

## **A step by step “how to” set up requests on Fsk3 guide**

### 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,...)

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

### **Information to have beforehand:**

1. What **type of experiment** do I want to use?
2. How **many samples (=libraries)** are planned?
3. What **output** do I want to target ?

### **Color code :**

Where on Fsk

Where to click & fill Fsk

Important / critical info.

Some extra comments

VBCF.NGS Fsk3
 Important Documents
 Projects
 Requests
 Samples
 Adaptors
 Custom Primers
 search
 Thomas Grentzinger
 Logout

+ New Request  
 Drafts  
 My Requests  
 Group Requests

1 Select new request

Submission Guidelines  
 New Request  
 Review Onhold Requests  
 Data Analysis  
 Data Download  
 Price List

+ Current Queue  
 + Announcements  
 + Requests/Samples  
 + Requested Lanes  
 + Notifications

v3.106.1 (20241212-2109) - Feedback Issues - API

**Request Data**

|                    |                                                                                                                                                                                                                                                                                                  |   |                              |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|------------------------------|
| Scientist/Group    | Thomas Grentzinger / NGS                                                                                                                                                                                                                                                                         | 1 | Name and group               |
| Project            | scRNA request                                                                                                                                                                                                                                                                                    | 2 | Project name (opt)           |
| Cost Assignment ?  | VBCF-NGS                                                                                                                                                                                                                                                                                         | 3 | Cost invoicing (ask your PI) |
| Submitted          | -                                                                                                                                                                                                                                                                                                |   |                              |
| Status             | Created                                                                                                                                                                                                                                                                                          |   |                              |
| Accepted           | -                                                                                                                                                                                                                                                                                                |   |                              |
| Completed          | -                                                                                                                                                                                                                                                                                                |   |                              |
| Reviewed           | No                                                                                                                                                                                                                                                                                               |   |                              |
| Priority           | Normal                                                                                                                                                                                                                                                                                           | 4 | Select Platform              |
| Platform           | Illumina                                                                                                                                                                                                                                                                                         |   |                              |
| Aviti Readmodes    | Please select "Illumina" as platform to find the Aviti readmodes!                                                                                                                                                                                                                                |   |                              |
| Readmode           | <div>5</div> <div>Select Prep/QC only or read mode</div> <div> <input checked="" type="radio"/> Prep and/or QC Only           <input type="radio"/> Select Readmode         </div>                                                                                                               |   |                              |
| Demultiplexing     | Please specify...                                                                                                                                                                                                                                                                                |   |                              |
| Alignment/Analysis | Finish editing and save this request - afterwards you can order <b>bioinformatics services</b> here.<br>This is only possible as long as the request is NOT completed. Please contact <a href="mailto:ngs-support@vbcf.ac.at">ngs-support@vbcf.ac.at</a> in case of a already completed Request. |   |                              |

Please to select your readmode

| Type/Read Length               | Expected Read Range                              | 50 bp                 | 100 bp                 | 150 bp    | 300 bp    | 10X          | 75 bp     | 200 bp    | 250 bp |
|--------------------------------|--------------------------------------------------|-----------------------|------------------------|-----------|-----------|--------------|-----------|-----------|--------|
| NextSeq2000P1                  | 100 - 120M reads                                 | > PE150 <             | > SR100 <              | > PE150 < | > PE300 < |              |           |           |        |
| NextSeq2000P2                  | 350 - 500M reads                                 | > PE150 <             | > SR100 <<br>> PE100 < | > PE150 < | > PE300 < | > PE200/90 < |           |           |        |
| NextSeq2000P3                  | 1000 - 1100M reads                               | > SR50 <<br>> PE150 < | > SR100 <<br>> PE100 < | > PE150 < |           |              |           |           |        |
| NovoSeqX_1.5B                  | 1.6B reads, full flowcell                        | > PE150 <             | > SR100 <<br>> PE100 < | > PE150 < |           |              | > SR200 < |           |        |
| NovoSeqX_10B                   | 10B reads, full flowcell                         | > PE150 <             | > SR100 <<br>> PE100 < | > PE150 < |           |              | > SR200 < |           |        |
| NovoSeqX_10B_XP                | 1.2B single lane                                 |                       |                        | > PE150 < |           |              |           |           |        |
| NovoSeqX_25B                   | 52B reads, full flowcell                         |                       |                        | > PE150 < |           |              |           |           |        |
| ElementBio_Aviti_Low_Output    | 250M                                             |                       |                        | > PE150 < |           |              |           |           |        |
| ElementBio_Aviti_Medium_Output | 500M (150 cycles, 300 cycles), 100M (600 cycles) |                       |                        | > PE150 < | > PE300 < |              | > PE75 <  |           |        |
| ElementBio_Aviti_High_Output   | 1B (150 cycles, 300 cycles), 300M (600 cycles)   |                       |                        | > PE150 < | > PE300 < |              | > PE75 <  |           |        |
| MISeq                          | 5 - 25M                                          |                       |                        | > SR150 < | > PE300 < |              | > PE75 <  |           |        |
| MISeqMicro                     | 4M                                               |                       |                        | > PE150 < |           |              |           |           |        |
| MISeqNano                      | 1M                                               |                       |                        | > PE150 < |           |              |           | > PE200 < |        |
| MISeqV2                        | 12 - 15M                                         |                       |                        |           |           |              |           |           |        |

