

# GUIDELINE OF NGS SAMPLE PREPARATION & ORDER SUBMISSION

This document provides guidelines on how to prepare, quantify, and submit Next-Generation Sequencing (NGS) samples to **Apical Scientific**. 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. If your samples do not meet these standards, or if you are unable to produce higher-quality samples, please consult our Customer Care Department at [ngs@apicalscientific.com](mailto:ngs@apicalscientific.com) before shipping your samples.

### **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. Please consult us for details.

## 1 Genome Sequencing

### 1.1 Human Whole Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
Human Whole Genome Library (350bp)	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
	Genomic DNA (PCR-free)	≥ 1.5 µg	≥ 20 µl	≥ 20 ng/µl	
	*FFPE DNA	≥ 800 ng	-	-	Fragments should be longer than 1500 bp.

\*FFPE: Formalin-Fixed, Paraffin-Embedded.

### 1.2 Exome Sequencing/ Target Region Capture

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
DNA Library (Human Exome or Human Target Region)	Genomic DNA	≥ 400 ng	≥ 20 µl	≥ 20 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
	*FFPE	≥ 800 ng	-	-	Fragments should be longer than 1000 bp.
	cfDNA/ctDNA	≥ 50 ng	-	-	Fragments should be in multiples of 170 bp, no genomic contamination.

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
Mouse Exome Library	Genomic DNA	≥ 400 ng	≥ 20 µl	≥ 20 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
	*FFPE	≥ 800 ng	-	-	Fragments should be longer than 1000 bp.

\*FFPE: Formalin-Fixed, Paraffin-Embedded.

### 1.3 Plant & Animal Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
Plant and Animal Whole Genome Library (≤ 500 bp)	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
	Genomic DNA (PCR-free non-350bp)	≥ 5 µg	≥ 20 µl	≥ 30 ng/µl	
	Genomic DNA (PCR-free 350bp)	≥ 1.5 µg	≥ 20 µl	≥ 20 ng/µl	

### 1.4 Microbial Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
Metagenomics Library	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
PCR Free Library (Amplicon)	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	
Microbial Whole Genome Library (350bp)	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	
Microbial Whole Genome Library (PCR-free 350bp)	Genomic DNA	≥ 1.5 µg	≥ 20 µl	≥ 20 ng/µl	

## 1.5 PCR Product Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
PCR Free Library	PCR Product	≥ 1.5 µg	≥ 20 µl	≥ 50 ng/µl	OD 260/280 = 1.8-2.0; no degradation, no contamination.
Library (with PCR)	PCR Product	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	

## 1.6 PacBio Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
PacBio Sequel II DNA CLR Library	*HMW Genomic DNA (Animal and Plant)	≥ 8 µg	≥ 50 µl	≥ 80 ng/µl	Fragment size: Majority above 40k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;
	*HMW Genomic DNA (Bacteria)	≥ 5 µg	≥ 50 µl	≥ 80 ng/µl	Fragment size: Majority above 20k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;
	*HMW Genomic DNA (Fungus)	≥ 8 µg	≥ 50 µl	≥ 80 ng/µl	Fragment size: Majority above 20k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;
PacBio Sequel II DNA Hifi Library	*HMW Genomic DNA	≥ 15 µg	≥ 50 µl	≥ 80 ng/µl	Fragment size: Majority above 30k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;

\*HMW: High Molecular Weight.

\*\*Nc/Qc: NanoDrop™ concentration/Qubit concentration.

## 1.7 Nanopore Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	DNA Integrity Number (Agilent 2200)	Purity (NanoDrop™ & Agarose Gel Assessment)
Nanopore DNA Library	*HMW Genomic DNA (Animal and Plant)	≥ 10 µg	≥ 100 µl	≥ 100 ng/µl	≥ 7.5, with flat base line.	Fragment size: Majority above 30k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;
	*HMW Genomic DNA (Bacteria and Fungus)	≥ 10 µg	≥ 100 µl	≥ 100 ng/µl	≥ 7.5, with flat base line.	Fragment size: Majority above 20k bp; OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; **Nc/Qc=0.95-3.00;
16s Full Length Amplicon Library	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	Not applicable	OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; no degradation, no contamination.
Rapid Barcoding Library	Plasmid DNA	≥ 1 µg	≥ 20 µl	Normalize to 50 ng/µl	Not applicable	OD 260/280=1.8-2.0; OD 260/230=1.5-2.6; no degradation, no contamination.
Native Barcoding Library	Pooled Amplicons (≤ 1 kb)	≥ 500 ng	≥ 50 µl	≥ 10 ng/µl	Not applicable	no degradation, no contamination.
	Pooled Amplicons (≥ 1kb)	≥ 1 µg	≥ 100 µl	≥ 10 ng/µl	Not applicable	no degradation, no contamination.

