

GROWTH POUCHES

FOR STUDYING BEAN ROOT ARCHITECTURE AND NODULATION

Clear sheet protectors, top loading
Paper towel or other absorbent paper
N free nutrient solution
2% Bleach solution
Water Agar Media
Grow light or growth chamber
YMB culture of *Rhizobium*

- 1) Cut toweling to fit into the sheet protector. Fold the top edge of the paper towel down approximately 2.5cm, then fold the edge back up to be even with the fold. Press to keep the folds from opening up, and cut two small slits evenly spaced from the edge and from each other. Wrap in foil and autoclave for 25 min at 25 psi.
- 2) Prepare 9 g of agar in 1 L RO water. Autoclave for 25 min at 25psi. Remove from autoclave and allow to cool in a sterile hood. Before media solidifies, but while cool enough to handle pour enough into a petri dish to cover the bottom. Allow to solidify.
- 3) Sterilize seeds by completely submerging them in 2.5% bleach solution for 2 min. Rinse twice in RO water for 2 min each.
- 4) Place sterilized seeds on water agar plates and gently push into the surface of the media. Place dishes in an incubator at 29 C. Germination should take place over the next day or two. When the radical is approximately 2.5 to 3cm long, the seedlings are ready for placement into the growth pouch.
- 5) At the same time seeds are placed on media to germinate, start a YMB culture of the desired *Rhizobium* sp.
- 6) Place the paper in the clear sheet protectors.
- 7) When seedlings have reached the appropriate stage, with sterile forceps under a sterile hood, place seedlings into the YMB culture. Open the fold and pull the cut open. With the forceps gently insert the seedling, root tip first into the hole, carefully to not break the root tip. Position the seedling so the root is pointing straight down. Repeat on other hole.
- 8) Once seedlings are in place, wet the paper with 40ml of final nutrient solution. To avoid washing off the rhizobia, do not pour the nutrient solution on the root.
- 9) With an indelible marker, mark the placement of the tip of the root on the front of the sheet protector. Label the sheet with genotype, *Rhizobium* strain, and date.
- 10) Place sheet protector in binder and place binder under grow lights.
- 11) Check seedlings daily to ensure proper emergence from the sheet protector, as growth of the hypocotyl may push the seedling out of place.
- 12) Evaluate roots to determine characteristics such as time to first visible nodules, time to change in color, placement on the roots, etc.