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Increased yield with isolation of a low-copy plasmid using Eppendorf Tubes® 5.0 mL compared to the 1.5 mL and 2 mL formats

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Abstract

Isolation of plasmid DNA is an important method in molecular biology, which plays a role in the analysis of recombinant clones, to name just one application. However, frequently the limited starting volume results in the problem of a low DNA yield. The isolation of plasmid DNA, using the Eppendorf Tubes® 5.0 mL in comparison with reaction tubes in 1.5 mL and 2.0 mL formats, is described below.
The results show that by increasing the starter culture to 5.0 mL in a larger tube, a considerably higher yield of plasmid DNA can be achieved. At the same time, all steps can be performed in the 5.0 mL tube as easily as in the smaller tubes without a higher contamination risk. Thus, it is not necessary to use several tubes for one sample in order to achieve a sufficient yield. The experiments performed here are representative of other applications for which larger volumes of culture medium are advantageous, or larger volumes are used in subsequent purification steps.

Introduction

Many applications exist in molecular biology which require volumes that exceed the capacity of the standard 1.5 mL or 2 mL reaction tubes. One example is the isolation of low-copy plasmids, which requires larger volumes of bacterial culture in order to achieve a DNA yield sufficiently high to accommodate subsequent applications. Especially during precipitation of DNA with ethanol, which requires 2 volumes of ethanol for each volume of DNA solution, the capacity of the tube is reached very quickly. Frequently, a smaller sample volume is therefore used, which carries the risk of isolating insufficient amounts of DNA, and the need to repeat the experiment.
When a larger amount of material is divided among several smaller tubes, the consumption of tubes and tips increases proportionally, and steps need to be performed multiple times. A further alternative are conical 15 mL polypropylene tubes. These, however, feature considerably lower centrifugation stability (approx. 8,000 – 15,000 x g) than microcentrifuge tubes (approx. 20,000 – 30,000 x g). It may be necessary to prolong the centrifugation time as the DNA yield following precipitation also depends on the g-force experienced during centrifugation [1].
The new Eppendorf Tube 5.0 mL offers the option of working comfortably with larger sample volumes. These tubes are suitable for all steps of DNA isolation, as they accommodate bacterial cultures up to 5 mL and can be centrifuged at high speed (max. 25,000 x g).
Below we will demonstrate the extent to which plasmid DNA yield may be increased when using Eppendorf Tubes 5.0 mL for the isolation of a low-copy plasmid in comparison with 1.5 mL and 2 mL tubes.

Materials and Methods

Minipreparation of plasmid DNA
Competent *E. coli* bacteria were transformed with the low-copy plasmid pBR322 using the Eppendorf Eporator® and subsequently cultured. The maximum volume of overnight culture was transferred into Eppendorf vessels of 1.5 mL, 2.0 mL and 5.0 mL formats, and mini preparation was performed with the QIAprep® Spin Miniprep Kit (Qiagen® GmbH) in triplicate, respectively. For all samples the same amounts of reagents were used.

DNA analysis
The DNA yield was determined with the Eppendorf BioPhotometer® plus. Furthermore, the respective samples were pooled and 3 µL each were loaded on an agarose gel for inspection.

Results and Discussion

The averages of the triplicate measurements for the plasmid DNA resulting from the different tube sizes are depicted in Figure 1. The average amounts of DNA of the respective tube types are 35.3 µg (5.0 mL Tube), 10.9 µg (2.0 mL Tube) and 6.6 µg (1.5 ml Tube), respectively. The yield of isolated plasmid DNA increased proportionally with increasing initial volume of bacteria culture when comparing smaller tube types (1.5 mL and 2.0 mL) with the Eppendorf Tube 5.0 mL.

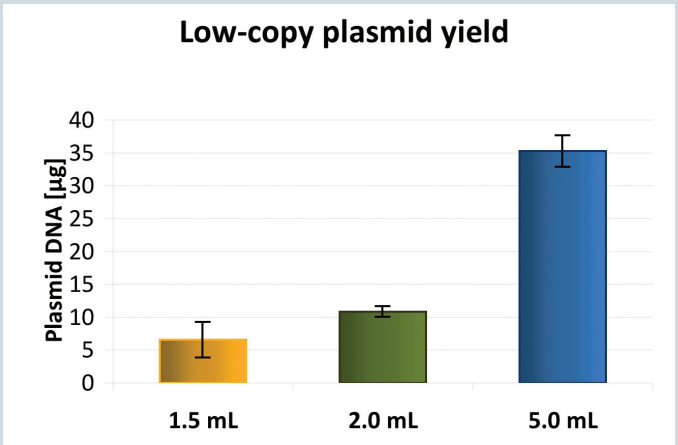

Figure 1: Yield of plasmid DNA: The diagram shows the average results of the triplicate plasmid DNA isolations from 1.5 mL, 2.0 mL, and 5.0 mL starting volume of the same bacterial culture.

