

## How to submit samples for Illumina/Short Read Sequencing (FGCZv10.4)

### Creating an order in B-Fabric for HT Sequencing/Short Read Sequencing: RNA and DNA Samples

**Important:** Please precisely follow the instructions below when submitting RNA or DNA samples for library preparation.  
If you are submitting **ready-made libraries** please follow the instructions [here](#) instead.

**Updates:** There is the possibility to copy and paste attributes from an Excel-Spreadsheet now during the order submission. Please refer to the section found [here](#).  
  
Samples can now be delivered either in tubes or in plates. **When delivering samples in plates please follow the instructions found [here](#).**

#### RNA samples

1. We need 300ng-2ug for standard library prep. Please check our application page for the requirements for the specific protocols <https://fgcz-intranet.uzh.ch/FullServiceSequencing>  
Dilute the samples to the same concentration. We prefer 100ng/ul, the lowest possible concentration is 2ng/ul for standard library prep. If this is not possible, please dilute to the highest concentration possible compared to your other samples.  
For low input RNA samples, please check with your coach as this will depend on the library prep kit.
2. Please dilute your samples in water or EB buffer.
3. Please use 1.5 ml Eppendorf tubes. **For large orders we encourage you to submit your samples in plates. The following plates (or similar) should be used:**
  - Eppendorf twin.tec®-PCR-Platte 96 LoBind semi-skirted or skirted
  - BioRad Hard-Shell® 96-Well PCR Plates

#### DNA samples


1. We need 100ng-500ng for standard library prep. Please check our application page for the requirements for the specific protocols <https://fgcz-intranet.uzh.ch/FullServiceSequencing>  
Dilute the samples to the same concentration. We prefer 100ng/ul, the lowest possible concentration is 2ng/ul for standard library prep. If this is not possible, please dilute to the highest concentration possible compared to your other samples.  
For low input DNA samples, please check with your coach as this will depend on the library prep kit.
2. Please dilute your samples in water or EB buffer.
3. Please use 1.5 ml Eppendorf tubes. **For large orders we encourage you to submit your samples in plates. The following plates (or similar) should be used:**
  - Eppendorf twin.tec®-PCR-Platte 96 LoBind semi-skirted or skirted
  - BioRad Hard-Shell® 96-Well PCR Plates
4. Please run your samples in a gel and attach the picture in the comments section of the order.



### 1.1) Order submission: step-by-step guide: **samples delivered in TUBES**

1. Please login to your project main page on B-Fabric. 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:

Project 2220 - Next Ger



<a href="#">Details</a>	
<a href="#">Log</a>	
<a href="#">Computer Login</a>	
<a href="#">Costs</a>	
<a href="#">Members</a>	15
<a href="#">Comments</a>	33
<a href="#">Samples</a>	505
<a href="#">Workunits</a>	321
<a href="#">Resources</a>	5744
<a href="#">Datasets</a>	308
<a href="#">Workflows</a>	0
<a href="#">Offers</a>	22
<a href="#">Orders</a>	113

3. Click on the “Create Order” button.
4. Under “**Service Area**”, click on “**High Throughput Sequencing**”.  
and fill up the form (select application, instrument, library protocol etc.) as best you can. If you indicate “*I don’t know*”, your coach will get in touch with you to clarify your order.

Select the option “**Tube**” for the Sample Submission and indicate the number of physical tubes you want to deliver.

Create Order

Service Type *	Service Area	Service Type
	No Filter	
	<input type="radio"/> Proteomics / Protein analysis	Amino Acid Analysis
	<input type="radio"/> Metabolomics (REFINE 2020)	Biophysics Lab Services
	<input type="radio"/> Metabolomics (REFINE 2020)	Biophysics User Lab
	<input type="radio"/> Genomics	Genome Informatics
	<input type="radio"/> Genomics	Genomics User Lab - Bench Access (QC, Library Prep, Sonication)
	<input type="radio"/> Genomics	Genomics User Lab - Walk-in Sequencing Access
	<input type="radio"/> Proteomics / Protein analysis	Glycan/Glycoprotein
	<input checked="" type="radio"/> Genomics	High Throughput Sequencing (NGS)
	<input type="radio"/> Proteomics / Protein analysis	Intact Mass Determination
	<input type="radio"/> Genomics	Long Read Sequencing
	Total: 18 / 18 Rows 1 2 10 CSV	

**required**

Selected Service Type High Throughput Sequencing (NGS)

Instruction Link [Please click here for order form instructions!](#)

Sequencing Application \* Whole Genome Sequencing

Library Protocol \* NEB Ultra II

Number of Samples \* 2 Please provide sample details later in the edit order items form!

