BECKMAN COULTER
Life Sciences

Scalable Plasmid Purification using CosMC Prep

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Summary

Plasmids are essential for most genetic engineering constructs. Plasmids are generally lysed using an alkaline lysis protocol, in which all DNA is denatured, and only smaller plasmid DNA is able to reanneal. The genomic DNA is then removed with the contaminating cellular debris, leaving purified plasmid DNA. A variety of different plasmid isolation kits utilize this chemistry; however, many of them either require extensive hands on time or have low input limits.

This application note demonstrates the flexibility of CosMCprep, a scalable plasmid purification kit. CosMCprep is shown here to work with volumes up to 5.5 mL purifying up to 300 µg of plasmid.

In its ability to not only isolate small amounts of small plasmid for use with general molecular techniques but the ability to extract larger amounts of plasmids for potential other uses the kit is flexible enough for most laboratory needs.

Materials and Methods

E. coli strain DH5α containing the plasmid pUC19 were grown up overnight in 1-6 mLs of 2xYT media (Boston BioProducts) containing carbenicillin (Fisher Scientific). After 17 hours of growth, the cultures were spun down at 10,000 x g for 2 min in 1.5 mL increments. The pellets were resuspended in various amounts of RE1 buffer and L2 buffer as described in Table 1. The cells were lysed for 5 minutes as described in the CosMCprep manual. At the end of the 5 minutes, the corresponding amounts of N3 (table 1) was added to neutralize the reaction. The total reactions were spun down at max speed for 2 minutes to pellet the flocculent. A proportion of the lysate was transferred as described in Table 2, and the amount of Pur4 + isoproponol Buffer was added as described in Table 2. The protocol was then followed as described in the CosMCprep kit with the recommended two ethanol washes and 40 µL of RE1 buffer for elution.

Overnight Volume (mL)	RE1 Buffer (µL)	L2 Buffer (µL)	N3 Buffer (µL)
0.8	100	100	100
2	100	200	200
5.5	200	800	800
10	500	1000	1000

Table 1. Lysis conditions and bind conditions for increasing overnight volumes

Overnight Volume (mL)	Supernatant to Transfer for Bind (µL)	Pur4 Buffer (µL)	Isopropanol (µL)
0.8	110	10	80
2	180	20	132
5.5	660	80	488
10	920	145	700

Table 2. Bind conditions for increasing overnight volumes

Results

At least three replicates were used for each starting volume. As the volume of overnight was increased the final yield of plasmid was increased as well (Table 3 and Figure 1). The yield of plasmid increases linearly with the increase in overnight volume. The yield of plasmid can be estimated for larger overnight volumes using a simple linear regression. In Figure 2 we have estimated the yield for a 10 mL overnight to be about 575 µg.

Volume of Overnight	Average Concentration	Average Yield (µg)	Average $_{260/280}$	Average $_{260/230}$
0.8	258	10.3	2.1	2.0
2	1893	74.0	2.2	2.8
5.5	2978	298.0	1.9	2.4

Table 3. The average yield and purity of pUC19 plasmid isolated from 3 different volumes of overnights. The averages were taken from three replicates of each volume. The purity shown for each overnight volume is sufficient for PCR based downstream applications. The plasmid's yield and purity was assessed using a NanoDrop (Thermo Fisher Scientific).

