UNIVERSITY OF HELSINKI

FuGU

CUSTOMER

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PRODUCT DESCRIPTION

ORDER DETAILS

Order number: 1500133 Smarter Ultra Low input Kit + Nextera XT + NextSeq Mid Output 2x150 bp

This order contains following services:
2 x Illumina NextSeq Mid Output 2 x 150 bp
2 x Bioanalyzer RNA Quality Control Assay
4 x Sequencing Library Prep

DETAILED PRODUCT INFORMATION

Sample	totRNA concentration ng/ul	RNA concentration, Qubit	Bioanalyzer total RNA RIN	Sequencing Library concentration
S1. Xylem tracheids	-	too low	2	4,13
S2. Whole sections A	3.96 ug/ml	62	7,8	1,48
S3. Whole sections B	5.3 ug/ml	75	8,6	0,984
S4. Ray cells	2 ug/ml	too low	7,2	0,974

Sample	Library size in bp	Sample pool	Index number	Index sequence
S1. Xylem tracheids	664	pool 1	N701	TAAGGCGA
S2. Whole sections A	893	pool 2	N702	CGTACTAG
S3. Whole sections B	817	pool 2	N703	AGGCGAA
S4. Ray cells	795	pool 1	N704	TCCTGAGC

Sample	Index 2 No	Index 2 sequence	Concentration for cluster generation (pM)	NextSeq Sequencing Run Number
S1. Xylem tracheids	S517	TCTTACGC	1	150605_NS500683_0013_AH2THCAFXX
S2. Whole sections A	S502	CTCTCTAT	1,3	150609_NS500683_0015_AH2TH5AFXX
S3. Whole sections B	S503	TATCCTCT	0	0
S4. Ray cells	S504	AGAGTAGA	0	0

Sample	Cluster Density	Clusters PF %	Reads PF (M)	Yield (G)

Sample	Cluster Density	Clusters PF %	Reads PF (M)	Yield (G)
S1. Xylem tracheids	152	84,7	110	35,3
S2. Whole sections A	154	83,2	110	34,8
S3. Whole sections B	0	0	0	0
S4. Ray cells	0	0	0	0

Sample	Q30 %
S1. Xylem tracheids	74,2
S2. Whole sections A	76,8
S3. Whole sections B	0
S4. Ray cells	0

PROTOCOLS

Clontech Laboratories, Inc. SMARTer® Ultra™ Low Input RNA Kit for Sequencing - v3 User Manual (120514)

Illumina Nextera XT DNA Library Preparation Guide, Part # 15031942 Rev. E January 2015

Illulmina NextSeq™ 500 System User Guide, Part # 15046563 Rev. F September 2014

METHOD DESCRIPTION

Since the concentration or quality of total RNA from Norwegian Spruce samples S1 and S4 could not be detected with Bioanalyzer nor Qubit all the sample provided by customer was used to generate the sequencing library. 10 ng of samples S2 and S3 was used for sample preparation.

The SMARTer Ultra Low Input RNA Kit for Sequencing - v3 was used to generate high-quality cDNA compatible with Illumina Nextera XT DNA Sample Preparation Kit. The SMARTer Ultra Low Input RNA Kit for Sequencing - v3 incorporates Clontech's patented SMART (Switching Mechanism at 5' End of RNA Template) technology. This technology uses the template switching activity of reverse transcriptase to enrich for full-length cDNAs containing the 5' end of the mRNA and directly add defined PCR adapters to both ends of the first-strand cDNA. The full-length cDNA output of the SMARTer Ultra Low Input RNA Kit for Sequencing - v3 was processed with the Illumina Nextera XT DNA Sample Preparation Kit which simultaneously fragments and tags the adapters to samples. Limited-cycle PCR adds the sequencing indexes and amplifies the tagged DNA.

Next generation sequencing (NGS) sequences millions of small fragments of DNA prepared from an entire genome, transcriptome, or smaller targeted regions in a single run of the instrument. The DNA is first fragmented to form a library of small segments, which are linked to adaptors that enable the fragments to bind to a flow cell in the sequencing instrument. The sequencer clonally amplifies each of these immobilized fragments to generate millions of molecular clusters. Each cluster contains ~1,000 copies of the same template. The sequencing utilizes sequencing-by-synthesis technology, where during every run cycle one fluorescent labelled nucleotide is added to the DNA strand and the fluorescence is detected by laser excitation and high-resolution camera. Two different fluorescent labels represent different nucleotides. Before the addition of another fluorescent labelled nucleotide the label of the previous nucleotide is cleaved. The NextSeq instrument performed a Mid Output 2 x 150 bp run, which should sequence all of the bases of each cluster.

PUBLICATION POLICY

According to our publication policy guidelines the Biomedicum Functional Genomics Unit (FuGU) should be acknowledged in any publication(s) that include results obtained from FuGU.

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DATA CONVERSION

Run folder: 150605_NS500683_0013_AH2THCAFXX
SampleSheet: 150605_NS500683_0013_AH2THCAFXX/SampleSheet.csv
FastQ files: 150605_NS500683_0013_AH2THCAFXX/Data/Intensities/BaseCalls/
Quality files: 150605_NS500683_0013_AH2THCAFXX/Data/Intensities/BaseCalls/FastQC_quality

Files for 150609_NS500683_0015_AH2TH5AFXX respectively.

.bcl ouput files of NextSeq500 [1] were converted with bcl2fastq2 [2] program to .fastq [3] files.
The default setting of the converter removes the adapters given in the SampleSheet.csv.
After conversion, the FastQC [4] quality tool was run for the files and the results saved to the quality folder as .html pages.
Guidance for interpreting the quality plots is provided with the FastQC Help [5].

[1] http://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/nextseq-500-system-guide-15046563-g.pdf
[2] http://support.illumina.com/downloads/bcl2fastq-conversion-software-v216.html
[3] http://en.wikipedia.org/wiki/FASTQ_format
[4] http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
[5] http://www.bioinformatics.babraham.ac.uk/projects/fastqc/Help/

EQUIPMENT

NanoDrop 1000 Spectrophotometer

Qubit Fluorometer
V.1.27

Illumina NextSeq Sequencing System
NextSeq Control Software 1.3.0

Agilent 2100 Bioanalyzer
2100 Expert B.02.08.SI648 (SR2)

CONTACT AND SUPPORTING INFORMATION

For additional information, please visit our web sites: www.helsinki.fi/fugu. You can contact us by email fugu-support@helsinki.fi or by phone +358 2941 25188.

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