RNA Quantification and Illumina Library Generation for RNA Seq

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RNA Sequencing Workflow

Cells → Tissue → BioFluid → RNA Extraction → QC and Quantification → Next Seq Sequencer → Illumina Library
Quantification Methods

• Check RNA Concentration using Qubit Fluorometer

• Check RNA Quality using Fragment Analyzer or Agilent Tape Station
Quantification Methods for RNA seq

- **Accurate reading**: It uses fluorescent dye selective for the bio molecule of interest
- **Incredible sensitivity**: It can be used as low as 10pg/ul

- **Lack of Accuracy**: It only measures purity of the sample and not the accurate concentration
- **Lack of Sensitivity**: It can’t be used with low concentration

**Qubit Fluorometer**

**Nanodrop Ultra Violet Specrophometer**

Not recommended for RNA seq
Checking RNA Quality to determine RNA Seq library preparation method

RNA Quality is measured by using

• Agilent instrument (Tape station)

• Fragment Analyzer
How to determine the RNA Quality

- RNA quality is measured by RNA Integrity Number (RIN) between 1 and 10 with 10 being the highest quality samples.
- RNA quality is measured by DV200 (Distribution Value) for highly degraded RNA samples which represents the percentage of RNA fragments that are > 200 nucleotides.
- Some examples in the next slides.
RNA Quality (RIN and DV200)

RIN 10

RIN 3

RIN 7

RIN<3 (DV200=%40)
**Library Generation Methods**

**Fresh RNA**
- RIN Score > 7
  - mRNA Seq
    - > 100ng: Illumina Truseq Stranded mRNA 100ng-1ug
    - < 10ng: Takara SMART HT Kit
    - > 100ng: Illumina Truseq Stranded TotalRNA 100ng-1ug
    - < 10ng: Takara SMARTer Stranded Total RNA 250pg-10ng
- RIN Score < 7
  - Total RNA Seq
    - > 100ng: Illumina Truseq Stranded TotalRNA 100ng-1ug
    - < 10ng: Takara SMARTer Stranded Total RNA 250pg-10ng

**FFPE RNA**
- Total RNA Seq
  - Illumina Truseq stranded TotalRNA 100ng -1ug
- Coding Transcriptome Seq
  - Illumina Truseq RNA Exome 20ng-100ng
**TotalRNA-Seq**

- rRNA depletion using biotinylated oligos combined with rRNA removal beads

**mRNA-Seq**

1. PolyA+ RNA captured
2. RNA fragmented and primed
3. First strand cDNA synthesized
4. Second strand cDNA synthesized
5. 3’ ends adenylated and 5’ ends repaired
6. DNA sequencing adapters ligated
7. Ligated fragments PCR amplified
TruSeq Stranded mRNA

Input
- Total RNA 0.1-1ug
- High quality RNA (RIN>7)

Protocol
- Stranded workflow
- Poly A selection
- Single index: 24-plex
- Dual index: 96-plex
- 9hrs hands-on time

Output
- Poly (A) RNA
TruSeq Stranded TotalRNA

**Input**
- Total RNA 0.1-1ug
- Degraded RNA and FFPE compatible
- Supports:
  - H/M/R
  - Gold (H/M/R)
  - Plant
  - Globin

**Protocol**
- Stranded workflow
- Ribo-Zero depletion
- Single index: 24-plex
- Dual index: 96-plex
- 8hrs hands-on time

**Output**
- mRNA & ncRNA
TruSeq RNA Exome

Total RNA Input

1. Total RNA Input
2. 3) First strand cDNA synthesized
3. 4) Second strand cDNA synthesized
4. 5) 3' ends adenylated and 5' ends repaired
5. 6) DNA sequencing adapters ligated
6. 7) Ligated fragments PCR amplified
7. 8) Pool Stranded RNA Seq Libraries in 4-plex
8. 9) Hybridize biotinylated probes to targeted regions
9. 10) Capture using Streptavidin beads
10. 11) Elute from beads
11. Coding RNA Enrichment

Probe
TruSeq RNA Exome

Input
- Low input
- FFPE compatible
- High Quality: 20-40ng
- Low Quality: 100ng (depending on DV200)
- Human

Protocol
- Coding RNA captured via sequence-specific probes
- Stranded workflow
- Single index: 24-plex
- Dual index: 96-plex
- 4-plex pooling pre-enrichment
- 11hrs hands-on time

Output
- Coding Transcriptome
Contact Information

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