## **Phosphate Limitation Carboy**

- Autoclave carboy
- Make 5L stock 10X salts for phosphate limitation:
  - Fill 6L carboy/flask with approx. 4L MilliQ H<sub>2</sub>O 0
  - Add with constant stirring: 0
    - CaCl<sub>2</sub><span style="font-family: Symbol;">·2H<sub>2</sub>O 5g</span >
      - NaCl 5g 25g </span >
    - . MgSO<sub>4</sub><span style="font-family: Symbol;">·7 H<sub>2</sub>O
    - $(NH_4)_2SO_4$ 250g
    - KCl\* 50g
  - Bring up to 5L with MilliQ H<sub>2</sub>O. 0
  - Aliquot into 2L bottles
  - Autoclave 0

To prepare 1 10L carboy:

- ٠ 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
  - $\circ$  1000x metals 10mL
  - 10 g/L KH<sub>2</sub>PO<sub>4</sub> (monobasic) 10mL\*\*
  - 200g\*\*\* • Dextrose
- ٠ 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 phosphate 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 ٠

## \*NOTE: this is the new, higher amount of potassium; 7/2005

\*\*NOTE: Limiting phosphate varies...specify when ordering...default is 10mg/L

>> previously 20: some strains might not be limited at 20<< modified 4/8/2011

\*\*\*NOTE: New, higher glucose level; 11/09

## Dunham, Maitreya. <a

href="http://genomics.princeton.edu/dunham/MDchemostat.pdf">http://genomics.princeton.edu/dunham/M Dchemostat.pdf</a>