

# Optimized Magnetic Bead-Based DNA Clean Up with Low Elution Volumes on the Hamilton Microlab® STAR™

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Omega Bioservices supports its full suite of laboratory services by establishing customized automated workflows on open platform liquid handlers, such as the Hamilton Microlab® STAR™. One of the newest services provided by Omega Bioservices is high throughput gut microbiome population identification from stool samples through 16S rRNA gene sequencing. Creating this high throughput automated workflow presented many challenges, but one of paramount importance was reliably cleaning up and concentrating PCR products in low elution volumes.

Optimization of the Mag-BIND® RXNPure Plus chemistry has yielded sequencable product in elution volumes as low as 15 µL. However, current automation methodology relies on standard magnetic separation products designed for reactions with a minimum elution volume 2X greater at 30 µL. This limitation is due to the way PCR labware nests in the ring magnets, resulting in the formation of a bead ring that is significantly elevated from the bottom of the well. Attempted elution at volumes lower than 30 µL resulted in non-uniform mixing of elution buffer, producing inconsistent product recovery and in some instances, nearly complete loss of elution product (Figure 1).

## PCR Recovery Using Standard Magnetic Plates

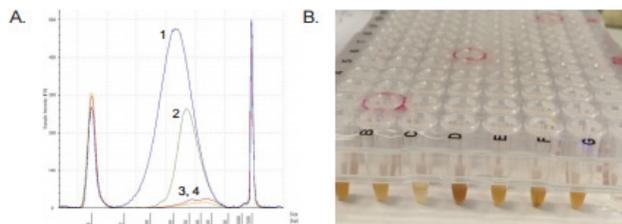


Figure 1. Inconsistent product recovery using standard magnet plate. a) Agilent TapeStation® 2200 chromatogram data peaks from sheared control sample (1) and purified products with proper mixing (2) and purified products with improper mixing (3 & 4). b) Wells visually observed in which magnetic beads did not resuspend into solution after mixing on the Hamilton STAR™.

In order to increase the reliability and reproducibility of our high throughput workflow, the Alpaqua Magnum FLX® Enhanced Universal Magnet Plate was inserted in place of our standard magnetic separator. The Magnum FLX addresses our primary problem as it is designed for elution volumes as low as 10 µL while simultaneously significantly decreasing separation time and allowing for an increased variation in acceptance labware. In order to accommodate the larger dimensions of the Magnum FLX, our Hamilton STAR™ was fitted with the SBS deep well carrier adapter, allowing the spring cushion technology of the Alpaqua plate to freely function and with adequate clearance for the pipette tips above labware.

## Improved Quality Using the Alpaqua Magnum FLX

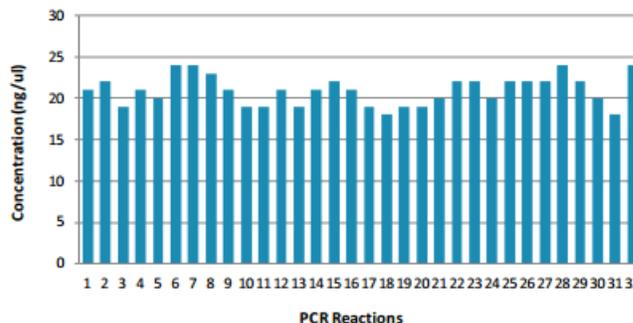


Figure 2. Highly consistent recovery using the Alpaqua Magnum FLX Magnet Plate. Measured concentration (ng/µL) of 32 cleaned up and recovered PCR products. Average total DNA per sample clean up = 362.6 ng. Average total DNA per sample post clean up = 313.5 ng. Average total DNA recovery = 86.5%.

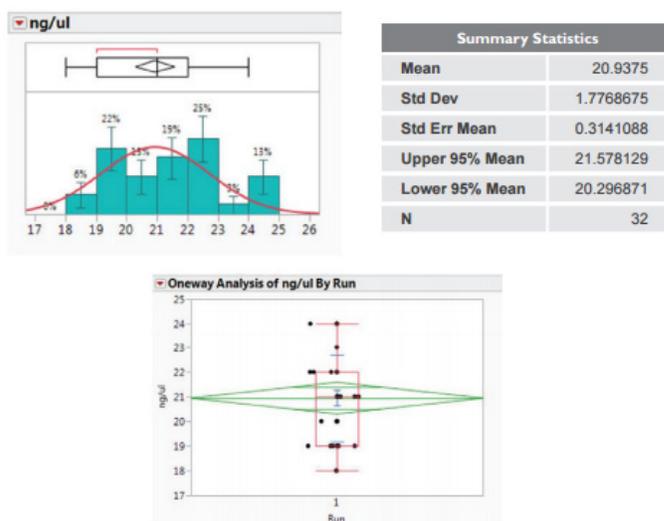


Figure 3. Statistical analysis of clean up quantification.

Efficiency of the Alpaqua Magnum FLX was tested in our PCR clean up workflow with 32 replicates on a 25 µL PCR reaction in a 96-well PCR plate. The amplicon product was cleaned up using our standard automated Mag-BIND® RXNPure Plus protocol adding 1.8 volumes of paramagnetic beads (45 µL) for initial binding. Two consecutive washes of 70% ethanol were following by elution in 15 µL or 10 mM Tris buffer. Following elution, sample concentrations were measured using Promega's Quantus™ system and DNA purity was measured using the NanoDrop™ 2000.

Table 1. Spectroscopic measurement of sample purity.

Sample Purity			
A260	A280	260/280	260/230
0.66 ± 0.03	0.33 ± 0.02	1.96 ± 0.04	2.57 ± 0.11

## Results

Complete recovery was seen across all 32 samples with exceptional consistency and sufficient ng/μL concentrations to proceed to sequencing (Figure 2). Measured concentration was on average 20.93 ng/μL with a standard deviation of 1.77 ng/μL (Figure 3).

Additionally, excellent sample purity results (Table 1) were achieved. Furthermore, the Alpaqua Magnum FLX plate was able to cut the PCR clean up magnetization time by 33%; from 12 minutes down to 8 minutes.

The testing demonstrated that method optimization needs to be extended to the technology of critical accessory components to achieve the most efficient and effective operations. The Alpaqua Magnum FLX plate and Omega Bio-tek’s Mag-BIND® RXNPure Plus provide a high throughput-capable option for laboratories desiring to automate their NGS workflows in which low elution volume DNA clean ups are required.

## Product Information

Company	Description	Product No.	Preps
Omega Bio-tek	Mag-BIND® RXNPure Plus	M1386-01	50 mL
		M1386-02	500 mL
Omega Bioservices	16S sequencing on Illumina MiSeq®	OBS-16S-100	-
Alpaqua Engineering	Magnum FLX® <i>Enhanced</i> magnet plate	A000400	96-well



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