

BGI Sample Testing Report

1. Project Information

Report No.: THKe21041608

Project Name	low input RNAseq_Benakis		Project No.	F21FTSEUHT0481			
Customer Name	Dr. Arthur Liesz		Customer Unit	AG Liesz, Institut für Schlaganfall- und Demenzforschung (ISD), Klinikum der Universität München			
Lab Sample Collector	hoonwing		Lab Sample Receiving Date	20210416			
Lab Sample Tester	siuhoiying, hoonwing		Lab Sample Testing Date	20210416			
Lab Name	BGI Hongkong Tech Solution NGS Lab		Lab Address	16 Dai Fu Street, Tai Po Industrial Estate, Tai Po, New Territories, Hong Kong.			
Reported by	hoonwing	Inspected by	Shijie ZOU	Approved by	Shijie ZOU	Report Date	20210416

2. Sample Test Method

- ①Method of concentration determination: ☐ QubitFluorometer, ☒ Agilent 2100, ☐ Fragment Analyzer, ☐ Fragment Analyzer, ☐ NanoDrop, ☐ MicroplateReader;
- ②Method of 28S/18S & 23S/16S test: ☒ Agilent 2100, ☐ Fragment Analyzer;
- ③Method of RIN or RQN test: ☒ Agilent 2100, ☐ Fragment Analyzer ;

3. Sample Test Result

No.	Sample Name	Sample Number	Tube No.	Concentration(ng/μL)	Volume(μL)	Total Mass(μg)	RIN	28S/ 18S	Library Type	Test Result	Remark
1	2	8522104003644	1	15.575	9	0.1402	3.9	2.1	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug
2	3	8522104003645	1	9.61	9	0.0865	3.2	1.4	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug
3	5	8522104003646	1	10.6	9	0.0954	3.6	1.2	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug
4	6	8522104003647	1	6.898	9	0.0621	3.1	1.1	DNBSEQ Transcriptome	Unqualified	RIN<7.0.2m<0.2ug
5	8	8522104003648	1	2.168	9	0.0195	3.2	0.0	DNBSEQ Transcriptome	Unqualified	RIN<7.0.2m<0.2ug
6	9	8522104003649	1	2.839	10	0.0284	3.3	1.3	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug
7	13	8522104003650	1	1.131	7	0.0079	3.6	1.6	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug
8	14	8522104003651	1	2.124	8	0.017	5.4	2.3	DNBSEQ Transcriptome	Unqualified	RIN<7.0.m<0.2ug

Note*

- The test result based on the 《RNA sequencing sample quality standards》 explains whether the testing sample meets the requirement of library construction.
 - Qualified means the sample meets the requirements of library construction and sequencing.
 - Risky means the sample does not totally meet the requirements of library construction and sequencing. BGI can try to construct library but sequencing quality is not guaranteed.
 - Unqualified means the sample does not meet the requirements of library construction and sequencing. BGI does not suggest using the sample.
- For Qualified samples, the first success rate of Transcriptome Library construction is above 95%, based on historical statistics in BGI.
- For Risky or Unqualified samples, Transcriptome Library construction has the following risk at least, based on historical statistics in BGI.:
 - Deficient or too Low Mass RNA: It may lead to library construction failure, too low library production to sequence or insufficient sequencing data amount; and it may affect data randomness and cause bias.
 - Sample Degradation: It may lead to library construction failure; may lead to high proportion of duplication and poor randomness of sequencing data ;may lead to inaccurate gene expression quantitation.
 - Concentration of RNA is too high , Sampling is inaccurate: It may lead to library construction failure.
 - 5S peak on the high side will affect the quantitative inaccuracy and conduce to the inaccuracy of loading amount and the poor data quality.
 - For plant, fungi and bacteria, the 260/280 and 260/230 absorbance ratio <1.8, It may have Impurity ,it may inhibit enzymatic reaction and affects the successful rate of library construction and insufficient sequencing data quantity. This kind of samples can have 90% successful rate in library construction.
- If COs insist on constructing library with Risky or Unqualified samples, COs shall take the responsibility and risk involved in this matter.
- The following conditions carry moderate risk, and we can try to construct libraries with them:
 - The RIN or RQN value is slightly under standard, but basic line is smooth.
 - The RIN or RQN value reaches standard, but basic line is slightly rise.
 - The basic line is smooth and RIN value reaches standard, but 5S peak is slightly high.
 - 28S/18S or 23S/16S value is slightly under standard, but the basic line is smooth.
 - Generally qualified, but total amount is below Qualified.
 - For plant samples, the OD 260/280, 260/230 is slightly under standard, and samples status is not copy.
 - For soil bacterial and ocean microorganism samples are not able for Meta Strand-Specific Transcriptome library construction.

