Automation of Small Volume PCR Purification

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Abstract

The expansion of small-scale laboratory processes to industrial-scale environments has necessitated the development of new instrument and product technologies. Aspects of importance include small volume sample recovery, compatibility with laboratory automation and the means to deliver a high throughput process. The polymerase chain reaction (PCR) is a key element of many current and emerging genomics applications, including sequencing, microarrays and genotyping. Each of these technologies is dependent upon rapid and robust post-PCR clean-up methods that effectively remove salts, primers and dNTPs. Building upon Millipore's size-exclusion based purification technology, the Montage™ $PCR_{\mu 96}$ plate has been developed specifically to meet the demands of modern PCR-based applications. The tapered well design of the Montage $PCR_{\mu 96}$ plate enables 20 µl recovery volumes for a wide range of input reaction sizes. The Montage $PCR_{\mu 96}$ plate rapidly delivers the high quality PCR products required for the most demanding of downstream applications while maintaining high DNA recovery. Full automation of the PCR clean-up is demonstrated using a Tecan Genesis® Te-MO™ Multi-Channel Pipetting Option, a stand-alone 96 channel pipettor.

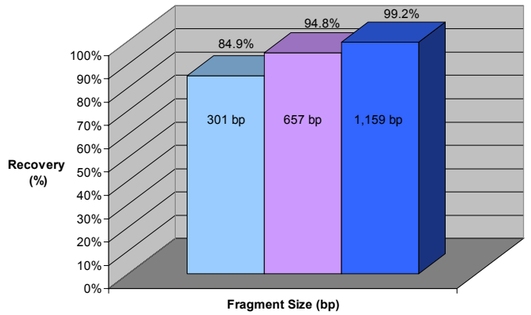
Materials and Method

- **20 µL aliquots of the PCR samples were diluted with 80 µL TE buffer**
- **100 µL from each well were then transferred to the Montage $PCR_{\mu 96}$ plate on the vacuum manifold**
- **Vacuum was applied at 20" Hg until the wells were dry (7 minutes)**
- **Samples were then resuspended in 20 µL TE buffer via incubation for 5 minutes and subsequent mixing of 15 µL for 75 cycles**
- **Purified samples were then transferred to a V-bottom collection plate**

***This protocol was carried out using two vacuum manifolds on the Te-MO deck.**

**** In all cases, some volume is lost to the membrane during resuspension and transfer to the collection plate (approximately 1 - 2 µL).**

PCR Product Recovery by SYBR® Quantitation and Primer Removal

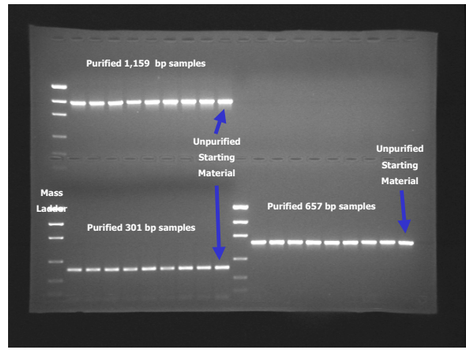


	Starting Concentrations		
	301 bp	**657 bp**	**1159 bp**
	8.6 ng/µL	25.5 ng/µL	21.7 ng/µL
	Percent Recovery		
	301 bp	**657 bp**	**1159 bp**
Avg	84.9%	94.8%	99.2%
CV	8.7%	8.5%	6.7%
n	96	96	96

Primer Removal	**>99%**
n	**183**

Recovery data was calculated using a fluorometric assay with SYBR Green I nucleic acid gel stain (Molecular Probes, Inc). Primer removal was performed by measuring the retention of a fluoresceinated 20-mer in the purified PCR samples.

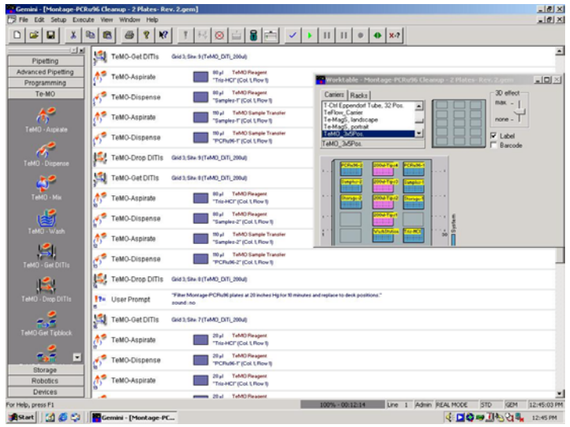
Agarose Gel Analysis of Purified PCR Samples



1.5% agarose gel analysis of PCR products after purification using Montage-$PCR_{\mu 96}$ on the Te-MO. 5 µL of samples loaded on gel.

The Montage $PCR_{\mu 96}$ Plate Designed for Automation

Montage $PCR_{\mu 96}$ Method in Gemini™ Version 4.0



Deck setup for PCR Cleanup on the Tecan Genesis Te-MO

Conclusions

- Fast processing time utilizing the Te-MO's 96-channel pipetting capabilities (under 20 minutes to purify two plates of 96 samples)
- Small volume recovery for sample concentration
- Efficient removal of PCR primers and salts
- Micro-well format compatible with sequencing, genotyping, and microarray production.

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