

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 Sequencing

| 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) | $\geq 6 \mu\text{g}$ | $\geq 3 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | OD260/280 = 1.8 – 2.0, no degradation, no contamination |
| | Genomic DNA (1.5G < Genome size ≤ 3.5 G) | $\geq 12 \mu\text{g}$ | $\geq 6 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | |
| ChIP-Seq | ChIP-Seq DNA | $\geq 100 \text{ ng}$ | $\geq 50 \text{ ng}$ | $\geq 10 \mu\text{L}$ | $\geq 20 \text{ ng}/\mu\text{L}$ | 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) | $\geq 2 \mu\text{g}$ | $\geq 1 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | ≥ 6.8 , smooth base line | OD260/280 ≥ 2.0 , OD260/230 ≥ 2.0 , no degradation, no contamination |
| | Total RNA (Plant and Fungus) | $\geq 2 \mu\text{g}$ | $\geq 1 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | ≥ 6.3 , smooth base line | |
| Prokaryotic RNA-Seq | Total RNA | $\geq 6 \mu\text{g}$ | $\geq 3 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | ≥ 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) | $\geq 6 \mu\text{g}$ | $\geq 3 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | ≥ 8 , smooth base line | OD260/280 ≥ 2.0 , OD260/230 ≥ 2.0 , no degradation, no contamination |
| | Total RNA (Plant and Fungus) | $\geq 6 \mu\text{g}$ | $\geq 3 \mu\text{g}$ | $\geq 20 \mu\text{L}$ | $\geq 50 \text{ ng}/\mu\text{L}$ | ≥ 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.