Secondary structure of *in vitro* transcribed RNA

Introduction

Selective 2′-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) is a chemical probing method that measures RNA flexibility at single nucleotide resolution. NAI adducts formed during probing induce a higher mutation rate by reverse transcriptase at the modified positions during the generation of cDNA (Fig. 1). SHAPE technology on IVT mRNAs measures local RNA flexibility in purified, deproteinized RNA *in vitro*, which can aid in the prediction of RNA secondary structure for the RNA formulation.

HIGHLIGHTS

Reactivities guide RNA fold by informing pairedness of each base along the RNA.

Structure of mRNA can be compared between samples with single nucleotide resolution.

Technology & Deliverables

eSHAPE *single RNA* is a method to obtain mRNA structure probing data for *in vitro* transcribed mRNA formulations. *In vitro* transcribed mRNAs are used as input for RNA structure probing with the NAI reagent. eSHAPE *single RNA* is available as a kit for end user usage, and as a full service offering with results delivered as a data package containing RNA structure information as mutations and reactivities.

.bam	eSHAPE *single RNA* DMSO reads aligned to the mRNA sequence of interest
.bam	eSHAPE *single RNA* NAI reads aligned to the mRNA sequence of interest
.shape	Reactivity values formatted for input to the RNAStructure algorithm to guide a fold prediction
.bedgraph	Reactivity score at each position of the mRNA with >1000x coverage for genome visualization
Metrics	Gene metrics table including alignment, total read and coverage stats
Plots	Coverage, mutation rate, and reactivity line plots across the gene and mutation rate box plot (.svg)

eSHAPE *single RNA* Workflow

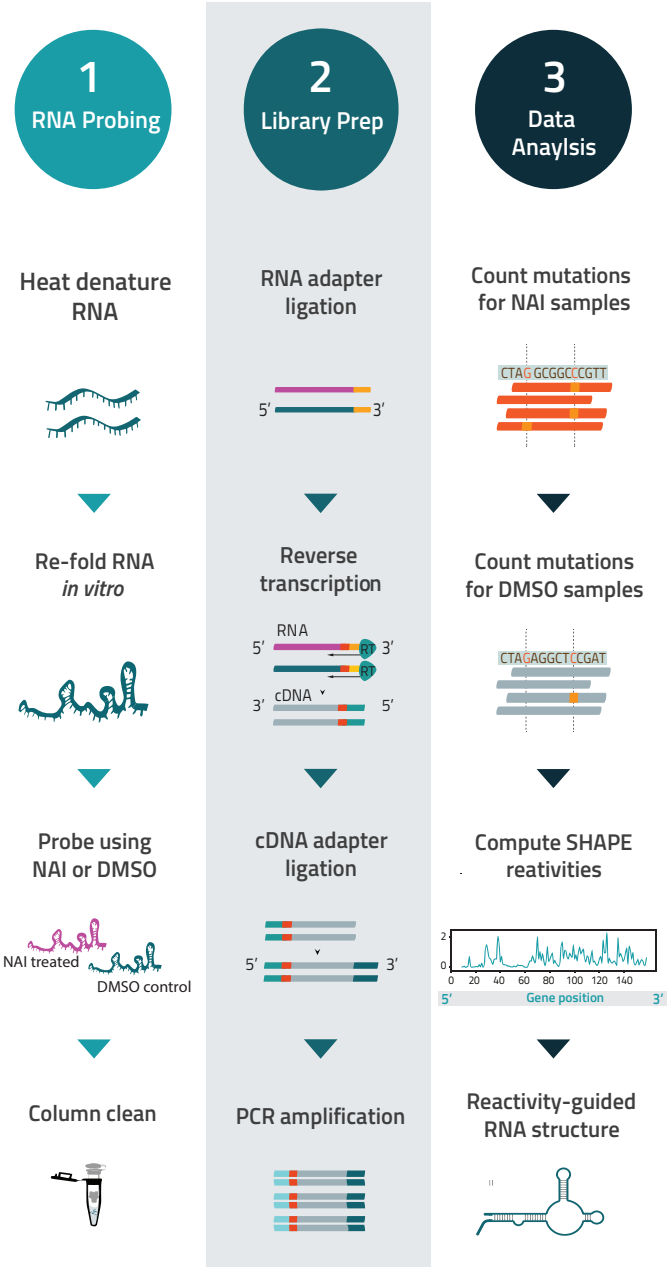

Figure 1 . *In vitro* transcribed mRNA fomulations are processed as shown above with replicates for each condition. mRNA is isolated, folded and probed (highlighted in purple). Once sequencing reads are aligned, mutations are counted for each position. NAI mutation rates are normalized to DMSO mutation rates, generating a SHAPE reactivity score for each position in the RNA.

Deep coverage from eSHAPE *single RNA*

An eSHAPE *single RNA* experiment was performed using 100 ng IVT 23S rRNA (2904 nt) as input. Six replicate samples from both NAI treated and DMSO treated controls were sequenced at approximately 2M reads each. Reads were aligned to the 23S rRNA sequence, and UMI deduplicated, yielding a deep coverage 23S rRNA SHAPE dataset (Fig. 2A). Mutation rates were calculated for all samples (Fig. 2B).