Return Select new Readmode

## Request Data

Scientist/Group: Thomas Grentzinger / NGS

Project: scRNA request

Cost Assignment: VBCF-NGS

Submitted: -

Status: Created

Accepted: -

Completed: -

Reviewed: No

Priority: Normal

Platform: Illumina

Aviti Readmodes: Please select "Illumina" as platform to find the Aviti readmodes!

Readmode: NovaSeqX 10B XP flowcell PairedEnd 150 - single lane

☒ Prep and/or QC Only ☐ Select Readmode ☐ Spikein Only

iSeq Test Run: No

Custom Readlength: Cycles per Read

Demultiplexing: Yes

Alignment/Analysis: Finish editing and save this request - afterwards you can order bioinformatics services here.

Demultiplexing - ask your Bioinformatician

Scroll down

Lane 1/Flowcell 1 - No samples! - Create

## 1 Select Lane options

Overview

Lane Options

Samples - Admin

Samples - Preparation

Samples - Required Fields

Multiplexing

Samples - Optional Fields

Status

Created

Sharing/Spikein Options

Please specify sharing options

Custom Primer

Please specify custom primer options

PhiX/Density

Please verify sample complexity

Library QC / Pooling Strategy

Please specify QC protocol and/or pooling strategy

Pick Old Samples/Multis

Remove Lane

+ Add Lane

Clone last Lane

Copy last Lane

2 Select lane sharing options

3 Specify custom primer

4 Select pooling strategy – otherwise request cannot be saved! (\*might need to be entered again after selecting prep options)

Lane 1 - No samples! - Created

## 1 Sample prep page

Overview

Lane Options

Samples - Preparation

Samples - Required Fields

Multiplexing

Samples - Optional Fields

Id Prep Kit Stranded Library Size Insert Size Description

Nothing but vast emptiness! But do not despair, this can be changed by adding samples.

+ Add New Sample

Pick Old Samples/Multis

Remove Lane

2 New sample button : 1 click = one sample created

+ Add Lane

Clone last Lane

Copy last Lane

Select preparation by user or facility

Sample description (needs to be unique)

Sample prep page

Type of experiment

Lane 1 - Create

Overview Lane Options Samples - Preparation Samples - Required Fields Multiplexing Samples - Optional Fields

| Id      | Prep               | Kit                          | Stranded       | Library Size | Insert Size | Description |
|---------|--------------------|------------------------------|----------------|--------------|-------------|-------------|
| NEW0001 | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 2           |             |
| NEW0002 | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 3           |             |
| NEW0003 | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 4           |             |
| NEW0004 | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 4           |             |

