Novogene

# Novogene Sample Requirements

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Advancing Genomics, Improving life

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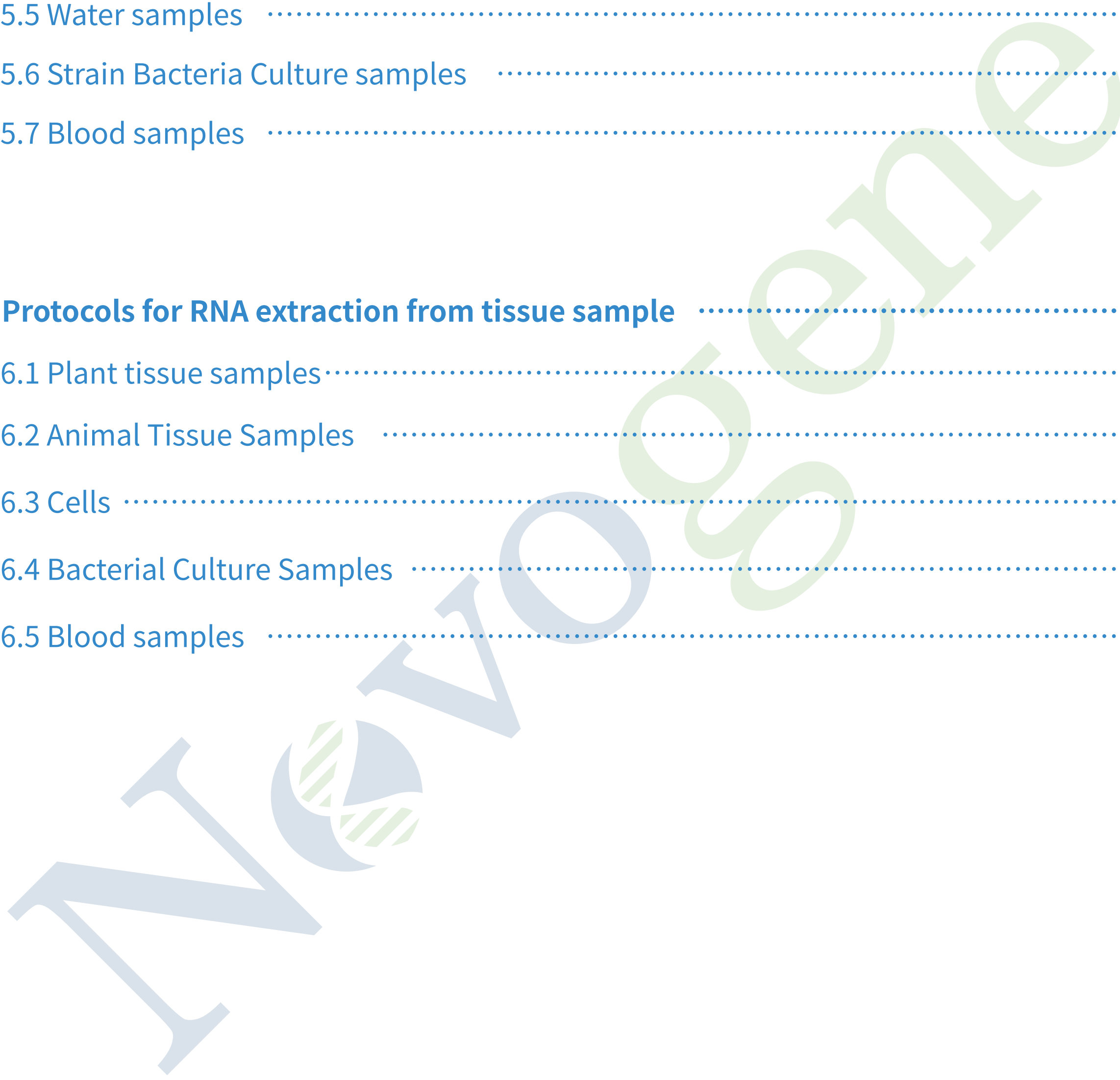

Note: If you have other library types and sample types not mentioned below, please contact your local representative. Double Sample Amount is recommended.

