# Cellulose Grinding Protocol

## Authorship

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## WARNINGS

* Make sure to not cross-contaminate 13C cellulose on equipment used to measure natural abundances of isotopes!
  + You should use Buckley lab equipment for handling the cellulose, especially the 13C cellulose.
  + We have equipment designated to hold just 12C or just 13C cellulose.
* Make sure to not mix up 13C and 12C cellulose!
  + Everything should be labeled 12C or 13C.
  + Only grind 12C or 13C at a time and keep the isotopes separate!

## Cellulose quality

The *Gluconoacetobacter xylinus* cultures should produce clear or white-ish pellicules that look smooth and don't have much of an odor. Culture contamination can be identified as a brownish color, funky vinegar/sweet odors, or fuzzy areas on the pellicule. We currently are still harvesting all cultures unless the contamination is very prominant (eg., fuzzy cultures). The cellulose is designated into 2 tiers:

* **'Prime' cellulose**
  + Cellulose from clear or white-ish pellicules that look smooth and don't have much of an odor
* **'Mediocre' cellulose**
  + Cellullose from tan/brown-ish pellicules that may have a funky odors.
  + Fuzzy cultures should be disposed of.

## Materials

1. Bacterial cellulose pellicules
2. Scissors
3. Bead beater
4. Bead beater canisters
   * There are canisters for just 12C or just 13C cellulose.
   * The canisters are marked with red and yellow tape, respectively.
5. Weighboats/weigh paper
6. Scale
7. 250 um sieves
   * There sieves for just 12C or just 13C cellulose.
8. Glass plates or large weight paper to collect the sieved cellulose.
9. Scintillation vials for holding ground cellulose.

## Procedure

1. Pre-weigh the scintillation vials so that you can measure the cellulose yield after grinding.
   * Record the weights.
   * **MAKE SURE:** Mark each vial as either 12C or 13C.
   * Also, mark each vial with the date and whether the cellulose is 'prime' or 'mediocre'.
2. Cut cellulose with sterilized scissors into small confetti pieces
3. Weigh out ~50 mg portions for grinding
   * Too much cellulose will cause clogging in the canister.
4. Add to appropriate bead beater canister (12C or 13C).
   * The canisters are marked with red and yellow tape, respectively.
   * **MAKE SURE:** Use the correct canister.
5. Place in bead beater machine (in Laurie Drinkwater lab) and bead for 1.5 min.
6. Place the sieve (use the appropriate sieve: 12C or 13C) on a glass plate or weigh paper.
7. Sift the ground cellulose through a 250 um sieve.
   * Anything that goes through the sieve gets put in a labeled scintillation vial.
   * Anything that remains in the sieve needs to be re-ground and re-sieved.
   * The sieving is to make sure the particle size is representative of particulate organic matter (POM) that microbes would experience in their natural environment.
8. Do until all cellulose confetti has been ground.
9. Weigh the vials containing cellulose to determine the total yeild of ground cellulose.
   * Record the cellulose yield.