Multiplexed RNA Modification sequencing

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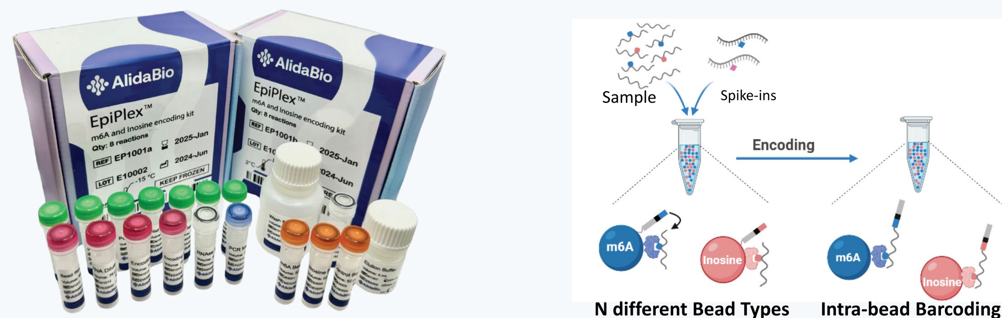
Introduction

The Baylor College of Medicine Genomic and RNA Profiling (GARP) core's mission is to facilitate cutting-edge genomics research by providing state-of-the-art equipment and expert assistance in strategic and experimental planning. Our services include next-generation sequencing on the Illumina iSeq 100, Nextseq500 and NovaSeq 6000 systems. We accept user-prepped samples and offer in-house library preparation for applications such as mRNA-seq, total RNA-seq, limited-Input RNA-seq, microRNA-seq, amplicon sequencing, whole-exome capture, whole genome bisulfite sequencing, epigenomics application including ChIP-seq, CUT&RUN, ATAC-seq, and long-read Oxford Nanopore sequencing. Our library prep automation system handles up to 96 samples per run and enables us to generate NGS libraries for low- and high-throughput projects with high reproducibility.

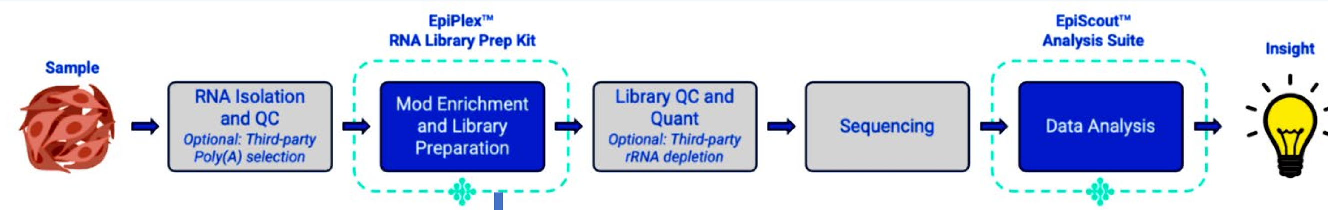
Recently, the GARP core partnered with the AlidaBio to beta-test the EpiPlex RNA Assay, which is designed for multiplexed detection of RNA modifications in the epitranscriptome. This assay can identify variations in both the total abundance and the specific locations of **N6-methyladenosine** (m6A) and **inosine** modification. The method is now available at GARP core and complements RNA modification detection by direct RNA-seq Nanopore sequencing.

