Technical Information

The impact of bacterial culture conditions on plasmid DNA yield

Introduction

Plasmid DNA is generally prepared from bacterial cultures grown in the presence of a selective agent, such as an antibiotic. A number of factors influence the DNA yield and quality, including plasmid copy number, host strain, culture volume and culture medium. This analysis was run to determine the impacts of certain factors on the plasmid DNA yield, with a view to identifying optimal conditions for 24-, 48- and 96-well culture. The plasmid DNA was prepared using the QIAGEN Plasmid *Plus* 96 Miniprep Kit.

Materials and Methods

Three experimental setups were used to assess the impact of altered culture conditions. They are listed in Table 1.

Table 1. Experimental setup for the culture condition–plasmid yield analyses

				Containers and volumes			
Experiment	**Host strain**	**Plasmid**	**Medium**	**500 ml flask, 100 ml medium**	**24-well plate, 5 ml medium**	**48-well plate, 2.5 ml medium**	**96-well plate, 1.25 ml medium**
1	DH5α	pUC-AV2-HCC	LB and 2xYT	Yes	Yes	Yes	Yes
2	DH5α and XL1Blue	pBluescript	LB and 2xYT	–	–	Yes	Yes
3	DH5α	pBluescript and pCMVβ	LB and 2xYT	–	Yes	Yes	Yes

In each case, the *E. coli* strains were plated on agar plates with the appropriate antibiotics and incubated overnight at 37°C. Starter cultures in flasks with the appropriate antibiotic were made using a single colony from each plate. These starter cultures were used to inoculate main cultures to an optical density of 0.05 at a wavelength of 600 nm (OD_{600} = 0.05). The culture media were LB (1% peptone, 0.5% yeast extract, 1% NaCl) or 2xYT (1.6% tryptone, 1% yeast extract, 0.5% NaCl).

Main culture incubation ran for 22 h at 37°C and 220 rpm. The cells were harvested by centrifugation and the obtained cell pellets from each well were processed using the standard protocol of the QIAGEN Plasmid *Plus* 96 Miniprep Kit. The volumes of the lysis buffers were increased slightly when a 5 ml culture was used, as indicated in the kit handbook: 350 µl of Buffer P1, Buffer P2 and Buffer S3 350 µl, and 300 µl of Buffer BB. Elution was performed in 120 µl of Buffer EB.

E. coli biomass production (cell densities) was measured based on OD_{600}. Plasmid DNA yield and quality (A260/280 ratios) were determined using a Tecan® Infinite® M200 spectrophotometer.

To verify the consistent performance of the chosen prep kit, a broadly based trial was performed with 880 individual *E. coli* Top10F cultures carrying high copy plasmids grown in 1.2 ml 2xYT medium in 96-well blocks. Plasmid DNA was prepared from the cultures using the standard protocol of the QIAGEN Plasmid *Plus* 96 Miniprep Kit.

Results

First setup: DH5α strain harboring pUC-AV2-HCC plasmid

Using 2xYT gave a 2- to 2.5-fold greater *E. coli* biomass production than with LB medium (Figure 1A). Culture volume and container form or size only had minor influence in this experimental setup, but the highest cell densities were clearly for cultures in the 48-well plates. This may be due to better aeration because of the asymmetric form of the wells.

The increase in *E. coli* biomass production in 2xYT medium resulted in up to 1.8-fold higher yields of plasmid DNA than the yield of cultures grown in LB medium (Figure 1B). In the wells of the multiwell plates, there was an almost linear relationship between the culture volume and the plasmid DNA yield. Plasmid quality was comparable and high for the two culture media (Table 2).

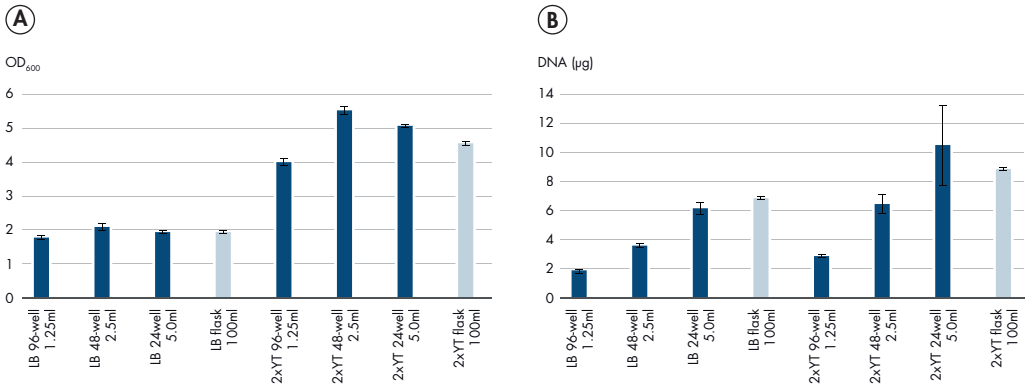

Figure 1. Culture medium and volume influence the plasmid DNA yield from *E. coli* cultures. A: Comparison of the OD_{600} values of *E. coli* DH5α cultures grown in different culture media, containers and volumes. **B**: Comparison of plasmid DNA yields from samples from the colonies.

