

Using Hamilton Automation to Reduce Turn-Around Times and Lower Costs at the Nevada Genomics Center

Jayden Feldman¹ and Andrew Gorzalski²

1. University of Nevada, Reno / College of Engineering / Honors College
2. University of Nevada, Reno / Research & Innovation



Abstract

Most labs must prepare their DNA samples for Sanger sequencing manually, which is inefficient and costly. The goal of this research is to study the feasibility of programming a Hamilton STARlet liquid handling device to reduce hands-on time, lower costs, and speed up procedures in the lab. After the method's initial implementation, we used an iterative approach to improve on previous versions; making small groups of changes at a time and observing the effect on the method during, and after, execution. The data shows that there is significant time that can be saved through usage of the Hamilton device, with other benefits such as lowered costs and improved sample quality. These findings show that Hamilton liquid handling machines can improve the Sanger reaction workflow, and there is potential for expansion into other reactions.

Introduction

The Hamilton STARlet Liquid Handling Device is underutilized in the Nevada Genomics Center. While the STARlet is programmed to perform infrequent library preparations, it has not been programmed to perform routine laboratory work (e.g., Sanger sequencing). If the STARlet was given this functionality, it would reduce costs and hands-on time at the Nevada Genomics Center.



The STARlet is equipped with 8 CO-RE channels, a 96-channel head, on-deck thermal cycler (ODTC), cooling rack, heated shaker, and iSWAP. The hardware on deck allows for the programming of many different laboratory procedures. Hamilton uses the VENUS 4 software to program methods on the STARlet. My project involved learning how to use VENUS 4, writing code in Hamilton Standard Language (HSL), and incrementally improving functionality.



My PREP objective was to program the STARlet to perform a Sanger sequencing clean-up reaction using Dynabeads™ Sequencing Clean-Up Kit (ThermoFisher #66101). This clean-up reaction is a necessary part of the Sanger sequencing process and is a cost-effective option over current reagents in use.

Methodology

Dynabeads Sequence Clean-Up Procedure

1. Add the Dynabeads™ Solution (see "Before you start" on page 1) to each sample.
 - For a 96-well plate, 20 µL Dynabeads™ solution is added to each 10 µL sample.
 - For a 384-well plate, 10 µL Dynabeads™ solution is added to each 5 µL sample.
2. Mix thoroughly by gentle pipetting. Do not vortex.
3. Incubate at room temperature for 5 minutes.

Note: Protect from light during incubation.

Note: Periodically pipette gently to help improve the binding of the extensions to the beads, which can increase signal.
4. Place the tube or plate on a magnet for 1 minute.
5. Keeping the tube or plate on the magnet, aspirate off the supernatant, then discard.

Note: Aspirate the supernatant from the opposite side of the well or tube to avoid the beads.
6. Add 70% ethanol to the Dynabeads™ with bound extension products.
 - a. Remove the tube or plate from the magnet.
 - b. Add 120 µL of 70% ethanol to the Dynabeads™ with bound extension products.
7. Mix thoroughly to resuspend the beads by gentle pipetting. Do not vortex.
8. Incubate at room temperature for 2 minutes.

Note: For a 384-well plate, use 35 µL of 70% ethanol wash and a total of 3 washes.
9. Place the tube or plate on a magnet for 1 minute.
10. Keeping the tube or plate on the magnet, aspirate off the supernatant, then discard.
 - a. Repeat step 6 through step 8 for a total of 2 washes.
 - b. Repeat step 6 through step 8 for a total of 2 washes.
11. Add 20 µL highly purified water or Hi-D™ Formamide.

Note: Eluates in water can evaporate over time. If running plates with many samples and long electrophoresis times, Hi-D™ Formamide should be used for eluting the sequencing product from the Dynabeads™.
12. Remove the tube or plate from the magnet.
13. Mix by pipetting (aspirating and dispensing the sample in the well) until beads are resuspended.
14. Incubate for 2 minutes at room temperature.
15. Place the tube or plate on a magnet for 1 minute.
16. Keeping the tube or plate on the magnet, transfer 10 µL of supernatant, containing the extension products, to a fresh plate.
17. The eluted products should be clear of beads for sequencing.

Note: If the elution buffer is not at the bottom of the tube or plate well or if any bubbles are present, briefly centrifuge the tube or plate for 5-10 seconds.
18. Install a plate septa (Cat. No. 4412614) on the plate, set the plate into an appropriate plate retainer, and load the plate with the purified reactions on the genetic analyzer. Run as directed in the genetic analyzer user manual with standard run modules (not BDX run modules).

Reagents: Template DNA, ddATP, ddTTP, ddGTP, ddCTP, Polymerase, dNTPs, Primers.