CosMCprep isolates more plasmid DNA with higher volumes of input

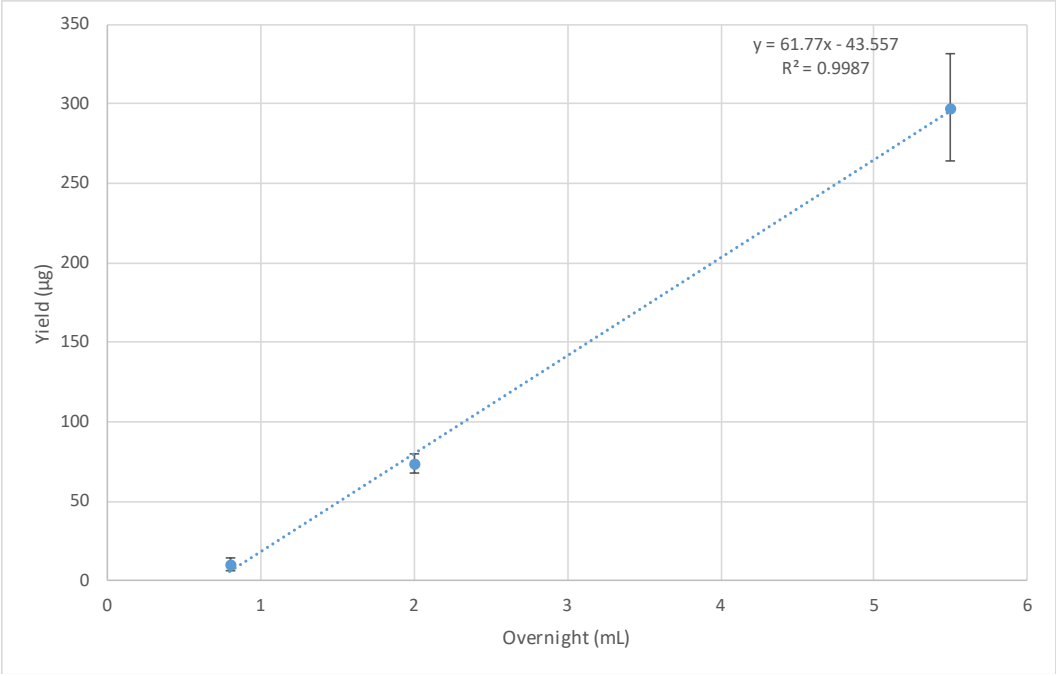

Figure 1. The average yield of three replicates for each volume of overnight is shown with the three dots and the standard deviation of the three replicates is also represented as error bars. A trend line was added that goes through the three replicates. The trend line has an R2 value of 0.9987.

Estimate the volume of overnight you need for your plasmid isolation needs

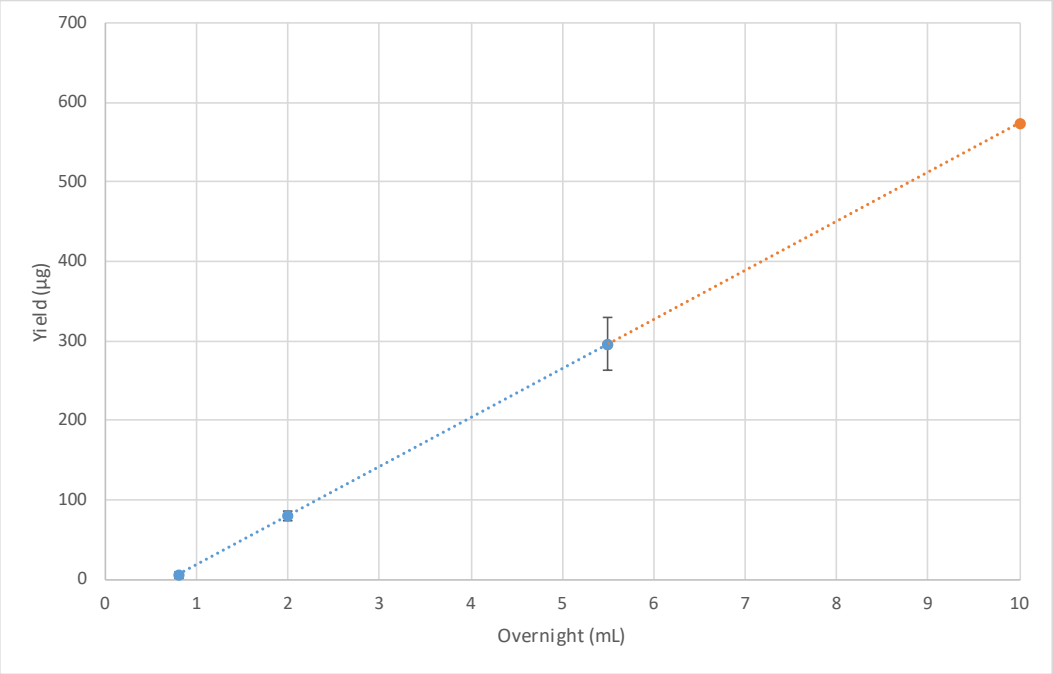

Figure 2. The estimation for the amounts of plasmid if larger volumes of overnight were used. Estimates were based off of the simple linear regression using the formula for the trend line in Figure 1.

Conclusions

This study demonstrates the flexibility of the CosMCprep kit. The kit is not only a great option for smaller amounts of molecular grade plasmids, but it can be scaled up to extract a larger amounts of molecular grade plasmids. The ability to do a mini prep as well as a maxi prep with one kit would allow for the freedom to choose the amount of plasmid that you want to extract without the hassle of having two or more kits on hand at any one time. Also by using the same chemistry for all plasmid extraction there is no need to learn more than one workflow or question if the chemistries affect your plasmid in different ways.

Please reference the current CosMCprep protocol for product information and a detailed description of use.

This protocol should be used if a high concentration of plasmid is desired.

Cells can be grown in a rich media (Either LB or 2xYT) for 17-22 hours in individual tubes with at least 1 mL of culture.

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