Moss blending updated: Aug 2014

Materials:

- BCDA agar plates (Following Cove et. al 2009)
- Moss tissue (a full growing plate can make about 10 new plates)
- Autoclaved cellulose cellophanes (A.A. packaging limited)
- Autoclaved water
- Autoclaved grinders (also single use- need to be autoclaved) (OMNI-INC 3_750)
- Large serological pipette tips (10 mL)
- Serological pipette pump
- Styrofoam tube brick
- Sterile tissue culture tubes

All steps of procedure must be performed in a Laminar flow hood using sterile techniques

1. Sanitize hood area and grinder motor with alcohol
2. Verify moss tissue plates are free of contamination
3. Prepare cellophane plates
    a. Label plate
    b. Place cellophane on plate, allow to rehydrate, smooth out ridges
4. Flame sterilize the mouths of water bottles and tissue culture tubes
5. Add sterile water to culture tube (approx. 1 mL per plate being made)
6. Gather moss tissue and put into culture tube (put the lid back on)
7. Attach autoclaved grinder to grinding motor
    a. Only unwrap the top of the grinder until time to actually blend
8. Remove tube lid and bottom aluminum foil covering grinder
9. Blend (until the mixture is even and there aren't moss chunks, but not too much!)
10. Pipette blended moss onto cellophane BCDA plate (1 mL per plate)
11. Allow plates to rest, in hood, for water to be absorbed into the plate (~10-15 min)
12. Tape up the plate (try to keep plate flat)
13. If grinding more than one strain/individual, use new pipette tips and grinder
14. Wash used grinders. Prepare grinders, water, and unused cellophanes to be autoclaved for next time
15. Clean hood