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GUIDELINE OF NGS SAMPLE PREPARATION & ORDER SUBMISSION

This document provides guidelines on how to prepare, quantify, and submit Next-Generation Sequencing (NGS) samples. Whether you are submitting DNA or RNA sample, it is essential that the appropriate instructions be followed to enable the successful completion of your project.

I. SAMPLE REQUIREMENTS

Sample quality directly impacts sequencing quality and subsequent bioinformatics analysis. Therefore, we have extensive sample quality control procedures to ensure submitted samples conform to requirements for downstream processing.

To guarantee the normal processing of your project, samples should meet the standards given below.

Notes:

1. Input quantity should be determined by Qubit® instead of NanoDrop™, and the final quantity and concentration should conform to our specifications.
2. Samples that do not meet these specifications will be classified as “sequencing at risk”, and will be subjected to full charges regardless of data output.

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1. Human Whole Genome/Exome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
		Strongly Recommended	Required			
Human Whole Genome/ Exome Sequencing	Genomic DNA	≥ 2 µg	≥ 1 µg	≥ 30 µL	≥ 50 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	PCR products of single-cell whole genome	≥ 2 µg	≥ 1 µg	≥ 30 µL	≥ 50 ng/µL	Fragments should be longer than 500 bp
	FFPE*	≥ 3 µg	≥ 1.5 µg	-	-	Fragments should be longer than 1500 bp

* Formalin-fixed, paraffin-embedded

2. Target Region Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
		Strongly Recommended	Required			
Target Region Capture	Genomic DNA	≥ 2 µg	≥ 1 µg	≥ 30 µL	≥ 20 ng/µL	**OD260/280 = 1.8 – 2.0, no degradation, no**
	PCR products of single- cell whole genome	≥ 2 µg	≥ 1 µg	≥ 30 µL	≥ 20 ng/µL	**Fragments should be longer 500 bp**
	FFPE	**≥ 3 µg**	**≥ 1.5 µg**	-	-	**Fragments should be longer 1500 bp**

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3. Plant & Animal Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
		Strongly Recommended	Required			
≤ 500 bp Insert	Genomic DNA	≥ 1.4 µg	≥ 700 ng	≥ 30 µL	≥ 50 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Mitochondrion/Chloroplast	≥ 1.6 µg	≥ 800 ng	≥ 30 µL	≥ 50 ng/µL	
Genotyping by	Genomic DNA	≥ 500 ng	≥ 300 ng	≥ 30 µL	≥ 50 ng/µL	
2 Kb Insert	Genomic DNA	≥ 30	≥ 15 µg	≥ 30 µL	≥ 50 ng/µL	
5 Kb Insert	Genomic DNA	≥ 30	≥ 15 µg	≥ 30 µL	≥ 50 ng/µL	
10 Kb Insert	Genomic DNA	≥ 50	≥ 25 µg	≥ 30 µL	≥ 50 ng/µL	
> 10 Kb Insert	**Genomic DNA**	**≥ 80**	**≥ 40 µg**	**≥ 30**	**≥ 50 ng/µL**	

4. Microbial Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/agarose gel)
		Strongly Recommended	Required			
≤ 500 bp Insert	Genomic DNA	≥ 1.6 µg	≥ 800 ng	≥ 30 µL	≥ 50 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
Meta Library	Genomic DNA	≥ 1.6 µg	≥ 800 ng	≥ 30 µL	≥ 50 ng/µL	Fragments should be longer than 500 bp
PCR-Free Library	Genomic DNA	≥ 800 ng	≥ 400 ng	≥ 30 µL	≥ 20 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
PCR-Free Library	PCR Products	≥ 400 ng	≥ 200 ng	≥ 30 µL	≥ 20 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination

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5. Epigenetics Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/ Agarose Gel)
		Strongly Recommended	Required			
Whole Genome Bisulfite Sequencing	Genomic DNA	≥ 6 µg	≥ 3 µg	≥ 30 µL	≥ 50 ng/µL	**OD260/280 = 1.8 – 2.0, no degradation, no contamination**
	Genomic DNA	≥ 12 µg	≥ 6 µg	≥ 30 µL	≥ 50 ng/µL	
ChIP-Seq	**ChIP-Seq DNA**	**≥ 100 ng**	**≥ 50 ng**	**≥ 20 µL**	**≥50 ng/µL**	**Main peak of 100 bp – 500 bp**

