The Single Cell Core Facility (SICOF) manual

Welcome to the Single cell Core (SICOF) facility! With this manual, we will help you submit your projects to us, as well as explain the working pipeline.

Changelog v1.4

- The acronym has been changed from ScCore to SICOF
- Added a clarification on the QC that SICOF performs
- General linguistic changes

Changelog v1.3

- From the registration page, the description on the Standing PO was removed. Registration is now easier for customers outside of KI
- The workflow flowchart was changed to reflect the changes in user communication. The stopping points after QC are now indicated. The text was changed in the flow chart to indicate that it is now the user who decides if to continue with a plate, regardless of QC result.
- A new section was added to clarify the stopping points and the classification of the plates by their quality.
- A priority list on submitted plates is now mandatory, the manual was updated to reflect this change.
- Minor changes to submission forms

Contents

Registration to iLab

SICOF uses the iLab registration system for all its projects. Everything from project submission up until billing is done through this system. Therefore, you need an iLab account when working with us. As iLab is still under development, we apologize for some of the cumbersome features of this portal. SICOF is not able to influence many of these steps.

As iLab is continuously changing, we would appreciate it if you would alert us when the sign-up procedure is different from this guide. SICOF is not always informed of changes in iLab. We will strive to keep this guide up to date with changes in iLab.

In case you work at an institute using the SWAMID (Most Swedish universities)

1. Navigate to the core page: https://karolinska.corefacilities.org/service_center/show_external/3693
2. In the upper-right-hand corner of the screen, select Sign In and use the 'Sign in using Karolinska Institutet credentials' option. Although it's suggesting these are KI credentials, it's actually SWAMID. SWAMID is the same as your KI-ID for KI users, for Uppsala University, it's called AKKA-ID, etc.

3. You will be directed to an authentication page where you will need to enter your SWAMID (KI-ID) credentials
4. Once you have entered your credentials, click the 'Login' button
5. You will be directed to an iLab registration page where you will need to select your PI/Lab and verify your contact information.
6. Once your registration has been submitted, your PI will receive a notification that you have requested membership to their lab in iLab. They will need to approve your membership and assign any Project numbers for your use.

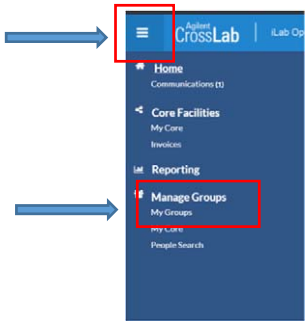
In case you don't work at an institute using SWAMID (foreign universities)

1. Complete the registration form on the sign-up page (see picture above)
2. Receive a Welcome Email from iLab (typically within one business day) with login credentials.
3. Log in to iLab with the provided credentials
4. You will be directed to an iLab registration page where you will need to select your PI/Lab and verify your contact information.
5. Once your registration has been submitted, your PI will receive a notification that you have requested membership to their lab in iLab. They will need to approve your membership and assign any Project numbers for your use.

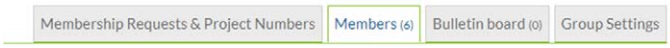
For PIs with a SWAMID (Swedish universities)

As PI, you may receive email requests from researchers wishing to join your group. The request email will have specific instructions on how to approve the request. In case you are interested in the process, we have pasted instructions below. If you would prefer to delegate these notifications/approvals to a financial manager or an administrator, please email ilab-support@agilent.com with the name & email of the person that will take care of this.

1. Click here to log-in: https://karolinska.corefacilities.org/service_center/show_external/3693
2. You will use your SWAMID (KI-ID) credentials to log into iLab (See above for pictures)
3. Once logged in, look for the link in the left-hand menu that says, 'my groups'. Hover-over and select your lab. Note that the menu is hidden under the symbol with the three horizontal lines



On the right side, you will see a few tabs where you can change all the settings as discussed below

