

Automated Sequencing Reaction Clean-up

The Promega Wizard® MagneSil™ Sequencing Reaction Clean-Up System on a Tecan Freedom Nucleic Acid Sample Preparation Workstation



Introduction

ABI PRISM® BigDye™ terminator chemistry is the method of choice for automated, high-throughput DNA sequencing today. However, certain sequencing reaction components interfere with data collection if not removed prior to analysis. Promega has developed a magnetic-based nucleic acid purification technology ideally suited for plasmid DNA. In the Wizard® MagneSil™ Sequencing Reaction Clean-Up System, paramagnetic particles are used for high-throughput purification of BigDye™ terminator DNA sequencing reactions. When automated on the Tecan Freedom Sample Preparation Workstation no user intervention is required for the entire purification process, from the time the 96-well amplification plate is placed on the instrument's worktable until the samples are loaded onto the DNA sequencer. Sequence quality is similar to manual methods and offers the distinct advantage of an automated protocol.

Purification Principle

The Promega Wizard® MagneSil™ Sequencing Clean-Up System is designed to purify BigDye™ Terminator sequencing reaction extension products. The entire process has been designed to use a magnetic separation procedure. The system removes unincorporated dye-labeled terminators and exchanges the buffer for gel loading solution. The kit is designed to work with the ABI PRISM™ 310, 3100, 377 and 3700 DNA sequencers and is based on a 96-microwell plate format.

Automation

Full automation of the clean-up process was achieved with a Tecan Freedom Sample Preparation Workstation. All pipetting and liquid mixing is performed by the instrument's liquid handling arm (LiHA). The liquid system is equipped with 1-ml syringes and washable tips. The PCR plate, containing the sequencing reactions, is placed on the instrument worktable

using MagnaBot® Adaptor T1 and the Plate Clamp 96 from Promega. The robotic manipulator arm (RoMa) moves the PCR plate between the plate stand and Promega's proprietary magnetic separation device (MagnaBot® II). 96 reaction product samples can be purified within 75 minutes. Easy upgrading to a multiple batch process can be realized by implementation of additional Tecan storage modules. Tecan Gemini™ software was used for programming of all process steps.

Worktable Layout

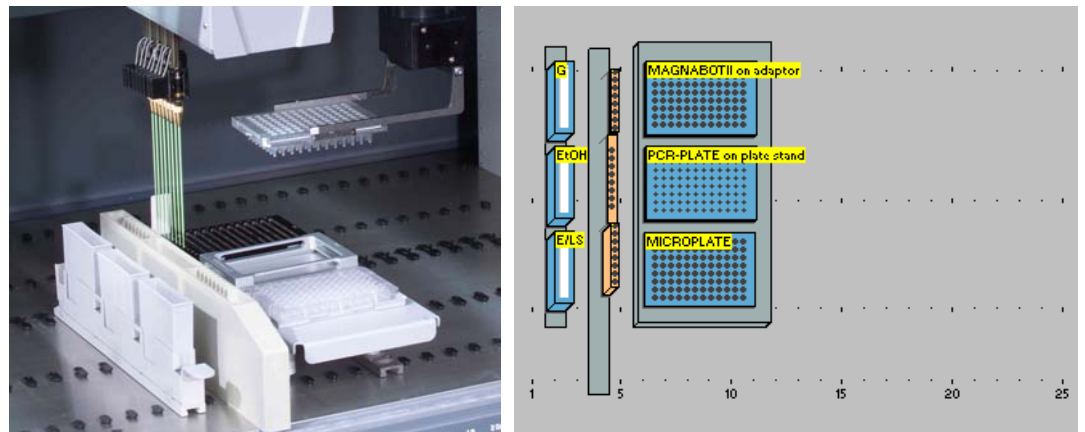


Fig. 1: Worktable layout

Typical worktable layout of Promega Wizard® MagneSil™ Sequencing Clean-Up System on a Tecan Sample Preparation Workstation (right: Software screenshot).