6. Transcriptome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic RNA-Seq	Total RNA (Animal)	≥2.6 µg	≥1.3 µg	≥ 30 µL	≥ 50 ng/µL	≥ 6.8, smooth base line	**OD260/280 = 1.8 - 2.2, OD260/230 ≥ 2.0, no degradation, no contamination**
	Total RNA (Plant and Fungus)	≥2.6 µg	≥1.3 µg	≥ 30 µL	≥ 50 ng/µL	≥ 6.3, smooth base line	
Prokaryotic RNA-Seq	**Total RNA**	**≥ 6 µg**	**≥ 3 µg**	**≥ 30 µL**	**≥ 50 ng/µL**	**≥ 6.0, smooth base line**	

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7. Small RNA Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic small RNA Sequencing	Total RNA (Animal)	≥ 6 µg	≥ 3 µg	≥ 30 µL	≥ 50 ng/µL	≥ 8, smooth base line	**OD260/280 = 1.8 - 2.2, OD260/230 ≥ 2.0, no degradation, no contamination**
	Total RNA (Plant and Fungus)	**≥ 6 µg**	**≥ 3 µg**	**≥ 30 µL**	**≥ 50 ng/µL**	**≥ 7.5, smooth base line**	

8. Long non-coding Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic Long non-coding RNA Sequencing	Total RNA (Animal)	≥ 5 µg	≥ 2.5 µg	≥ 30 µL	≥ 50 ng/µL	≥ 6.8, smooth base line	**OD260/280 = 1.8 - 2.2, OD260/230 ≥ 2.0, no degradation, no contamination**
	Total RNA (Plant and Fungus)	**≥ 5 µg**	**≥ 2.5 µg**	**≥ 30 µL**	**≥ 50 ng/µL**	**≥ 6.3, smooth base line**	

9. Self-Prepared Library

(1) Volume requirement:

Required Sequencing Data	Library Volume*
< 30 Gb	**≥ 20 µL**
≥ 30 Gb	**≥ 30 µL**

*Self-prepared library with high concentration should be diluted before order submission.

(2) Library concentration quantified by Qubit® 2.0 (Life Technologies): ≥ 0.5 ng/uL

(3) Insert size: dilute to 1 ng/µL before checking the insert size by Agilent 2100 Bioanalyzer.

(a) Insert size: insert + adapters (120 bp) ± 50 bp (Not applicable for small RNA library)

(b) Main peak present, no multiple peaks, no adapter contamination and no primer dimers.

(4) Library concentration quantified by qPCR:

Platform	Concentration
HiSeq/ Novaseq	2 nM – 30 nM
MiSeq	4 nM – 30 nM
HiSeq X	3 nM – 30 nM

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II. OVERVIEW OF SAMPLE QUALITY CONTROL (QC)

Before order submission, we encourage customers to examine the sample quality using one of the following methods: Qubit®, NanoDrop™, agarose gel electrophoresis, or Agilent 2100.

We recommend samples to go through a **quick check** using **Qubit®/ PicoGreen® / gel electrophoresis** (with quantity indicator), so that the result will correspond more closely to our QC results.

If NanoDrop™ is used to estimate the sample quantity before order submission; we strongly recommend that you send more DNA/ RNA for processing than the amount given above.

For gel electrophoresis, the following conditions are recommended:

DNA: 1.0% agarose gel; 1x TAE buffer; 100V for 60 min
RNA: 1.0% agarose gel; 1x TAE buffer; 100V for 60 min

Note:
Different electrophoresis conditions may generate a different and potentially misleading QC report on your samples. Therefore, before order submission, it is highly recommended that you provide us the agarose gel photo of your samples by adhering to the conditions recommended above.

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III. EXAMPLE OF QUALIFIED DNA & RNA SAMPLES

1. Information of DNA Markers

The following molecular size DNA markers (in bp) were used in our original sample QC.