\*HMW: High Molecular Weight.

\*\*Nc/Qc: NanoDrop™ concentration/Qubit concentration.

## 1.8 Nanopore Virus Whole Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Titration of the Targeted Virus (qPCR)
Rapid Barcoding or Native Barcoding Library	Total RNA Contains SARS-CoV2 Virus	Not applicable	≥ 40 µl	Not applicable	≤ 25.0
	gDNA Contains African Swine Flu Virus (ASFV)	≥ 2 µg	≥ 50 µl	≥ 40 ng/µl	≤ 25.0

## 2. RNA Sequencing

### 2.1 Transcriptome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
Eukaryotic mRNA Library (Poly A Enrichment)	Total RNA (Animal)	≥ 400 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.8, with flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Total RNA (Plant and Fungus)	≥ 400 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.3, with flat base line.	
	Total RNA (Blood)	≥ 800 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.8, with flat base line.	
	Amplified cDNA (double strand)	≥ 100 ng	≥ 10 µl	≥ 10 ng/µl	Fragments distributing between 400bp-5000bp, with the main peak at ~2000bp;	OD 260/230 ≥ 2.0; no degradation, no contamination.
Eukaryotic Directional mRNA Library (Poly A Enrichment)	Total RNA (Animal)	≥ 800 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.8, with flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Total RNA (Plant and Fungus)	≥ 800 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.3, with flat base line.	
	Total RNA (Blood)	≥ 800 ng	≥ 20 µl	≥ 20 ng/µl	≥ 6.8, with flat base line.	
Prokaryotic RNA Library	Total RNA	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 6.0, with flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
Meta-transcriptome Library	Total RNA	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 6.5, with flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.

## 2.2 Eukaryotic Small RNA Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
Small RNA Library (with 18~40 bp insert)	Total RNA (Animal)	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 7.5, with smooth base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Total RNA (Plant and Fungus)	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 7, with smooth base line.	
	Exosome RNA	≥ 20 ng	≥ 10 µl	-	Fragments distributing between 25-200nt (by high sensitive Agilent 2100 Bioanalyzer), FU > 10, with no peak > 2000nt.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.

## 2.3 Eukaryotic Long Non-coding RNA Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
Directional RNA library (rRNA removal)	Total RNA (Animal)	≥ 500 ng	≥ 10 µl	≥ 50 ng/µl	≥ 6.5, flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Total RNA (Plant and Fungus)	≥ 500 ng	≥ 10 µl	≥ 50 ng/µl	≥ 6, flat base line.	
	Exosome RNA	≥ 20 ng	≥ 10 µl	-	Fragments distributing between 25-200nt (by high sensitivity Agilent 2100 Bioanalyzer), FU > 10, with no peak > 2000nt.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.

## 2.4 Eukaryotic CircRNA Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
CircRNA Library	Total RNA (Animal)	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 7, flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Total RNA (Plant and Fungus)	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 6.5, flat base line.	

## 2.5 Ribosome Profiling

Library Type	Sample Type	Amount	Volume	Concentration	Main Peak	Purity (Agarose Gel Assessment)
Ribo Library	RPF Sample	≥ 2 µg	≥ 10 µl	≥ 200 ng/µl	25-38 nt	No degradation, no contamination.

## 2.6 PacBio Iso-seq

Library Type	Sample Type	Amount	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
PacBio Sequel II RNA library	Total RNA	≥ 2 µg	≥ 50 µl	≥ 30 ng/µl	≥ 7, flat base line.	OD 260/280=1.8-2.2; OD 260/230=1.3-2.5; *Nc/Qc ≤ 2.5;

\*Nc/Qc: NanoDrop concentration/Qubit concentration



## 2.7 Nanopore Direct RNA Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™ & Agarose Gel Assessment)
Direct RNA Library	Total RNA (Eukaryotes)	≥ 2 µg	≥ 20 µl	≥ 50 ng/µl	≥ 7, flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.
	Poly(A)-tailed RNA (Prokaryotes)	≥ 200 ng	≥ 20 µl	≥ 20 ng/µl	≥ 7, flat base line.	OD 260/280 ≥ 2.0; OD 260/230 ≥ 2.0; no degradation, no contamination.

