

ABSTRACT

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

INTRODUCTION

Automation in a laboratory setting has the potential to lead to "increased productivity, efficiency, reliability and confidence[1]." In particular liquid handling robots offer the ability to ... key steps of the pipeline for taking sample DNA, creating a library, and performing a capture before passing it off for sequencing. As an example the Princess Margeret Genome Centre has a QIAGEN QIAcube and a Hamilton Star.

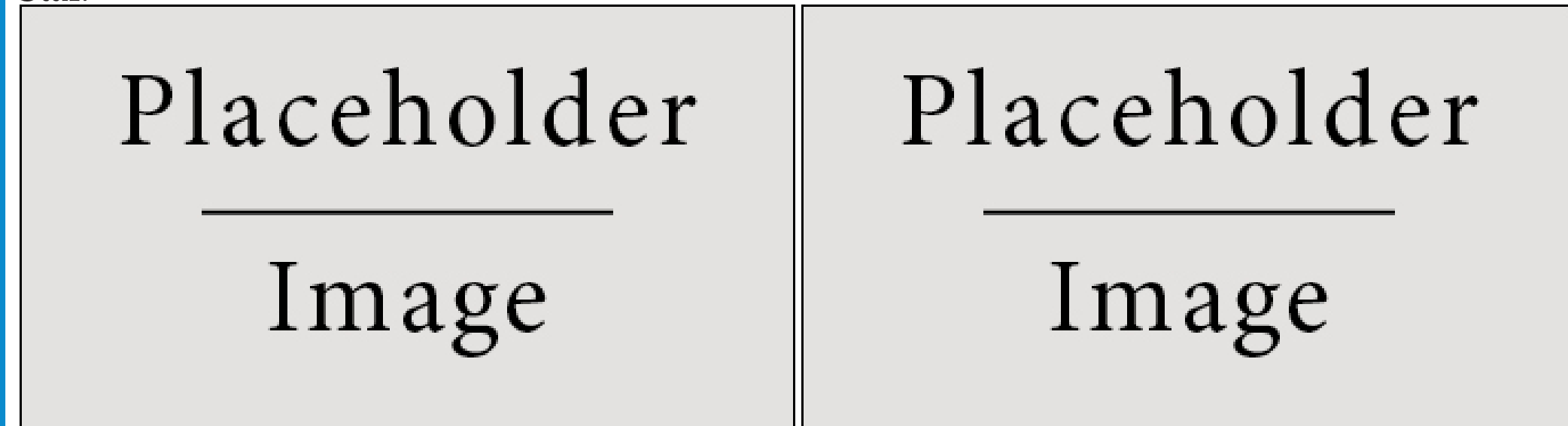


Figure 1: QIAcube

Figure 2: Hamilton Star

Besides the difference in footprint these robots differ in several key ways. The QIAcube is a self contained unit which includes an integrated centrifuge. Protocols are designed to work to with QIAGEN kits and come preinstalled. The robot can process up to 12 samples at once.

In contrast the Star requires an attached computer to program methods and to actually control it. Its features include Air Displacement Pipetting, Anti-Droplet Control, Total Aspiration and Dispense Monitoring, Monitored Air Displacement, and Liquid Level Detection. Additional modules like centrifuges, incubators, and heater/shakers can be added after. Any protocols must be written from scratch using a drag and drop interface. The robot can process at least 96 samples at once if set up accordingly.

DNA library construction is a key step in processing biological samples for DNA sequencing. It consists of[2]

1. End repair and A-tailing, which produces end-repaired, 5'-phosphorylated, 3'-dA-tailed dsDNA fragments
2. Adapter ligation, during which dsDNA adapters with 3'-dTMP overhangs are ligated to 3'-dA-tailed molecules
3. Library amplification (optional), which employs highfidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends

Portions of the library can be selected for further PCR amplification using a capture to select regions of interest by

1. Blocking the sequencing adaptors
2. Binding capture probes to the target regions
3. Incubate with streptavidin beads
4. Use magnet to isolate target
5. Elute off beads or save supernatant if doing a reverse capture

