LB (Lysogeny Broth/Agar)

Lysogeny broth, commonly shortened to LB, is the most commonly used rich medium for bacterial culture, especially for *E. coli*. It is a solution of tryptone, yeast extract, and NaCl.
The main carbon source is the oligopeptides from the two peptones (protein sources): tryptone and yeast extract. Tryptone is the result of digestion of bovine milk casein with the pancreatic protease trypsin. Yeast extract is the autolysate of yeast, where the yeast proteome from ruptured cells is digested by endogenous proteases. *E. coli uses* oligopeptide permeases and proteases to import and digest tryptone and yeast extract peptides into amino acids, which are utilized as primary carbon sources in order of the strain's preference [(1)] . As a result, growth in LB does not acidify the medium as with growth on most sugar/carbohydrate carbon sources; LB is alkalified to pH 9 at saturation [(1)] at which point exhaustion of a carbon source arrests growth (not the alkaline pH) [(1)] . Yeast extract is a complex, widely used hydrolysate of yeasts that provides amino acids/peptides, nucleotides, water-soluble metabolic cofactor "B vitamins", some carbohydrates, and other growth enhancers, which can enable growth of diverse microbes with differing nutrient needs. Yeast extract content was found to be the main determining factor for plasmid yield in *E. coli*, resulting in a higher yeast extract version of LB called LB^{30} (4,6).
LB has limitations; no conclusions about *E. coli* physiology ought to be made in LB [(2)] , but rather in a rationally-designed defined medium, like M9 or MOPS minimal [(3)] .

For improving low- and high-copy plasmid production efficiency/yield, Shyam Bhakta devised LB ^{30} and variants based on the literature (Notes).

Note: The term "LB" is used both for the broth and the agar "lysogeny agar", originally LA, and it is often incorrectly(2,12) expanded to "Luria broth" or "Luria-Bertani medium". The common formulation differs from Bertani(12) and Lennox's(13) broths by the omission of 1 g/L glucose, and only LB-Lennox maintains only 5 g/L NaCl, whereas the most common LB-Miller keeps Bertani's original 10 g/L.

1. Combine the following in an appropriate sized flask or bottle:
    a. **For broth:**
        - 25 g/L LB powder
          or from separate components:
            - 10 g/L tryptone
            - 5 g/L yeast extract
            - 10 g/L NaCl for LB-Miller (common). 5 g/L for LB-Lennox. 0.5 g/L for LB-Luria.
    b. **For agar:**
        - 40 g/L LB agar powder
          or from separate components:
            - 10 g/L tryptone
            - 5 g/L yeast extract
            - 10 g/L NaCl for LB-Miller (common). 5 g/L for LB-Lennox. 0.5 g/L for LB-Luria.
            - 15 g/L agar
    c. **For LB^{30}:** Add an additional 25 g/L yeast extract to either recipe for a final 30 g/L.
    d. **Enhancements:**
        i. **$MgCl_2$** (or $MgSO_4$): add 5–10 mM from an autoclaved 1–2 M stock. Improves growth yield and rate, reduces cell stress and protein acetylation.(5,Notes). Prefer Cl salt (*Not es*).
        ii. **Buffer**: add 1× M9 salts or TB salts from an autoclaved 5× or 10× stock. Reduces cell stress, purportedly. Alternative: roughly 100 mM final Na/K phosphate buffer.
        iii. **Carbohydrate**: add pre-autoclaved 5 g/L (0.5% $_{m/V}$) glucose or 10 g/L (7.94% $_{V/V}$) glycerol after autoclaving. Improves cell yield, reduces cell stress. Glycerol catabolism is less acidifying than glucose. May not improve plasmid yield [(4)] . Only use in combination with Mg and buffer (5,*Notes*).
    e. **Water:** Milli-Q water or deionized water up to final volume.
2. Close and shake to partially combine, or stir with stir bar and remove. Flasks can be closed with parafilm or gloved hand for shaking. Remove stir bar from broth with a magnet.
   For agar, adding a stir bar is advised for quicker and uniform cooling prior to pouring, unless using a 50–60° water bath.
3. Sterilize by autoclaving for 15–20 min, 15 psi on the liquid cycle; 15 min for 1 L volume(s) and 20 min for >1 L volume(s).
4. Allow the medium to cool to 50–55°C. Add any antibiotics or other thermosensitive additives, *e.g.* inducers, *only* when the vessel's temperature has fallen to 50–55°C. Swirl thoroughly to distribute additives if not using a stir bar.

