APPLICATION NOTE No. 427

Optimization of Bacterial Culture and Plasmid Purification with Eppendorf Conical Tubes 25 mL

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Abstract

Plasmid purification from bacterial culture is a commonly performed protocol in molecular biology and life science laboratories. For medium preparations 15 mL conical tubes with screw caps are typically used which often pose handling drawbacks and don't enable achieving optimal productivity of bacterial culture and maximal yields of DNA. Eppendorf Conical Tubes 25 mL with snap cap address these disadvantages. In this application note, we show that the bacterial culture productivity and DNA yields using 25 mL Tubes were much higher when compared to standard 15 mL conical tubes and at the same time improved handling and performance of the workflow.

Introduction

Bacterial culture and subsequent plasmid purification are undoubtfully one of the most commonly performed protocols in molecular biology and life science laboratories. Despite increasing availability and affordability of plasmid purification kits from numerous providers, the standard method of alkaline lysis [1] is still widely used and remains mainstream in the academic sector. It is cost effective, scalable and typically produces high yields of pure plasmid DNA, which can be directly used in various down-stream applications like DNA digestion, cloning or sequencing. For medium scale preparations, 15 mL conical tubes with screw caps are typically used which offer tight lid closure and good centrifugation stability, but often pose handling disadvantages (screw cap handling, cross contamination, difficulty in reaching sample at the bottom of the tube) and often don't enable the optimal productivity of a bacterial culture and maximized yields of DNA - particularly of low copy plasmids.

These disadvantages have been specifically addressed by Eppendorf Conical Tubes 25 mL with snap cap (SnapTec™), which markedly improve one-hand handling, while offering the same safety and intermediate volume range for applications between 15 mL and 50 mL.

In this application the bacterial culture and subsequent purification of low copy plasmid DNA using standard alkaline lysis protocol were compared using Eppendorf Conical Tubes 25 mL and standard 15 mL conical tubes. The bacterial culture productivity and plasmid DNA yields using 25 mL Tubes was much higher and at the same time provided improved handling and overall performance of the workflow.

Materials and Methods

Bacterial Culture

Escherichia coli bacteria (DH5α™, Invitrogen) transformed with low copy plasmid DNA (pBR322™, Invitrogen) were cultured in LB medium with ampicillin in triplicates for 16 hours (37°C, 250 rpm, Innova® S44i shaker, Eppendorf). Cell growth was evaluated by optical density at 600nm (OD_{600}) using Eppendorf BioSpectrometer® and *E. coli* cell number was estimated using the following conversion formula:

OD_{600} of 1.0 ≈ 5 x 10^8 cells/mL.

Low Copy Plasmid DNA Extraction

Plasmid DNA extraction was performed using standard alkaline lysis protocol: 7.5 mL of bacterial cultures were centrifuged at 10,000 x g (5 min, RT) and pellets resuspended in 1.5 mL of solution 1 (50 mM glucose; 10 mM EDTA; 25 mM Tris pH 8.0; 100 µg/mL RNase A). After 5 min incubation, 3 mL of solution 2 (0.2 NaOH; 1 % SDS) was added and samples were mixed by inverting and further incubated for 10 min on ice. After addition of 2.25 mL of solution 3 (3M KOAc, pH 5.4), samples were mixed and centrifuged at 17,000 x *g* (30 min, 4°C). The supernatants were transferred to new tubes, precipitated with same volumes of isopropanol, mixed and centrifuged at 17,000 x *g* (30 min, 4°C). Pellets were rinsed twice (70 % ethanol/centrifugation), dried and resuspended in 200 µL of TE buffer. For 15 mL bacterial culture the alkaline lysis buffers were respectively scaled up by factor 2. All centrifugation steps were performed using Eppendorf centrifuge 5810R with FA-45-6-30 rotor and respective tube adapters. Plasmid yield was estimated by absorbance measurement at 260 nm (BioSpectrometer, Eppendorf). Additional absorbance measurements at 280 nm and 320 nm were used to evaluate the DNA purity.

Results and Discussion

Comparison of bacterial culture growth and productivity is depicted in figure 1. Bacterial culture density and total cell number was higher for both 7.5 mL and 15 mL culture volumes incubated in Eppendorf Conical Tubes 25 mL with snap cap. This indicates better growth rates and overall productivity due to more efficient aeration and mixing properties in Eppendorf Conical Tubes 25 mL as compared to standard 15 mL Tubes.

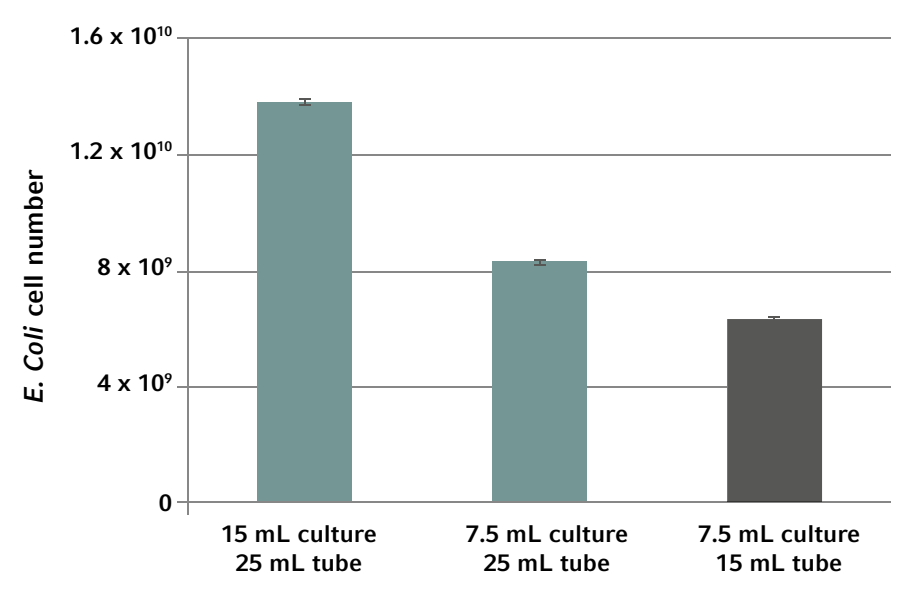

Figure 1: Total cell number of *E. coli* cultures incubated in Eppendorf Conical Tubes 25 mL with snap cap and standard 15 mL conical tubes.