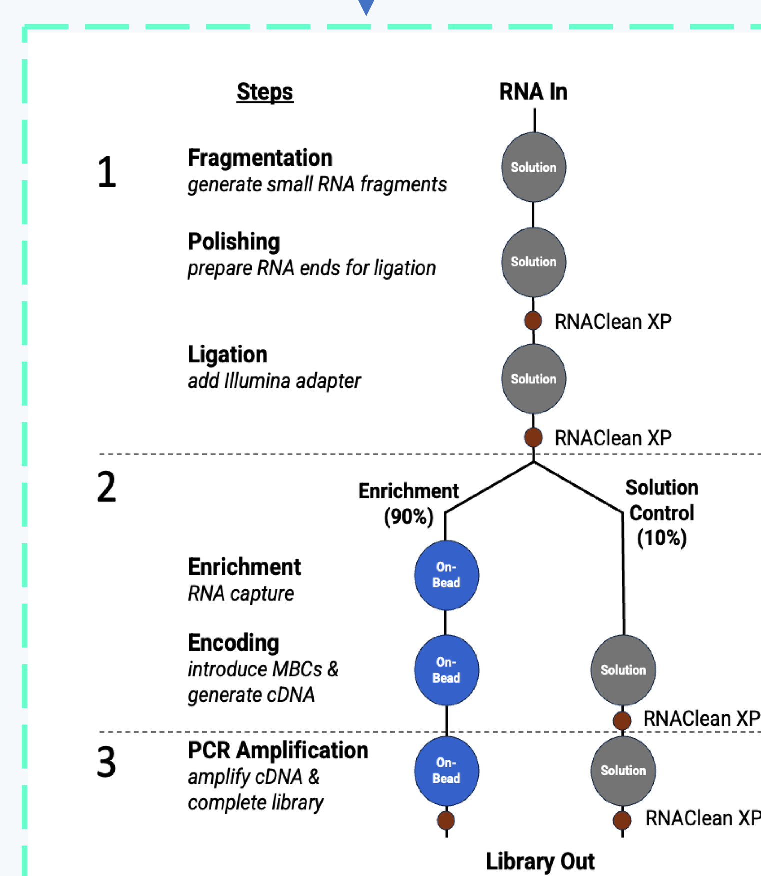
Overview

The AlidaBio EpiPlex Assay generates libraries that identify the presence of RNA modifications (mods) within an RNA fragment. A proximity barcoding technology are employed to record the presents of modifications on fragmented RNA. Molecular recognition of the mods using custom binding agents enriches modified fragments onto beads specific to each mod types. The EpiPlex Kit interrogates m6A and inosine which are significant mods of mRNA and ncRNA. Intra-bead barcoding chemistry appends a mod-specific barcode during library preparation, recording the identify of each mod in the fragment. For each sample, a non-enriched solution control is included to ensure accurate determination of mod-enriched loci.



Workflow





Description

- Multiplexed detection of m6A and I in a one-pot reaction
- Spike-in standards enabling quantification of relative differences across samples
- Low input requirements
- Input: total RNA or poly(A) enriched mRNA.
- 8 hours workflow
- Integrated bioinformatic pipeline (EpiScout™)

Methods

➢ **Application Kit:** The Alida Biosciences EpiPlex RNA library Prep Kit

➢ **Input requirements**:

Specification	Total RNA	PolyA+ mRNA
Quantity	≥250 ng	≥20 ng
Minimum Concentration (Qubit)	≥18 ng/uL	≥1.5 ng/uL
RIN score (Tapestation or equivalent)	≥4 (8 recommended)	n/a
Recommended Buffer	10mM Tris pH 7.0, 0.1mM EDTA (Low TE)	
DNAse I Treatment	Required	

➢ **Procedure**:

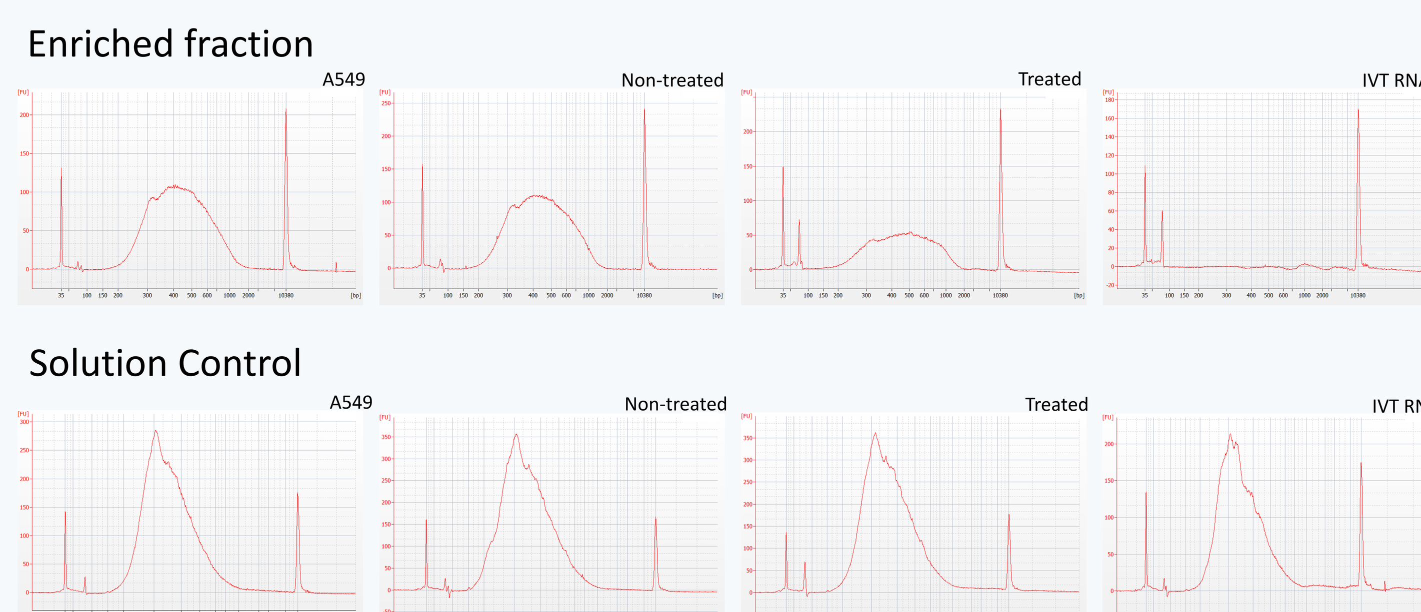
- Sample RNA is combined with Alida's controls, fragmented in solution, and ligated to an adapter
- The RNA fragments are enriched on beads and encoded specific to their mod content. 10% of the RNA pool bypasses the enrichment step to serve as un-enriched solution control.
- Both the bead encoded fraction and solution control are amplified by PCR, adding sequences that complete the library structure.

➢ **Sequencing:** Novaseq 6000, PE100. Read depth: 25M/sample.

➢ **Tested samples**:

Sample 1. A549 (+ Ctrl)
Sample 2. Non-treated
Sample 3. Treated with STM2457 RNA methyltransferase METTL3 inhibitor
Sample 4. *In vitro* transcribed (IVT) RNA (- Ctrl)

