APh 162 Size/Rate of Things general protocols

LB is the standard medium used to grow bacteria, principally *Escherichia Coli*. The original intent of the creators was for LB to stand for "lysogeny broth"; however, it has also been known as Luria broth, or Luria-Bertani medium or Lennox broth. Bertani was the inventor of LB and was a student with Max Delbruck at Caltech. They were investigating phage lysis of E. Coli.

-LB for bacteria, both solid and liquid.

Per liter, add:
10 g tryptone
5g yeast extract
5g NaCl
1mL 1 N NaCl (optional)

Add ingredients to water in a 2L flask, and heat with stirring until dissolved. Autoclave 25 min. Do not dilute.

M9 salts are used for making minimal media; the salts provide basic ionic buffering for the cells, and also provide an environment with comfortable osmotic properties. However, there are no nutrients.

-5x M9 salts for bacteria.

Per Liter:
30g Na2HPO4
15g KH2PO4
5g NH4Cl
2.5g NaCl
15mg CaCl2 (optional)

Add ingredients to water in 2L flast and heat with stirring until dissolved. Pour into bottles with loosened caps and autoclave 15 min at 15 lb/in^2. Cool to < 50 deg C before adding nutritional supplements and antibiotics. Tighten caps and store concentrated media indefinitely at RT.

Before use, dilute concentrated media to 1x w/ sterile water and add the following sterile solutions, per liter to make minimal (MGC) media:

-MGC (this is the minimal medium I use for cells I'm gonna look at under the scope).
1X M9 minimal media (1X salts + 1mL 1M MgSO4)
0.2% glycerol
.01% casamino acids
.15 ug/mL biotin
1.5 uM thiamine

for 500 mL, mix together
100 mL sterile 5X M9 salts
1mL 1M $MgSO_4$
2mL 50% glycerol
1mL 5% casamino acids
500 uL 150 ug/mL biotin
125 uL 6mM thiamine
+ ~400 mL water to 500 mL total
mix together, filter sterilize

If one is making bacteria with an antibiotic resistance plasmid, a good way to prevent contamination of anything else is to add antibiotics to kill everything else. These should be added after autoclaving, to a solution of media that has cooled to below 50 C (hot to the touch, but bearable).

-Ampicilin, Kanamycin concentrations (both working and stock solutions).

Additives:
ampicillin to stock: 4 mg/mL, working: 50 microg/mL
tetracycline, in 70% ethanol to stock: 12 mg/mL, working: 12 microg/mL
note: store tetracycline in the dark

YPD medium is for growing yeast.

-YPD for yeast, both solid and liquid.

Per liter:
10g yeast extract
20g peptone
20g dextrose

Final concentration:
1% yeast extract
2% peptone
2% dextrose

Dissolve in 1 L water, mix until completely dissolved, autoclave in 100-500mL bottles.
Or: filter sterilize

If solid media is desired, to the above liquid media (LB, MGC, YPD), add the following:

Solid:
1. Follow procedure above
2. Add 20g agar, a pellet of NaOH

After:
1. Add a stir bar for mixing after autoclaving.
2. After autoclaving, leave at RT for ~45-60 min until ~50 deg C.
(Now add drugs, etc.)
3. Mix on stir plate until contents are homogeneous (~5 min)
4. Pour the plates (~30 mL)
5. If bubbles are in the plates, flame lightly.
6. One Liter --> ~30-35 plates (for 30 mL plates, natch)

-How to make plates. An important thing, especially for long term storage of plates, is to make sure all moisture has evaporated out of the dishes – this may involve temporarily storing the plates at high temperatures or waiting a few days for the plates to dry.

LB:
0. Add water to ingredients to 1 L. Autoclave 25 min.
1. Autoclave 25 min
2. Pour 32-40 mL of medium/plate.
3. Dry plates 2-3 days at RT, or 30 min with lids slightly off at 37 deg C. or in a laminar flow hood. Store dried plates wrapped at 4 deg C.

For M9 salts:
1. Autoclave 15g agar in 800mL water for 15 min.
2. Add 200mL sterile 5x M9 and carbon source.
3. Cool to 50 deg C. and add antibiotics.
4. Pour 32-40 mL medium/plate for ~25-30 plates/liter.

Once we have the plates, we should use them to grow something:

-How to plate cells and inoculate medium.
Use either disposable toothpicks or inoculating loops. For the latter, sterilize by applying flame until it is red hot. Cool by touching to surface of agar plate.

Plating cells:
1. Pipet 0.05 to 1 mL of culture onto a plate
2. Dunk a spreader in ethanol and flaming the ethanol off. After flame flames out, touch to surface of agar to cool.
3. spread using circular motion
4. Incubate at 37 deg C with plate lid ajar until completely dry

Alternative:
1. Streak an inoculum of culture across one side of plate
2. Resterilize loop and streak a sample from first sample across fresh part of plate.
3. Repeat until plate is covered
3. Incubate at 37 deg C.

The following two protocols are useful for growing up some cells for visualization on microscope slides.

Growing cultures:
1. Transfer 5mL of medium into a sterils 16-18 mm culture tube
2. Inoculate with single bacterial colony on an inoculating loop by dipping and shaking the loop in the medium.
3. Cap the tube and grow at 37 deg C. to saturation (~6hr) in a shaker or on a roller drum, 60 rpm.

Alternative:
1. Dilute overnight cultures 1:100 in Erlenmeyer flask that is >5x volume of culture (or 20x, without the agitation).
2. Grow at 37 deg C. with vigorous agitation, ~300 rpm.