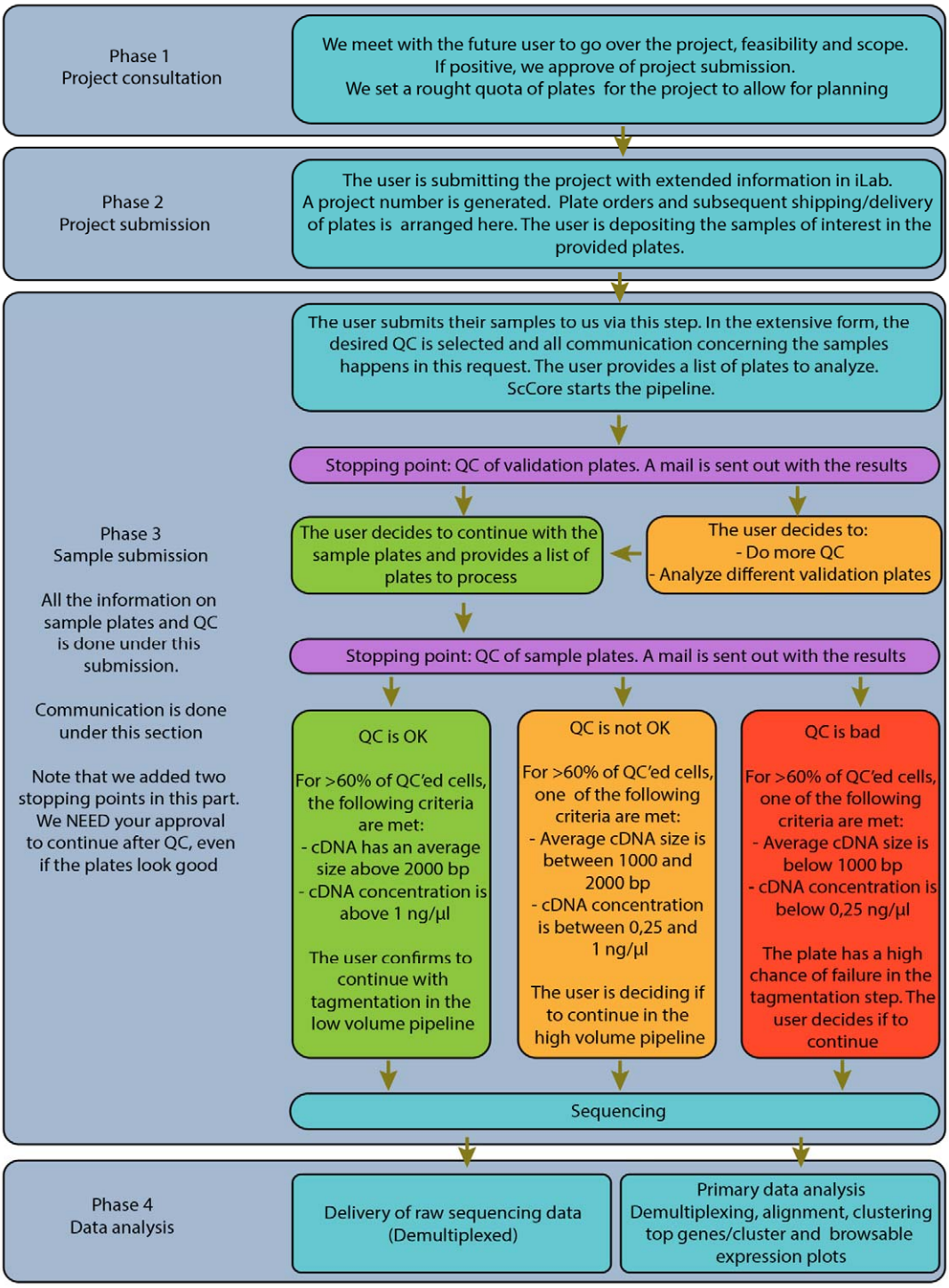


4. Set the auto-approval amount if you do not wish to approve service requests below a certain amount. To do this, select the 'Members' panel and enter an amount in the 'Auto Pre-Approval' amount and click 'save settings.' The default setting for the pre-approval is 10 000 SEK.
5. To approve lab membership requests, select the 'Membership Requests & Project numbers' tab. New membership requests will show at the top of this page. Click "Approve" to accept a member into your lab. Click "Reject" if they are not a member of your lab.
6. To assign a Project number to a member of your lab, find the member in the above list where it says, 'Manage Project numbers.' Select the checkbox(es) to the right of their name for the Project number(s) you wish to assign them.

We apologize for the cumbersome steps of registering, but this is something you will only need to do once.

How to use iLab for interaction with SICOF

Now that you can access iLab, we can go over the workflow that we are using at our facility. In summary, there are 4 phases in each project, of which phase 3 is the most interactive. A flow chart of the pipeline is illustrated below. This flow chart is specific for plate-based Smart-seq2/3 sequencing, which is the bulk of the projects we perform.



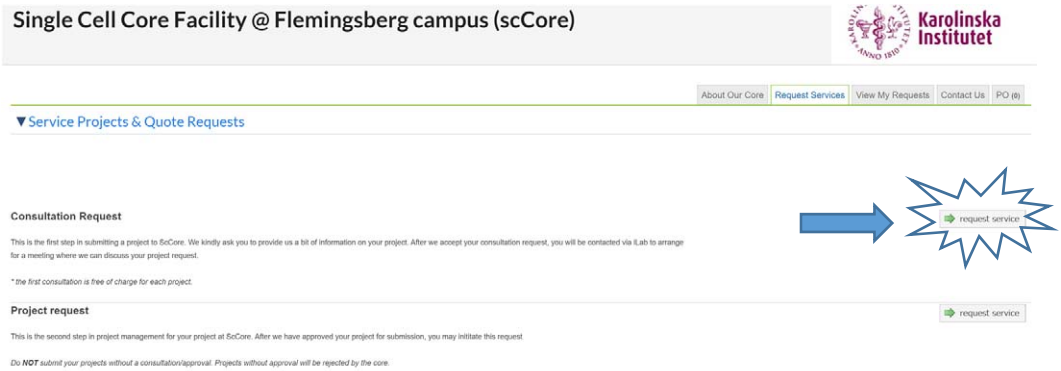
On the next page, we will describe the phases for your project in more details.

