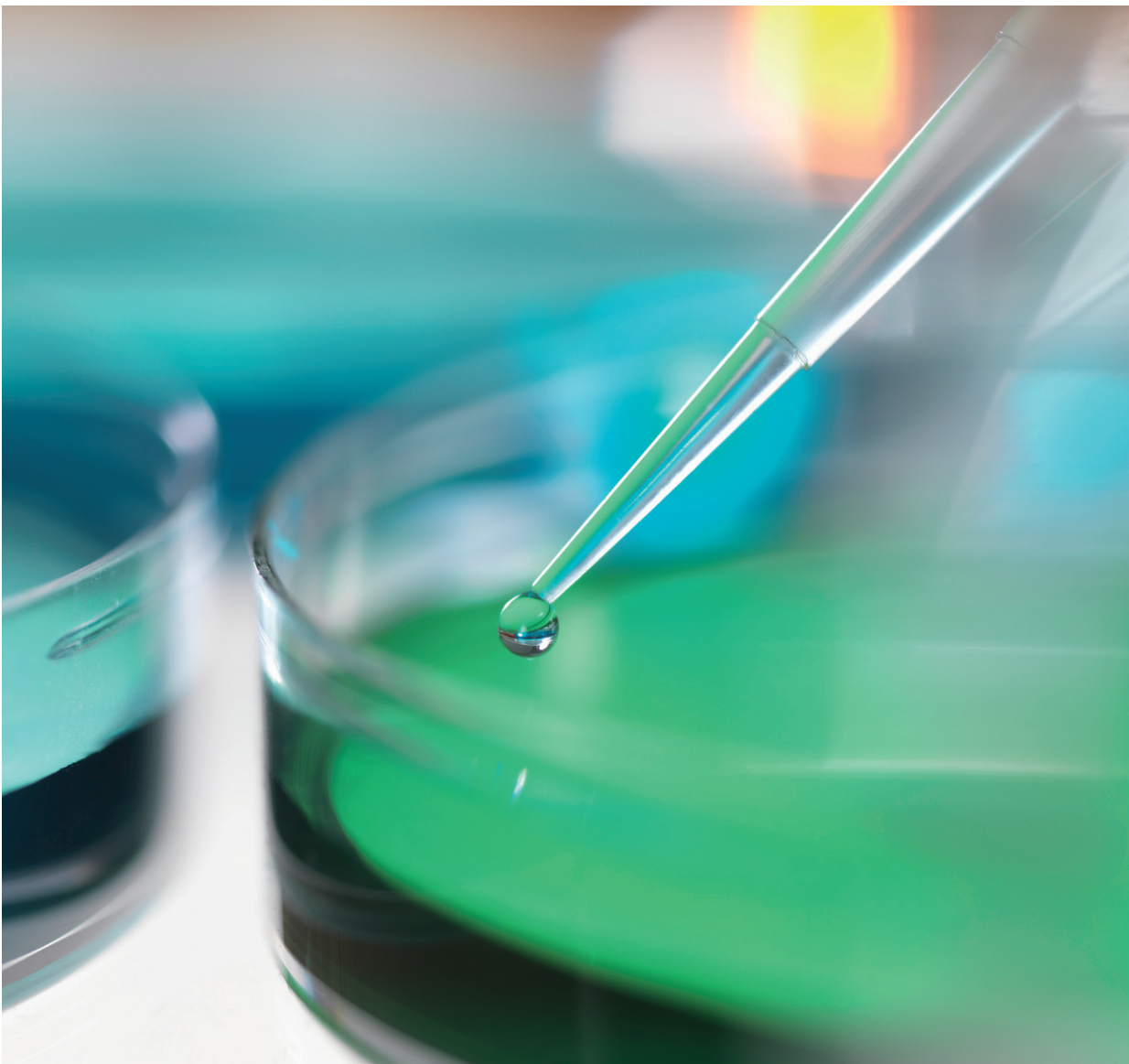




Novogene Sample Requirements



Leading Edge Genomic Services & Solutions

