

Automated High-Throughput Plasmid Purification

Eppendorf Perfectprep® Plasmid 384 Kit on a Tecan Freedom Nucleic Acid Sample Preparation Workstation



Introduction

For isolating plasmid DNA, various methods are available today, ranging from traditional manual alcohol precipitation to kit-based vacuum extraction procedures performed in 96 well format. However, when it comes to high sample numbers further miniaturization is required to increase throughput and reduce costs per sample. The Eppendorf Perfectprep® Plasmid 384 Kit provides a suitable format for real high throughput automation.

Extraction Principle

The Perfectprep® Plasmid 384 Kit is a membrane-based system for high-quality plasmid purification. The combination of ultra-thin membrane with minimized dead volume and optimized chemistry allows highly-concentrated plasmid DNA to be obtained from just 0.3 ml of bacterial culture.

The entire process from bacterial cultures to plasmid elution can be performed in the 384-well format. Harvesting bacterial cells, direct binding of the plasmid DNA to the membrane, washing and elution steps are performed by centrifugation.

The elution volume can be chosen from 10 µl to 15 µl, the quantity of buffer used being virtually completely recovered. The eluted DNA can be used immediately in any downstream application (e.g. manual and automatic sequencing, using fluorescent stains, PCR and digestion with restriction enzymes).

Process Automation

Bacterial cultures are grown according to the instructions in the Eppendorf Perfectprep® Plasmid 384 Kit manual. All required liquid handling steps can be performed automatically on a Tecan Freedom Sample Preparation Workstation equipped with the Tecan Te-MO Multi-channel pipetting Option. Gemini™ 4.0 is used for software control of the process.

Up to 768 plasmid extractions (2x384) can be completed within 60 minutes when working in a two-plate batch mode. Even higher throughput can be achieved by running a four-plate batch mode. A complete walk-away system is achieved by the use of the robotic manipulator arm (RoMA), an integrated centrifuge and hotels for plate storage. Blotting of filterplate trip directors can be performed automatically by the robotic arm.

Instrumentation

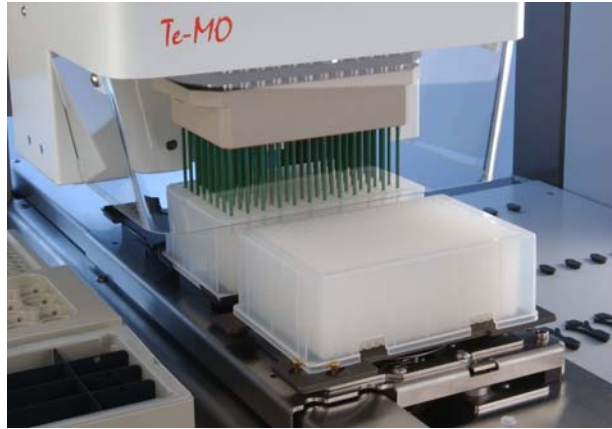


Fig. 1: Tecan Multi-channel pipetting Option (Te-MO)

The Tecan Te-MO enables ultrafast sample transfers and buffer dispenses into the Eppendorf 384 well plates. A specific wide-bore tip block can be used to mix bacterial pellets from high density cultures.

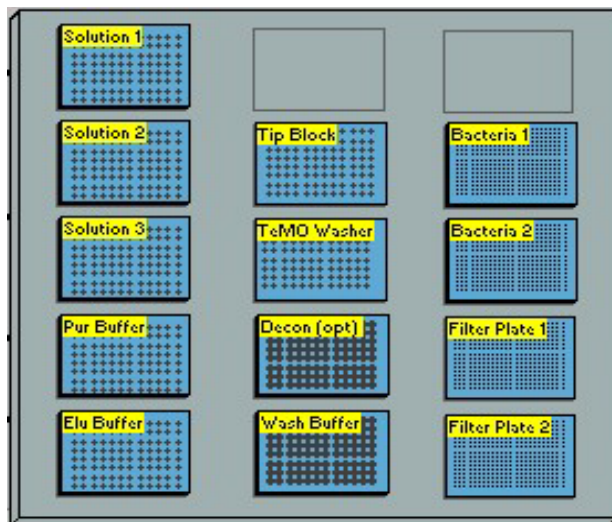


Fig. 2: Te-MO Slide Layout

Two culture plates (768 samples) and all required buffer troughs can be placed simultaneously on the Te-MO 3/5. For automation of multiple batches (or batches of 4 culture plates), intermediate plate exchange is performed by the robotic manipulator arm (RoMA).