Library Fragment Size Distribution

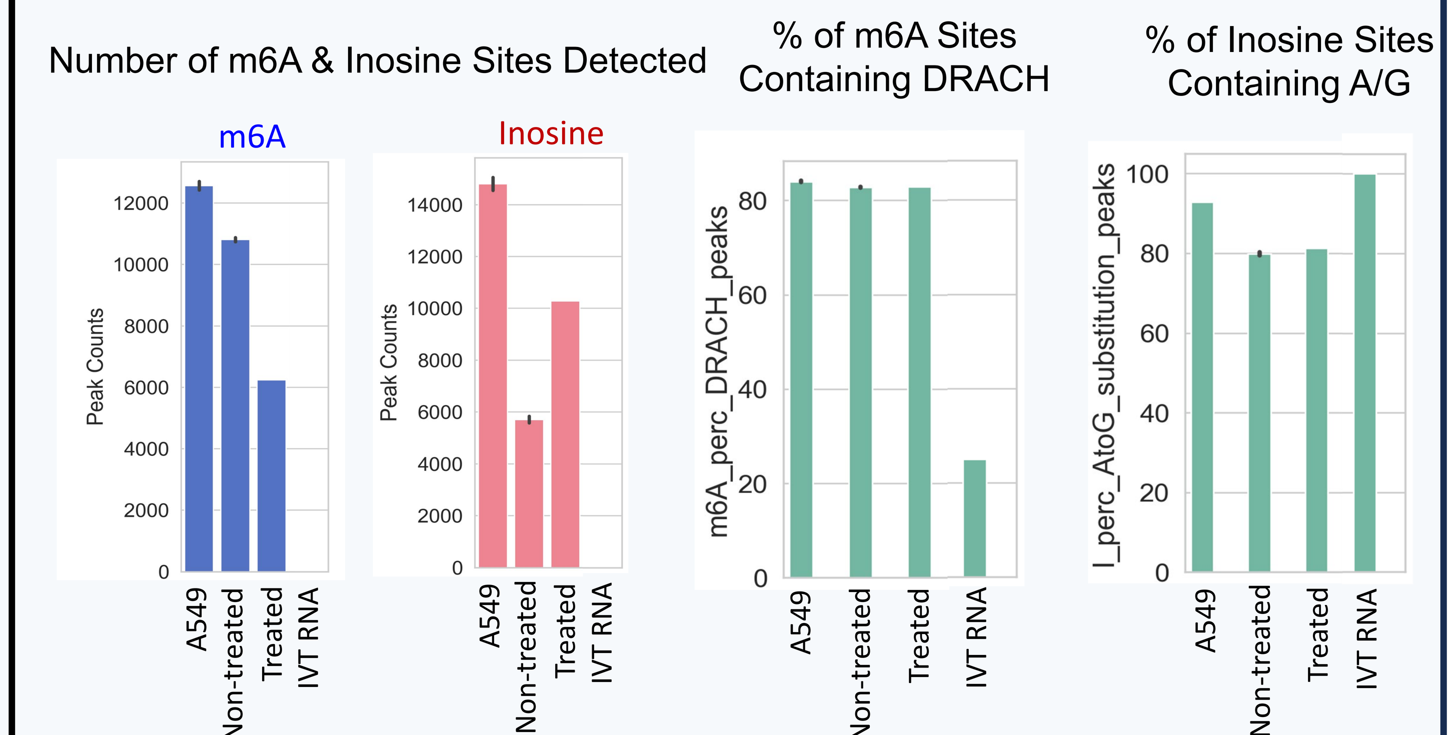


Post-sequencing QC summary

	Enrichment				Solution control			
	A549	Non-treated	treated	IVT RNA	A549	Non-treated	treated	IVT RNA
Total reads	34,498,982	40,629,335	39,466,691	2,062,166	40,915,025	42,670,431	35,828,816	38,873,342
Mapped Reads genome	26,941,421	32,384,007	29,558,027	704,715	28,508,627	30,178,897	25,826,027	27,227,990
% Mapped Reads	78.09	79.71	74.89	34.17	69.68	70.73	72.08	70.04
% duplicates genome	49.26	54.42	69.90	68.23	43.02	48.50	42.67	59.04
% usable reads	39.63	36.33	22.55	10.86	39.70	36.43	41.32	28.69

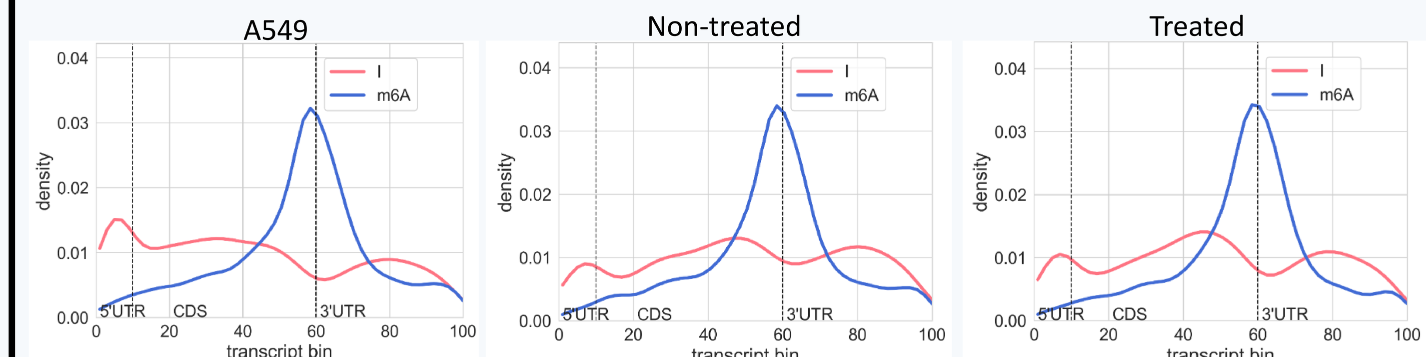
- The sequenced libraries are high quality and pass quality control.