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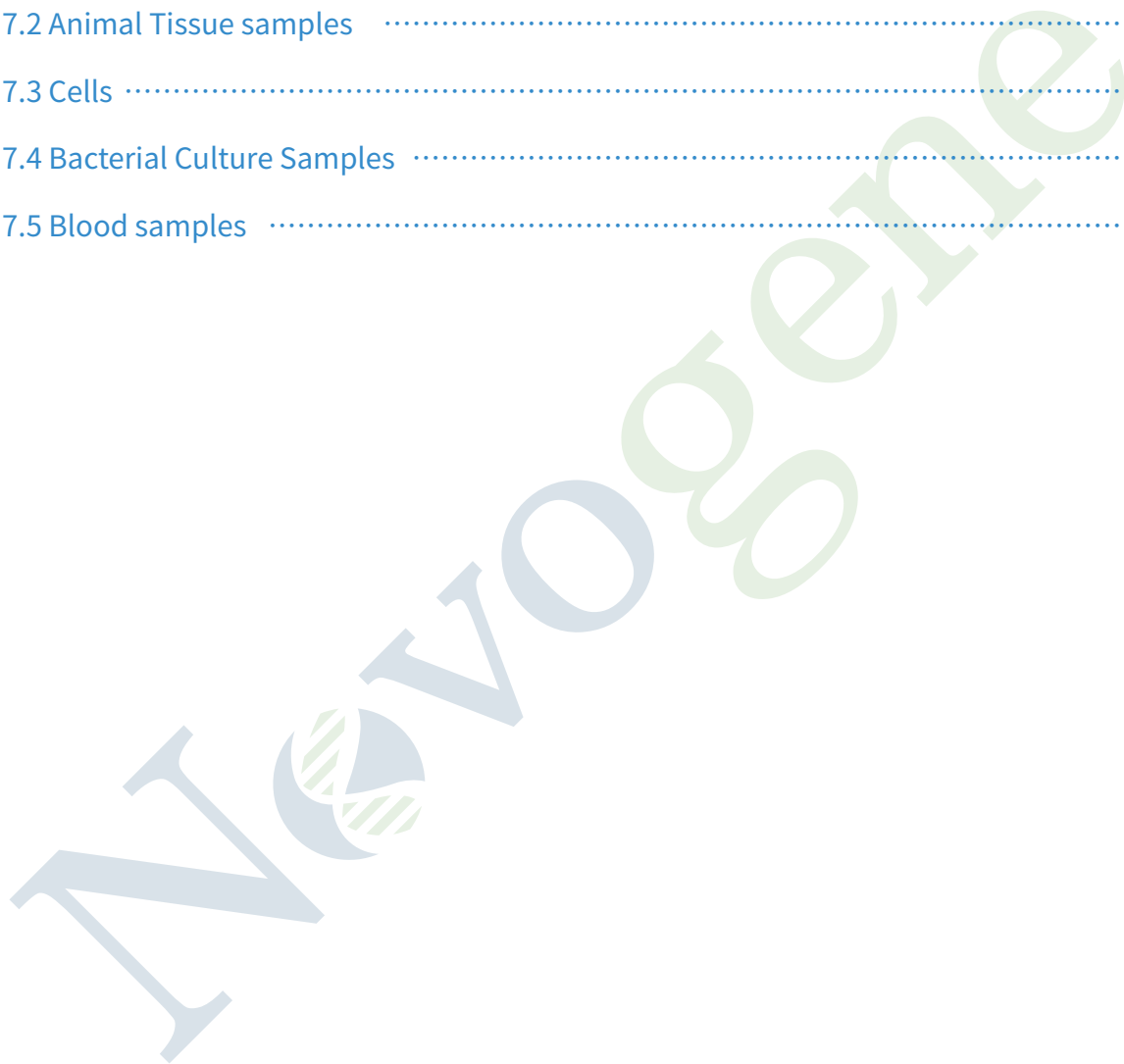
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1 DNA Samples