Insert Size (nt)

Instrument Illumina Novaseq 6000

Data Package/Run Unit \* Data Package (~200M Reads)

Total Number of Units \* 1

Read Configuration \* Paired End 150 bp

Data Storage Model \* ☒ Data Delivery Only (without bioinformatics analysis and support)  
☐ Bioinformatics Analysis and Support (including data delivery)

Remarks

Note: Please indicate any special instruction in the “Remarks” field.

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**.



- A table will open with fields for the number of samples you have chosen. If you have more samples, add any number of the fields by using the “Add Item(s)” option. Would you like to add already existing samples from previous orders within the same project, please click on “Add Item(s) Using Samples.”

Edit Order Items

Delete	Tube Id	Sample Name *	Species *	Concentration (ng/μl) *	Extraction Protocol *	Source Type *	Barcode/Index	Total Amount (μg)	Volume (μl)	Description
Delete	p2220_6155/1	abc	Mus musculus (house m	100	custom	Genomic DNA		10000	100	
Delete	p2220_6155/2	gzt	Mus musculus (house m	100	custom	Genomic DNA		10000	100	
Total: 2 / 2 Rows										
1 Add Item(s) Add Item(s) Using Samples Create Annotation										
Save Cancel										

- Enter the information about your samples like **Sample Name**, **Species**, **Concentration**, **Extraction Protocol** etc.

### Update: Assign sample attributes by copy and paste from a local spreadsheet

If you would like to assign sample attributes in bulk, you may copy/paste values from a local spreadsheet (see below):

Add/Edit Items, i.e., the sample and service details of this order Please

Delete	Clone	Tube Id	Sample Name *	Species *
Delete		p2220_26384/1	Select Item	Select Item
Delete		p2220_26384/2	Select Item	Select Item
Delete		p2220_26384/3	Select Item	Select Item
Delete		p2220_26384/4	Select Item	Select Item
Delete		p2220_26384/5	Select Item	Select Item
Delete		p2220_26384/6	Select Item	Select Item
Delete		p2220_26384/7	Select Item	Select Item
Delete		p2220_26384/8	Select Item	Select Item
Delete		p2220_26384/9	Select Item	Select Item
Delete		p2220_26384/10	Select Item	Select Item

1 Add Item(s) Add Item(s) Using Samples **Excel Edit**

Save Cancel

Edit Sample Details

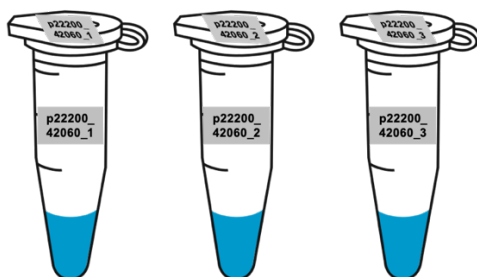
	Tube Id	Name	Species	Concentration (ng/μl)	Extraction Protocol	Source Type
1	p2220_26384/1					
2	p2220_26384/2					
3	p2220_26384/3					
4	p2220_26384/4					
5	p2220_26384/5					
6	p2220_26384/6					
7	p2220_26384/7					
8	p2220_26384/8					
9	p2220_26384/9					
10	p2220_26384/10					
11	p2220_26384/11					
12	p2220_26384/12					
13	p2220_26384/13					
14	p2220_26384/14					
15	p2220_26384/15					
16	p2220_26384/16					

Apply Cancel

After clicking on “Excel Edit”, a pop-up window will open. You must then copy the attributes from your local spreadsheet, navigate to the pop-up window, and press Ctrl + V to assign your values directly to the samples.

The pop-up table works very similar to Excel and can be auto-filled the same way.

- Please leave the **Tube Id** unchanged and use these numbers (project ID + Order ID) when labeling your tubes. Please label your tubes on **top and on the side** with the Tube ID.



- Click on “Save”



10. Click on “Submit Order” button on the bottom of the page.
11. 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.

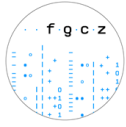
Once you are at the door of the FGCZ you can call the **Genomics Sample Delivery number (reachable from 09:00-17:00)**.

