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APPLICATION NOTE No. 234

Centrifugation at 30,000 x *g* in Plasmid DNA Precipitation Allows Better Recovery Rates and Shorter Centrifugation Times

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Abstract

Using the Eppendorf 30,000 x *g* system comprising of Safe-Lock Tubes and Centrifuge 5430 R with high-speed rotor (Figure 1), the effect of relative centrifugal force (rcf), duration of centrifugation and the amount of isopropanol on the recovery rate of plasmid DNA from alcohol precipitation were investigated. All three factors play a role, with rcf having the greatest effect. At 30,000 x *g*, nearly 90% of DNA could be recovered from a 5 minutes centrifugation.
Besides shortening the centrifugation time by 75%, the amount of isopropanol could be reduced by 40% with comparable precipitation yield.

Introduction

Isolation and purification of plasmid DNA are routine experiments in most molecular biology laboratories. In plasmid preparation, alcohol precipitation is a common technique to concentrate diluted plasmid DNA solution or to purify plasmid DNA from high content of salt. In this procedure, alcohol and salt (if required) are added to the DNA aqueous solution, which forces the DNA to precipitate out from the solution. The precipitated DNA is pelleted in a subsequent centrifugation, followed by one or more 70% ethanol wash steps before being resuspended in water or buffer.
It has been known for many years that the duration of centrifugation has a strong influence on the recovery rate of precipitated DNA [1]. However, prolonged centrifugation times extend the experimental process. In this Technical Report, the effect of relative centrifugal force (rcf) on plasmid precipitation yield was investigated, to find out the possibility to reduce centrifugation times by increasing the rcf, while achieving comparable recovery rates. To this end, three rcfs (15,000 x *g*, 20,000 x *g*, 30,000 x *g*) in combination with three centrifugation times (5, 10 or 20 minutes) were tested. In addition, the conditions were tested using two different concentrations of isopropanol (0.6 v/v and 1.0 v/v).

Figure 1:
a) Eppendorf LoBind® Safe-Lock tube 1.5 mL
b) High-speed rotor (FA-45-24-11-HS) for Eppendorf Centrifuge 5430/5430 R
c) Eppendorf Centrifuge 5430 R

Materials and Methods

Competent *E. coli* were transformed with plasmid vectors pGEM®-T easy. The plasmids were isolated from an overnight culture originated from a single colony using QIAprep® Spin Miniprep Kit (Qiagen).
The amount and purity of the isolated plasmid was determined with Eppendorf BioPhotometer® D30. Only samples with an absorbance ratio 260/280 above 1.6 and an absorbance ratio 260/230 above 2.0 were used for the following precipitation step (Figure 2).

Plasmid precipitation was performed in the Eppendorf 1.5 mL Safe-Lock tubes DNA LoBind (Figure 1 a) which reduce sample-to-surface binding and allow maximum sample recovery [2]. Centrifugation was done with the high-speed rotor (Figure 1 b) in the Eppendorf Centrifuge 5430 R (Figure 1 c). Six replicates were tested in each condition, and the tests were performed three times. The recovery rates for each condition, represented by means of three tests, are shown in Figure 3 and 4.