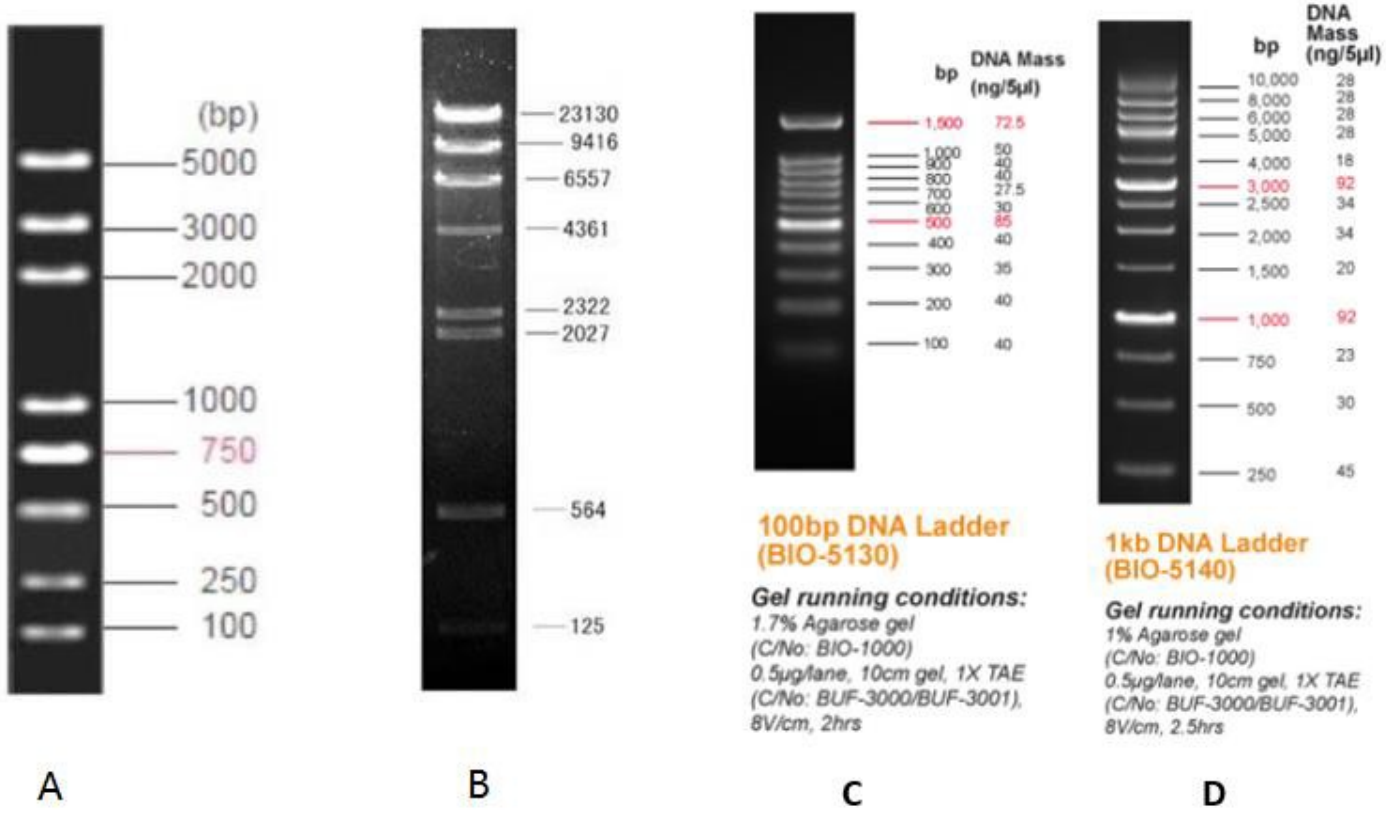

Fig. 1. (A) Trans2K™ Plus DNA Marker; (B) λ/HindIII DNA Marker; (C) 1st BASE 100bp DNA Marker; (D) 1st BASE 1kb DNA Marker

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2. Example of Qualified DNA Samples