Fig. 3: Integrated Centrifuge

The integrated centrifuge enables fully walk-away working mode. Plates are transferred from the Te-MO g6 pipettor directly into the automated centrifuge by the robotic manipulator arm (long version).

Results

The Eppendorf Perfectprep® Plasmid 384 Kit provides supercoiled plasmid DNA free from proteins, chromosomal DNA and RNA.

Typically up to 0.8 µg high-copy plasmid DNA (pGEM; Promega) could be purified from 200 µl XL1-Blue culture ($A_{600}=2.0$). Consistent yields

were obtained, and across the plate, the CV of yields was lower than 15% as determined by Picogreen® quantification. High purity was confirmed by UV determination. A consistent A_{260}/A_{280} ratio between 1.7 - 1.9 was achieved. Sequencing of pGEM plasmid DNA resulted in more than 650 bp confirmed sequence data (Fig. 5).

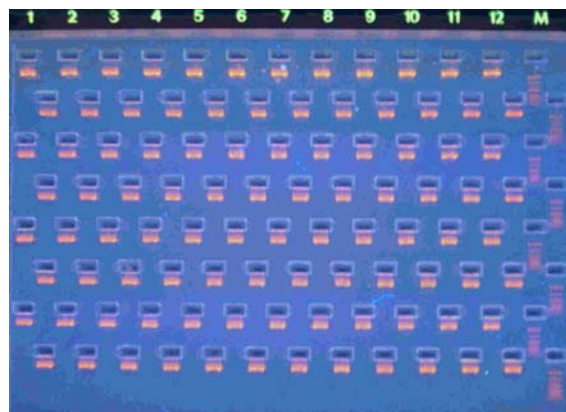


Fig. 4: Homogeneous yields

Agarose gel electrophoresis of pGEM plasmid extracted from homogeneous bacterial pellets (XL1-Blue). Purification was performed with the Eppendorf Perfectprep® Plasmid 384 Kit on the Tecan Freedom Workstation with Te-MO. (96 of 384 samples shown; randomly chosen from elution plate, 10 µl volume per lane)

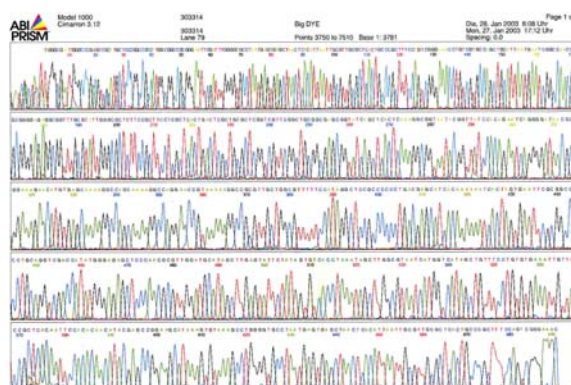


Fig. 5: Excellent reading length

Typical sequencing result (ABI PRISM™ 377) of pGEM plasmid analyzed after automated purification with the Eppendorf Perfectprep® Plasmid 384 Kit on the Tecan Freedom Workstation.

Conclusion

Using the Tecan Multi-channel pipetting Option (Te-MO 3/5) on a Tecan Freedom Sample Preparation Workstation, ultrafast plasmid extraction can be performed with the Eppendorf Perfect prep® Plasmid 384 Kit. All liquid transfers are performed by Te-MO. Up to 768 samples (2x384) can be completed within 60 minutes when working in a two-plate batch mode. Even higher

throughput can be achieved by running a four plate batch mode. A complete walk-away system is enabled by the use of a long robotic manipulator arm (RoMa) and an integrated centrifuge. Yield and purity of extracted plasmids clearly fulfill the kit manufacturers specifications as shown by agarose gel electrophoresis, Picogreen® quantification as well as cycle sequencing.

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