Application of NGS Analysis:

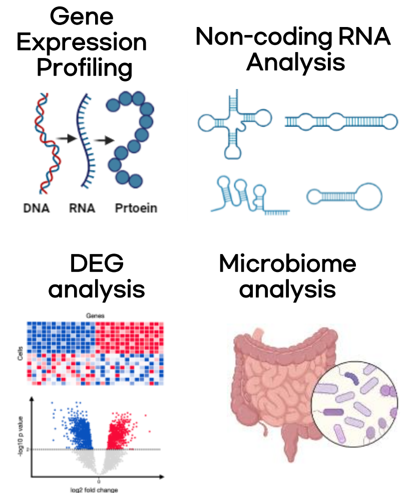
Optimal Solution for High-Quality RNA Purification in RNA-Seq Analysis

iNtRON Biotechnology

RNA sequencing

RNA-Seq (RNA Sequencing) is a technology that analyzes the entire transcriptome within a cell at high resolution, allowing for the exploration of gene expression levels and RNA variants. After reverse transcribing RNA into cDNA, the nucleotide sequence is analyzed through next-generation sequencing (NGS). This allows for gene profiling, which is used to understand the transcriptome of cells or tissues. Additionally, through the analysis of non-coding RNA, researchers can study the expression of various RNA molecules, such as miRNA and lncRNA, in addition to mRNA. This provides valuable information related to their biological functions. In differential expression gene (DEG) analysis, exploring gene expression differences between conditions is useful for identifying genes that change under specific conditions. This provides crucial insights into disease mechanisms. Moreover, in microbiome research, RNA-Seq enables the analysis of gene expression within microbial communities, allowing for the study of microbial interactions and their effects on the host. With its high accuracy and sensitivity, RNA-Seq is widely utilized across various fields of life sciences. It is a powerful tool for precisely analyzing complex gene expression patterns.

Applications of RNA-Seq



Genomic Research Using RNA-Seq (Example)

Ex) Gene Analysis Research Method for Virus-Infected Plants

1. Library Construction and Sequencing

- After extracting total RNA and constructing library from tangerine leaves from 3 regions, Jeju, Wando, and Dangjin, transcriptome data is generated through sequencing.

2. Virus Identification

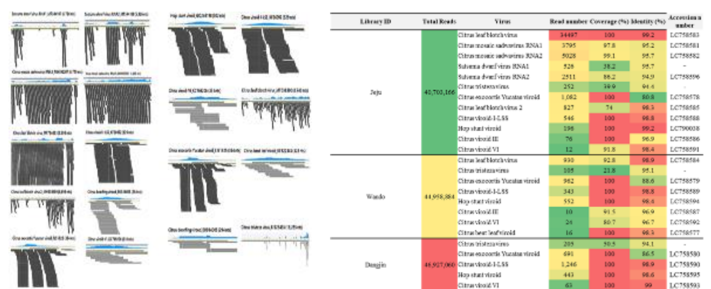
- Identify viruses through BLAST search by using the obtained transcriptome data.
- After mapping the reference genome of the identified viruses, create a heatmap.

3. Phylogenetic Analysis

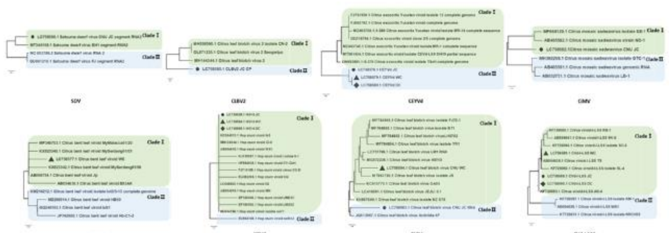
- It is possible to perform phylogenetic analysis by investigating the evolutionary relationships of the viruses identified in the three regions through RNA-Seq.

Summary of sequencing data

Sample	Yield	Amount of data (bases)	Error (%)	Q30 (%)
Jeju	40,703,166	6,105,474,900	98.7	93.6
Wando	44,958,884	6,743,832,600	98.68	93.26
Dangjin	46,927,060	7,039,059,000	98.69	93.16

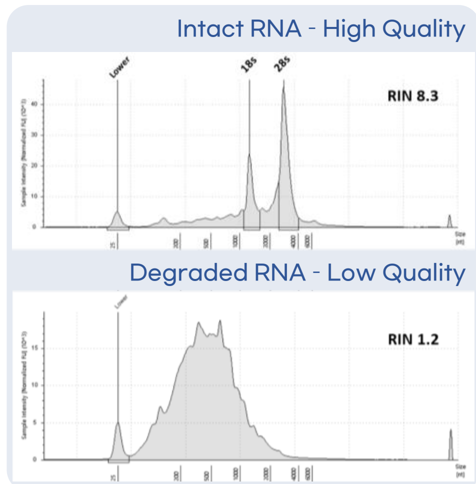


Genome assembly of identified viruses



Phylogenetic analyses of identified viruses and viroids.