Phase 1: Project consultation

This is where your interaction with SICOF begins. **All projects, regardless of prior interaction with our core (pre-2021), need to go through a consultation before a project submission can begin.** This is important for us to keep track of all the projects we have in iLab and facilitate our future interaction with SICOF.

Project submissions (see phase 2) that do not get approved in phase 1 will be rejected by the core.

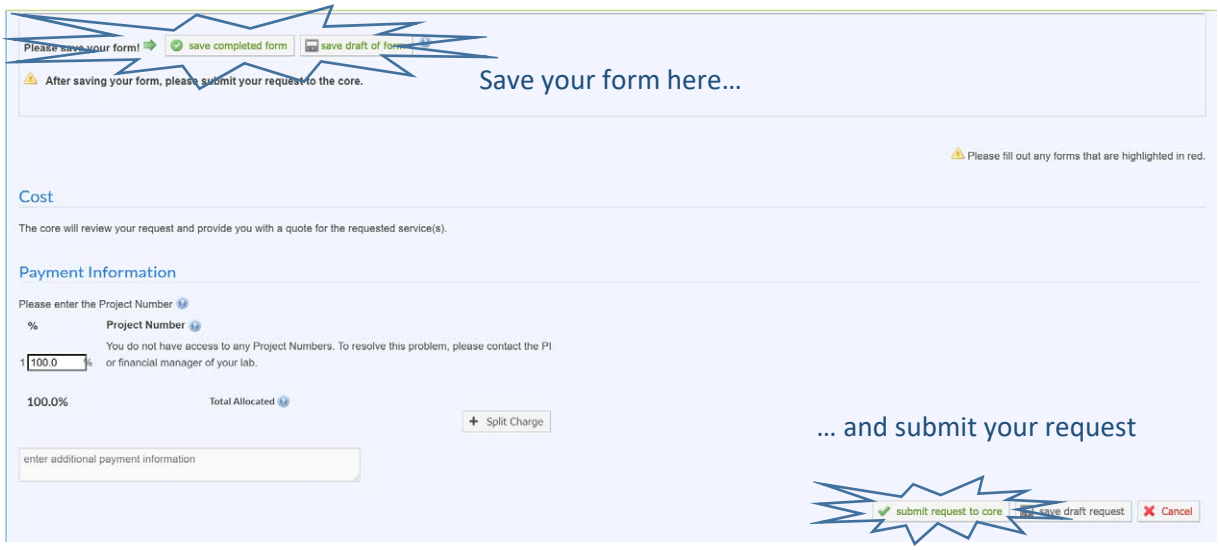
After logging into iLab, please click on the "Request Services" tab and click on the "Request service" tab on the right of "Consultation request"



Next, you are asked to which lab you belong (which is the one you signed up with, or are associated with via SWAMID). Afterwards, you are asked to fill in a small form as detailed below:

You may change your request name on top or let the system generated an automated name, it doesn't matter. Your project description does not have to be long, just so we have an idea what it is about.

There is a strange mechanic in iLab you need to understand for all requests that you are placing: <u>You always need to do two actions when submitting a request: 1) save the form and 2) submit the request.</u> If you forget to save your form while submitting the request, we will only see an empty form and miss critical information. This is important and therefore we are repeating this several times in this manual.



If your registration was done correctly, you should be able to select a project to be used for billing. **However, we will NOT charge you anything at this point. Project consultations are free for new projects.** We can't hide the payment window in iLab unfortunately.

After you have submitted your consultation request, we will contact you with a suggestion for a meeting time (most likely over Zoom). After we have discussed your project, the SICOF team will add a message to the consultation request, stating that the project is ready for submission (phase 2).

You are welcome to ask for additional consultations during your project, but we ask that you kindly keep this to a minimum.

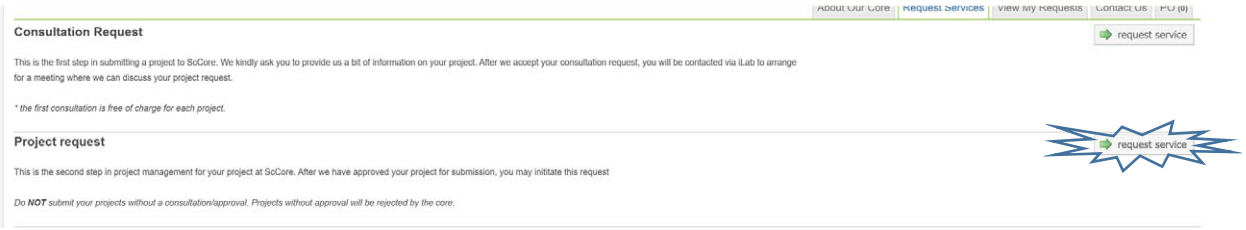
If you are part of KI, you will be asked to fill in your project number and billing details as below

If you are not a KI member, you will need to provide a PO number or payment reference, see below

