Start up a MiSeq sequencing project

1. Buy necessary kits and reagents

Sample prep kits are bought and delivered to your own lab. The sample prep will be performed at your own lab.

In order to avoid transporting the sequencing reagents up and down with risk of thawing, make sure to use our delivery address and contact person for sequencing kits: (UNIV ANTWERPEN-CHARLOTTE CLAES, DEPARTMENT OF MEDICAL GENETICS, PRINS BOUDEWIJNLAAN 43, EDEGEM, 2650, BELGIË).

Please forward the mail with your order and shipping notification to ngs@uantwerpen.be . This way we know what to expect. We'll take care of the package as soon as it arrives and store it in the correct way. For Illumina kit and pricing information, contact customerservice@illumina.com.

2. Schedule your experiment

Book a run with the online reservation tool (http://ngs.uantwerpen.be/booking/). Contact ngs@uantwerpen.be to create a new account.

- Log in/ create a new account
- my account – profile: contains your personal information
- my account – notification preferences: settings for email notifications
- schedule – resource calendar: overview of all planned runs, can be filtered by equipment
- schedule – my calendar: overview of all your personal runs
- To make a reservation: schedule – bookings:
✓ Select the preferred equipment on an available date, a reservation screen opens.
✓ Choose start and end point (take into account the min/ max reservation time). Please take the following runtimes into account when making equipment reservations

MiSeq run time	
Read Length	**Total Time**
MiSeq Reagent Kit v2	
1 × 36 bp	~4 hrs
2 × 25 bp	~5.5 hrs
2 × 150 bp	~24 hrs
2 × 250 bp	~39 hrs
MiSeq Reagent Kit v3	
2 × 75 bp	~24 hrs
2 × 300 bp	~65 hrs

✓ Entitle the run with a clear name containing your institute and project.
✓ Complete the entire form
✓ After saving, your reservation will be send for approval to the administrators.
✓ Twice a week, administrator will approve the reservations.

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3. Perform sample prep

Check you've all the user-supplied materials, instruments and reagents available.

4. Create sample sheet

Create a sample sheet in <u>Illumina Experiment Manager (v1.9-11)</u> using the correct settings.
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Create sample sheet-> MiSeq -> other -> fastq only
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Don't use: ',;-. or spaces in the samplesheet, only _ is allowed.

OPTION 1: For 'Sample Project', fill in the correct data destination folder for each sample For

Data destination	Sample Project	Notification
FTP server	Real_Name@F	Automatic email notification will be send when run or analysis is finished.
Exome pipeline only for registered Galaxy users	Real_Name@E	
CLC Genomics Workbench only for registered CLC users	Real_Name@C	
Galaxy only for registered Galaxy users	Real_Name@G	
Galaxy en CLC only for registered galaxy and CLC users	Real_Name@B	
UZA SeqPilot only for registered UZA users	Real_Name@S	
/home folder only for command line users	Real_Name@H	

For correct "Real_Name", contact NGS team

Data destination	Sample Project	Notification
FTP server	Real_Name@F	Automatic email notification will be send when run or analysis is finished.
CLC Genomics Workbench	Real_Name@C	
Galaxy	Real_Name@G	
Galaxy en CLC	Real_Name@B	
UZA SeqPilot	Real_Name@S	

For correct "Real_Name", contact NGS team

OPTION 2: For 'Investigator Name', fill in your email address. Data will be send to FTP server.

Deliver the sample sheet to us (<u>ngs@uantwerpen.be</u> or USB key) <u>at least 24h before the run starts</u>.
The responsible will copy the sheet to a server. No USB keys are plugged into MiSeq!

5. Quantification of the library

An accurate quantification of the library is very important. For MiSeq applications we prefer <u>Qubit measurement.</u> The delivered library has to be diluted to 2nM (v2 or v3) or 4nM (v3).
As NexteraXT protocols include a normalization step, no concentration measurement is needed.
Multiplicom MASTR Plus libraries need to be measured with nanodrop because of an interfering component in one of the Qubit reagents.

6. Actual sequencing at CMG

Deliver your library <u>at least 24h before the run starts</u>. Only trained people can start up the MiSeq sequencer.

If you're not trained starting up the sequencer will be done by one of the responsible for Miseq of CMG. Depending on the need, we organize MiSeq training from time to time. Keep in mind, only people who handle MiSeq at least once in 6 months will retain their authorization.

7. Data extraction

A notification will be send to you, when your run is finished. After downloading and storing your data, please delete them on the FTP server.

By default, data will be deleted from our servers automatically after 3 months.