

Yields of different plasmid DNA from *E. coli* cultures using the QuickPick™ Plasmid DNA kit

SANNA PIETILÄ, M.Sc., PEKKA MATTSSON, Ph.D

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. Plasmid yields are dependent on the type of plasmid used as well as growth conditions and other factors. The purification 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 plasmids to the magnetic particles in the presence of Plasmid DNA Binding Buffer. PickPen® 1-M is used to capture the magnetic particles with bound plasmids, and to carry out a subsequent wash to remove contaminants. Finally, plasmid DNA is eluted from the particles using Plasmid DNA Elution Buffer, and is ready for use in downstream applications. The protocol, starting from the cell culture and ending with purified plasmids takes less than 20 minutes. Throughput can be further increased by using PickPen® 8-M which allows processing in 96-well plates.

MATERIALS & METHODS

Different plasmids were transformed into *E. coli* XL1-Blue cells (Table 1). 1.5 ml aliquots of overnight grown cultures were used as sample materials. Samples were purified following the QuickPick Plasmid DNA kit insert protocol and with PickPen® 1-M. The sample cells were centrifuged in microtubes into pellets and the culture mediums were discarded. The cell pellets were resuspended into Buffer A and Buffer B was added to lyse the cells. Chromosomal DNA was precipitated by using Buffer C and removed with residual cell debris by centrifugation. The plasmid DNA was retrieved from the supernatant using magnetic particles, according to the kit protocol. The yields and the purities were determined spectrophotometrically (Table 1).

RESULTS

Table 1. Plasmid DNA yields from 1.5 ml cell culture

Plasmid	Typical yield	Purity
pUC19 (2,7 kb)	4,0 – 5,0 µg	≥ 1.8
pGEM (2,8)	1,5 – 1,8 µg	≥ 1.8
pBAT4* + 1.9 kb insert (6,3kb)	7,4 – 11 µg	≥ 1.8
pBR322 (4,4kb)	1,6 – 2,4 µg	≥ 1.8
pGEX4T-3 (5,2 kb)	2,0 – 3,7 µg	≥ 1.8

*Peränen et al, (1996) *Anal. Biochem.*, **236**:371-373.

DNA yields vary depending on the plasmid type and growth conditions. Up to approximately 11 µg of plasmid has been obtained from a sample of 1.5 ml cell culture. The purified plasmids were of high quality as shown by spectrophotometric measurement in Table 1, and verified on 1 % agarose gel (results not shown).