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CUSTOMER

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PRODUCT DESCRIPTION

ORDER DETAILS

Order number: 1500072 Smarter Ultra Low input Kit + Nextera XT + NextSeq Mid Output 2x150 bp

This order contains following services:
2 x Sequencing Library preparation
1 x NextSeq Mid Output 2x150 bp

DETAILED PRODUCT INFORMATION

Sample	Sequencing Library concentration	Library size in bp	Index number	Index sequence
Ray Cell 1	2,11	557	N701	TAAGGCGA
Xylem trach.12.2.15	3,70	584	N702	CGTACTAG

Sample	Index 2 No	Index 2 sequence	Concentration for cluster generation (pM)	NextSeq Sequencing Run Number
Ray Cell 1	S501	TAGATCGC	1,0	150417_NS500683_0007_AH2HGLAFXX
Xylem trach.12.2.15	S502	CTCTCTAT	1,0	150417_NS500683_0007_AH2HGLAFXX

Sample	Cluster Density	Clusters PF %	Reads PF (M)	Yield (G)
Ray Cell 1	173	86	127	40,7
Xylem trach.12.2.15	173	86	127	40,7

Sample	Q30 %
Ray Cell 1	76,8
Xylem trach.12.2.15	76,8

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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

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

METHOD DESCRIPTION

Concentration or quality of total RNA from Norwegian Spruce samples could not be detected with Bioanalyzer nor Qubit. All of the sample provided by customer was used to generate the sequencing library.

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.

DATA CONVERSION

Run folder: 150417_NS500683_0007_AH2HGLAFXX
SampleSheet: 150417_NS500683_0007_AH2HGLAFXX/SampleSheet.csv
FastQ files: 150417_NS500683_0007_AH2HGLAFXX/Data/Intensities/BaseCalls/
Quality files: 150417_NS500683_0007_AH2HGLAFXX/Data/Intensities/BaseCalls/FastQC_quality

.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/

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Genome Profiling Services: +358 50 442 0228

EQUIPMENT

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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