Cloning Broth: LB^{30} $_{enh}$

Enhances low- and high-copy plasmid yields [(4)] and is more volume-efficient. Even better with 5–10 mM Mg and (maybe) 1× M9 salts, Shyam's standard cloning broth.

LB^{30}(regular) just has added yeast extract compared to LB; LB ^{30} $_{Mg}$ has additional Mg; LB ^{30} $_{enh}$ has additional Mg and buffering (Shyam, 6).

- 878 mL/L Milli-Q or deionized **water**
    - *Add half volume first. Rinse bottle sides with remaining half at end.*
    - *Using scale for water saves you from measuring volume.*
- 25 g/L **LB powder**
  or from separate components:
    - *10 g/L tryptone*
    - *5 g/L yeast extract*
    - *10 g/L NaCl*
- 25 g/L **yeast extract** (additionally, for final 30 g/L)
    - This can probably be increased.
- *Autoclave 15 min for 1 L volumes.*
  After cool enough to handle, add:
- 10 mM **$MgCl_2$** (autoclaved, preferred). Or $MgSO_4$. 20 mL/L 2 M stock.
  Swirl to mix; then add:
- 1× M9 Salts or TB Salts or other **buffer** (100 mL/L 10× stock). *S wirl to mix.*
    - *I have been using old 10× M9 salts from previous lab members. Has not been tested if buffering improves "neutral" plasmid yields or just more toxic plasmid yields.*
- *opt.* Carbohydrate: *when needed, 4–10 g/L glucose or glycerol can be added for improving growth of strains with toxic constructs. Buffering and Mg supplementation should be used with simple carbon sources [(5, Notes)] . May not improve plasmid yield [(4, Notes)] .*

Cloning broth: YEMS

***Y**east **E**xtract, **M**agnesium, **S**alts. Since tryptone in LB seems to be pointless (see Notes on C/N source), this broth ought to outperform LB^{30} formulations above for plasmid production. Still in trial.*

- 40 g/L **yeast extract**
    - *Gibco might be better than Bacto. See Notes.*
    - *Might be able to increase even more.*

- *Cooling can be sped and made uniform by stirring with a stir bar added prior to autoclaving. Cooling rate can be maximized to 10 min for 1 L volumes by immersing the flask/bottle with stir bar in a small autoclave bin filled with water and set over a stir plate, stirring rapidly. Stir bar is essential, or else agar will congeal.*
- *For agar, monitor temperature closely using a tape thermometer held against the vessel surface over the medium, or an infrared thermometer pointed at a dry vessel surface in contact with the medium.*
- *Plates ought to be poured soon after medium reaches 50°C to allow time for pouring before agar congeals.*
- *Immersing the vessel in a 50°C water bath, as made using a "sous vide" immersion circulator clipped to the side of an autoclave bin filled with deionized water, will not maximize the cooling rate of the medium but will allow the most time flexibility when dispensing agar, as the agar will cool to the proper temperature and remain so until one is ready to add additives and until one is done dispensing the agar.*
- *Polystyrene Petri dishes may deform if agar is hotter than 70°C.*

5. **For agar**, dispense 15–20 mL into each Petri dish. Work quickly to avoid agar congealing in the vessel.
    a. *Pouring is fastest, but less accurate: stop pouring when ¾ dish is covered; then let the remainder fill, slightly swirling the dish or stack of dishes if needed.*
    b. *For more accuracy, use a 50 mL pipette to dispense, though slower and more prone to premature congealing and pipette clogging.*
    c. *Stacks of plates can be efficiently filled from the bottom up: fill a dish with one hand while holding its lid below the remainder empty stack above it in the other hand.*
    d. *Bubbles can be eliminated by very briefly (<1 s) passing a flame over the surface.*

