

INTEGRATING SEQUENCING REACTION PURIFICATION WITH THE ABI PRISM 3700 DNA SEQUENCER

Jack Leonard, Joe Gabriels, Constance MacDonald, and Masaharu Mabuchi

Millipore Corporation, Life Sciences Division, Danvers, MA USA

ABSTRACT

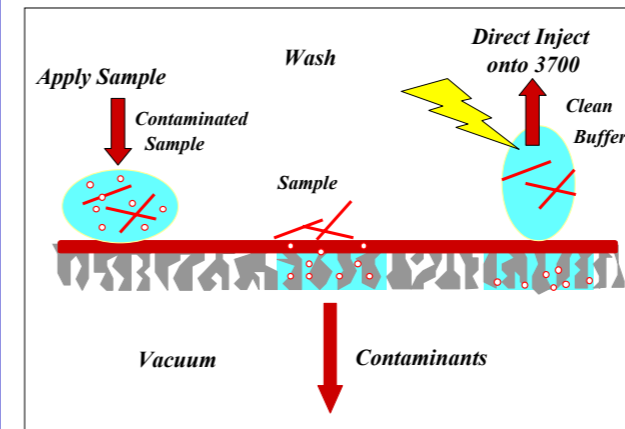
The ABI PRISM 3700 capillary sequencer, combined with the new BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems) provides the most high throughput, automated and robust solution for production DNA sequencing available. As a consequence, labor-intensive sample preparation methods upstream of sequencing can quickly become the most costly and rate-limiting steps as genomics facilities scale-up. One time-tested and reliable way to increase productivity is to integrate sample processing steps with analysis. The new Millipore Direct-Inject holder was designed to fit on the deck of the ABI 3700, to eliminate processing steps, and to fully realize the quality and throughput enhancements associated with the new sequencing chemistry. Montage SEQ384 plates (Millipore) were used to purify 1/8th scale BigDye v3.0 sequencing reactions on a Beckman Multimek or Biomek FX liquid handler. After filtration, a small volume of injection solution was dispensed to each well of the Montage SEQ384 plate, the plate was sealed with tape, and transferred to the Direct-Inject holder. The Direct-Inject holder was placed at one position on the ABI 3700 deck, and the purified sequencing products were automatically transferred from the Montage SEQ384 plate by the ABI 3700 onto the loading bar, and electrokinetically injected into the capillary array. Four Montage SEQ384 plates containing purified sequencing reactions can be loaded onto "Direct-Inject holders on the ABI 3700, and sixteen consecutive runs can be carried out without user intervention. Details of the new sample preparation process, and an analysis of the quality of the DNA sequencing results will be shown.

BigDye Terminator v3.0 Reaction Clean-up Using Montage SEQ₃₈₄

Methods

- The Montage SEQ₃₈₄ is a novel 384-well filter bottom plate using a proprietary membrane that retains DNA at the membrane surface while allowing contaminants to pass.
- Add 15 µl of Injection Solution to reactions in 384 thermal cycling plate
- Transfer the diluted reactions to Montage SEQ₃₈₄
- Vacuum to emptiness (4 minutes)
- Add 25 µl of Injection Solution
- Vacuum to emptiness (5 minutes)
- Add 20 µl of Injection Solution
- Direct-Inject onto 3700

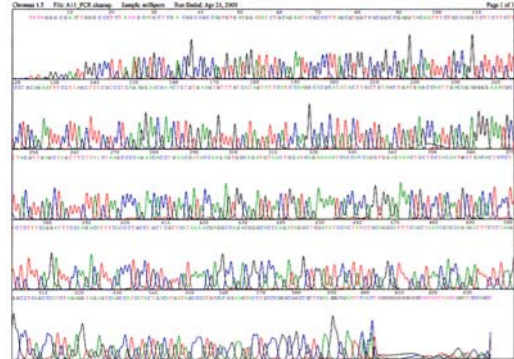
384-WELL CLEANUP AUTOMATED LIQUID HANDLING