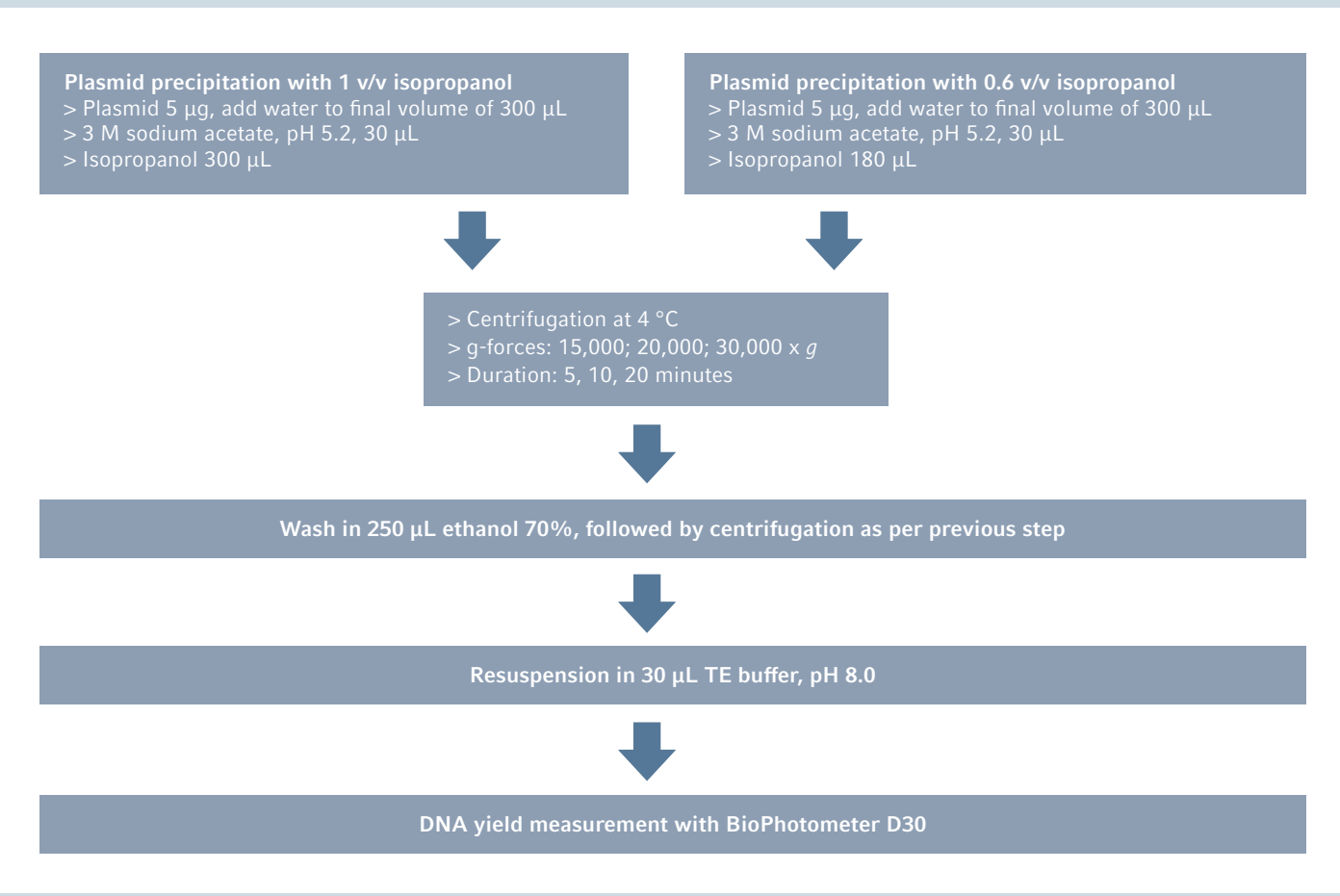

Figure 2: Plasmid precipitation in two different concentrations of isopropanol (0.6 and 1 v/v) at different centrifugation forces and times.

Results and Discussion

The results of the experiments, using 1 v/v isopropanol, are shown in Figure 3. Consistent with previous reports, longer centrifugation times contribute to higher recovery rates [1], shown by the higher recovery rates at all rcfs tested for 20 minutes compared to 5 and 10 minutes. The effect of g-force on DNA recovery was especially pronounced. A full recovery was achieved when the samples were centrifuged at 30,000 x *g* for 20 minutes after precipitation. The recovery rate was close to maximum even when the centrifugation time was reduced, with almost 100% after 10 minutes and nearly 90% after 5 minutes. At lower g-force, the DNA recovery rate was much reduced when the centrifugation time was shortened. At 20,000 x *g*, there was only 70% DNA yield after 5 minutes of centrifugation. Centrifugation at 15,000 x *g* resulted in even lower recovery, with a poor recovery rate of less than 40% after 5 minutes. The results show that high plasmid recovery can be achieved in very short time when samples are centrifuged at 30,000 x *g*.

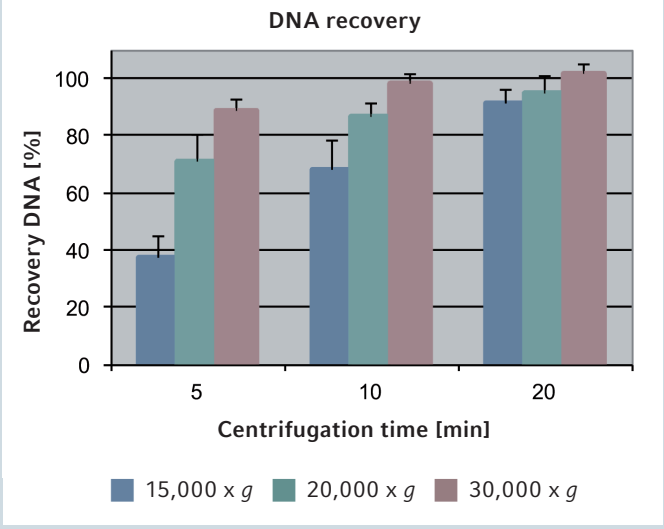

Figure 3: Percentage of recovery of plasmid DNA following precipitation with 300 µL isopropanol at different g-forces and centrifugation times. Error bars represent the standard error.

We have also investigated the amount of plasmid recovered when precipitated with 0.6 v/v and 1 v/v isopropanol at different g-forces. Figure 4 shows the comparison of the DNA recovery at centrifugation time of 5 minutes using 0.6 v/v and 1 v/v isopropanol. When samples were centrifuged at 30,000 x *g*, both concentrations resulted in almost similar DNA recovery, with a recovery difference of about 6 percentage points. There were huge differences in DNA recovery between the two concentrations at lower g-force though.