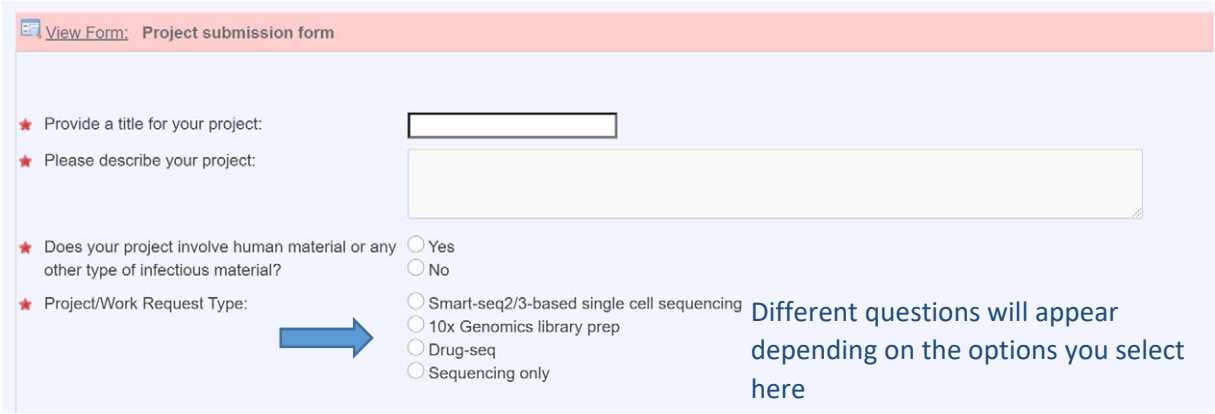
In case of problems (for example, no project numbers showing up as a KI member), please mail cfm-support@ki.se , they can help you further.

Phase 2: Project submission.

Now that your project is accepted, you are welcome to submit the project into the system. The submission system for phase 2 is similar to the other phases, just press the “request service” button next to “project request”.



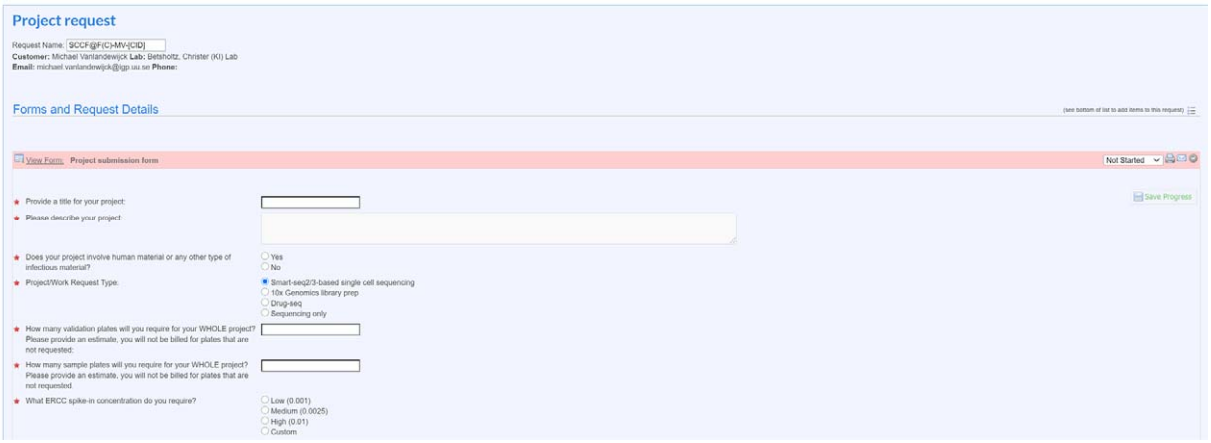
On the next page, you need to confirm your lab that the request is for. On the next page, you have a form to fill in, that will look different depending on some of the options you select under Project/Work request type.



After you are done with the project submission, don’t forget to save the form, and submit the request (in a similar way as to submitting a consultation request). You will always need to do these two steps when doing a request, this is just how iLab works and we can’t change it.

For users working with Smart-seq2/3

If you are doing plate-based single cell sequencing with us, you will need sample plates and validation plates containing lysis buffer that are provided by SICOF. When we discuss your project during the consultation phase, we will have discussed a rough quota of plates needed for your project. It is in this project submission that you are able to order plates from us.

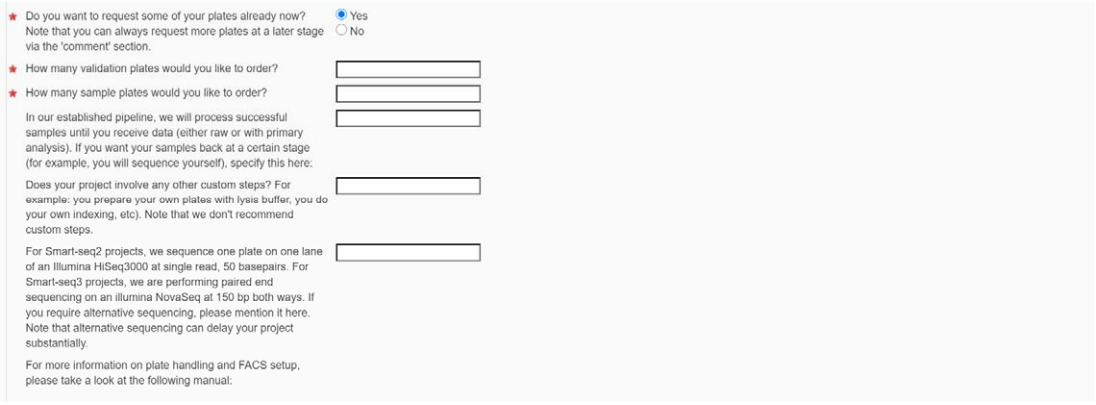


We ask that you extend your project description a bit here.

