**Table 1:**

Summary of m6A methylation sites and transcripts detected in the sequencing data before and after filtering. The first column shows the sample names. The second column shows the total number of transcripts with detected methylation. The third column shows the total number of methylation sites. Columns four to six display the number of methylated sites retained after each filtering step in sequence.

**Figure 1:**

*Workflow of nanopore direct RNA-sequencing data analysis.*

The workflow starts with sequencing data (pod5) transformed into nucleotide sequences (BAM). From this point, two complementary analyses are performed: analysis of RNA m6A modifications using modification count data, and differential expression analysis using transcript and gene expression count data.

**Figure 2:**

*m6A modification patterns across cell lines and conditions.*

A) Distribution of modification rates at sites (adenosine bases) shared across samples, retained after initial filtering.

B) PCA on methylation rates for all samples.

C) Methylation rates of four sites belonging to four transcripts out of 3,228 sites with detected methylation in 10 transcripts of METTL3.

**Figure 3:**

*Significant differential methylated sites (58,192) and their associated motifs and genes between control and knock-down conditions in HCT116 and DLD-1 cell lines.*

A) Distribution of significant differential methylated sites across conditions, retained with a p-value < 0.05 (two proportions z-test).

B) PCA on significant methylated sites across all samples.

C) Volcano plot of significant methylated sites; dot size indicates p-value, and color represents conditions. Top 10 motifs (5-mers) are shown based on smallest p-values.

D) Volcano plot similar to (C) with gene annotations.

E, F) Consensus motif (E) and base proportions (F) for control condition.

G, H) Consensus motif (E) and base proportions (F) for knock-down condition.

I) Significant GO terms associated with genes linked to significant methylated sites in the knock-down group.

**Figure 4:**

*Association of DRACH motif with METTL3.*

A) Consensus DRACH sequence for control group representing 6,650 sites, and base proportions at each DRACH site. The top three motifs are shown, with GGACU being the most frequent.

B) Consensus DRACH for knock-down group representing 1,020 sites, with base proportions at each site; top motifs include AAACA.

C) Distribution of significantly different methylated DRACH sites (7,670) between conditions, with overall methylation rate differing significantly.

D) Volcano plot of significant DRACH methylated sites, with dot size indicating p-value, color for conditions, and top 10 motifs.

E) Significant GO terms for genes (3,604) associated with significantly methylated DRACH sites in the control group.

**Figure 5:**

*DEGs and their enrichment in genes with significant methylation at DRACH motifs.*

A) PCA on gene expression data for control and knock-down conditions in HCT116 and DLD-1 cell lines.

B) Similarity matrix of samples; darker indicates higher similarity.

C) Expression of METTL3 across conditions.

D) Top three significant DEGs upregulated in control and knock-down groups.

E) Volcano plot showing 61 DEGs (p-value < 0.05); dots represent genes, with color for conditions and grey dots for 13 DEGs (upregulated in control) within DRACH methylated genes.

F) Volcano plot of 2,141 DEGs (log2FC >= 0.25, no p-value cutoff); color represents conditions, and grey dots are 223 DEGs in DRACH methylated genes.

G) Fisher’s exact test for enrichment of DEGs in DRACH gene set: 13 out of 29 DEGs (p-value < 0.05) upregulated in control group are in DRACH genes, with no significant enrichment; 223 out of 1,089 DEGs (log2FC > 0.25) show significant enrichment.

H) Significant GO terms for 223 DEGs upregulated in control and present in DRACH gene set.

**Figure 6:**

*Significant DEGs (adjusted p-value < 0.05) from the simulation study of DLD-1 cell line and their enrichment in DRACH genes.*

A) PCA on gene expression data for six samples: three control and three knock-down of DLD-1, with two replicates simulated from original counts.

B) Top three significant DEGs upregulated in control group.

C) Volcano plot of 699 DEGs; dots represent genes with size indicating p-value, color for conditions, and grey dots for 84 DEGs in DRACH methylated genes.

D) Fisher’s exact test for enrichment in DRACH gene set: 84 out of 373 DEGs upregulated in control are in DRACH genes, showing significant enrichment.

**Figure 7:**

*Differential transcript usage (DTU) across conditions.*

A) Proportion of transcript usage for METTL3 across control and knock-down conditions.

B) Top three genes showing varying proportions of transcript usage across conditions.