At 15,000 x *g*, the DNA yield for 0.6 v/v was only 20%, half of the yield obtained from 1 v/v isopropanol. The recovery rate for 0.6 v/v was 40%, compared to 70% obtained from 1 v/v for 20,000 x *g* centrifugation. Thus, centrifugation at 30,000 x *g* allows reduction of isopropanol consumption by 40%, with comparable DNA recovery.

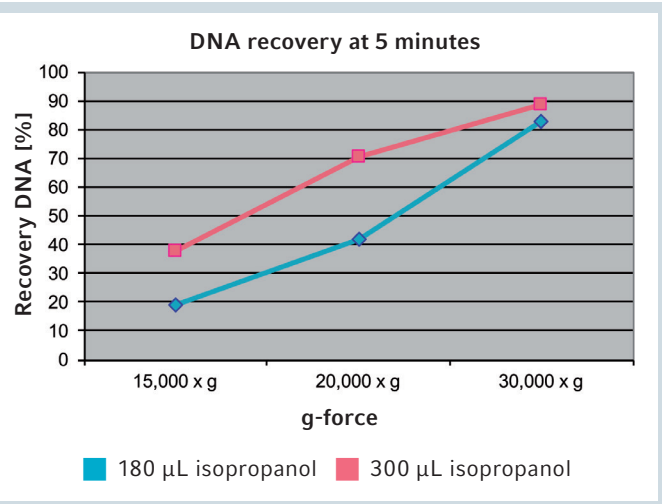

Figure 4: Percentage of recovery of plasmid DNA following precipitation with 180 µL (0.6 v/v) and 300 µL (1 v/v) isopropanol at different g-forces

The results show that the centrifugal force, centrifugation time and the concentrations of isopropanol have influence on the efficiency of the plasmid precipitation. The greatest effect is exerted by the centrifugal force of 30,000 x *g*, with nearly 90% recovery achieved for a centrifugation time of 5 minutes. The DNA yield remained relatively stable following precipitation with 0.6 v/v or 1 v/v isopropanol at 30,000 x *g*. In comparison, the recovery rate fell sharply when samples were centrifuged at 15,000 x *g* or 20,000 x *g*. Based on the results, centrifugation at 30,000 x *g* after DNA precipitation offers flexibility on the amount of isopropanol used, which offers the option to process higher sample volumes. Additionally, it allows noticeably faster performance.
By decreasing the centrifugation time from 20 to 5 minutes, total time required for the protocol can be reduced by up to 30 minutes (precipitation and washing step) at a constant high yield of 90% or above.

Conclusion

We have shown that the 30,000 x *g* system by Eppendorf, consisting of the Centrifuge 5430 R and the 1.5 mL Safe-Lock Tubes (DNA LoBind), is a robust system capable of high yield plasmid precipitation. Using the system, the whole precipitation process can be shortened compared to using 20,000 x *g* and 15,000 x *g* by 50% or 75%, respectively. At the same time, the amount of isopropanol for precipitation can be reduced by 40% without compromising the DNA recovery, thereby reducing costs and amount of chemical waste.
The prerequisites for this efficient centrifugation are a centrifuge that can perform at 30,000 x *g* and tubes with correspondingly high centrifugal stability (e.g. the Eppendorf Safe-Lock Tubes) [3].

References

[1] Zeugin JA and Hartley JL. Ethanol precipitation of DNA. Focus 1985; 7 (4):1-2
[2] Eppendorf Tubes - General technical data - Application notes for LoBind Tubes (http//:www.eppendorf.com)
[3] Eppendorf Application Note 164: Eppendorf Safe-Lock Tubes – Outstanding performance for centrifugation and incubation of samples in the laboratory (http//:www.eppendorf.com)

Ordering information

Description	Order no. International	Order no. North America
Eppendorf Safe-Lock TubeTM 1.5 mL, 1,000 tubes	0030 120.086	022363204
Eppendorf Safe-Lock TubeTM 1.5 mL, PCR clean, 1,000 tubes	0030 123.328	022363212
Eppendorf Safe-Lock TubeTM 1.5 mL, DNA LoBind, 250 tubes	0030 108.051	022431021
High Speed rotor FA-45-24-11-HS, incl. rotor lid, aerosol tight, PTFE-coated	5427 710.000	022654080
Eppendorf BioPhotometer® D30	6133 000.001	613000010
Centrifuge 5430 with 30 x 1.5/2.0 mL aerosol tight fixed-angle rotor FA-45-30-11		
Keypad	5427 000.216	022620509
Knob	5427 000.410	
Centrifuge 5430 without rotor		
Keypad	5427 000.011	022620584
Knob	5427 000.615	
Centrifuge 5430 R (refrigerated) with 30 x 1.5/2.0 mL aerosol tight fixed-angle rotor FA-45-30-11		
Keypad	5427 000.216	022620601
Knob	5427 000.410	022620623
Centrifuge 5430 R (refrigerated) without rotor, 230 V / 50 - 60 Hz		
Keypad	5427 000.011	022620667
Knob	5427 000.615	022620689

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