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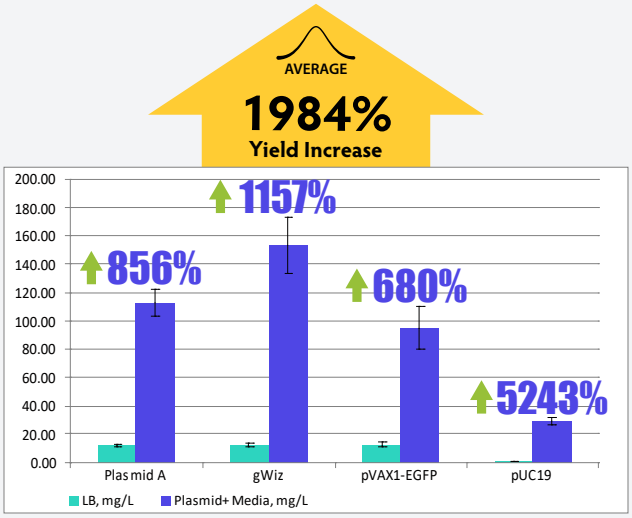
# Improved DNA Protocol for *E.coli* with PLASMID+® Media




## Description

PLASMID+® liquid media is an enriched media specifically designed for plasmid DNA production. PLASMID+® supports much higher cell densities and plasmid yields than LB media. Optimal shake flask yields are achieved using Ultra YieldTM Flasks (Thomson Instrument Company), which facilitates maximum culture aeration. PLASMID+® media may also be used in a bioreactor with continuous aeration and agitation.

## Storage

Store PLASMID+® liquid media at room temperature for up to 12 months.

## Bacteria Strains

E. coli DH5α is the preferred host strain for use with PLASMID+® media. E. coli XL1-Blue also produces high quality plasmid DNA and may improve plasmid DNA yields with plasmids smaller than 3kb.

## Seed Culture

A seed culture is recommended for culture volumes larger than 50mL. Cultures less than 50mL may be inoculated directly from a glycerol stock or plate. To create a seed culture, use 50mL of Plasmid+® in a 250mL Ultra Yield® Flask sealed with an Enhanced AirOtop® Seal. Cultures less than 50mL may be inoculated directly from a glycerol stock or plate. To prepare a seed culture, use a glycerol stock or plate to inoculate 1/100th of the final culture volume of PLASMID+® + appropriate antibiotic (e.g. 100 µg/mL ampicillin; 50 µg/mL kanamycin) and grow to saturation with shaking at 37°C.

## Ultra Yield Flask Cultures

Using aseptic technique, add PLASMID+® media and appropriate antibiotic (e.g. 100µg/mL ampicillin; 50µg/mL kanamycin) to one or more Ultra Yield® Flasks inoculate the media, place an AirOtop seal on the flask, and grow at 37°C with shaking at 350rpm for 16-18 hours.

## Plasmid DNA Purification Adjustments for PLASMID+® Cultures

Plasmid DNA may be purified from PLASMID+® cultures by the common methods (e.g. Qiagen* Mega/Giga kits, etc.). The maximum loading capacity for Mega Kits is 5mg of DNA and 20mg of DNA for Giga Kits. PLASMID +® media typically yields are 5-10 times higher when compared to LB media. The increased cell mass and plasmid DNA content must be taken into consideration to insure efficient lysis and to avoid overloading purification columns. When using plasmid purification kits, the culture volume per purification should be decreased by a factor of 5 with respect to the recommended LB culture volume.

## Plasmid+® Media: Maximum Column Loading

We recommend resuspending the cell pellet using 10 mL of P1 buffer per gram of cell pellet. If preferred, using a volume of P1 buffer equivalent to half of the Plasmid+® culture volume is acceptable , ensure the P2 lysis buffer volumes are appropriate for higher cell density.

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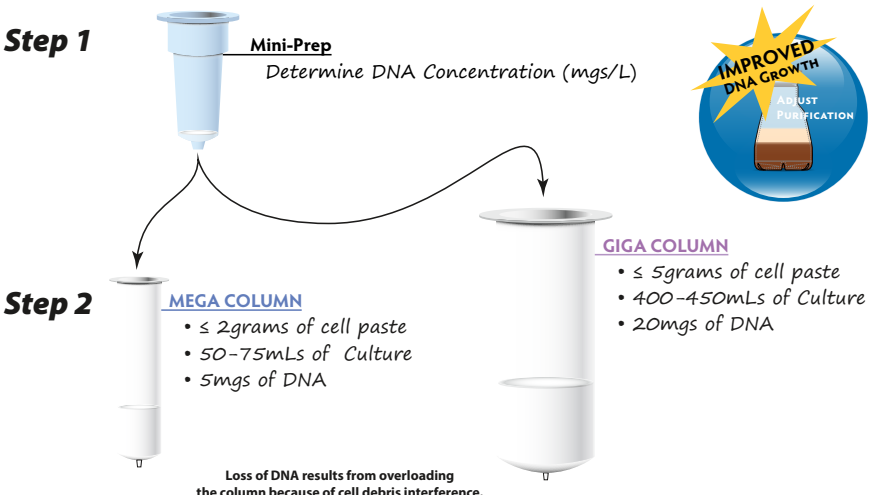