MultiScreen₃₈₄-SEQ Performance

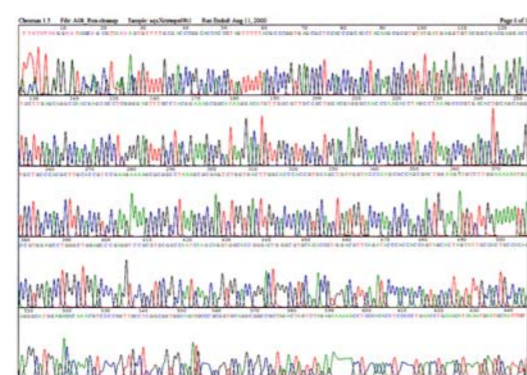
- ◆High quality sequencing reactions are prepared for the ABI 3700 and the MegaBACE 1000 as measured by PHRED analysis.
- ◆384, 5 or 10 µL, sequencing reactions are purified in 15-20 minutes.
- ◆Vacuum filtration eliminates the centrifuge.
- ◆The proprietary membrane allows high recovery of sequencing products and the elimination of labeled ddNTP's and salts.
- ◆Patents pending.

MultiScreen₃₈₄-PCR: Template Quality ABI 3700



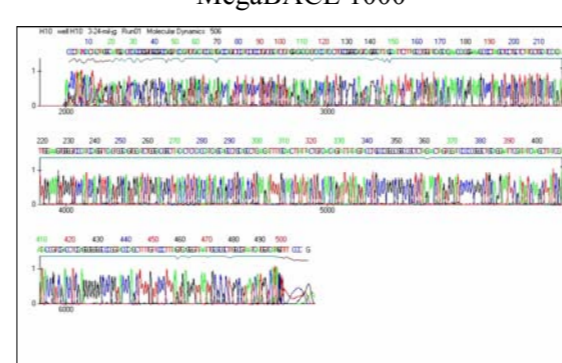
MultiScreen₃₈₄-PCR purified samples provide clean templates for BigDye terminator reactions run on the ABI 3700 after sequencing reaction cleanup on MultiScreen₃₈₄-SEQ. 88% of the first 400 bases are PHRED 20 or greater.

MultiScreen₃₈₄-SEQ: BigDye ABI 3700



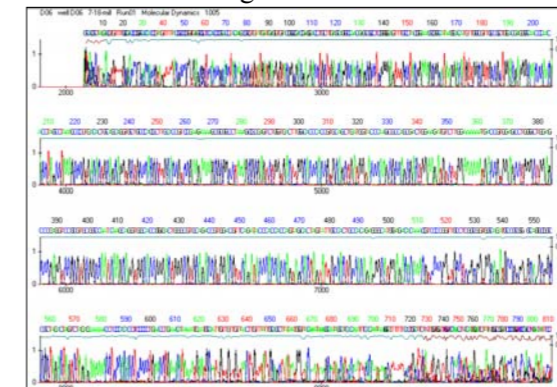
MultiScreen₃₈₄-SEQ purified BigDye terminator sequencing reactions from a plasmid template analyzed on the ABI 3700. 94% of the first 400 bases are PHRED 20 or greater.

MultiScreen₃₈₄-PCR: Template Quality MegaBACE 1000



MultiScreen₃₈₄-PCR purified samples provide clean templates for BigDye terminator reactions run on the MegaBACE 1000 after sequencing reaction cleanup on MultiScreen₃₈₄-SEQ (PHRED 20 = 436).

MultiScreen₃₈₄-SEQ: BigDye MegaBACE 1000



MultiScreen₃₈₄-SEQ purified BigDye terminator sequencing reactions from a plasmid template analyzed on the MegaBACE 1000 (Phred 20 = 683).

CONCLUSIONS

- ◆The MultiScreen₃₈₄ filter plates can be used for reproducible, PCR sequencing template generation and sequencing reaction cleanup before capillary electrophoresis.
- ◆PCR products purified on MultiScreen₃₈₄ filter plates serve as excellent templates for BigDye (PE-Biosystems) and ET (APBiotec) terminator sequencing as measured by PHRED analysis.
- ◆This novel 384 platform readily accommodates small reaction volumes, and thereby aids in the reduction of reagent consumption.
- ◆Neither PCR purification, nor sequencing reaction cleanup requires centrifugation.
- ◆These rapid, vacuum-based filtration protocols are compatible with the Cyberlab C250, the Beckman MultiMek 96 channel pipettor, and with other comparable liquid handlers.