Sequencing at the Research Sequencing Facility

Thank you for considering sequencing at our facility! This letter contains a workflow to make communication as clear as possible and a list of requirements of your NGS libraries in order to guarantee quality.

Workflow of sequencing

Contact the Research Sequencing Facility:

Send an e-mail to Diana Spierings (research.sequencing.facility@umcg.nl) and mention:

1. What type of sequencing run you want us to perform (*e.g.* high-output single read 75 bp).
2. If you have a custom primer and/or protocol.
3. How much PhiX you want to spike-in (minimum of 5% is required by Illumina).
4. When you can have your sequencing pool ready.
5. Whether you will need assistance with bioinformatics analysis.

Please let us know if we can help you with making these decisions.
Diana will schedule a run date for you.

Library pool transfer:

1. The library pool needs to be delivered before 12 o'clock on the day of sequencing, but preferably a day in advance. Please contact Diana as soon as the appointment cannot be met. Depending on our personal schedule we can still start the run at a later time point or decline the run scheduled on that day. Depending on how busy the sequencing pipeline is at that moment, the run might be delayed for a week, therefore the 4 nM dilution and pooling may need to be redone.
2. The library pool can be given directly to one of our technicians or by putting it in the 'ready to sequence' box in the -20°C freezer at the 1st floor lab (shown to you by one of our technicians).
3. Besides the library pool, you need to submit a filled-in and signed '***Checklist Before Sequencing***' form together with the quality & quantity measurement information. This can be done on paper but preferably by e-mail.
4. If the sequencing facility will do the demultiplexing, please provide us with a sample sheet that contains the unique names of the samples and the corresponding readed sequences of used primers. Please note the following:
    - Each unique index needs a unique sample name; a sample name cannot be linked to multiple indexes.
    - Sample name has a maximum length of 30 characters (incl. space).

After sequencing:

1. We will make the data available for you via a sftp site.
2. If the library pool needs to be returned, please contact one of our technicians within two weeks after the sequencing run. By default, we throw pools away after 6 months.

Requirements of library pools

To guarantee high sequencing quality, the quality and concentration of your libraries need to be determined as accurately as possible using a capillary electrophoresis system (*e.g.* Agilent TapeStation) and quantitative assay (*e.g.* Qubit dsDNA HS) respectively, as described in our "***NGS Library Quality and Quantity Control***".

If some of these requirements cannot be met, please contact the Research Sequencing Facility about this in advance and we will try to adjust it to make it work anyway.

1. The library pool should to be approximately 4 nM, (range of 1,5 nM - to 8 nM).
2. The minimum total volume of the library pool should be 10 µl.
3. The pool should be made less than a week before the sequencing day and stored at -20°C until sequencing.
4. The library pool fragment size (including extended adapter sequences) should be between 200 bp to 1000 bp, measured using a capillary electrophoresis system (if this is not the case, please mention this in advance).
5. There should be no adapter-dimer contamination (maximum of 10% is allowed).
6. Samples that are included in the pool require a post-PCR clean-up. This is standard in most library preparation kits, but not in all.
7. Label the tube (use a printed label) with pool name, molarity, and date, all in accordance to the submitted checklist.

Please let us know if you need help with making the libraries, quality assessment, quantification or pooling. We can help or teach you if necessary.

Kind regards,

The Research Sequencing Facility team

umcg.nl/nl/web/research/w/research-genome-center

research.sequencing.facility@umcg.nl