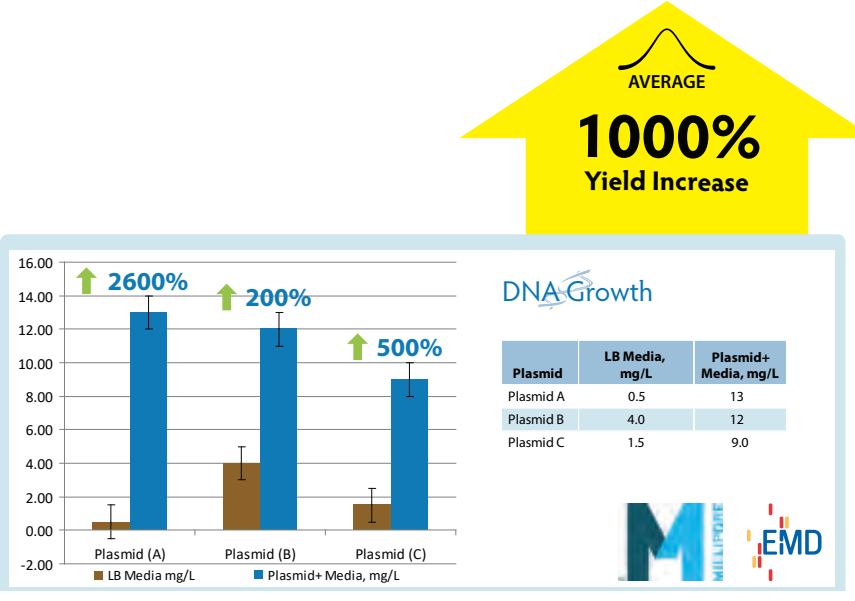
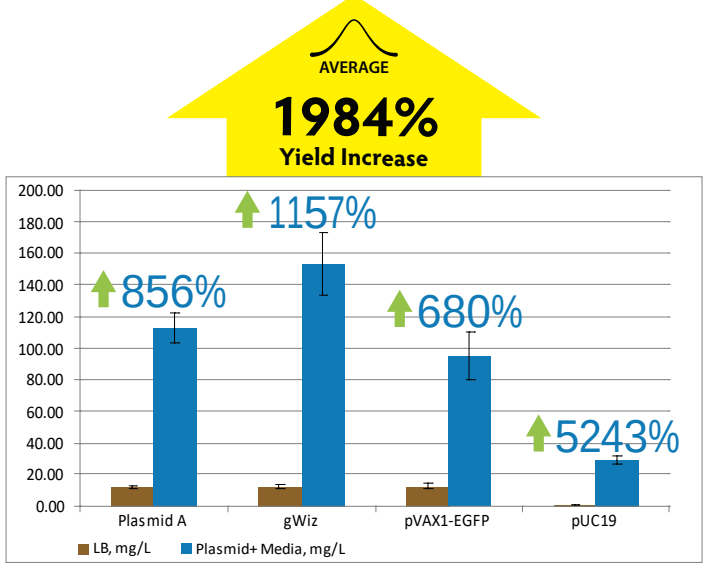
THOMSON Solutions ™ At Work
INSTRUMENT COMPANY

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



Thomson Ultra Yield Flask™ for DNA Growth

Data for this graph can be found on our website htslabs.com in the Technical Library



Improved DNA Protocol for E. coli

Storage

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

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.

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)

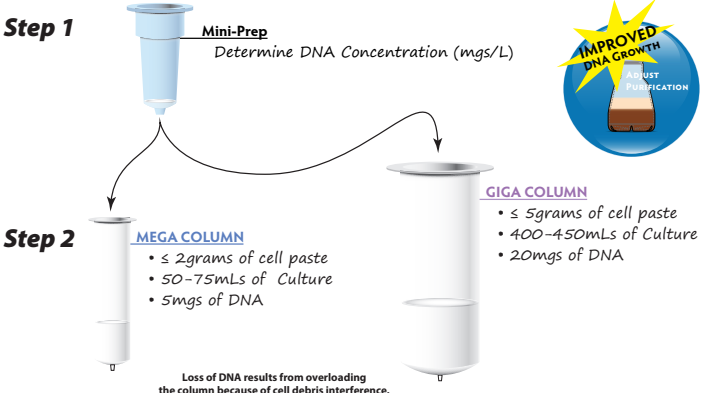
to one or more Ultra Yield™ Flasks inoculate the media, place an AirOtop seal on the flask, and grow at 37°C with shaking at 350rpm for 16-18 hours.

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. For the full protocol please see our Technical Library at htslabs.com



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 • Insure proper culture aeration. Use the recommended media volumes in Ultra YieldTM Flasks with shaking at 350 rpm • Increase the growth time (for up to 48 hours) • Use a starter culture for final culture volumes > 50mL • 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 • Use enough resin for higher quantity yields