2.1 Comparison of Qualified DNA Sample

A qualified DNA sample is compared with some common types of unqualified DNA samples (Fig. 2):

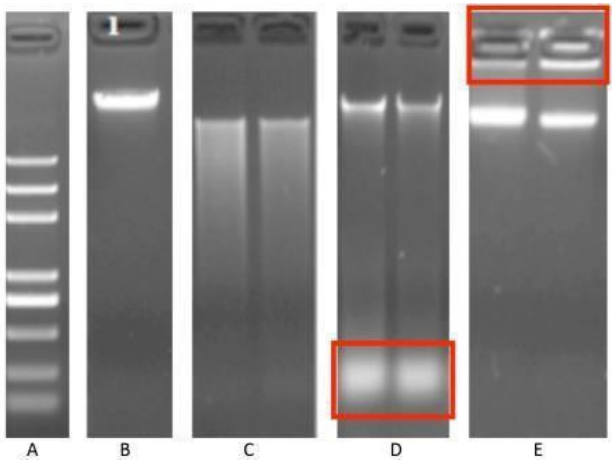

Fig. 2. Comparison of DNA sample quality. Red boxes denote areas of contamination.

(A) Trans2K™ Plus DNA Marker;

(B) Qualified DNA Sample;

(C) Degraded DNA Sample;

(D) DNA Sample contaminated with RNA;

(E) DNA Sample contaminated with protein.

2.2 DNA Samples with Degradation

The gel picture illustrates DNA samples with degradation. Severe degradation can impact the quality of the prepared library and subsequent bioinformatics analysis (Fig. 3):

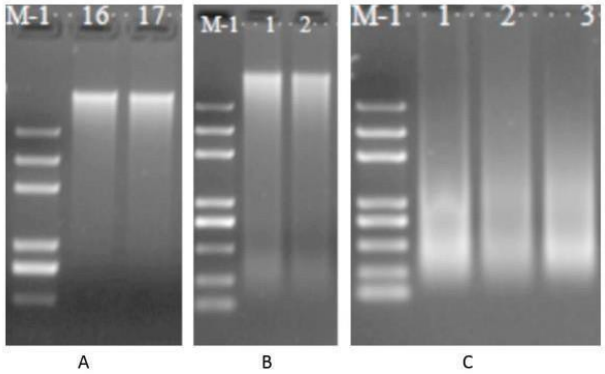

Fig. 3. DNA samples with degradation. Panels A, B, and C demonstrate increasing levels of DNA degradation. M-1, Trans2K™ Plus DNA Marker.

2.3 DNA Samples with RNA Contamination

RNA contamination of DNA samples (Fig. 4) can impede the library construction process. It is strongly recommended to clean your DNA samples with RNase during the process of DNA extraction.

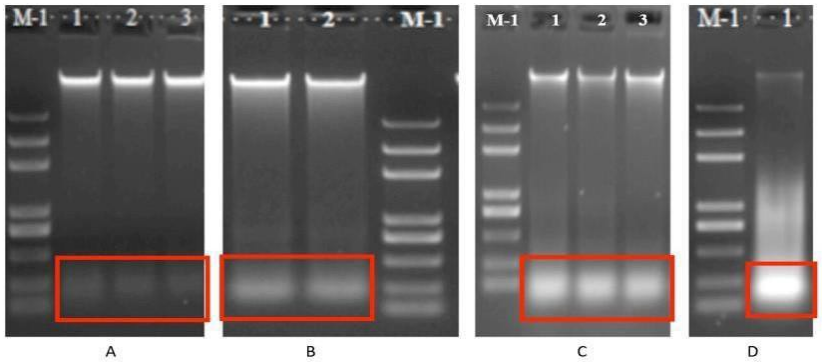

Fig. 4. DNA samples contaminated with RNA. Panels A – D demonstrate increasing levels of RNA degradation. Red boxes denote areas of contamination. M-1 = Trans2K™ Plus DNA Marker.

2.4 DNA Samples with Protein Contamination

DNA samples can be contaminated by proteins (Fig. 5) during the process of DNA extraction from raw materials. The protein-contaminated DNA samples can be purified using affinity column, though it will lead to some loss of DNA.