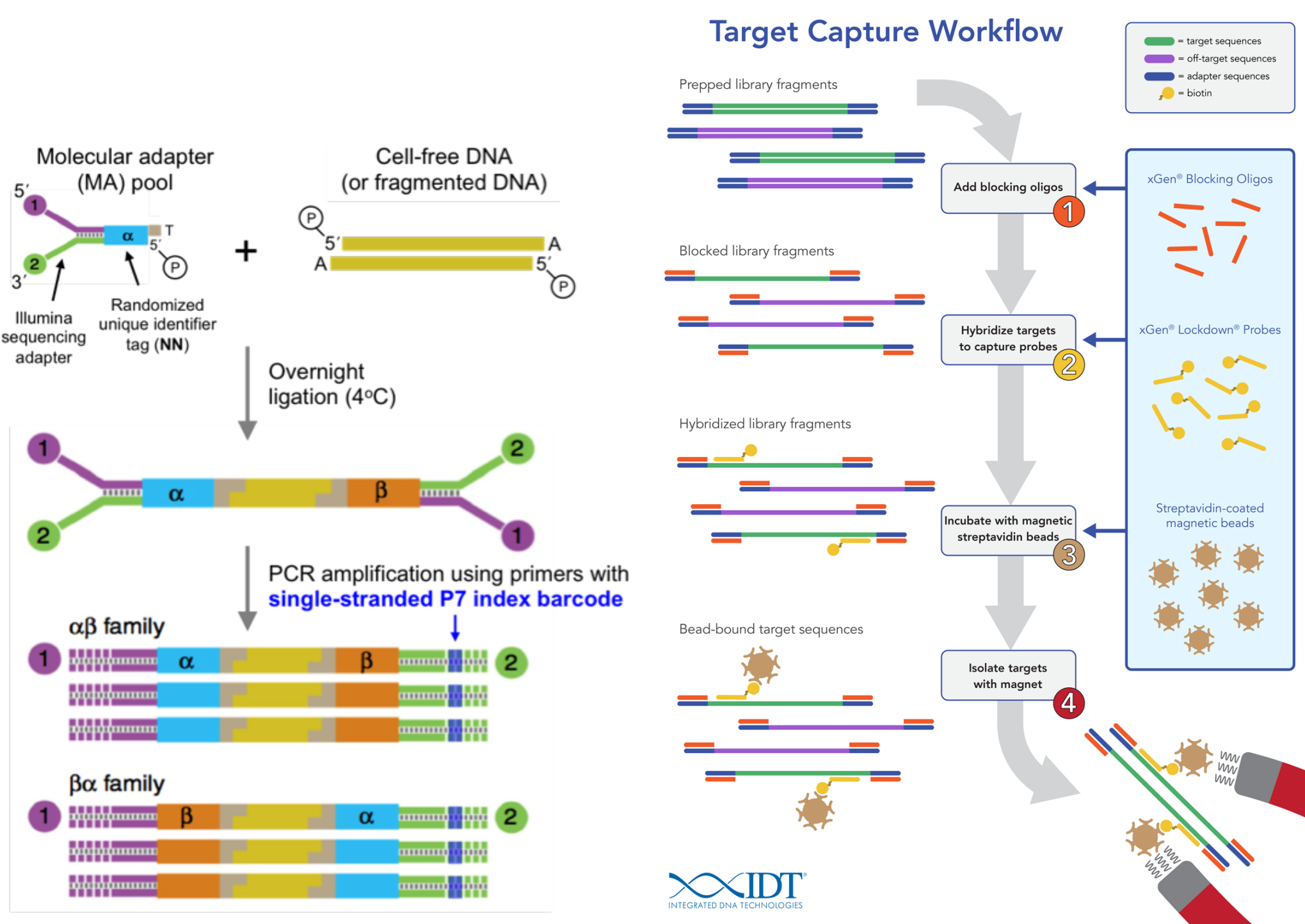


Figure 3: Library Construction Workflow

Figure 4: Target Capture Workflow

OBJECTIVES

- Validate the QIAcube DNA/RNA extraction protocol
- Create a custom 3 step capture protocol for the Hamilton Star
- Validate the Hamilton Star Kapa HyperPrep protocol

MATERIALS & METHODS

- The QIAcube DNA/RNA extraction protocol was run according to the manufacturers instructions on 51 samples as was 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

QIACUBE RESULTS

The QIAcube successfully extracted DNA/RNA from 51 samples with yields of 5543.13 ± 2248.41 ng 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 ± 2099.30 ng for RNA and 7693.23 ± 3406.96 ng for DNA.