Material and Methods

Plasmid DNA (pGem and pBluescript) was chosen for standard cycle sequencing reactions (20 µl) using ABI PRISM® BigDye™ terminator chemistry. Working solutions for the magnetic clean-up procedure were prepared according to the instructions in the Promega Wizard® MagneSil™ Sequencing Reaction Clean-Up System Technical Bulletin No. 287. Comparative clean-up was performed using a standard ethanol precipitation procedure.

Final elution volumes were chosen according to the requirements of the respective DNA sequencer. 3-6 µl Formamide/blue dextran/EDTA were used for ABI 377.

Results

Sequencing reaction products purified with the Promega Wizard® MagneSil™ Sequencing Reaction Clean-Up System on the Freedom Sample Preparation Workstation can be used for automated DNA sequencing with the ABI PRISM™ sequencing system. pGEM plasmid DNA on the ABI PRISM™ 377 DNA sequencer results in more than 580 bp confirmed sequence data (Fig. 2). The quality is equal to the pGEM sequence analyzed in parallel after a standard manual ethanol precipitation.

Conclusions

The Freedom Sample Preparation Workstation provides an ideal platform for fully automated processing of the Promega Wizard® MagneSil™ Sequencing Reaction Clean-Up System. Electropherograms of purified plasmid sequencing extension products show high quality results that are equivalent to manual and semi-automated methods.

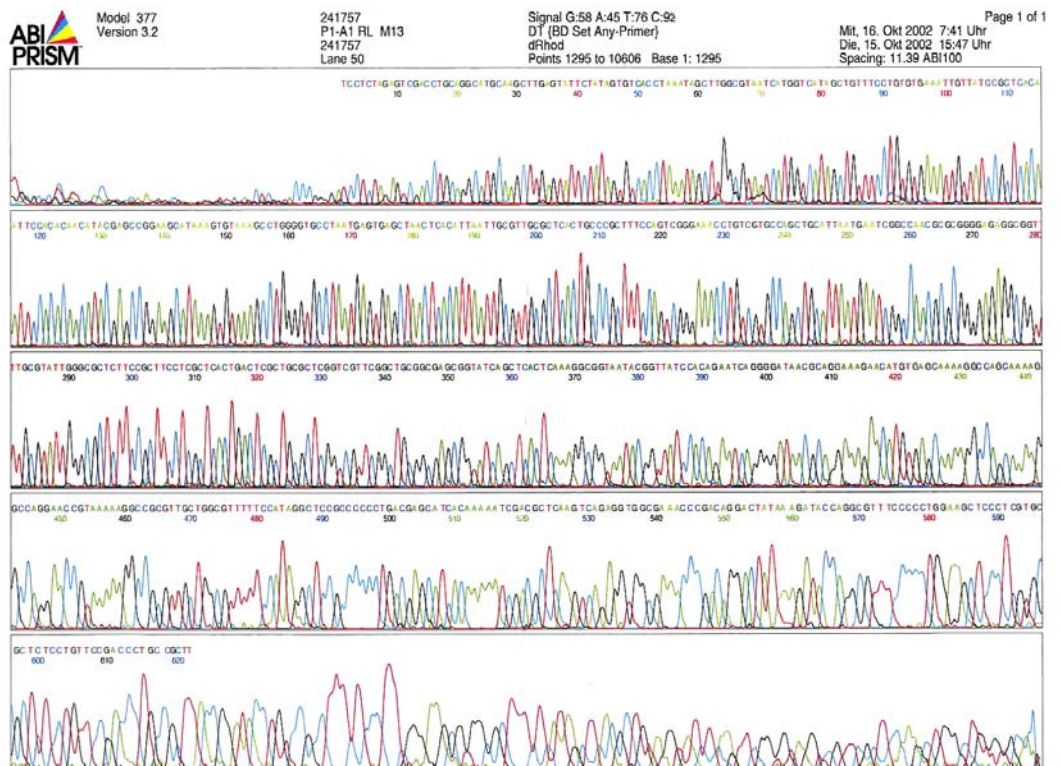


Fig 2: Sequencing results

Electropherogram of pGEM plasmid sequencing reactions analyzed on an ABI PRISM® 377 DNA Sequencer. Reactions were purified with the Promega Wizard® MagneSil™ Sequencing Clean-Up System on a Tecan Sample Preparation Workstation

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