Application Note

Omega Bioservices
Your lab partner in research

OMEGA bio-tek

ALPAQUA
Accelerating Genomic Discovery™

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

Michael Farrell[1], Henry She[1], Travis Butts[1], Jeffrey Roeder[1], and Keith McKenna[2].
[1]Omega Bio-tek, Norcross, GA, [2]Alpaqua Engineering, Beverly, MA

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 15µl. However, current automation methodology relies on standard magnetic separation products designed for reactions with a minimum elution volume 2X greater at 30µl. This limitation is due to the way the 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 30µl 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 Magnet Plates

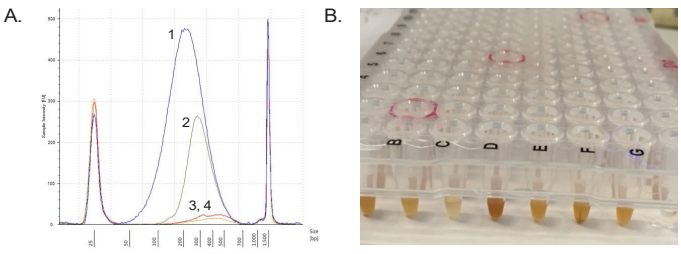


Figure 1: Inconsistent Product Recovery using Standard Magnet Plate. ***A.*** *Agilent TapeStation 2200 chromatogram data peaks from sheared control sample (1) and purified products with proper mixing(2) and purified products with improper mixing (3&4).* ***B.*** *Wells visually observed in which magnetic beads did not resuspend into solution after mixing on the Hamilton Microlab 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 was 10µl while simultaneously

Greatly Improved Consistency & Recovery using the Alpaqua Magnum FLX

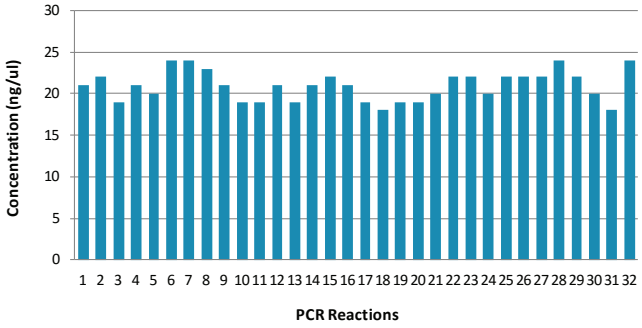
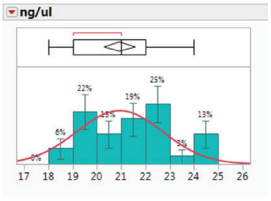


Figure 2: Highly Consistent Recovery using the Alpaqua Magnum FLX Magent Plate. *Measured concentration (ng/µL) of 32 cleaned-up and recovered PCR product. Average total DNA per sample clean-up = 362.6 ng. Average total DNA per sample post clean-up = 313.5 ng. Average total DNA recovery = 86.5%.*

Improved Quality using the Alpaqua Magnum FLX



Summary Statistics	
Mean	20.9375
Std Dev	1.7768675
Std Err Mean	0.3141088
Upper 95% Mean	21.578129
Lower 95% Mean	20.296871
N	32

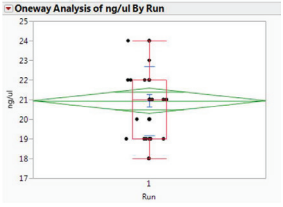


Figure 3: Statistical Analysis of Clean-up Quantification.

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

Table 1: Spectroscopic Measurement of Sample Purifty.

significantly decreasing separation time and allowing for an increased variation in acceptable labware. In order to accommodate the larger dimensions of the Magnum FLX our Hamilton Microlab 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.

Efficiency of the Alpaqua Magnum FLX was tested in our PCR clean-up workflow with 32 replicates of a 25µl 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 (45µl) for initial binding. Two consecutive washes of 70% ethanol were followed by elution in 15µl of 10mM Tris buffer. Following elution, sample concentrations were measured using Promega's Quantus™ system and DNA purity was measured using the NanoDrop™ 2000.

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 20.93ng/µl with a standard deviation of 1.77ng/µl (Figure 3).

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

This 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 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.

Ordering Information

Company	Product Number	Description
Omega Bio-tek	M1386-01	Mag-Bind® RxnPure Plus (50 mL)
Omega Bio-tek	M1386-02	Mag-Bind® RxnPure Plus (500 mL)
Alpaqua	A000400	Magnum FLX® *Enhanced* Universal Magnet Plate (96-well)
Omega Bioservices	OBS-16s-100	16s Sequencing on Illumina® MiSeq®

Omega Bioservices
Your lab partner in research

www.omegabioservices.com
info@omegabioservices.com

OMEGA bio-tek

www.omegabiotek.com
info@omegabiotek.com

ALPAQUA®
Accelerating Genomic Discovery™

Tel: (800) 690-1620
Outside US: +1 (978) 878-9489
Fax: (978) 865-9499
www.alpaqua.com
info@alpaqua.com