General Guidelines for nCounter Core Facility

Starting a project

To start a project at nCounter Core Facility you should first register on our [iLabs nCounter Core Facility site](#) and ask for a consultation:

To register for an account:
To get started, you must register for an account:
1. Navigate to the core page:
https://eu.ilabsolutions.com/service_center/show_external/3574
2. In the upper-right-hand corner click 'SIGN-UP' You should be navigated to a screen that will walk you through the registration steps.
3. Submit the completed registration form.
4. Receive a *Welcome Email* from iLab (typically within one business day) with login credentials and basic instructions on using the system at this time.

To Create a Consultation Request:
Using your iLab login credentials, you are supposed to first of all place a consultation request.
1. Navigate to the core page:
https://eu.ilabsolutions.com/service_center/show_external/3574
2. In the upper-right-hand corner click 'LOGIN'.
3. Enter your email address and password, and sign in.
4. Select the *Request Services* tab and click on the *'Request Consultation'* button next to the service of interest.

Based on that, will setup a meeting with nCounter Core Facility staff to review the planned project, how we can best serve you and to discuss how to proceed. Thereafter, you place a service request into the system and download the *Terms of Use* sheet, sign it and upload it to the system.

To Create a Service Request:
Using your iLab login credentials, you can place a service request.
1. Navigate to the core page:
https://eu.ilabsolutions.com/service_center/show_external/3574
2. In the upper-right-hand corner click 'LOGIN'.
3. Enter your email address and password, and sign in.
4. Select the *Request Services* tab and click on the *'Request Service'* button next to the service of interest.
5. You will be asked to complete a form before submitting the request to the core.
6. Your request will be pending review by the core. The core will review your request and either Agree to the work or they will ask for more information if needed.

All further steps will be handled via the iLabs platform. Once your samples are ready to be submitted, please upload all information in the *Sample Submission Form*, sign this as well and upload it in your service request in iLabs.

What analyses options do I have?

Expression analyses can be performed from total RNA (miRNA, mRNA, ncRNA), protein or crude cell lysates. Analyses of copy number variations (CNVs) are performed using genomic DNA samples, we offer also karyotyping and ChIP-based DNA analysis. We can also profile miRNA and mRNA or RNA and protein from one sample. Within the 3D Biology approach we can even profile on DNA, RNA and protein level from one specimen. Current analysis formats are 12, 24 up to 72 samples in parallel in one experiment.

What is the nCounter workflow?

The diagram below outlines the workflow. After the iLabs based consultation and project planning and discussion with us, you will design and order your CodeSet/oligo set/ or pre-designed panel from nanoString (www.nanostring.com) directly. In order to speed up things, we ask you to let ship the nanoString consumables to our Core Facility. You will only have to deliver the DNA/RNA/ protein material or crude cell lysate together with a signed hard copy of the *Sample Submission Form* to the facility.

Furthermore, all information has to be uploaded in your service request in iLabs.

As outlined above, please be aware that you have to register for an experiment iLabs nCounter Core Facility site in advance. After sample and CodeSet/ElementsTagSet/ nCounter Panel receipt, we will perform a quality control (QC) step of your samples. If these pass QC, we will proceed with the analysis. The QC report will also be uploaded in your project in iLabs.

Our service includes sample QC, hybridization, samples processing, nCounter analysis, data QC and temporary storage and data delivery.

Data analysis by the nCounter Core Facility includes QC. For detailed data analysis nanoString provides the nSolver™ Analysis Software. This programme enables quick and easy QC, normalisation, and analysis of data without the need to purchase additional software packages. Furthermore, data visualisation including box plots, heat maps, scatter plots, violin plots and histograms is possible. We offer nSolver™ Analysis Software trainings once a year, please ask for the next opportunity to get trained.
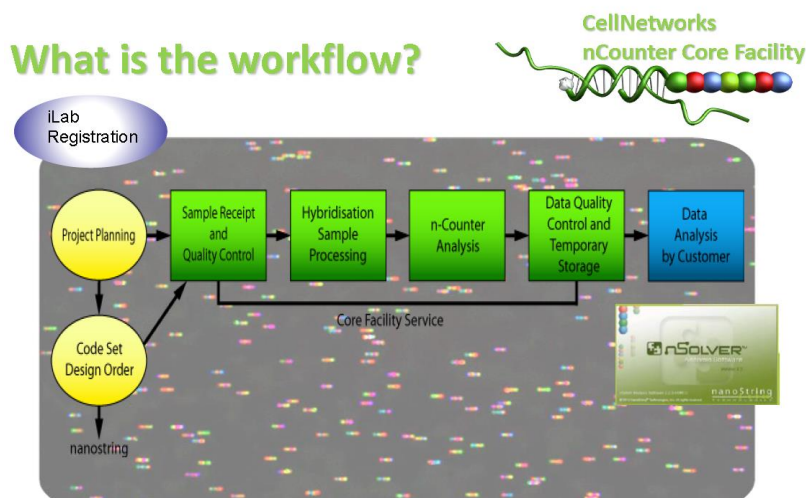
How are samples checked prior to the experiment?