Table 2. Quality of plasmid DNA purified from *E. coli* DH5α cultures

Culture container	Culture volume	LB medium	2xYT medium
		Average A260/280 ratio	
96-well plate	1.25 ml	1.84	1.83
48-well plate	2.5 ml	1.82	1.83
24-well plate	5 ml	1.83	1.84
500 ml flask	100 ml	1.83	1.83

Second setup: DH5α and XL1-Blue strains harboring pBluescript plasmid

2xYT medium gave higher *E. coli* biomass production and higher plasmid DNA yield than LB medium (Figure 2A). The plasmid DNA yield from cultures grown in 2xYT medium was up to 2-fold higher than that from cultures grown in LB medium (Figure 2B). No significant difference in plasmid DNA yield was observed between the two host strains. As in the first setup, the highest cell densities were for cultures in the 48-well plates and the DNA yield was higher from the 48-well plate cultures than from the 96-well plate cultures. Plasmid quality was comparable and high for the two culture media (Table 3).

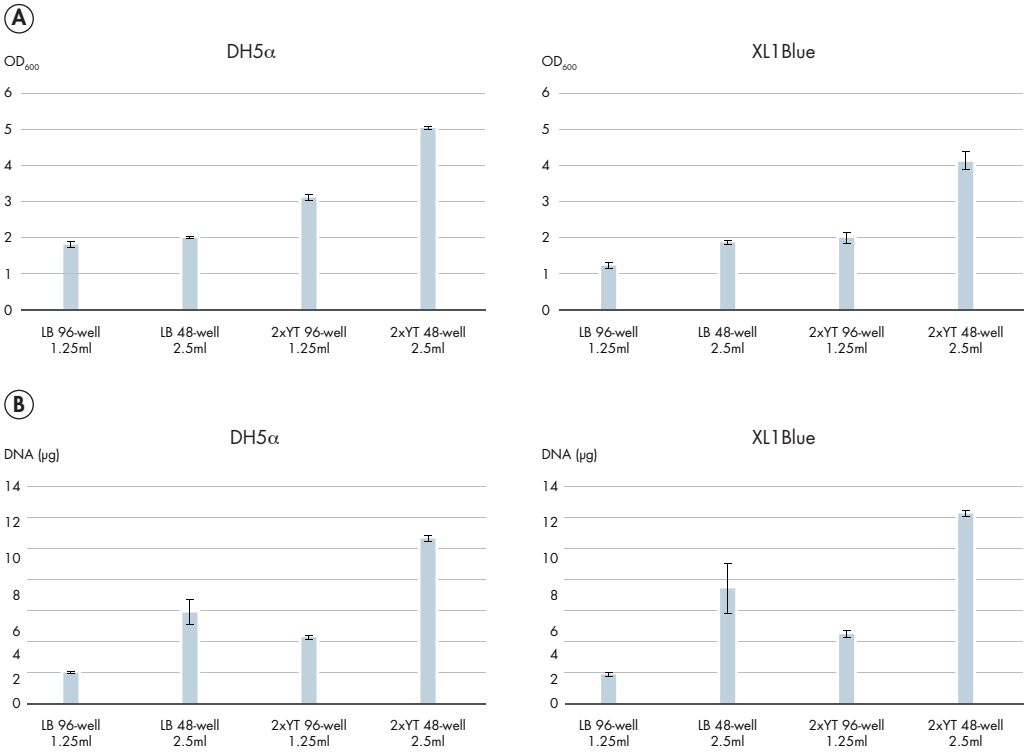

Figure 2. Host strain, culture medium and volume influence the plasmid DNA yield from *E. coli* cultures. A: Comparison of the OD_{600} values of *E. coli* DH5α and XL1Blue cultures grown in different culture media and volumes. **B**: Comparison of plasmid DNA yields from samples from the colonies.

