

Leucine Limitation Carboy for Disome Strains

- Autoclave carboy
- Make 20L stock 10X MDS:
 - Fill plastic carboy with approx. 17L MilliQ H₂O
 - Add with constant stirring:
 - CaCl₂·2H₂O 20g
 - NaCl 20g
 - MgSO₄·7 H₂O 100g
 - KH₂PO₄ (monobasic) 200g
 - (NH₄)₂SO₄ 1000g
 - Bring up to 20L with MilliQ H₂O
 - Aliquot into 2L media bottles
 - Autoclave

To prepare 1 10L carboy:

- Measure out ~500ml of water and add while stirring
 - If necessary for auxotrophs:
 - Adenine sulfate (5mg/mL) 100mL
 - Uracil (2mg/mL) 100mL
 - Tryptophan (5mg/mL) 100mL
 - Lysine (10mg/mL) 100mL
 - Methionine (5mg/mL) 40mL
 - 1g of geneticin
- necessary for all leu auxotrophs:
 - 1000x vitamins 10mL
 - 1000x metals 10mL
 - Dextrose 200g*
 - Leucine (10mg/mL) 15mL
- Add contents of flask to separate carboy with approx. 7L of MilliQ H₂O
- Rinse flask into carboy
- Measure 1L 10X MDS, add and rinse into to carboy
- Bring up to 10 L with MilliQ H₂O
- Mix by stirring for 5 minutes
- Filter sterilize into autoclaved carboy

Dunham, Maitreya. <http://genomics.princeton.edu/dunham/MDchemostat.pdf>

*Modified 11/09; new, higher glucose level compensates for glucose wasting.