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A WORKFLOW FOR miRNA DISCOVERY USING sRNA SEQUENCING

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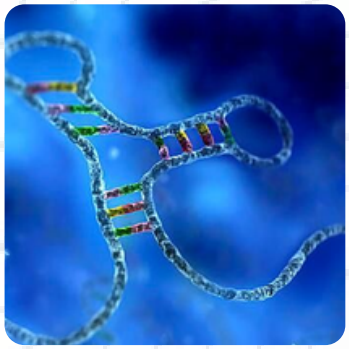

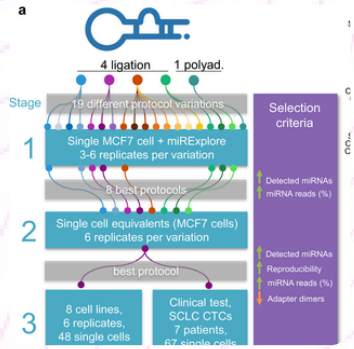

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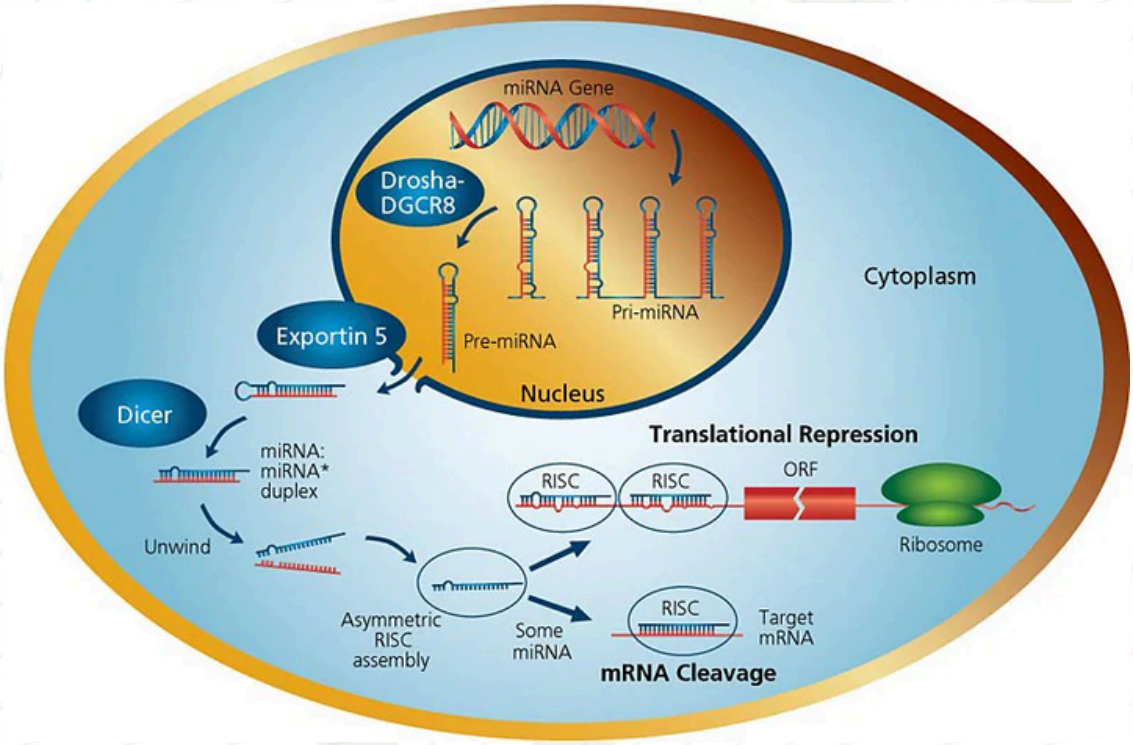
WHAT IS sRNA SEQUENCING?

The sRNA-seq targets RNA fragments between 18–30 nucleotides in length, capturing a range of small RNAs including miRNAs, siRNAs, and piRNAs. The primary goal is often to identify known and novel miRNAs and understand their expression profiles under different biological conditions.

