

A Novel System for Automated RNA Isolation

“Increasing Through-put without Increasing Footprint”

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Abstract

The High Throughput Biology (HTB) department at GlaxoSmithKline is developing *in vitro* models to better predict the efficacy of compounds in the clinic. The development and progression of disease is often associated with characteristic changes in gene expression. These “transcriptional profiles” (mRNA gene expression patterns associated with a given disease) can be used as biomarkers to monitor disease states. Therefore, we are utilizing transcriptional profiling as a way to understand diseases and the effects of pharmaceuticals on those diseases. Transcriptional profiling has numerous advantages over other techniques including highly sensitive and highly quantitative assays, simple assay development, and accessibility of nearly the entire genome to transcriptional readouts. Our transcriptional profiling strategy involves isolation of total RNA from samples followed by real-time quantitative PCR assays (Taqman_® assays).

Current automated systems allow for the isolation of total RNA from one 96-well plate at a time on a Biomek_® FX taking approximately 1 hour per plate. The HTB Automation Team recently developed an automated system to increase through-put and reduce reagent waste for 96-well RNA isolation extractions. This system can isolate RNA from ten 96-well plates in approximately four hours. An additional goal was to create a system on one stand-alone Biomek_® FX. Beyond simultaneous processing of plates and custom deck layout we designed and co-developed with Beckman a NestedTip design that allows the stacking of five P20 tip boxes on one Automated Labware Positioner (ALP) location. This poster describes the nested tip functions, robot functions, and assay performance of a higher through-put automated RNA isolation workstation.

RNA Procedure Overview

The SV 96 Total RNA Isolation System (Promega, Inc., Madison, WI) purifies total RNA from cell lysates using 96 well vacuum filtration. Total RNA is purified by binding cell lysates to a silica-based 96-well RNA binding plate, performing DNase I digestion, washing and eluting RNA into water. The department of HTB chose the Promega SV 96 because of its adaptability to automation and its ability to isolate RNA from a wide range of different cell types. The automated steps are outlined in Figure 1.

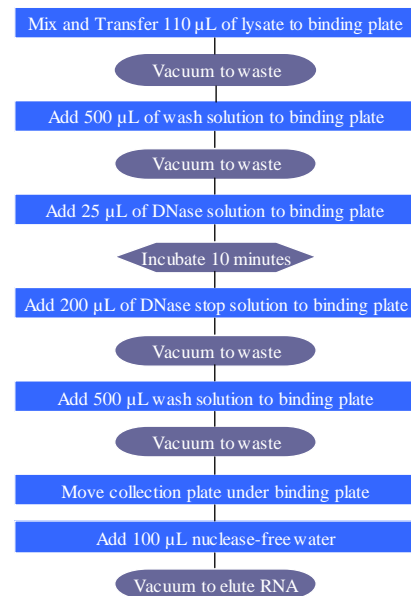


Figure 1: RNA Isolation Automated Steps

System Overview

The goal of the Automation Team was to develop a walk-away workstation that could perform the RNA isolation assay in an off-the-shelf format. Specific goals included a system that could process 10 sample plates with one setup, reduce overall reagent waste, and increase through-put without increasing footprint.

Although the Beckman Coulter Biomek_® FX contained the desired pipetting functionality the system required some customization to achieve the through-put goals. Figure 2 shows the current high through-put RNA isolation system. The system is comprised of a Biomek_® FX single pod 96-well pipette head with a custom 3x4 ALP, dual vacuum system, and NestedTips (discussed later). Figure 3 shows the system layout as it appears in the Biomek_® FX software. Key hardware and software concepts of the system are as follows:

- Custom 3x4 ALP - The ALP is shorter than standard ALPs. This allows consumables to be stacked higher, specifically the binding and sample plates (Figure 2).
- Dual vacuum systems - The dual systems process sample plates in parallel.
- P20 NestedTips - The NestedTips reduce the need for an additional external storage hotel. The tips are used for transferring lysates from sample to binding plates.
- P250 Tips - The tips are designated for specific reagents (DNase, Wash, DNase Stop, and RNA free water) and reused throughout the process.
- Single program - One program was developed utilizing “If” statements, single assay loop, and variables for performing the assay on a range of 4 to 10 plates. Figure 3 shows the Biomek_® FX programming screen.