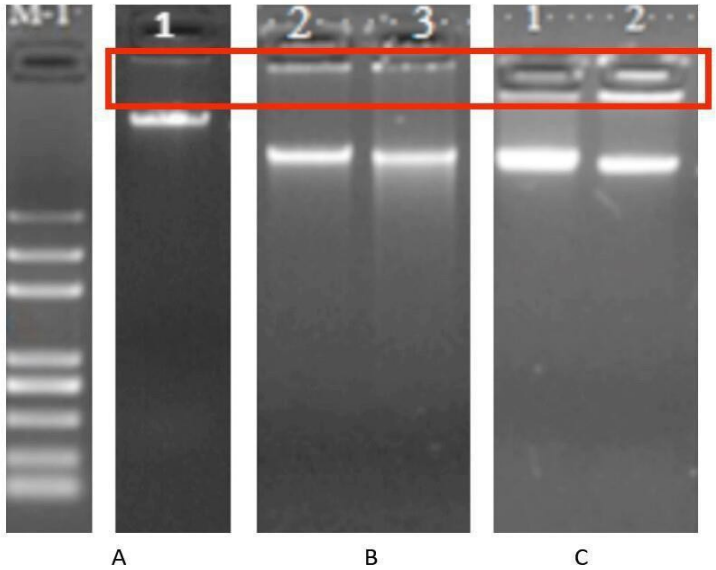

Fig. 5. DNA samples contaminated with protein. Panels A – C demonstrate increasing levels of protein contamination. Red boxes denote areas of contamination. M-1, Trans2K™ Plus DNA Marker.

3. Example of Qualified RNA Samples

3.1 Comparison of Qualified RNA Sample

A qualified RNA sample is compared with some common types of unqualified RNA samples (Fig. 6):

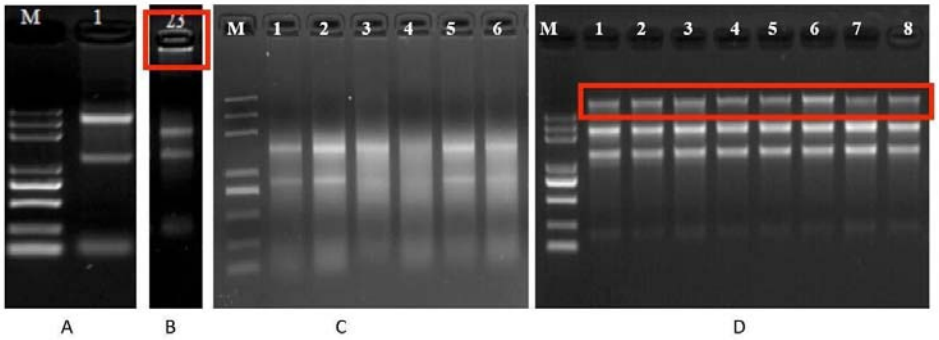

Fig. 6. Comparison of RNA sample quality. Red boxes denote areas of contamination. DNA Marker = Trans2K™ Plus DNA Marker.

(A) Qualified RNA sample;

(B) RNA Sample with protein contamination;

(C) RNA Samples with degradation;

(D) RNA samples with genomic DNA contamination.

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3.2 RNA Samples with Protein Contamination