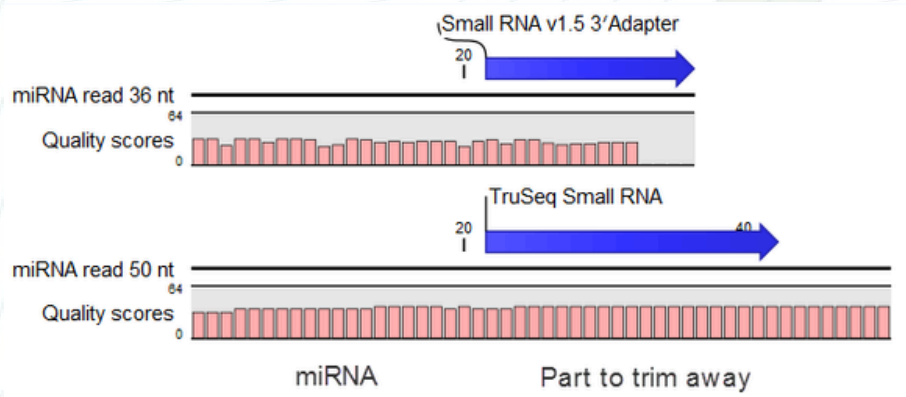


Small RNA (sRNA) sequencing has transformed our understanding of gene regulation by enabling the identification and quantification of microRNAs (miRNAs)—short, non-coding RNA molecules that regulate gene expression at the post-transcriptional level. From plant biology to cancer research and developmental studies, predicting miRNAs from sRNA sequencing data has become a critical step in uncovering regulatory mechanisms. Below is an overview of the typical workflow used in miRNA analysis.

KEY STEPS IN miRNA PREDICTION

1. Quality Control & Preprocessing

- Tools: FastQC, Cutadapt, Trimmomatic
- Remove adapter sequences, low-quality reads, and filter reads by length (typically 18–26 nt for miRNAs).



2. Read Length Distribution Analysis

- Why it matters: miRNAs are generally 21–24 nucleotides long, and the read length distribution can provide a quick overview of the RNA species present in your sample.
- A sharp peak around 21–22 nt often indicates high-quality and expression of miRNA data, whereas broader or irregular distributions may signal contamination from other RNA types (e.g., degraded rRNA or tRNA fragments).
- Tools: FastQC, custom scripts, or plotting tools like R/ggplot2 can be used to visualize the read length histogram.
- Tip: Filtering for specific lengths (18 to 24 nt) based on the distribution plot helps enrich true miRNA candidates.

KEY STEPS IN miRNA PREDICTION

3. Read Alignment

- Tools: Bowtie (Short read aligner)
- Align cleaned reads to a reference genome or transcriptome with strict mismatch (preferably no mismatch allowed) criteria to maintain specificity.

4. miRNA Prediction

- Known miRNAs: Use databases like miRBase for annotation.
- Novel miRNAs: Predict using tools like:
  - miRDeep2: Combines alignment, secondary structure prediction, and scoring models.
  - sRNAWorkbench (miRcat)
  - miRPlant, ShortStack: Designed for plant and complex libraries.

5. Estimation of abundances/expression read counts

- Quantify the expression levels of both known and novel miRNAs by counting mapped reads.
- This step provides the basis for downstream analyses like differential expression.

