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Procedure to extract DNA from tissue using the Macherey-Nagel NucleoMag® Tissue kit

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Procedure to extract DNA from tissue using the Macherey-Nagel NucleoMag® Tissue kit

Scope and Purpose:
To isolate genomic DNA from marine turtle, cetacean and pinniped skin, muscle, and internal organ tissue.

Summary of Method:
Marine animal tissue is digested with the active digestive enzyme Proteinase K. Genomic DNA is then isolated and purified from this digested material using magnetic beads and a series of washes. This is a kit based 2 day procedure, about 2-3 hours each day depending on the number of samples (full plate is 96 samples).

Health and Safety Warnings:
- Wear latex or nitrile gloves, closed-toe shoes and a lab coat.
- This procedure uses chemicals that may be harmful. Please read Appendix A (at end of SOP) before performing this protocol.
- Be careful handling razor blades (see razor blade safety in Appendix A).
- If using frozen tissues, equilibrate tissues to room temperature for razor blade safety.
- Rinse forceps in MilliQ water after bleach rinse to prevent corrosion of forceps.

Quality Control:
- Wear latex or nitrile gloves, closed-toe shoes and a lab coat.
- Wear latex or nitrile gloves throughout extraction protocol.
- Wipe down all pipettes and bench surface with 10% bleach squirt bottle followed by 70% EtOH squirt bottle before placing bench paper down.
- Always use a new razor blade, forceps, and weighing dish when cutting up subsequent tissue and rinse each forceps with bleach and water after each use.
- Always use filtered tips and change them each time when adding reagents to samples if tissue is already inside the wells.
- Include negative controls in the lysis tube plate, ideally one per column.

Equipment and Supplies: (see Reagent list in SOP Binder for reagent locations)
1. Bench paper
2. Gloves
3. Pipettes (10ul, 100ul, 1000ul)
4. Pipette tips (10ul, 100ul, 1000ul)
5. Multi-channel Pipette (200ul and 1000ul)
6. New razor blades (1 per sample)
7. Plastic weighing dishes (1 per sample)
8. Cold beads and ice bucket
9. Forceps
10. Bleach
11. MilliQ water
12. Two beakers
13. MilliQ squirt bottle
14. Printed out extraction grid with order of samples
15. Lysis Tubes Strips, Rack and Cap Strips (1.2 mL wells)

16. Aluminum sealing foil
17. Vortemp machine
18. Vortex
19. Plate spinning centrifuge
20. Square-well Block Separation plate (96-well, 1.2 ml square wells)
21. 10ml Serological Pipettes
22. Serological Pipette Controller
23. Reagent Reservoirs and Base
24. Strip tubes and dome caps
25. Reagents:
26. NucleoMag® SEP (magnetic plate/separation system)
27. Reagents:
    - NucleoMag® B-Beads
    - Lysis Buffer T1
    - Binding Buffer MB2
    - Wash Buffer MB3
    - Wash Buffer MB4
    - Wash Buffer MB5
    - Elution Buffer MB6
    - Proteinase K
    - Proteinase Buffer PB

Procedures:

Day one –Digestion (Approximate time: 3 hrs for 96 samples)

1. Create extraction grid (see SOP PR-010.00)
2. Clean the bench top with bleach and ethanol and cover with new bench paper.
3. Make a 10% bleach solution using MilliQ water in one beaker and fill the other beaker with MilliQ water for rinsing.
4. Set up razor blades, weighing dishes (to cut on), forceps and lysis tube rack.
5. Label plate with your initials and date, and mark each extraction negative by outlining the top of the wells with a sharpie.
6. Add 200µl of T1 lysis buffer to each tube in the lysis rack.
7. For each sample, use clean forceps, remove tissue from vial, rinse with MilliQ squirt bottle if it was stored in a preservative and place on a new weighing dish.
8. Using a new razor blade cut a small piece of tissue approximately 2mm/2mm square, and put into the appropriate well according to your extraction grid. Be careful not to touch any of the other wells with the forceps.
9. Place forceps into bleach-rinse beaker after every sample, and then place into the MilliQ water rinse beaker to use for next sample. Place used razor blades in sharps container. Double check sample grid often, to verify that you are in the right well.
10. Add 25µl of Proteinase K to every well using new pipette tips for every well to avoid contamination.
    - To make Proteinase K add 2.6 mL of Proteinase Buffer PB to a 75 mg vial of Proteinase K (lyophilized) from the kit. Mix well (do not vortex). Store in -20 °C.
11. Place cap strips on wells, and lightly vortex samples to mix.
12. Incubate in a Vortemp at 37° C overnight. Make sure it is set to 0 RPM. Do not digest at higher temperatures as caps will pop off and the liquid will evaporate.

Day two –Isolating and Purifying DNA

1. ***Lyse Samples***
   1.1. Centrifuge the rack containing your samples for 5 min at full speed (4500 x g) on a plate centrifuge to pellet the leftover tissue. Remove cap strips.
   1.2. **Transfer as much of the supernatant/cleared lysate (equilibrated to room temperature) as you can** to a Square-well Block. Typically about 200 µL, and you might suck up tissue which is okay. Do not moisten the rims of the well.
   1.3. Label the block with your initials and date of extraction.

