Urban Tilth Soil and Root Microbiome Workflow

Valencerina, A., Florero, C., Downing, R., Coffelt, J, Comandao, R., Ford, D., Hyun, R., Serafin, R., Krolikowski, K.

Soil Microbiome Project, Biotechnology Dept., Contra Costa College

Main Goal

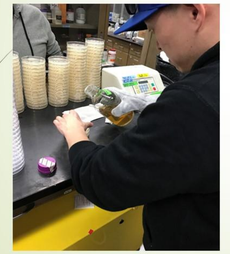
- The main goal of this project is for the CCC Biotech program to contribute information about the living microorganisms that Urban Tilth is nurturing in their soil to turn barren land into a significantly productive food source for the community.
- To accomplish this goal we will be using cutting-edge DNA extraction, sequencing, and bioinformatics techniques along with more standard biotech lab skills.

Lab Safety Rules

Rules to follow as proper safety precautions

SOP 7.1
MS Plates

Agar plates are created as a source for plants to be grown free of unknown microbes or particles that could affect any data



Things to do:

- MS media recipe
- Pouring agar plates
- Label plates appropriately
- Store at 4 degree Celsius
- Cleaning work area

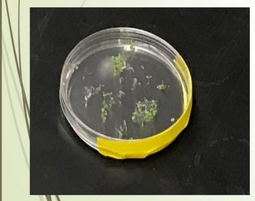
SOP 7.2
Sterilization and Plate Seeding

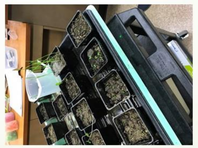
Process to aseptically plant Arabidopsis thaliana in agar plates

- Plate seeding of *Arabidopsis thaliana*
- Use of micropipettes for controlling seed amount placed onto agar plates
- Place seeds in a row having an adequate space between them
- Place seeded plates under light

SOP 7.3
Arabidopsis thaliana transplantation

Seedlings grown from sterilized seeds sown on agar plates are transplanted onto soil sample pots





- Place growing plants onto a designated pot from areas A-F
- Transfer 5 plants into one pot at a time
- Place all pots under light and water frequently

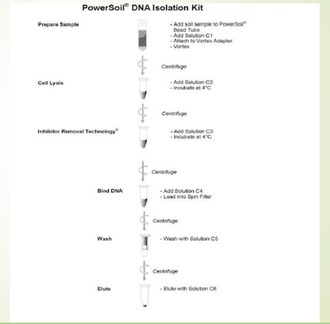
7.4 Root and Soil Cleaning

To isolate Arabidopsis thaliana roots by carefully removing them from its original soil to further examine the microbes that are present in both the roots and the soil

- Observe and note important information about soil, roots, and plants (e.g. length, durability, etc.)
- Use bins and gloves to avoid cross contamination
- Save 14 mL of soil, roots, and rhizosphere
- Store samples in freezer

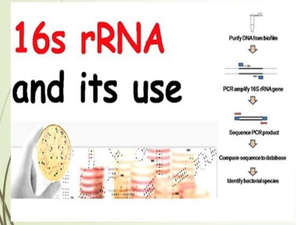
7.5 Bacterial DNA Extraction

To extract DNA from root and soil samples for the identification of microbes



Polymerase Chain Reaction (PCR) Gene Sequencing

To set up and run PCR reactions with appropriate primers to detect GFP constructs



- PCR allows for making a large amount of a particular DNA fragment
- Amplification of 16S rRNA gene
- Sequencing of PCR product
- Comparing sequences of the gene (16S DNA) to a database
- Identification of bacterial species

In a Nutshell

- Always follow lab safety rules as lab safety precautions
- Sterile techniques are essential for all lab procedures
- Have a plan and orderly strategies for a more efficient workflow
- Keep detailed and clear data on lab notebook for future reference