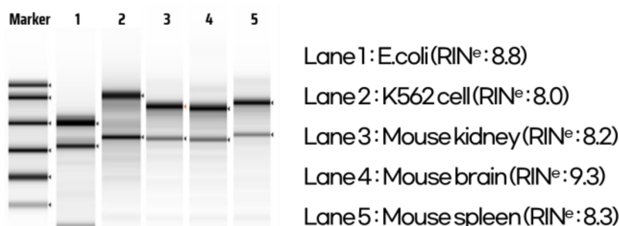
RNA Quality Analysis Criteria for RNA-Seq



The purity of RNA is generally assessed using a spectrophotometer by measuring the A260/A280 ratio (nucleic acid to protein ratio, with 2.0 indicating pure RNA) and the A260/A230 ratio (nucleic acid to contaminants such as phenol, with 2.0-2.2 indicating pure RNA). However, purity and quality are distinct; even if the purity is high, the quality can be rated low if the RNA is degraded. The integrity of RNA is assessed using the RNA Integrity Number(RIN) measured by the Bioanalyzer from Agilent Technologies. The RIN value is provided on a scale from 1 to 10 based on the RNA band pattern, the 28S:18S (or 23S:16S) rRNA ratio, and background noise (degraded RNA fragments). For RNA-Seq, it is generally recommended for RNA to have a minimum RIN value of 7.0 or higher and a concentration of at least 1 μg. If RNA is degraded, sequencing accuracy can decrease, and gene expression levels may be distorted; therefore, high-concentration and high-quality RNA purification is essential.

Optimal Solution for High-Quality RNA Purification : easy-spin™ Total RNA Extraction Kit

Validation of RNA Extraction Performance for Various Samples

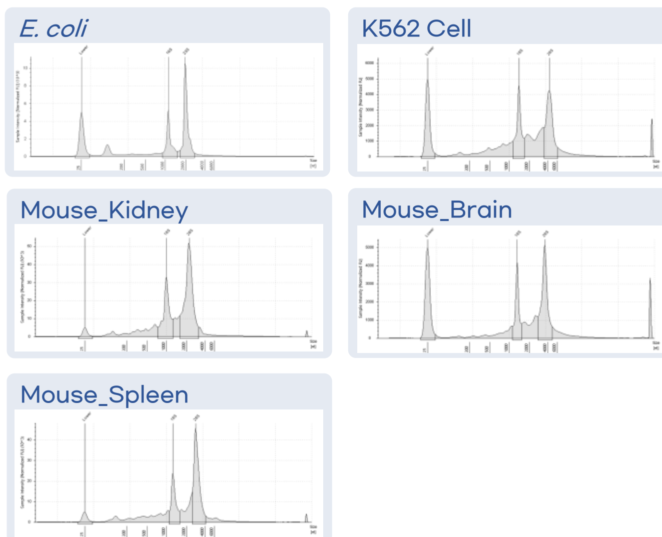


A260/A230 & A260/A280 ratio:

- The A260/A230 and A260/A280 ratios of *E. coli*, K562 cells, and three types of mouse tissue samples (kidney, brain, spleen) all fell within the acceptable range, confirming pure RNA purification results.

RIN value:

- The RIN values of *E. coli* and all four sample types are above 8.0, which meets the recommended RIN value (7 or higher) for RNA-Seq, indicating effective high-quality RNA purification.
- This product efficiently extracts high-quality RNA from various biological samples and is suitable for genomic analysis research.



Sample	A260/A230	A260/A280	RIN
E. coli	2.2	2.0	8.8
K562 Cell	2.2	2.0	8.0
Mouse_Kidney	2.1	2.0	8.2
Mouse_Brain	2.2	2.1	9.3
Mouse_Spleen	2.2	2.0	8.3

Citation

- Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews genetics, 10(1), 57-63.
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- Kim, H. J., Choi, S. R., Cho, I. S., & Jeong, R. D. (2024). Viral Metatranscriptomic Analysis to Reveal the Diversity of Viruses Infecting Satsuma Mandarin (Citrus *unshiu*) in Korea. The Plant Pathology Journal, 40(2), 115.