4. Appendix

Appendix 1: Test results of Agilent 2100 or Fragment Analyzer

Appendix 2: Original information of sample

5. Statement

- 1. The results shown in this report refer only to the sample of the report unless otherwise stated.
- 2. This test report cannot be copied partly without the prior written permission of the Lab.

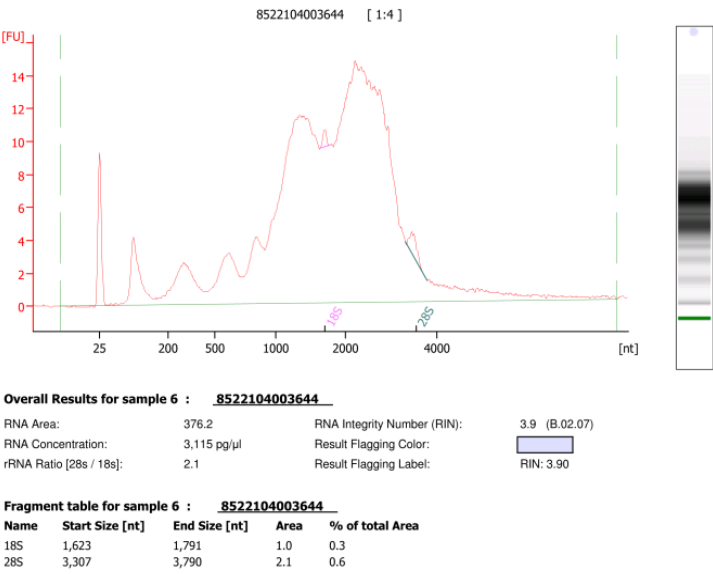
Appendix 1: Test results of Agilent 2100 or Fragment Analyzer

1. Pre-treatment

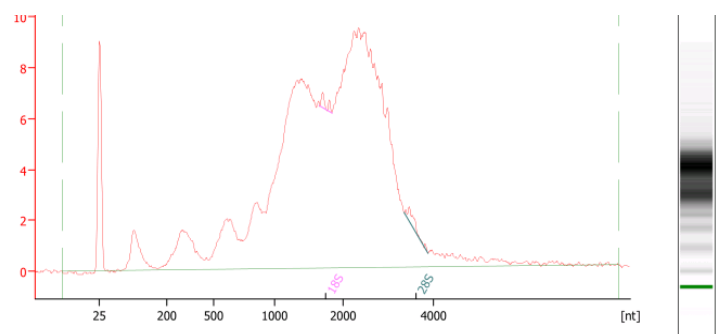
After the sample melted the ice, centrifuged and fully mixed, take appropriate samples for testing.

2. Test Result

(1) Sample name: 2



(2) Sample name: 3



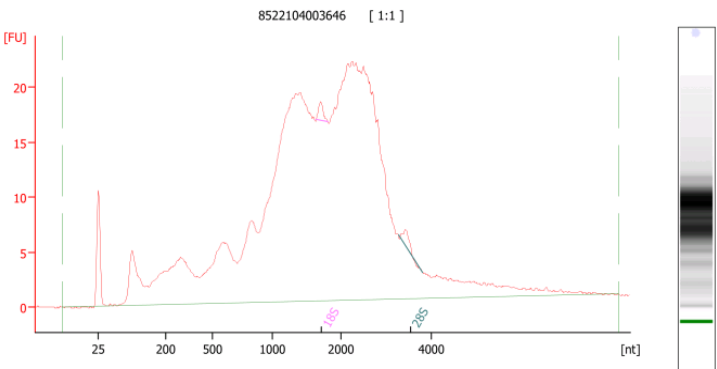
Overall Results for sample 7 : 8522104003645

RNA Area:	232.1	RNA Integrity Number (RIN):	3.2 (B.02.07)
RNA Concentration:	1,922 pg/µl	Result Flagging Color:	<div></div>
rRNA Ratio [28s / 18s]:	1.4	Result Flagging Label:	RIN: 3.20