For human/infectious material, we ask that you provide us with a risk assessment. I.e., we need to know what the risk is for us in dealing with such material. Your plates will be disinfected upon arrival and the material will be inactivated for 5 minutes at 95 degrees. This will not impact your sample quality.

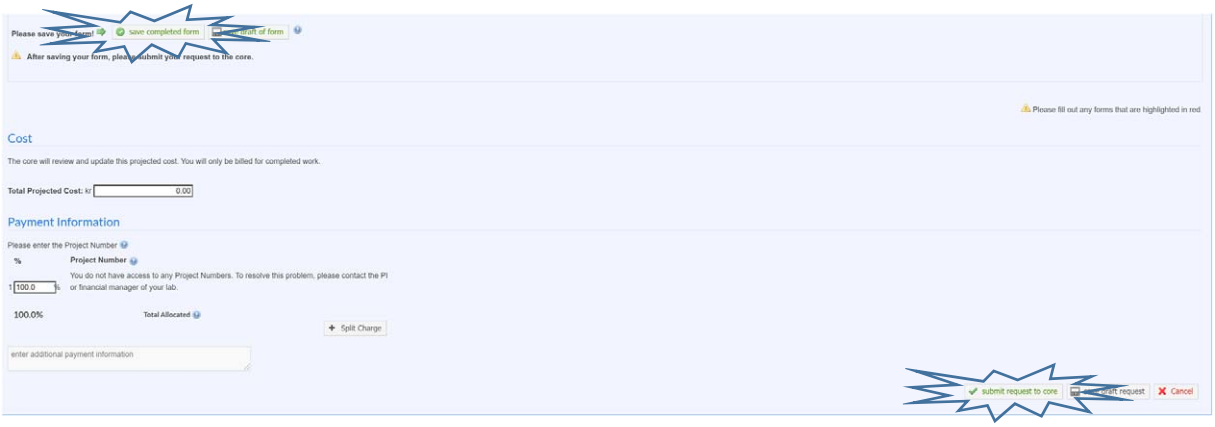
For the estimation of amount of validation and sample plates for your WHOLE project, please fill in the numbers as discussed during phase one. That way we can keep track of your project size and prevent projects from ballooning out of proportion, risking to clog the facility for other users. Note that this estimate is a soft ceiling that can always be re-negotiated. It is only in rare cases that we will not allow this estimate to change.

The ERCC spike-in depends on the mRNA content of your cells. The lower the content, the lower the ERCC. When in doubt, pick low.



You can order plates already when you are submitting your project, but **you don't need to order all your project plates at once.** As mentioned in the form, you can always request more plates using the comment section in your project submission page. For more information on the comment section, see lower.

The last three fields are optional in case you have special requests. As mentioned in the form, some special requests may delay your project substantially.



As always, please remember to **save your form** and **submit your request.** Your request is now submitted to SICOF and visible to us.

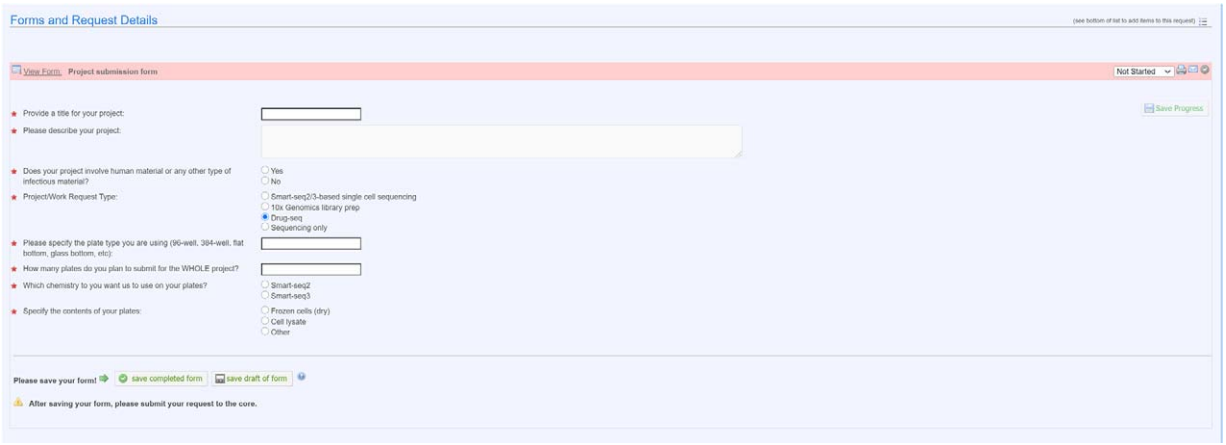
For users working with 10x Genomics droplet sequencing

We need very little information at this stage. Please fill in the fields in the form

Your number of samples is once more not binding, it just gives us a rough idea about the size of the project. As always, save the form, and submit your request.