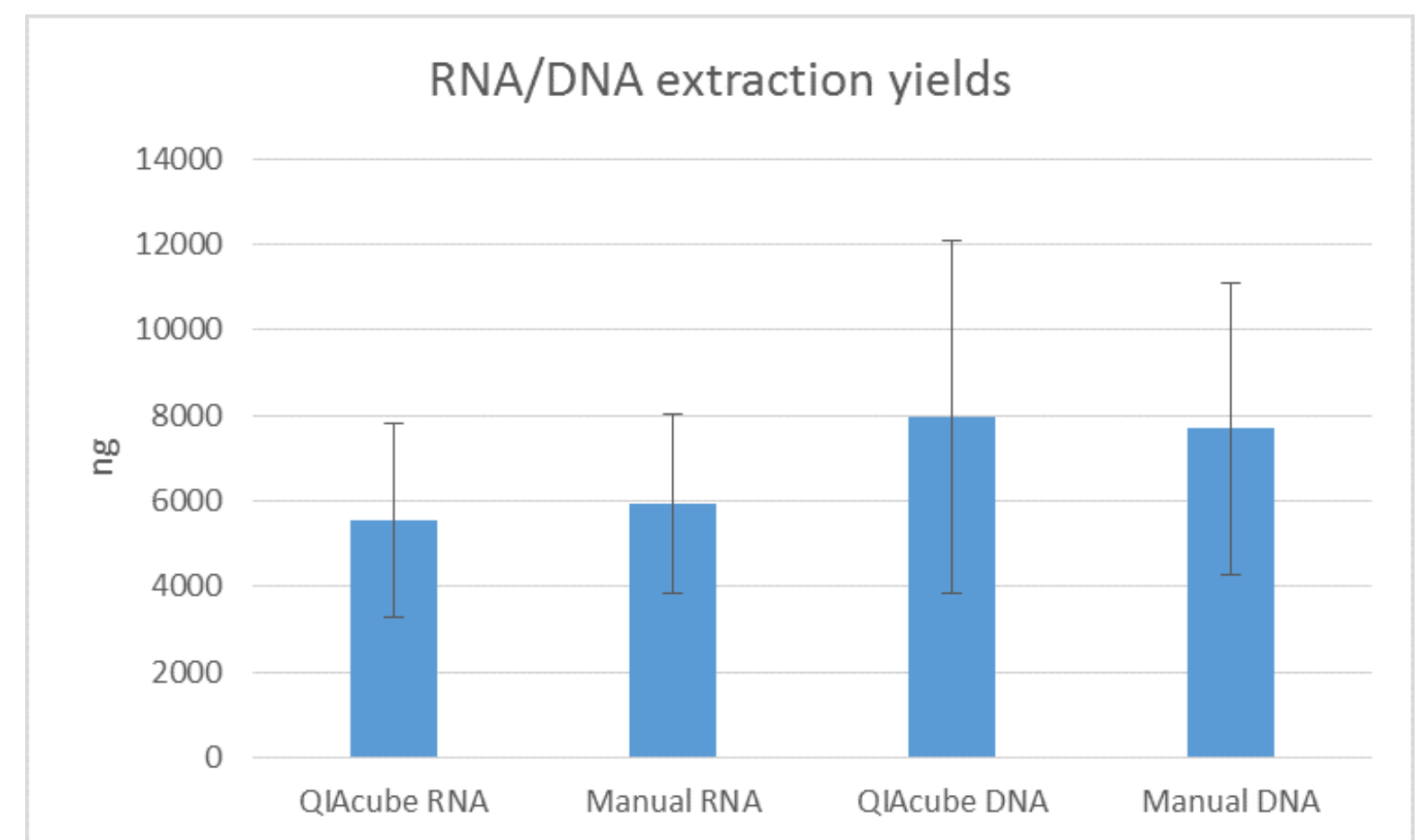


Figure 5: QIAcube vs Manual Yields

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

CUSTOM CAPTURE PROTOCOL RESULTS

It took us several weeks to become familiar with the Hamilton Star control software. During this time we wrote several libraries to add features lacking in the control software. For example being able to dynamically choose the best tips for a given volume.

We then spent time coding the 3 step capture and running water tests on it.

Ultimately it did not work very well and we moved on to testing the Kapa library prep protocol.

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 though.

An example problem was that the plate shaking speed was too fast during one step and some of the samples jumped out and potentially cross mixed. The robot also ran out of PCR primer (even with 20% excess) and failed to aspirate any of the additional PCR primer

we added.

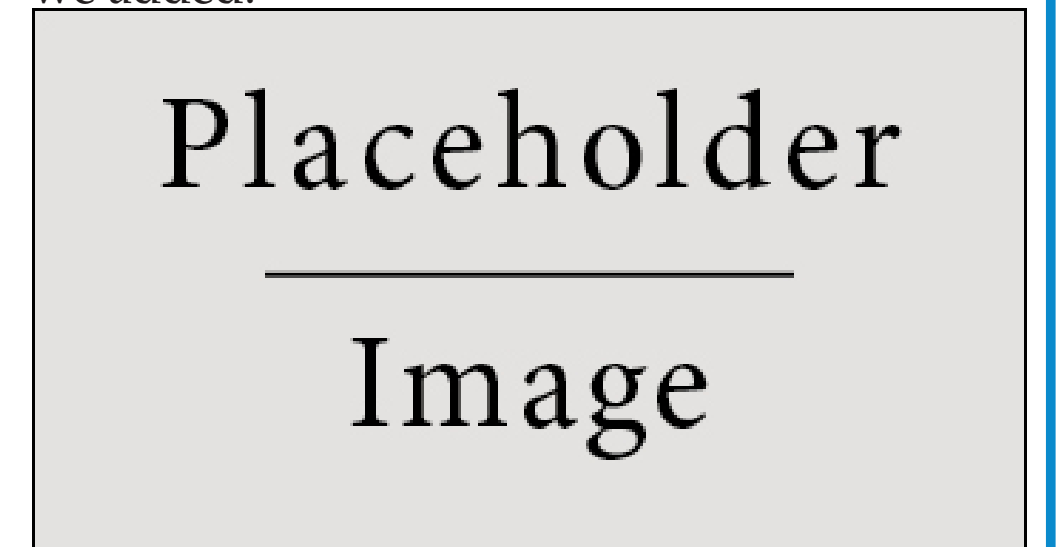


Figure 6: DNA Trace

CONCLUSION

- The QIAcube can increase productivity but can not be used for all samples indiscriminately
- The Hamilton Star is a powerful liquid handling robotics system with very capable hardware but the programming interface is a bottle neck to creating protocols
- The Kapa protocol on the Hamilton Star works overall but a few things need to be fixed 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

REFERENCES

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