**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.**

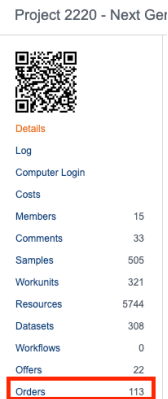
12. The signed confirmation form means that you agree to our terms and conditions.
13. You will receive status updates every time a milestone is reached in the processing of your order. The current state can always be checked on the order page.

**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: step-by-step guide: **samples delivered in PLATES**

1. Please login to your project main page on B-Fabric. 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 “**High Throughput Sequencing**” and fill up the form (select application, instrument, library protocol etc.) as best you can. If you indicate “*I don’t know*”, your coach will get in touch with you to clarify your order.

Select the option “**Plate**” for the Sample Submission (see below) and indicate the number of physical plates you want to deliver.

Selected Service Type High Throughput Sequencing (NGS)

Technologies \* ☐ General ☒ Genomics/Transcriptomics ☐ Metabolomics/Biophysics ☐ Proteomics

Instruction Link [Please click here for order form instructions!](#)

Sample Submission \* ☒ Plate ☐ Tube

Sequencing Application \* Whole Genome Sequencing

Library Protocol \* Illumina Truseq Nano

Number of Plates \* 2 Please provide plate details later in the edit order items form!

Insert Size (nt)

Instrument Illumina Novaseq 6000 #1

Data Package/Run Unit \* Data Package (200M reads) (~200M Reads)

Total Number of Units \* 1.00

Read Configuration \* Paired End 150 bp

Data Storage Model \* ☒ Data Delivery Only (without bioinformatics analysis and support) ☐ Bioinformatics Analysis and Support (including data delivery)

Note: Please indicate any special instruction in the “Remarks” field.

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 plates you have chosen. If you have more plates, add any number of the fields by using the “Add Item(s)” option.



Add/Edit Items, among others provide unique names for the submitted plates and samples. Please click here for order form instructions!

Delete	Plate Name	Given Plate Name	Plate Layout	Sample Assignment Order	Samples *
Delete	p2220_27218/1	Plate_xyz	96-well	Column	Assign Samples (3)
Total: 1 / 1 Rows					

1 Add Item(s)

Save Cancel

Assign Samples To Plate

Position	Name
1 A1	a
2 B1	b
3 C1	c
4 D1	
5 E1	
6 F1	
7 G1	

- Define the **Plate Name**, **Plate Layout** (Standard: *96-well*) and **Sample Assignment Order** (Standard: *Column-wise*).

Click on “**Assign Samples**” and copy and paste your sample names from an excel spreadsheet. **Please make sure that the plate positions and sample names match!**

- Click on “**Save**”.
- You will be forwarded to the screen seen below. Click on “**Edit Samples**” for each plate and fill out the mandatory additional fields for the samples on that plate. Please note that you can also use mock values for fields like “*Concentration*” in case this is not relevant for you.

Order Items (2)

This order is NOT submitted yet: DO NOT send or bring any samples!

Samples on Plate	Order Item Id	Plate Name	Plate Layout	Sample Assignment Order	Charges	Created By	Created	Modified By	Modified
Edit Samples (96)	161362	PlateTest1	96-well	Column	0	simongruter	2021-11-01 14:36:39	simongruter	2021-11-01 14:36:39
Edit Samples (96)	161363	PlateTest2	96-well	Column	0	simongruter	2021-11-01 14:36:40	simongruter	2021-11-01 14:36:40
Total: 2 / 2 Rows									

1 CSV

Edit Order Items Show Samples on Plate

- Please use the **Plate Name** and the **Tube Id** (project ID + Order ID, e.g. p2220\_26381/1) when labeling your plates. Please label your plates on **top and on the side**.
- Click on “**Submit Order**” button on the bottom of the page.
- 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)**.

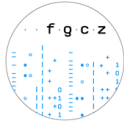
**Do not bring the samples if your order has not been accepted by your coach!**

**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.**

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



## Creating an order in B-Fabric for HT Sequencing/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 follow the instructions [here](#) instead.