+ Add New Sample -

Pick Old Samples/Multis Remove Lane

+ Add Lane Clone last Lane Copy last Lane

Specify experiment

Sample required field page

Your favorite biological model organism

Overview Lane Options Samples - Preparation Samples - Required Fields Multiplexing Samples - Optional Fields

| Id      | Exptype | Organism     | Library Size | Insert Size | Description                                    |
|---------|---------|--------------|--------------|-------------|------------------------------------------------|
| NEW0001 | 10x RNA | Homo Sapiens |              |             |                                                |
| NEW0002 | 10x RNA | Homo Sapiens |              |             | 3' NextGEM V3.1, 10 000 cells target, sample 2 |
| NEW0003 | 10x RNA | Homo Sapiens |              |             | 3' NextGEM V3.1, 10 000 cells target, sample 3 |
| NEW0004 | 10x RNA | Homo Sapiens |              |             | 5' NextGEM V3.1, 5 000 cells target, sample 4  |
| NEW0005 | 10x RNA | Homo Sapiens |              |             | 5' NextGEM V3.1, 7 000 cells target, sample 5  |

+ Add New Sample -

Pick Old Samples/Multis Remove Lane

Do you need more reads?

optional

Add another lane

Clone lane to keep settings, sample IDs, all changes will be transferred

Copy lane to create new sample and multiplex IDs, with same settings

Lane 1/Flowcell 1 - Created

Overview Lane Options Samples - Admin Samples - Preparation Samples - Required Fields Multiplexing Samples - Optional Fields

| Id      | Prep               | Kit                          | Stranded       | Library Size | Insert Size | Description |
|---------|--------------------|------------------------------|----------------|--------------|-------------|-------------|
| NEW0002 | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 2           | 1           |
|         | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 2           | 2           |
|         | DNA - DNA:Facility | Ligation - sheared/short DNA | Please specify | 200-800      | 2           | 3           |

+ Add New Sample -

Pick Old Samples/Multis Remove Lane

+ Add Lane Clone last Lane Copy last Lane

heinz - 2018-12-19 09:18

Invalid! Save

If system doesn't allow you to save - press "Run Checks"

Run Checks

Invalid!

Save

## Lane option page

Lane 1 - Created

Overview Lane Options Samples - Preparation Samples - Required Fields Multiplexing Samples - Optional Fields

Sharing/Spikein Options regular - Full lane

Custom Primer Custom primers are not available for facility prepared samples!

PhiX/Density Please verify sample complexity Custom PhiX Ratio (> 1%)

Library QC / Pooling Strategy qpcr - Multiplex pooling by qPCR, more accurate

Pick Old Samples/Multis Remove Lane

Add Lane Clone last Lane Copy last Lane

Save

Save button

WBCF.NGS Fsk3 Important Documents Projects Requests Samples Adaptors Custom Primers

Request 18375

Request ID

Request list Create New Request Edit Submit Abort Delete Clone Show Versions

Have Gels or other Measurements?

You can upload gels, Fragment Analyzer or other data under Attachments

This request is not yet submitted to the facility.

You can still add/remove samples etc. Once you are satisfied, click on

## Cost estimate page

Samples 5 Cost Estimate Attachments 0 Lab data 0/0 Sequencing Runs 0 Tickets 0 History

Estimate

Check cost estimate

| Code      | Item                                              | Price  | Disc | Count | Total  |
|-----------|---------------------------------------------------|--------|------|-------|--------|
| NGS24PI00 | Library QC (1-23 samples)                         | 0.00 € | 0    | 5     | 0.00 € |
| NGS24PI28 | 10X Genomics scRNA-seq ST, 3' or 5' (2-8 samples) | 0.00 € | 0    | 5     | 0.00 € |
| NGS24SI33 | NovaSeqX 10B 300 cycles Lane                      | 0.00 € | 0    | 1     | 0.00 € |
| NGS24BI01 | Demultiplexing                                    | 0.00 € | 0    | 1     | 0.00 € |
| Total     |                                                   |        |      |       | 0.00 € |

WBCF.NGS Fsk3 Important Documents Projects Requests Samples Adaptors Custom Primers

Request 18375

Request list Create New Request Edit Submit Abort Delete Clone Show Versions

Have Gels or other Measurements?

You can upload gels, Fragment Analyzer or other data under Attachments

This request is not yet submitted to the facility.

