SAMPLE PREPARATION & SHIPPING INSTRUCTIONS

This document provides guidelines on how to prepare, quantify, and submit samples to Novogene. Whether you are submitting DNA or RNA samples, 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, Novogene has 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 you are unable to produce higher-quality samples, please consult with your Novogene Project Manager before shipping your samples.

Notes:

1) Input quantity should be determined by Qubit® instead of by NanoDrop™, and the final quantity and concentration should conform to Novogene's specifications.
2) Samples not meeting these specifications should be designated as "at risk" by the customer, and will be subject to billing regardless of data quality. Please consult the Project Manager for further details.

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 / Target Region	Genomic DNA	≥1 μg	≥ 0.5 μg	≥ 20 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 3 μg	≥ 1.5 μg	≥ 20 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	PCR products of single-cell whole genome	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	Fragments should be longer than 500 bp

Capture	FFPE*	≥ 2 μg	≥ 1 μg	-	-	Fragments should be longer than 1500 bp
	Single cell	≥ $5x10^7$	≥ $5x10^6$			Dissolved in Trizol

* Formalin-fixed, paraffin-embedded

2. 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 μg	≥ 500 ng	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 3 μg	≥ 1.5 μg	≥ 20 μL	≥ 20 ng/μL	
	Mitochondrion/Chloroplast DNA	≥ 1.6 μg	≥ 800 ng	≥ 20 μL	≥ 50 ng/μL	
Genotyping by Sequencing	Genomic DNA	≥ 500 ng	≥ 300 ng	≥ 10 μL	≥ 50 ng/μL	
2 Kb Insert	Genomic DNA	≥ 30 μg	≥ 15 μg	≥ 20 μL	≥ 50 ng/μL	
5 Kb Insert	Genomic DNA	≥ 30 μg	≥ 15 μg	≥ 20 μL	≥ 50 ng/μL	
10 Kb Insert	Genomic DNA	≥ 50 μg	≥ 25 μg	≥ 20 μL	≥ 50 ng/μL	
> 10 Kb Insert	Genomic DNA	≥ 80 μg	≥ 40 μg	≥ 20 μL	≥ 50 ng/μL	

3. Microbial Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/agarose gel)
		Strongly Recommended	Required			
≤ 500 bp Insert Re sequencing/ Meta Library	Genomic DNA	≥ 1.6 μg	≥ 800 ng	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 3 μg	≥ 1.5 μg	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
PCR-Free Library for	Genomic DNA	≥ 300ng	≥ 150ng	≥ 30 μL	≥ 5ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination

Amplicon	PCR Products*	≥ 400 ng	≥ 200 ng	≥ 10 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	PCR Products**	≥ 200 ng	≥ 100 ng	≥ 10 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination

* One PCR product for one library
** Multiple PCR products for one library (at least 2 different PCR products)

4. 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	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Single cell	≥ $5x10^7$	≥ $5x10^7$			Dissolved in Trizol, excluding of RNAlater
ChIP-Seq	ChIP-Seq DNA	≥ 100 ng	≥ 50 ng	≥ 10 μL	≥20 ng/μL	Main peak of 100 bp – 500 bp

5. Transcriptome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity （NanoDrop™）
		Strongly Recommended	Required				
Eukaryotic RNA-Seq	Total RNA (Animal)	≥ 2 μg	≥1 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.8, smooth base line	OD260/280≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.3, smooth base line	
Prokaryotic RNA-Seq	Total RNA	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.0, smooth base line	

6. Small RNA Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity （NanoDrop™）
		Strongly Recommended	Required				
Eukaryotic small RNA Sequencing	Total RNA (Animal)	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	≥ 7.5, smooth base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	≥ 7, smooth base line	
	Single cell	≥ $5x10^7$	≥ $5x10^7$				Dissolved in Trizol, excluding of RNAlater

7. Long non-coding Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity （NanoDrop™）
		Strongly Recommended	Required				
Eukaryotic Long non-coding RNA Sequencing	Total RNA (Animal)	≥ 4 μg	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.8, smooth base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 4 μg	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.3, smooth base line	

8. Sequel

DNA

Sample Type	Remarks	Amount (Qubit)	Concentration	Purity
Genomic DNA**	Strongly Recommended	⩾20 μg	⩾100 ng/μL	OD260/280=1.8-2.0 OD:260/230=2.0-2.2

	Required	⩾10 μg		No degradation (main band ⩾ 23kb) Nc/Qc: 1.5~1.9 No impurity No fluorochrome No RNA & protein contamination

*Please avoid any circumstances and chemicals may result in DNA damage, such as repeated freeze-thaw cycles, ultraviolet and fluorescent dyes. Freshly prepared DNA and low-temperature transport are recommended.