- 10 mM **$MgCl_2$** (autoclaved, preferred). Or $MgSO_4$. 5 mL/L 2 M stock.
- **Salts**: NaCl or a buffer
    - 10 g/L NaCl, or
    - 1× M9 Salts or TB Salts or 60–100 mM K/Na phosphate buffer pH 7
      If using a buffer, NaCl should perhaps be omitted, since the buffer has plenty of salt.
- ***opt.* Carbohydrate**: *when needed, 4–10 g/L glucose or glycerol can be added for improving growth of strains with toxic constructs. Buffering and Mg supplementation should be used with simple carbon sources* [(5, Notes)] *. May not improve plasmid yield* [(4, Notes)] *.*

Notes

Autoclaving

It has been classically suggested to separately autoclave phosphate buffers and agar, and recently it was found that phosphate and agar react to form H_2O_2 [(9)] , but *E. coli* are less, if at all, significantly impacted due to catalase expression. Commercial mixes do not separate the two, yet instruct autoclaving. However, phosphate also induces precipitation of trace metals at high temperatures [(8)] , another reason to add phosphate buffer separately after components are cool. There is also reason for separately autoclaving carbohydrate sources separately, as they partake in Maillard reactions with tryptone [(8)] , though glycerol probably less so than sugars do due to lack of carbonyls. Maillard reactions sequester nutrients and form products that decompose into growth-inhibitory compounds [(8)] .

Autoclaving 1 L volumes for 15 min as per CSH protocols has been sufficient for sterility. Minimizing autoclaving time reduces Maillard browning reactions that decompose nutrients and darken the medium. 1–1.5 L volumes benefit from 20 min autoclaving; agar remains cloudy with 15 min autoclaving.

Magnesium and Buffering

Magnesium is deficient in LB [(2,5)]. It has been found that in peptide broths, supplemental Mg^{2+} is necessary for optimal growth, especially when a carbohydrate carbon source such as glucose is provided [(5)] . 1 mM $MgCl_2$ supplementation improves *E. coli*, *V. fischeri*, and *B. subtilis* growth by prolonging exponential phase [(5)] . Contaminant Mg^2 in phosphate salts or other peptide broth components is evidently insufficient. In the modified Table 2 below from (5), note how supplemental Mg and buffering yield 68% higher *E. coli* cell density. Also note how glucose can hardly increase yield without supplemental Mg, which when added allows successful glucose utilization and drives the pH too low and arrests growth, *except* when additionally buffered (in

Notes

Carbon and Nitrogen Sources

Yeast extract was found in a study to be the main determinant of plasmid yield, with plasmid yield of LB matching that of terrific broth (TB) when yeast extract concentration was matched [(4)] . Neither the buffering of TB nor the better glycerol carbon source were found to improve plasmid yield. 30 g/L yeast extract was the maximum tested in this study, yielding the "LB^{30}" above.

PDMR, a cheaper medium than $LB^{30}{}_{enh}$ likewise designed to maximize plasmid yield, has yet to be tested in our lab [(7)] , but since Terrific Broth (TB) only underperforms to PDMR ~25% in volumetric plasmid yield [(7)] a nd LB^{30} outperforms TB [(4)] , LB^{30} must perform even more closely to PDMR's plasmid production efficiency. TB was found to give fourfold the pUC19 plasmid volumetric yield relative to LB but at 65% the cost, also better in both respects relative to SOC [(4)] . PDM(R) was, however, twice as cost-effectiveness as TB for a quantity of plasmid.

4–10 g/L glucose or glycerol does seem to be useful in alleviating growth inhibition of toxic DNA constructs while cloning them. It would be interesting to independently verify that adding such increases plasmid yield in general for nontoxic constructs, and does not only increase cell yield. In both experiments and cloning, magnesium and buffering ought to be used with glucose, as discussed in the *Magnesium* section, and the same may be extrapolated for glycerol or other carbohydrates out of caution.

E. coli oligopeptide permeases can only uptake peptides 650 Da [(2)]. Yeast extracts have much more of their peptides+amino acids in this *E. coli*-usable range than tryptone and peptone [(11, fig 2 below)]. This might explain why Shyam found faster and slightly higher growth in a version of LB that replaces the tryptone with additional yeast extract [(fig 1 below)]. Superior to Bacto yeast extract ought to be Gibco yeast extract, which the manufacturer data shows is 13% higher in low-weight peptides in the

LB7), enabling full glucose utilization and a whopping 11.6 final OD, 2.8-fold more than plain LB.

