

How to submit samples for Illumina/Short Read Sequencing (FGCZv10.2)

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.
- **Update**: There is the possibility to copy and paste sample names from an Excel-Spreadsheet now. Please refer to the section found <u>here</u>.

RNA samples

- We need 300ng-2ug for standard library prep. Please check our application page for the requirements for the specific protocols <u>https://fgcz-intranet.uzh.ch/FullServiceSequencing</u> 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 tubes.

DNA samples

- We need 100ng-500ng for standard library prep. Please check our application page for the requirements for the specific protocols <u>https://fgcz-intranet.uzh.ch/FullServiceSequencing</u> 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 tubes.
- 4. Please run your samples in a gel and attach the picture in the comments section of the order.

Order submission: step-by-step guide

- 1. Please login to your <u>project main page</u> B-Fabric. If you have more than one project at the FGCZ, please make sure you are in the correct project page.
- 2. Click on the "<u>Orders</u>" button listed on the left part of the screen:



Or by clicking on the following icon on top of the screen:

	My Orde										
Project 1001	My Proje	All your	kilos orde	ws and Data Analy	rsis						
Project 1001 - G	My Sam	ples kunits	an	nd Data Anal	lysis						
Details	My Data	isets	ł.	Status 0			Service Type 0			Service Area 0	Coach 0
Costs	All Order	rs	11	No Filter	•						
Members	All Proje	icts	E	canceled		High Th	roughput Sequenci	ng (NGS)	Genomics		Hatakeyama
Comments	All Same	oles		canceled		High Th	roughput Sequenci	ng (NGS)	Genomics		Hatakeyama
Commenta				canceled		High Th	roughput Sequenci	ng (NGS)	Genomics		Hatakeyama
Samples	All Work	units		canceled		High Th	roughput Sequenci	ng (NGS)	Genomics		Hatakeyama
Workunits	All Datas	sets		canceled		Novased	3		Next Gene	eration Sequencing	Hatakeyama
Resources	5500	19311		canceled		HiSeq 4	000		Next Gene	aration Sequencing	Rehrauer
Datasets	404	16433		canceled		Bioinform	natics		Next Gene	eration Sequencing	Dimitrieva Janes
Orders	20	14144		canceled		Hiseq25	00		Next Gene	eration Sequencing	Opitz
Charges	0	14001		canceled		Hiseq25	00		Next Gene	eration Sequencing	
Crimillian	0	13120		canceled		Hiseq25	00		Next Gene	eration Sequencing	
Bookings	0										
Instrument Reservations	1	Create									
Reviews	2										
Timeline											

3. Click on the "<u>Create Order</u>" button.

Create Order

Under "Service Area", click on "High Throughput Sequencing".
 and fill up the form (select application, instrument, library protocol ...) as best you can. If you indicate "I don't know", your coach will get in touch with you to clarify your order. See Example below.

e Order			
Service Type *	Service Area \$	Service Type 🗢	
	O Proteomics / Protein analysis	Amino Acid Analysis	
	 Metabolomics (REFINE 2020) 	Biophysics Lab Services	
	 Metabolomics (REFINE 2020) 	Biophysics User Lab	
	Genomics	Genome Informatics	
	O Genomics	Genomics User Lab - Bench Access (QC, Library Prep, Sonication)	
	O Genomics	Genomics User Lab - Walk-in Sequencing Access	
	Proteomics / Protein analysis	Glycan/Glycoprotein	
	Genomics	High Throughput Sequencing (NGS)	
	O Proteomics / Protein analysis	Intact Mass Determination	
	Genomics	Long Read Sequencing	
	Totac 18 / 18 No	ws 1 = 1 2 + 1 10 CSV	
Relected Service Ture	High Throughout Sequencing (NGS)		
Instruction Link	Please click here for order form instructional		
Sequencing Application *	Whole Genome Sequencing		
Library Protocol *	NEB Ultra II		
Number of Samples *	2	Please provide sample details l	ater 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 a	analysis and support)	
	Bioinformatics Analysis and Support (includ	ling data delivery)	
		4	

<u>Note</u>: Please indicate any special instruction in the "<u>Remarks</u>" 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.