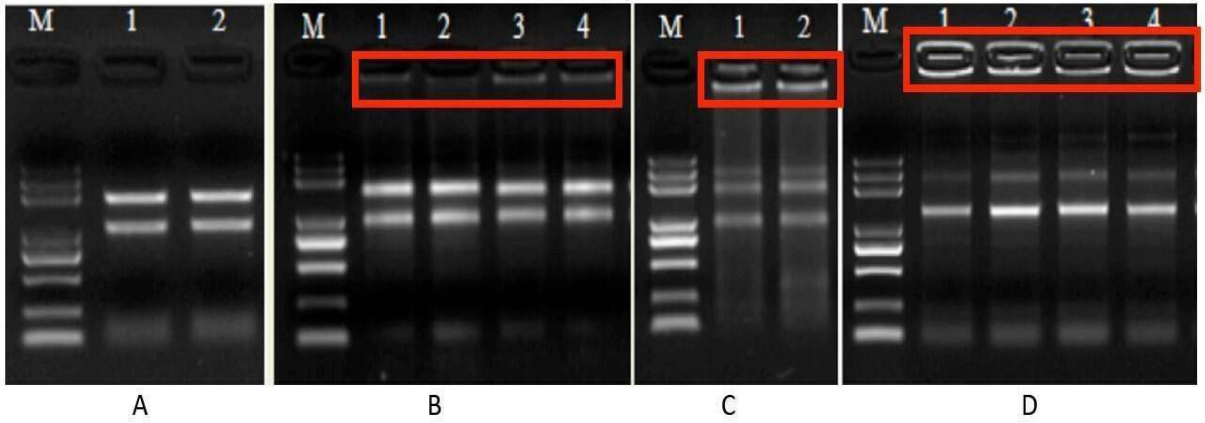

Fig. 7. RNA samples with protein contamination. Red boxes denote areas of contamination. DNA Marker = Trans2K™ Plus DNA Marker. Panels A to D demonstrate increasing levels of protein contamination.

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3.3 Comparison of RNA samples on Agarose Gel and Agilent 2100 Analysis

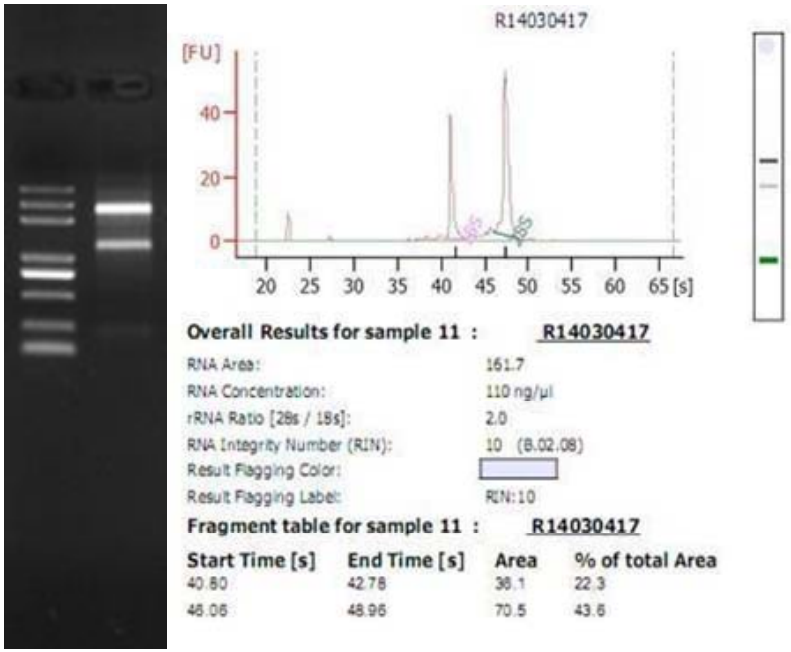

Fig. 8. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **qualified RNA sample.**