For users working with Drug-seq

Similar to above, we need little information at this stage. Please fill in the form.



The question on plate number is once more for us to estimate the workload associated to the project, it does not have to be exact.

If you ship us frozen cells, you will see an additional question about cell number/well. If you send lysed cells, you will need to provide a concentration of RNA. If you don't know the RNA concentration, state the cell number before lysing the cells so that we can estimate the amplification cycles needed.

Save your form, submit your request.

Sequencing only projects

These projects are usually rather straightforward and we need little information at this stage.

Under sequencing parameters, please specify as detailed as possible. Also note that the HiSeq3000 can't run partially, so if you have very specific sequencing needs, you will need to fill the flow cell completely and pay for a full run.

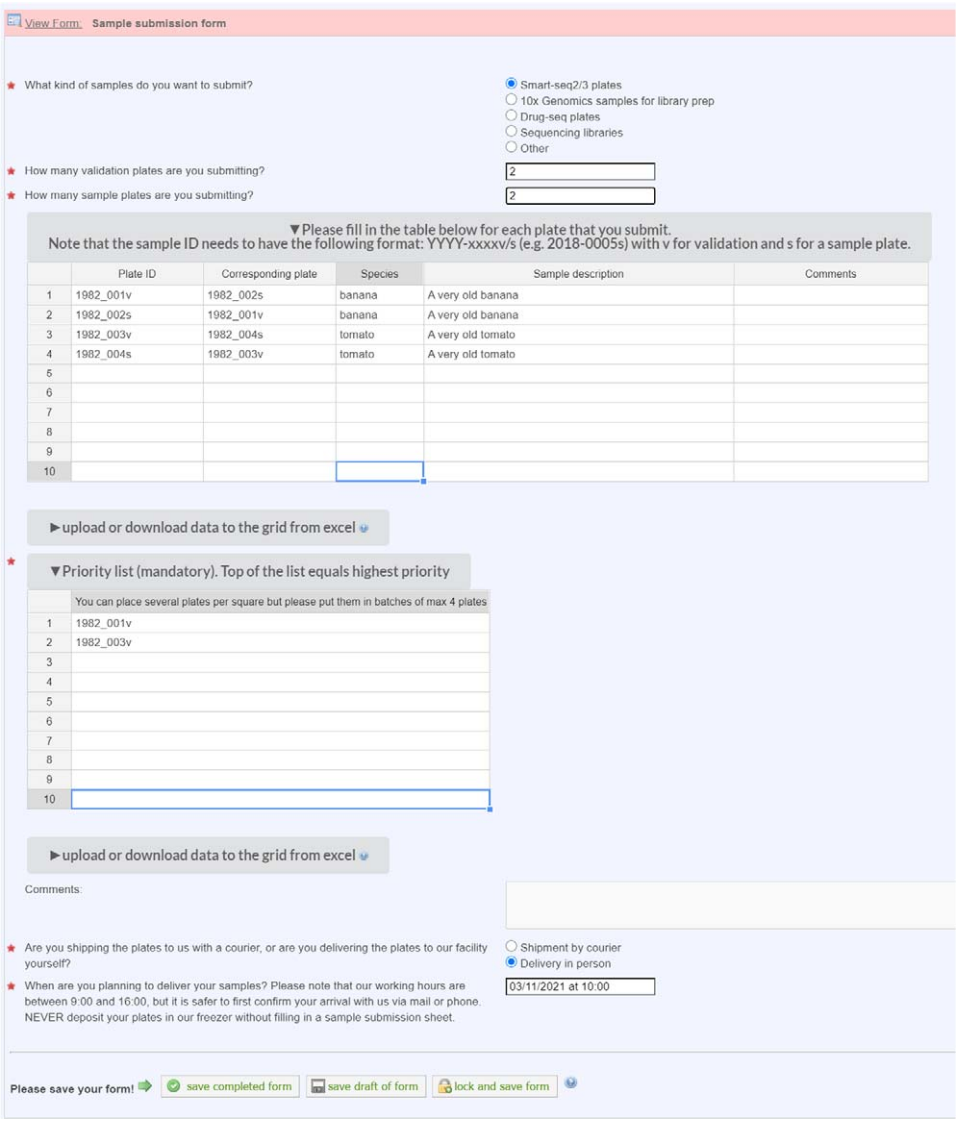
Save your form, submit your request.

Phase 3. Sample submission

Note that you can also select the “other” option if the standard choices don’t fit the default options

Smart-seq2/3

This is where the bulk of the interaction with SICOF happens. You will need to file a request each time you are delivering samples to us. Please fill in the form as shown below.



For Smart-seq2/3 we will need detailed plate information. Make sure you are typing the correct plate numbers **(Including year)** to prevent any mix-up of plates. You also need to provide a list of plates that you want us to amplify and QC first.

Save the form, submit the request.

A note on QC and stopping points.

