BACTERIOLOGY

1. Bacterial Media

LB Medium

10 g Bacto-tryptone
5 g Bacto-yeast extract
10 g NaCl
-dissolve in ~900 ml H_20
-adjust pH to 7.5 w/NaOH
-q.s. to 1L with H_2O

NZCYM

10 g NZ amine
5 g NaCl
5 g yeast extract
1 g casamino acids
2 g $MgSO_4 \cdot 7H_20$
-dissolve in ~900 ml
-adjust to pH 7.5 w/NaOH
-q.s. to 1L with H_2O

Media should be autoclaved minimum of 20 mins. on liquid cycle.

Plates and Top Agarose

Plates of 1.5% agar/LB medium are commonly used to grow bacteria. Antibiotics can be included in the medium, to use for specific selection procedures.

Preparation of 1.5% agar/LB medium plates:

i. Include 15 g. agar per liter medium before autoclaving. Agar will not dissolve until medium is autoclaved.

ii. To include antibiotics cool the autoclaved medium to ~55°C (a temp at which flask containing medium is cool enough to be held in hand, yet hot enough so it remains liquid). Add appropriate amount of a sterile antibiotic solution to achieve desired conc. Antibiotic solutions are sterilized by filtration through 0.22 μm filter (see CSH manual p.444 for making stocks).
Working concs.: Amp. = 50 μg/ml.
Tet. = 15-25 μg/ml

iii. After agar has cooled to ~55°C plates may be poured. This is done on the bench, using flame to keep media bottle sterile. Approx. 40-50 plates can be poured from 1 liter (10 cm sterile petri dishes). Flame to remove bubbles.

Top agarose - used in plating bacteriophage g-infected bacteria.
Preparation - 0.7% agarose/LB medium
Include 7 g agarose/liter medium before autoclaving. Store in 100-250 ml aliquots and melt in microwave before using.

2. Growing bacteria

1. Overnight culture

   Transfer LB sterilely to a sterile tube. Pick a single colony from a streaked plate and inoculate a 5 or 10 ml LB aliquot. The loop used for inoculation should be flamed (and briefly cooled) before it touches the colony and after the inoculation. Grow the culture overnight at 37°C with vigorous shaking.

2. Streaked Plate

   Bacteria can be picked from a frozen culture by scratching the sterile loop across the surface of the culture or they can be picked from a liquid culture by immersing loop in it. In either case the loop is used to streak out the cells on the surface of a 1.5% agar/LB plate. Colonies will be visible after 12-16 hours growth at 37°C. Plates should be inverted in the incubator to prevent condensation from dripping on the colonies.

3. Storage

1. Bacteria can be stored several weeks on agar plates at 4°C if plates are wrapped in parafilm and stored inverted.

2. Medium-term storage in stab cultures
   -Inoculate a small vial containing 2-3 ml 1.5% agar/LB with a sterile, straight wire which was dipped into an overnight culture.
   -Grow stab culture o.n. at 37°C with the lid loose.
   -Tighten lid, wrap in parafilm, and store in dark at room temp.

3. Long. term storage at -70°C in 15% glycerol
   -Mix 150 µl sterile glycerol and 850 ul overnight culture
   -store cultures at -20°C for few years or at -70°C for many years.

Reference: Molecular Cloning, T. Maniatis, E.F. Fritsch, and J. Sambrook (1982) pp. 61-62, 68, 442, 444.