# 1 Genome Sequencing

## 1.1 Human Whole Genome Sequencing

| Library Type | Sample Type | Amount (Qubit®) | Volume | Concentration | Purity (NanoDropTM/Agarose Gel) |
|---|---|---|---|---|---|
| Human whole genome library (350bp) | Genomic DNA | ⩾ 200 ng | ⩾ 20 μL | ⩾ 10 ng/μL | OD260/280 = 1.8-2.0; no degradation, no contamination |
| | Genomic DNA (PCR-free) | ⩾ 1 μg | ⩾ 20 μL | ⩾ 20 ng/μL | |
| | FFPE* DNA | ⩾ 300 ng | - | - | Fragments should be longer than 1500 bp |

* FFPE: Formalin-Fixed, Paraffin-Embedded
Recommended suspension buffer: TE, EB and TB

## 1.2 Exome Sequencing/ Target Region Capture

| Library Type | Sample Type | Amount (Qubit®) | Volume | Concentration | Purity (NanoDropTM/Agarose Gel) |
|---|---|---|---|---|---|
| DNA library (Human Exome or Human Target region) | Genomic DNA | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | OD260/280 = 1.8-2.0; no degradation, no contamination |
| | FFPE* | ⩾ 300 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) |
|---|---|---|---|---|---|
| Mouse Exome Library | Genomic DNA | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | OD260/280 = 1.8-2.0; no degradation, no contamination |
| | FFPE* | ⩾ 300 ng | - | - | Fragments should be longer than 1000 bp |

* FFPE: Formalin-Fixed, Paraffin-Embedded
Recommended suspension buffer: TE, EB and TB

## 1.3 Plant & Animal Genome Sequencing

| Library Type | Sample Type | Amount (Qubit®) | Volume | Concentration | Purity (NanoDrop™/Agarose Gel) |
|---|---|---|---|---|---|
| Plant and animal whole genome library ( ⩽ 500 bp) | Genomic DNA | ⩾ 200 ng | ⩾ 20 µL | ⩾ 10 ng/µL | OD260/280 = 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 µg | ⩾ 20 µL | ⩾ 20 ng/µL | |

Recommended suspension buffer: TE, EB and TB

## 1.4 Microbial Genome Sequencing

| Library Type | Sample Type | Amount (Qubit®) | Volume | Concentration | Purity (NanoDrop™/Agarose Gel) |
|---|---|---|---|---|---|
| Metagenomics library | Genomic DNA | ⩾ 200 ng | ⩾ 20 µL | ⩾ 10 ng/µL | OD260/280 = 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 µg | ⩾ 20 µL | ⩾ 10 ng/µL | |

Recommended suspension buffer: TE, EB and TB

## 1.5 PacBio Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | Purity |
|---|---|---|---|---|---|
| PacBio Sequel II DNA CLR library | HMW Genomic DNA (Animal and Plant) | ⩾ 7 µg | ⩾ 50 µL | ⩾ 80 ng/µL | A260/280=1.7~2.0; A260/230=1.5~2.6; NC/QC=0.95~3.00 Fragments should be ⩾ 30K |
| | HMW Genomic DNA (Bacteria and Fungus) | ⩾ 4 µg | ⩾ 50 µL | ⩾ 70 ng/µL | A260/280=1.7~2.0; A260/230=1.3~2.6; NC/QC=0.95~3.00 Fragments should be ⩾ 20K |
| PacBio sequel II/IIe DNA HiFi library | HMW Genomic DNA (Animal and Plant) | ⩾ 15 µg | ⩾ 50 µL | ⩾ 80 ng/µL | A260/280=1.8~2.0; A260/230=1.5~2.6; NC/QC=0.95~3.00 Fragments should be ⩾ 30K |
| | HMW Genomic DNA (Bacteria and Fungus) | ⩾ 15 µg | ⩾ 50 µL | ⩾ 70 ng/µL | A260/230=1.3~2.6; A260/280=1.7~2.0; NC/QC=0.95~3.00 Fragments should be ⩾ 20K |

**HMW: High Molecular Weight
***NC/QC: NanoDrop concentration/Qubit concentration
Recommended suspension buffer: EB

## 1.6 Nanopore Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | Purity |
|---|---|---|---|---|---|
| Nanopore PromethION DNA library | HMW Genomic DNA (Animal and Plant) | ⩾ 8 µg | ⩾ 50 µL | ⩾ 60 ng/µL | OD260/280=1.75-2.0;<br>OD260/230=1.4-2.6;<br>NC/QC=0.95~3.00<br>Fragments should be ⩾ 30K |
| | HMW Genomic DNA (Bacteria and Fungus) | ⩾ 6 µg | ⩾ 50 µL | ⩾ 60 ng/µL | OD260/230=1.4-2.6;<br>OD260/280=1.75-2.0;<br>NC/QC=0.95~3.00<br>Fragments should be ⩾ 20K |