## 3. Epigenetics Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	Purity (NanoDrop™ & Agarose Gel Assessment)
Whole Genome Methylation (WGBS) Library	Genomic DNA	≥ 200 ng	≥ 20 µl	≥ 10 ng/µl	0 < OD260/230 < 3; no degradation, no contamination.
Reduced Representation Bisulfite Sequencing (RRBS) Library	Genomic DNA	≥ 1.5 µg	≥ 20 µl	≥ 20 ng/µl	0 < OD260/230 < 3; no degradation, no contamination.
ChIP-seq Library	Enriched DNA	≥ 50 ng	≥ 20 µl	≥ 2 ng/µl	Main peak of 100 bp-500 bp.
RIP-seq Library	Enriched DNA	≥ 100 ng	≥ 20 µl	≥ 3 ng/µl	Without fragmentation; fragments should be longer than 1000 bp.

## 4. Pre-made Library Sequencing

### 4.1 Volume Requirement

#### PE150-HiSeq

Data Amount	Volume Requirement*
Lane Sequencing	≥ 20 µl (additional 10 µl for one more lane).

#### PE150-NovaSeq

Data Amount	Volume Requirement*
< 20 Gb	≥ 15 µl
20 Gb ≤ X ≤ 100 Gb	≥ 25 µl
100 Gb < X < 400 Gb	≥ 50 µl
Lane Sequencing	≥ 50 µl (additional 40 µl for one more lane).

#### NovaSeq PE250 & SE50 & PE50

Data Amount	Volume Requirement*
X ≤ 20M reads	≥ 15 µl
20M reads < X ≤ 50M reads	≥ 25 µl
50M reads < X < 150M reads*	≥ 50 µl
Lane Sequencing	≥ 100 µl (additional 100 µl for one more lane).

**\*Notes:**

High concentration libraries should be diluted before shipping.

Pre-made libraries should be colorless.

## 4.2 Library Concentration

≥ 0.5 ng/μL, quantified by Qubit® 2.0 (Life Technologies).

2 nM-30 nM, quantified by qPCR.

## 4.3 Library Size

Library size: insert + adapters (120 bp) ± 50 bp (this size estimation does not apply to small RNA library).

Single main peak, no multiple peaks, no adapter contamination, and no primer dimers.

Sequencing Strategy	Library Size (insert + adaptors (120 bp)+/- 50 bp for Optimal Results)
SE 50/ PE 50	130 bp~650 bp
PE 150	320 bp~520 bp
PE 250	400 bp~650 bp
Lane Sequencing	≥ 100 μl (additional 100 μl for one more lane).

## 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<sup>®</sup>, NanoDrop<sup>™</sup>, agarose gel electrophoresis, or Agilent 2100.

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

If NanoDrop<sup>™</sup> 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.

