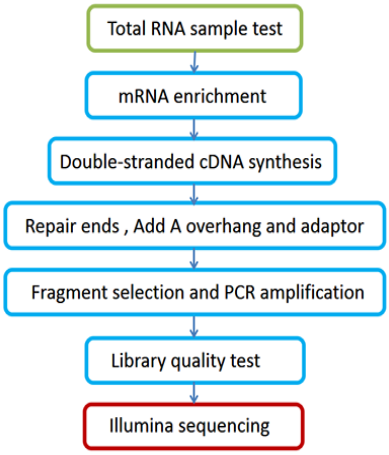
A. Library Preparation and Sequencing

From the RNA samples to the final data, each step, including sample test, library preparation, and sequencing, influences the quality of the data, and data quality directly impacts the analysis results. To guarantee the reliability of the data, quality control (QC) is performed at each step of the procedure. The workflow is as follows:



1 Sample Quality Control

There are three main methods of QC for RNA samples:

(1) Nanodrop: Preliminary quantitation

(2) Agarose Gel Electrophoresis: tests RNA degradation and potential contamination

(3) Agilent 2100: checks RNA integrity and quantitation

2 Library Construction and Quality Control

After the QC procedures, mRNA from eukaryotic organisms is enriched using oligo(dT) beads. For prokaryotic organisms or eukaryotic organisms' long-non-coding libraries, rRNA is removed using the Ribo-Zero kit that leaves the mRNA. First, the mRNA is fragmented randomly by adding fragmentation buffer, then the cDNA is synthesized by using mRNA template and random hexamers primer, after which a custom second-strand synthesis buffer (Illumina) , dNTPs, RNase H and DNA polymerase I are added to initiate the second-strand synthesis. Second, after a series of terminal repair, A ligation and sequencing adaptor ligation, the double-stranded cDNA library is completed through size selection and PCR enrichment.

The quality control of library consists of three steps:

(1) Qubit 2.0: tests the library concentration preliminarily.

(2) Agilent 2100: tests the insert size.

(3) Q-PCR: quantifies the library effective concentration precisely.

The workflow chart is as follows: