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Novogene AMEA Sample Requirements

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- If you require extraction services that are not covered in this document, or have sample or library types that are not listed, please reach out to marketing_amea@novogeneait.sg or your local sales representative.
- For guidance on how to prepare DNA or RNA samples from various sources, please refer to our Sample Preparation Guide.
- We recommend doubling the sample amount whenever it is feasible to do so.

1. Genome Sequencing

It is recommended to suspend DNA samples in Tris-EDTA (TE) buffer, elution buffer (EB) or Tris-Borate (TB) buffer. High Molecular Weight (HMW) DNA samples should be in EB buffer.

1.1 Human Whole Genome Sequencing (WGS)

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human whole genome library preparation (350bp)	Genomic DNA	≥200 ng	≥20 μL	≥10 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination
	FFPE* DNA	≥400 ng	≥20 μL	≥15 ng/μL	Fragments longer than 1,000 bp
Human PCR-free library (350bp)	Genomic DNA	≥1.2 μg	≥20 μL	≥50 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination

* FFPE: Formalin-Fixed, Paraffin-Embedded

1.2 Whole Exome Sequencing (WES)

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human WES library	Genomic DNA	≥300 ng	≥20 μL	≥15 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination Main peak is above 3000bp
	FFPE* DNA	≥400 ng	≥20 μL	≥15 ng/μL	Fragments longer than 1000 bp
	cfDNA/ctDNA	≥40 ng	≥20 μL	≥1 ng/μL	Fragments of 170 bp or multiples, no genomic DNA contamination
Mouse WES library	Genomic DNA	≥300 ng	≥20 μL	≥15 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination

* FFPE: Formalin-Fixed, Paraffin-Embedded

1.3 Plant & Animal Whole Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Plant & Animal whole genome library preparation (350bp)	Genomic DNA	≥200 ng	≥20 μL	≥10 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination
Plant & Animal PCR-free library (350 bp)	Genomic DNA	≥1.2 μg	≥20 μL	≥50 ng/μL	
GBS (Genotyping by sequencing) library	Genomic DNA	≥600 ng	≥20 μL	≥20 ng/μL	OD260/280 = 1.8-2.0; no degradation, no RNA contamination

1.4 Microbial Whole Genome Sequencing & Metagenomics

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Bacteria / Fungi whole genome library (350bp)	Genomic DNA	≥200 ng	≥20 μL	≥10 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination
Bacteria / Fungi PCR-free library (350bp)	Genomic DNA	≥1.2 μg	≥20 μL	≥50 ng/μL	
Shotgun-based metagenomics library (350bp)	Total DNA	≥200 ng	≥20 μL	≥10 ng/μL	OD260/280 = 1.8-2.0; no contamination; Main peak is above 500bp
Shotgun-based metagenomics PCR-free library (350bp)	Total DNA	≥1.2 μg	≥20 μL	≥50 ng/μL	
Amplicon-based metagenomics	Total DNA	≥200 ng	≥20 μL	≥10 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination

1.5 PacBio DNA Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity
PacBio Revio DNA HiFi library	HMW* Genomic DNA (Human/ Plant/ Animal)	≥ 9 μg (Additional 5 ug per sample per Cell)	≥ 50 μL	≥ 70 ng/μL	OD260/280=1.75~2.0; OD260/230=1.5~2.6; NC/QC**=1.00~2.20 Fragments should be ≥ 30K
PacBio DNA HiFi library (Sequel II/IIe)	HWM* Genomic DNA (Human/ Plant/ Animal)	≥ 9 μg (Additional 5 ug per sample per Cell)	≥ 50 μL	≥ 70 ng/μL	OD260/280=1.75~2.0; OD260/230=1.5~2.6; NC/QC**=1.00~2.20 Fragments should be ≥ 30K
	HMW* Genomic DNA (Fungus/ Metagenomics)	≥ 8.5 μg	≥ 50 μL	≥ 70 ng/μL	OD260/280=1.75~2.0; OD260/230=1.3~2.6; NC/QC**=1.0~2.2 Fragments should be ≥ 20K
PacBio DNA CLR library	HWM* Genomic DNA (Bacteria & Fungus)	≥2 μg	≥40 μL	≥50 ng/μL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC**=0.95~3.00 Fragments should be ≥ 15K
PacBio Full-length 16S library	Genomic DNA	≥300 ng	≥30 μL	≥10 ng/μL	Clear main band; No degradation; No contamination