### 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.

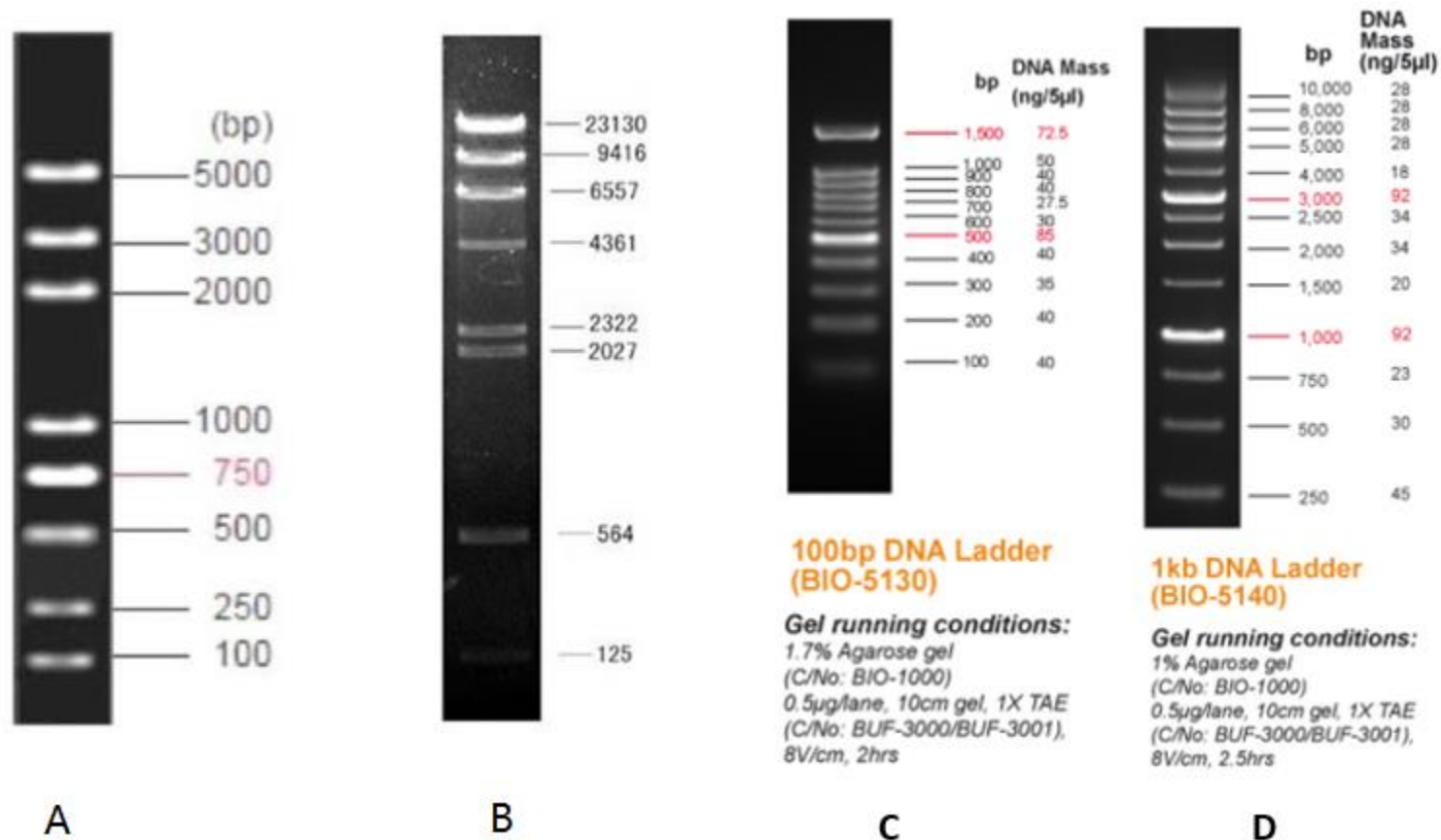


Fig. 1. (A) Trans2K™ Plus DNA Marker; (B)  $\lambda$ /HindIII DNA Marker; (C) 1st BASE 100bp DNA Marker; (D) 1st BASE 1kb DNA Marker

## 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):

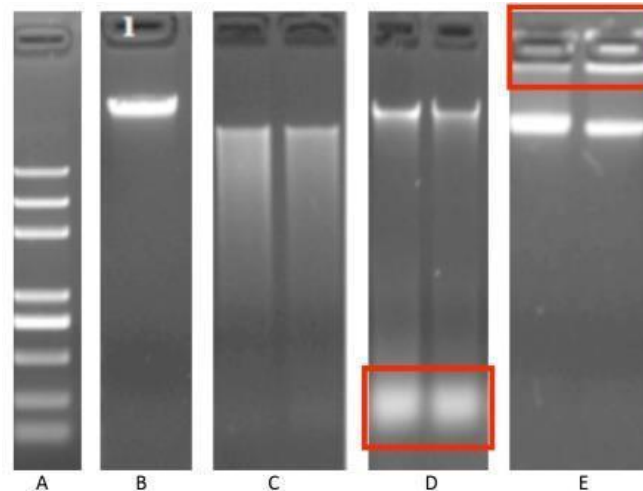


Fig. 2. Comparison of DNA sample quality. Red boxes denote areas of contamination.

- (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):

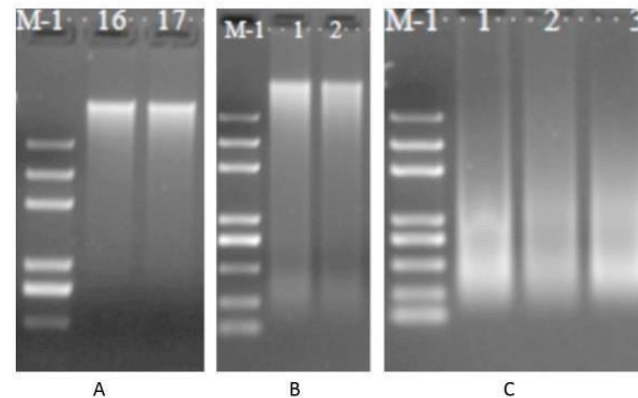


Fig. 3. DNA samples with degradation. Panels A, B, and C demonstrate increasing levels of DNA degradation. M-1, Trans2K™ Plus DNA Marker.