Figure 2: Biomek_® FX

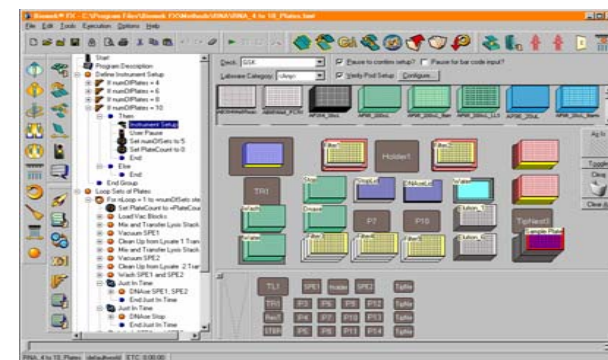


Figure 3: RNA Isolation Program

NestedTips

The NestedTip system was designed to expand the through-put and efficiency of Biomek_® FX deck layout. GlaxoSmithKline prototyped the original concept and design for the NestedTip system and further developed the product in partnership with Beckman Coulter. Currently the NestedTip system consists of NestedTip Racks (NTRack) and NestedTip Base (NTBase) and is only functional with P20 pipette tips. Future designs could incorporate additional tip sizes.

Figures 4 and 5 show the NestedTip system in the stacked configuration. The NTRacks can be stacked up to 5 racks high on modified ALPs. Notice the NTBase can also be stacked on top the NTRack. This allows further optimization of the deck layout by not needing a specific ALP to store the base when not in use. As shown in Figure 6, during the loading process the base is placed on the tip loader and the pod is used to move an NTRack onto the NTBase. The tips are fetched and removed in the same manner as a standard box of P20 tips.

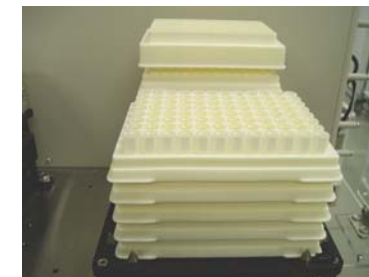


Figure 4: NestedTip Rack

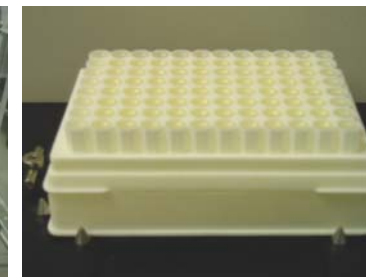


Figure 5: NestedTip Base and Rack



Figure 6: Grippers with NestedTip Rack

Discussion

In order to test the reproducibility of the 10 plate Biomek_® FX RNA isolation protocol, we prepared RNA using the Promega SV 96 Total RNA Isolation System. In this experiment, 2 x 10⁵ HUVEC cells/well were seeded in ten 96 well tissue culture plates. Total RNA was purified from all 10 plates, as outlined in Figure 1, using the Biomek_® FX RNA isolation protocol. RNA samples were converted to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems) for use in real time quantitative PCR (Taqman_®) assays. Taqman_® assays were performed to quantitate 18S RNA levels for each sample (Figure 7) and the average %CV was 3% (Table 1). These results demonstrate that the RNA samples are of high enough quality for subsequent Taqman_® analysis.

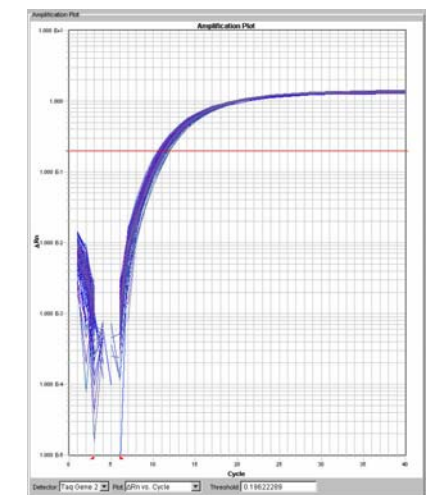


Figure 7: Ct Determination via Taqman

Variability in 18S rRNA Quantitative RT-PCR										
Plate	1	2	3	4	5	6	7	8	9	10
%CV	2.4	3.4	2.4	2.7	2.6	2.8	3.1	3.3	3.6	1.8

Table 1: Plate to Plate Variability

Conclusions

The unique design of our high through-put RNA Isolation system has increased our through-put, reduced reagent waste, provided consistent results, and decreased operator involvement. Automation of the RNA Isolation procedure by the Biomek_® FX increased efficiency by four-fold when compared to manual manipulation of 10 x 96-well plates. This procedure also reduced reagent waste by 10-fold per 10 plates processed over manual purification. The results (see above) demonstrated that the high through-put RNA isolation system consistently produced high quality RNA suitable for quantitative gene expression analysis. The automated procedure also improves scientist efficiency by allowing the operator to “walk away” during the run.

Acknowledgements:

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