Isolation of plasmid DNA (pGEX-4T-3) from *E. coli* XL1-Blue culture using the QuickPick™ Plasmid DNA kit

KEY WORDS: plasmid DNA, magnetic particle separation, purification, PickPen®

ABSTRACT

The QuickPick Plasmid DNA purification kit provides a fast and simple means of isolating plasmid DNA from bacterial cultures. The technique does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The purified plasmid DNA is of high quality, suitable for downstream applications such as restriction enzyme digestion, ligation, DNA sequencing, *E. coli* transformation, PCR and cloning.

PRINCIPLE OF QuickPick Plasmid DNA kit

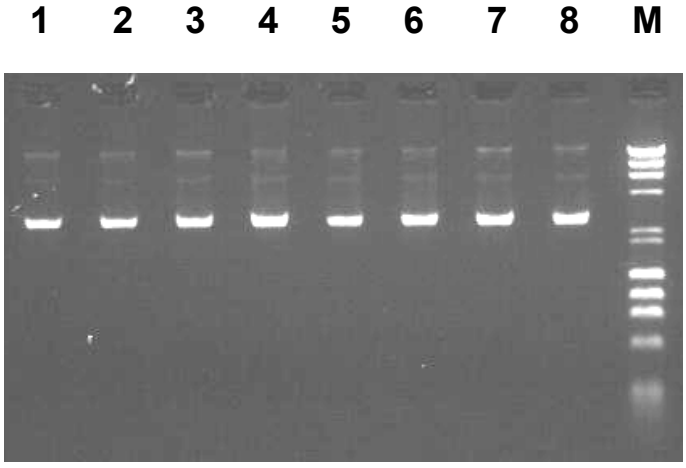
The purification of plasmid DNA is based on a modified alkaline lysis procedure followed by the specific binding of plasmid DNA to the magnetic particles in the presence of Plasmid DNA Binding Buffer. PickPen® 1-M is used to capture the magnetic particles with bound DNA, and to carry out a subsequent wash to remove contaminants. Finally, DNA is eluted from the particles using Plasmid DNA Elution Buffer, and DNA is ready for use in downstream applications. The protocol, starting from the cell culture and ending with purified DNA takes less than 20 minutes. Throughput can be further increased by using PickPen® 8-M which allows processing in 96-well plates.

MATERIALS & METHODS

8 aliquots of 1.5 ml each were withdrawn from an *E. coli* XL1-Blue overnight culture transformed with high-copy pGEX-4T-3 plasmid. The 8 samples were purified following the QuickPick Plasmid DNA kit insert protocol and with PickPen® 1-M. First the samples were prepared using Buffers A, B and C. The sample cells were centrifuged into a pellet, and Buffer A was used to resuspend the cells, Buffer B was used to lyse the cells, and Buffer C was used to precipitate the chromosomal DNA. The plasmid DNA was then retrieved using magnetic particles, according to the kit protocol.

RESULTS

The eluate containing isolated DNA was loaded onto a 1% agarose gel. To each well 1 µl Sybr Green, 1 µl TD loading buffer, 1 µl eluate and 7 µl distilled water were added. As the gel picture shows, plasmid DNA was isolated from all samples with good reproducibility, the average yield per preparation was 1.5 ± 0.3 µg.



M = Marker

1-8 = Plasmid DNA isolated from 1.5 ml of *E. coli* culture