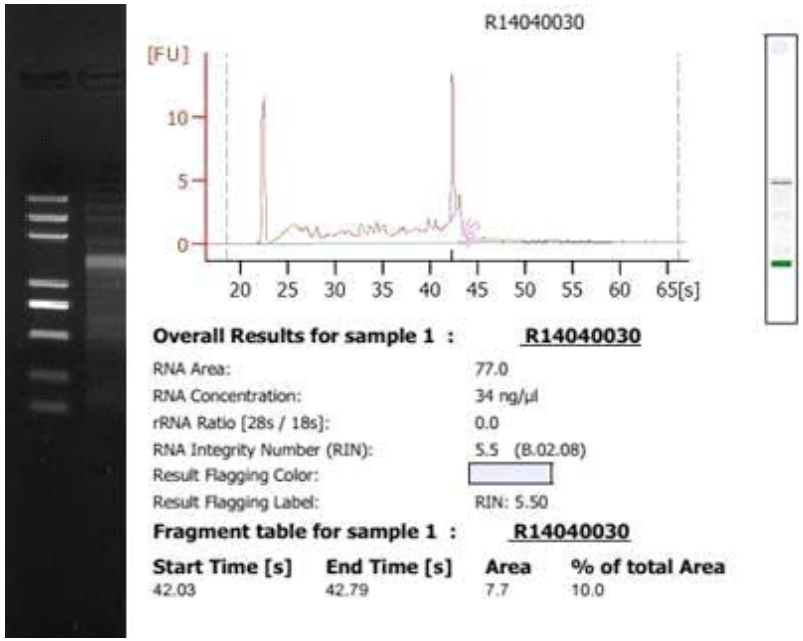

Fig. 9. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **degraded RNA sample**.

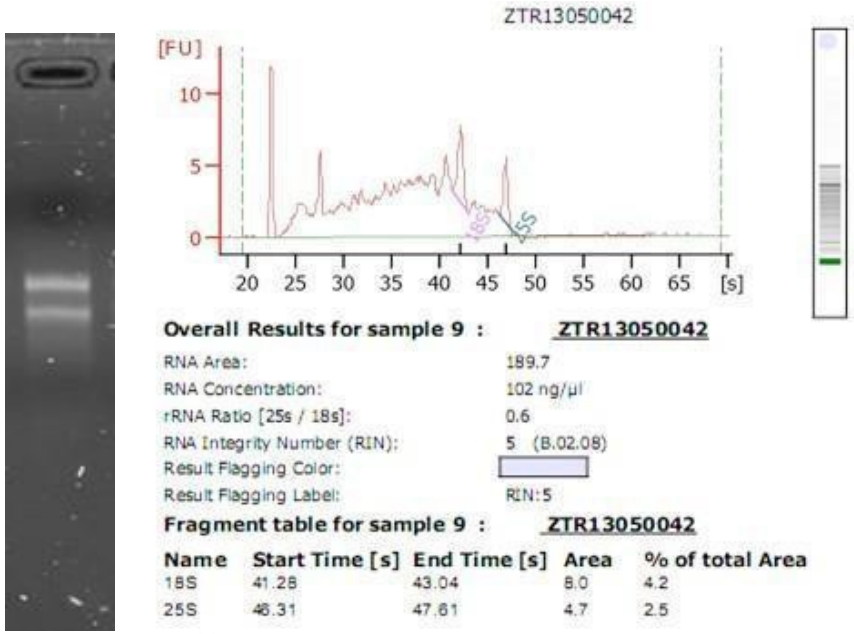

Fig. 10. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **RNA sample with solvent/ salt contamination (high viscosity).**

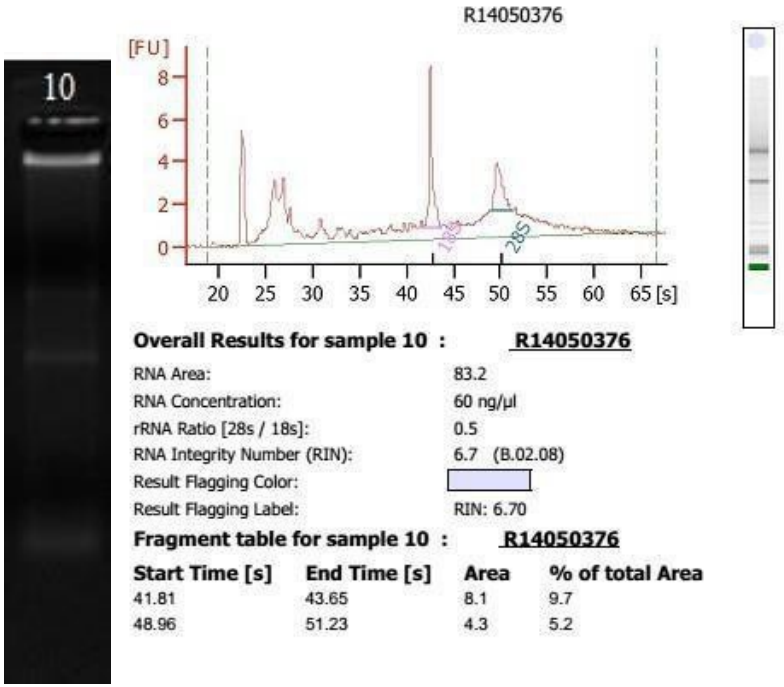

Fig. 11. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **RNA sample with protein contamination.** The RNA Labchip would not able to show DNA contamination.