**HMW: High Molecular Weight
***NC/QC: NanoDrop concentration/Qubit concentration
Recommended suspension buffer: EB

## 1.7 PCR Product Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | Purity (NanoDropTM/Agarose Gel) |
|---|---|---|---|---|---|
| PCR free library | PCR product | ⩾ 1 µg | ⩾ 20 µL | ⩾ 50 ng/µL | OD260/280 = 1.8-2.0;<br>no degradation, no contamination |
| Library (with PCR) | PCR product | ⩾ 200 ng | ⩾ 20 µL | ⩾ 10 ng/µL | |

Recommended suspension buffer: TE, EB and TB

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# 2 RNA Sequencing

## 2.1 Transcriptome Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | RNA Integrity Number (Agilent 2100) | Purity (NanoDrop™) |
|---|---|---|---|---|---|---|
| Eukaryotic mRNA library (poly A enrichment) | Total RNA (Animal) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 4.0, with flat base line | OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation, no contamination |
| | Total RNA (Plant and Fungus) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 4.0, with flat base line | |
| | Total RNA (Blood) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 5.8, with flat base line | |
| | Amplified cDNA (double-strand) | ⩾ 100ng | ⩾ 10 μL | ⩾ 10 ng/μL | Fragments distributing between 400bp-5000bp, with the main peak at ~2000bp; | OD260/230 ⩾ 2.0; no degradation, no contamination |
| Eukaryotic Directional mRNA library (poly A enrichment) | Total RNA (Animal) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 5.8, with flat base line | OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation, no contamination |
| | Total RNA (Plant and Fungus) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 5.8, with flat base line | |
| | Total RNA (Blood) | ⩾ 400 ng | ⩾ 20 μL | ⩾ 20 ng/μL | ⩾ 5.8, with flat base line | OD260/280 ⩾ 2.0, OD260/230 ⩾ 2.0, no degradation, no contamination |
| Prokaryotic RNA library | Total RNA | ⩾ 2 μg | ⩾ 20 μL | ⩾ 50 ng/μL | ⩾ 6.0, with flat base line | OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation, no contamination |
| Meta-transcriptome library | Total RNA | ⩾ 2 μg | ⩾ 20 μL | ⩾ 50ng/μL | ⩾ 6.5, with flat base line | OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation, no contamination |

Recommended suspension buffer: RNase-free ddH2O

## 2.2 Eukaryotic Small RNA Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | RNA Integrity Number (Agilent 2100) | Purity (NanoDrop™) |
|---|---|---|---|---|---|---|
| Small RNA library (with 18~40 bp insert) | Total RNA (Animal) | ≥ 2 μg | ≥ 20 μL | ≥ 50 ng/μL | ≥ 7.5, with smooth base line | OD260/280 ≥ 2.0; OD260/230 ≥ 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 | OD260/280 ≥ 2.0; OD260/230 ≥ 2.0; no degradation, no contamination |

Recommended suspension buffer: RNase-free ddH2O

## 2.3 Eukaryotic Long Non-coding RNA Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | RNA Integrity Number(Agilent 2100) | Purity (NanoDrop™) |
|---|---|---|---|---|---|---|
| Directional RNA library (rRNA removal) | Total RNA (Animal) | ≥ 500 ng | ≥ 10 μL | ≥ 50 ng/μL | ≥ 6.5, flat base line | OD260/280 ≥ 2.0; OD260/230 ≥ 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 sensitive Agilent Bioanalyzer 2100), FU>10, with no peak>2000nt | OD260/280 ≥ 2.0; OD260/230 ≥ 2.0; no degradation, no contamination |

Recommended suspension buffer: RNase-free ddH2O

## 2.4 Eukaryotic CircRNA Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | RNA Integrity Number(Agilent 2100) | Purity (NanoDrop™) |
|---|---|---|---|---|---|---|
| CircRNA library | Total RNA (Animal) | ≥ 2 μg | ≥ 20 μL | ≥ 50 ng/μL | ≥ 7, flat base line | OD260/280 ≥ 2.0; OD260/230 ≥ 2.0; no degradation, no contamination |
| | Total RNA (Plant and Fungus) | ≥ 2 μg | ≥ 20 μL | ≥ 50 ng/μL | ≥ 6.5, flat base line | |