Fragment table for sample 7 : 8522104003645

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,656	1,839	0.7	0.3
28S	3,351	3,860	1.0	0.4

(3) Sample name: 5



Overall Results for sample 8 : 8522104003646

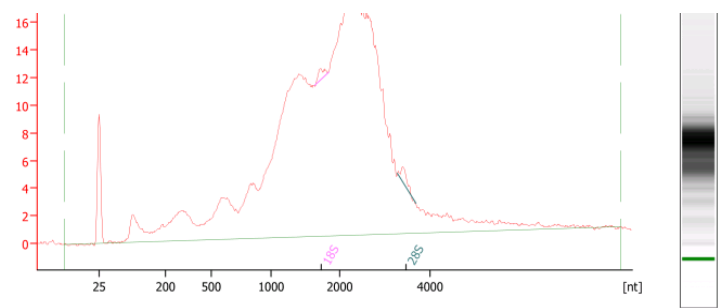
RNA Area:	640.0	RNA Integrity Number (RIN):	3.6 (B.02.07)
RNA Concentration:	5,300 pg/µl	Result Flagging Color:	<div></div>
rRNA Ratio [28s / 18s]:	1.2	Result Flagging Label:	RIN: 3.60

Fragment table for sample 8 : 8522104003646

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,644	1,814	1.7	0.3
28S	3,277	3,809	2.0	0.3

(4) Sample name: 6





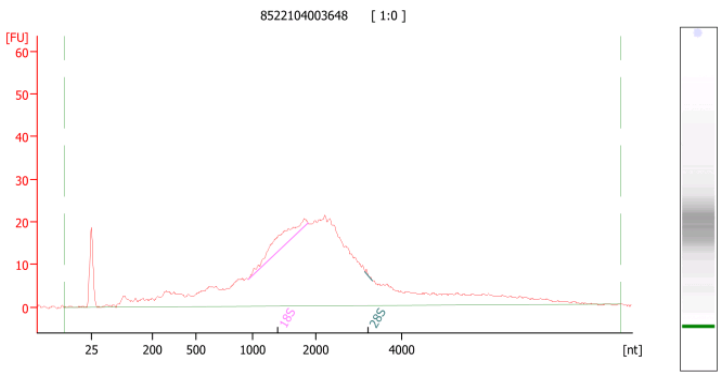
Overall Results for sample 9 : 8522104003647

RNA Area:	416.5	RNA Integrity Number (RIN):	3.1 (B.02.07)
RNA Concentration:	3.449 pg/ul	Result Flagging Color:	<div></div>
rRNA Ratio [28s / 18s]:	1.1	Result Flagging Label:	RIN: 3.10

Fragment table for sample 9 : 8522104003647

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,633	1,847	1.2	0.3
28S	3,294	3,701	1.3	0.3

(5) Sample name: 8



Overall Results for sample 7 : 8522104003648

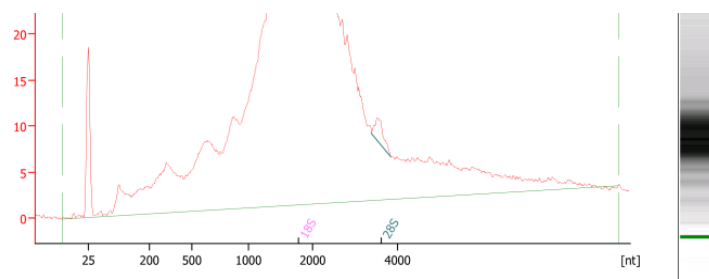
RNA Area:	626.2	RNA Integrity Number (RIN):	3.2 (B.02.07)
RNA Concentration:	2.168 pg/ul	Result Flagging Color:	<div></div>
rRNA Ratio [28s / 18s]:	0.0	Result Flagging Label:	RIN: 3.20

Fragment table for sample 7 : 8522104003648

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	965	1,870	27.3	4.4
28S	3,137	3,294	0.4	0.1