## 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.

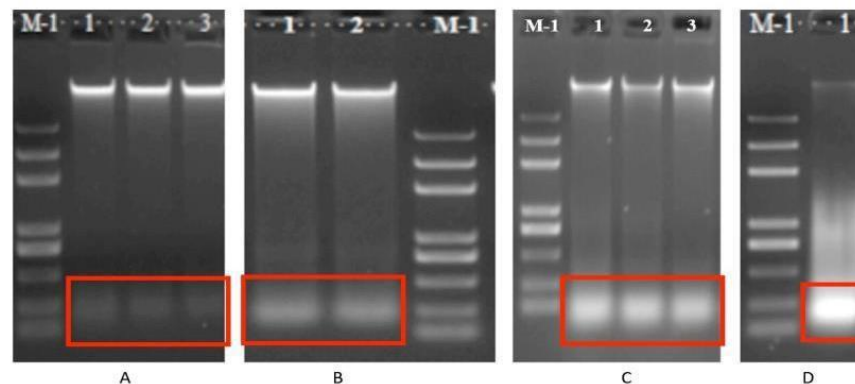


Fig. 4. DNA samples contaminated with RNA. Panels A – D demonstrate increasing levels of RNA degradation. Red boxes denote areas of contamination. M-1 = Trans2K™ Plus DNA Marker.

## 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.

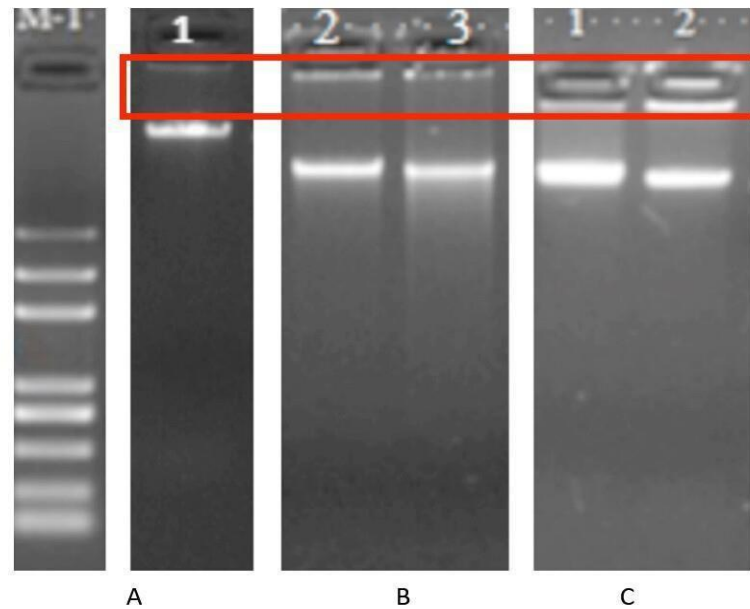


Fig. 5. DNA samples contaminated with protein. Panels A – C demonstrate increasing levels of protein contamination. Red boxes denote areas of contamination. M-1, Trans2K™ Plus DNA Marker.



### 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):

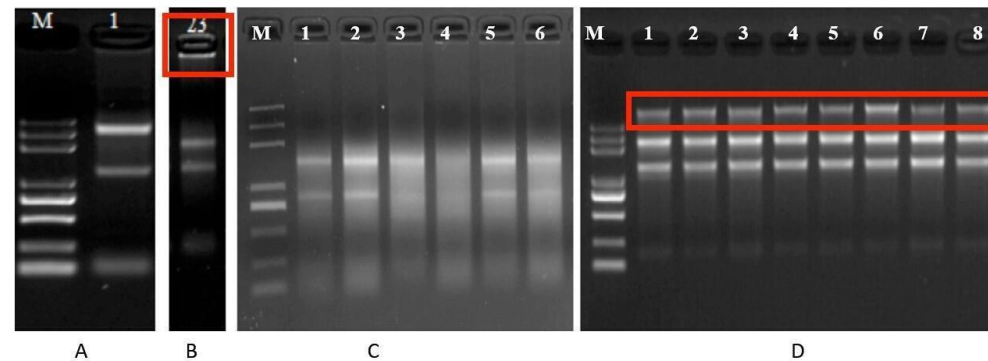


Fig. 6. Comparison of RNA sample quality. Red boxes denote areas of contamination. DNA Marker = Trans2K™ Plus DNA Marker.

(A) Qualified RNA sample;

(B) RNA Sample with protein contamination;

(C) RNA Samples with degradation;

(D) RNA samples with genomic DNA contamination.