Table 3. Quality of plasmid DNA purified from *E. coli* DH5α and XL1Blue cultures

Culture container and medium	Culture volume	DH5α strain	XL1Blue strain
		Average A260/280 ratio	
LB medium, 96-well plate	1.25 ml	1.84	1.83
LB medium, 48-well plate	2.5 ml	1.83	1.83
2xYT medium, 96-well plate	1.25 ml	1.84	1.81
2xYT medium, 48-well plate	2.5 ml	1.83	1.83

Third setup: DH5α harboring pBluescript and pCMVß plasmids

Using 2xYT medium gave higher biomass production and plasmid DNA yield than with LB medium (Figure 3). As in the first setup, the highest cell densities were for cultures in the 48-well plates and there was an almost linear relationship between the culture volume and the plasmid DNA yield. Plasmid quality was comparable and high for the two plasmid types (Table 4).

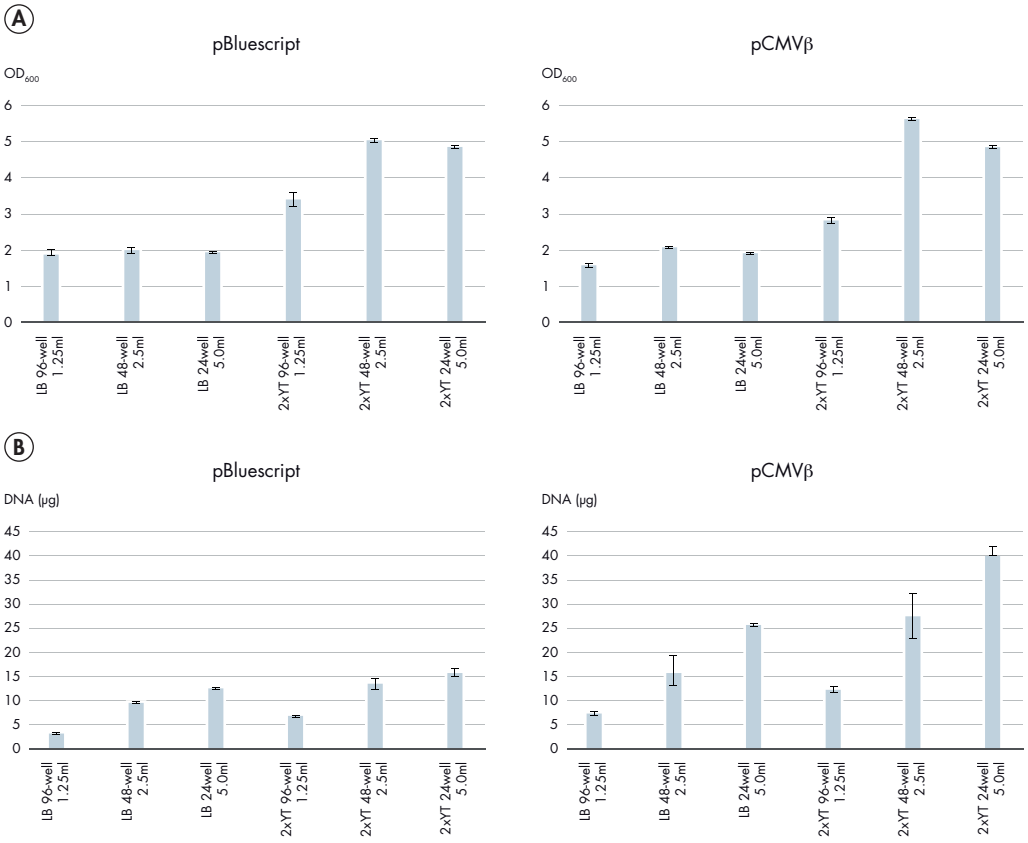

Figure 3. Plasmid type, culture medium and volume influence the plasmid DNA yield from *E. coli* cultures. A: Comparison of the OD_{600} values of *E. coli* DH5α cultures harboring pBluescript or pCMVβ grown in different culture media and volumes. **B**: Comparison of plasmid DNA yields from samples from the colonies.

Table 4. Quality of plasmid DNA purified from *E. coli* DH5α cultures

Culture container and medium	Culture volume	pBluescript	pCMVβ
		Average A260/280 ratio	
LB medium, 96-well plate	1.25 ml	1.89	1.88
LB medium, 48-well plate	2.5 ml	1.88	1.88
LB medium,24-well plate	5 ml	1.86	1.86
2xYT medium, 96-well plate	1.25 ml	1.88	1.87
2xYT medium, 48-well plate	2.5 ml	1.88	1.86
2xYT medium,24-well plate	5 ml	1.85	1.85

QIAGEN Plasmid *Plus* 96 Miniprep Kit performance

The median yields per single well and plate from the assessment of performance consistency for the QIAGEN Plasmid *Plus* 96 Miniprep Kit are shown in Figure 4. Cultivation in a 96-well block using 2xYT medium and subsequent plasmid preparation in the 96-well format consistently delivered ~30 µg of plasmid DNA per well.

