

Genome Analysis Platform REPORT

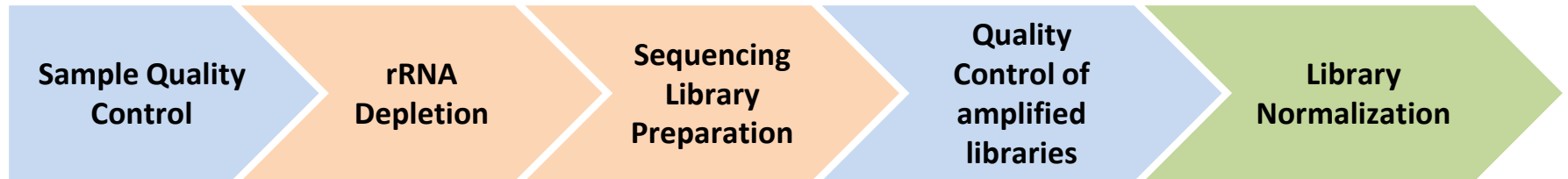
MG-04_TotalRNA

Illumina® Stranded Total RNA Library preparation

➤ Summary of materials and methods

- The quantity and quality of the RNAs were evaluated using Qubit RNA HS Assay Kit (Thermo Fisher Scientific, Cat.# **Q32855**) and Agilent RNA 6000 Nano Chips (Agilent Technologies, Cat.# **5067-1511**), respectively. Sequencing libraries were prepared using “**Illumina® Stranded Total RNA Prep, Ligation with Ribo-Zero Plus**” kit (Illumina, Inc. **Part # 20040525**), following “**Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus Reference Guide**” (**Document # 1000000124514 v02**)”.
- Starting from **1000** ng of total RNA, rRNA was depleted and remaining RNA was purified, fragmented and primed for cDNA synthesis. cDNA first strand synthesis was carried out for 10 min at 25°C, 15 min at 42°C, 15 min at 70°C and pause at 4°C and cDNA second strand was synthesized at 16°C for 1 hour. Following A-tailing, pre-index anchors were ligated to the ends of the double-stranded cDNA fragments to prepare them for dual indexing. A subsequent PCR amplification step to add the index adapter sequences (30 sec at 98°C; **10** cycles of 10 sec at 98°C, 30 sec at 60°C, 30 sec at 72°C; 5min at 72°C and pause at 4°C) was performed.
- After a final library clean-up, libraries were visualized on an Agilent 2100 Bioanalyzer using Agilent High Sensitivity DNA kit (Agilent Technologies, Cat. # **5067-4626**) and quantified using Qubit dsDNA HS DNA Kit (Thermo Fisher Scientific, Cat. # **Q32854**).

➤ **Illumina® Stranded Total RNA Prep, Ligation with Ribo-Zero Plus: Library Preparation Steps**



➤ Sample Quality Control conclusions

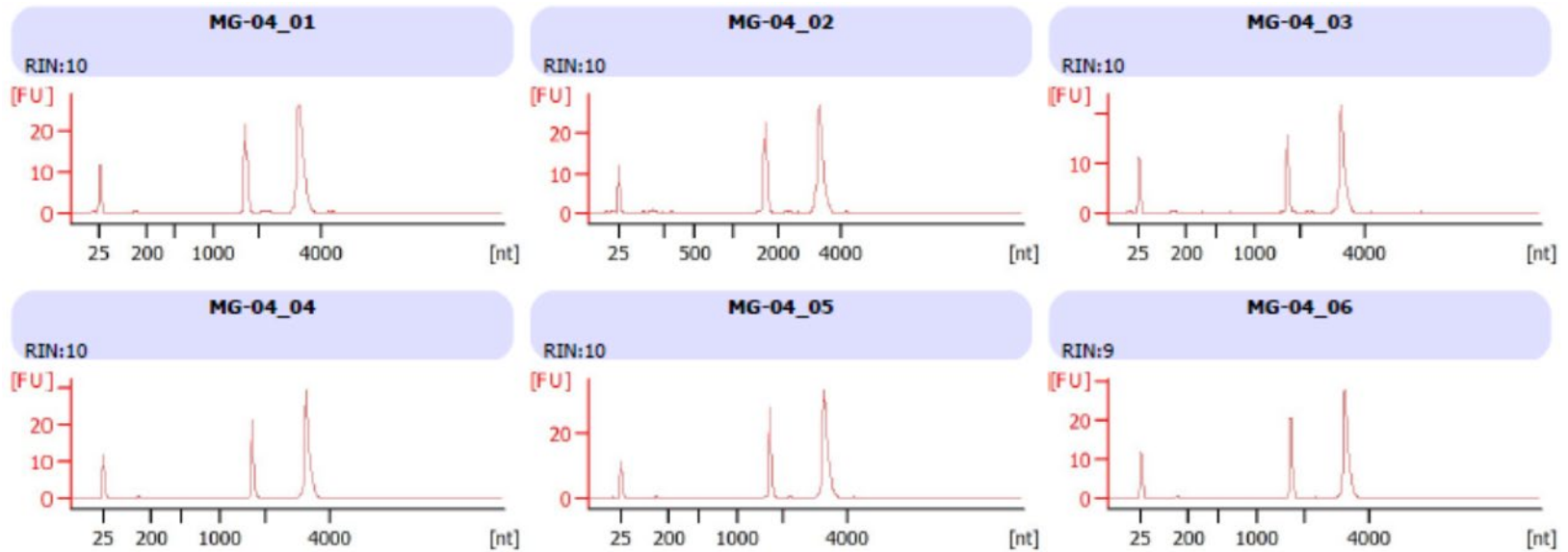
1. The RNA concentration measured in Qubit 2.0, with Qubit RNA assay kit (Thermo Fisher Scientific, Cat.# **Q32855**) showed to be enough to start the experiment (*see results on page 5*) with **1000ng** of total RNA.
2. The quality of all assayed RNAs (RNA integrity characterization by Agilent 2100 Bioanalyzer using an Agilent RNA 6000 Nano Chips Cat. # **5067-1511**) is optimal (*see results on page 6*).