### 3.2 RNA Samples with Protein Contamination

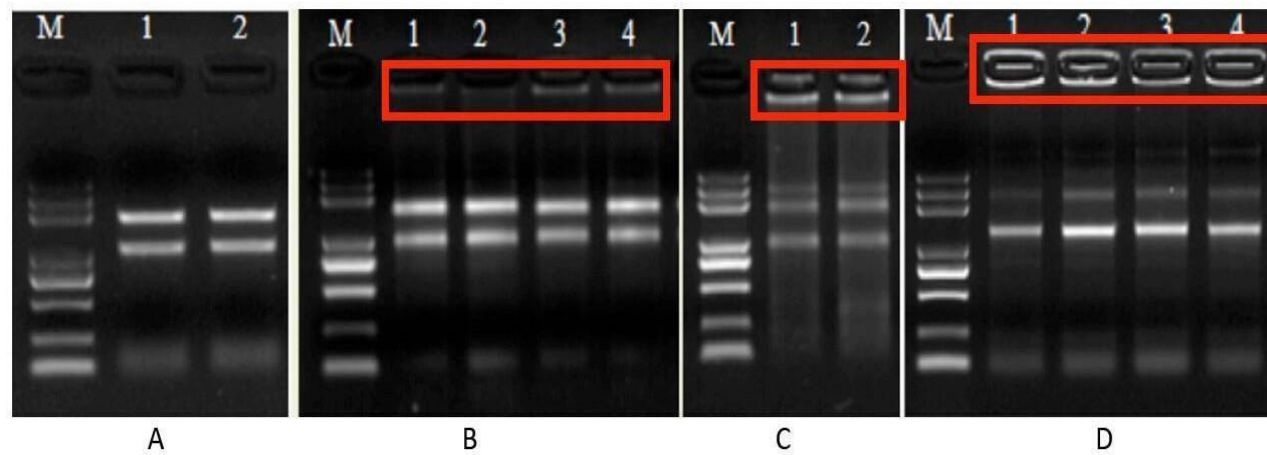


Fig. 7. RNA samples with protein contamination. Red boxes denote areas of contamination. DNA Marker = Trans2K™ Plus DNA Marker.

Panels A to D demonstrate increasing levels of protein contamination.

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

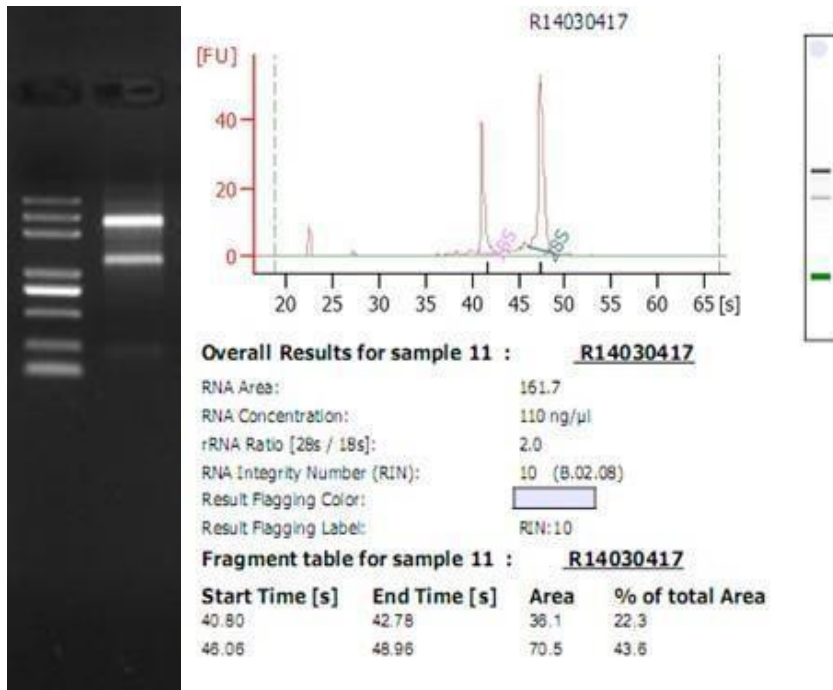


Fig. 8. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **qualified RNA sample**.

