Extraction of DNA from root samples for *Phytopthora sojae* and *Phytophthora sansomeana*

I. Reagents and equipment

- DNeasy Plant Mini kit (Qiagen Cat No. 69104)
- Lysing Matrix A tubes (MPBio Cat No. 116910)
- Razor blades
- Microcentrifuge tubes 1.5-1.7 mL
- Towel paper
- FastPrep instrument or similar
- Microcentrifuge
- Heat block or water bath

II. Preparation before DNA extraction

- Recommended number of samples per extraction range from 20 to 24 to avoid desiccation of samples during the process and it also depends on the capacity of the centrifuge rotor.

- For the DNeasy Plant Mini kit:
    - Add the required ethanol 200 proof to buffers AW1 and AW2 for new kits
    - Buffer AP1 may form precipitates, if necessary redissolve at 42ºC

- Label all necessary tubes and columns before the extraction process (six sets).
    - Set 1: Lysing matrix A tubes (orange cap)
    - Set 2: QIAshredder columns (lilac columns from DNeasy kit)
    - Set 3: Microcentrifuge tubes 1.5 – 1.7 mL
    - Set 4: DNeasy columns (white columns from DNeasky kit)
    - Set 5: Collection tube (supplied in from DNeasy kit)
    - Set 6: Microcentrifuge tubes 1.5 – 1.7 mL

- Start the water bath or heat block at 65ºC

III. DNA extraction process

1. Wash the root system using tap water to remove soil and rhizosphere adhered to the tissue. Pat the root tissue dry on the towel papers.

2. Collect root tissue with symptoms of root rot, taking samples if possible from the margin between necrotic tissue and healthy tissue. The sample can include lateral and tap roots, or stem for *Phytophthora sojae*.

3. Cut the tissues into small pieces less than 0.5 cm using the single-use razor blade and collect approximately 100mg of tissue for DNA extraction.
    a. Reusable implements must be sterilized with alcohol in between samples.

4. Place the 100mg of root tissue in a Lysing matrix A tube, and add 400 µL AP1 lysis buffer and 4 µL of RNaseA.

5. Place the tubes in a FastPrep bead-beater machine and homogenize samples at speed 6 for 40s.

6. Remove the tubes from the bead-beater and incubate at 65ºC for 15 min, mix or vortex tubes three times during incubation.

7. Add 130 µL of buffer P3, mix and incubate for 5 min on ice.

8. Centrifuge samples for 5 min at 20,000 x g (14,000 rpm).

9. Pipet lysate (supernatant) to the QIAshredder columns placed in 2mL collection tube. Centrifuge for 2 min at 20,000 x g (14,000 rpm).

10. Discard the column (top part) and transfer the flow-through into a new microcentrifuge tube without disturbing the pellet if present.

11. Estimate the volume of the flow-through in the new tube and add 1.5 volumes of buffer AW1 and mix carefully by pipetting up and down.

12. Transfer 650µL of the mixture into a DNeasy Mini spin column placed in a 2mL collection tube.

13. Centrifuge for 1 min at ≥6000 x g (≥8000 rpm). Discard the flow-through and repeat this step with remaining volume from step 11.

14. Place the DNeasy column in a new 2mL collection tube and add 500 µL of buffer AW2, and centrifuge for 1 min at ≥6000 x g (≥8000 rpm).

15. Discard the flow-through and add another 500 µL of buffer AW2. Centrifuge for 2 min at 20,000 x g (14,000 rpm).

16. The collection tube can be discarded and remove the DNeasy column carefully and place it in a new microcentrifuge tube.

17. Add 50 µL of buffer AE and incubate for 5 min at room temperature. Centrifuge for 1 min at ≥6000 x g (≥8000 rpm).

18. Add another 50 µL of buffer AE, incubate again for 5 min and centrifuge for 1 min at ≥6000 x g (≥8000 rpm).

19. Store DNA at -20ºC for downstream applications.

IV. References

- Qiagen instruction manual for the DNeasy® Plant Handbook, section Plant Tissue – Mini Protocol, version October 2012.
- USDA-APHIS 2004. Sudden oak death: *Phytophthora ramorum*. In: Pest Detection and Management Programs. L. Levy and V. Mavrodieva, eds. USDA Animal Plant Health Inspection Service, USA.