

Standard Operating Protocol for Transgenic Plant Growth

Intended Use

Seeds from transgenic *Medicago truncatula* plants will be sterilized using sulfuric acid and cold treated for 3 days in the dark. After the cold treatment seeds will be germinated in sterile petri dishes overnight in growth chambers. These seedlings will be grown in turface and vermiculite mix for 1 week and will then be inoculated with *Sinorhizobium meliloti* WT at an OD600 of 0.02-0.05. After 3-4 weeks, the inoculated transgenic plants will be uprooted and nodules counted or morphology noted as pink/white. Additional phenotypes such as N-fixation rates will also be tested if any interesting phenotypes are discovered. At the same time, DNA will be collected from leaves to confirm transgene (UBIQUITIN:promoter fusion constructs) and the gene interrupted (for Tnt1 insertion lines) and confirmed by PCR.

To obtain more seeds from these lines, occasionally the plants will be allowed to grow to full maturity in controlled spaces such as growth chambers and seed pods harvested.

Plant Growth Space

All transgenic material will be grown in growth chambers at the Tennessee State University main campus in Nashville, TN.

Labelling

All plants are labelled with detailed information related to Name of the plant material, sowing date, personnel name, lab affiliation. Different colored stakes are used to distinguish Wild Type (unmodified - white) plants from transgenic plant material (orange).

Personnel PPE

While handling these transgenics, care is taken that students and other lab personnel wear lab coats and disposable gloves that will be autoclaved and heat killed to prevent pollen dispersal.

Plant Material Disposal

All transgenic plant material and plastic consumables that will come in contact with this material will be collected in red autoclave bags and heat killed before disposal.

Permits

All transgenic plant material will be transported via FedEx, UPS or USPS and will be accompanied by a BRS (Biotechnology Regulatory Services (BRS) - USDA APHIS) permit obtained prior to shipment be it seeds (10-100 seeds) or pods (5-500 seed pods). This will be attached to the outer packaging material and will be labelled as such to ensure security of shipment against unauthorized release of the organism. These material will only be opened and used within controlled laboratory conditions within the designated laboratory at Tennessee State University.

Packaging Material and Shipping Container Disposal

Seeds provided in manila envelopes or plastic microcentrifuge tubes will be autoclaved before disposal. If the envelopes/tubes are shipped in metal containers, they will be emptied and thoroughly cleaned with alcohol. All external packaging will also be autoclaved before disposal.

Release into the environment

No transgenic material used in the lab for research purposes will be released into the environment. All experiments will be carried out in controlled environmental conditions and in the rare case that is planned for, all permissions will be obtained in prior, in written from USDA and the US government.

International Movement and Importation

For any transgenic material being imported into the United States, we will follow all packaging and labelling information found in 7 CFR 340.5(m). The responsible person (Lead PI Roy) is aware of, understands, and will comply with the permit conditions. All shipping containers will be accompanied by documents including the names and contact details for the sender and recipient, information regarding the nature and quantity of the contents. In addition, the country and localities where the material were collected, developed, manufactured, reared, cultivated, and cultured and the permit number authorizing the importation will also be included. For organisms imported under permits by mail, the container will be addressed to a plant inspection station listed in the USDA Plants for Planting Manual.