(6) Sample name: 9





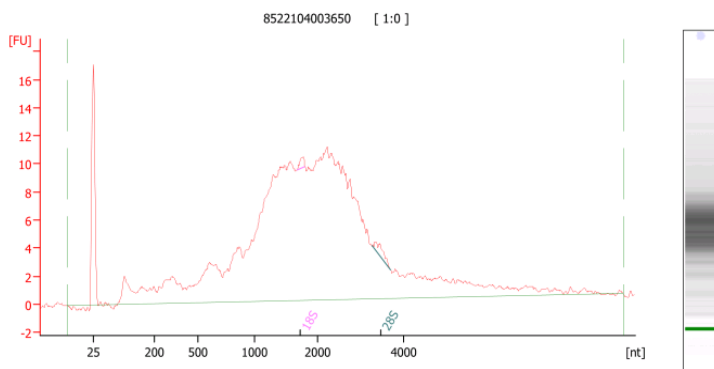
Overall Results for sample 8 : 8522104003649

RNA Area:	820.0	RNA Integrity Number (RIN):	3.3 (B.02.07, Anomaly Threshold(s) manually adapted)
RNA Concentration:	2,839 pg/ul	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.3	Result Flagging Label:	RIN: 3.30

Fragment table for sample 8 : 8522104003649

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,698	1,892	3.8	0.5
28S	3,389	3,836	4.9	0.6

(7) Sample name: 13



Overall Results for sample 9 : 8522104003650

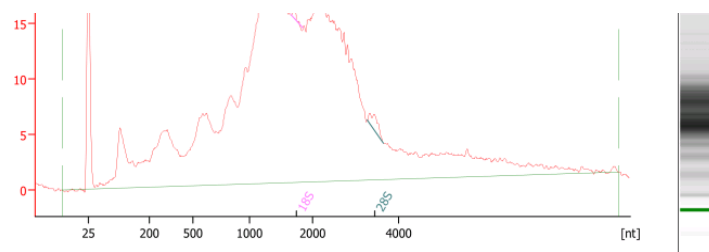
RNA Area:	326.6	RNA Integrity Number (RIN):	3.6 (B.02.07)
RNA Concentration:	1,131 pg/ul	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.6	Result Flagging Label:	RIN: 3.60

Fragment table for sample 9 : 8522104003650

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,667	1,794	0.9	0.3
28S	3,263	3,675	1.5	0.5

(8) Sample name: 14





Overall Results for sample 10 : 8522104003651

RNA Area: 613.4 RNA Integrity Number (RIN): 5.4 (B.02.07)
 RNA Concentration: 2,124 pg/μl Result Flagging Color: RIN: 5.40
 rRNA Ratio [28S / 18S]: 2.3 Result Flagging Label:

Fragment table for sample 10 : 8522104003651

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,644	1,840	1.0	0.2
28S	3,263	3,643	2.3	0.4

Sample Name	Test Instrument	Test Kit	Dilution Ratio(×)	Test Concentration(ng/μL)	Concentration of original sample(ng/μL)	RIN/RQN	28S/18S	Remark
2	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	5	3.115	15.575	3.9	2.1	
3	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	5	1.922	9.61	3.2	1.4	
5	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	2	5.300	10.6	3.6	1.2	
6	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	2	3.449	6.898	3.1	1.1	
8	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	1	2.168	2.168	3.2	0.0	
9	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	1	2.839	2.839	3.3	1.3	
13	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	1	1.131	1.131	3.6	1.6	
14	Agilent 2100 Bioanalyzer	Agilent RNA 6000 pico Reagents Part 1	1	2.124	2.124	5.4	2.3	

Appendix2: Original information of sample

Sample Type:									
Total RNA									
Sample status:									
溶于无RNase水(或DEPC水)									
Further Information:									
Sample Name	Species	No. of Tubes	Concentration (ng/μL)	Volume (μL)	Total Quantity (μg)	28S/18S	OD260/280	OD260/230	Remark
2	mouse	1	8.00	15.00	0.12		1.68	0.63	stroke
3	mouse	1	6.90	15.00	0.1	3.40	1.7	0.93	sham
5	mouse	1	9.20	15.00	0.14	2.90	1.56	1.16	stroke
6	mouse	1	5.30	15.00	0.08		1.62	0.65	sham
8	mouse	1	3.90	15.00	0.06	2.60	1.57	0.24	stroke
9	mouse	1	2.80	15.00	0.04		1.59	0.44	sham
13	mouse	1	2.30	15.00	0.03	2.60	1.53	0.41	stroke
14	mouse	1	2.80	15.00	0.04	3.40	1.68	0.37	sham

----- End of Report -----