For project planning, we take quality and quantity of your samples into account. Therefore, we ask you to share not only DNA/RNA concentration but also absorption spectra data, that is OD_{230}, OD_{260} and OD_{280} as well as respective ratios $OD_{260/230}$ and $OD_{260/280}$ as retrieved from e.g. nanodrop system. If not available, we will also perform this step.

All total RNA samples are subsequently checked for their concentration and integrity using the Agilent Bioanalyzer 2100 and Qubit system. Therefore, we need an extra amount of input material for QC (see table below).

If samples fail to meet incoming quality checks they will be rejected with a given reason.

Please be aware that we have to charge you an extra QC fee.

What about storage of my Sample?

As with many labs, freezer space is limited. We will therefore not be able to store your samples and CodeSets/TagSets/nCounter Panels indefinitely. As a consequence, we will retain samples and CodeSets/TagSets/nCounter Panels for a maximum of 6 months; it is your responsibility to collect any remaining samples and CodeSets/TagSets/nCounter Panels from us once we have released the data, or in the worst case if there is a preparation problem and we cannot continue. You will receive an e-mail informing you that the samples, cartridges and CodeSets/TagSets/nCounter Panels will be discarded if not picked up. There will be no exceptions. In case of shipping back you have to take over the shipment costs.

What data do I receive?

A zip folder containing the nCounter raw data RCC files, the respective RLF file (This file contains all information's about your CodeSet), the QC Bioanalyzer data as pdf file and the Qubit and nanodrop data as excel file will be uploaded in iLabs.

What is the name of my file?

- The separate raw data files are included in a zip folder named in the following manner:
- iLabs_ID.nCounterResults_RawData_CustomerID.ZIP
- Raw data files are named in the following manner:
- Sample Name_Lane ID.RCC
- Sample's name is that which you registered your sample with.
- Run ID is the tracking number we provide.

How long do you store my data?

All data will be stored for 6 months after the respective CodeSets/TagSets/nCounter Panels is finished. It is your responsibility to save and back up your data, we cannot do this for you. After the 6 months your data will be automatically deleted.

Sample requirements

nCounter analysis requires on average 25-150 ng of total RNA depending on the application per biological replicate for gene expression and miRNA assays and 300 ng of genomic DNA per biological replicate for CNV assays (for detailed description see tables below).

All total RNA samples are subsequently checked for their concentration and integrity using the Agilent BioAnalyzer 2100 and Qubit system. Therefore, we need **an extra amount of 2.3 µl RNA/DNA**. Based on our experience, nanodrop measurements often differ compared to the more sensitive Agilent BioAnalyzer 2100 and Qubit analysis.

All samples must have a concentration between 25-100ng/µL (RNA) or 85-600ng/µl(DNA). For miRNA assays, the minimum concentration is 33ng/µL. For CNV assays the minimum DNA concentration is 85ng/µl.

RNA QC: To ensure optimal assay performance, NanoString recommends purified RNA have a $OD_{280/260}$ of 1.9 or greater and an $OD_{260/230}$ of 1.8 or higher.

Storage Solution: The storage solution should be RNAse free aqueous solution and can be water, TE, Tris, etc.

Technical Replicates: If you would like to have a sample run more than 1 time, please indicate the number of replicates you desire.

Sample Type	nCounter Analysis System (GEN1/GEN2)	nCounter *SPRINT* Profiler
FFPE-derived RNA	300 ng	150 ng
Total RNA (CodeSets < 400 genes)	100 ng	50 ng
Total RNA (CodeSets > 400 genes)	50 ng	25 ng
Fragmented DNA	300 ng	150 ng
ChIP DNA (unamplified)	10 ng	5 ng
ChIP DNA (whole genome amplification)	100 ng	50 ng
Single Cell or Low Input Material	up to 8 µL of amplified sample	up to 5 µL of amplified sample

Initial Number of Cells	Recommended Lysis Buffer	nCounter Analysis System (GEN1/GEN2)	nCounter *SPRINT* Profiler	Sample Volume (either system)
50,000 cells or more	RLT or other buffer with a high concentration of guanidine isothiocyanate	~6,500 cells/µL	~3,500 cells/µL	Up to 1.5 µL
50,000 cells or less	iScript™ RT-qPCR Sample Preparation Reagent or other detergent/chemical lysis buffer	~2,000 cells/µL	~1,000 cells/µL	Up to 5 µL

Citing our Service

Please cite our service in the acknowledgements section of your publication in case we have just performed the standard service. The following statement may be used:

"We thank the nCounter Core Facility Heidelberg for providing the nCounter system and related services."