Novogene | Advancing Genomics, Improving Life

NOVOGENE AMERICA SAMPLE SUBMISSION GUIDELINES

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Contents

- 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 feasible, in case library re-construction is needed.

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)

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Human 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
	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	≥ 3 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 IIe 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)	≥ 1.5 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 Sequel IIe and 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
	HMW genomic DNA (Bacteria & Fungi)	≥ 5 μg	≥ 50 μL	≥ 70 ng/μL	OD260/280=1.75~2.0; OD260/230=1.3~2.6; NC/QC=1.00~2.20 Fragments should be ≥20K
PacBio DNA Low-input HiFi library	HMW genomic DNA (Human)	≥ 1 μg	≥ 35 μL	≥ 30 ng/μL	OD260/280=1.75~2.0; OD260/230=1.5~2.6; NC/QC=0.95~3.00 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
PacBio PCR product library	PCR product	≥ 2 μg	≥ 40 μL	≥ 50 ng/μL	OD260/280=1.75~2.0; OD260/230=1.4~2.6; NC/QC=0.95~3.00; Single band (PacBio library fragments distributed above 1k)

*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
Nanopore Ultra-long DNA Library	uHMW*** Genomic DNA (plant and animal tissues)	≥ 20 μg	≥ 50 μL	≥ 133 ng/μL	OD260/280=1.7-2.0; OD260/230=1.3-2.6; NC/QC=0.95-3.00; Fragments should be ⩾ 100k, no fragments below 30k.
	uHMW Genomic DNA (blood and cells)	≥ 30 μg	≥ 50 μL	≥ 40 ng/μL	OD260/280=1.7-2.0; OD260/230=1.3-2.6; NC/QC=0.95-3.00; Fragments should be ⩾ 300K, no fragments below 30k.
Nanopore PCR product library	PCR product	≥ 2 μg	≥ 40 μL	≥ 50 ng/μL	OD260/280=1.75~2.0; OD260/230=1.4~2.6; NC/QC=0.95~3.00; Single band

*HMW: High Molecular Weight
**NC/QC = NanoDrop concentration/Qubit concentration
***uHMW: Ultra-high Molecular Weight
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 (ddH_2O).

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)	≥ 100 ng	≥ 10 μL	≥ 10 ng/μL	≥ 4.0, with flat baseline	OD260/280≥2.0; OD260/230≥2.0; no degradation, no contamination
	Total RNA (blood)	≥ 400 ng	≥ 20 μL	≥ 20 ng/μL	≥ 5.0, with flat baseline	
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	
	Ultra-low total RNA（human, mouse, rat)	≥ 25ng	≥ 15 μL	≥ 1 ng/μL	⩾5.5, with flat baseline	
	Ultra-low total RNA（blood)（human, mouse, rat)	≥ 120ng	≥ 15 μL	≥ 10 ng/μL	⩾5.5, with flat baseline	
	Exosome RNA (human, mouse)	≥ 5ng	≥ 15 μL	≥ 1 ng/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, fungui)	≥ 2 μg	≥ 25 μL	≥ 50 ng/μL	≥ 7.0, with flat baseline	
	Exosome RNA	≥ 10 ng	≥ 15 ul	≥ 1 ng/μL	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 & Kinnex 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

2.7 SINGLE CELL TRANSCRIPTOME SEQUENCING

Service	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity or fragment size (NanoDrop™/Agarose Gel)/Sample Status
10x Single Cell Transcriptome Sequencing	Single Cell Suspension	Minimum ⩾ 500K cells/sample;	/	/	/	Cell viability ⩾ 80%
10x Single Cell Transcriptome Sequencing	Tissue*	Minimum >=50mg	/	/		Dry ice packaging; Minimized froze-thaw cycles

*Tissue amount requirement varies according to the species and tissue types, please consult your Sequencing Specialist for details.

3. EPIGENETICS SEQUENCING

It is recommended to suspend RNA samples in RNAse-free double-distilled water (ddH2O), and DNA samples (except EM-seq) in Tris-EDTA (TE) buffer, elution buffer (EB) or Tris-Borate (TB) buffer. For Enzymatic Methyl Sequencing (EM-seq) projects, please prepare DNA samples in a buffer that **does not** contain EDTA.
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)/Sample Status
Whole Genome Bisulfite Sequencing (WGBS)	Genomic DNA	≥ 200 ng	≥ 20 μL	≥ 10 ng/μL	0 < OD260/230 < 3; no degradation, no contamination
Enzymatic Methyl Sequencing (EM-seq)	Genomic DNA	⩾ 50ng	≥ 20 μL	⩾ 2.5 ng/μL	Fragments are above 5000 bp, and mainly above 13000 bp, no degradation, no contamination, **no EDTA**
	cfDNA	⩾ 50ng	≥ 20 μL	⩾ 2.5 ng/μL	Agilent 2100 peak at 170bp and integer multiples, no genomic contamination, no contamination, **no EDTA**
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	≥ 30 ng (50ng is recommend) ≥ 100 ng for rRNA depletion	≥ 20 μL	≥ 1.5 ng/μL	Fragments longer than 80 nt
ATAC-Seq	Frozen Tissue	≥ 50mg (100mg is recommended)	/	/	Transport under dry ice conditions
	Cryopreserved cell	≥ 500k cells (1M is recommended)	/	/	Transport under dry ice conditions; Cells in Single Cell Suspension; ⩾60% viability

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 X Plus Partial Lane Seq	50G or 100G	≥ 30 μL
		100 G < X ≤ 400 G	≥ 50 μL
		400 G < X ≤ 1000 G	≥ 130 μL
	NovaSeq 6000 S4	Lane sequencing (800G/lane)	≥ 50 μL (additional 40 μL for one more lane)
	NovaSeq X Plus 10B	Lane sequencing (375G/lane)	≥ 50 μL (additional 40 μL for one more lane)
	NovaSeq X Plus 25B	Lane sequencing (1000G/lane)	≥ 130 μL per lane
SE50 PE50	NovaSeq 6000 SP	Flow cell sequencing	≥ 200 μL per flow cell
PE250	NovaSeq 6000 SP	Flow cell sequencing	≥ 200 μL per flow cell

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