* HMW: High Molecular Weight.
** NC/QC: NanoDrop concentration/Qubit concentration

1.6 Nanopore Sequencing

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity
Nanopore PromethION DNA library	HWM* Genomic DNA (Human/ Plant/ Animal)	≥8.5 μg	≥50 μL	≥100 ng/μL	OD260/280=1.75~2.0; OD260/230=1.4~2.6; NC/QC**=0.95~3.00 Fragments should be ≥30K
	HWM* Genomic DNA (Bacteria & Fungus)	≥6.5 μg	≥50 μL	≥60 ng/μL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC**=0.95~3.00 Fragments should be ≥15K
	HWM* Genomic DNA (Metagenomics)	≥5.5 μg	≥50 μL	≥80 ng/μL	OD260/280=1.7~2.5; OD260/230=1.1~2.6; NC/QC**=0.95~4.00 Fragments should be ≥10K

* HMW: High Molecular Weight.
** NC/QC: NanoDrop concentration/Qubit concentration

1.7 PCR Product Sequencing

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
PCR-free library	PCR product	≥1.5 μg	≥20 μL	≥60 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination
Library with PCR	PCR product	≥200 ng	≥20 μL	≥10 ng/μL	

2. RNA Sequencing

It is recommended to suspend RNA samples in RNase-free double-distilled water (ddH2O).

2.1 Transcriptome Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic mRNA library (polyA enrichment)	Total RNA (animal)	≥200 ng	≥20 μL	≥10 ng/μL	≥4.0, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no contamination
	Total RNA (plant/ fungus)	≥200 ng	≥20 μL	≥10 ng/μL	≥4.0, with flat baseline	
	Total RNA (blood)	≥400 ng	≥20 μL	≥20 ng/μL	≥5.0, with flat baseline	
	Double stranded cDNA	≥100 ng	≥20 μL	≥5 ng/μL	Fragments between 400 bp-5000 bp, main peak at ~2000 bp	
Eukaryotic mRNA library (Globin removal & polyA enrichment)	Total RNA (human blood)	≥400 ng	≥20 μL	≥20 ng/μL	≥5.0, with flat baseline	
Eukaryotic strand-specific mRNA library (polyA enrichment)	Total RNA (animal)	≥400 ng	≥20 μL	≥20 ng/μL	≥5.0, with flat baseline	
	Total RNA (plant/ fungus)	≥400 ng	≥20 μL	≥20 ng/μL	≥5.0, with flat baseline	
Eukaryotic strand-specific mRNA library (Globin removal & polyA enrichment)	Total RNA (human/ mouse blood)*	≥400 ng	≥20 μL	≥20 ng/μL	≥5.0, with flat baseline	
Prokaryotic strand-specific RNA library (rRNA depletion)	Total RNA	≥500 ng	≥20 μL	≥25 ng/μL	≥6.0, with flat baseline	
Dual RNA library (double rRNA depletion)	Total RNA	≥800ng	≥20 μL	≥25ng/μL	≥6.5, with flat baseline	
Metatranscriptome library (double rRNA depletion)	Total RNA	≥ 500 ng	≥20 μL	≥25 ng/μL	≥5.8, with flat baseline	

* Globin clear applies to human blood only, Ribo-Zero Globin applies to human/mouse blood.