From March 2021, we implemented stopping points after QC to enhance our communication to our users. This means that you **NEED** to confirm how you want to proceed after QC each time we send you the results, even if the QC looks perfect. If we do not receive a reply to the email/message with the QC results, we can’t proceed with your plates.

When we do a QC of your plates, we primarily look at two parameters. 1) The size of the cDNA and 2) the concentration. The former is an indication on degradation of the sample, while the latter provides information on the amount of cDNA present. Low amounts of cDNA can indicate that cell fragments were sorted instead of whole cells. Based on the result, we classify the plates in three categories: Green, yellow and red.

Green indicates that all parameters look fine. We recommend that we proceed with the low volume cheaper pipeline and foresee no problems in library prep. In the unlikely case that a library prep would fail for a green sample plate, SICOF will stand for the cost of reattempting library prep for a total of 2 additional attempts.

Cost clarification:

Successful library prep = the user pays for the library prep service

Failed library prep which succeeded on the 2^{nd}/3^{rd} attempt = the user pays for one library prep service

Yellow indicates that the quality is sub-optimal. We will not be able to use our low input pipeline for library prep but can use the classical, more expensive high-volume pipeline. We foresee that the plate will yield a usable library, but can't guarantee success. If the library prep would fail, we will attempt one more time without additional cost. If the library prep fails a second time, another attempt can be made but the user will stand for the library prep cost of the third attempt regardless of the result.

Cost clarification:

Successful library prep after 1^{st}/2^{nd} attempt = the user pays for one library prep service

Successful/failed library prep on the 3^{rd} attempt = the user pays for two library preps.

Red indicates that the quality is very low and success can't be guaranteed. However, we have had several cases of successful library preparation of red plates. In our experience, low concentration is less detrimental compared to degraded RNA. The user decides if to attempt a library prep and the library prep service costs will be charged to the customer regardless of the outcome.

Cost clarification:

Successful/failed library prep after x attempts: The user pays for x library prep services, with x referring to how many times SICOF has tried to prepare the library

Disclaimer: We will always clearly communicate the additional costs for extra attempts.

This section will be updated at a later point with QC examples of green, yellow and red plates.

Types of QC

At the moment, SICOF provides QC on nucleotides using Agilent BioAnalyzers and an Agilent Fragment Analyzer. The latter has the same sensitivity but superior cost-efficiency. We therefore will always use the Fragment Analyzer for QC unless you specify that you would like to have a BioAnalyzer analysis.

Note that we are charging always for the total of samples as stated in the service description. E.g. if you submit 15 samples for BioAnalyzer analysis, we charge for 22 samples (2 x 11). If you submit 40 samples for Fragment Analyzer analysis, we charge for 48 samples (3 x 16).

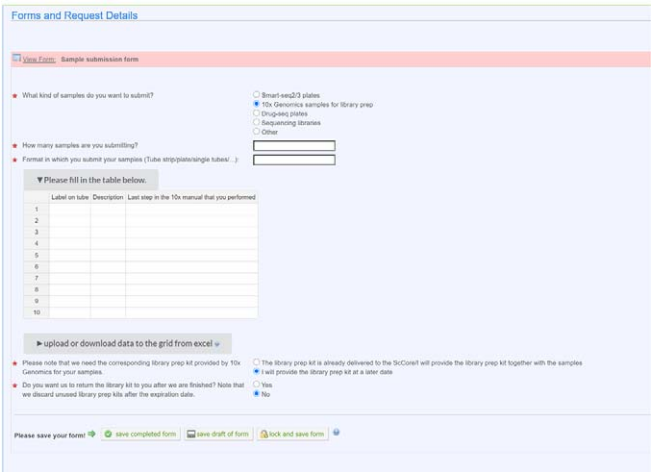
A BioAnalyzer QC will be done using the High Sensitivity DNA Kit (cat: 5067-4626). For the Fragment Analyzer, the HS NGS Fragment Kit (cat: DNF-474-0500) is used. If you would like to run another assay

on one of these instrument, SICOF can run that assay for you, but you will need to provide the reagents. The leftovers of the reagents will be returned to you. The cost for this service will be decided upon beforehand.

10x Genomics

Currently, we are unable to help you with loading your cells onto a 10x controller. We would kindly refer you to the Bioinformatics and Expression Analysis Core Facility (BEA) for this: http://www.bea.ki.se/

We however can help you prepare libraries. You will need to provide the 10x Genomics kit to us though, which we will deliver back to you after your libraries are prepared.