Peak counts

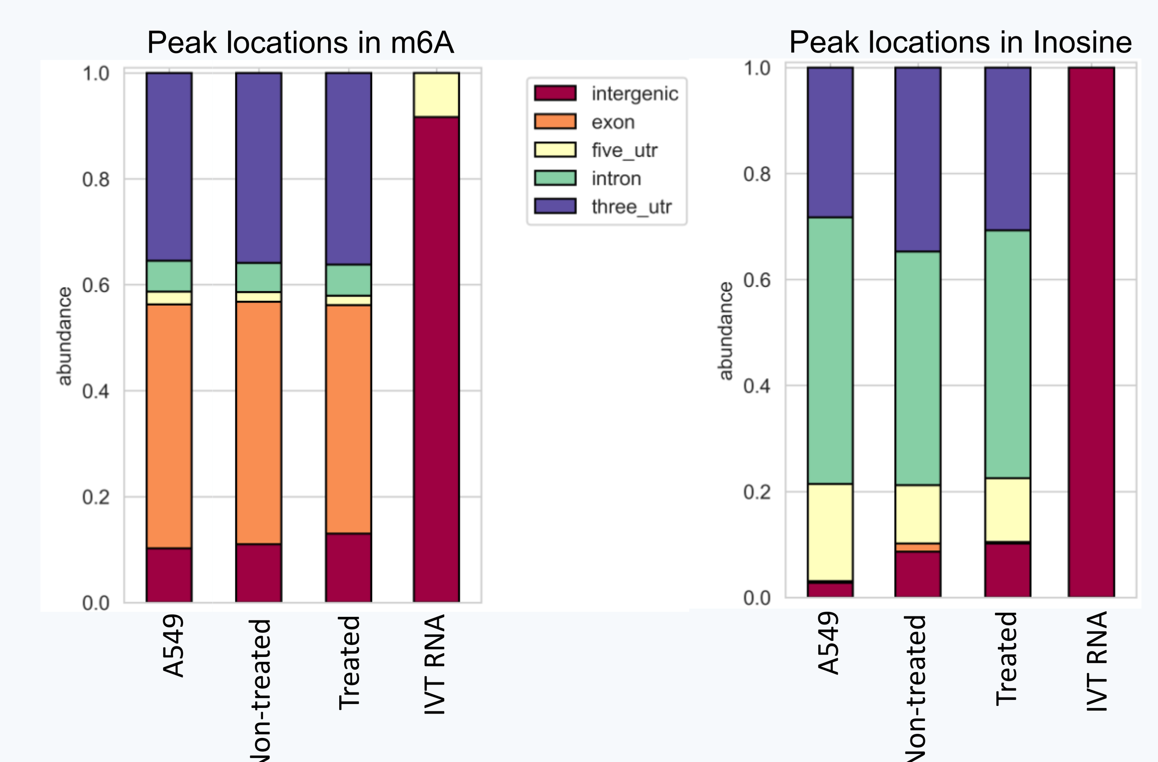


m6A and Inosine distribution

➢ Peak distribution across transcript



➢ Peak distribution across gene features



- m6A was found to be enriched in the CDS/3′-UTR junction near stop codon as expected, whereas inosine was generally present within intronic regions of the CDS in all samples

Conclusion

- 20ng of poly A enriched mRNA is sufficient input.
- The AlidaBio EpiPlex RNA library prep kit provides high quality RNA modification sequencing which identifies N6 methy-adenosine (m6A) and inosine (i) concurrently.
- m6A peak calls were significant reduced in the cells treated with STM2457 RNA methyltransferase inhibitor expectedly (1).

References

1. Yankova E, Blackaby W, Albertella M, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature. 2021;593(7860):597–601.
2. Cerneckis J, Ming GL, Song H, He C, Shi Y. The rise of epitranscriptomics: recent developments and future directions. Trends Pharmacol Sci. 2024 Jan;45(1):24-38.
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4. Dominissini, D., Moshitch-Moshkovitz, S., Schwartz, S., et al. (2012). Comprehensive analysis of m6A RNA methylation reveals enrichment in 3′ UTRs and near stop codons. Nature, 485(7397), 201-206.