Figure 2 shows the agarose gel, which was loaded with uncleaved DNA. The lower dominant band contains supercoiled plasmid DNA which demonstrates that plasmid isolation has resulted in a good yield of intact DNA. Additional visible bands above which are weaker represent further conformations of the plasmid. They make the difference of yield between smaller tube types (1.5 mL and 2.0 mL) and the 5.0 mL tube particularly obvious.

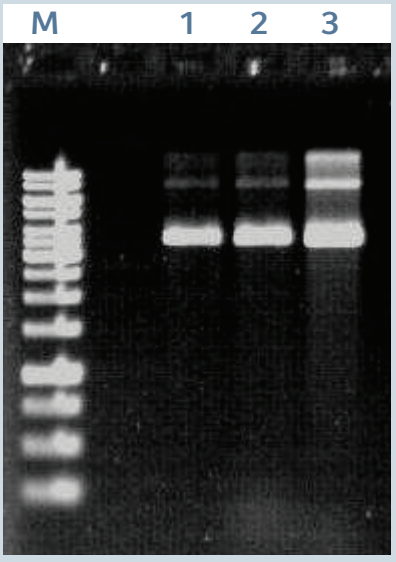

Figure 2: Distribution of the DNA on a 1 % agarose gel, stained with ethidium bromide:
M: 1 kb DNA ladder
1: Eppendorf Safe-Lock Tube 1.5 mL
2: Eppendorf Safe-Lock Tube 2.0 mL
3: Eppendorf Tube 5.0 mL

The results have confirmed that DNA yield may be increased considerably by choosing a larger starting volume. Despite the larger initial volumes of bacterial culture, no changes needed to be made to the protocol, and all sub-steps of the lysis and the precipitation could be performed in the convenient Eppendorf Tube 5.0 mL. Their use in Eppendorf Centrifuges and Thermomixers is secured by appropriate accessories. Handling is comparable to 1.5 mL and 2.0 mL Eppendorf Tubes. A significant advantage is the fact that contamination-free work can be carried out in the same fashion as with the smaller vessels: despite the larger volume, the pipette cone does not reach into the tube.
The 5 mL tube helps avoid the need for division of the sample into several smaller vessels, thus eliminating the need for additional consumables, saving reagent costs and simultaneously simplifying the workflow.

Conclusion

The Eppendorf Tube 5.0 mL thus presents a very good alternative for isolation of plasmid DNA with higher sample volume. The convenient handling is advantageous when sample volumes between 2-5 mL are used, so that the tube serves as a useful link between the established standard tube sizes of 2.0 mL and 15 mL.

Literature

[1] Eppendorf Application Note 234: Centrifugation at 30,000 x g in plasmid DNA precipitation allows better recovery rates and shorter centrifugation times (www.eppendorf.com)

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Tubes® 5.0 mL, Eppendorf Quality, 200 tubes	0030 119.401	0030119401
Eppendorf Tubes® 5.0 mL, PCR clean, 200 tubes	0030 119.460	0030119460
Eppendorf Tubes® 5.0 mL, Sterile, 200 tubes	0030 119.487	0030119487
Eppendorf Tubes® 5.0 mL, Eppendorf Biopur®, 50 tubes (individually wrapped)	0030 119.479	0030119479
Eppendorf Protein LoBind Tubes 5.0 mL, PCR clean, 100 tubes	0030 108.302	0030108302
Eppendorf DNA LoBind Tubes 5.0 mL, PCR clean, 200 tubes	0030 108.310	0030108310
Tube Clip 5.0 mL, 10 pcs., secures lid for boiling	0030 119.509	0030119509
Starter Pack Eppendorf Tubes® 5.0 mL, PCR clean, 400 tubes, 2 racks (16 spaces), white, 8 universal adapters for rotors with bore for 15 mL conical tubes	0030 119.380	0030119380
Eppendorf Safe-Lock Tubes 1.5 mL, Eppendorf Quality, (international: 1000 tubes, North America: 500 tubes)	0030 120.086	022363204
Eppendorf Safe-Lock Tubes 1.5 mL, PCR clean, (international: 1000 tubes, North America: 500 tubes)	0030 123.328	022363212
Eppendorf Safe-Lock Tubes 2.0 mL, Eppendorf Quality, (international: 1000 tubes, North America: 500 tubes)	0030 120.094	022363352
Eppendorf Safe-Lock Tubes 2.0 mL, PCR clean, (international: 1000 tubes, North America: 500 tubes)	0030 123.344	0030123344
Eppendorf Eporator®, for bacteria and yeast	4309 000.019	4309000019
Eppendorf BioPhotometer® plus	6132 000.008	952000006

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