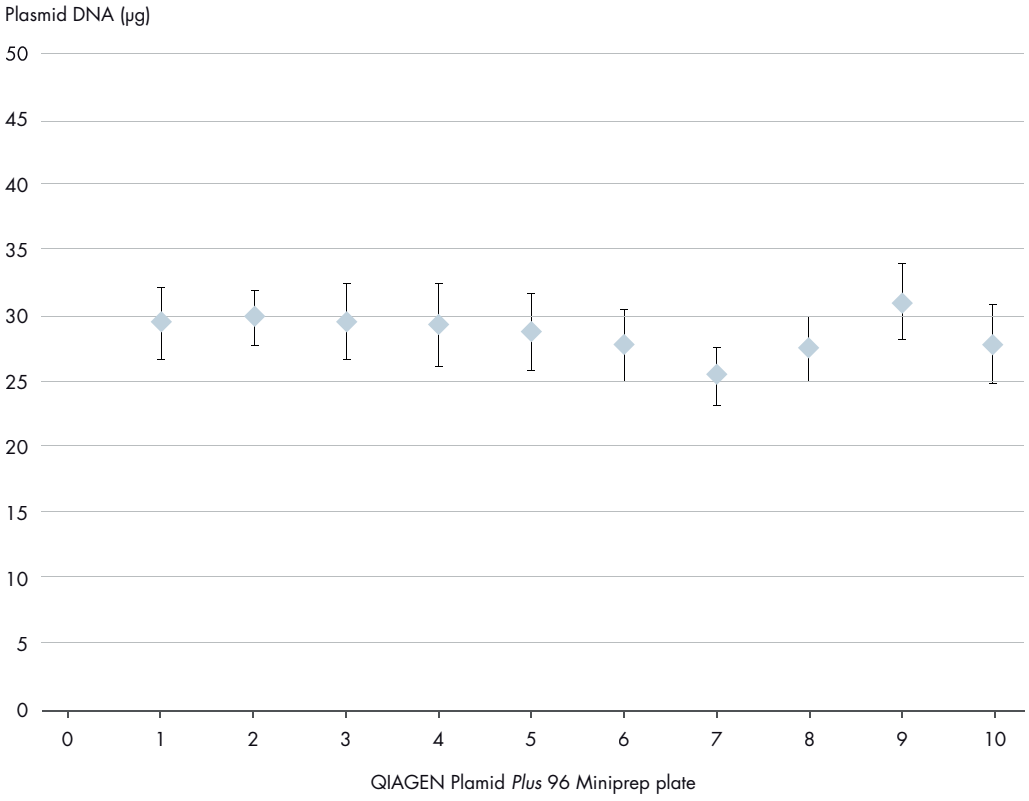

Figure 4. The QIAGEN Plasmid *Plus* 96 Miniprep Kit delivers consistently high yields of plasmid DNA. Median total yield per well from 10 x 96-well purifications of 880 individual *E. coli* Top10F cultures carrying a high copy plasmid are shown.

Conclusion

We analyzed the influence of culture medium, container and volume, *E. coli* host strain, and plasmid type on the bacterial biomass and plasmid DNA yields. The chosen plasmid DNA prep was the QIAGEN Plasmid *Plus* 96 Miniprep Kit, which was demonstrated to deliver highly consistent yields in an experiment with a sample set of 880 bacterial cultures. The main factor influencing biomass and plasmid DNA yield in these experiments was the choice of culture medium. Using 2xYT medium in the 24-, 48- or 96-well format consistently gave 1.5- to 2-fold higher yields of plasmid DNA than with LB medium. Thus we recommend the use of rich 2xYT medium for the culture and performance of 96-well plasmid preparations with the standard QIAGEN Plasmid *Plus* 96 Miniprep Kit protocol using slightly increased lysis buffer volumes.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Plasmid *Plus* 96 Miniprep Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid *Plus* 96 Plates, Buffers, Reagents, Flat-Bottom Blocks, S-Blocks, and Elution Microtubes; requires use of QIAvac 96 and Elution Microtube Adapter (available from QIAGEN Technical Services), or a centrifugation system suitable for 96-well blocks	16181

To find out more about the QIAGEN Plasmid *Plus* 96 Miniprep Kit, visit **qiagen.com/Plasmid-Plus**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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