Recommended suspension buffer: RNase-free ddH2O

## 2.5 Whole Transcriptome Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | RNA Integrity Number (Agilent 2100) | Purity (NanoDrop™) |
|---|---|---|---|---|---|---|
| lncRNA library & Small RNA library | Total RNA | ⩾ 3 μg | ⩾ 30 μL | ⩾ 50 ng/μL | Animal ⩾ 7.5, Plant ⩾ 7, with smooth baseline | OD260/280 ⩾ 2.0; OD260/230 ⩾ 2.0; no degradation, no contamination |
| lncRNA library & Small RNA library & CircRNA library | Total RNA | ⩾ 5 μg | ⩾ 50 μL | ⩾ 50 ng/μL | | |

Recommended suspension buffer: RNase-free ddH2O

## 2.6 Ribosome Profiling

| Libray Type | Sample Type | Amount | Volume | Concentration | Main Peak | Purity |
|---|---|---|---|---|---|---|
| Ribo library | RPF Sample | ⩾ 2 μg | ⩾ 10 μL | ⩾ 200 ng/μL | 25-38 nt | No degradation, no contamination |

## 2.7 PacBio Iso-seq

| Library Type | Sample Type | Amount | Volume | Concentration | RIN (Agilent 2100) | Purity (Nanodrop™/ Agarose Gel) |
|---|---|---|---|---|---|---|
| PacBio sequel II/IIe RNA Library | Total RNA | ⩾ 800 ng | ⩾ 50 μL | ⩾ 60 ng/μL | ⩾ 6.5 | A260/280=1.8-2.2; A260/230=1.3-2.5; NC/QC ⩽ 2.5 |

*NC/QC: NanoDrop concentration/Qubit concentration
Recommended suspension buffer: RNase-free ddH2O

# 3 Epigenetics Sequencing

| Library Type | Sample Type | Amount | Volume | Concentration | Purity (NanoDrop™/Agarose Gel) |
|---|---|---|---|---|---|
| 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 | ⩾ 30 ng | ⩾ 20 μL | ⩾ 2 ng/μL | Main peak of 100 bp-500 bp |
| RIP-seq library | Enriched RNA | ⩾ 100 ng | ⩾ 20 μL | ⩾ 3 ng/μL | Without fragmentation; fragments should be longer than 1000 bp |

Recommended suspension buffer:
TE, EB and TB for DNA and enriched DNA
RNase-free ddH2O for enriched RNA

# 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) |

## 4.2 Library concentration

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

2 nM-30 nM, quantified by Q-PCR

*Notes:

High concentration samples should be diluted before delivery.

