Preparation of media for bacterial growth, according to the methods of Studier[1]

The paper cited gives recipes for optimised media for the growth of bacteria for plasmid preparation, working stocks (which can be kept in the cold room for weeks without loss of viability), expression testing, high-yield expression, and the preparation of labelled proteins for X-ray crystallography or NMR. The use of these methods has an excellent potential for improving the efficiency of our work, reducing risks, and eliminating waste of materials. My experience of using these systems has been extremely positive and I commend them to all users of bacterial expression systems.

The recipes here are for the media that Studier recommends in his paper, and states that his laboratory and colleagues routinely use for their work. It may be that some of these media will in time be superseded: if so, let me (and everybody else) know!

"ZYM-5052": media for high-level expression of auto-induced proteins

This media is suitable for the expression of proteins from T7 promoters in BL21 (DE3). The container for the media should be filled to one-tenth to one-quarter of its total volume (i.e. 200-500 ml in a 2-litre flask). Filling the flask more than this will lead to the growth and expression being limited by the availability of oxygen, and may reduce the overall yield (apart from being extremely bad microbiological practice).

To make 500 mL, add:
- 5 g Tryptone (or other casein digest)
- 2.5 g Yeast Extract
- demineralised water to 475 mL
- Autoclave

Then add the following sterile stocks:
- 12.5 mL of 40X "M" solution
- 0.5 mL of 2 M $MgSO_4$
- 10 mL of 50X "5052" solution
- 100 μL of 1000X trace metals solution
- Antibiotic(s) appropriate for your cells

Cells should be grown at 20-30° with shaking at 250 rpm (or even 350 rpm). Generally, expression is slightly better at 20°C, but it may be necessary to grow at 37°C for a few hours to get the growth going as the doubling time is much longer at 20°C. Approximately 24 hours growth (at 30°C, or 20°C with an initial growth at higher temperature) or 48 hours growth (at 20°C) should yield the highest cell density and expression levels.

[1] Studier FW (2005) Protein production by auto-induction in high-density shaking cultures, *Protein Expression and Purification*, **41**, 207-234.