**Genomic DNA should be dissolved in TE buffer or EB buffer

RNA

Sample Type	Remarks	Amount (Qubit)	Concentration	RIN value	Purity
RNA	Strongly Recommended	⩾10 μg	⩾300 ng/μL	RIN⩾9	OD260/280=1.8-2.2 OD:260/230⩾1.8 Nc/Qc⩽2.0 No impurity No fluorochrome No DNA & protein contamination
	Required	⩾5 μg			

9. **RSII**

Sample Type	Remarks	Amount (Qubit)	Concentration	Purity
Genomic DNA**	Strongly Recommended	⩾30 μg	⩾40 ng/μL	OD260/280=1.8-2.0 OD:260/230=2.0-2.2 No degradation (main band ⩾ 23kb) No impurity No fluorochrome No RNA & protein contamination
	Required	⩾15 μg		

10. **Pre-prepared library**

Library volume requirement:

Data Amount	Volume Requirement*
< 30 G	≥ 10 μL
≥ 30 G	≥ 20 μL

*High concentration samples should be diluted before delivery

(2) Library concentration: 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 (Does not apply to small RNA library)

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

(4) Library concentration quantified by Q-PCR:

Platform	Concentration Requirement
HiSeq X /Nova	3 nM – 30 nM

II. PRE-QUALITY CONTROL (QC) INSTRUCTIONS

Customers had better provide the sample quality analysis results obtained using one of the following methods: Qubit®, NanoDrop™, agarose gel electrophoresis, or Agilent 2100. It is recommended samples be analyzed by Qubit/PicoGreen/gel electrophoresis (with quantity indicator), so that the results will correspond more closely to Novogene QC results. NanoDrop™ quantification is NOT recommended. If NanoDrop™ is utilized for pre-QC quantification,

Novogene strongly recommends that you send more DNA/RNA for processing than the amounts given above.

For gel electrophoresis, the following conditions are recommended:

DNA: 1.0% agarose gel; 1.0% TAE solution; 100V for 40 min

RNA: 1.0% agarose gel; 0.5× TBE solution; 180V for 16 min

Note:

Different electrophoresis conditions may generate a different, and potentially misleading, QC report on your samples. Therefore, it is highly recommended that you adhere to the conditions recommended above for the initial check, and that you provide Novogene with a picture of the gel.

III. DEMONSTRATIONS OF QUALIFIED DNA/RNA SAMPLES

1. Demonstration of Markers Used

Novogene utilizes the following molecular size markers for sample quality control testing (Fig. 1).

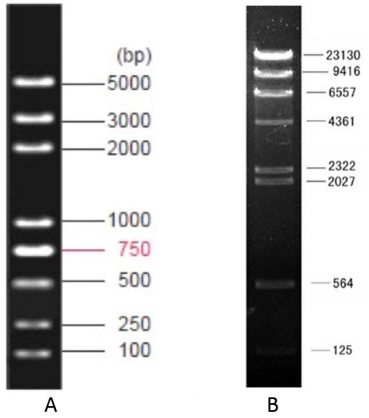


Fig. 1. (A) Trans2K^{TM} Plus DNA Marker, (B) λ/HindIII DNA Marker, bp.

2. Demonstrations of DNA sample quality

2.1 Main types of sample quality

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

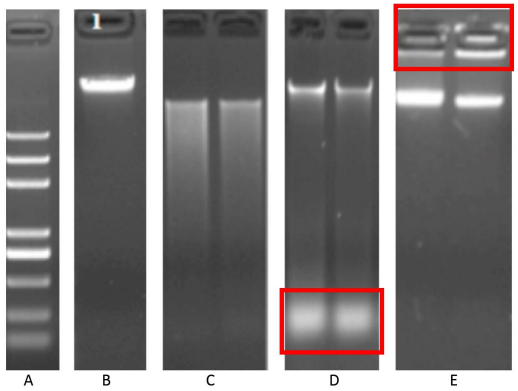


Fig. 2. Examples of DNA quality. (A) Trans2K™ Plus DNA Marker, (B) qualified sample, (C) degraded sample, (D) sample contaminated with RNA, (E) sample contaminated with protein. Red boxes denote areas of contamination