- Therefore, we proceed with the library preparation as detailed below

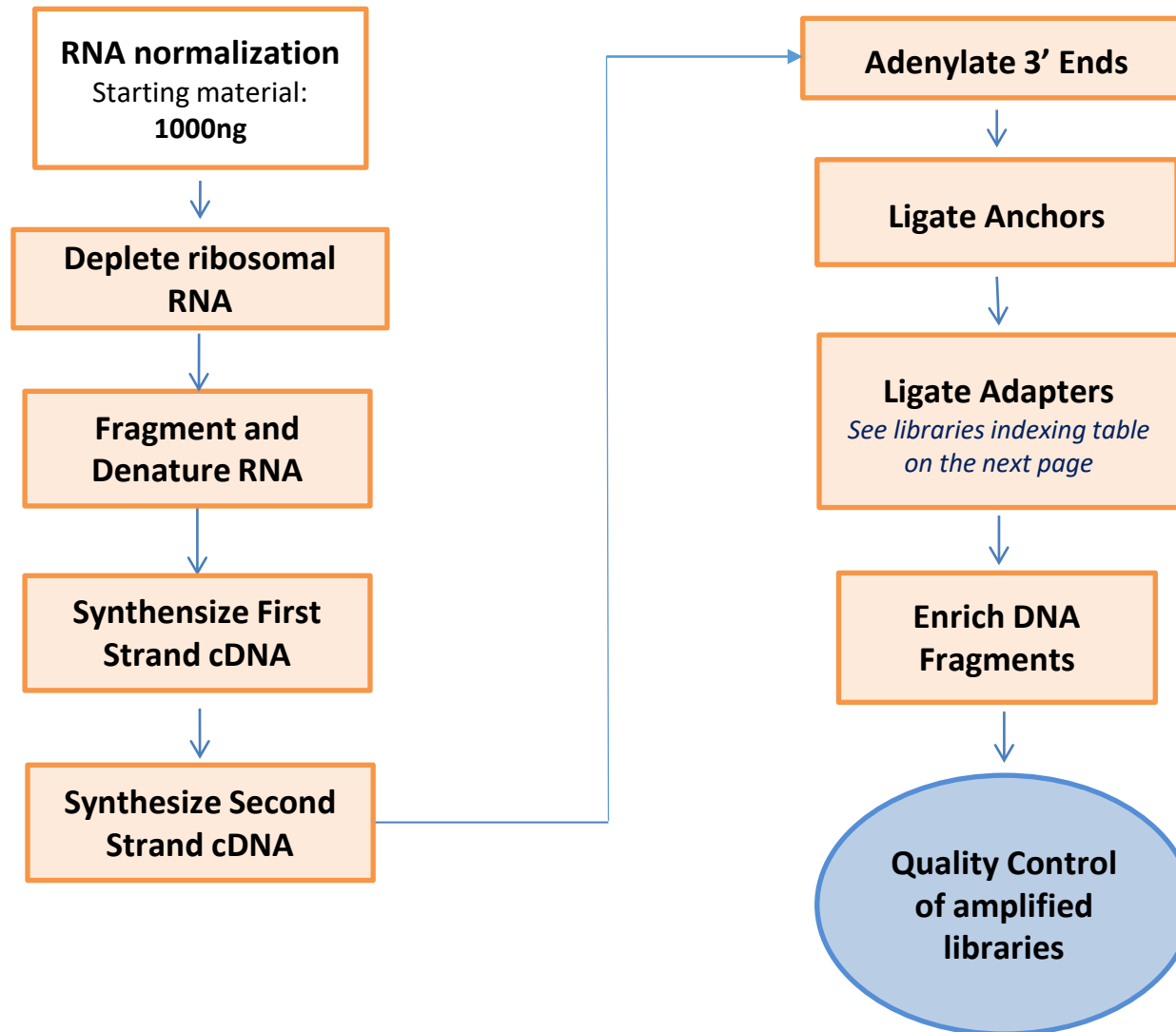
➤ **Total RNA quantification with Qubit RNA HS Assay Kit and RNA integrity numbers (RINs) obtained with Agilent 2100 Bioanalyzer**