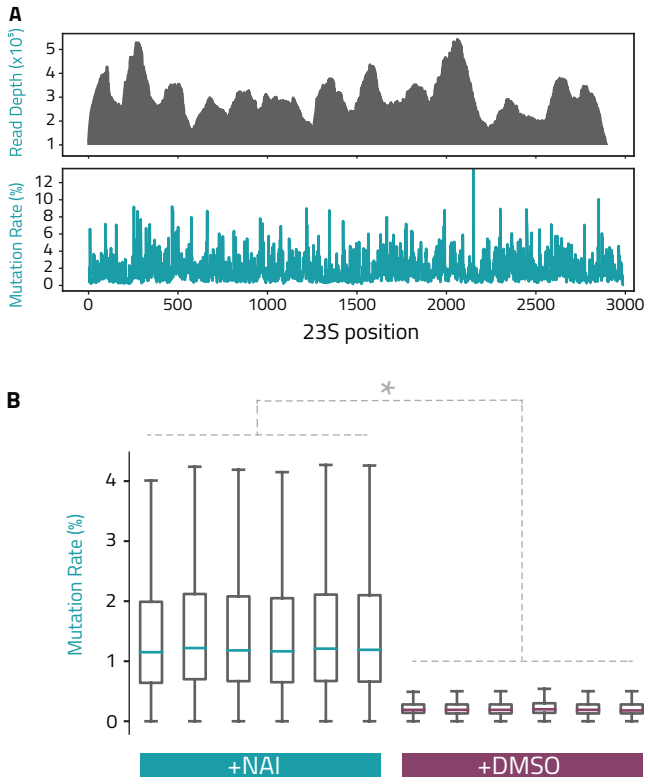

Figure 2. 23S E. coli rRNA (A) Merged coverage across the length of 23S rRNA (in vitro transcribed). Note that the lowest coverage points are all >10,000X coverage. Mutation rate line plot across the 23S rRNA for NAI samples. (B) Mutation rate box plot for all 6 replicates of +NAI or +DMSO control (*p-value of 10^293 by KS-test). Figure shows significant elevation of mutation rates in NAI samples versus DMSO samples, indicating succesful adduct formation by NAI. Mutations in DMSO samples are considered background and will be subtracted from NAI mutation rates to compute reactivities.

Input Specifications

Input	Starting Material	Read Depth	PE/SE
1ug RNA	in vitro transcribed RNA	8M Reads/ Sample	Single End 100

Detect RNA structural changes due to single nucleotide variants

The 5′ UTR of FTL (Ferritin Light Chain) contains a stable structure, the iron response element (IRE) (Fig. 3A). Certain genetic variants have been shown to disrupt the IRE structure and can lead to human disease. Using IVT mRNA we created three mutant 5′ UTRs of FTL - one of which (U22G) disrupts the IRE, and causes a change in reactivity (Fig. 3B).

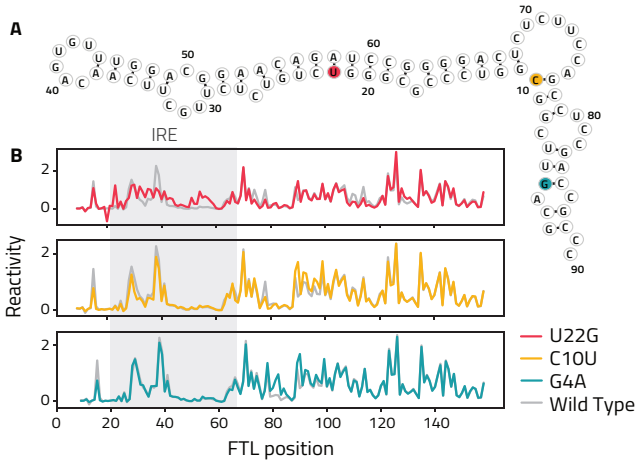

Figure 3. (A) FTL IRE fold with point mutations indicated in red, yellow and teal. (B) Mutation reactivity profiles (red, yellow, teal) versus wild-type (grey). Grey shadow box indicates IRE. U22G is the only mutant that causes a change in reactivity versus wild-type.

Reactivities of high sensitivity & specificity

High RNA folding sensitivity and specificity is achieved, consistent with other methods (Fig. 3).

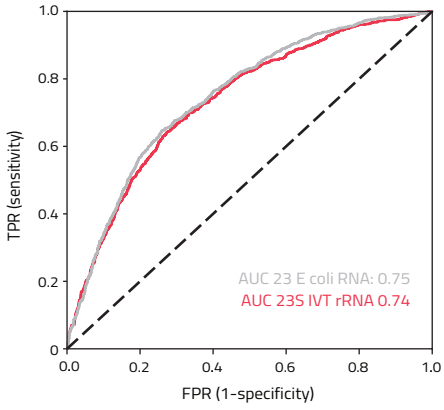

Figure 4. IVT 23S rRNA reactivity scores were compared with the paired/unpaired status of each nucleotide in the accepted reference 23S rRNA structure using Receiver Operating Characteristic (ROC) analysis and area under the curve (AUC) metric. A similar AUC is observed for the 23S rRNA reactivities obtained from a whole transcriptome E. coli RNA experiment.