2.2 Samples with degradation

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

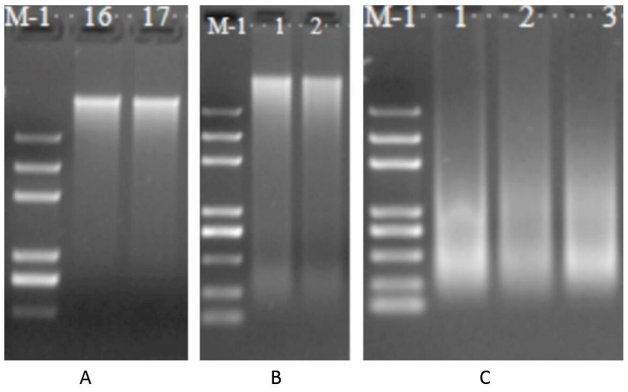


Fig. 3. DNA samples with degradation. Panels A, B, and C demonstrate increasing levels of DNA degradation. M-1, Trans2K™ Plus DNA Marker.

2.3 Samples with RNA contamination

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

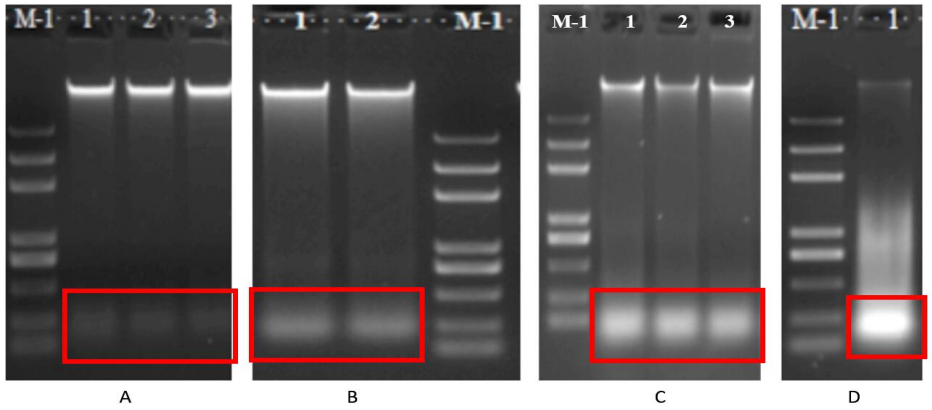


Fig. 4. DNA samples contaminated with RNA. Panels A – D demonstrate increasing levels of RNA degradation. Red boxes denote areas of contamination. M-1, Trans2K™ Plus DNA Marker.

2.4 Samples with protein contamination

DNA samples can be contaminated by proteins, as illustrated in Fig. 5. It is recommended that you purify protein-contaminated DNA samples by affinity column. Please note that column purification will lead to some loss of DNA.

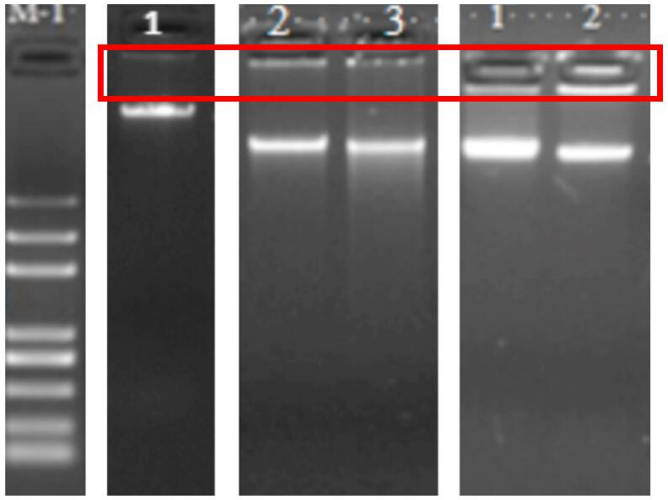


Fig. 5. DNA samples contaminated with protein. Panels A – C demonstrate increasing levels of protein contamination. Red boxes denote areas of contamination. M-1, Trans2K™ Plus DNA Marker.

