# Cellulose Production

1. Plate *Gluconoacetobacter xylinus* glycerol stock onto Herstin and Schram agar plates.
   * Incubation temp: 30oC
   * Colonies should form within ~7 days
   * Only a few plates of media should be needed.
   * The rich media is used to get a healthy stock which is needed to grow well on the minimal Heo and Son media
2. Streak *Gluconoacetobacter xylinus* from single colonies grown on the Herstin and Schram media to Heo and Son 0.5% glucose agar plates.
   * Media contains 0.5% v/v 12C-glucose and no inositol (Heo and Son 2002)
   * Incubation temp: 30oC
3. Autoclave media and 10 foil covered 1L flasks
   * **Note:** This recipe is for 1L of media, alter number of 1L flasks according to the amount of media you need.
4. Add all of the filter-sterlized components of the media **EXCEPT** for FeSO4
   * FeSO4 will produce a precipitant, which is hard to aliquot to all 1L flasks evenly
5. Aliquot 100 mL of media to each 1L flask
   * 1L flasks allows for a large liquid surface area for cellulose production
6. Add filter-sterlized FeSO4 to each 1L flask.
7. Inoculate each aliquot of media with three isolated colonies of *Gluconoacetobacter xylinus* from Heo and Son plate.
8. Keep flasks static in the dark at 30oC for 2-3 weeks until thick cellulose pellicule forms.
   * **WARNING:** Any disturbance to the flasks may prevent/stop pellicule production

# Cellulose Harvest

1. Pour off excess culture from cellulose growth flask
2. Make 1% Alconox solution
3. Add Alconox 2:1 with cellulose+ residual media
   * i.e., 200 mL of 1% alconox for every 100mL media culture
4. Autoclave for 30 minutes
5. Combine the pullicules into a large bucket/beaker
6. Rinse under high pressure DI water faucet
   * ~10 times, or until no suds being produced
   * Use a screen to hold over beaker to pour out water each time.
   * **Note:** the pellicules should be clear/white in color. Other coloring is probably due to contamination.
7. At end of rinsing, fill the bucket/beaker with DI H2O and cover.
8. Let sit for 12 hours at room temp, then rinse 3 times under high pressure DI.
   * Use >= 1L water for each rinse.
9. Fill with water and let sit for another 12 hours. Repeat this for 2 days
   * i.e., ~4 rinsings total
10. On the third day (after the 4th 12 hour soak):
    1. Rinse the cellulose another 3 times
    2. Decant all liquids and put only the cellulose on a pre-weighed weighboats
       * 1 weighboat per full-sized pellicule (this helps with drying)
11. Cover the weighboats with another weight to prevent contamination (especially of streptomyces spores)
12. Place the weighboats in a drying oven overnight
13. Weigh dried pellicules and calculate difference (from weighboat)
    * Record the pellicule weight

# Media

## Hestrin and Schram

* 2% glucose
* 0.5% peptone
* 0.5% yeast extract
* 0.27% Disodium phosphate anhydrous
* 0.115% Citric acid monohydrade
* 0.05% MgSO4 x 7H2O
* 2% agar
* adjust the pH to ~6

## Heo and Son

* See HeoAndSonMinimalMedia.xls
* **NOTE:** A precipitant will form shortly after adding the FeSO4
  + The precipitant will look 'fluffy'

# Notes

* Yield from 13C-cellulose farming in 2014: **~24%**

# References

1. [Moon-Soo Heo and Hong-Joo Son, Biotechnol Appl Biochem (2002) 36: 41-45](http://onlinelibrary.wiley.com/doi/10.1042/BA20020018/full)