# 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. Rinse under high pressure DI water faucet
   * ~10 times, or until no suds being produced
   * Use screen to hold over beaker to pour out water each time.
6. At end of rinsing, fill beaker with DI H2O and cover.
7. Let sit for 12 hours at room temp, then rinse each beaker 3 times under high pressure DI.
8. Fill with water and let sit for another 12 hours. Repeat this for 2 days
   * i.e., ~4 rinsings total
9. On the third day (after the 4th 12 hour soak), rinse cellulose about 3 times, then decant all liquids and put only the cellulose on a pre-weighed weighboat.
10. Cover weighboat with another weight to prevent contamination (especially of streptomyces spores)
11. Place in drying oven overnight
12. Weigh dried pellicules and calculate difference (from weighboat)

# 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)