**Note on isolation of rhizobia and DNA extraction**

The formation of symbiotically effective legume root nodules by rhizobia is often expected *ex gratia* by growers in many countries. However, it is clear that isolation of unique rhizobial strains for culture *ex situ* and then used as an inoculum may be an inexpensive and effective approach to ensure nodulation and good levels of biological nitrogen fixation. Towards that end, is important that Standard Operating Procedures (SOPs) are available for the following activities:

1. “Isolation the rhizobia”, preferable from freshly harvested legume nodules- see the SOP titled, “*Isolation of bacteria from root nodules*”
2. The extraction of DNA for use in molecular tests - see SOP

“*Nodule Bacteria\_DNA Extraction and PCR*”:

* 1. Ensure the purity and identity of each isolate: “***16S-PCR***”. This sequence data may also be used for molecular diversity analysis.
  2. Diversity may also be assess using this additional generic approach which provides “dominant marker” data that may be used to characterises the core-genome: for example using, “***ERIC-PCR***”.
  3. Diversity may also be assessed using sequence data gathered for key symbiotic genes such as “***nodD-PCR***” and “***nodA-PCR***”, and we have used these predominantly for typing isolates for *Rhizobium leguminosarum* biovar *viceae*, symbiont of wild and crop species of the “LPV complex” (*Lathyrus, Pisum, Vicia*) species which are all very closely related phylogenetically).

Molecular diversity analysis and any associated statistical testing are not straightforward and there are many different types of software available. Therefore, we would advise that those new to this field seek the help of someone who is experienced in these disciplines. We are happy to receive any queries you may have on this aspect pf rhizobial analyses, and we will do our best to help: please contact [pete.iannetta@hutton.ac.uk](mailto:pete.iannetta@hutton.ac.uk).