Automated, High Throughput Sample Purification and Cleanup for Low Elution Volume and High Viscosity Sample Applications using Alpaqua's Specialized LE and Magnum Magnet Plates.

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ALPAQUA® Liquid Handling Products

Abstract

Newer genomic applications such as exome capture, library construction and next generation sequencing, continue to rely on magnetic bead-based reagents due to their high throughput nature and amenability to liquid handling automation. Additionally, evolution of existing bead-based protocols, such as 'with-bead' SPRI purification, seek to further reduce reaction volumes and sample handling. New application development and protocol refinement are frequently met with technical challenges that must be overcome through the design &/or optimization of specialized equipment and consumables. Alpaqua offers a line of magnet plates including 24-well, 96-well and 384-well formats that are designed and optimized for a variety of genomic application needs. In this poster, we demonstrate the use of two Alpaqua 96-well magnet plates, the LE Magnet Plate for low elution volume needs, and the Magnum Plate for increased speed of bead sequestration or for use with higher viscosity samples. Our data show that the unique architecture of the Alpaqua LE Magnet Plate allows for high yield elution of samples in as little as 8-10µls. Use of the LE Magnet Plate produces highly concentrated samples without sacrificing nucleic acid recovery. Additionally, we show that the Alpaqua Magnum Plate can sequester magnetic beads up to 40% faster than standard 96-well magnet plates as well as demonstrate its compatibility with higher viscosity samples.

Materials & Methods

- AMPure® XP PCR Purification System (Beckman Coulter P/N: A63880)
- NoLimits™ 1000 bp DNA Fragment (Fermentas P/N: SM1671)
- 96-Well Non-Skirted PCR Microplates (Axygen P/N: PCR-96-C).
- Biomek® FXP Laboratory Automation Workstation (Beckman Coulter PN: A31844)
- Qubit® dsDNA HS Assay Kit (Life Technologies P/N: Q32851)
- Qubit® 2.0 Fluorometer (Life Technologies P/N: Q32866)

96S Super Magnet Plate (Alpaqua P/N: A001322)

96M Magnum™ Plate (Alpaqua P/N: A000250)

LE Magnet Plate (Alpaqua P/N: A000350)

DNA Purification:
A DNA solution containing 144ng of a 1,000bp fragment in 20µL of TE was added to 18 wells of a PCR plate and purified using AMPure XP. Purifications were conducted on the Biomek FXP Laboratory Automation Workstation. Briefly, the protocol was based on AMPure XP PCR Purification Protocol 000387v001 and is as follows:

1. 36µL of AMPure XP (1.8x) was added to each well and mixed.
2. After incubation with the beads, the PCR plate was moved to the Alpaqua 96S or LE Magnet Plate. The PCR plate remained on magnet plates for 5 minutes to allow bead settling.
3. Supernatant was removed and discarded.
4. Beads were washed 4 times with 80µL of fresh 70% ethanol.
5. PCR plates were removed from the magnet plates and TE added to beads for DNA elution (40µl for 96S plates, 8µl for LE plates).
6. PCR plates were placed back on the magnet plates and incubated for 5 minutes.
7. Eluates were transferred to a new 96-well PCR plate.

Determination of DNA Concentration Factor:
DNA concentration was determined for each purified DNA sample using the Qubit dsDNA HS Assay Kit and the Qubit 2.0 Fluorometer following standard protocols. Total DNA was calculated. DNA concentration factor was determined using the ratio of the purified DNA concentration to the starting DNA concentration.

Time Lapse Photography:
AMPure beads were diluted 1.8x with TE at the noted volumes and added to microplate wells and plates placed on 96S, 96M or LE Magnet Plates. Images were taken at approximately 1 minute intervals. Date and time stamp is noted in the lower right corner of each image. For images of 'high viscosity' samples, undiluted AMPure beads were used.

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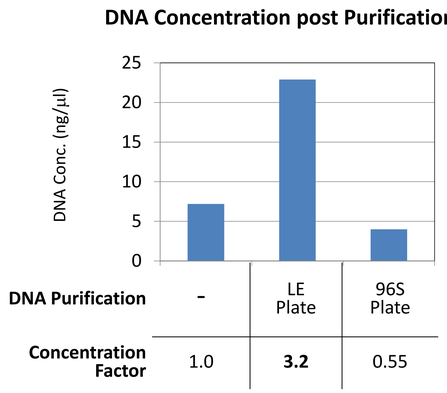
Low Volume Elution Produces Highly Concentrated DNA

