**003 Reagents**

Complete Media (1X CM)

1. 700ml of ddH20 (left tap on sink) that has been autoclaved (in a 1 Liter container).
2. 100ml 20% glucose (glucose = dextrose)
3. 100ml 10X YNB (stored in fridge)
4. 100ml 10X CM dropout mix (stored in fridge)
5. Store in fridge (long-term) or on bench (short-term)

20% Dextrose (D-glucose) (1L)

* + 1. **200g** Dextrose (D-glucose)
    2. Add diH2O up **to 1L**
    3. Autoclave

Notes:

* + Can be stored for at least 1 year in refrigerator
  + Be sure to check bottom for sediment – could be a slow growing contaminant
  + It is normal for there to be a few particles, but there should not be too much
  + Avoid batch effects – ensure that there will be enough to last through an entire experiment. Do not want to have to make more part way through.

10X YNB (1L)

* 1. **17g** Yeast Nitrogen Base without amino acid and ammonium sulfate
  2. **50g** Ammonium sulfate
  3. add diH2O up **to 1L**
  4. Autoclave

Notes:

* + Can be stored for at least 1 year in refrigerator
  + Be sure to check bottom for sediment – could be a slow growing contaminant
  + It is normal for there to be a few particles, but there should not be too much
  + Avoid batch effects – ensure that there will be enough to last through an entire experiment. Do not want to have to make more part way through.

10X CM dropout mix (1L)

* 1. **13g** CM dropout powder
  2. Add diH2O up **to 1L** (use autoclaved diH2O if possible, but not necessary)
  3. Filter sterilize

Notes:

* + Can be stored for at least 1 year in refrigerator
  + Be sure to check bottom for sediment – could be a slow growing contaminant
  + It is normal for there to be a few particles, but there should not be too much
  + Avoid batch effects – ensure that there will be enough to last through an entire experiment. Do not want to have to make more part way through.

Complete Media + Tartaric Acid (1X CM + TA)

* + 1. 800ml 1X CM
    2. 400ml 150 mM tartaric acid (sodium) buffer solution (pH=2.9) in 0.5L 1x CM
    3. 400ml 150 mM tartaric acid (sodium) buffer solution (pH=4.2) in 0.5L 1x CM
    4. Adjust pH to 3.52 using 150 mM tartaric acid (sodium) buffer solution (pH=2.9) in 0.5L 1x CM

150 mM tartaric acid (sodium) buffer solution (pH=2.9) in 0.5L 1x CM

1. 500mL 1x CM
2. 56.47 mmol (8.475g) Tartaric acid (M.W. = 150.09)
3. 18.91 mmol (4.35g) Sodium tartrate dehydrate (M.W. = 230.08)

150 mM tartaric acid (sodium) buffer solution (pH=4.2) in 0.5L 1x CM

1. 500mL 1x CM
2. 14.54 mmol (2.8125g) Tartaric acid (M.W. = 150.09)
3. 56.26 mmol (12.945g) Sodium tartrate dehydrate (M.W. = 230.08)

Chemical Stressors

**NaCl**

1x working solution

Concentration: 280 grams/Liter NaCl in 1x CM

Recipe:

1. 1000ml diH20 (autoclaved)
2. 280g NaCL

100% lethal solution

concentration: 20.006 grams/Liter

Recipe:

71.45 mL NaCl 1x working solution

928.55 mL 1x CM

**CuSO4**

1x working solution

Concentration: 100uM CuSO4 in 1x CM

Recipe:

First need to make 1M CuSO4 stock solution in diH20

1. 1000ml diH20 (autoclaved)
2. 1M (159.609 g) CuSO4 anhydrous

Next dilute the 1M stock

1. dilute 1M stock 1:100 for a 0.01M intermediate solution
2. dilute 0.01M intermediate solution 1:100 for a 0.0001 or 100um 1x working solution.

100% lethal solution

concentration: 8uM

Recipe:

80 mL cuSO4 1x working solution

920 mL 1x CM

**Na2SO3**

1x working solution

Concentration: 100mM Na2SO3 in 1x CM + TA

Recipe:

1000ul 1x CM + TA

0.1M (12.60 g) Na2SO3

100% lethal solution

concentration: 0.8mM

Recipe:

8.00 mL Na2SO3 1x working solution

992.00 mL 1x CM + TA