1. Primer annealing and chain extension
2. ddNTP binding and chain termination
3. Fluorescently labeled DNA sample
4. Capillary gel electrophoresis and fluorescence detection
5. Sequence analysis and reconstruction

Sanger Clean-up Step

Calculate the needed volumes based on number of samples entered (line 21) and skip the timers if the skip timers' box was selected (23) during setup. Display the values in a debug trace (24-27) and in a loading dialog, along with their locations (28).

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20 Grouping
21 Calculate times and volumes
22 Use the needed volumes
23 Use the timers
24 Trace 24 of Traceout
25 Trace 24 of Traceout
26 Trace 24 of Traceout
27 Trace 24 of Traceout
28

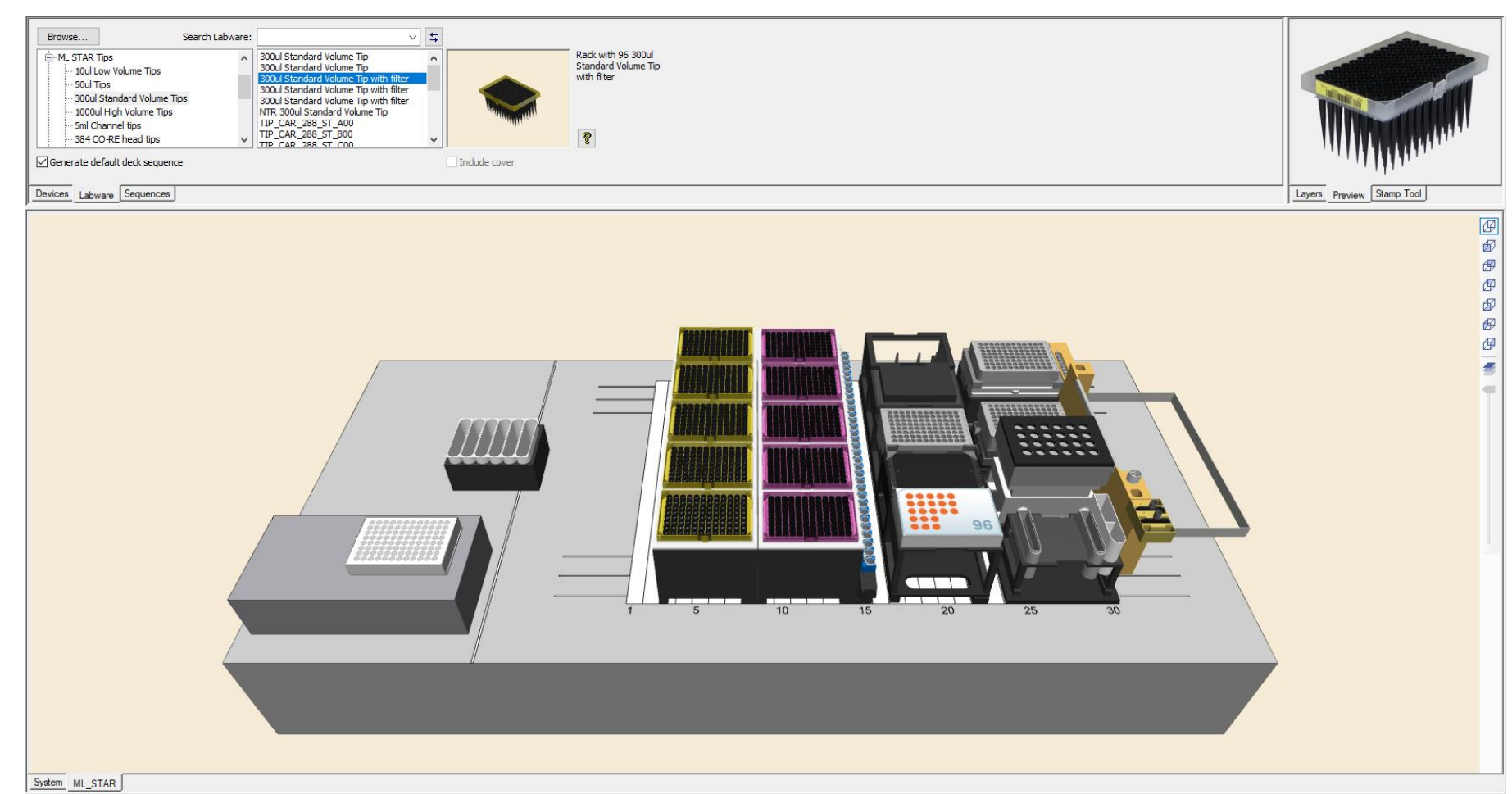
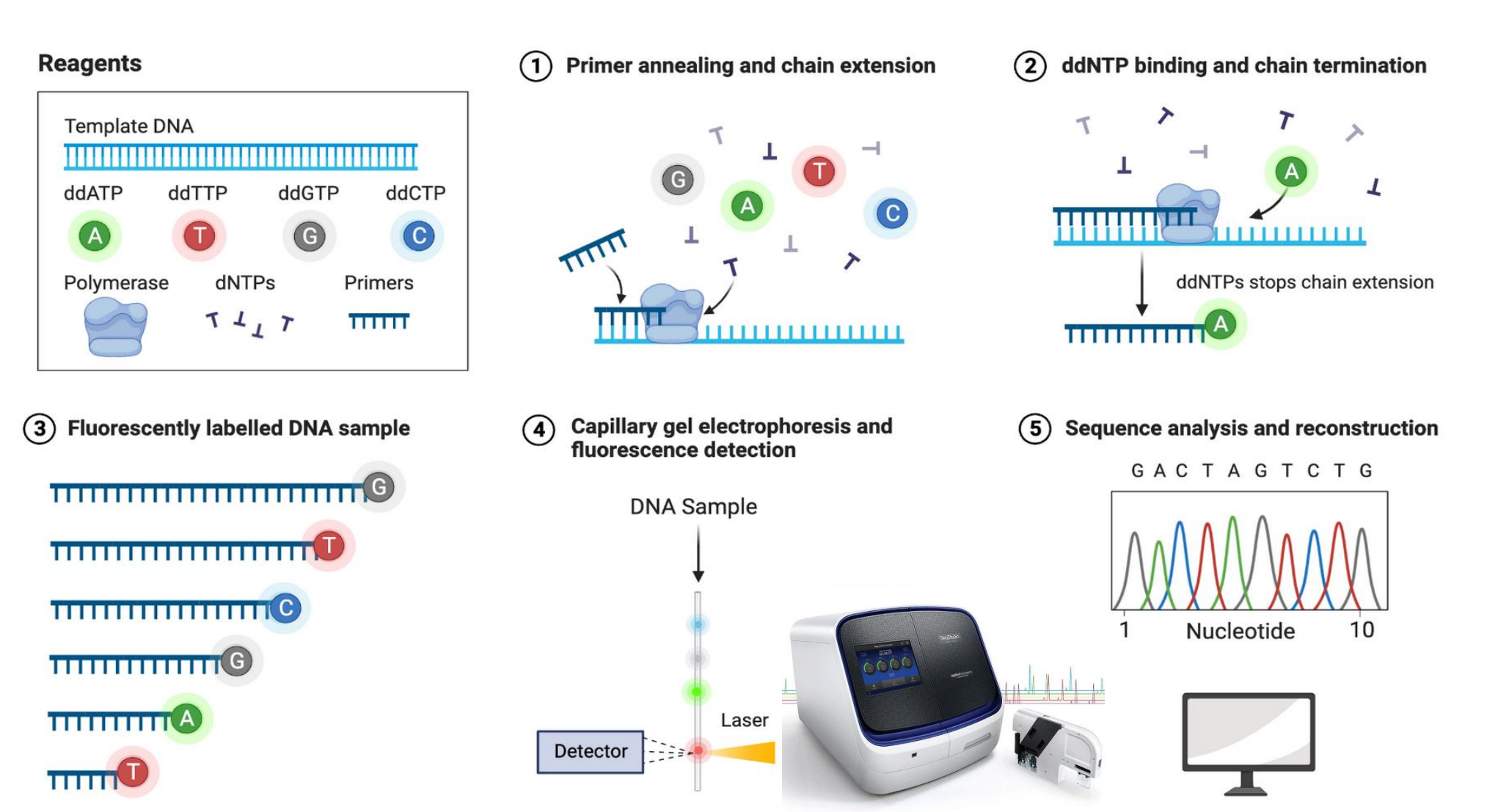
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A commented snippet of the EtOH wash steps, with the user-defined (starting with t_) variables shown on the right. The shown code moves the plate to the heated shaker, adds 70% EtOH to each sample, shakes at high speed for 1 minute, and then logs the time.

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The deck editor screen, which shows where your equipment should be located on the deck and allows you to rearrange it. The yellow and pink columns hold 300uL and 50uL filtered tips respectively. The column of blue containers holds the Dynabeads. The row to its right holds the sample plate in the middle position, and the elution plate in the front position. The back position of the last column holds the heated shaker, the second to back position holds the magnet plate, and the front position holds the reagent troughs. The troughs, from left to right, are 70% EtOH, the elution buffer, and the liquid waste trough.