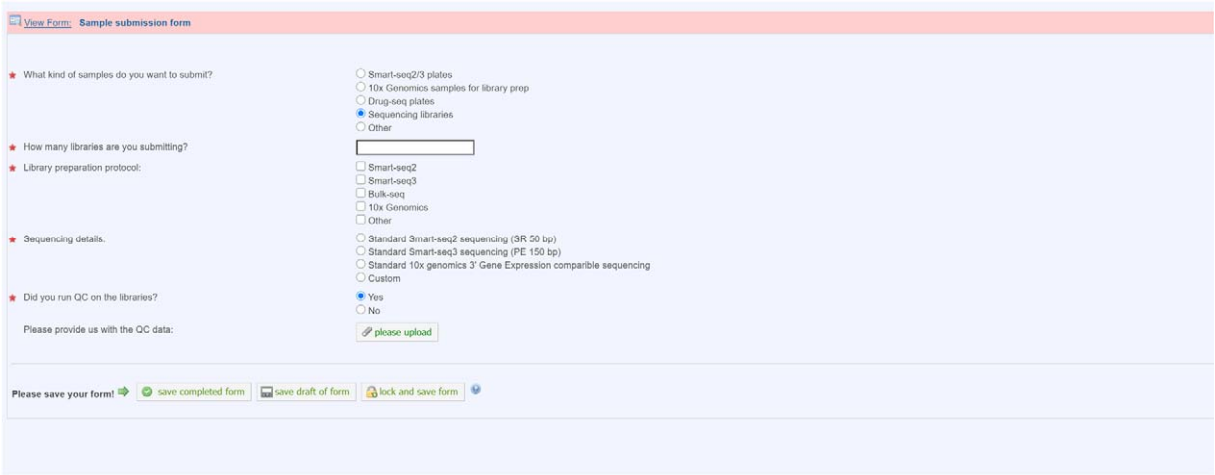


It is critical that you inform us of the last step that you performed in the 10x library prep protocol, we can't discriminate the sample by eye. Save the form, submit the request.

Drug-seq

This sample submission is very similar to submission of Smart-seq2/3 plates, please see that section.

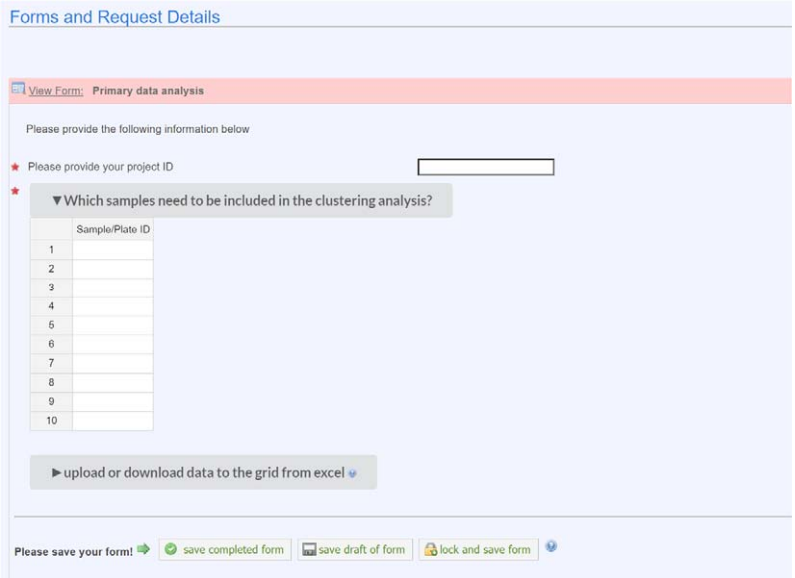
Sequencing libraries



For "Standard 10x Genomics" sequencing, we will forward your samples to the BEA core facility to be sequencing on a Nextseq500/2000. The HiSeq3000 of SICOF is not ideal for sequencing 10x libraries. Standard sequencing will aim for 50.000 reads/cell.

Phase 4: Primary data analysis

At SICOF, we aim to provide you with meaningful data that can be browsed by scientists without the need for a bioinformatician. We will however only be able to provide you with the data according to our pipeline. We are at the moment unable to assist in customized data analysis, such as specific graphs, color changes, detailed comparisons outside of the main pipeline, etc. We strive to expand and enhance the details on our pipeline continuously and will keep it up to date with new clustering algorithms.



You will need to provide us with the plates/samples to be included in the analysis. At the moment, we can't provide you with the option to divide samples by genotype/time point/patient group etc., but we are working on including this. It should be live in the near future.

Communication with SICOF (please read).

This chapter is very important, as it describes the way that we communicate with you the status and results of your experiments. We try to limit the use of emails as much as possible.

On each submitted request, you will see a comment section. It is not easy to spot though:

We know, iLab hides it well… Note that you can only comment on submitted requests, not on drafts.

On the left side, you have the comment section, where you can find a history of the communication with SICOF. On the right side, you have the option to attach files or URL's. This is where we will provide links to download your data, as well as QC results.

Most of your commenting will be in the Sample submission request (Phase 3), where we will discuss QC results and steps to proceed. It is here where the decision on the pipeline to be used is taken.

For your project, you will usually see the same person communicating with you. However, **the person who replies to your messages is not necessarily the person who performs the experimental work on your samples**. We work continuously as a team and there is never a single person that is responsible for a single step in our pipeline. It is therefore important that you never email a single person in the core, as that information might get lost. Always use the comment section in iLab.

Authorship

As a core facility, we don't demand co-authorship on publications that contain data generated by SICOF, unless you as a costumer thinks it is warranted. However, we need to register and document all projects in order to secure funding for our operation. Therefore, we request that users include the following statement in the acknowledgement section of their publications:

"We would like to acknowledge the Single cell core Facility @ Flemingsberg campus (SICOF), Karolinska Institute, for their single cell sequencing services."

You may adjust the last part of the statement depending on the services that we provided.