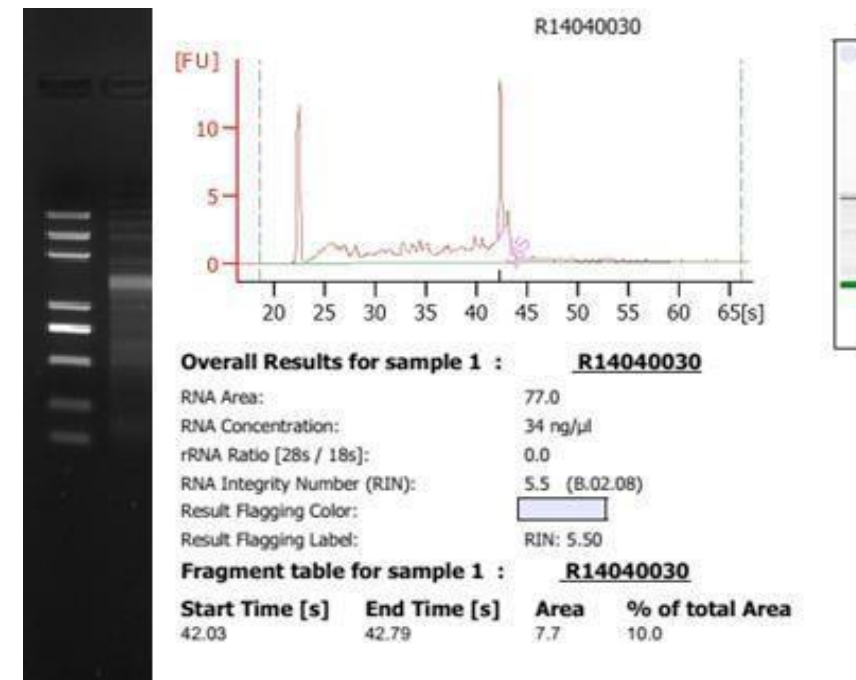


Fig. 9. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **degraded RNA sample**.

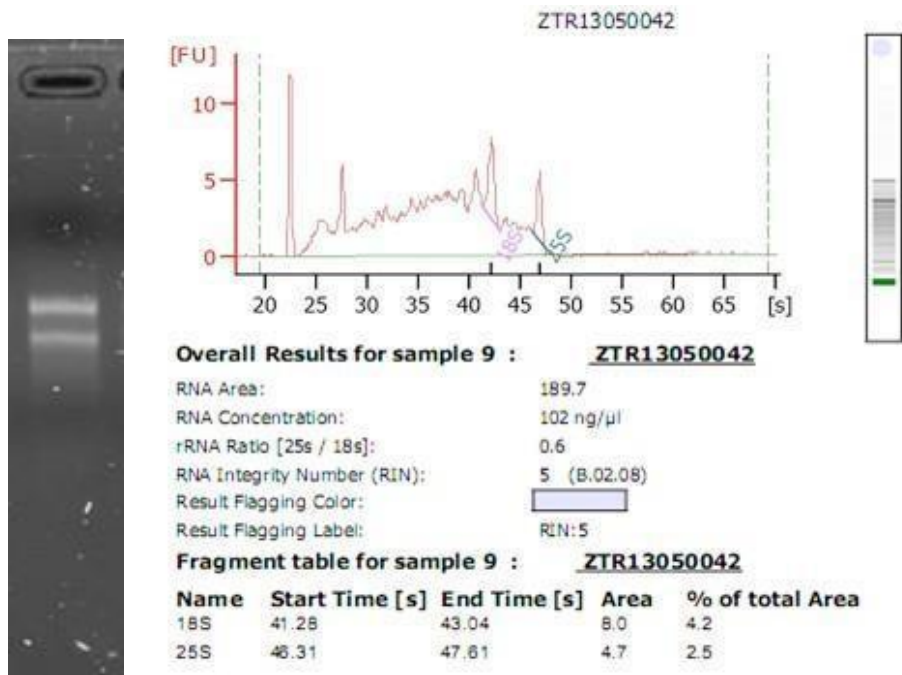


Fig. 10. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **RNA sample with solvent/ salt contamination (high viscosity)**.

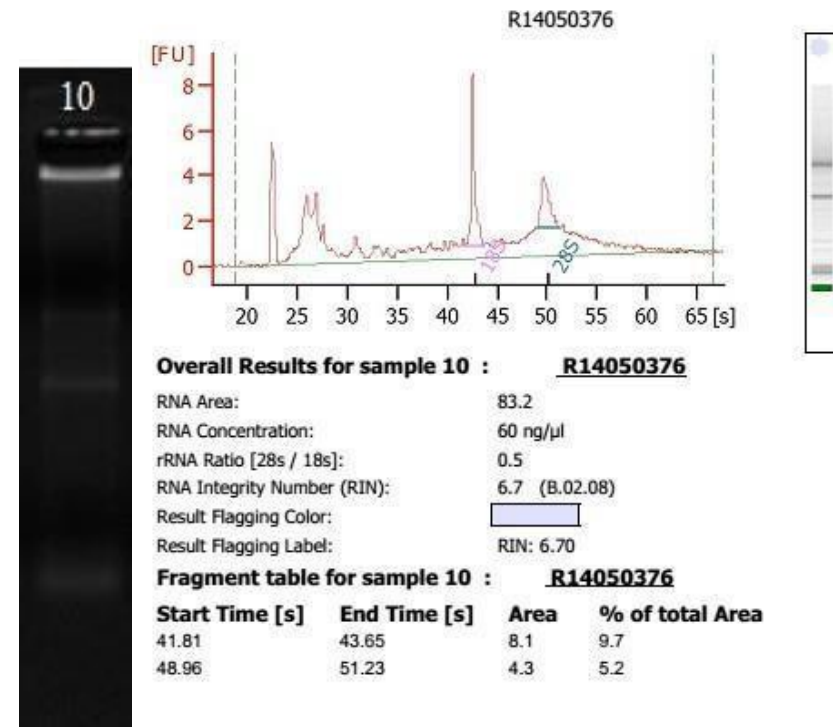


Fig. 11. An example of gel electrophoresis (left), and Agilent 2100 results (right), for a **RNA sample with protein contamination**. The RNA Labchip would not be able to show DNA contamination.