1.1 Human Whole Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human Whole Genome 350 bp Insert DNA Library	Genomic DNA	≥ 0.2 µg	≥ 20 µL	≥ 10 ng/µL	OD260/280 = 1.8~2.0, no degradation, no contamination
	Genomic DNA (PCR-free low input)	≥ 1.5 µg	≥ 20 µL	≥ 20 ng/µL	
	FFPE* DNA	≥ 0.8 µg	-	-	Fragments should be longer than 1500 bp

* FFPE: Formalin-Fixed, Paraffin-Embedded

1.2 Whole Exome Sequencing / Target Region Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human Exome Library	Genomic DNA	≥ 0.4 µg	≥ 20 µL	≥ 20 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	MDA* / Single Cell Amplified DNA	≥ 1 µg	≥ 20 µL	≥ 20 ng/µL	Fragments should be longer than 500 bp
	FFPE**	≥ 0.8µg	-	-	Fragments should be longer than 1000 bp
	cfDNA/ctDNA	≥ 50 ng	-	-	Fragments should be in multiples of 170bp, no genomic contamination

*MDA: Multiple Displacement Amplification

**FFPE: Formalin-Fixed, Paraffin-Embedded

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Mouse Exome Library	Genomic DNA	≥ 0.4 µg	≥ 20 µL	≥ 20 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	FFPE*	≥ 0.8 µg	-	-	Fragments should be longer than 1000 bp

1.3 Plant & Animal Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
≤ 500 bp Insert DNA Library	Genomic DNA	≥ 0.2 µg	≥ 20 µL	≥ 20 ng/µL	OD260/280 = 1.8 ~2.0, no degradation, no contamination
	Genomic DNA (PCR-free non-350-bp library)	≥ 5 µg	≥ 20 µL	≥ 30 ng/µL	
	Genomic DNA (PCR-free low input-350 bp)	≥ 1.5 µg	≥ 20 µL	≥ 20 ng/µL	
	Mitochondrion/Chloroplast DNA	≥ 0.8 µg	≥ 20 µL	≥ 30 ng/µL	

1.4 Microbial Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Shotgun 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	≥ 20 ng/µL	
PCR product Library	PCR product	≥ 1.5µg	≥ 20µL	≥ 50ng/ul	
Standard WGS Library for Illumina Platform	Genomic DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	
WGS Library for illumina platform(PCR-free)	Genomics DNA	≥ 1.5µg	≥ 20µL	≥ 50ng/µL	
De novo Library for Illumina Platform	Genomic DNA	≥ 1 µg	≥ 20 µL	≥ 20 ng/µL	
De novo Library for PacBio Platform	Genomic DNA (*HMW)	≥ 10 µg	≥ 50 µL	≥ 100 ng/µL	

*HMW: High Molecular Weight

1.5 Nanopore Sequencing

Sample Type	Amount (Qubit®)	Volume	Concentration (Qubit®)	Note
*HMW Genomic DNA	≥ 10µg	≥ 50 µL	≥ 100 ng/µL	OD260/280=1.8-2.0; OD260/230=2.0-2.2; no degradation or RNA contamination mainbands should be longer than 40K