## How Many Mega Kits or Giga Kits Are Needed?

| Plasmid Run | Qiagen® Mega-Kits | Qiagen® Giga Kits |
|---|---|---|
| Plasmid A | 46 | 12 |
| gWiz | 63 | 16 |
| pVAX1-EGFP | 38 | 10 |
| pUC19 | 12 | 3 |
| Plasmid (A) | 6 | 2 |
| Plasmid (B) | 5 | 2 |
| Plasmid (C) | 4 | 1 |

## Troubleshooting

| | |
|---|---|
| Low Protein Yield | • Check that the proper antibiotic and concentration is used<br>• Insure proper culture aeration. Use the recommended media volumes in Ultra YieldTM Flasks with shaking at 350 rpm<br>• Increase the growth time (for up to 48 hours)<br>• Use a starter culture for final culture volumes > 50mL<br>• Protein may be toxic, try growth at 16°C. Growth time may need to be increased at 16°C |
| Low Recovery From Purification | • Make sure resuspension of cell pellet is complete<br>• Use enough resin for higher quantity yields |

## PLASMID+® vs Circlegrow®

| Plasmid | Circlegrow® mg/L | Plasmid+® mg/L |
|---|---|---|
| 250mL UYF (A) | 6.40 | 50.86 |
| 250mL UYF (B) | 4.60 | 54.86 |
| 2.5L UYF (A) | 3.27 | 53.43 |
| 2.5L UYF (B) | 6.13 | 53.71 |

## Plasmid DNA Purification and Yield Determination

1. Pellet an appropriate volume of fresh fermentation culture by centrifuging at about 13,000 x g for 5 minutes. Remove supernatant completely and analyze immediately or store at -80°C to -20°C for later yield determination. It is good to take multiple cell pellets for each sample point that can be frozen and saved if it is necessary to repeat the analysis. Determination of plasmid yield using the QIAprep* Spin columns is accurate for cell pellet samples containing 10 to 20µg of pDNA; beyond

20µg the columns will be overloaded and plasmid yield will be underestimated.

**Note:** 50µL is about the minimum volume of a dense culture that can be accurately pipetted. Smaller volumes of culture can be lysed, if necessary, by adding the culture directly to the P1 buffer, or by using a smaller fraction of a resuspended pellet. Culture volumes ≤20µL may be added directly to the 250µL Buffer P1 without pelleting (such small volumes may be required to prevent column overloading with very high yielding fermentations).

2. Resuspend pelleted bacterial cells in 250µL Buffer P1 and transfer to a microcentrifuge tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
3. Add 250µL Buffer P2 and mix thoroughly by inverting the tube 4–6 times. Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Allow the lysis reaction to proceed for 3 minutes
4. Add 350µL Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.
5. Centrifuge for 10 min. at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge. A compact white pellet will form.
6. Apply the supernatants from Step 5 to the QIAprep* spin column by pipetting. Be sure to collect as much of the supernatant as possible without disturbing the pellet.
7. Centrifuge for 60 seconds. Discard the flow-through.
8. Wash QIAprep* spin column by adding 0.75mL Buffer PE and centrifuging for 60 seconds.
9. Discard the flow-through, and centrifuge for an additional 1 min. to remove residual wash buffer. Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. Place the QIAprep* column in a clean 1.5mL microcentrifuge tube. To elute DNA, add 50µL Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of each QIAprep* spin column, let stand for 1 min., and centrifuge for 1 min. Add another 50µL Buffer EB to the center of each QIAprep* spin column, let stand for 1 min, and centrifuge for 1 min. The total elution volume for each spin column will be about 100µL. Performing the elution twice with 50µL Buffer EB insures complete recovery of plasmid DNA.
11. Determine the plasmid concentration and purity by triplicate measurement of A260 and A280 of a 40 fold dilution with TE of the EB elution. Calculate the plasmid concentration in the EB elution by correcting for the dilution (multiply by 40) and multiplying by the DNA concentration factor for A260 (1 OD at 260 nm corresponds to 0.050 mg/mL double stranded DNA): pDNA mg/mL = A260 x 40 x 0.050 mg/mL. The ratio A260/A280 should be between 1.8 and 2.0. If this ratio to too high or low repeat the reading.
12. Calculate the plasmid yield in the culture. The plasmid yield in mg pDNA per liter culture is back calculated by dividing the amount of pDNA obtained from the miniprep by the volume of culture that was used for the miniprep:

**Plasmid mg/L = (100µL) x (miniprep concentration, µg/µL) / (mL of culture pelleted)**

Finally, make sure that the total amount of pDNA from each miniprep is 10 to 20µg for the most accurate yield determination; if necessary, repeat the yield analysis with a different volume of culture.