# Auto-tRNAseq: Driving throughput in tRNA library generation with firefly®

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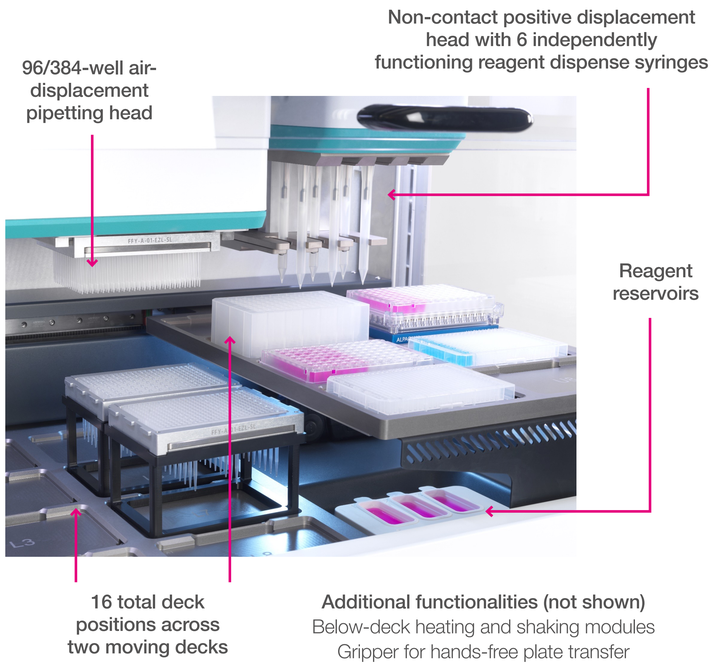
## Introduction

Transfer RNAs (tRNAs) are the critical adaptor molecules that translate genetic information into functional protein sequences. While the genome defines a static set of tRNA gene sequences, the functional tRNA pool depends on the expression and aminoacylation levels of different tRNA species, as well as many modifications that fine-tune tRNA activity.[1,2] Dynamically regulated tRNA pools dictate the fidelity of the translation process and proteome integrity of a cell. Considering the central role of tRNA in translation regulation, investigating the cellular composition of tRNA pools provides a deeper understanding of the translational state of the cell. High-resolution sequencing-based methods for tRNA sequencing have provided transformative insights into the physiological impact of tRNA expression, modification, and aminoacylation levels on cellular stress and/or disease conditions.[3–6] More recently, large-scale genome engineering efforts have also employed tRNA sequencing to recapitulate the translational state of engineered cells.[7]

While tRNA sequencing offers comprehensive insights into the cellular state, it has not been readily employed in research across the globe. Current library generation workflows for tRNA sequencing are time-consuming, arduous, and rely on multiple gel purification steps that incorporate experimental variability and limit experimental throughput. In this poster, for the first time we propose a high-throughput automated library generation workflow for tRNA sequencing using SPT Labtech's firefly liquid handling platform.

## Automating genomics liquid handling with firefly

firefly has been designed specifically to streamline NGS library preparation by bringing together multiple liquid handling capabilities.




## Method

### Manual tRNA library generation

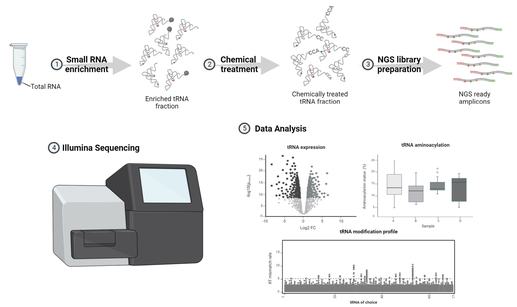



Figure 1. Manual workflow for generation of tRNA sequencing data. Chemical treatment steps and bead-based cleanups result in a highly specialized and labor-intensive workflow.

### Automated tRNA library generation

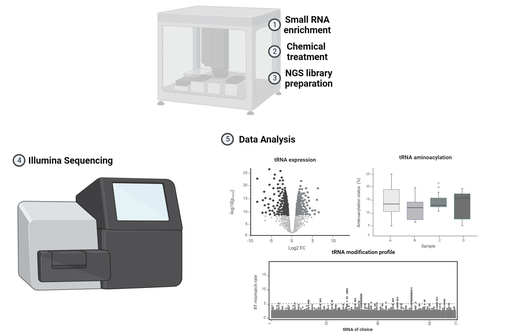



Figure 2. Automated workflow for generation of tRNA sequencing data. Chemical treatment steps and bead-based cleanups are performed on an automated liquid-handler, improving consistency and allowing for higher-throughput experiments

### Bead-based RNA purification

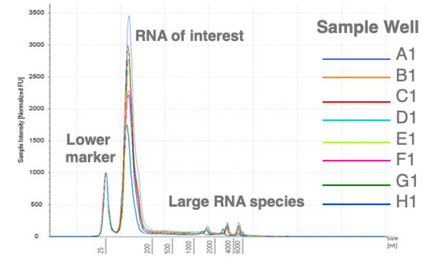
firefly was used to perform all dispense, aspiration and mixing steps in a 96-well PCR plate (Biorad Hard Shell Plate). The non-contact dispense head was used to dispense RNA purification beads (SPRIselect, Beckman Coulter), 20-45% isopropanol or ethanol in water and elution buffer (RNase-free water). The pipetting head was used to perform the initial RNA transfer, all mixing steps, and all transfers of supernatant to waste. A 96-well plate magnet (MagnumFLX, Alpaqua) was used for all bead separation steps.

AutotRNAseq_worflow1_part1: (a) RNA size selection: Using the SPRI beads with 7.5% PEG + 20% isopropanol to remove large RNA molecules > Increase isopropanol concentration (~45%) to bind small RNA molecules > (b) Chemical treatment: Add chemical reaction buffer I > Incubate for 15 mins > Add reaction buffer II.


Figure 3. Execution of tRNA size-selection on firefly

## Results

### Small RNA enrichment on firefly



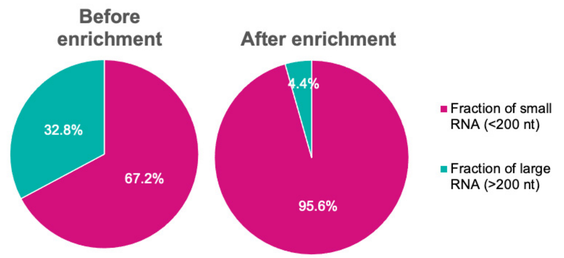




Figure 4. TapeStation results showed successful and consistent size selection of tRNA between all wells in a column. Larger RNA species were removed from the mixture to leave behind only the smaller RNA species of interest.

## Conclusions

- firefly can successfully be used to automate bead-based RNA purification, a key component of Next Generation Sequencing (NGS) workflows, with a uniform fragment size distribution demonstrated for samples across a 96-well plate.
- firefly completed size selection of RNA for the purposes of tRNA sequencing and modification detection.

The versatility of firefly with its two distinct liquid handling heads is well-suited to develop customized bead-based RNA size selection methods to circumvent gel purification techniques. In this work, we have prototyped the crucial short RNA (<200 nt) enrichment step with consistent final yield from the same starting material of total RNA input, with minimal well-to-well variation.

## References

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