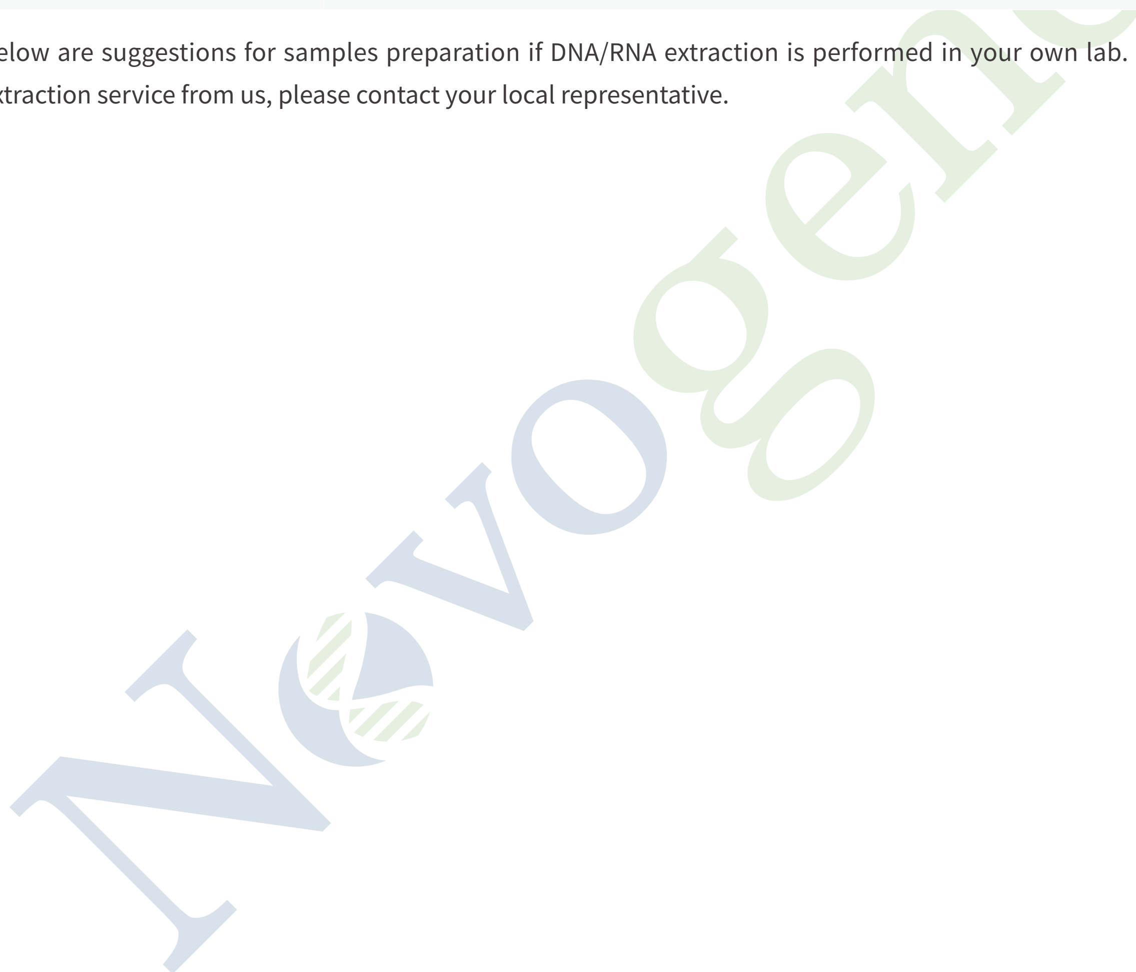
Pre-made libraries should be colorless.

## 4.3 Library size

Library size: insert + adapters (120 bp) ± 50 bp (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) |

Below are suggestions for samples preparation if DNA/RNA extraction is performed in your own lab. If you need extraction service from us, please contact your local representative.



# 5. DNA extraction notice

## 5.1 Plant tissue samples

5.1.1 Freshly collected tissues are preferred. Select tissues with relatively high nucleic acid content, such as young parts of plants.

5.1.2 If cryopreservation is needed, it is recommended to freeze samples with liquid nitrogen immediately after separation. Prior to freezing, wipe collected samples with sterile water or 75% ethanol.

5.1.3 Absorbent paper can be used to blot the sample surface. Cut sample into small pieces of about 100 mg, and freeze immediately with liquid nitrogen.

5.1.4 Place frozen sample in pre-chilled cryopreservation tubes, 15 mL/50 mL centrifuge tubes or Ziplock bags, and store at-80°C . If tissue samples are packaged with foil, the texture must be followed when folding, and the foil should be placed in a Ziplock bag to avoid spilling the sample when the foil is opened.

5.1.5 When collecting samples, it is recommended that the samples should be stored in separate tubes according to the amount of primary extraction, to avoid sample degradation due to secondary separation and merging during extraction.

## 5.2 Animal tissue samples

5.2.1 Freshly collected samples for DNA extraction is preferred. It is recommended to select tissues with high content of nucleic acid (such as animal liver or other tissues).

5.2.2 It is recommended to rinse collected sample with cold saline to remove blood and contaminants Unwanted tissues (such as connective tissue, hair) should be removed.

5.2.3 Divide the samples into small pieces of about 50 mg (the smaller the tissue is, the better preservation effect it has). Cut tissues into smaller pieces (~50 mg) on ice, to improve preservation.

5.2.4 If cryopreservation is needed, the fresh samples collected from animals should be frozen immediately with liquid nitrogen. Samples are to be put into 1.5 mL or 2.0 mL precooled EP or cryopreservation tube with screw cap, sealed with sealing film and stored at-80°C . Please avoid overfilling the container with sample to prevent cracking and sample contamination during freezing.

5.2.5 If nucleic acid stabilizer is used for preservation of tissue samples, please operate strictly in accordance with the requirements of the reagent specification. Do keep tissue block within the size range required by the reagent, so as to ensure samples can be fully penetrated by the reagent to avoid degradation.

