**NM medium** (minimal glucose medium lacking histidine)

For 500 ml:

Mix these components together in the order listed:

418 ml ddH2O

50 ml 10 x M9 salts

10 ml 20% glucose

5ml 20 mM adenine HCl

15 ml amino acid mixture (see recipe below)

0.5 ml 1 M MgSO4

0.5 ml 10 mg/ml thiamine

0.5 ml 10 mM ZnSO4

0.5 ml 100 mM CaCl2

5 ml 20 mM uracil

filter sterilize and store at 4 °C,

add proper antibiotics, IPTG, and 3-AT as desired

if adding histidine, include at a final conc of 0.1%

For 500 ml agar:

Autoclave 418 ml ddH2O, 7.5 g bacto-agar, and a stir bar; while agar cools to about 65 °C, mix the following components in the order listed:

50 ml 10 x M9 salts

10 ml 20% glucose

5ml 20 mM adenine HCl

15 ml amino acid mixture (see recipe below)

0.5 ml 1 M MgSO4

0.5 ml 10 mg/ml thiamine

0.5 ml 10 mM ZnSO4

0.5 ml 100 mM CaCl2

5 ml 20 mM uracil

filter sterilize

add proper antibiotics, IPTG, and 3-AT as desired

add the above mixture to the agar while stirring and then pour plates.

**Amino acid solution for NM medium**

Solution I (200x):

Phe (0.99%) 0.99 g, Lys (1.1%) 1.1 g, Arg (2.5%) 2.5 g, dissolve to 100 ml water;

Solution II (200x)

Gly (0.2%) 0.2 g, Val (0.7%) 0.7 g, Ala (0.84%) 0.84 g, Trp (0.41%) 0.41 g, dissolve to 100 ml water;

Solution III (200x)

Thr (0.71%) 0.71g, Ser (8.4%) 8.4 g, Pro (4.6%) 4.6 g, Asn (0.96%) 0.96 g, dissolve to 100 ml water;

Solution IV (200x)

First make up 9.1 ml 36.5% HCl, add 80 ml water, then dissolve Asp (free acid) (1.04%) 1.04 g, Gln (14.6%) 14.6 g to 100 ml water;

Solution V (200x)

Dissolve K.Glu (18.7%) 18.7 g in 80 ml water, then add Tyr (0.36%) 0.36 g, + 4 g NaOH pellets, add water to 100 ml;

Solution VI (200x)

Ile (0.79%), 0.79 g, Leu (0.77%) 0.77 g, dissolve to 100 ml water.

These 6 solutions have 17 of the 20 amino acids (except His, Met, and Cys).

Mix equal volumes of all 6 solutions to get 100/3 X

amino acid mixture and store at 4 °C.