Finnish Functional Genomics Centre – FFGC

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Approved for use

Project

Project number	200022 Ruuskanen
Project title	Glyphosate and DNA methylation
Number of samples	100
Application	Reduced representation bisulfite sequencing
Sample material	Genomic DNA
Low quality sample form filled	No

Sample Quality

The quality of the samples was ensured using Agilent Bioanalyzer 2100 or Advanced Analytical Fragment Analyzer. Sample concentration was measured with Qubit® Fluorometric Quantitation, Life Technologies, and/or Nanodrop ND-2000, Thermo Scientific. General guide lines for reference values of each measurement are described at the end of this document.

Bioanalyzer	N/A	
Fragment Analyzer	Pass	GQN 9.0-9.9
Qubit	Pass	
Nanodrop	N/A	
Sample QC file attached	no	
Exception	-	

Library Preparation

Library preparation was performed according to the library preparation protocol.

Library preparation protocol 1	Reduced representation bisulfite sequencing, Boyle et al, 2012
Library preparation protocol 2	N/A
Starting amount	500 ng
FFGC positive control	Coriell Institute NA12878
Library preparation kit 1	Reduced representation bisulfite sequencing (Custom)
Library preparation kit 2	MethylCode Bisulfite Conversion Kit (Invitrogen)
Index kit	TruSeq DNA Index Kit (Illumina)
Exception	-

Library Quality

The quality of the samples was ensured using Agilent Bioanalyzer 2100 or Advanced Analytical Fragment Analyzer. Sample concentration was measured with Qubit® Fluorometric Quantitation, Life Technologies, and/or KAPA Library Quantification kit for Illumina platform, KAPA Biosystems. General guide lines for reference values of each measurement are described at the end of this document.

Bioanalyzer	N/A	
Fragment Analyzer	Pass	Average fragment size 345 bp
Qubit	Pass	
qPCR	N/A	
Library QC file attached	no	
Exception	-	

Next Generation Sequencing

Sequencing run was performed using Illumina NovaSeq 6000 or MiSeq instrument. Sequencing data yield and quality is dependent on the library quality. FFGC quarantees only the technical quality of the run which is not dependent of the biological nature of or quality of the samples.

Instrument / run type	NovaSeq 6000 SP NovaSeq 6000 S1
Read length	1 x 100 bp
Number of lanes	6
Number of pools	5
PhiX v3 Control spike-in	5%
Expected Q30 values	≥ 85% bases higher than Q30 at 2 × 50 bp NovaSeq 6000 run
Typical data yield	650-800 M reads/run (2 lanes) from NovaSeq 6000 SP run 1300-1600 M reads/run (2 lanes) from NovaSeq 6000 S1 run

Data Extraction

Raw data delivery format	fastq
Automatic adapter trimming	Yes
Base calling	bcl2fastq2 conversion software (NovaSeq 6000)

Publications

Please acknowledge the use of our services in any resulting publications with the statement: “This study was supported by Finnish Functional Genomics Centre, University of Turku and Åbo Akademi and Biocenter Finland”. Please inform the Finnish Functional Genomics Centre about the publication.
More information about Illumina next-generation sequencing from http://www.illumina.com/.

General guidelines for sample quality

DNA/RNA concentration

The expected concentration varies from sample to sample and the concentration needed for downstream analysis varies from application to application. Usually Qubit concentration measurement is used for library preparation. NanoDrop and Fragment Analyzer concentrations are used only for comparison purposes.

RNA purity and integrity

Integrity is based on ribosomal RNA subunit peaks 28S and 18S. Intact RNA measures Bioanalyzer RIN/ Fragment Analyzer RQN value of 10. Range 8-10 is considered high quality, values >7.0 are acceptable. Total RNA purity is evaluated using Nanodrop absorbance ratios, A260/A280 and A260/A230. Values are normally close to 2.0. Impurities and degradation lower the values effectively.
For low quality total RNA samples RIN/RQN value is < 7.0, but all samples should be similar and equally degraded. Only fragments above 150 bp can be used for library preparation. FFGC does not guarantee results for samples with low RNA quality.

DNA purity and integrity

Fragment Analyzer software calculates the percentage of DNA above the threshold, producing a GQN value of 0–10. A low GQN value (< 2.5) represents sheared or degraded DNA. A high GQN (> 9) represents undegraded DNA. There is no strict GQN value cutoff for deciding sample acceptability. If NanoDrop measurements are available, the ratio of A260/280 and A260/A230 can be used as an indication of sample purity. Values of 1.8–2.0 indicate relatively pure DNA.

General sample quality within the project

If there are considerable quality differences between the DNA/RNA samples, it may lead to false results in further analysis, e.g. degraded samples will give more false negative results than intact samples. Thus, all samples that are to be compared to each other should be approximately of equal quality.

General guidelines for library quality

Library concentration and fragment size

Library validation is based on the library quantitation and quality check. Ready-made individual libraries should have a concentration of at least 1-5 nM and 10 µl volume depending on the library type. Library fragments should have compatible size distribution with selected read length and sequencing instrument according to library preparation protocol's recommendations.

Adapter dimer and secondary peaks

Adapter dimer peak is a sharp peak at ~100-150 bp libraries (size may vary based on the library type). If adapter peak is seen in the library QC run results, the final bead purification of the library preparation protocol should be repeated.
In some library types it is normal to see a secondary peak, as a concatemer of the library. Depending on the library preparation protocol, this type of library might require qPCR quantitation in some cases.

General library quality within the project

All library concentrations and fragment profiles should be similar within the sample set. If library profile fragment size distribution vary significantly compared to the other libraries within the project, library is prepared again from the beginning. If one or more libraries have lower concentration, but have good quality and enough material for sequencing, library preparation is not repeated.