## 5.3 Microbial samples

5.3.1 Use an alcohol-sterilized shove to dig and collect 5~20 cm depth of soil, remove visible roots and filter the soil with 2 mm sieve. Each sample is collected from three different sampling points and pooled together.

5.3.2 Sample is collected in sterile centrifuge tubes and placed below 0°C , for transportation back to the laboratory for DNA extraction. If the extraction cannot be carried out immediately, the soil samples should be frozen in the freezer at-80°C or-20°C .

5.3.3 It is strongly recommended to use 5 mL EP or 15 mL/50 mL centrifuge tube for transporting soil samples. Seal tubes with parafilm. Note that the use of self sealing bags has great risk of sample cross contamination.

5.3.4 Aliquot samples into various tubes to avoid sample degradation due to repeated freeze-thawing.

5.3.5 For plant root microbial research please refer this paper: Edwards J, Johnson C, Santos-Medellín C, et al. Structure, variation, and assembly of the root-associated microbiomes of rice[J]. Proceedings of the National Academy of Sciences, 2015, 112(8).

5.3.6 If you do not use the commercial kit for extraction, please follow the below papers: Clegg C D, Ritz K, Griffiths B S. Direct extraction of microbial community DNA from humified upland soils[J]. Letters in Applied Microbiology, 1997, 25(1):30-33. Researchers in this paper got enough amount and high-quality microbial DNA from humic soil.-DNA amount is 30 µg per kg dry soil-OD260/280 & OD260/230 is between 1.6~2.0.

5.3.7 If client want to use commercial kit we recommend PowerSoil @DNA Isolation Kit of Mobio. Reference website: http://www.mobio.com/soil-dna-isolation/powersoil-dna isolation-kit.html

## 5.4 Stool samples

5.4.1 For detail step, please check: Kyle G. Bowel care. Part 3--obtaining a stool sample[J]. Nursing times, 2007, 103(44):24-25. The specific steps are as follows:

a. Prepare feces container, wash hands and put on gloves;

b. Collect the sample with a spoon (1 g can be collected and packed separately in each tube) and put into 5 mL EP or 15 mL, 50 mL centrifugal tube. After collecting, screw the cap tightly and seal with parafilm. Keep the sample in an anaerobic environment, if possible, with an anaerobic bag;

c. Discard the feces container and gloves and store the samples at-80°C to avoid repeated freezing and thawing. If fecal samples are too large or cannot be collected immediately, the collection shall be completed within 4 hours at the latest.

5.4.2 Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

5.4.3 If you do not use the commercial kit for fecal extraction, please refer to the following literature: Furet J P, Firmesse O, Gourmelon M, et al. Comparative assessment of human and farm animal fecal microorganisms using real-time quantitative PCR. FEMS Microbiology Ecology, 2009.

5.4.4 If a commercial kit is used: recommended kit for DNA extraction is powerfecal @DNA isolation kit. (Mobio) Reference website: http://www.mobio.com/fecal-dna-isolation/powerfecal-dna isolation-kit.html

## 5.5 Water samples

5.5.1 Filter collected water through filter membranes. According to the turbidity of the water samples, the filter membranes with corresponding pore sizes can be selected. For turbid water, 0.45 μm pore size filter membrane can first be used to remove suspended particles. Filter membranes with small pore sizes (0.22 μm) is then selected to filter sediment-free water samples. Filter 10 L of clear water to obtain microbial-enriched filter membrane.

5.5.2 After filtering, the microbial enriched filter membrane can be installed in a 50 mL centrifugal tube (the area of the filter membrane should not be too large, take the part with a diameter of 3~4 cm rich in microorganisms) and storage. If clients want to use commercial kit, we recommend PowerWater® Sterivex ™ DNA Isolation Kit from Mobio. Please check this reference: http://www.mobio.com/water dna-isolation/powerwater-sterivex-dna-isolation-kit.html

## 5.6 Strain Bacteria Culture samples

5.6.1 For bacterial samples, it is recommended to collect bacteria during the phase of logarithmic growth.

5.6.2 Collect the strains by centrifugation at low speed. Discard supernatant. Wash bacterial pellet with sterile water or PBS buffer (1~3 times). Store sample in 1.5/2 mL EP tube. Seal with sealing film, and stored at-8°C .

5.6.3 Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

## 5.7 Blood samples

5.7.1 Collect whole blood samples with EDTA anticoagulation blood collection tubes (Avoid heparin anticoagulant).

5.7.2 Blood samples should not be subjected to repeated freeze-thaw cycles. Fresh blood can be placed at 4°C for 12~24 hours. However, the frozen samples must be stored at low temperature.