*HMW: High Molecular Weight

2 RNA Samples

2.1 Transcriptome Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RNA Integrity Number(Agilent 2100)	Purity (NanoDrop™)
Eukaryotic RNA-Seq (cDNA library)	Total RNA(Animal)	≥ 0.4 µg	≥ 20 µL	≥ 20 ng/µL	≥ 6.8, with flat base line	OD260/280 ≥ 2.0; OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 0.4 µg	≥ 20 µL	≥ 20 ng/µL	≥ 6.3, with flat base line	
	Total RNA(Blood)	≥ 0.8 µg	≥ 20 µL	≥ 20 ng/µL	≥ 6.8, with flat base line	
	Amplified cDNA(double-stranded) or total RNA(single cell)	≥ 100ng	≥ 20 µL	≥ 10 ng/µL	Fragments distributing between 400 bp~5000 bp, with the main peak at ~2000bp	OD260/230 ≥ 2.0; no degradation, no contamination
Eukaryotic RNA-Seq (strand specific library)	Total RNA(Animal)	≥ 0.8 µg	≥ 20 µL	≥ 20 ng/µL	≥ 6.8, with flat base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA(Plant and Fungus)	≥ 0.8 µg	≥ 20 µL	≥ 20 ng/µL	≥ 6.3, with flat base line	
Prokaryotic RNA Library	Total RNA	≥ 3 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	≥ 2.5 µg	≥ 20 µL	≥ 50 ng/µL	≥ 6.5, with flat base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination

Regarding of RNAsstable (not recommended), please follow the relevant product manual.

2.2 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 25~200 nt (by high sensitive Agilent 2100 Bioanalyzer), FU> 10, with no peak > 2000nt	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination

2.3 Long Non-coding Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RNA Integrity Number(Agilent 2100)	Purity (NanoDrop™)
LncRNA Library (250~300 bp insert strand specific library with rRNA removal)	Total RNA(Animal)	≥ 2 µg	≥ 20 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)	≥ 2 µg	≥ 20 µL	≥ 50 ng/µL	≥ 6, flat base line	
	Exosome RNA	≥ 20 ng	≥ 10 µL	-	Fragments distributing between 25~200 nt (by high sensitive Agilent 2100 Bioanalyzer), FU> 10, with no peak > 2000nt	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination

2.4 Circular RNA Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RNA Integrity Number(Agilent 2100)	Purity (NanoDrop™)
CircRNA Library (250~300 bp insert strand specific library with rRNA & linear RNA removal)	Total RNA (Animal)	≥ 5 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)	≥ 5 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
Ribo-seq Library	RPF Sample	≥ 2 µg	≥ 10 µL	≥ 200 ng/µL	25~38 nt	No degradation, no contamination

3 Epigenomics Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Whole Genome Bisulfite Sequencing Library	Genomic DNA	≥ 2.5 µg	≥ 20 µL	≥ 20 ng/µL	0 < OD260/230 < 3, no degradation, no contamination
Reduced Representation Bisulfite Sequencing Library	Genomic DNA	≥ 1.5 µg	≥ 20 µL	≥ 20 ng/µL	0 < OD260/230 < 3, no degradation, no contamination
ChIP Library	ChIP-Seq DNA	≥ 50 ng	≥ 10 µL	≥ 2 ng/µL	Main peak of 100 bp ~500 bp
RIP Library	RIP-Seq RNA	≥ 100 ng	≥ 20 µL	≥ 3 ng/µL	Without fragmentation; fragments should be longer than 1000 bp

4 PacBio Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration (Qubit®)	Purity (NanoDrop™/Agarose Gel)
PacBio Library	Genomic DNA	≥ 10 µg	≥ 50 µL	≥ 100 ng/µL	mainbands should be longer than 40K; no contamination; non-viscous; no EDTA contained in DNA elution OD260/280=1.8~2.0, OD260/230=2.0~2.2, no degradation, no contamination
	PCR Products	≥ 5 µg	≥ 50 µL	≥ 100 ng/µL	no color; no contamination; non-viscous; no multiple or smearing bands on Gel
	Full-length Amplicon	≥ 200ng	≥ 20 µL	≥ 10 ng/µL	
	Total RNA	≥ 5 µg	≥ 20 µL	≥ 300 ng/µL	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination RIN>9

5 Pre-made Library Sequencing

5.1 Sample requirements

Volume requirement (PE150-HiSeq)

