Automation of Qiagen's Miniprep Plasmid Purification Process

Natalie Barker
AstraZeneca

Overview

- **Purpose:** Produce a liquid handling enabled method for high-throughput plasmid purification. Requirements included:
  - Consistently generating high-yield and high-quality DNA.
  - Reducing FTE hands-on-time.
  - Maximising sample throughput.
- **Method:** We developed an automated Qiagen miniprep plasmid purification method that features:
  - A Hamilton STAR with an integrated positive pressure manifold [MPE]^2.
  - Custom 3D printed holders for ensuring reliable plate placements with a robotic arm (iSwap).
  - Custom [MPE]^2 plate adapter enabling use of Qiagen labware.
- **Results:** Overall, this method generates both a good yield and high-quality DNA. A total of 4 plates are processed in 1hr 52mins.
  - Mean DNA Yield = 20.3 µg.
  - Mean A260/280 = 1.87 µg.
  - Mean A260/230 = 2.25 µg.

Introduction

- AstraZeneca's (Biologics Engineering) antibody production pipeline relies heavily upon the use of manual filtration steps for DNA preparation.
- Previous automated methods have been rigorously tested and rejected as viable alternatives to manual processes:
  - **Automated vacuum filtration failed** to consistently process samples, due to a single vacuum applied across the whole plate. The vacuum also caused significant cross contamination between samples.
  - **Automated magnetic bead methods proved unreliable** and failed to produce high-quality and high-yield DNA.
- To solve the above issues, attention was turned to optimising a new manual process using a positive pressure manifold [MPE]^2.
- The [MPE]^2 applies positive pressure to each well of a 96w plate, eliminating inconsistent sample processing seen via vacuum filtration and preventing cross contamination. As a result, high-quality/yield DNA was produced for each sample during validation.
- The aim of this work was to **produce a liquid handling enabled method for high-throughput plasmid purification** by adapting the manual [MPE]^2 process.
- The method requirements included, consistently generating high-yield and high-quality DNA, reducing FTE hands-on-time and maximising sample throughput.

Methods

- The Hamilton STAR method (Figure 1) was adapted from a protocol supplied by Qiagen with the QIAprep ® 96 *Plus* Miniprep Kit (Cat. No. 27291)[1].

Figure 1: Process Map of the Automated Qiagen Miniprep Plasmid Purification Process. Green = Automated Step, Red = Manual Step.

- During method development we encountered numerous challenges including:
  - **Lack of deck space** for required throughput.
  - **Inconsistent plate placements** due to millimetre-accuracy required by the [MPE]^2.
  - **[MPE]^2 Incompatible labware** provided by the QIAprep ® 96 *Plus* Miniprep Kit.
  - **Residual PE wash buffer** droplets which reduced the DNA quality.
- To maximise throughput (4x 96w sample plates) we **3D printed a reagent trough holder** (Figure 3a) which allowed us to use an empty NTR carrier position (Track 39, position 5) (Figure 2).
- To enable consistent placement of both the PlasmidPlus® and TurboFilter® plates, we **3D printed plate holders** (Figure 3b, 3c). This prevented on-deck plate movement, facilitating reliable plate placement for the [MPE]^2 system.
- To enable the use of the labware provided by the QIAprep ® 96 *Plus* Miniprep Kit, a new plate adapter was milled with alterations made to the internal width (Figure 4).
- Multiple attempts were made to eliminate the residual PE wash buffer droplets containing ethanol:
  - Testing various pressure and flow parameters to optimise reagent processing within the [MPE]^2.
  - Using firmware commands to 'tap' the PlasmidPlus® plate onto absorbent tissue in an off-deck position to force excess liquid out.
  - A manual centrifugation step was finally introduced to resolve the issue.

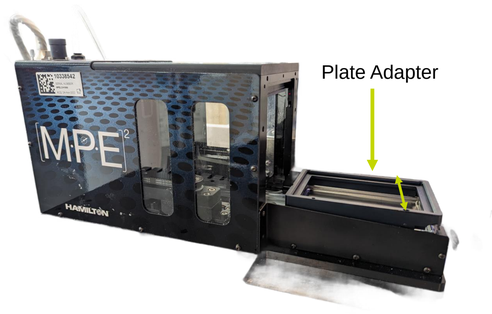
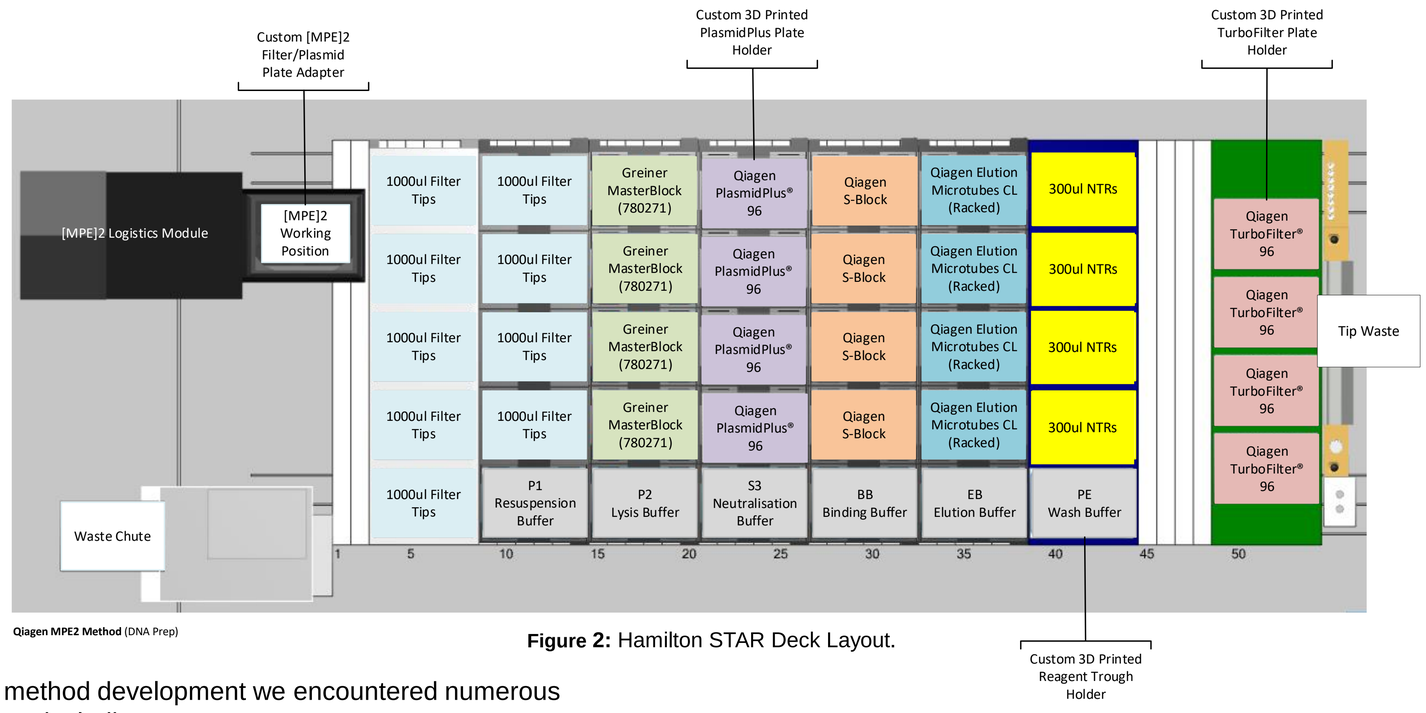