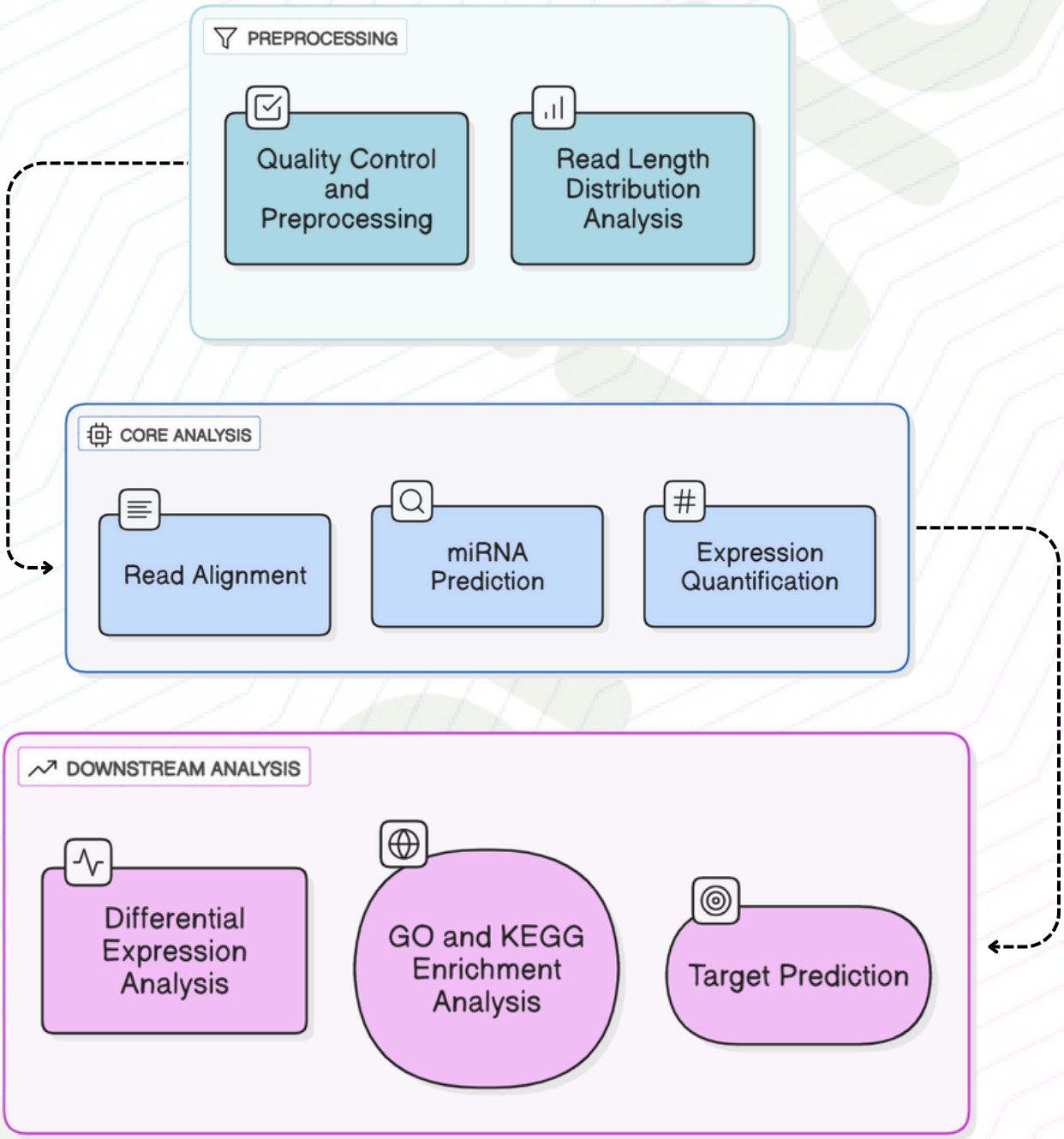
6. Differential Expression Analysis (Optional)

- Tools: DESeq2, edgeR
- Identify miRNAs significantly up- or down-regulated under different conditions.

7. Target Prediction (Optional)

- Tools: psRNATarget, TargetScan, miRanda
- Predict potential mRNA targets of identified miRNAs to infer biological functions.

WORKFLOW


PREPROCESSING
Quality Control and Preprocessing
Read Length Distribution Analysis
CORE ANALYSIS
Read Alignment
miRNA Prediction
Expression Quantification
DOWNSTREAM ANALYSIS
Differential Expression Analysis
GO and KEGG Enrichment Analysis
Target Prediction

BEST PRACTICES

- Validate novel miRNAs experimentally using qRT-PCR or northern blotting.
- Carefully examine the read length distribution to ensure enrichment of miRNA-sized fragments.
- Use biological replicates and proper normalization for reliable differential expression analysis.
- Integrate prediction with downstream functional annotation to derive meaningful insights.

CONCLUSION

miRNA prediction from sRNA-seq data provides critical insights into gene regulatory networks. Early assessment of read length distribution ensures data quality, while a streamlined bioinformatics pipeline enables accurate identification and quantification of miRNAs. With appropriate tools and biological context, sRNA-seq can reveal novel regulatory molecules with significant functional relevance.

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