

## Superbroth recipe and growing HB101

Adapted from protocol by Michael Koelle and Tory Herman, by Erik Andersen May 20, 2011

### Superbroth (2.5 L autoclaved in a 6 L flask)

30 g Bactotryptone  
60 g yeast extract  
20 mL 50% glycerol stock solution  
2.25 liters DDW

Autoclave 30 minutes. Cool to  $< 60$  °C.

Prepare 0.17M  $\text{KH}_2\text{PO}_4$ , 0.72M  $\text{K}_2\text{HPO}_4$  (46.2 g  $\text{KH}_2\text{PO}_4$ , 250.8 g  $\text{K}_2\text{HPO}_4$  per 2 liters),  
Aliquot 250 mL to a 500 mL bottle and autoclave.

When the superbroth is cooled, add 250 mL of sterile 0.17M  $\text{KH}_2\text{PO}_4$ , 0.72M  $\text{K}_2\text{HPO}_4$  to each flask.

### GROWING HB101 (or other *E. coli* strain)

1. Prepare 2.5 L of Superbroth in a 6 L flask
2. Grow 5 mL of HB101 overnight for each 6 L flask.
3. Inoculate 5 mL of HB101 into each 6 L flask
4. Grow for 24 hours at 37° C with shaking
5. Spin down the bacteria
6. Do two washes using S basal by resuspending the bacterial pellet.
7. Pellet and either (1) weigh the pellet to get mg of bacteria to make w/v sample, or (2) add defined volume of S medium to make v/v sample.
8. Aliquot or store at 4°C for immediate use.