			* 🗹 I agree to the	e Terms and Co	nditions and conf	irm that the billing	address above is	s correct
Sav	ve	Cancel						
6	To a	add sample	s that you wa	ant to subr	hit to your o	order, click (on the "Add	order items"

 To add samples that you want to submit to your order, click on the "<u>Add order items</u>" button.



Please enter the number of samples you would like to submit.
 Would you like to add new samples please click on <u>"Add Item(s)"</u>
 Would you like to add already existing samples from previous orders within the same project, please click on <u>"Add Item(s) Using Samples.</u>



8. 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 "<u>Add Item(s)</u>" option.

		Generale	Set Value for Column \$	Set Value for Column 🖪	Set Value for Column	Set Value for Column	¢	Set Value for Column			
olete	Tube Id	Sample Name*	Species *	Concentration (ng/µl)*	Extraction Protocol *	Source Type *		Barcode/Index	Total Amount (µg)	Volume (µl)	Description
elete	p2220_6155/1	abc	Mus musculus (house m \$	100	custom	Genomic DNA	\$		10000	100	
loto	p2220_6155/2	gzt	Mus musculus (house m \$	100	custom	Genomic DNA	٥		10000	100	
\$	Add Item(s) A	dd Item(s) Using Samples	Create Annotation	Tr	stat: 2 / 2 Rows	1 10 0	CS	/			



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

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

If you would like to assign sample names in bulk, you may copy/paste your own sample names from a local spreadsheet (seen below):

Add/Edit i	tems, i	.e., the sample	e and service detail	s of this orde	r Please cli	ck here for or	der form instruc	ctions!			
			(Generate	Assign	Set Value fo	r Column 🗸 🗸	Set Value for	Column	Set Value for Column	
Delete	Clone	Tube Id	San	nple Name *		Spec	es * 🗄	Concentr	ation (ng/µl)) * Extraction Protocol *	
Delete		p2220_2569	0/1		T	Select item	~				
Delete		p2220_2569	0/2			Select item	~				
Delete		p2220_2569	0/3			Select item	\sim				
Delete		p2220_2569	0/4			Select item	~				
Delete		p2220_2569	0/5			Select item	~				
4					Total: 5 / 5	5 Rows 1	14 <4	1 >> >=	10 Y CS	SV	
1	÷ /	Add Item(s)	Add Item(s) Using	Samples							
Save		Cancel									

After clicking assign, a pop-up window will open where you may paste your own sample names. You must then copy the sample names from your local spreadsheet, navigate to the pop-up window, and press Ctrl + V to assign your sample names directly to the samples (seen below):





You must then click "Assign" and fill out the rest of the required fields (indicated with a red *).

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 "<u>Submit Order</u>" button on the bottom of the page.

Status

	Status	Created By	Created
	pending	patrig	2018-02-16 14:20
C	Submit C	Order Ca	rcel Order

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. **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.



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 or 1 lane of HS2500).
- 2. Pool samples together that will be sequenced on the same lane.
- 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 3 nM of your pooled libraries and use the following volumes:

SP/S1	150ul
S2	200ul
S4	400ul

Order submission: step-by-step guide

- 1. Please login to your <u>project main page</u> B-Fabric. If you have more than one project at the FGCZ, please make sure you are in the correct project page.
- 2. Click on the "<u>Orders</u>" button listed on the left part of the screen:

	Details	
	Members	3
	Comments	0
	Samples	0
	Extracts	0
	Workunits	0
	Resources	0
	Experiment Definitions	0
C	Orders	0
C	Orders Charges	0 0
C	Orders Charges Bookings	0 0 0
C	Orders Charges Bookings Instrument Reservations	0 0 0
C	Orders Charges Bookings Instrument Reservations Reviews	0 0 0 0
C	Orders Charges Bookings Instrument Reservations Reviews Mails	0 0 0 0

Or by clicking on the following icon on top of the screen:

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	My Orde							101			
Project 1001	My Proje	All you	aflows	and Data Anal	ysis						
Project 1001 - G	My Sam	ples units	and	Data Ana	lysis						
Details	My Data	sets	1	Status 0			Service Type 0			Service Area D	Coach C
Costs	All Order	15	100	No Filter							
Members	All Proie	cta		anceled		High T	hroughput Sequend	ing (NGS)	Genomi	26	Hatakeyama
Contraction of Contra	411 0		c	anceled		High T	hroughput Sequence	ing (NGS)	Genomi	-15	Hatakeyama
Comments	Air Samp	2005	0	anceled		High T	hroughput Sequence	ing (NGS)	Genomi	35	Hatakeyama
Samples	All Work	units	0	anceled		High T	hroughput Sequence	ing (NGS)	Genomi	25	Hatakeyama
Workunits	All Datas	iets	0	anceled		Novas	PR		Next Ge	neration Sequencing	Hatakeyama
Resources	5500	19311		anceled		HiSeq	4000		Next Ge	neration Sequencing	Rehrauer
Datasets	404	16433	c	anceled		Bioinfo	rmatics		Next Ge	neration Sequencing	Dimitrieva Ja
Orders	20	14144	0	anceled		Hiseq2	500		Next Ge	neration Sequencing	Opitz
01		14001	0	anceled		Hiseq2	500		Next Ge	neration Sequencing	
Charges	0	13120	c	anceled		Hiseq2	500		Next Ge	neration Sequencing	
Bookings	0										
Instrument Reservations	1	Create									
Reviews	2										
Timeline											
Timeline											
100											

3. Click on the "<u>Create Order</u>" 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 ...) 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 s	ome it	ems, 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.



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.

		* I agree to the Terms and Conditions and confirm that the billing address above is correct
Save	Cancel	
\smile		

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

			Generate		×	Set Valu	ue for Column	~	Set Value for Column	Set Value for Column	Set Valu	e for Column	~	Set Value for Colum	Set Value for C
elete C	Clone	Tube Id	Sample Name*		Library Pooled	s	species * 🖸		Concentration (ng/µl	Extraction Protocol	Sou	rce Type * 🖸		Total Amount (µg	Barcoo
elete		p2220_23350/0001		1	Assign Barcodes (0)	Select if	em	~			Select its	em	~		
elete		p2220_23350/0002		1	Assign Barcodes (0)	Select it	em	~			Select its	em	~		
elete		p2220_23350/0003		~	Assign Barcodes (0)	Select it	em	~			Select its	em	~		
‡ Ac	dd Iter	m(s) Add Item(s) Us Cancel	sing Samples				Assign Barco Number	odes ' of Sa	To Pooled Library p2 mples in Pool 5	220_23350/0001 (maximum: 1000) Name		Ba	rcoo	de1	Barcode2
‡ Ac	dd Iter	m(s) Add Item(s) Us	sing Samples				Assign Barco Number	odes ' of Sa	To Pooled Library p2	220_23350/0001 (maximum: 1000)					
C Ac	dd Iter	m(s) Add Item(s) Us Cancel	sing Samples				Assign Barco Number	odes [•] of Sa	To Pooled Library p2 mples in Pool 5 3350/0001#1 Se	220_23350/0001 (maximum: 1000) Name mple 1		Ba	rcoo	de1	Barcode2
C Ac	dd Iter	m(s) Add Item(s) Us Cancel	sing Samples				Assign Barco Number	odes ¹ of Sa 220_2 220_2	To Pooled Library p2 mples in Pool 5 3350/0001#1 Se 3350/0001#2	220_23350/0001 (maximum: 1000) Name mple1		Ba GATCATCG	rcoo	de1 ATC	Barcode2 GGATC
Save	dd Iter	m(s) Add Item(s) Us Cancel	sing Samples				Assign Barco Number 1 p22 2 p22 3 p23	odes [•] of Sa 220_2: 220_2: 220_2:	To Pooled Library p2 mples in Pool 5 3350/0001#1 Sa 3350/0001#2 3350/0001#3	220_23350/0001 (maximum: 1000) Name mple1		Ba GATCATCG	rcoo	le1 ATC	Barcode2 GGATC
2 Ac	dd Iter	m(s) Add Item(s) Us Cancel	sing Samples				Assign Barco Number 1 p22 2 p22 3 p22 4 p22	odes ¹ of Sa 220_2: 220_2: 220_2: 220_2:	To Pooled Library p2 mples in Pool 5 3350/0001#1 St 3350/0001#2 3350/0001#3 3350/0001#4	220_23350/0001 (maximum: 1000) Name mpie1		Ba GATCATCG	rcoo	le1 ATC	Barcode2 GGATC

- 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.
- Enter the information about your pools like Sample Name, Species, Concentration,
 Extraction Protocol
- 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 "<u>Submit Order</u>" 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. **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.