1 mM $MgCl_2$ produces a slight 8% more cell yield than 1 mM $MgSO_4$ (5: fig S1) . Glucose was used at 4 g/L, and buffering by 100 mM KHPO pH 7. Mg supplementation was found to reduce protein acetylation by means of rerouting carbon from fermentation to biomass production (5: fig 6) .

TABLE 2

Effect of buffering on final cell density and pH

Supplement	LB		LB7	
	OD	pH	OD	pH
None	4.09 ±	8.73 ±	3.88 ±	7.50 ±
Magnesium	5.71 ±	8.71 ±	6.90 ±	7.35 ±
Glucose	4.76 ±	6.28 ±	4.12 ±	7.16 ±
Magnesium + glucose	5.93 ±	5.06 ±	11.56 ±	7.26 ±

[a]Averages of 9 replicates ± standard deviation.

Another study found that 95% of ribosome biogenesis and purine metabolism genes are downregulated by low Mg^2 conditions (*e.g.* LB), and 95% of flagellar genes are downregulated by sufficient/high-magnesium conditions (thus less biofilmy) (10: fig7) . Studies find "ATP synthesis is tightly coordinated with Mg^2 availability… cells will scavenge Mg^2 from ribosomes at the expense of protein synthesis before allowing intracellular Mg^2 to fall to levels that are insufficient to support ATP chelation. (14)"

Conclusion: 1–10 mM $MgCl_2$ or $MgSO_4$ can be supplemented to any peptide broth for higher cell yield and ATP and protein synthesis with less cell stress and biofilm.

Buffering Salts

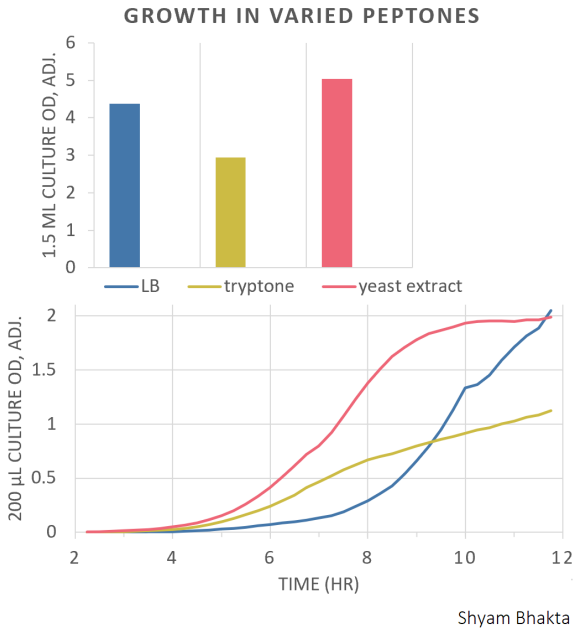
M9 salts: See M9 Medium

TB (Terrific Broth) salts, 10×: 23.1 g/L KHPO (monobasic), 125.4 g/L KHPO (dibasic).

<600 Da range that *E. coli* can eat, compared to Bacto tryptone (11). And the free amino acid data in the manual shows the Gibco YE has equal or significantly more of each free amino acid compared to Bacto YE. Also, Gibco YE has a fifth the sodium. Casamino acids are nearly completely hydrolyzed bovine casein, and though it has the most peptides in the *E. coli*-usable range, it has the disadvantage of being absent in tryptophan, asparagine, and glutamine, destroyed during acid hydrolysis.

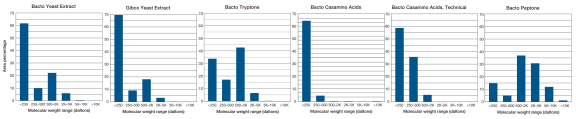
Growth of *E. coli* DH10B in three media, all supplemented with 5 mM $MgCl_2$ (and chl^{25} to maintain a plasmid):

- "LB"-Miller (5 g/L Bacto yeast extract, 10 g/L Bacto tryptone, 10 g/L NaCl)
- "tryptone" (15 g/L tryptone, 10 g/L NaCl)
- "yeast extract" (15 g/L yeast extract, 10 g/L NaCl).



Shyam Bhakta

From the ThermoFisher (Gibco/Bacto) *Technical guide to peptones, supplements, and feeds* (11)



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