

NOVOGENE AMERICA SAMPLE SUBMISSION GUIDELINES

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- If you need extraction service from us, have any other sample types or library types not covered in this document, please [contact us](#) or your local sales representative.
- If you need guidelines on how to prepare DNA or RNA samples from different sources, please check our [Sample Preparation Guide](#).
- It is recommended to double the sample amount when possible.

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)

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human WGS (350 bp insert size)	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	≥ 20 ng/µL	Fragments longer than 1500 bp
PCR-free human WGS	Genomic DNA	≥ 1.1 µg	≥ 20 µL	≥ 20 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination

*FFPE: Formalin-Fixed, Paraffin-Embedded

1.2 WHOLE EXOME SEQUENCING (WES) / TARGET REGION SEQUENCING (TRS)

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human WES/TRS	Genomic DNA	≥ 300 ng	≥ 15 µL	≥ 15 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination
	FFPE* DNA	≥ 400 ng	≥ 20 µL	≥ 20 ng/µL	Fragments longer than 1000 bp
	cfDNA/ctDNA	≥ 35 ng	≥ 20 µL	≥ 0.5 ng/µL	Fragments of 170 bp or its multiples, no genomic DNA contamination
Mouse WES	Genomic DNA	≥ 300 ng	≥ 15 µL	≥ 15 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination
	FFPE DNA	≥ 400 ng	≥ 20 µL	≥ 20 ng/µL	Fragments longer than 1000 bp

*FFPE: Formalin-Fixed, Paraffin-Embedded

1.3 PLANT & ANIMAL WHOLE GENOME SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Plant & Animal WGS (350 bp insert size)	Genomic DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination
PCR-free Plant & Animal WGS (350 bp insert size)	Genomic DNA	≥ 1.1 µg	≥ 20 µL	≥ 20 ng/µL	
PCR-free Plant & Animal WGS (custom size, ≤500 bp)	Genomic DNA	≥ 5 µg	≥ 20 µL	≥ 30 ng/µL	

1.4 MICROBIAL WHOLE GENOME SEQUENCING & METAGENOMICS

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™ / Agarose Gel)
Microbial WGS	Genomic DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination
Shotgun-based metagenomics	Total DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	
PCR-free WGS / PCR-free shotgun-based metagenomics	Genomic DNA / total DNA	≥ 1.1 µg	≥ 20 µL	≥ 20 ng/µL	
Amplicon-based metagenomics*	Total DNA	≥ 200 ng	≥ 40 µL	≥ 5 ng/µL	

*The recommended concentration of the sample for 16S and 18S is 5–15 ng/ul.

*The recommended concentration of the sample for ITS is 5–30 ng/ul.

*It is suggested to dilute your samples before submitting them if the sample concentration is too high.

*gDNA for Amplicons Metagenomics should be colourless; otherwise, enzymatic activity will be lower and affect PCR amplification process.

1.5 PACBIO SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity
PacBio Sequel II DNA CLR library	HMW* genomic DNA (Plant & Animal)	≥ 5 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.75~2.0; OD260/230=1.5~2.6; NC/QC**=0.95~3.00 Fragments should be ≥30K
	HMW genomic DNA (Bacteria & Fungi)	≥ 2 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC=0.95~3.00 Fragments should be ≥20K
PacBio Sequel II/IIe DNA HiFi library	HMW genomic DNA (Plant & Animal)	≥ 5 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.75~2.0; OD260/230=1.5~2.6; NC/QC=0.95~3.00 Fragments should be ≥30K
	HMW genomic DNA (Bacteria & Fungi)	≥ 5 µg	≥ 50 µL	≥ 70 ng/µL	OD260/280=1.7~2.2; OD260/230=1.3~2.6; NC/QC=0.95~3.00 Fragments should be ≥20K
PacBio Revio DNA HiFi library	HMW genomic DNA (Plant & Animal)	≥ 5 µg	≥ 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 Full - Length 16S/18S/ITS	Total DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination

*HMW: High Molecular Weight

**NC/QC = NanoDrop concentration/Qubit concentration

Recommended suspension buffer: EB

1.6 NANOPORE SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity
Nanopore PromethION DNA library	HMW* genomic DNA (Plant & Animal)	≥ 8 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
	HMW genomic DNA (Bacteria & Fungi)	≥ 6 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 ≥20K

*HMW: High Molecular Weight

**NC/QC = NanoDrop concentration/Qubit concentration

Recommended suspension buffer: EB

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 EUKARYOTIC MESSENGER RNA SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic mRNA (polyA enrichment)	Total RNA (animal, plant, and fungi)	≥ 200 ng	≥ 10 µL	≥ 20 ng/µL	≥ 4.0, with flat baseline	OD260/280≥2.0 ; OD260/230≥2.0 ; no degradation, no contamination
	Total RNA-low Input (animal, and plant, fungi)	≥ 100 ng	≥ 10 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	≥ 10 µL	≥ 10 ng/µL	Fragments between 400 bp-5000 bp, main peak at ~2000 bp	
Strand-specific Eukaryotic mRNA (polyA enrichment)	Total RNA (animal, plant, and fungi)	≥ 400 ng	≥ 20 µL	≥ 20 ng/µL	≥ 5.0, with flat baseline	
	Total RNA (blood)	≥ 400 ng	≥ 20 µL	≥ 20 ng/µL	≥ 5.0, with flat baseline	

2.2 TRANSCRIPTOME SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic strand-specific lncRNA (rRNA depletion)	Total RNA (animal)	≥ 500ng	≥ 15 µL	≥ 50 ng/µL	≥ 5.5, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no contamination
	Total RNA (plant, fungi)	≥ 500ng	≥ 15 µL	≥ 50 ng/µL	≥ 5.5, with flat baseline	
	Total RNA (blood)	≥ 500ng	≥ 15 µL	≥ 50 ng/µL	≥ 5.5, with flat baseline	
	Exosome RNA (human, mouse)	≥ 5ng	≥ 15 µL	-	Fragments between 25-200nt, FU* >10	
Prokaryotic strand-specific RNA (rRNA depletion)	Total RNA	≥ 500 ng	≥ 10 µL	≥ 50 ng/µL	≥ 6.0, with flat baseline	
Dual RNA (double rRNA depletion)	Total RNA	≥ 1 µg	≥ 20 µL	≥ 50ng/µL	≥ 6.5, with flat baseline	
Metatranscriptome (double rRNA depletion)	Total RNA	≥ 1 µg	≥ 20 µL	≥ 50ng/µL	≥ 5.8, with flat baseline	

*FU = Fluorescent unit

2.3 EUKARYOTIC SMALL RNA SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic small RNA (18-40 bp insert)	Total RNA (animal)	≥ 2 µg	≥ 25 µL	≥ 50 ng/µL	≥ 7.5, with flat baseline	OD260/280≥2.0 ; OD260/230≥2.0 ; no degradation, no contamination
	Total RNA (plant, fungi)	≥ 2 µg	≥ 25 µL	≥ 50 ng/µL	≥ 7.0, with flat baseline	
	Exosome RNA	≥ 10 ng	≥ 15 ul	-	Fragments between 25-200nt, FU* >10	

*FU = Fluorescent unit

2.4 EUKARYOTIC CIRCULAR RNA SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic circRNA (rRNA and linear RNA depletion)	Total RNA (animal)	≥ 2 µg	≥ 25 µL	≥ 50 ng/µL	≥ 7.0, with flat baseline	OD260/280≥2.0 ; OD260/230≥2.0 ; no degradation, no contamination
	Total RNA (plant, fungi)	≥ 2 µg	≥ 25 µL	≥ 50 ng/µL	≥ 6.5, with flat baseline	

2.5 EUKARYOTIC WHOLE TRANSCRIPTOME SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
Eukaryotic lncRNA & small RNA	Total RNA	≥ 3 µg	≥ 40 µL	≥ 50 ng/µL	≥ 7.5, with flat baseline (animal) ≥ 7.0, with flat baseline (plant, fungi)	OD260/280≥2.0 ; OD260/230≥2.0 ; no degradation, no contamination

2.6 LONG READ TRANSCRIPTOME SEQUENCING

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	RIN (Agilent 2100)	Purity (NanoDrop™)
PacBio ISO-seq (polyA enrichment)	Total RNA	≥ 600 ng	≥ 15 µL	≥ 40 ng/µL	≥ 6.5 with flat baseline	OD260/280=1.8-2.2; OD260/230=1.3-2.5; NC/QC* ≤2
Nanopore RNA (polyA enrichment)	Total RNA	≥ 100 ng	≥ 10 µL	≥ 10 ng/µL		

*NC/QC = NanoDrop concentration/Qubit concentration

3. EPIGENETICS SEQUENCING

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

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity or fragment size (NanoDrop™/Agarose Gel)
Whole Genome Bisulfite Sequencing (WGBS)	Genomic DNA	≥ 200 ng	≥ 20 µL	≥ 10 ng/µL	0<OD260/230<3; no degradation, no contamination
Reduced Representation Bisulfite Sequencing (RRBS)	Genomic DNA	≥ 1 µg	≥ 20 µL	≥ 20 ng/µL	0<OD260/230<3; no degradation, no contamination
ChIP-seq	Enriched DNA	≥ 20 ng	≥ 20 µL	≥ 2 ng/µL	OD260/280=1.8-2.0 No degradation, no contamination Main peak within 100 bp and 500 bp
RIP-seq	Enriched RNA	≥ 100 ng	≥ 20 µL	≥ 3 ng/µL	Fragments longer than 80 nt



4. PREMADE LIBRARY SEQUENCING

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

4.1 LIBRARY VOLUME

Sequencing Strategy	Sequencing Platform	Sequencing data amount	Volume requirement
PE150	NovaSeq 6000	< 30 Gb	≥ 15 µL
		30 Gb ≤ X ≤ 100 Gb	≥ 25 µL
		100 Gb < X ≤ 800 Gb	≥ 50 µL
	NovaSeq 6000 & NovaSeq X Plus	Lane sequencing	≥ 50 µL (additional 40 µL for one more lane)
SE50 PE50 PE250	NovaSeq 6000	Lane sequencing	≥ 100 µL (additional 100 µL for one more lane)

4.2 LIBRARY CONCENTRATION

- ≥ 2 ng/µL, quantified by Qubit® 2.0 (Life Technologies)
- 2 nM-30 nM, quantified by qPCR

4.3 LIBRARY SIZE

- Library Size = Insert Length + Adapters (120 bp) ± 50 bp
- The above calculation does not apply to small RNA libraries or small libraries.
- Libraries should have a single main peak, no multiple peaks, no adapter contamination, and no primer dimers.

Sequencing Strategy & Sequencing Platform	Library Size for Optimal Results	Library Size with Risks
PE150 (NovaSeq 6000 and NovaSeq X Plus)	320 bp ~ 650 bp	300 ~ 320 bp, 650 ~ 700 bp
SE50/PE50 (NovaSeq 6000)	130 bp ~ 650 bp	120 ~ 130 bp, 650 ~ 700 bp
PE250 (NovaSeq 6000)	400 bp ~ 650 bp	370 ~ 400 bp, 650 ~ 750 bp

5. Quantitative Proteomics

It is recommended to dilute extracted proteins in 6 M Urea.

Service	Sample Type	Amount	Concentration	Notes
Label Free	Total Protein	30 µg	0.5 µg/µL	Purification or concentration will be required when samples are diluted in detergent*, or the concentration falls below 0.5 µg/µL, in which case, the protein amount needs to be doubled.
TMT/ iTRAQ	Total Protein	110 µg	0.5 µg/µL	
DIA	Total Protein	70 µg	0.5 µg/µL	

* Detergent includes SDS, triton X-100, NP40, CHAPS, etc.

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