

## Sulfur Limitation Carboy

- Autoclave carboy
- Make 5L stock 10X salts for sulfur limitation:
  - Fill 6L carboy/flask with approx. 4L MilliQ H<sub>2</sub>O
  - Add with constant stirring:

▪ CaCl <sub>2</sub> -2H <sub>2</sub> O	5g
▪ NaCl	5g
▪ MgCl <sub>2</sub> -6 H <sub>2</sub> O	20.6g
▪ NH <sub>4</sub> Cl	202.5g
▪ KH <sub>2</sub> PO <sub>4</sub> (monobasic)	50g
  - Bring up to 5L with MilliQ H<sub>2</sub>O
  - Aliquot into 2L media bottles
  - Autoclave

To prepare 1 10L carboy:

- Add the following to 1L of MilliQ H<sub>2</sub>O in a 1L flask and mix with magnetic stirrer until glucose is completely dissolved:

◦ 1000x vitamins	10mL
◦ 1000x metals	10mL
◦ 2 g/L (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	15mL
◦ Dextrose	200g*
- Add contents of flask to separate carboy with approx. 7L of MilliQ H<sub>2</sub>O, rinse flask into carboy
- Measure out 1L of 10X sulfur limited salts and add to carboy, rinse grad. cylinder into carboy
- Bring up to 10 L with MilliQ H<sub>2</sub>O
- Mix for 5 minutes with magnetic stirrer
- Filter sterilize into autoclaved carboy

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

Modified, S. Silverman 6/2006

\*Modified 11/09, new, higher level of glucose