Automating Laboratory Liquid Handling

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Abstract

The Hamilton Star and the QIAGEN QIAcube are liquid handling robots (robots for doing automated pipetting) designed to automate standard laboratory protocols. Using these robots we validated a RNA/DNA extraction protocol on the QIAcube, created a 3 step capture protocol for T cell receptor genome sequences on the Star, and validated a Kapa DNA library preparation protocol on the Star. The RNA/DNA extraction protocol had similar yields to manual extractions and was overall faster, the 3 step capture protocol needs work, and the library preparation protocol needs more work before it can be run unattended.

Objectives

- Validate the QIAcube DNA/RNA extraction protocol
- Create a custom 3 step capture protocol for T cell receptors for the Hamilton Star
- Validate the Hamilton Star Kapa DNA library preparation protocol

Materials & Methods

- The QIAcube DNA/RNA extraction protocol was run according to the manufacturers instructions on 51 samples as were the manual comparison on 41 samples from the same batch
- The custom capture protocol was designed and tested using the manufacturers software
- The Kapa Prep protocol on the Hamilton Star was tested with tap water and two samples following the instructions provided in the protocol as it ran

Introduction

Automation has the potential to lead to "increased productivity, efficiency, reliability and confidence[1]" in a laboratory setting. Specifically liquid handling robots offer genomics labs the ability to streamline key steps of the sequencing pipeline.

Sequencing Pipeline

1. Sample RNA/DNA extraction
2. Library preparation
3. Target capture
4. Sequencing

Each of the pre-sequencing steps benefits from automation by enabling multiple samples to be processed at once increasing user productivity and efficiency.

DNA/RNA Extraction

The QIAGEN kit for RNA/DNA extraction binds the RNA/DNA to a column and then elutes it off in a later step.

Library Preparation

Library preparation takes fragmented DNA, repairs the sticky ends, adds a terminal deoxyadenosine 5′-monophosphate (dAMP) to the 3′ now blunt end, and adds adaptors. The final product can be PCR amplified and size selected if necessary.

Target Capture

Target capture is used to enhance a specific region of the genome by blocking the adaptors added during the library preparation step, binding the DNA regions of interest to streptavidin beads, and capturing them using magnets, and then eluting the DNA back off after the supernatant has been discarded (a reverse capture saves the supernatant and throws out the beads). PCR can then be used to amplify this selected DNA.

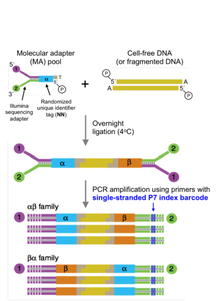

Figure 1: Library PRep Workflow

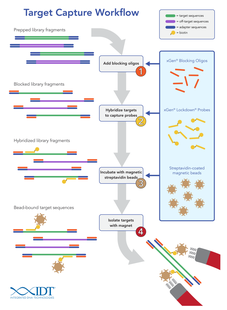

Figure 2: Target Capture Workflow

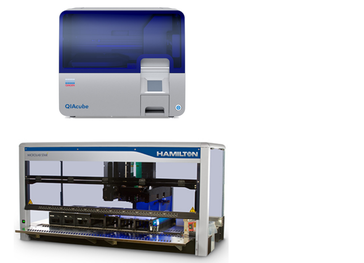

Figure 3: QIAcube & Hamilton Star at the Princess Margaret Genome Center

QIAcube and STAR Features

QIAcube

- Self contained with integrated centrifuge
- Protocols are based on QIAGEN kits
- Protocols are not user editable
- Can process 1-12 samples at once

STAR

- Computer needed to edit/run protocols
- Can process up to 96 samples
- centrifuges, incubators, heater/shakers can be added
- Air Displacement Pipetting with Anti-Droplet Control
- Total Aspiration and Dispense Monitoring
- Liquid Level Detection

QIAcube Results

The QIAcube successfully extracted RNA/DNA from 51 samples with yields of 5543.13 ± 2248.41ng for RNA and 7950.58 ± 4117.03 ng for DNA. In comparison 41 samples from the same batch were extracted by hand and had yields of 5933.82 ± 2099.30ng for RNA and 7693.23 ± 3406.96ng for DNA.

