CD Genomics
The Genomics Services Company

RNA-Seq Library Preparation:

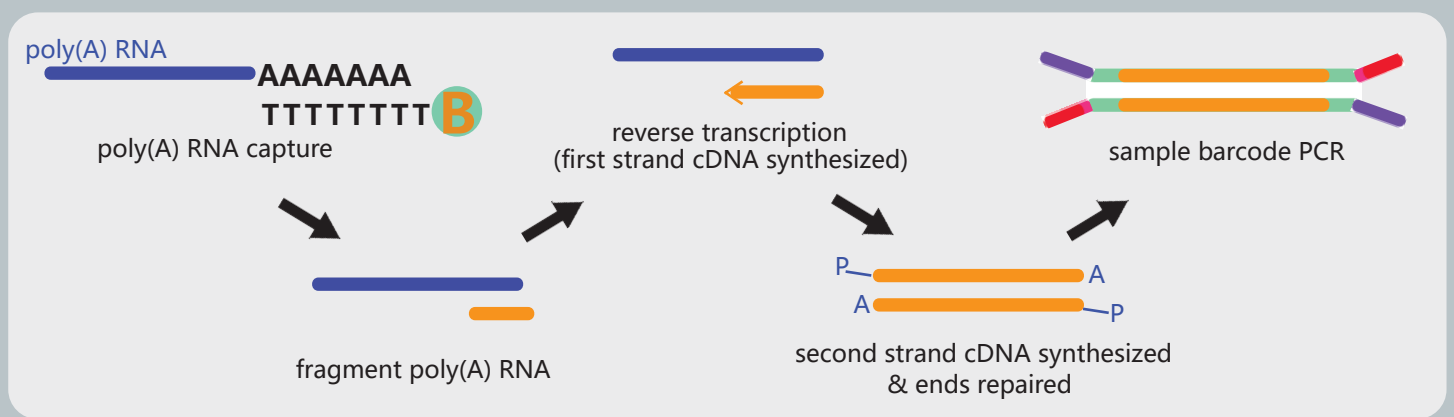
Comparing the rRNA Depletion Methods

The great advance in next-generation based RNA sequencing (RNA-Seq) has widened the scope of scientific investigation into gene expression and regulation. RNA-Seq reveals the qualitative and quantitative information of RNA in biospecimens at certain conditions and depicts the dynamic states of metatranscriptome. Hence RNA-Seq is widely used in transcriptome profiling, detecting novel coding and non-coding genes, alternative splicing events, single nucleotide variants, gene fusions, and so forth.

RNA-Seq yields abundant data of both coding and non-coding RNA such as mRNA, small RNA, circRNA, and lncRNA. However, highly abundant ribosomal RNA (rRNA) occupies approximately 90% of the total RNA, which would greatly limit data quality of the reads from other RNAs of interest especially that requires high total read depth if rRNAs were not depleted. This makes the removal of rRNA necessary to an economical RNA-Seq protocol. Poly(A) selection, physical rRNA removal, and targeted amplification are typical rRNA depletion methods for library preparation.