3. Demonstrations of RNA sample quality

3.1 Main types of sample quality

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

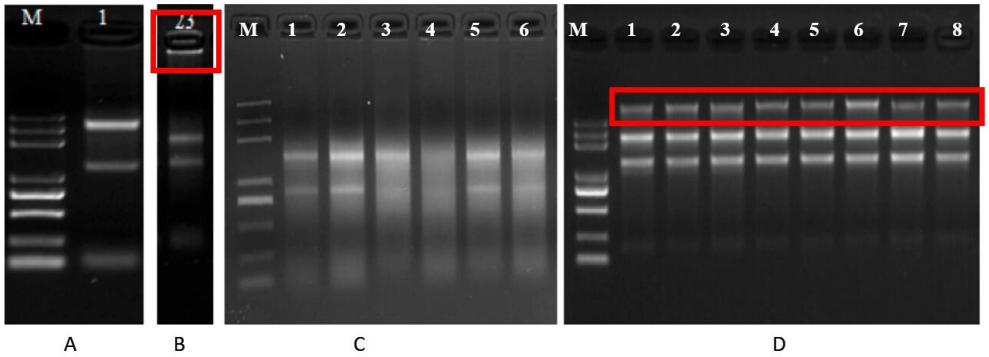


Fig. 6. Examples of RNA quality. (A) qualified sample, (B) sample with protein contamination, (C) samples with degratdation, (D) samples with genomic DNA contamination. Red boxes denote areas of contamination. M, Trans2K^{TM} Plus DNA Marker.

3.2 Samples with protein contamination

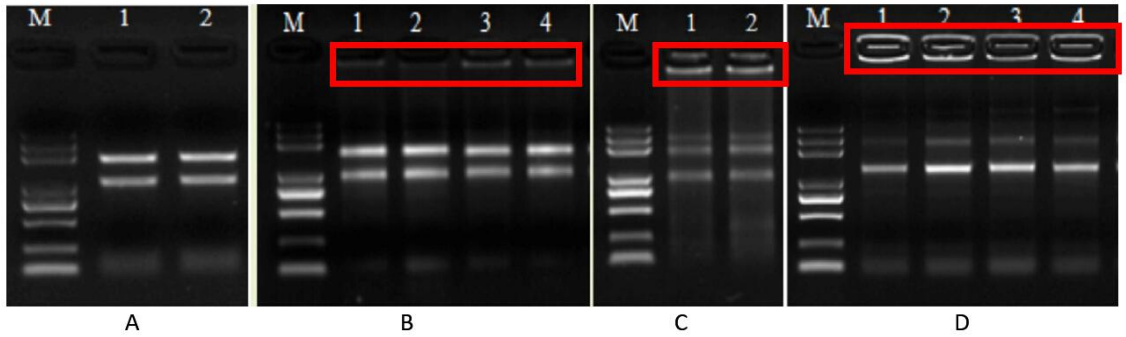


Fig. 7. RNA samples with protein contamination. Panels A – D demonstrate increasing levels of protein contamination. Red boxes denote areas of contamination. M, Trans2K^{TM} Plus DNA Marker..

3.3 Agarose gel and Agilent 2100 analysis of RNA samples

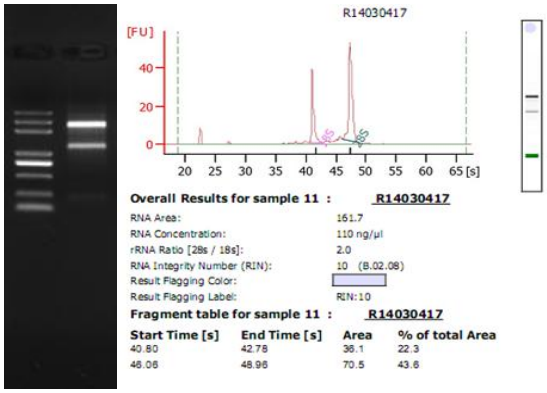


Fig. 8. An example of gel electrophoresis (left), and Agilent 2100 (right), results for an acceptable total RNA sample.

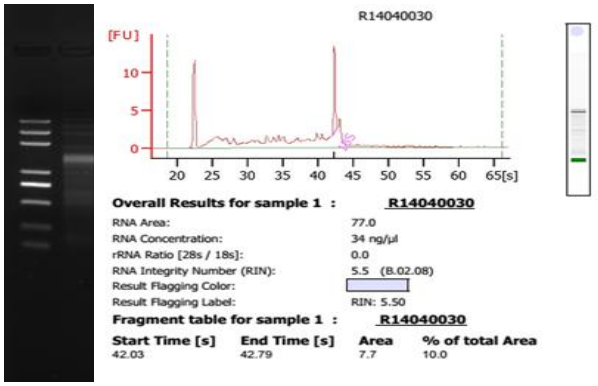


Fig. 9. An example of gel electrophoresis (left), and Agilent 2100 (right), results for a degraded total RNA sample.