You can still add/remove samples etc. Once you are satisfied, click on

Submit button

Samples 5 Cost Estimate Attachments 0 Lab data 0/0 Sequencing Runs 0 Tickets 0 History

Estimate

| Code      | Item                                              | Price  | Disc | Count | Total  |
|-----------|---------------------------------------------------|--------|------|-------|--------|
| NGS24PI00 | Library QC (1-23 samples)                         | 0.00 € | 0    | 5     | 0.00 € |
| NGS24PI28 | 10X Genomics scRNA-seq ST, 3' or 5' (2-8 samples) | 0.00 € | 0    | 5     | 0.00 € |
| NGS24SI33 | NovaSeqX 10B 300 cycles Lane                      | 0.00 € | 0    | 1     | 0.00 € |
| NGS24BI01 | Demultiplexing                                    | 0.00 € | 0    | 1     | 0.00 € |
| Total     |                                                   |        |      |       | 0.00 € |

Request 18375

Request ID

Sample ID  
How to label your tubes

Adaptors: 10X Dual Index Kit TT Set A Workflow A [Display Index Balance](#) [Pooling: qpcr](#)

| Id     | Status              | Tag         | Ratio | Prep                                      | Description                                                            |
|--------|---------------------|-------------|-------|-------------------------------------------|------------------------------------------------------------------------|
| 340083 | Created - Submitted | auto / auto | 16.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 1; 10x RNA ; Homo Sapiens |
| 340084 | Created - Submitted | auto / auto | 16.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 2; 10x RNA ; Homo Sapiens |
| 340085 | Created - Submitted | auto / auto | 33.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 3; 10x RNA ; Homo Sapiens |
| 340086 | Created - Submitted | auto / auto | 8.5   | Cells, stranded, kit: 10X 5' st scRNA-seq | 5' NextGEM V3.1, 5 000 cells target, sample 4; 10x RNA ; Homo Sapiens  |
| 340087 | Created - Submitted | auto / auto | 23.7  | Cells, stranded, kit: 10X 5' st scRNA-seq | 5' NextGEM V3.1, 7 000 cells target, sample 5; 10x RNA ; Homo Sapiens  |

5 samples

Download button

Download the document  
(will also be sent by email.)

- Sequencing Data
- Request Sheet
- Regenerate Request Sheet
- CSV Export
- Report

Lane 1 - M20068 5 samples - Created

Overview

Adaptors: 10X Dual Index Kit TT Set A Workflow A [Display Index Balance](#) [Pooling: qpcr](#)

| Id     | Status              | Tag         | Ratio | Prep                                      | Description                                                            |
|--------|---------------------|-------------|-------|-------------------------------------------|------------------------------------------------------------------------|
| 340083 | Created - Submitted | auto / auto | 16.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 1; 10x RNA ; Homo Sapiens |
| 340084 | Created - Submitted | auto / auto | 16.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 2; 10x RNA ; Homo Sapiens |
| 340085 | Created - Submitted | auto / auto | 33.9  | Cells, stranded, kit: 10X 3' st scRNA-seq | 3' NextGEM V3.1, 10 000 cells target, sample 3; 10x RNA ; Homo Sapiens |
| 340086 | Created - Submitted | auto / auto | 8.5   | Cells, stranded, kit: 10X 5' st scRNA-seq | 5' NextGEM V3.1, 5 000 cells target, sample 4; 10x RNA ; Homo Sapiens  |
| 340087 | Created - Submitted | auto / auto | 23.7  | Cells, stranded, kit: 10X 5' st scRNA-seq | 5' NextGEM V3.1, 7 000 cells target, sample 5; 10x RNA ; Homo Sapiens  |



## VBCF NGS Project Agreement 18375

### Lanes

Use 10X Dual Index Kit TT Set A Workflow A adaptors.

5 Samples

| Id     | Tag  | Exptype | Ratio | Prep            | Multiplex |
|--------|------|---------|-------|-----------------|-----------|
| 340083 | auto | 10x RNA | 16.9  | Cells, stranded | M20068    |
| 340084 | auto | 10x RNA | 16.9  | Cells, stranded | M20068    |
| 340085 | auto | 10x RNA | 33.9  | Cells, stranded | M20068    |
| 340086 | auto | 10x RNA | 8.5   | Cells, stranded | M20068    |
| 340087 | auto | 10x RNA | 23.7  | Cells, stranded | M20068    |

Double check the document

### Estimated Costs

| Description                                       | Price | Count | Total |
|---------------------------------------------------|-------|-------|-------|
| Library QC (1-23 samples)                         | 0.00  | 5     | 0.00  |
| 10X Genomics scRNA-seq ST, 3' or 5' (2-8 samples) | 0.00  | 5     | 0.00  |
| NovaSeqX 10B 300 cycles Lane                      | 0.00  | 1     | 0.00  |
| Demultiplexing                                    | 0.00  | 1     | 0.00  |
| Total                                             |       |       | 0.00  |

Here I can spot an issue with the estimated costs -> contact NGS

**Signatures**

**And get it signed**

Scientist ..... Date .....