Load Volumes

Please load the following volumes to their indicated locations:

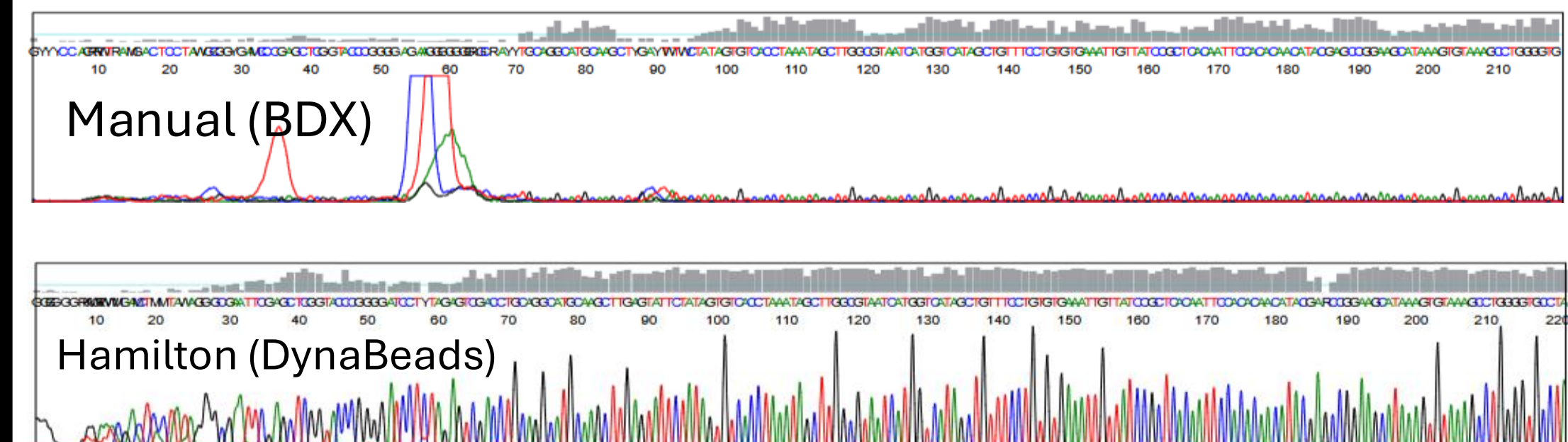
Magnetic Beads: 210 uL
 70% Ethanol: 1970 uL
 Eluate: 210 uL
 Volume Per Sample: 10 uL

Loading dialog showing needed volumes & respective locations. Alternative text appears when hovering, giving labels if the image is too difficult to read.

Results/Discussion

After the method was initially implemented and proven to run successfully, we began to iterate over versions to improve it. Notable improvements include:

- Making the system function when processing anywhere from 1 to 96 samples at a time
- Adding display screens for loading volumes, labware, and total times
- Adding a running log for time taken to reach certain milestones in the method, allowing for identification of the most time-consuming portions and tracking time saves throughout iterations
- Significantly speeding up the program through minor optimizations in the method (e.g., beginning incubation immediately after first column is ready, picking up tips while waiting for the shaker/incubation/magnet, etc.)



Comparison of raw Sanger sequences of manual (BigDye Xterminator) and Hamilton method. The manual method does a fairly poor job removing all fluorescently labeled artifacts (personal communication), while the Hamilton produced clean reads. The difference in clean-up allowed 70 additional quality nucleotides to be parsed from the data (an improvement of 15%).

Reagent	Method	\$ per Sample	Time for 12 rxns (hands-on time)	Time for 24 rxns (hands-on time)	Sequence length (nt)
BigDye Xterminator	Manual	\$0.98	30 min (6 min)	32 min (8 min)	551
DynaBeads	Manual	\$0.72	35 min (35 min)	40 min (40 min)	617
DynaBeads	Hamilton	\$0.72	29 min (1 min)	34 min (1 min)	629

Automating Sanger clean-up reactions reduces cost per sample (\$0.72 versus \$0.98) as well as shortens the amount of hands-on time required (1 minute versus 6 minutes). The overall time performing the clean-up was not significantly different either. By implementing automated liquid handling at the Nevada Genomics Center, I was able to reduce cost and hands-on time for routine laboratory work.

Future Directions

In the future, we would like to implement the cycle sequencing (PCR) step prior to the rest of the method, so that we can perform the entire Sanger sequencing process on the STARlet. We would also like to implement more advanced/time-consuming methods (RNA-Seq library preparation) onto the machine to make use of its versatility.

Acknowledgements

This project is funded by PREP. Thank you to the Hamilton company for assistance, especially Will Hutchens.