2. ***Bind DNA to NucleoMag® B-Beads***
   2.1. Shortly vortex the NucleoMag® B-Beads until completely resuspended.
   2.2. **Add 24 µL of NucleoMag® B-Beads** and **360 µL Buffer MB2** to each well of the Square-well Block.
   2.3. Mix by pipetting up and down 10 times and incubate for 5 min at room temperature.
   2.4. Separate the magnetic beads against the side of the wells by placing the Squarewell Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.
      - Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

3. ***Wash With Buffers MB3, MB4, and MB5***
   3.1. Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
   3.2. **Add 600 µL Buffer MB3** to each well and resuspend the beads by pipetting up and down until the beads are resuspended completely (about 15 times).
   3.3. Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.
   3.4. Remove the Square-well Block from the magnetic separator.
   3.5. **Add 600 µL Buffer MB4** to each well and resuspend the beads by pipetting up and down until the beads are resuspended completely (about 15 times).
   3.6. Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.
   3.7. Leave the Square-well Block on the magnetic separator.
   3.8. **Gently add 900 µL Buffer MB5** to each well and incubate for 45–60 s while the beads are still attracted to magnets. Then remove and discard the supernatant by pipetting.
      - Note: Do not resuspend the beads in Wash Buffer MB5. This step is to remove traces of ethanol and eliminates a drying step!

4. ***Elution***
   4.1. Remove the Square-well Block from the NucleoMag® SEP magnetic separator.
   4.2. **Add desired volume of Buffer MB6 (50–100 µL)** to each well and resuspend the beads by pipetting up and down until the beads are resuspended completely (about 15 times). Incubate for 5-10 minutes at room temp.
      - Alternatively, for better yield seal the top of the block with aluminum sealing foil and place it in the vortemp at 56 °C and 40 RPM, for 5-10 min.

4.3. Separate the magnetic beads by placing the Square-well Block on the magnetic separator. Wait 5 min until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified genomic DNA to strip tubes. DNA is ready to use.
4.4. Cap/seal, label, and store the used lysis tubes and square well block in the cabinet until the negatives have been determined clean.
4.5. Enter extractions into extraction database (see SOP PR-010.00).

Waste Management:
Dispose of used razor blades in a designated sharps container. Bleach solution and water should be rinsed down the sink. Plastics and other liquids can be thrown in the trash. If there are questions on handling chemicals contact the Safety Officer or Lab Manager. Spills, cuts or other accidents should be reported to the Safety Officer and Lab Manager.

References:
Macherey-Nagel SDS webpage, Use reference code 7443 to look up NucleoMag® Tissue kit https://www.mn-net.com/Support/LiteratureBioanalysis/MSDS/tabid/10658/language/en-US/Default.aspx
Macherey-Nagel NucleoMag® Tissue User Manual, https://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/Genomic%20DNA/UM_gDNATissue_NMag96.pdf
For SDS information, go to C:\\Users\ljuser\Desktop\MMTD Genetics SDS (see Lab Manager for designated computers).
Hazardous substance information: http://epa.gov/ceppo/pubs/title3.pdf

Appendix A: Chemical Health and Safety Warnings

T1
No SDS needed

Proteinase K
Sensitizer and irritant. Irritating to the eyes, respiratory system and skin. It may cause sensitization by inhalation and skin contact.
First Aid
Eyes: rinse immediately with plenty of water and seek medical advice.
Inhalation: If experiencing respiratory symptoms seek medical attention.

PB (Proteinase Buffer)
No SDS needed

B-Beads
No SDS needed

MB2
Flammable liquid and vapor. Keep away from heat and ignition sources. Harmful if swallowed.
First Aid
Eyes: flush with water
Skin: wash with water thoroughly after handling.
Ingestion: Wash out mouth completely with water if person is conscious. Seek medical attention immediately (POISON CENTER or doctor) if you feel unwell.

MB3
Flammable liquid and vapor. Keep away from heat and ignition sources. Harmful if swallowed.
First Aid
Eyes: flush with water
Skin: wash with water thoroughly after handling.
Ingestion: Wash out mouth completely with water if person is conscious. Seek medical attention immediately (POISON CENTER or doctor) if you feel unwell.

MB3
Flammable liquid and vapor. Keep away from heat and ignition sources. Harmful if swallowed.
First Aid
Eyes: flush with water
Skin: wash with water thoroughly after handling.
Ingestion: Wash out mouth completely with water if person is conscious. Seek medical attention immediately (POISON CENTER or doctor) if you feel unwell.

MB5
No SDS needed

MB6
No SDS needed

Additional Safety Information

Blade Safety

Our most common lab accident is razor blade and scalpel cuts. These range in severity from a minor cut with a clean blade to severing tendons with a dirty blade. Since we are often working with dead mammal tissue, infection from a dirty blade is almost a guarantee. Usually you are started on antibiotics, and sometimes stitches or even surgery are required. We can avoid these accidents if we really take our use of blades seriously and follow a few good practices.

When to use a blade:

- Razor blades and scalpels are for working with small pieces of tissue. If your sample is large use an appropriate knife.
- Only use a blade on tissue that requires very little force applied (the amount you might apply to cut a piece of toast). If you have to press hard you've got the wrong tool and are endangering yourself.

Good practices with a blade:

- Cut away from yourself. I know you know, but we all cheat on this one and if we didn't there would be a lot less accidents …and in the lab environment the accidents can be severe.
- Use something other than your hand/fingers to hold the sample in place. Since it is a small sample you should be able to use things like forceps, a toothpick, a needle, etc.
- Let your piece thaw enough to allow you to cut it easily (again like toast).
- Look at the blade before you cut. There have been times when people have pushed down on the wrong edge of a razor blade, or grabbed the wrong end of the scalpel.
- Never apply force at an angle. Cut straight down etc. Blades can snap when oblique force is applied and the pieces go airborne.
- Wear safety glasses.
- Report all cuts to the Lab Manager and Safety Officer