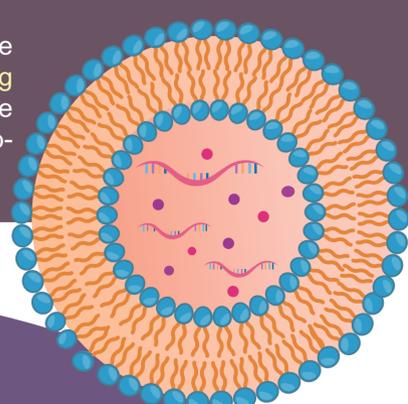


Exosome RNA Sequencing

– From Exosome Isolation to Bioinformatics Analysis

Exosomes are nanoscale extracellular vesicles featuring a double-layer lipid membrane structure, 30-150 nm in size. In both physiological and pathological conditions, nearly all types of cells can release exosomes into body fluids, which play important roles in cell-to-cell communication and epigenetic regulation by transporting crucial protein and genetic materials such as miRNA, siRNA, mRNA, and DNA. The delivery of their cargo RNA molecules is a mainstream hypothesis to explain biological effects. Exosomes hold high potential in several ongoing preclinical and clinical studies, such as disease biomarkers development, early diagnosis, recurrence detection, drug resistance detection, and drug use guidance.

Sequencing technology has advanced successfully to become one of the most used routine tools for life science research. Transcriptome profiling that uses sequencing technologies can supply valuable information. Here we bring you a standardized workflow and protocol for the isolation of exosomes and downstream analysis of exosomal RNA.



Workflow for RNA-Seq of Exosomes



Sample Preparations

Exosomes have been detected in almost all eukaryotic fluids, including blood, urine, saliva, and cell culture media.



Exosome Enrichment and Isolation

Exosome isolation is based on the size, density, and content of surface proteins. The two most frequently used approaches for the isolation of exosomes involved extraction kits and ultracentrifugation. Commercial extraction kits provide a simple and straightforward method for exosome isolation. Ultracentrifugation allows the achievement of highly purified exosomes, while it is a lengthy and difficult process.



The Characterizations of Exosomes

Exosome identification is necessary to distinguish exosomes from other vesicles and macromolecular protein complexes. Exosome identification mainly uses transmission electron microscopy (TEM) depending on its morphological characteristics, nanoparticle tracking analysis (NTA) relying on sizes and concentrations, and Western blot (WB) for detecting signature proteins.



RNA Sequencing

Exosomes contain microRNAs, mRNAs, lncRNAs, circRNA, and ncRNAs in different subsets. Long RNA, including lncRNA and mRNA, can be detected simultaneously by whole transcriptome sequencing. Small RNA sequencing can detect miRNA, siRNA, and other endogenous small RNA. It starts with library construction for sequencing, including exosomal small RNA library, long RNA sequencing library, and exosomal total RNA library.



Bioinformatics Analysis

Exosomal RNA length distribution and quantitative analysis, differential exosomal RNA expression analysis, exosomal RNA and binding site prediction, GO and KEGG pathway analysis, interactive network analysis, coding-non-coding genes co-expression network analysis, and lncRNA-miRNA-mRNA network analysis.

The Broad Prospects of Exosome Transcriptome Research

Exosome Biogenesis & Uptake Research

Exosomes are derived from the endosomal system, and the molecular mechanisms of biogenesis include ESCRT-dependent and -independent biogenesis processes. It has been found that certain exosomes are highly enriched in ncRNA and are involved in initiating the process of exosome biosynthesis, release, and uptake. In some cases, exosomes are transported to more distant sites even if neighboring cells can interact with or utilize them. Therefore, it is necessary to analyze the differences in exosomal RNA content to study various mechanisms involved in exosome biogenesis and uptake.



The Heterogeneity of Exosomes

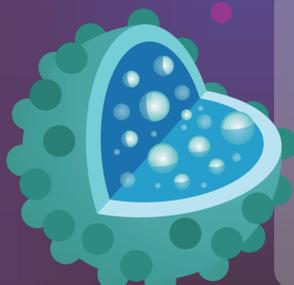
Different exosome populations are highly diverse in their cellular origins, biogenesis, sizes, functions, cargos, or membrane markers, suggesting distinct roles in biology and therapeutic applications. Studying the intrinsic heterogeneity of exosomes may facilitate the study of exosome subpopulation classification and interactions.

The Functions of Exosomes

Exosomes represent a novel mode of cell communication, involved in material transport, signal transmission, and gene expression. Studies have found that ncRNAs in exosomes such as miRNA can mediate communication between diverse cell types, including immune cells. Cellular exchanges of RNA via exosomes contribute to adaptive and innate immune responses, influencing biological reproductive developmental processes and disease onset, including cancer, neurodegenerative diseases, and viral infections.

Clinical Application of Exosomes

Exosome-encapsulated RNAs have been shown to act as highly specific biomarkers for a variety of pathological conditions and may contribute to tumorigenesis and metastasis. Diagnostic development and liquid biopsy biomarker screening and validation studies are now frequently facilitated by content analysis of exosomal RNAs (various small non-coding RNA, lncRNA and mRNA fragments) from a large number of samples.



CD Genomics is a preeminent service provider specialized in exosome RNA sequencing and bioinformatics analysis. Based on cutting-edge platforms, we deliver end-to-end services with more usable reads from low input RNA and in-depth scientific and technical support. Our scientists help you discover new classes of exosome-based RNA biomarkers and the hidden potential in biological data, predict potential functions and patterns of interactions between pathways, and reveal intercellular signaling communication mechanisms.