 **User signature**

Group leader ..... Date .....

 **Group leader signature**

# add old samples to request

Scroll  
down

Lane 1 - No samples! - Created

Overview Lane Options Samples - Preparation Samples - Required Fields Multiplexing Samples - Optional Fields

Adaptors: Display Index Balance Custom Primer: ((not defined!)) Share Status: Pooling:

Id Status Tag Ratio Prep Description

No data to display

0 samples

Pick old samples button

Pick Old Samples/Multis Remove Lane

Titta, alla dina fina prover!

| Samples  Multiplexes |                         | id list |              | Exptype                                   |                                                                    | Organism   |           | Description                                    |  |
|----------------------|-------------------------|---------|--------------|-------------------------------------------|--------------------------------------------------------------------|------------|-----------|------------------------------------------------|--|
| id                   | Owner                   | Exptype | Organism     | Prep                                      | Adaptor                                                            | Received   | Status    | Details                                        |  |
| 340087               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 5' st scRNA-seq | 10X Dual Index Kit NN Set A Workflow A auto                        | 2025-01-16 | Submitted | 5' NextGEM V3.1, 7 000 cells target, sample 5  |  |
| 340086               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 5' st scRNA-seq | 10X Dual Index Kit NN Set A Workflow A auto                        | 2025-01-16 | Submitted | 5' NextGEM V3.1, 5 000 cells target, sample 4  |  |
| 340085               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A auto                        | 2025-01-16 | Submitted | 3' NextGEM V3.1, 10 000 cells target, sample 3 |  |
| 340084               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A auto                        | 2025-01-16 | Submitted | 3' NextGEM V3.1, 10 000 cells target, sample 2 |  |
| 340083               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A auto                        | 2025-01-16 | Submitted | 3' NextGEM V3.1, 10 000 cells target, sample 1 |  |
| 327212               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A 708CGAAGTATAC 508GAACCTTGAG | 2024-12-12 | Prepared  | PIP 323948 FIXED on Bif beads                  |  |
| 326970               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A 795ACATCGATC 595TGACGGAGTG  | 2024-12-11 | Ready     | PIP 3224798 FIXED                              |  |
| 326969               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, stranded, kit: 10X 3' st scRNA-seq | 10X Dual Index Kit TT Set A Workflow A 796TGATGATCA 596GTAGGAGTGG  | 2024-12-11 | Ready     | PIP 322478 FIXED                               |  |
| 326741               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, kit: 10X 3' st scRNA-seq           | 10X Dual Index Kit TT Set A Workflow A auto                        | 2024-12-09 | Prepared  | LT 322479 Fixed                                |  |
| 326740               | Thomas Greentzinger NGS | 10x RNA | Homo Sapiens | Cells, kit: 10X 3' st scRNA-seq           | 10X Dual Index Kit TT Set A Workflow A auto                        | 2024-12-09 | Prepared  | LT 322478 Fixed                                |  |
| 324764               | Thomas Greentzinger NGS | 10x RNA | Mus musculus | RNA, stranded, kit: NEB, poly-A           | Unique NEB auto                                                    | 2024-11-27 | Accepted  | NB13-11-4                                      |  |
| 324763               | Thomas Greentzinger NGS | 10x RNA | Mus musculus | RNA, stranded, kit: NEB, poly-A           | Unique NEB auto                                                    | 2024-11-27 | Accepted  | NB13-7-2                                       |  |

5 selected / 122 total

1 Select the samples you want to sequence in this request

Not that button !  
Except if you want all your NGS libs.  
re-sequenced...in that case contact NGS first.

2 Add selected button

Add 5 Samples Add All