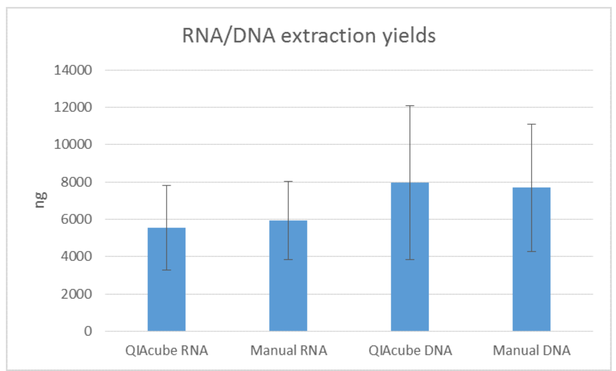

Figure 4: RNA/DNA Extraction : QIAcube vs Manual Yields

A skilled technician can process around 20 samples a day compared to 36 (3 runs of 12) on the QIAcube making the robot faster. But a downside of the QIAcube is that it can't be used with samples with very little starting material since the robot protocol can't be user edited. It also currently can't be used to run the entire protocol since it only transfers 600ul of lysed material (even if there is more).

Custom Capture Protocol Results

We created a 3 step capture protocol (a standard capture followed by a reverse capture followed by a final standard capture) for T cell receptor genome sequences based on a written protocol in consultation with technicians in the Pugh lab. Here we focus on the first standard capture.

We broke the written protocol down into several key steps.

- Pool samples
- Block samples
- Add probe
- Prepare & Wash Streptavidin beads
- Bind hybridized samples to beads
- Remove unbound DNA
- Prepare PCR amplification
- DNA purification

Each of these steps was implemented as a function to enable code reuse and to make the overall protocol more flexible. After testing the protocol with water for reagents and samples we enlisted user feedback. Based on the feedback we updated the protocol and moved the Streptavidin beads preparation off robot and plan to make pooling samples a separate stand alone protocol and to use the robots ability to shake plates to mix samples instead of pipette mixing thereby reducing the number of tips required.

Kapa Protocol Tests Results

The Kapa HyperPrep protocol was written by Kapa for a generic Hamilton Star and modified for our robot's deck layout. When run with real samples it successfully completed its run with only a few errors which must be addressed before more samples can be run with it. The DNA trace results look promising since the primary peak is where it should be with minor amounts of adaptor dimers left.

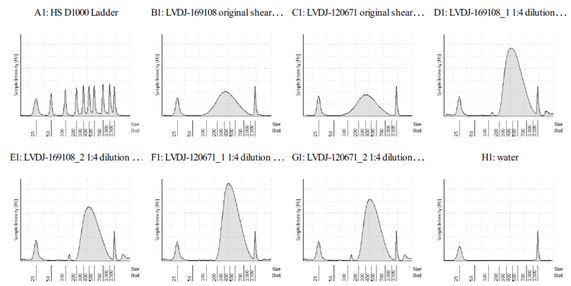

Figure 5: DNA Traces : Original samples & Post Cleanup

One of the problems encountered was that the plate shaking speed was too fast during one step and some of the solutions splashed and potentially cross mixed. This can be fixed by changing the shaking speed. The robot also ran out of PCR primer (even with 20% excess) and failed to aspirate any of the additional PCR primer we added. This will require further investigation to determine the cause.

Conclusion

- The QIAcube can increase productivity but can not be used for all samples indiscriminately
- More work needs to be done to make the 3 step capture protocol usable
- The Kapa protocol on the Hamilton Star needs tweaking before samples are routinely run with it

Future Research

- Finish testing/improving the Kapa protocol so it reliably runs without user intervention.
- Write generic capture and reverse capture protocols for the Hamilton Star
- Write a sample pooling protocol for the Hamilton Star.

References

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