# 6. RNA extraction notice

## 6.1 Plant tissue samples

6.1.1 Freshly collected tissues are preferred. Tissues with relatively high nucleic acid content is advised to be selected, such as young parts of plants.

6.1.2 It is recommended to quickly wipe or rinse freshly collected plant sample with RNase free water, and blot dry with absorbent paper.

6.1.3 Quickly cut tissues into smaller pieces (~100 mg) on ice. To ensure RNA preservation, it is recommended to complete this step within 3 minutes after tissue separation.

6.1.4 Freeze sample with liquid nitrogen and store in a pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with parafilm and stored at -80°C immediately.

6.1.5 Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

## 6.2 Animal Tissue Samples

6.2.1 Using freshly collected samples for RNA extraction is preferred. For sample collection, it is recommended to select tissues with high content of nucleic acid (such as animal liver or other tissues).

6.2.2 It is recommended to rinse freshly extracted tissue sample from living animals immediately, with precooled RNase-free water. Ensure removal of blood, unwanted tissues (such as hair and connective tissue) and other contaminants.

6.2.3 Quickly cut tissues into smaller pieces (50~100 mg) on ice and freeze using liquid nitrogen. Store sample in pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with sealing film and stored at-80°C immediately. To ensure RNA preservation, it is recommended to complete this step within 3 minutes upon tissue removal from living body. Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

6.2.4 Note: If using commercial RNA protection reagent (such as RNAlater), please follow manufacturer recommendation.

## 6.3 Cells

6.3.1 Cell suspensions: Rinse suspended cells with PBS buffer. Add TRIzol reagent (1 mL per $5\times10^6$ cells). Aspirate the cell suspension using syringe to break up any obvious clumps until the suspension turns clear. Store in -80 °C freezer.

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6.3.2 Adherent cells: Rinse adherent cells with PBS buffer. Add TRIzol reagent (1 mL per 10cm2 of culture area=a well of 6-well plate=a 35 mm diameter petri dish). Repeat pipetting to ensure TRIzol to contact all cell surface. Transfer suspensions to RNase-free 1.5 mL or 2 mL centrifuge tubes. Aspirate the cell suspension using syringe to break up any obvious clumps until the suspension turns clear. Store in-80°C freezer.

6.3.3 Collect cell samples into the centrifuge tube and remove the culture medium. Rinse cell pellets with PBS buffer, and remove the supernatant by centrifuge. Quickly freeze with liquid nitrogen. No lysate is needed. Store in-80°C freezer.

6.3.4 Do not use RNAlater for cell samples because it' s difficult to remove RNAlater reagents by centrifuge as RNAlater has a high density.

6.3.5 Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

## 6.4 Bacterial Culture Samples

6.4.1 For bacterial samples, it is recommended to collect bacteria during the phase of logarithmic growth.

6.4.2 Collect the strains by centrifugation at low speed and discard supernatant. Wash pellet with sterile water or PBS buffer (1~3 times). Store sample in pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with sealing film and stored at -80 °C immediately. Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

6.4.3 It' s recommended not to send samples utilizing TRIzol lysate because TRIzol method fails to the extraction of nucleic acid from some of bacteria.

6.4.4 It is not recommended to store samples with low bulk density in RNAlater and other tissue with RNA protection reagents. Because the density of RNAlater is a little high which increase the difficulty of collection by centrifugation for extraction.

## 6.5 Blood samples

6.5.1 Sample preparation by TRIzol method

(1) Add 6 mL TRIzol and 2 mL fresh blood (TRIzol: blood=3: 1) together in a 15 mL tube. Repeat pipetting to ensure TRIzol to contact all cell surface.

(2) Intensely shake and mix the sample for one to two minutes until the floc is completely dissolved.

(3) Incubate at room temperature for 5 minutes.

(4) Aliquot and store sample into 4 cryovials (2 mL). Seal with film and store at-80°C .

Note: RNAlater is not recommended.

6.5.2 BD PAXgene Blood RNA Tube

(1) Collect blood

Product name: BD 762165 PAXgene Blood RNA Tube

Note: Please refer to the manufacture’ s introduction for more detailed information.

(2) Sample Extraction

The special kit from Qiagen is always used to extract total RNA from blood samples Collected.

Kit name: PAXgene Blood miRNA Kit (Qiagen) (50 times)

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