Montage® $PCR_{\mu 96}$ and SEQ_{96}: A Total Solution for Sequencing PCR-derived Templates

Joseph Hitti, Peter J. Rapiejko, Rosalind Parry, and Masaharu Mabuchi
Millipore Corporation, Life Sciences Division, Danvers, MA 01923, USA

ABSTRACT

The application of laboratory processes to industrial-scale environments has necessitated the development of technologies which promote the automated manipulation of small volumes and provide a cost-effective means of delivering high throughput data. As the post-genomic research era evolves towards an understanding of the relationship between genetic variation and disease, the development of new technologies and the adaptation of existing technologies continues apace. Large-scale population-based studies designed to correlate candidate single-nucleotide polymorphisms (SNPs) with specific disease states and/or drug treatment regimens can be readily approached via sequencing of PCR products derived from either genomic DNA or plasmid sources. This straightforward approach has several advantages over other technologies, including accessibility, flexibility and high accuracy. Sequencing of PCR-derived templates is therefore a cost-effective method for reliable high throughput genotyping analysis. Successful sequencing of PCR-amplified template DNA requires a rapid, robust and automatable PCR cleanup method capable of quantitatively removing primers, salts and dNTPs in high-throughput. Purification of PCR products using Montage $PCR_{\mu 96}$ plates achieves these goals. The unique micro-well design of the $PCR_{\mu 96}$ plate enables significant reaction miniaturization that, when used in concert with the Montage SEQ_{96} kit in a single process, results in significant savings in both reagents and processing time. The Montage SEQ_{96} kit virtually eliminates dye blobs and is compatible with the latest BigDye chemistries. We have developed a single protocol that accommodates PCR products of 300-700 bp and requires as little as 2-20 ng (10-100 fmol) of template DNA for efficient sequencing. This approach is applicable to all phases of SNP research, facilitating the transition from discovery to validation and ultimately to high-throughput genotyping. By providing a unique micro-well platform that enables both small volume PCR and sequencing reaction cleanup, Millipore's Montage line of plates and kits facilitates the cost-effect adoption of high throughput sequencing of PCR template DNA.

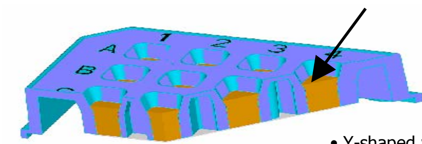
Platform Features

Montage $PCR_{\mu 96}$ Plates

Montage SEQ_{96} Kit

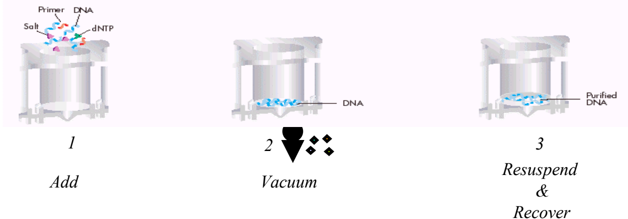
- **96-well PCR & SEQ purification plates with unique "micro-well" design**
- **Ideal for processing small volume samples**
- **One piece design meets current SBS guidelines**
- **Superior sequencing of PCR fragments**

Micro-well design

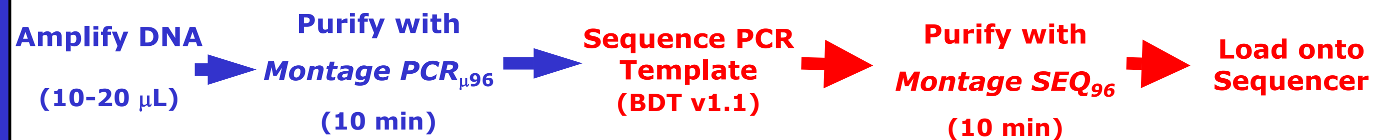


- Y-shaped well
- Load up to 150 µl
- Recover in as little as 20 µl

Easy 3-step clean-up process

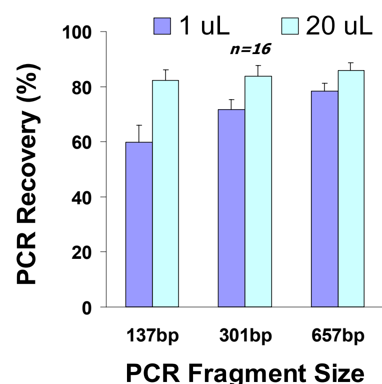


Schematic for Sequencing PCR Products



Montage $PCR_{\mu 96}$ - Robust Recovery of Small Fragments from as little as 1 µL of PCR

Excellent Recovery of Small Volume Samples



Highly Purified Samples (>99% Primer Removal)

Sample size	Primer Removal (*n*=24)
5uL	100 ± 0.8%
10uL	99.9 ± 1.6%
20uL	99.6 ± 3.0%

