Soybean Lima Bean Media (SBLB)
Used to isolate, maintain and increase the FLS pathogen in the lab.

Ingredients:

1. Cut up soybean stalks
   Any soybean variety is grown in the greenhouse for 4-12 weeks, cut, leaves removed and stalks cut up into 1 inch pieces. This is stored in the freezer for up to a year.

2. Lima Beans
   Frozen food section at grocery store

3. Granulated Agar

To Make:

- Place 283 grams lima beans in 2L glass jar, and cover with dH2O, (approx 600 ml).

- Weigh out 100 grams soybean stalk, and place in a table top blender with approx 600 ml dH20

- Blend for 30 seconds or until the stalk is finely shredded, then place into a 1L glass bottle.

- Autoclave both bottles until sterile, at least 45 minutes.

- After the bottles cool, (can be the next morning), use cheesecloth to strain the cooked lima beans rinsing out the bottle through the cheesecloth. Gently squeeze out any remaining liquid from the lima beans. Discard the lima bean pellet.

- Into the lima bean juice, strain the soybean stalk juice, washing the bottle through the cheesecloth. Gently squeeze out any remaining liquid from the stalks. Discard the pellet.

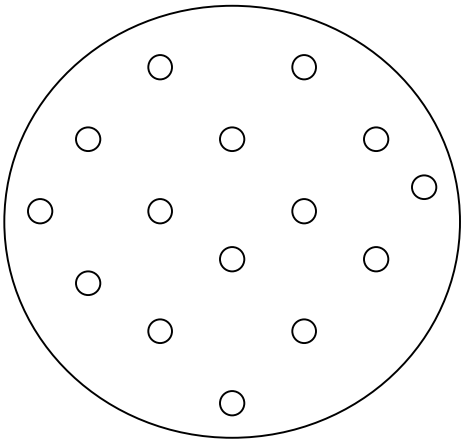
- Bring the volume of the juice to 2L and pour back into the 2L bottle.

- Add 40 g granulated agar and a stir bar. Autoclave until sterile, at least 40 minutes.

- When the agar is cool, mix and use a sterile beaker to pour plated thin, approx 15ml/plate.

Increasing FLS pathogen
Sterilize toothpicks in small glass tubes for this procedure. One toothpick/5 plates is needed.

- Remove a sterile toothpick and touch it to a sterile SBLB plate to moisten the end.
- Dip the sterile toothpick into a large actively growing FLS colony.
- Touch the toothpick onto the sterile plate 12-15 times, similar to the drawing below, repeat onto 5 plates. Discard toothpick.
- Repeat onto five plates with a new toothpick, first touching the sterile plate then the colony, then onto 5 plates. .



Isolating FLS pathogen from leaves:

- Place the infected leaves in a ziplock bag and add a moist paper towel.
- After 1-2 days, the mycelium from the lesion will begin to grow and produce spores on the under and upper leaf. I collect from the underside of the leaf.
- Take a sterile toothpick and tap it on a sterile Soybean Lima Bean plate and touch it to the actively growing lesion. Touch the plate with the toothpick 5-6 times in different locations. (Also you can use a sterile scapula to gently scrape off the mycelium onto a plate.).
- Do this again with a new toothpick, picking from different sites on the leaflet.
- Incubate 22C 14-hour light.
- Contamination is usually minimal; transfer 2 times to clean up plates. I have not had problems isolating *Cercospora*, it produces abundant spores in the moist environment.