2.2 Eukaryotic Long Non-coding RNA Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Directional RNA library (rRNA depletion)	Total RNA (animal)	≥500 ng	≥20 μL	≥25ng/μL	≥5.5, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no genomic contamination
	Total RNA (plant/ fungus)	≥500 ng	≥20 μL	≥25 ng/μL	≥5.5, with flat baseline	
	Exosome Total RNA	≥10 ng	≥10 μL	-	Fragments between 80-200nt, no peaks> 2000nt, FU*>10	

* FU: Fluorescent unit

2.3 Eukaryotic Small RNA Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic small RNA library (18-40 bp insert)	Total RNA (animal)	≥2 μg	≥20 μL	≥50 ng/μL	≥7.5, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no contamination
	Total RNA (plant/ fungus)	≥2 μg	≥20 μL	≥50 ng/μL	≥7.0, with flat baseline	
	Exosome Total RNA	≥20 ng	≥20 μL	-	Fragments between 25-200nt	

2.4 Eukaryotic Circular RNA Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic circRNA directional library (rRNA and linear RNA depletion)	Total RNA (animal)	≥2 μg	≥20 μL	≥50 ng/μL	≥7.0, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no contamination
	Total RNA (plant)	≥2 μg	≥20 μL	≥50 ng/μL	≥6.5, with flat baseline	

2.5 Eukaryotic Whole Transcriptome Sequencing

Library Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic lncRNA & small RNA library	Total RNA	≥2.5 μg	≥30 μL	≥50 ng/μL	≥7.5, with flat baseline (animal) ≥7.0, with flat baseline (plant/ fungus)	OD260/280≥2.0; OD260/230≥2.0; no degradation, no genomic contamination
Eukaryotic lncRNA & small RNA & circRNA library	Total RNA	≥4.5 μg	≥50 μL	≥50 ng/μL		

2.6 Long Read Transcriptome Sequencing

Service	Sample Type	Amount**	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
PacBio ISO-seq	Total RNA	≥1.2 ug	≥30 μL	≥40 ng/μL	≥6.5 with flat baseline	OD260/280=1.8-2.2; OD260/230=1.3-2.5; NC/QC*≤2

* NC/QC: NanoDrop concentration/Qubit concentration
** Highly recommend sending above 2ug if don't order per Cell.

3. Epigenetics Sequencing

It is recommended to suspend DNA samples in Tris-EDTA (TE) buffer, elution buffer (EB) or Tris-Borate (TB) buffer, and RNA samples in RNase-free double-distilled water (ddH2O).
RIP-seq input controls should be rRNA-depleted prior to sample shipment.

Library Type	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity or fragment size (NanoDrop™/Agarose Gel)
Whole Genome Bisulfite Sequencing (WGBS)	Genomic DNA	≥200 ng	≥20 μL	≥5 ng/μL	0<OD260/230<3; no degradation, no contamination
Reduced Representation Bisulfite Sequencing (RRBS)	Genomic DNA	≥800 ng	≥20 μL	≥40 ng/μL	0<OD260/230<3; no degradation, no contamination
ChIP-seq	Enriched DNA	≥20 ng	≥20 μL	≥1 ng/μL	Main peak within 100 bp-500 bp
RIP-seq	Enriched RNA	≥80 ng	≥20 μL	≥3 ng/μL	Recommend fragments size 250-300bp

4. Premade Library Sequencing

Premade libraries should be colourless. Sub-libraries must be pooled together prior to library shipment.

4.1 Library Volume

Sequencing platform & sequencing strategy	Sequencing data amount	Volume requirement
NovaSeq X Plus PE150	Lane sequencing	≥70 μL (additional 70 μL for one more lane)
NovaSeq 6000 PE150	<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 6000 SE50/PE50/PE250	<20 M reads	≥15 μL
	20 M reads ≤ X ≤ 50 M reads	≥25 μL
	50 M reads ≤ X ≤ 150 M 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 qPCR

4.3 Library Size

Library size includes: insert + adapters (120 bp) ± 50 bp (Does not apply to small RNA library)
Libraries should have a single main peak, no multiple peaks, no adapter contamination, and no primer dimers.

Sequencing platform & sequencing strategy	Library size
NovaSeq X Plus PE150	> 300bp
NovaSeq 6000 PE150	> 300bp
NovaSeq 6000 SE50/PE50	130 bp~650 bp
NovaSeq 6000 PE250	400 bp~650 bp