Silage DNA extraction procedure

Using the 'Fast DNA SPIN Kit for Soil' by MP Biomedicals, LLC

This Procedure is accompanied by a Video demonstration that can be downloaded at:
http://animalscience.ucdavis.edu/faculty/robinson/videos.html

Materials

Equipment and Chemicals for DNA extraction (not in kit)

- Pipette (200µL, 1000µL)
- Pipette tips
- Bead beater
- Centrifuge tubes (50mL)
- Micro centrifuge tubes (2mL)
- Rack for micro centrifuge tubes
- Micro centrifuge
- Ice and mini cooler
- Heat block/water bath
- Ethanol (100%)
- Paper towels
- Cryogenic Gloves
- Heat resistant gloves
- Vortex
- Autoclavable containers (a box and small containers)
- Autoclave tapes
- Liquid nitrogen holding container
- Tweezer and/or small spoon

Equipment and Chemicals for DNA extraction (in kit)

- Lysing Matrix A tube (can be other types)
- Matrix beads
- Sodium Phosphate buffer
- MT Buffer
- PPS Solution
- Binding Matrix
- SPIN Filter tubes
- Catch tubes
- Concentrated SEWS-M
- DES
- BBS Loading dye

Equipment and Chemicals for NanoDrop

- DES
- Pipette (20µL)
- Pipette tips
- KimWipes
- DI water

Equipment preparation (Autoclaving)

Ascertain the location of the autoclave.

****Remember: Ask for hands-on instruction if it's the first time you to use the machine.***

Equipment that needs to be autoclaved includes Pipette tips and micro centrifuge tubes

1. Put pipette tips and micro centrifuge tubes into an autoclavable container (*i.e.,* heat resistant plastic or glass container) and place in an autoclavable box.
2. Put autoclave tape on each container (to make sure everything is sterilized after autoclaving, autoclave tape changes its color when exposed to high temperature).
3. Place the box inside the autoclave.

****Wear heat resistant gloves in all the steps after this step***

4. Close the door of the autoclave. Make sure the door is closed tightly and not loose.

5. Start the autoclave.
6. Put your name, PI's name, contact number and time on the sign-up sheet if applicable.
7. Run 30 min.
8. After 30 min, stop the autoclave and confirm that the pressure inside the machine decreases on the gauge.
9. After the pressure has dropped, open the door of the autoclave.

Be careful of steam coming out when opening the door.

10. Take out the autoclave box and let the equipment cool.

Sample preparation

1. Prepare several sets of clean mortars and pestles.
2. Label 15 centrifuge tubes of 50 mL.
3. Fill a foam cooler with ice and keep silage samples on ice to keep them cold - always.
4. Get liquid nitrogen.

   Always wear cryogenic gloves and face protector while handling liquid nitrogen.

   Place the nozzle inside the liquid nitrogen container and carefully turn the handle to open the liquid nitrogen tank. When finished, turn the handle of the tank fully to close. Put your name, PI's name, date and amount of liquid nitrogen on the sign-up sheet if applicable.
5. Put ~5 g of silage sample in mortar, add 10 to 15mL of liquid nitrogen until all material is covered. Grind the sample until it becomes fine particles. Start with a slow pace when silage is covered with liquid nitrogen and speed up as it evaporates.

   Do not forget to wear cryogenic gloves and eye goggles when handling liquid N.

   Do not grind too hard as the mortar can crack.
6. Put ground samples into a labeled 50mL centrifuge tube using a tweezer or a small spoon, store on ice or at -20C° until DNA extraction.

Clean up

1. Wipe mortars and pestles with paper towel.
2. Spray with DNA erase.
3. Wait for at least a minute.
4. Rinse everything with DI water.

Equipment preparation (DNA extraction)

1. Clean up the bench, spray with 70% EtOH and wipe with paper towels.
2. Arrange DNA kit, pipette, autoclaved pipette tips and micro centrifuge tubes on the bench.
3. Prepare a gas burner and a striker to use during the DNA extraction process.
4. Add 100 mL of 100% ethanol to 12 mL of concentrated SEWS-M wash solution. Mark the date and your name on the bottle.
5. Fill a foam cooler with ice and place the 50 mL centrifuge tubes with silage samples on ice to keep them cold during the procedure.
6. Label Lysing Matrix A tubes.

Make sure to label on top and side of the tubes.

DNA extraction protocol

1. Add ~ 500 mg of ground silage sample to a Lysing Matrix A tube using a clean tweezer or a small spoon. Tare the tube before weighing the samples.
2. Add 978µL of Sodium Phosphate Buffer to sample in Lysing Matrix A tube.
   - Note: Sodium phosphate is a reagent with very high buffering capacity. It protects DNA together with MT Buffer upon cell lysis.
3. Vortex 10 to 15 seconds.
4. Add 122µL MT Buffer. Shake vigorously to mix, then vortex 10 to 15 seconds.
   - Note: MT Buffer is a lysis solution, which was developed to protect and solubilize nucleic acids and proteins upon cell lysis. It allows extraction of DNA with minimal RNA contamination.
5. Add another lysing bead to a Lysing Matrix A tube.
   - Note: This is dependent on the DNA kit used.
6. Homogenize for 3 min using a bead beater.
7. Centrifuge at 14,000 x*g* for 15 min.
8. While waiting, label 2 sets of 2.0 mL micro centrifuge tubes for the next step.
9. Transfer supernatant (~500 to 600µL) from Lysing Matrix A tube to a clean 2.0 mL micro-centrifuge tube.
10. Add 250 µL PPS (protein precipitation solution) and mix by shaking the tube by hand 10 times, then incubate at room temperature for 10 minutes. ***DO NOT vortex.***

- Note: PPS is a lysis solution component which is used as a stabilization agent during sample lysis.