GAP_ID	Sample ID	Sample name	Species	Sample Group	Vol. (ul)	Conc. (ng/ul)	Total RNA (ng)	RNA RIN
MG-04_01	1	1	Human	1	20	188.4	3,579.6	10
MG-04_02	2	2	Human	1	20	183.6	3,488.4	10
MG-04_03	3	3	Human	1	20	187.8	3,568.2	10
MG-04_04	4	4	Human	2	20	145.8	2,770.2	10
MG-04_05	5	5	Human	2	20	181.8	3,454.2	10
MG-04_06	6	6	Human	2	20	156.6	2,975.4	9

➤ **RNA Integrity Number (RIN) characterization by Agilent 2100 Bioanalyzer using Agilent 2100 expert (Agilent RNA 6000 Nano kit)**



➤ **rRNA Depletion and Sequencing Library preparation steps using: “Illumina® Stranded Total RNA Prep, Ligation with Ribo-Zero Plus” kit (Illumina Inc.)**



➤ Libraries indexing table

						Index Name	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases in Adapter	i5 Bases for Sample Sheet	i5 Bases for Sample Sheet
Library ID	GAP_ID	Sample ID	Sample name	Species	Sample Group					NovaSeq 6000 with v1.0 reagent kits, MiSeq, HiSeq 2000/2500, NextSeq 2000 (Sample Sheet v2)	iSeq, NovaSeq 6000 with v1.5 reagent kits, MiniSeq, NextSeq 500/550, HiSeq 3000/4000/X, NextSeq 2000 (Sample Sheet v1)
MG-04_L01	MG-04_01	1	1	Human	1	UDP0141	GGCCAATAAG	CTTATTGGCC	GATAACAAGT	GATAACAAGT	ACTTGTATC
MG-04_L02	MG-04_02	2	2	Human	1	UDP0142	CAGTAGTTGT	ACAACTACTG	AGTTATCACA	AGTTATCACA	TGTGATAACT
MG-04_L03	MG-04_03	3	3	Human	1	UDP0143	TTCATCCAAC	GTTGGATGAA	TTCCAGGTAA	TTCCAGGTAA	TTACCTGGAA
MG-04_L04	MG-04_04	4	4	Human	2	UDP0144	CAATTGGATT	AATCCAATTG	CATGTAGAGG	CATGTAGAGG	CCTCTACATG
MG-04_L05	MG-04_05	5	5	Human	2	UDP0190	GATTAAGGTG	CACCTTAATC	TTAGGATAGA	TTAGGATAGA	TCTATCCTAA
MG-04_L06	MG-04_06	6	6	Human	2	UDP0191	CAACATTCAA	TTGAATGTTG	CGCAATCTAG	CGCAATCTAG	CTAGATTGCG

➤ Quality control of amplified libraries

- Amplified libraries concentration was determined with Qubit fluorometer using the Qubit®dsDNA HS assay kit (Thermo Fisher Scientific, Cat.# **Q32854**) and their size distribution was assessed running an aliquot on an Agilent Technologies 2100 Bioanalyzer, using an Agilent High Sensitivity DNA Chip (Agilent Technologies, Cat.# **5067-4626**).

Conclusions:

Obtained Libraries seem to have the expected size and its concentration is appropriate for sequencing.

(See library profile qualification by Agilent High Sensitivity DNA on the next page(s))

Library average size: 438 bp

➤ Libraries QC: Bioanalyzer

