

Long Lab, Stanford University  
<http://cmgm.stanford.edu/biology/long/>

### **Isolation of bacteria from nodules**

- 1) Cut nodule from root leaving a short piece of root on each side.
- 2) Sterilize in about 1 ml 20% Clorox for 5 minutes. Invert several times to make sure nodule is completely wetted.
- 3) Wash two times with 1 ml of water.
- 4) Add 1 ml of LB with 0.3 M glucose.
- 5) Use sterile pestle for Eppendorf tube and crush nodule. Serially dilute bacteria in LB with 0.3 M glucose. Plate 100  $\mu$ l of 10<sup>0</sup> to 10<sup>-3</sup> dilutions on selective medium.

#### Alternatively:

- 4) Add 1 ml of LB.
- 5) Remove nodule with sterile forceps, crush with forceps, and streak out on selective medium.

#### Notes:

Several nodules can be sterilized in one tube. The forceps don't reach the bottom of Eppendorf tubes. 2 ml tubes work better.