## IV. LABELING OF YOUR SAMPLE TUBES

1. It is important to have a good labelling 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 labelling 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 match 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 recommend placing the sample tubes in a container, such as a 50-ml Falcon tube. Cotton/ absorbent papers/ 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.

5. Any raw materials that require DNA/ RNA extraction services before sequencing, please confirm with us the sample submission method (e.g. amount and size of raw materials, replicates and shipping temperature) before arranging the shipment to us. Other information can be found at <https://base-asia.com/downloads/products/NGS/Sample-Submission-Guidelines-gDNA-RNA-Extraction.pdf>

## 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.
- ✓ Kindly **label your NGS Project ID** on the parcel or the Ziplock bag that secures your samples.
- ✓ Remember to provide a copy of gel photo 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.
2. Before submitting and ship the order, notify us at [ngs@apicalscientific.com](mailto:ngs@apicalscientific.com) to email the required order form and gel photo.
3. Select a reliable courier and choose the priority option for international shipments.
 

For overseas customers, we recommend FedEx (<http://fedex.com/>), UPS ([www.ups.com](http://www.ups.com)), DHL ([www.dhl.com](http://www.dhl.com)), TNT ([www.tnt.com](http://www.tnt.com)), USPS ([www.usps.com](http://www.usps.com)), and World Courier ([www.worldcourier.com](http://www.worldcourier.com)).

Please ensure the selected carrier can facilitate the importation of DNA or RNA samples, and dry ice packing (if applicable). You will need to provide us the **Air Way Bill (AWB) number** of your selected courier service in the Order Form.
4. Sample transportation options: *(Please pay attention to other notes on next page)*

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



**Notes:**

- ✓ It is highly recommended that RNA samples be shipped in dry ice packaging. Other packaging/ transportation methods may 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**.

5. For overseas customers to fulfill the requirement of customs, your local courier office will need you to provide a **Proforma Invoice** to attach with the shipment. Please complete the Pro-forma Invoice using the following information:

- i. RNA Samples or DNA Samples for Research Use Only
- ii. Non-Dangerous, Non-Infectious
- iii. No Commercial Value, Value for Customs Only
- iv. Declare the value of the goods for customs [i.e. \$1.00 (USD)]
- v. Number of samples and volumes [the # of samples, and the estimated volume in  $\mu\text{L}$ ]
- vi. Dimension of container (Height x Width x Length in cm)

**Notes:**

- ✓ It is not necessary to mention the source of nucleic acid on shipping documents (e.g. Proforma Invoice or Airwaybill).
- ✓ Please exclude sensitive words such as "Human, Tissue, Cell, blood, dry ice and etc." on shipping documents to avoid delay in custom clearance.
- ✓ Clearly state your parcel are "RNA Samples or DNA Samples for Research Use Only" is sufficient.

6. Attach the completed NGS Order Form and any pre-QC data or quick check of your NGS sample (if available). Pack the DNA and RNA samples according to the transportation options listed in (4) above. **Send the package** to the address below:

**Attn: Customer Care Department / NGS Division**

**Apical Scientific Sdn. Bhd.**

No. 7-3, Jalan SP 2/7,

Taman Serdang Perdana, Seksyen 2,

43300 Seri Kembangan, Selangor, Malaysia.

Tel: +603-8943-3252 Fax: +603-8943-3243

7. Email NGS Order Form to [ngs@apicalscientific.com](mailto:ngs@apicalscientific.com) before ship or submit the order.

8. Once the shipment is activated, email us the information below. We will assist you to **track the parcel** upon receiving the delay notification from the Courier Company or custom clearance.

- a. The NGS ID (stated in your quotation; or within the subject of the email conversation between you and our sales department);
- b. Tracking information of your airway bill (AWB) or local courier.

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 a timely update to you about the progress of your order.

Qubit is a trademark of Life Technologies and Thermo Fisher Scientific.

NanoDrop is a trademark of NanoDrop Technologies LLC.

Agilent 2100 Bioanalyzer or Agilent 2200 TapeStation is a trademark of Agilent Technologies.

Trans2K Plus is a trademark of TransGen Biotech.

1st BASE is a trademark of Apical Scientific Sdn. Bhd.