SAMPLE PREPARATION GUIDE

This document provides guidelines on how to prepare, quantify, and submit samples to LifeSct. Whether you are submitting DNA or RNA samples, it is essential that the appropriate instructions be followed to enable the successful completion of your project.

I. SAMPLE REQUIREMENTS

Sample quality directly impacts sequencing quality and subsequent bioinformatics analysis. Therefore, LifeSct has extensive sample quality control procedures to ensure submitted samples conform to requirements for downstream processing.

To guarantee the normal processing of your project, samples should meet the standards given below. If your samples do not meet these standards, or you are unable to produce higher-quality samples, please consult with your LifeSct Project Manager before shipping your samples.

Notes:

1) Input quantity should be determined by Qubit® instead of by NanoDrop™, and the final quantity and concentration should conform to LifeSct's specifications.
2) Samples not meeting these specifications should be designated as "at risk" by the customer, and will be subject to billing regardless of data quality. Please consult the Project Manager for further details.

1. Human Whole Genome/Exome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
		Strongly Recommended	Required			
Human Whole Genome/ Exome Sequencing / Target Region Capture	Genomic DNA	≥ 2μg	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	PCR products of single-cell whole genome	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	Fragments should be longer than 500 bp
	FFPE*	≥ 3 μg	≥ 1.5 μg	-	-	Fragments should be longer than 1500 bp

* Formalin-fixed, paraffin-embedded

2. Plant & Animal Genome Squencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/ Agarose Gel)
		Strongly Recommended	Required			
≤ 500 bp Insert	Genomic DNA	≥ 1.4 μg	≥ 700 ng	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 20 ng/μL	
	Mitochondrion/Chloroplast DNA	≥ 1.6 μg	≥ 800 ng	≥ 20 μL	≥ 50 ng/μL	
Genotyping by Sequencing	Genomic DNA	≥ 500 ng	≥ 300 ng	≥ 10 μL	≥ 50 ng/μL	
2 Kb Insert	Genomic DNA	≥ 30 μg	≥ 15 μg	≥ 20 μL	≥ 50 ng/μL	
5 Kb Insert	Genomic DNA	≥ 30 μg	≥ 15 μg	≥ 20 μL	≥ 50 ng/μL	
10 Kb Insert	Genomic DNA	≥ 50 μg	≥ 25 μg	≥ 20 μL	≥ 50 ng/μL	
> 10 Kb Insert	Genomic DNA	≥ 80 μg	≥ 40 μg	≥ 20 μL	≥ 50 ng/μL	

3. Microbial Genome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/agarose gel)
		Strongly Recommended	Required			
≤ 500 bp Insert Re sequencing/ Meta Library	Genomic DNA	≥ 1.6 μg	≥ 800 ng	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (PCR-free)	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 50 ng/μL	
PCR-Free Library for Amplicon	Genomic DNA	≥ 10 μg	≥ 5 μg	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	PCR Products*	≥ 400 ng	≥ 200 ng	≥ 10 μL	≥ 20 ng/μL	
	PCR Products**	≥ 300 ng	≥ 150 ng	≥ 10 μL	≥ 20 ng/μL	

* One PCR product for one library **Multiple PCR products for one library (at least 2 different PCR products)

4. Epigenetics Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/ Agarose Gel)
		Strongly Recommended	Required			
Whole Genome Bisulfite Sequencing	Genomic DNA (Genome size ≤ 1.5 G)	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	OD260/280 = 1.8 – 2.0, no degradation, no contamination
	Genomic DNA (1.5G < Genome size ≤ 3.5 G)	≥ 12 μg	≥ 6 μg	≥ 20 μL	≥ 50 ng/μL	
ChIP-Seq	ChIP-Seq DNA	≥ 100 ng	≥ 50 ng	≥ 10 μL	≥20 ng/μL	Main peak of 100 bp – 500 bp

5. Transcriptome Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic RNA-Seq	Total RNA (Animal)	≥ 2 μg	≥1 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.8, smooth base line	OD260/280≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 2 μg	≥ 1 μg	≥ 20 μL	≥ 50ng/μL	≥ 6.3, smooth base line	
Prokaryotic RNA-Seq	Total RNA	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50ng/μL	≥ 6.0, smooth base line	

6. Small RNA Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic small RNA Sequencing	Total RNA (Animal)	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	≥ 8, smooth base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 6 μg	≥ 3 μg	≥ 20 μL	≥ 50 ng/μL	≥ 7.5, smooth base line	

7. Long non-coding Sequencing

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity (NanoDrop™)
		Strongly Recommended	Required				
Eukaryotic Long non-coding RNA Sequencing	Total RNA (Animal)	≥ 4 μg	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.8, smooth base line	OD260/280 ≥ 2.0, OD260/230 ≥ 2.0, no degradation, no contamination
	Total RNA (Plant and Fungus)	≥ 4 μg	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL	≥ 6.3, smooth base line	

8. Pre-prepared library

(1) Library volume requirement:

Data Amount	Volume Requirement*
< 30 G	≥ 10 μL
≥ 30 G	≥ 20 μL

*High concentration samples should be diluted before delivery

(2) Library concentration: library concentration quantified by Qubit® 2.0 (Life Technologies): ≥ 0.5 ng/uL

(3) Insert size: dilute to 1 ng/μL before checking the insert size by Agilent 2100 Bioanalyzer.

a) Insert size: insert + adapters (120 bp) ± 50 bp (Does not apply to small RNA library)

b) Main peak present, no multiple peaks, no adapter contamination and no primer dimers.

(4) Library concentration quantified by Q-PCR:

Platform	Concentration Requirement
HiSeq 2500	2 nM – 30 nM
MiSeq	2 nM – 30 nM
HiSeq X	3 nM – 30 nM

II. PRE-QUALITY CONTROL (QC) INSTRUCTIONS

Customers must provide the sample quality analysis results obtained using one of the following methods: Qubit®, NanoDrop™, agarose gel electrophoresis, or Agilent 2100. It is recommended samples be analyzed by Qubit/PicoGreen/gel electrophoresis (with quantity indicator), so that the results will correspond more closely to LifeSct QC results. NanoDrop™ quantification is NOT recommended. If NanoDrop™ is utilized for pre-QC quantification, LifeSct strongly recommends that you send more DNA/RNA for processing than the amounts given above.

For gel electrophoresis, the following conditions are recommended:

DNA: 1.0% agarose gel; 1.0% TAE solution; 100V for 40 min

RNA: 1.0% agarose gel; 0.5× TBE solution; 180V for 16 min

Note:

Different electrophoresis conditions may generate a different, and potentially misleading, QC report on your samples. Therefore, it is highly recommended that you adhere to the conditions recommended above for the initial check, and that you provide LifeSct with a picture of the gel.