

The Isolation of *Rhizobium leguminosarum* from root nodules on clover plants

- 1) Using a pair of scissors cut off a root section which has a nodule on it. Make sure there is root either side of the nodule
- 2) Transfer your root section to the tube marked A, this has ethanol in it to surface sterilise the root section. Close the lid and shake it a few times and then leave it for 2 minutes (use your timer)
- 3) Take a pair of forceps and remove the root section from tube A and place into tube B. This has sterile water in it to wash away the ethanol. Close the lid, shake it a few times then leave it for 2 minutes.
- 4) Take a pair of forceps and remove the root section from tube B and place into tube C. This has more sterile water in it to wash the root. Close the lid, shake it a few times then leave it for 2 minutes.
- 5) Put 50 μ l of sterile water in the bottom of your small Petri dish
- 6) With your forceps transfer the root section from Tube C to this drop of water.
- 7) Using your forceps crush the root nodule. You should see some milky fluid coming out.
- 8) Place your Petri dish full of agar onto the template provided for you with the lid facing upwards.
- 9) Using your pipette take up as much of the milky liquid as you can. Remove the lid of the Petri dish and put it on agar plate where indicated from the template beneath.
- 10) Using your loop streak lines across the agar plate as indicated by the template underneath.
- 11) The plates will be taken back to the University and incubated at 28°C for 5 days.