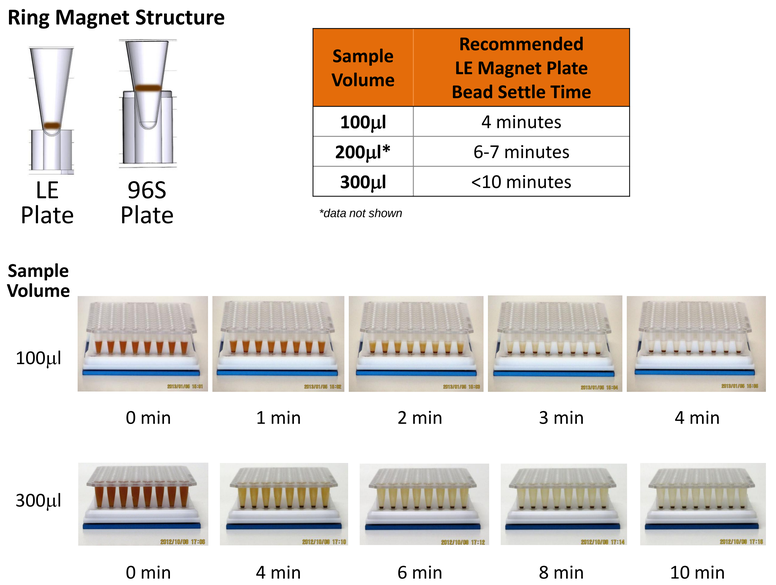
Elute Samples in as Little as 8µl



	LE Magnet Plate	96S Magnet Plate
Starting [DNA]	7.2 ng/µl	7.2 ng/µl
Starting Volume	20µl	20µl
Elution Volume	8µl	40µl
Final [DNA]	22.9 ng/µl	4.0 ng/µl
Std Dev.	±3.1 ng/µl	±0.4 ng/µl
Conc. Factor	**3.2**	**0.55**

Figure 1/Table 1. Low volume elution produces high concentration DNA without sacrificing yield. Side-by-side DNA purifications were conducted (*see Materials and Methods*) using either the 96S or LE Magnet Plate. DNA concentrations were evaluated of samples before and after purification using both magnet plates. Both plates purified 100% of the starting DNA indicating that DNA yield was not affected by low elution volume plate architecture. The LE Magnet Plate samples were over 3 times more concentrated than the starting sample, while the 96S Magnet Plate samples were less concentrated (0.55) than starting sample. The average DNA concentration for LE purified DNA was over 5.5 times more concentrated than DNA purified using the 96S magnet plate.

Magnetic Bead Settle Times Dependent on Sample Volume



Sample Volume	Recommended LE Magnet Plate Bead Settle Time
100µl	4 minutes
200µl*	6-7 minutes
300µl	<10 minutes

**data not shown*

Figure 2. Bead Settle Times for Initial Sample Separation are Unaffected by LE Magnet Plate Architecture. The Alpaqua LE Magnet Plate has a unique architecture that sequesters magnet beads lower in the PCR well bottoms than the 96S Magnet Plate while maintaining the sequestration of beads in a ring at the well perimeter. This enables sample elution in volumes as little as 8µl. Time lapse photography was used to examine the effects of the unique architecture of LE Magnet Plates on bead settle times. AMPure beads diluted with TE (1.8/1.0 ratio) and 100, 200, or 300µl dispensed per well in a 96-well PCR plate. PCR plates were placed on the LE Magnet Plate (time 0) and images taken over time. As expected, bead settle times are affected by sample volume. The recommended settle times vs. sample volume can be found in the table. LE settle times do not significantly differ from 96S settle times (*data not shown*).

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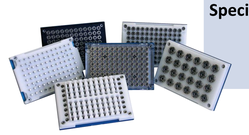

96M Magnum Plate: Reduce Bead Settle Times by Up to 40%

Ideal for High Viscosity Samples

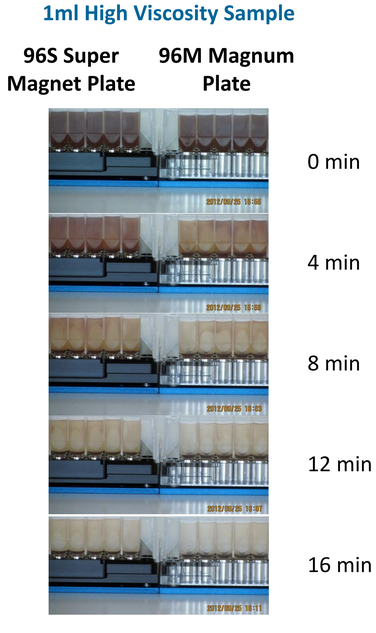

Figure 3. Faster Bead Sequestration for High Viscosity Samples using the 96M Magnum Plate. Side-by-side bead separations were conducted using 96S Super Magnet and 96M Magnum Plates. 1 ml of undiluted AMPure beads were used to represent high viscosity samples. Time lapse photography is shown from 0 to 16 minutes at 4 minute intervals. Samples were followed for a total of 1 hour (*data not shown*) to determine the time of maximal bead settle. Bead settle times for larger volume, viscous samples were 40% faster on the 96M Magnum Plate than the 96S Plate.

Summary

- The Alpaqua LE (low elution) Magnet Plate has a unique ring structure that enables sample elution in 8µl.
- DNA purification using the LE Magnet Plate produces DNA over 5x more concentrated than DNA purified using the 96S Super Magnet Plate.
- The LE Magnet Plate retained high DNA yield when compared to the 96S plate.
- The Alpaqua 96M Magnum Plate sequesters magnetic beads up to 40% faster than the 96S plate. Reduction in settle times are dependent on the sample viscosity, with higher viscosity samples showing the greatest reduction in settle time.
- Both the LE and Magnum Magnet Plates have ring magnets and integrated spring cushion technology that maximizes sample recovery while minimizing tip occlusions.

	MagPlate 24	96S	Magnum	LE	384
Format	24	96	96	96	384
Magnet Type & Strength	Ring – N48	Ring – N48	Ring – N50	Ring – N48	Post – N38
Sample volume	200µl – 10ml	40µl – 2ml	40µl – 2ml	15µl – 300µl	20µl - 39µl
Minimum Elution volume	100µl	30µl	30µl	8µl	15µl
Specialty Use	Large volume	General needs	Rapid separation, high viscosity samples	Low elution for high [DNA]	Highest throughput