NGS High Throughput Genomics Core at BRCAS

中央研究院 生物多樣性研究中心 新世代基因體定序核心實驗室

NGS Sample Preparation Guide 樣品送件須知

A. Preparation of Nucleic Acid

1. Nucleic acid extraction by either organic method or commercial kit requires at least twice ethanol wash steps. Do additional spin to collect residual solution at tube bottom and pipet to remove it. Air dry on benchtop (preferred over speedvac). Chemicals and contaminants (e.g. CTAB, polysaccharides, lipids, pigment, phenolic compound, etc) should be removed to avoid inhibition on library construction. **Please note an additional charge will occur for sample purification by NGS core lab should there be concern of contamination.**
2. We recommend including a DNase step (in the RNA isolation) or a RNase step (in the DNA isolation), followed by phenol:chloroform purification, to increase the sample purity. Degraded RNA may be present after RNase treatment and visualized by 2% gel check. Some commercial kits provide on-column digest to help wash away degraded RNA.
3. **RNase inhibitor is generally NOT recommended.** But if necessary, please add only at low concentration (e.g. half or quarter of the commercial suggestion) into the RNA sample, and indicate the brand name and the usage amount on the Sample Submission Form.
4. Please dissolved **RNA sample in RNase-free water**, and **DNA sample in nuclease-free molecular grade water or EB buffer (10 mM Tris-HCl, pH 8.0)**, and make sure to be free of contamination or salt.

B. Quantification

1. The accurate input of nucleic acid for successful library preparation relies on the precise quantification.
2. **Spectrophotometer** (e.g. NanoDrop) detects all matters which absorbs light per given wave length, so both DNA and RNA are detected at OD260, and may lead to over estimation of concentration, especially for DNA. All gDNA samples are recommended to submit at 2-3X higher than required input amount due to frequent issues of concentration over estimation by spectrophotometer.
3. The **fluorometric method** is recommended to determine the amount of nucleic acid. In NGS core lab, we use **Qubit Assays (Invitrogen)** to determine the specific concentration per nucleic acid type, and decide whether the sample reaches the requirement of the input amount.
4. **Please see the quantity requirement of samples for Illumina Sequencing in Table 1 and for PacBio Sequel Sequencing in Table 2.**

C. Quality Assessment

1. For successful library construction, it is important to use high-quality nucleic acid as the starting material.
2. **Absorbance ratios OD260/280 and OD260/230** are used to indicate the sample purity. Generally both ratios are around 1.8-1.9 for pure DNA, and 1.9-2.0 for pure RNA.
3. **Agarose gel analysis** is required to assess the sample integrity and potential cross-contamination between DNA/RNA. TAE buffer is recommended for electrophoresis, and gels should be run at maximum 8V per cm of gel length. Gel percentage should be adjusted depending on the sample type (0.6-0.8% for gDNA; 1.5-2% for RNA and amplicons <1.5kb). Molecular weight markers should cover 100bp~12kb range. All gels should be stained only AFTER electrophoresis in order to have even staining across the whole range.
4. **Bioanalyzer 2100 (Agilent)** provides a visual assessment with an electropherogram and gel-like image to describe the intactness of samples. The rRNA ratios and RIN values are used to infer RNA integrity.
5. **Please see the quality requirement of samples for Illumina Sequencing in Table 1 and for PacBio Sequel Sequencing in Table 2**, and attach the supplemental material (e.g. gel images or BioA traces) to the NGS Sample Submission Form for the reference.

D. Packaging and Shipping

1. Please submit your samples in **clearly labelled 1.5-ml or 1.75-ml DNase/RNase-free microcentrifuge tubes**. Low retention tube is highly recommended (e.g. Eppendorf LoBind Microcentrifuge Tubes). Autoclaved tubes should be avoided.
2. Sample volume should be corresponding to the volume indicated on the NGS Sample Submission Form.
3. To reduce freeze-thaw damage to nucleic acid, please ship your sample with surplus amount of **ice**, **dry ice**, or **gel freezer packs**.
4. RNA samples may be accepted in the pellet form in absolute ethanol for long-distance transfer, with 2-3X amount over the required quantity.
5. Please follow the procedure below to complete the NGS sequencing application.
   a. Contact NGS core manager **Dr. Mei-Yeh Lu** to inquire service application of interest, and provide sample information for visual quality inspection
   b. **Log-in LIMS** and complete on-line application
   c. Download **Sequencing Application Form** and **Sample Submission Form**, and have the form signed by PI for project approval
   d. Make an appointment and submit your samples to NGS core lab (see contact information below)

