User QC

Introduction

User QC is an application used for projects where the user wants to save money when preparing 96 samples with either Nextera XT or ThruPlex sample preparation. The decreased library preparation cost can be obtained when skipping incoming QC and individual library QC.

Please note that the user will be asked to pay for the project regardless of the results. However, if library prep completely failed there will not be any sequencing cost.

Laboratory setup

The users prepare the reaction plate themselves, by sending in

- 1 ng in exactly 5 µl EB/MQ (Nextera XT)

or

- 1-10 ng in exactly 10 µl EB/MQ (ThruPlex),

in each well of a 96 wells plate. This will be enough material for one preparation.

Average fragment lengths are somewhat fixed. Nextera XT can only generate libraries with in average around 450-600 bp and ThruPlex 150-400 bp.

Samples will be prepared according to relevant protocol (Nextera XT or ThruPlex) and finished DNA libraries will be pooled with an equal volume before sequencing. QC will only be made on the pool.

Sequencing setup

Pool is sequenced according to the order, and the ordered number of total reads will be delivered. However, user is not guaranteed to receive the QC-limit of number of reads per sample. If some samples don't receive any reads, they will not be re-sequenced.

Results from an example project

We prepared 63 bacteria samples using Nextera XT, pooled them by taking equal volumes of each (see Figure 1 for the Bioanalyzer trace) and sequenced them on a HiSeq Rapid Mode lane, 2x150 bp.
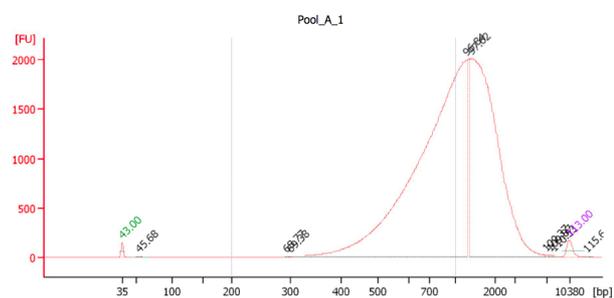

Fig 1. Bioanalyzer HS electropherogram of the pool with the 63 samples. The smear has an average fragment length of 1200 bp.

The pool received the number of ordered reads, however the number of reads per sample varied. Sequencing evenness of the pool can be seen in figure 2. As can be seen, two samples had far fewer reads than the others and this might be commonly observed in the User QC project setup. We can't guarantee an even number of reads per sample.
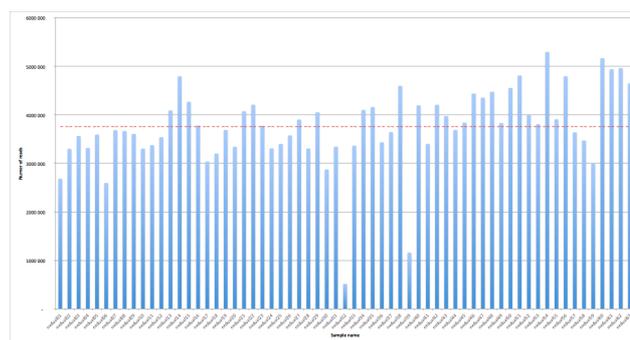

Fig 2. Sequencing results displaying the evenness of the pool, y-axis shows the number of reads and x-axis shows the samples. The red dotted line is the average number of reads per sample in the pool.

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