

Converting NGS (Next-Generation Sequencing) reads into RNA reactivity profiles

a specialized process used to infer RNA structure and dynamics. This is particularly relevant in techniques like SHAPE-Seq, DMS-Seq, and icSHAPE, which combine chemical probing with high-throughput sequencing to map RNA flexibility and structure at single-nucleotide resolution.

RNA Reactivity Profiles

RNA reactivity profiles indicate how accessible or flexible each nucleotide is in an RNA molecule. High reactivity suggests unpaired or flexible regions (like loops), while low reactivity points to structured or base-paired regions (like stems).

Pipeline: From NGS Reads to Reactivity

1. Chemical Probing

RNA is treated with reagents (e.g., SHAPE, DMS, CMCT) that modify flexible/unpaired nucleotides.

These modifications cause reverse transcriptase to stop or misincorporate during cDNA synthesis.

2. Library Preparation and Sequencing

Modified RNA is reverse-transcribed into cDNA.

Libraries are prepared and sequenced using NGS platforms (e.g., Illumina).

3. Read Alignment

Raw reads are quality-filtered and aligned to a reference transcriptome or genome.

Tools like Bowtie, STAR, or HISAT2 are commonly used.

4. Mutation/Stop Detection

Sites of reverse transcription stops or mutations are identified.

These correspond to chemically modified (i.e., reactive) nucleotides.

5. Reactivity Calculation

Reactivity scores are computed by comparing treated vs untreated samples.

Normalization methods (e.g., boxplot normalization) are applied to scale the data.

6. Profile Generation

A per-nucleotide reactivity profile is generated.

These profiles can be visualized or used as input for RNA secondary structure prediction tools like RNAstructure, ViennaRNA, or RNAStructure.