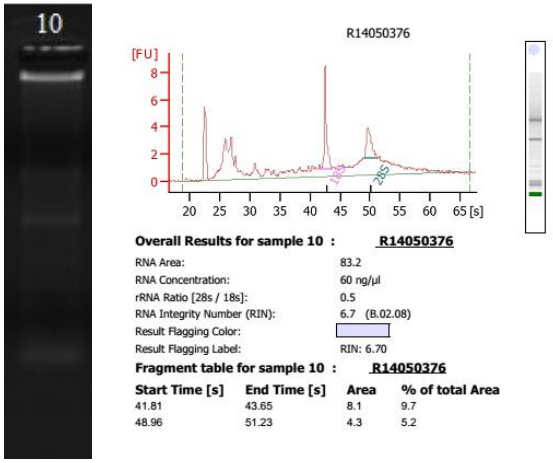


Fig. 10. An example of gel electrophoresis (left), and Agilent 2100 (right), results for an RNA sample with contamination.

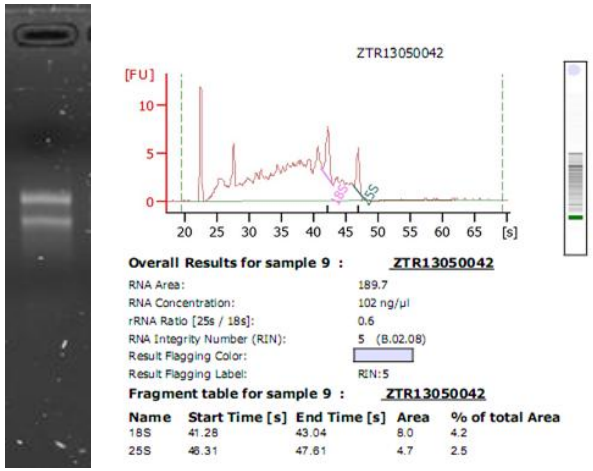


Fig. 11. An example of gel electrophoresis (left), and Agilent 2100 (right), results for a viscous total RNA sample.

IV. SAMPLE LABELING RECOMMENDATIONS

1. It is important to prevent the sample labels from being dissolved by solvents and from falling off the tubes. Use waterproof marker pen to write directly on the tube wall or lid is recommended strongly. Or you can also write the sample information on a paper/plastic label, stick the label onto the tube wall, and then secure the label to the tube by wrapping with clear, adhesive tape (e.g. Scotch tape) completely around the tube.
2. Please fill out and attach the Sample Information Form provided by Novogene in the email before shipping the samples. Please make sure that the sample information on the Sample Information Form matches the labels on the tubes.

