please consider the number of spots covered by each of your sample sections on the Visium slide, since it affects the required minimum number of reads and therefore the ratio for pooling your sample libraries. The required minimum number of reads differs for libraries from FFPE and fresh frozen tissue. Please see the following instructions for details on how to calculate the number of covered spots and the minimum reads required for your samples.

FFPE: Sequencing Visium FFPE - Official 10x Genomics Support

Fresh frozen: Sequencing Visium Fresh Frozen - Official 10x Genomics Support

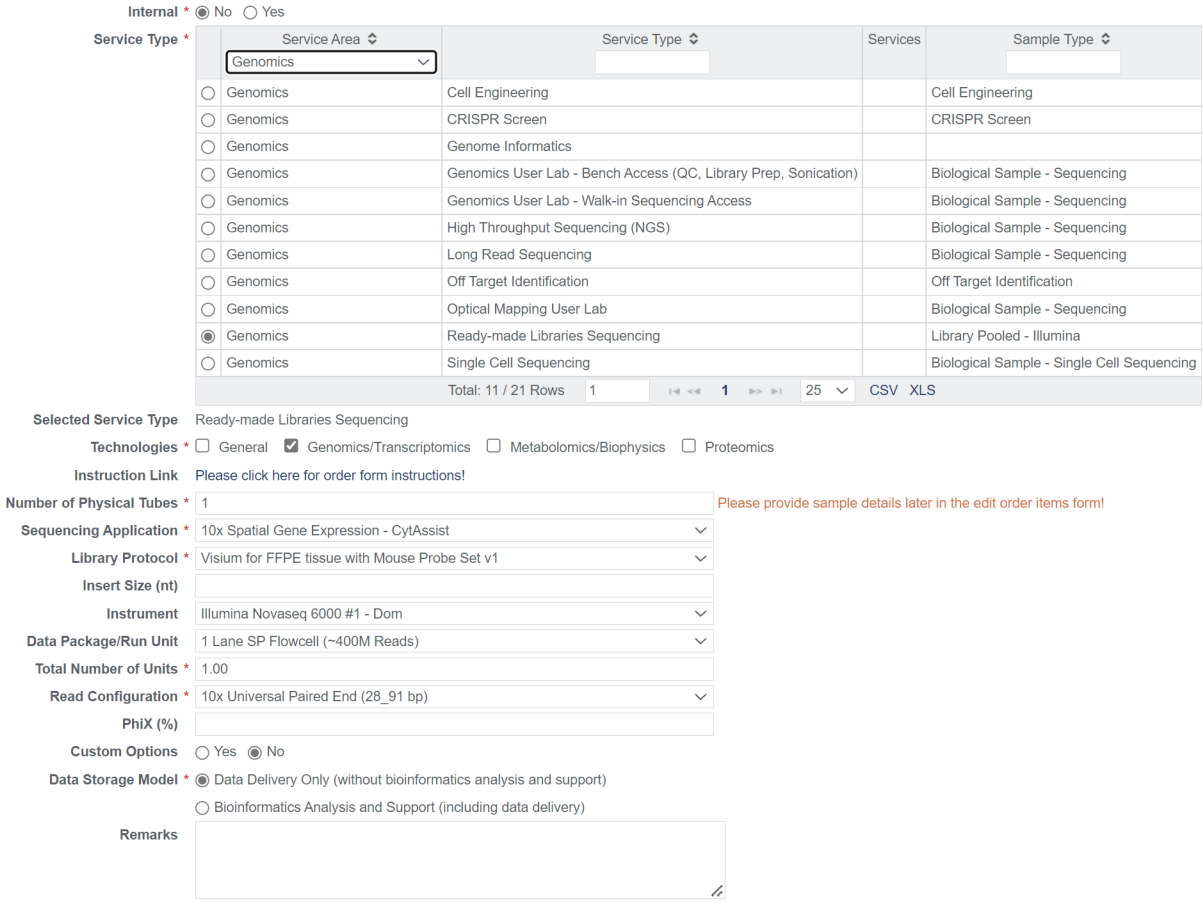
Once you have prepared your pool and calculated the minimum required number of reads, please consider our sequencing offers (s. table 1) to choose the right sequencing option. If you have problems to decide, which sequencing option is the best, you can select "I don't know, please suggest the best option" as an option for the 'Instrument' during order creation.

Table 1: Suitable sequencing options for 10x Genomics Visium libraries at FGCZ.