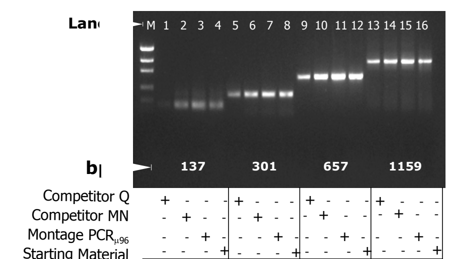
Consistent Product Performance for Small PCR Fragments

n=16	20 µL, 137bp PCR Reactions	
	PCR Recovery	Primer Removal
Plate #1	72 ± 6%	99 ± 1%
Plate #2	77 ± 5%	100 ± 1%
Plate #3	75 ± 5%	98 ± 1%

PCR Recovery & Primer Removal Assays

Pooled PCR products were diluted to 100 µL with TE buffer prior to filtration. Quantitation was by a SYBR® Green Assay (Molecular Probes). Primer removal was determined using a fluorescein-labeled oligonucleotide (20 bases).

Outperforms Competitive UF PCR Clean up Methods

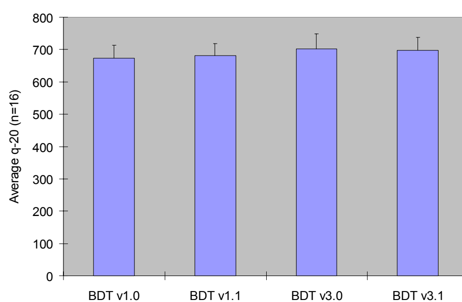


1.5% agarose gel analysis of PCR products after purification using Montage $PCR_{\mu 96}$ or devices from competitors Q & MN.

Montage SEQ_{96} - High Quality Sequence Data Enables Reaction Miniaturization

Montage SEQ_{96} is Compatible with Big Dye™ Chemistries

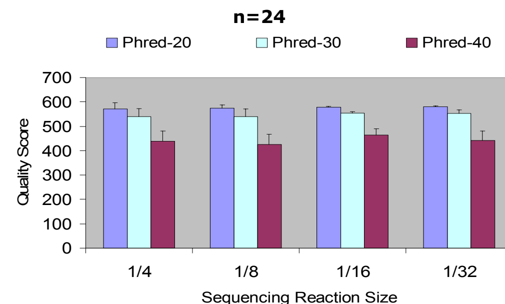
Consistently High Phred-20 (*n*=16)



SEQ_{96} clean up of 1/8 x BDT reactions (plasmid DNA) analyzed on an ABI-3700

Sequencing of a $PCR_{\mu 96}$-purified 657 bp PCR Product using BDT ver 1.1 on ABI-3700

Quality Maintained Over a Broad Range of Reaction Sizes n=24



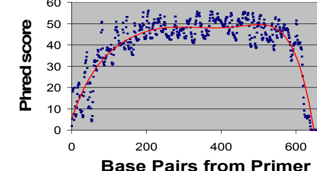
Well-to-well consistency with Sequence Reads starting at Primer plus-1

Reaction Size	Sequence Quality Analysis (*n*=24)			
	Average Phred-30	Pass Rate (Phred 20 > 300)	Start of Read (Distance from Primer)	% of Sequences Displaying Primer Plus 1
1/4	540 ± 33	100%	2.1	75%
1/8	540 ± 31	100%	1.2	96%
1/16	555 ± 4	100%	1.0	100%
1/32	552 ± 15	100%	1.8	79%

PCR reactions (10 µL) were purified using Montage $PCR_{\mu 96}$. Aliquots (2 µL, approx. 50 fmoles) of the PCR template were sequenced in 5 µL reactions and purified with Montage SEQ_{96}.

Electropherogram of 1/16 x BD Reaction (5 µL)

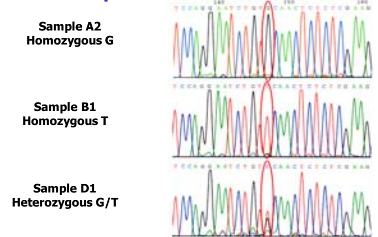
Mapping of q-scores over a sequencing read



Since q-40 scores begin after~150 bases, optimized PCR primers are needed for accurate SNP identification by sequencing.

The Integrated Platform is Ideal for SNP Genotyping

SNP Scoring of Human Triglyceride Lipase - Raw Data



Genotyping of Individuals using $PCR_{\mu 96}$ and SEQ_{96}

A 301 bp PCR product was amplified from 100 ng genomic DNA and sequenced (1/8x BDT) using Montage $PCR_{\mu 96}$ and SEQ_{96}. Genotypes of different individuals are readily distinguished by this approach.

Conclusions

- **Montage $PCR_{\mu 96}$ and Montage SEQ_{96} together provide an ideal platform for applications requiring sequencing of PCR templates.**
- **The microwell format enables reaction miniaturization of both PCR and Sequencing reactions, significantly reducing reagent consumption.**
- **High quality sequencing data within one base of the primer can be achieved using this platform and is suitable for the most demanding applications.**

MILLIPORE