Data Amount	Volume Requirement*
< 20 G	≥ 15μL
20G < X ≤ 50G	≥ 25 μL
50G < X < 120G	≥ 45 μL
Lane Sequencing	≥ 20 ul (additional 10ul for one more lane)

*Notes:

1. Pre-made libraries should be colorless.
2. High concentration samples should be diluted before delivery.

Volume requirement (PE150-Nova)

Data Amount	Volume Requirement*
< 20 G	≥ 15 μL
30 G < X ≤ 100 G	≥ 25 μL
100 G < X < 400 G	≥ 50 μL
Lane Sequencing	100μL(Additional 100μL for one more lane)

*Note: High concentration samples should be diluted before delivery.

Volume requirement (Nova PE250&SE50&PE50)

Data Amount	Volume Requirement*
X ≤ 20 Mb	≥ 15 uL
20 Mb < X ≤ 50 Mb	≥ 25 uL
50 Mb < X < 150 Mb*	≥ 50 uL
Lane sequence	≥ 100 uL (additional 100 uL for one more lane)

*Note: High concentration samples should be diluted before delivery.

5.2 Library concentration:

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

5.3 Insert size:

- Library size: insert + adapters (120 bp) \pm 50 bp (Does not apply to small RNA library)
- Single main peak, no multiple peaks, no adapter contamination and no primer dimers.

*Dilute to 1 ng/ μ L before detection by NGS3K.

Sequencing strategy	Library size (insert + adaptors (120bp) \pm 50 bp for optimal results)
SE 50	130 bp ~650 bp
PE 150	320 bp~520 bp
PE 250	400 bp ~650 bp

5.4 Library concentration

2nM-30nM, quantified by Q-PCR.

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.

6. Protocols for DNA extraction of tissue samples

6.1 Plant tissue samples

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

6.1.2 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.

6.1.3 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.

6.1.4 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.

6.1.5 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.

6.2 Animal tissue samples

6.2.1 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).

6.2.2 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.

6.2.3 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.

6.2.4 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.

6.2.5 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.

6.3 Microbial Samples

6.3.1 Use an alcohol-sterilized shovel 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.

6.3.2 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 .

6.3.3 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.

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

6.3.5 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).

6.3.6 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-OD260/28 & OD260/230 is between 1.6~2.0.

6.3.7 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>

6.4 Stool samples

6.4.1 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.

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

6.4.3 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.

6.4.4 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>

6.5 Water samples

6.5.1 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.

6.5.2 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>

6.6 Strain Bacteria Culture Samples

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

6.6.2 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 .

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

6.7 Blood Samples

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

6.7.2 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.

7. Protocols for RNA extraction from tissue sample

7.1 Plant tissue samples

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

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

7.1.3 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.

7.1.4 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.

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

7.2 Animal Tissue Samples

7.2.1 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).

7.2.2 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.

7.2.3 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.

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

7.3 Cells

7.3.1 Cell suspensions: Rinse suspended cells with PBS buffer. Add TRIzol reagent (1 mL per 5×10^6 cells). Aspirate the cell suspension using syringe to break up any obvious clumps until the suspension turns clear. Store in -80 °C freezer.

7.3.2 Adherent cells: Rinse adherent cells with PBS buffer. Add TRIzol reagent (1 mL per 10cm^2 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.

7.3.3 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.

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

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

7.4 Bacterial Culture Samples

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

7.4.2 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 RNasefree 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.

7.4.3 It' s recommended not to send samples utilizing TRIzol lysate because TRIzol method fails to the extraction of

nucleic acid from some of bacteria.

7.4.4 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.

7.5 Blood samples

7.5.1 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.

7.5.2 BD PAXgene Blood RNA Tube

- (1) Collect blood

Product name: BD 762165 PAXgene Blood RNA Tube

Chinese name: BD vein vacuum blood collection tube (whole blood RNA tube)

Reference price: 158 RMB/tube

Volume: 2.5 mL

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 totalRNA from blood samples Collected.

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