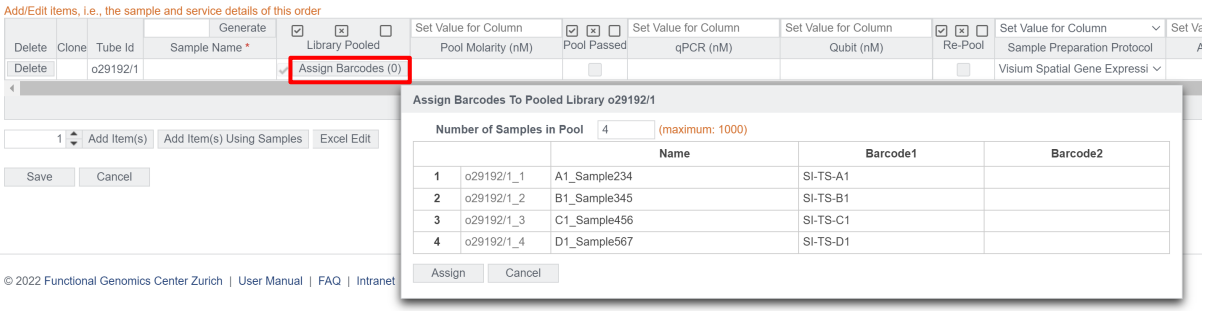
Sequencing Options	**Expected Read Count**	**Required volume of 10 nM pool**
Novaseq SP 100cycles 1 Lane	400 Million	25 µl
Novaseq SP 100cycles FC	800 Million	50 µl
Novaseq S1 100cycles FC	1.4 Billion	50 µl
Novaseq S2 100cycles FC	3.4 Billion	70 µl
Novaseq S4 100cycles FC	8.0 Billion	150 µl

Once you have decided for the best sequencing options, please follow this step-by-step instruction for creating an order in B-fabric. Please also have a look at the more detailed list at the beginning of this guide in case anything is unclear.

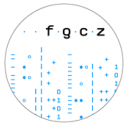
1) Please login to your project main page on B-Fabric (https://fgcz-bfabric.uzh.ch/bfabric/). If you have more than one project at the FGCZ, please make sure you are on the correct project page.
2) Click on the "Orders" button listed on the left part of the screen:
3) Click on the "Create Order" button.
4) Under "Service Area" select "Genomics" and choose "Ready-made Libraries Sequencing" as a Service Type



5) Number of Physical Tubes will be the number of pools, which you want to submit. This is **not** the number of samples in the pool.
6) For “Sequencing Application” select “10x Spatial Gene Expression” with or without CytAssist depending on the workflow you used for your samples
7) Specify in the “Library Protocol” option which specific protocol you used for library generation
8) Select the “Instrument” and “Run Unit” according to your calculations for the pool’s read requirements
9) For “Read Configuration” select “**10x Universal Paired End (28_91 bp)**” for either library protocol
10) 10x Genomics recommends to use 1% PhiX as a sequencing run control. Unless specified otherwise, this is what will be used for your run.
11) Once you have entered all the required information, you can click on “Save” at the bottom of the page
12) For each of your pools, you can click on “Assign Barcodes”, which opens a pop-up window to enter the sample information of your pool. Adjust the “Number of Samples in Pool” accordingly and fill out the “Name” and enter the index barcode as specified by 10x Genomics as “Barcode1” (For FF e.g.: SI-TT-A1; for FFPE e.g.: SI-TS-A1). Please note, that only samples with unique barcodes can be pooled.



13) Click on “Assign” and then on “Save”
14) Once you have reviewed your order, you can click on the green “Submit Order” button
15) Please label the tube with your pool on the lid as well as on the side with the tube Id (in our example: o29192/1
16) After your coach accepted the order, you can bring your pool together with the signed order conformation form to FGCZ
17) If you want bioinformatics support, please upload the corresponding image files for your order according to the instructions in the Wiki and fill out the Excel-template, which you can download there: https://fgcz-intranet.uzh.ch/tiki-index.php?page=10X_Visium_UserInfos



Revision History

Version	Date	Description of Change
10.5.2	05.01.2023	Added additional instructions for 10x Visium. Changed naming of read configuration to "*10x Universal Paired End (28_91 bp)*" for Single Cell Seq.
10.5.3	19.07.2023	Minor changes to "10x Ready-made libraries" section