Sequencing at ERIBA 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 ERIBA 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 bioinformatic analysis.

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

Delivery of library pool:

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 '***ERIBA Checklist Before Sequencing***' form together with a sample sheet and the quality & quantity measurement information. This can be done on paper but preferably by e-mail.
4. The sample sheet contains the names of the samples and their index sequences. Please note that there is a maximum length of 30 characters (incl. space).

After sequencing:

1. We demultiplex your samples and 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 Bioanalyzer) and Qubit dsDNA HS assay respectively, as described in our "***ERIBA NGS Library Quality and Quantity Control***".

If some of these requirements cannot be met, please contact Diana 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. 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 ERIBA Sequencing Facility team

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