Figure 4: Hamilton [MPE]^2 System [2]. The double-sided arrow indicates the width of the plate adapter which required alteration.



Qiagen MPE2 Method (DNA Prep)

Figure 2: Hamilton STAR Deck Layout.

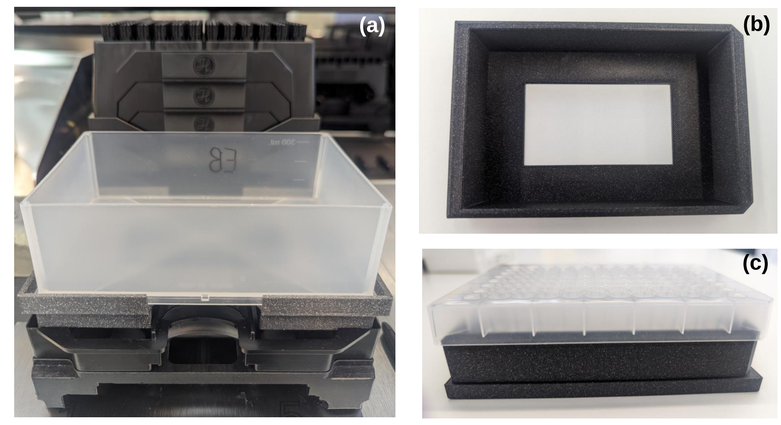


Figure 3: (a) 3D Printed Reagent Trough Holder Attaches to an Empty NTR Carrier Space, (b) 3D Printed Plate Holder (Top View), (c) 3D Printed Plate Holder (Side View).

Results

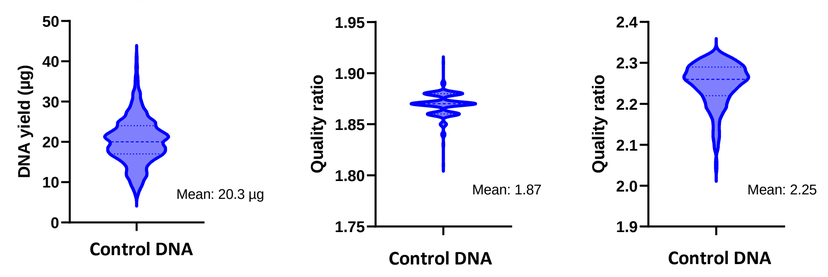


Figure 5: Automated Method Results n = 383, (a) DNA Yield, (b) A260/A280 Ratio, (c) A260/A230 Ratio.

- 4x 96w plates are processed from a centrifuged pellet to eluted DNA in 1hr 52mins. When carried out manually, this process takes two FTEs constant hands-on time of 2.5hrs. This results in an almost three-fold time saving.
- The method generates good yield (Figure 5a) which is above the minimum 6 µg required for a repeat. As well as high-quality DNA (Figure 5b, 5c) which falls within the required A260/280 and A260/230 parameters.

Conclusions

- We have successfully validated a method which utilises a Hamilton STAR with an integrated positive pressure manifold system [MPE]^2.
- Overall, this method generates both a good yield (Mean: 20.3 µg) and high-quality DNA (Mean A260/280: 1.87 µg, Mean A260/230: 2.25 µg).
- Custom 3D printed plate holders facilitated reliable plate placements with a robotic arm (iSwap).
- A new design for the plate adapter enabled the use of Qiagen labware with the [MPE]^2.
- Droplet removal could only be resolved using manual centrifugation; however, automated centrifugation and therefore full method automation will be introduced through the installation of an integrated BioSero platform (Q1 2025).
- We are currently investigating tip-based purifications as an alternative to using positive pressure manifolds.

References

1.Quick-START Protocol QIAprep ® 96 *Plus* Miniprep Kit 2019, QIAGEN, https://www.qiagen.com/us/resources.

Acknowledgements

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