

Increasing Throughput and Quality of Sequencing by Automation Of MultiScreen³⁸⁴™ Filter Technology on the Biomek FX.



ABSTRACT

As Genomic applications move towards the support of Drug Discovery technologies, the need for processing large numbers of samples in an automated fashion continues to be extremely important. Specifically, DNA sequencing technology is a very important tool for looking at the molecular basis of disease. In this poster we describe techniques which were successful in improving sequence quality and the automation of these processes.

The purity of a DNA template contributes significantly to the overall quality of a sequence. When sequencing from PCR products, we observe quantitative improvements in number of successful sequences and read length if we purify the PCR reactions on the MultiScreen³⁸⁴-PCR plate prior to its use as a template. This procedure is automated for use on a Biomek FX liquid handling station.

We compare the use of ethanol precipitation to Millipore's MultiScreen³⁸⁴-SEQ filter plate for sequencing reaction cleanup. Several improvements were seen when using Millipore's vacuum filtration based assay versus centrifugal based ethanol precipitation when analyzed on the ABI 3700 DNA sequencer. These improvements include throughput, sequence quality and read length.

Ease of automation is an attribute of this filtration-based assay. Samples are introduced, purified and recovered from the surface of the membrane using an automated liquid handler. All filtrate is directed to waste eliminating the need for manifold assembly/disassembly which is time consuming and difficult to automate. We have automated both MultiScreen³⁸⁴ assays on the Biomek FX using two manifolds simultaneously to meet our aggressive throughput needs. Two plates of 384 PCR or SEQ can be processed in approx 45 minutes without intervention.

David S. Wexler¹, Cristina Melero¹, and Marcy Engelstein²

¹AGY Therapeutics, Inc., South San Francisco, CA 94080

²Millipore Corporation, 17 Cherry Hill Drive, Danvers, MA 01923



Figure 1: Biomek FX workstation set-up to run a two plate purification using the MultiScreen³⁸⁴-SEQ filters.

RESULTS

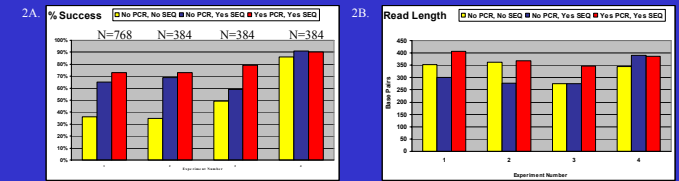


Figure 2: Comparison of sample preparation techniques for "Difficult" and "Easy" templates. Difficult sequencing templates are represented in experiments 1, 2 and 3. Experiment 4 represents easily sequenced clones. Figure 2A shows the percentage of successful sequences out of the total processed while figure 2B shows the average read length for the same set of sequences. In each experiment, the same clones were utilized across each condition, but individual experiments used unique clones. Experiment 1 was run in duplicate and the number of samples in each experiment is indicated on the graph. 'Yes PCR' indicates the use of MultiScreen³⁸⁴-PCR filter plates, 'Yes SEQ' indicates the use of MultiScreen³⁸⁴-SEQ filter plates and 'No SEQ' indicates use of ethanol precipitation to purify sequencing reactions. A successful sequence is defined as having a minimum 100 base pair region where no 6 contiguous bases have less than a Phred10 score. Read length is trimmed sequence (removal of vector sequence etc.) that has an overall average of at least Phred10.

MATERIALS AND METHODS

Biomek FX Protocol Outlines



Purify PCR products using the MultiScreen³⁸⁴-PCR filter plate

1. Pipette 30µl of H₂O into the filter plate.
2. Transfer 2x20µl of PCR reactions from replicate source plates to MultiScreen³⁸⁴-PCR plate and mix 5 times.
3. Move filter plate from the static ALP to the vacuum manifold.
4. Vacuum liquid to waste at 10" Hg for 15 minutes.
5. Pipette 20µl of H₂O into the filter plate.
6. Mix the H₂O in the MultiScreen plate to resuspend the samples (15µl, 30 cycles, 30% speed).
7. Transfer resuspended samples to a clean thermocycler plate for storage.



Purify Cycle Sequencing Reactions using the MultiScreen³⁸⁴-SEQ filter plate

See figure 1 for deck layout.

1. Add 15µl of 0.3mM EDTA into 10µl cycle sequencing reaction in thermocycler plate and mix.
2. Transfer all 25µl of EDTA/sequence reaction mix to MultiScreen³⁸⁴-SEQ filter plate.
3. Move filter plates from the static ALP to the vacuum manifold.
4. Vacuum liquid to waste at 24" Hg for 5 minutes.
5. Add 25µl of 0.3mM EDTA to the filter plate.
6. Vacuum liquid to waste at 24" Hg for 7 minutes.
7. Add 20µl of H₂O to the filter plate.
8. Mix the H₂O in the MultiScreen plate to resuspend the samples (15µl, 30 cycles, 15% speed).
9. Transfer resuspended samples to a clean thermocycler plate for loading onto the ABI 3700 DNA sequencer.

Test Conditions for Different Templates

1. No purification of PCR products. Sequence reactions purified by ethanol precipitation. (No PCR, No SEQ).
2. No purification of PCR products. Sequence reactions purified by MultiScreen³⁸⁴-SEQ filter plate. (No PCR, Yes SEQ).
3. Purification of PCR products by MultiScreen³⁸⁴-PCR filter plate. Sequence reactions purified by MultiScreen³⁸⁴-SEQ filter plate. (Yes PCR, Yes SEQ).

Sequencing Reaction Specifications

1/4 Reaction, Total Volume = 10µl

- 1 µl Template
- 1 µl Primer (conc.)
- 1 µl 5X buffer (ingred)
- 2 µl Big Dye V 2.0
- 5 µl H₂O

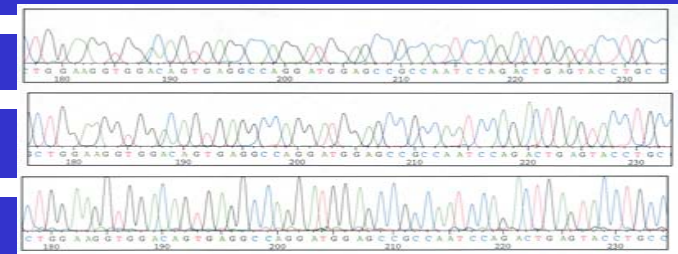
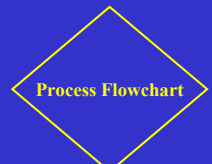


Figure 3: Examples of chromatograms from a single clone prepared by the three different methods described in this poster. (A) No PCR purification, sequence cleanup with ethanol precipitation; No PCR, No SEQ. (B) No PCR purification, sequence cleanup with MultiScreen³⁸⁴-SEQ; No PCR, Yes SEQ. (C) PCR cleanup with MultiScreen³⁸⁴-PCR and sequencing cleanup with MultiScreen³⁸⁴-SEQ; Yes PCR, Yes SEQ.

CONCLUSIONS

- Increased throughput by purifying 384 samples simultaneously. Reduction of both process time and process steps.
- Increased 'walk-away' time by automation on the Biomek FX.
- Filtration based assays allow for simple automation. No filtrate collection required, only top access to membrane necessary.
- Observed improvements in both sequence success and read length due to the high purity of samples recovered from combination of MultiScreen³⁸⁴ plates.