11. Centrifuge at 14,000 x*g* for 5 min to precipitate a pellet.
12. Transfer supernatant (~600 to 800µL) to a clean 2.0 mL micro centrifuge tube.
13. Add an equal amount of Binding Matrix (as the amount of supernatant transferred in step 12) to the micro centrifuge tube.
    - Note: Binding Matrix is a silica slurry that binds DNA from lysates for further purification.
14. Shake gently by hand to mix, then place on a rocker or invert by hand for 3 to 5 min to allow binding of DNA to the matrix.
    - Note: If there is no rocker, place all the tubes on rack and cover them with tapes to make it easy to invert
15. While waiting, label a set of 2 mL SPIN filter tubes.
16. Mix the contents of micro centrifuge tube by pipetting up and down several times to dissolve the precipitate.
17. Transfer half of the solution to a clean, marked, SPIN filter tube.
18. Centrifuge at 14,000 x*g* for 5 min.
19. Remove filter and empty catch tube.
20. Repeat mixing, transferring and centrifuging for the remaining solution to the same used SPIN Filter tube, discard the flow-through but keep the filter with solid material.
    - Note: DNA bound to Binding Matrix remains in the tube filter while residual material is discarded as flow-through.
21. Add 500 µL of previously prepared SEWS-M cleaning solution to the SPIN Filter tube.
22. Shake gently by hand to or flick the tube to mix.
    - Note: SEWS-M is EtOH based wash solution. Purified DNA is eluted from the Binding Matrix after washing with the SEWS-M solution.
23. Centrifuge at 14,000 x*g* for 5 min.
24. Empty the catch tube but keep the filter with solid material.
25. Centrifuge again at 14,000 x*g* for 5 min to remove residual ethanol.
26. While waiting, label a set of 2 mL catch tubes.
27. Turn on the heat block and set the temperatures to 55°C.
28. Transfer the SPIN Filter to a clean, marked, catch tube.

29. Air dry the SPIN Filter for 5 minutes at room temperature (while inside the marked catch tube).
30. Add 100 µL of DES (DNA elution solution) to the SPIN Filter tube and gently re-suspend the pellet by finger flicking.
    - Note: DES is added to elute DNA bound to the filter.
31. Incubate SPIN Filter tubes at 55°C in a heat block/water bath for 5 min.
32. Centrifuge at 14,000 x*g* for 2 min.
33. Discard the SPIN Filter and store liquid at -20°C until use.
    - Note: The remaining liquid is Pure DNA.

NanoDrop 2000 protocol

Remember to always read official protocol of NanoDrop if it's the first time for you to use the machine

1. Put small amount of DI water on a KimWipe and gently wipe the sampling arm and measurement pedestal before, after and between each sample.

   Do not squirt water directly onto the machine.

2. Turn on your PC and log in.
3. Click the previously installed NanoDrop icon to start the program.
4. Select 'Nucleic acid'.
5. Let NanoDrop do a wavelength verification (it starts automatically). When it is ready, a 'Blank' button will show up on top left of the screen.
6. Measure the blank by placing 2 to 3 µL of blank solution on the measurement pedestal. Lower the sampling arm.
   - Note: Blank solution is usually the solvent in which the DNA is dissolved - in this case, DES solution.
7. Select the 'Blank' button on the program.
8. Once the blank measurement is complete, the 'Measure' button will appear next to the 'Blank' button on the program.
9. Clean the measurement pedestal and sampling arm with DI water and KimWipe to remove blank solution.
10. Measure samples by placing 2 the 3 µL of DNA solution on the measurement pedestal. Lower the sampling arm.

11. Type the sample ID into the program and select the 'Measure' button.
12. When complete, DNA concentration, A260, A280, 260/280 ratio, 260/230 ratio appear on the screen.
    - A260 and A280 show the absorbance of light by a sample at wavelength of 260 nm and 280 nm respectively.
    - Nucleic acids, including DNA, RNA and oligonucleotide, have distinct UV spectrum features and usually have a prominent peak at 260 nm. NanoDrop determines the DNA concentration in samples based on A260. However, if there is any other substance in solution other than DNA, such as peptides, it is difficult to measure the true concentration of DNA in the sample, therefore 260/280 ratios are used to measure the purity of the DNA.
    - As proteins have an absorbance peak at 280 nm, high A280 and a low 260/280 ratio indicate the purity of DNA is low. A 260/280 ratio of ~1.8 is generally accepted as 'pure' for DNA.
    - If DNA is not pure enough, repeat the DNA extraction procedure for those samples.

For more detailed information about each parameter, please refer to the Thermo Fisher Scientific webpage and other papers

- *https://tools.thermofisher.com/content/sfs/brochures/TN52607-E-0914M-Oligonucleotides-Mweb.pdf*
- *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3346308/pdf/jove-45-2565.pdf*

13. Clean the measurement pedestal and sampling arm with DI water and KimWipe to remove sample solution.
14. Repeat steps 10 to 13 until all samples are measured.
15. Select the 'Results' icon on lower left of the screen and 'Export' the excel file with the data generated. Save it in your USB or cloud website.

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