IV. LABELING OF YOUR SAMPLE TUBES

1. It is important to have a good labeling on sample tubes. The label on tubes must always tally with the sample name indicated in the order form. Use waterproof permanent marker pen for labeling to keep labels from fading upon receipt. You should label on both sides of the tube lid and its tube body.

2. According to the tube label, tally matches the sample name in the provided NGS Order Form before submission.

V. PACKING OF YOUR ORDER

1. For DNA and RNA samples, use 1.5 mL or 2.0 mL screw-cap DNase and RNase-free micro-centrifuge tubes. Use Parafilm to seal each tube before packaging. We do not recommend shipping samples in organic solvents (such as absolute ethanol or isopropanol) because the solvents will leak during shipping, which could cause cross-contamination between samples, as well as losing sample from further process.

2. In order to minimize the micro-centrifuge tubes from damage during shipping, we highly recommend placing the sample tubes in a container, such as a 50-ml Falcon tube or a box with interior racks/ holders. Cotton, absorbent papers or dunnage can be used to prevent tubes from moving around inside the container.

3. We recommend dry ice shipment for majority of NGS samples. Genomic DNA is optional to ship with blue ice (4°C). Saliva samples that require DNA extraction services before sequencing must be shipped at room temperature.

4. 96-well plate and PCR strips tubes are NOT accepted for shipping (See picture below).

Fig. 12. Recommended and prohibited tubes for sending samples

VI. COMPLETING THE ORDER FORM

- ✓ Before submitting the order, the ordering information must be completed using NGS Order Form.
- ✓ Send us the ELECTRONIC COPY of the completed order form via email.
- ✓ For outstation or overseas customer, kindly attach the HARD COPY in the shipment.
- ✓ Please also provide a copy of Gel Electrophoresis picture or Bioanalyzer trace if available.

VII. SUBMITTING THE ORDER

Disclaimer: The information below only constitutes a recommendation for shipping samples classified as "non-regulated materials" to our facility. At the time this document was prepared, gDNA and total RNA were not defined as a diagnostic specimen in the International Air Transport Association (IATA) packing instructions, and therefore no special packaging requirements are listed. Due to the constant changes in import/ export regulations, customers should always check with their safety office and/or shipping department to comply with their local regulatory.

1. Ensure all samples follow our quality standards and they are prepared and packaged according to the guidelines given above.

DNA	1. Lyophilize the DNA to ship on ambient temperature
	2. Pack with ice packs/blue ice (2-8 °C)
	3. Use the cold-chain transportation system (2-8 °C) of the courier
	4. DNAstable (Liquid format, Biomatrica) and ship on 2-8 °C
	5. Pack in dry ice (-60 °C – -80 °C) ***Strongly Recommended.**
RNA	1. Lyophilize the RNA to ship on dry ice
	2. Suspend RNA in 75% ethanol and ship on dry ice
	3. RNAstable (Biomatrica) and ship on 2-8 °C
	4. Pack in dry ice (-60 °C – -80 °C) *** Strongly Recommended.**

Note:

- ✓ It is highly recommended that RNA samples be shipped in dry ice packaging. Other packaging/ transportation methods may add impurities or cause slight degradation of the total RNA.
- ✓ The required quantity of dry ice and ice bags varies with seasons (i.e., weathers), transit time and the thickness of Styrofoam box and receptacle. Please contact your local courier office to **estimate the transit time**, so that you can estimate the amount of dry ice or blue ice required to ship your order. Normally, **dry ice** is consumed (sublimates) at a rate of **5 kg per day**.

9. Once the parcels reach our facility, all samples will be **stored in -80 °C** ultra-low temperature freezer **before QC**. Our Customer Care Specialist will provide timely update to you about the progress of your order.