RNA THREE WAYS

agrf
Empowering Australian Genomics

RNA Sequencing is an NGS method for transcriptome analyses. Complementary DNA (cDNA) generated from RNA is sequenced to provide a quantitative view of the RNA present within a sample.

mRNA Seq

TruSeq Stranded mRNA Library Prep with Unique Dual Indexing (input 200-1000 ng of Total RNA):

- protocol enriches mature, poly adenylate mRNA using oligo d(T) beads
- enrichment is highly efficient (<5% rRNA sequences remaining)
- 10-20 million reads recommended for expression analysis in mammalian transcriptomes

Whole Transcriptome

TruSeq Stranded Total RNA Library Prep with Unique Dual Indexing (input 200-1000 ng of Total RNA):

- protocol includes depletion of ribosomal RNA via hybrid capture (Illumina Ribo-Zero)
- rRNA depletion leaves a mix of mature mRNAs and non-coding/regulatory RNAs
- rRNA depletion reagents for mammals, plant and bacteria are available
- rRNA depletion is required for all prokaryotic (bacterial) samples
- 20-40 million reads recommend for expression analysis of mammalian transcriptomes

Micro RNA

Small RNA library prep NEBNext Small RNA Library Prep (input 1 µg of Total RNA or 10 ng of pre-enriched small RNA):

- protocol targets mature miRNA and other small RNAs with 3'OH group through ligation of RNA adapters
- further specificity for miRNA achieved through size selection of cDNA via electrophoresis (Pippin Prep)
- 5-10 million short reads (<75 bp) per sample recommended

Strand-specific RNA Seq

- strand specificity is achieved through "strand marking" with dUTP, which is used in place of dTTP for 2nd strand synthesis
- final library is amplified with uracil sensitive polymerase (only the 1st strand is amplified)
- final library has "RF" (reverse forward) orientation, meaning Read 1 = anti-sense strand & Read 2 = sense strand

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NCRIS
National Research Infrastructure for Australia
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BIOPLATFORMS AUSTRALIA

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