      Core Manager: Dr. Mei-Yeh Lu 呂美曄 博士

      Email: biofuel@gate.sinica.edu.tw

      Phone: (02) 2787-2218

      Location: Interdisciplinary Research Building, Room A603 跨領域科技研究大樓 A603 室

Table 1. Information and Requirements for Illumina Sample

Sample Type	Suitable Application	Recommend Quantity	Volume & Concentration (fluorometric measurement)	Quality Assessment
Genomic DNA	Methylation DNA	**1 – 2 ug**	in 25 ul **(≥ 40 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. 0.6% gel analysis (1X TAE; post-run staining) - Include λ-HindIII ladder or Invitrogen 1KB Extension DNA Ladder (cover 100bp~12kb range) - Intact chromosome band above 23 kb - No degradation/smear observed 3. RNase treated (< 10% RNA out of DNA) 4. DNase I digestion test (recommended) - Treat 0.5 ug of gDNA with 0.1U of DNase I (25˚C for 5 min and 95˚C for 5 min), and check the digestion pattern on 3% agarose gel (1X TAE; post-run staining)
	Paired-End DNA (0.2-0.7 kb)	**2 – 3 ug**	in 50 ul **(≥ 40 ng/ul)**	
	Long Insert PE DNA (1-2 kb)	**2 – 3 ug**	in 50 ul **(≥ 40 ng/ul)**	
	Mate-Pair DNA (1-40 kb)	**10 – 20 ug**	in 100 ul **(≥ 100 ng/ul)**	
	Synthetic Long-Read DNA	**1 – 2 ug**	in 50 ul **(≥ 20 ng/ul)**	
	Nextera DNA	**5 – 500 ng**	in 20 ul **(≥ 0.25 ng/ul)**	
	Human Exome Capture	**1 – 2 ug**	in 50 ul **(≥ 20 ng/ul)**	
Total RNA	Small RNA (miRNA)	**8 – 10 ug**	in 10 ul **(≥ 200 ng/ul)**	1. OD260/280 ≥ 1.9; OD260/230 ≥ 1.8 2. 2% gel analysis (1X TAE; post-run staining) - Clear rRNA bands (28S:18S ~ 2:1) 3. DNase treated (< 10% DNA out of total RNA) 4. BioAnalyzer traces (recommended) - rRNA ratio (28S/18S) ≥ 1.8 - RIN value ≥ 8
	Degradome	**100 – 150 ug**	in 100 ul **(≥ 1000 ng/ul)**	
	Stranded RNA (Poly-A)	**4 – 6 ug**	in 50 ul **(≥ 80 ng/ul)**	
	Stranded RNA (Ribo-Zero)	**4 – 6 ug**	in 50 ul **(≥ 80 ng/ul)**	
	Low-Input Stranded RNA	**5 – 100 ng**	in 25 ul **(≥ 0.2 ng/ul)**	
Amplicon	Indexing PCR (2nd Step PCR)	**≥ 300 ng**	in 20 ul **(≥ 15 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. 2% gel analysis (1X TAE; post-run staining) 3. BioAnalyzer traces (recommended) - Size range 200 – 700 bp
ds cDNA	Nextera DNA	**5 – 500 ng**	in 20 ul **(≥ 0.25 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. BioAnalyzer traces (recommended)
ChIP DNA	ChIP Seq	**5 – 10 ng**	in 25 ul **(≥ 0.2 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. BioAnalyzer traces (recommended) - Size range 100 – 500 bp after shearing; major at ~250 bp 3. qPCR with gene-specific primers (recommended)
Ready-to-Seq Library	Ready-to-Seq		in 20 ul **(≥ 10 nM or ≥ 10 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. 2% gel analysis (1X TAE; post-run staining) 3. BioAnalyzer traces (recommended) 4. qPCR result (recommended)

Table 2. Information and Requirements for PacBio Sequel Sample

Sample Type	Suitable Application	Recommend Quantity	Volume & Concentration (fluorometric measurement)	Quality Measurement
Genomic DNA	Genome Sequencing	**30 – 60 ug**	in 200 ul **(≥ 150 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. Qubit/Nanodrop ratio = 0.9~1.0 3. 0.6% gel analysis (1X TAE; post-run staining) - Include λ-HindIII ladder or Invitrogen 1KB Extension DNA Ladder (cover 100bp~12 kb range) - Intact chromosome band above 23 kb - No degradation/smear observed 4. RNase treated (< 10% RNA out of DNA) 5. DNase I digestion test (recommended) - Treat 0.5 ug of gDNA with 0.1U of DNase I (25˚C for 5 min and 95˚C for 5 min), and check the digestion pattern on 3% agarose gel (1X TAE; post-run staining)
Amplicon	Full Length Amplicon Sequencing	**5 – 10 ug**	in 50 ul **(≥ 100 ng/ul)**	1. OD260/280 = 1.8~1.9; OD260/230 ≥ 1.8 2. 2% gel analysis (1X TAE; post-run staining) 3. BioAnalyzer traces (recommended)
Total RNA	Isoform Sequencing (Iso-Seq)	**5 – 10 ug**	in 10 ul **(≥ 500 ng/ul)**	1. OD260/280 ≥ 1.9; OD260/230 ≥ 1.8 2. 2% gel analysis (1X TAE; post-run staining) - Clear rRNA bands (28S:18S ~ 2:1) 3. DNase treated (< 10% DNA out of total RNA) 4. BioAnalyzer traces (recommended) - rRNA ratio (28S/18S) ≥ 1.8 - RIN value ≥ 8