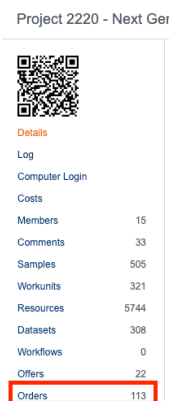
1. Minimum order is 1 physically separated lane. (*1 lane of the Novaseq for SP, S1, S2 and half an S4 flowcell*).
2. Pool samples together that will be sequenced on the same lane. Please use 1.5 ml Eppendorf tubes.
3. Dilute your samples to 10nM or an agreed upon concentration. We still need to QC and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

### 2.1) Order submission: step-by-step guide: **ready-made libraries**

1. Please login to your project main page on B-Fabric. 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 “**High Throughput Sequencing**”.  
Select “**Ready-made Libraries**” under “**Sequencing Application**” and fill up the remaining form (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.

Internal \* ☒ No ☐ Yes

Selected Service Type High Throughput Sequencing (NGS) (Note: Since the order already contains some items, only service types associated with the sample type 'Biological Sample - Sequencing' are listed)

Technologies \* ☐ General ☒ Genomics/Transcriptomics ☐ Metabolomics/Biophysics ☐ Proteomics

Instruction Link Please click here for order form instructions!

Sequencing Application \* Ready-made Libraries

Library Protocol \* Custom

**Number of Physical Tubes \* 3** Please provide sample details later in the edit order items form!

Insert Size (nt)

Instrument Illumina Novaseq 6000

Data Package/Run Unit \* 1 Lane SP Flowcell (~400M Reads)

Total Number of Units \* 1.00

Read Configuration \* Paired End 150 bp

Data Storage Model \* ☒ Data Delivery Only (without bioinformatics analysis and support)  
☐ Bioinformatics Analysis and Support (including data delivery)

Remarks

**Note:** Please indicate any special instruction 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 don't assume that we know how to sequence your libraries. We really prefer for you to be as specific as possible.

- 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**.
- A table will open with fields for the **number of physical tubes / pools** you have chosen previously.
- By default, the “**Library Pooled**” option will be selected on the “Edit order items” page. Click on “**Assign Barcodes**” and define the number of samples in each pool (see figure below).

Add/Edit items, i.e., the sample and service details of this order Please click here for order form instructions!

Delete	Clone	Tube Id	Generate	Library Pooled	Species	Set Value for Column Concentration (ng/ul)	Set Value for Column Extraction Protocol	Set Value for Column Source Type	Set Value for Column Total Amount (µg)	Set Value for Column Barcode1
Delete		p2220_23350/0001	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Select Item			Select Item		Disabled
Delete		p2220_23350/0002	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Select Item			Select Item		Disabled
Delete		p2220_23350/0003	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Select Item			Select Item		Disabled

1 Add Item(s) Add Item(s) Using Samples

Save Cancel

Assign Barcodes To Pooled Library p2220\_23350/0001

Number of Samples in Pool 5 (maximum: 1000)

	Name	Barcode1	Barcode2
1	p2220_23350/0001#1	Sample1	GATCATCG
2	p2220_23350/0001#2		ATCGGATC
3	p2220_23350/0001#3		
4	p2220_23350/0001#4		
5	p2220_23350/0001#5		

Assign Cancel

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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.

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

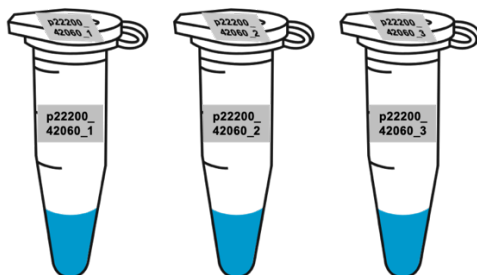
GATCGGAAGAGCACACGTCTGAACTCCAGTCAC [i7] ATCTCGTATGCCGTCTTCTGCTTG

Index 2 (i5) Adapters

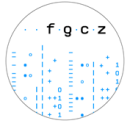
AATGATACGGCGACCACCGAGATCTACAC [i5] ACACTCTTTCCCTACACGACGCTCTTCCGATCT

Index Name	i7 Bases in Adapter	Barcode1		Barcode2	
		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	GCTTGTC	GCTTGTC	GAACATAC	GAACATAC	GTATGTTC
UDI0007	CAAGCTAG	CAAGCTAG	ACATAGCG	ACATAGCG	CGCTATGT
UDI0008	TGGATCGA	TGGATCGA	GTGCGATA	GTGCGATA	TATCGCAC

9. Enter the information about your pools like **Sample Name, Species, Concentration, Extraction Protocol** etc.
10. Please leave the **Tube Id** unchanged and use these numbers (project ID + Order ID) 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.

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