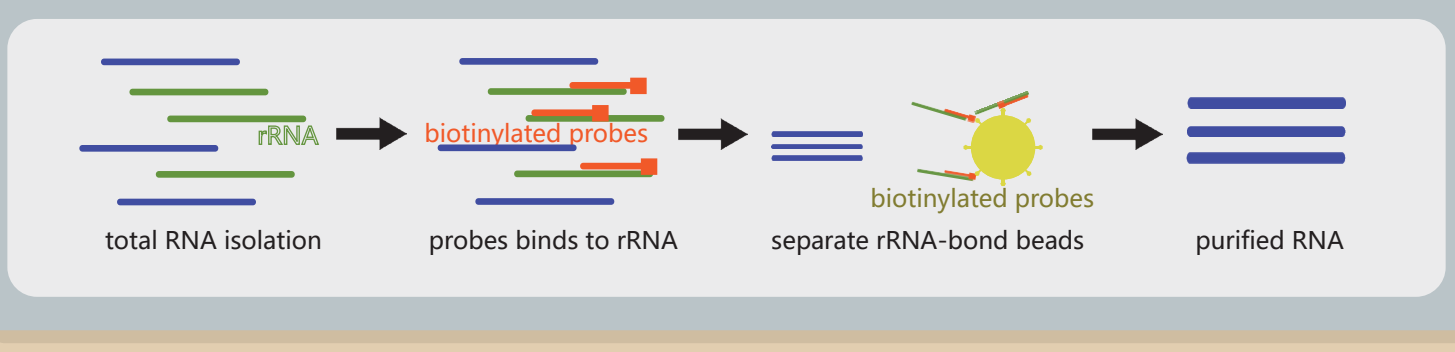
Poly(A) Selection

Poly(A) selection, the enrichment for the PolyAdenylated mRNA transcripts with oligo (dT) primers, is a standard solution for mRNA-Seq library preparation. The oligo (dT) primers are attached to a solid support such as magnetic beads to isolate mRNA transcripts. However, this method excludes all non-poly(A) RNAs along with rRNAs, hence it is not an ideal method for non-poly(A) transcripts and when RNAs are degraded or partially degraded. Meanwhile, since prokaryote mRNA is less stable and lacks poly(A) tail, poly(A) selection is not suitable for sequencing bacteria and archaea. Despite this, poly(A) selection might still be an efficient method when eukaryote protein-coding genes are the focus.



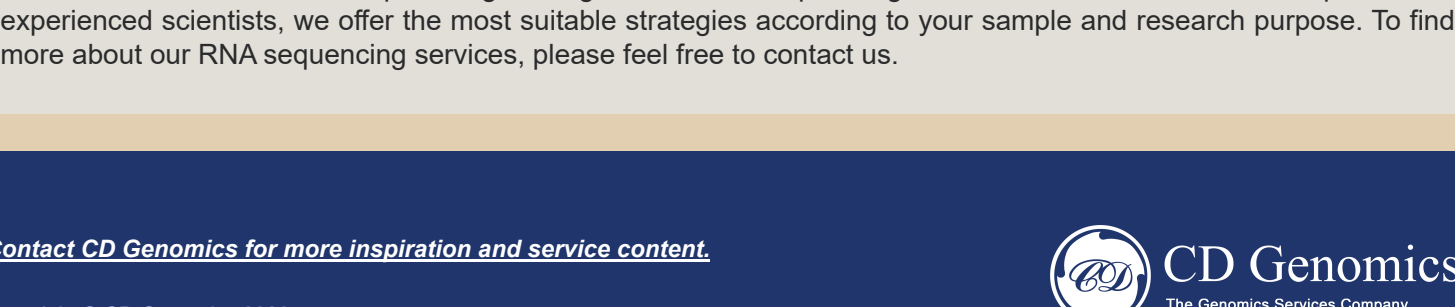
Physical rRNA Removal

Compared to polyA selection methods, physical rRNA removal enables detection of non-poly(A) transcripts, allowing for the detection of both long and short transcripts with less of a 3′ bias than poly(A) selection as well as offering a cost-effective solution for bacterial RNA-seq and FFPE samples alike. By using targeted probes, physical rRNA removal offers greater flexibility of depleting abundant RNAs according to experiment needs, but needs to produce more intronic/intergenic reads, which indicates greater sequencing depth.



Targeted Amplification

In targeted RNA-Seq assays, targeted amplification can work as an alternative method to deplete rRNA in a “not so random” manner, which is a highly accurate method for selecting and sequencing specific transcripts of interest with a decreased affinity for rRNA during first-strand cDNA synthesis. Targeted amplification is effective for low input material though also comes at a cost of greater sequencing depth.



A Comparison Among rRNA Depletion Methods

	Advantages	Disadvantages
Poly(A) capture	◆Lower sequencing depth needed ◆Greater exonic coverage	◆Only detects poly(A) mRNA transcripts ◆Less information on immature transcripts ◆Unsuitable for degraded RNA or FFPE samples ◆Bias towards 3' end of transcripts ◆Cannot be used for prokaryotes
Physical rRNA removal	◆Able to detect small and non-poly(A) RNAs ◆No bias towards 3' end of transcripts ◆Suitable for degraded RNA or FFPE samples ◆Applicable for prokaryotes ◆Can be used for other abundant RNA	◆Greater intronic reads ◆Greater sequencing depth required
Targeted amplification	◆Able to detect small and non-poly(A) RNAs ◆Suitable for degraded RNA or FFPE samples ◆Applicable for prokaryotes ◆Suitable for low input RNA ◆Qualitative and quantitative measurement for specific transcripts	◆Bias towards 3' end of transcripts ◆Greater sequencing depth required ◆Greater intronic reads

Ribo-depletion is a critical step to RNA-Seq library preparation. All methods have some degree of non-specificity and detection bias. Further variability can also arise from different sequence alignment methods in RNA-Seq data analysis. The choice for ribo-depletion methods varies according to different contexts.

CD Genomics is dedicated to providing the highest level of sequencing services. With various solution options and experienced scientists, we offer the most suitable strategies according to your sample and research purpose. To find more about our RNA sequencing services, please feel free to contact us.

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