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	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	Genomic DNA	≥ 200 ng	≥ 20 μL	≥ 10 ng/μL	OD260/280 = 1.8-2.0; no degradation, no contamination
PCR-free Plant & Animal WGS	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 and Revio DNA HiFi library	HMW genomic DNA (Plant & Animal)	≥ 3 μg	≥ 40 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)	≥ 3 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)	≥ 300ng	≥ 6 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)	≥ 300ng	≥ 6 μL	≥ 50 ng/μL	≥5.5, with flat baseline	
	Total RNA (blood)	≥ 300ng	≥ 6 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, fungi)	≥ 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 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; No genomic contamination
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)	Sample status and ship condition
10x Single Cell Transcriptome Sequencing	Fresh cell	Minimum ⩾ 500K cells/sample	/	/	/	Cell viability⩾70%, Transport with ice pack.
10x immune profiling (5' RNA and TCR or/and BCR)						
Multiome (ATAC + RNA) sequencing		Minimum ⩾ 10M cells/sample	/	/		
10x Single Cell Transcriptome Sequencing	Frozen cell	Minimum ⩾ 500K cells/sample	/	/		Cell viability⩾70%, Transport with dry ice.
10x immune profiling (5' RNA and TCR or/and BCR)						
Multiome (ATAC + RNA) sequencing		Minimum ⩾ 10M cells/sample	/	/		
10x Single Cell Transcriptome Sequencing	Fresh Tissue*	Varies by region	/	/		Transport with ice pack.
10x immune profiling (5' RNA and TCR or/and BCR)						
10x Single Cell Transcriptome Sequencing	Frozen Tissue*	Minimum >=50mg	/	/		Transport with dry ice.
Multiome (ATAC + RNA) sequencing						

*Tissue amount requirent varies by the species and tissue types, please consult your Sequencing Specialist for details.

2.8 SPATIAL TRANSCRIPTOME SEQUENCING

Service	Sample Type	Amount	Preservation	RNA QC	Shipping
10x Visium HD Spatial Transcriptome Sequencing	FFPE block (Human/Mouse)	1 block, contained on plastic dehydrating box.	After embedding store at 4°C, protected from light	DV200%≥ 30 %	4°C or Room Temperature
	FFPE slide (Human/Mouse)	5-10 FFPE (10μm thickness) scrolls in tube for RNA QC; 2-4 FFPE (5μm thickness) tissue sections on glass slides, store in Slide Mailer, for library prep*	Dry and sealed, storage time at 4°C < 14 days		4°C or Room Temperature
Stereo-seq OMNI	FFPE block (Animal with reference genome)	1 block, contained on plastic dehydrating box.	After embedding store at 4°C, protected from light		4°C or Room Temperature
	FFPE section (Animal with reference genome)	5-10 FFPE (10μm thickness) scrolls in 1.5ml tube for RNA QC; 4-6 FFPE (5μm thickness) tissue sections in 50ml centrifuge tube, for library prep*	Dry and sealed, storage time at 4°C < 14 days		4°C or Room Temperature

*From FFPE section to library prep, try to keep the interval within 14 days as much as possible.

3. EPIGENETICS SEQUENCING

It is recommended to suspend RNA samples in RNAse-free double-distilled water (ddH2O), and DNA samples (except Enzymatic Methyl Sequencing) in Tris-EDTA (TE) buffer, elution buffer (EB) or Tris-Borate (TB) buffer. For Enzymatic Methyl Sequencing 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	≥ 100 ng	≥ 20 μL	≥ 5 ng/μL	0 < OD260/230 < 3; no degradation, no contamination
Enzymatic Methyl Sequencing	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	≥ 800 ng	≥ 20 μL	≥ 24 ng/μL	0 < OD260/230 < 3; no degradation, no contamination
ChIP-seq	Enriched DNA	≥ 10 ng	≥ 20 μL	≥ 0.5 ng/μL	OD260/280=1.8-2.0 No degradation, no contamination Main peak within 100 bp and 500 bp
RIP-seq	Enriched RNA	≥ 20 ng (50ng is recommend) ≥ 100 ng for rRNA depletion	≥ 20 μL	≥ 1 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)	/	/	Cryopreserved cells (in single cell suspension) should be shipped on dry ice.
	Fresh Cell		/	/	Fresh cells stored in MACS Cell Storage Buffer, kept in single-cell suspension; Transport on ice packs (no dry ice for fresh cells)

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 not contain 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. OLINK PROTEOMICS

5.1 OLINK REVEAL

Sample Type	Sample Volume	Others
Serum/Plasma	≥ 20 μL	Store sample at -80°C immediately after extraction, as hemolysis may affect analysis results.

*Please include sufficient amount of dry ice and send by frozen delivery to avoid sample thawing during shipping.
**Please contact us for more information about any other sample types.