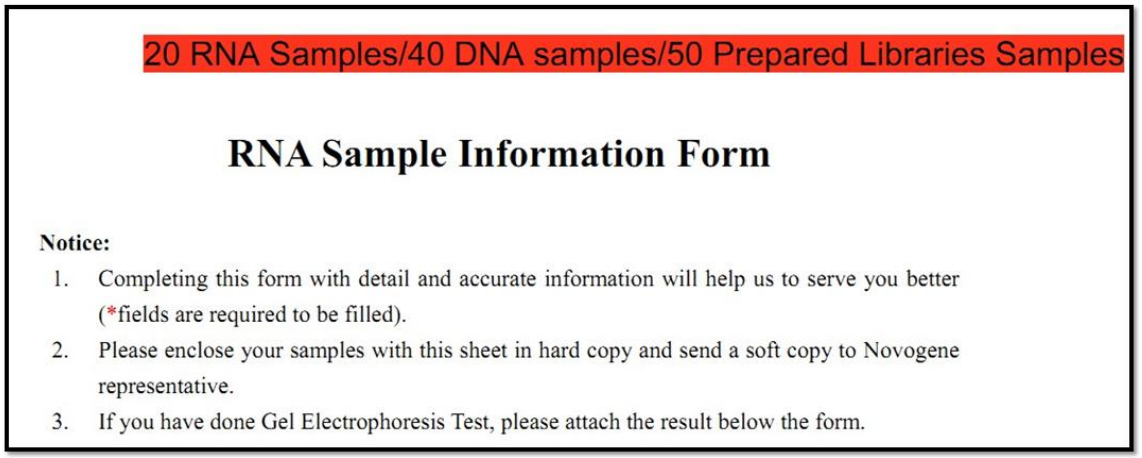
V. SAMPLE PACKING RECOMMENDATIONS

1. For DNA and RNA samples, Novogene recommends 1.5 ml or 2 ml screw-cap DNase- and RNase-free microcentrifuge tubes. Please use Parafilm to seal each tube before packaging. Novogene does not recommend shipping samples dissolved in organic solvents (such as absolute ethanol or isopropanol) because the solvents may cause leakage of the samples, which can result in cross-contamination between samples. If it is unavoidable to ship samples in organic solvents, please use screw-cap tubes and seal the opening of the tube with at least 10 layers of Parafilm.
2. In order to avoid crushing during shipping, Novogene highly recommends placing the sample tubes in a container such as a 50-ml tube or a box with interior racks/holders. Cotton and absorbent papers can be used to prevent tubes from moving around inside the container.
3. RNA samples should be kept in dry ice during shipment. Genomic DNA samples should be kept in blue ice during shipment. Saliva samples should be shipped at room temperature.
4. **In order to stick with our high quality control standards, 96-well plates and PCR stripe tubes are NOT acceptable containers for your sample shipping. The only container we allow for sample shipping is 1.5 ml or 2 ml tube. (See picture below).**

Fig. 12. Recommended and prohibited tubes for sending samples

VI. COMPLETING THE SAMPLE SUBMISSION FORM

A Sample Information Form must be submitted for each sequencing service project. All information on the forms should be filled out carefully. Please submit the completed ELECTRONIC COPY via email to our local sales representative and enclose a HARD COPY in the shipment. In both copies, please make sure you mark the samples summary (Sample types and number) at the top of the Form (Fig. 13)


20 RNA Samples/40 DNA samples/50 Prepared Libraries Samples

RNA Sample Information Form

Notice:

1. Completing this form with detail and accurate information will help us to serve you better (*fields are required to be filled).
2. Please enclose your samples with this sheet in hard copy and send a soft copy to Novogene representative.
3. If you have done Gel Electrophoresis Test, please attach the result below the form.

Fig. 13. Sample summary (top) and Sample Information Form

VII. SHIPPING SAMPLES TO NOVOGENE

1. Ensure that all samples conform to our quality standards and that they are prepared and packaged according to the guidelines given above.
2. Please make sure to notify a Novogene representative and to send the required documents before shipping your samples.
3. Carry the samples to Zhuhai(珠海), choose the SF express to send the sample to our sequencing center and tell the sale the tracking number.
4. Sample transportation options:

DNA	Lyophilize the DNA for shipping at ambient temperature
	Pack with ice packs/blue ice (2-8 °C)
	Use the cold-chain transportation system (2-8 °C) of the courier
	DNA Stable (Liquid format, Biomatrica)
	Pack in dry ice (-60 °C – -80 °C)
RNA	Lyophilize the RNA for shipping at 2-8 °C or ambient temperature
	Suspend RNA in 75% ethanol and ship on dry ice
	RNAstable (Biomatrica)
	Pack in dry ice (-60 °C – -80 °C)

Note:

1) 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 RNA.
2) The quantity of dry ice and ice bags needed varies with seasons (i.e., room temperature), transit time, and the thickness of Styrofoam box and receptacle. Please contact your local courier office for estimated transit time. **Normally, dry ice is consumed (sublimates) at a rate of 5 kg per day**.

5. Package the samples with (1) a completed and detailed Sample Information Form; and (2) include any QC data for the samples if available (Qubit/Nanodrop/agarose gel electrophoresis/Agilent 2100). Pack the DNA and RNA samples according to the above options, and send the package to the address below: (Note: the sale will inform you which address you need send sample to)

Contact：收样组

地址：天津武清区，创业总部基地 B07

Address: Building B07, Venture Headquarter Base, Tianjin Wuqing Development Area, China

Phone number: 18526699442, 18502628672

Contact：南京样本组

地址：南京浦口区扬子科创中心 A 栋三楼诺禾致源

Address: Third floor, Building A, Yangzi Science and Technology Centre, Pukou District, Nanjing

Phone number: 17701147686, 17701147586

6. Email the Sample Information Form and Purchase Order (PO) to the Novogene sales representative/project manager assigned to your project (indicated in the official quotation). Use the Sample Tracking Quote# xxx as the subject line in the email, and include the tracking information (courier name and tracking number) in the body of the email to help ensure that the samples arrive safely and without any delay.
7. After arriving at the Novogene site, samples will be stored in -80 °C freezer. The Project Manager will be responsible for providing timely feedback to you on the progress of your project.