Low Copy Plasmid DNA Extraction

Bacterial cultures were directly used for extraction of low copy plasmid DNA (pBR322™, Invitrogen) using standard alkaline lysis extraction protocol. Notably, in this method several high-speed centrifugation steps (up to 17.000 x *g*), as well as mixing and phase collection steps take place. Tight cap closure and safety of Eppendorf Conical Tubes 25 mL was equal to standard 15 mL conical tubes with screw caps. In addition, snap caps allowed faster tube handling and reduced the cross-contamination risk, which may occur when numerous screw cap tubes are processed in parallel.

As shown in the figure 2, the plasmid DNA yields obtained using Eppendorf Conical Tubes 25 mL were substantially higher, than those obtained with standard 15 mL conical tubes. For 7.5 mL and 15 mL bacterial culture volumes the yield was higher by 70% and 400%, respectively, indicating high culture density and improved growth parameters allowing larger production of plasmid DNA. OD ratios indicated highly pure DNA preparations: A260/280 > 1.9 and A260/230 > 2.0.

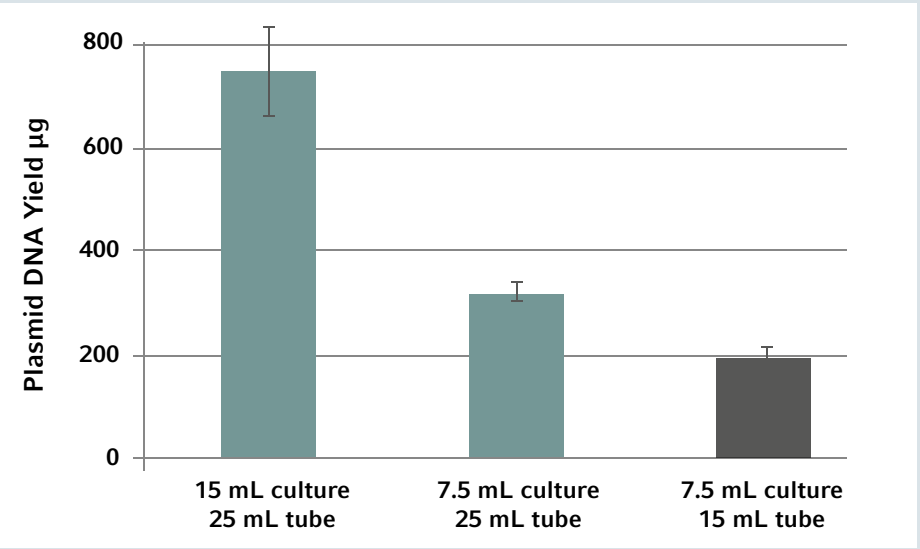

Figure 2: Total yield of low copy plasmid DNA (PBR 322) purified from bacterial cultures incubated in Eppendorf Conical Tubes 25 mL with snap cap and standard 15 mL conical tubes.

Conclusion

The results indicate that both the bacterial culture productivity and the resultant plasmid DNA yields obtained in Eppendorf Conical Tubes 25 mL with snap cap (SnapTec™), were much higher (70% to 400%) as compared to standard 15 mL conical tubes. The significantly better growth rates obtained in 25 mL tubes were due to more efficient aeration and mixing properties of the bacterial culture.

Tight lid closure and safe handling, as well as centrifugation stability of the Eppendorf Conical Tubes 25 mL was equal as compared to 15 mL conical tubes with screw caps at the same time providing markedly improved handling for intermediate volume range applications between 15 mL and 50 mL.

In addition, Eppendorf Conical Tubes 25 mL allowed better sample accessibility and the risk of cross contamination in the purification workflow was reduced, which offers significant handling improvements in various molecular biology and life science protocols.

Literature

[1] Birnboim, H C. A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 1983; 100: 243–255.

Ordering information

Description	Order no.
Eppendorf Conical Tubes 25 mL with snap cap, Eppendorf Quality™, colorless, 200 tubes, (5 bags x 40 tubes)	0030 118 405
Eppendorf Conical Tubes 25 mL with snap cap, PCR clean, colorless, 200 tubes, (5 bags x 40 tubes)	0030 118 413
Eppendorf Conical Tubes 25 mL with snap cap, sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, colorless, 150 tubes, (6 bags x 25 tubes)	0030 118 421
Eppendorf Conical Tubes 25 mL with screw cap, Eppendorf Quality™, colorless, 200 tubes, (4 bags x 50 tubes)	0030 122 410
Eppendorf Conical Tubes 25 mL with screw cap, PCR clean, colorless, 200 tubes, (4 bags x 50 tubes)	0030 122 429
Eppendorf Conical Tubes 25 mL with screw cap, sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, colorless, 200 tubes, (8 bags x 25 tubes)	0030 122 437

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