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DNA extraction from Salton Sea

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We use this protocol and it's working

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Abstract

To extract DNA from environmental microorganisms, we used GenEluteTM Bacterial Genomic DNA KIT (Sigma- Aldrich, Missouri, USA). For field samples, we used the modified gram-positive extraction procedure described by the manufacture. After filtration and sonication, we centrifuged samples for 2 min at 12,000 RPM without the overnight enrichment step recommended by the manufacturer, to preserve original concentrations. Pelleted cells were resuspended in 200 mL of lysozyme solution and incubated for 30 min at 37 °C. We then added 20 µL of Proteinase K solution to the sample, followed by 200 µL of lysis solution, then incubated at 55 °C for 10 min after thorough vortexing. Columns were prepared by adding 500 µL of the column preparation solution to each pre-assembled GenElute Miniprep Binding Column and centrifuging for 1 min at 12,000 RPM. We then added 200 µL of 95 % ethanol to the lysate and mixed thoroughly. The entire lysate was transferred into the binding column and centrifuged at 12,000 RPM for 1 min, after which the column was placed in a new collection tube and 500 µL of wash solution # 1 was added to the column and centrifuged for 1 min at 12,000 RPM. After discarding the eluate, we placed 500 µL of wash solution # 2 into the binding column and centrifuged for 3 min at 12,000 RPM. For DNA elution, we poured 200 µL of elution solution onto the column and allowed it to incubate for 5 min at room temperature, then centrifuged it for 1 min at 8,000 g. DNA concentration was estimated using a NanodropTM 1000 (Thermo-Scientific, Ramsey, Minnesota).

Equipment and Supplies

1 **Equipment**

- Microcentrifuge (2 mL tube, rotor equipped)
- 1.5 mL microcentrifuge tube
- 37 °C water bath or heating block
- 55 °C water bath or heating block
- Pipette tips (aerosol barrier recommended)
- Vortex mixer
- Incubator
- Pipettes
- NanoDrop 1000 spectrophotometer

Supplies

- NA2100 DNA extraction Sigma (https://www.sigmaaldrich.com/US/en/product/sigma/NA2100)
- Gloves
- Ethanol (95%–100%)

Collection and Storage

2 **Sample Collection and Storage of Sample**

1. Collect samples from surface water without introducing contamination.
2. Store in refrigerator.

Storage and preparation of DNA extraction kit

1. Store at room temperature.
2. If any reagent from the kit forms a precipitate, warm it at 55–65 °C until it dissolves and allow it to cool to room temperature before use.
3. Dilute the wash solution concentrate with 10 mL (10 prep package), 80 mL (70 prep package), or 360 mL (350 prep package) of 95–100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of the ethanol.
4. Dissolve the powder in one bottle of Proteinase K in 0.25 mL of water to obtain a 20 mg/mL stock solution.

- **Note:** The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature. The Proteinase K solution must be added directly to each sample every time. Do not combine the Proteinase K Solution and Lysis Solution for storage.

Option if using enrichment

3 **Steps**

1. Prepare sterile **Trypticase Soy Broth** (TSB) according to **instructions.**
2. Add 30 mL of sterile TSB in 50ml centrifuge tube.
3. Shake sample on vortex for 30 seconds and then add 1 mL of sample to the TSB solution.
4. Loosen caps and insert into shaker incubator at 37 °C for overnight at 50 rpm.
5. Harvest cells by forming a pellet.
6. To form a pellet, collect 1.5 mL of the overnight bacterial broth culture using a pipette.
7. Place 1.5 ml in centrifuge tube.
8. Centrifuge for 2 minutes at 12,000-16,000 x g.
9. Pour off the liquid supernatant and use the pellet in the next extraction steps.

- **Note-** If bacteria are propagated in rich media such as Terrific broth (**T9179**), reducing the starting material volume to 0.5 mL of an overnight bacterial broth culture to avoid overloading the GenElute™ columns will be necessary. See Appendix 2 for more information.

Option if filtering (concentration)

4 **Filtering steps:**

1. Filter Salton Sea water through 0.45µM HA filter using vacuum and millipore disposable filter holder with included HA filter. The **Nalgene™ Single Use Analytical Filter Funnels** includes 47mm diameter cellulose nitrate membrane. *Can also use a **Millipore glass filter funnel and clamp set** with sterile 0.47mm 0.45µM HA filter.*
2. Filter as much volume as you can through the HA filter. Usually, 200 mL can be filtered through. Mark the tube to indicate how much sample was filtered. Samples with heavy algal or biological growth may take several hours to filter. Filter in increments of 50 mL and stop if necessary.
3. When filtering completes, use two forceps and fold the HA filter with the brown biological side on the outside of the filter. Use forceps to tear off small sections of the filter and insert into bead tube.
4. Use the Machery-Nagel **2ml bead tubes type A** with a yellow top. These contain 0.6–0.8 mm ceramic beads. It may also be possible to use other types of bead tubes with garnet beads.
5. Label the tube with sample ID and volume that was filtered.

5 **Bead Beating preparation steps:**

1. Prepare**Lysozyme stock solution** from MP Biomedicals LLC cat no. 100034 1GM. Mix 10 mg of powder in microcentrifuge tube with 1ml of IDTE water. This is a lysozyme stock solution to be used for this extraction. Aliquot into several tubes to prevent freeze/thaw destruction.

2. Prepare the Bead Tube
3. Add 200 µL of IDTE water
4. Add 200 µL of lysozyme solution in a Bead tube with a filter. This is from the Genelute kit.
5. Add 6.5 µL Beta-mercaptoethanol (Beta-ME) to each Bead tube.

Note

NOTE: the similar, but different, 2-mercapto-ethanol/DTT breaks disulphide bonds.https://openwetware.org/wiki/SDS . Also, please use the Beta-mercaptoethanol under an exaust hood.

Bead Beat:

1. Use the flush cutter snippers and cut the skirt off of the MN bead tubes so that they fit in the Bead Bug
2. Use the bead bug on full speed 400 x g at full time of 320 seconds.
3. Incubate the bead tube for 30 min at 37 °C
4. Pipette as much volume as possible from the bead tube (only liquid, avoid beads and filter pieces) into a 2 mL collection tube and label them.

DNA Extraction (Gram-Negative)

6 **1. Resuspend Cells-** Use 180 µL of Lysis Solution T (Buffer STL for GenElute™ Mammalian Genomic DNA Kit (B6678)) to resuspend the cells using a pipette.

Optional RNase A treatment- If residual RNA is not a concern, continue with the next step. If RNA-free genomic DNA is required, add 20 µL of RNase A Solution (**R6148**), mix, and incubate for 2 minutes at room temperature, then continue with the next step.

7 **2. Prepare for Cell Lysis-** Add 20 µL of the Proteinase K solution to the sample. Mix and incubate for 30 minutes at 55 °C .

8 **3. Lyse Cells-** Add 200 µL of Lysis Solution C (B8803), vortex thoroughly for about 15 seconds, and incubate at 55 °C for 10 minutes.

(A homogeneous mixture is essential for efficient lysis!)

9 **4. Column Preparation-** Place each pre-assembled GenElute™ Miniprep Binding Column in a 2 mL collection tube. Add 500 µL of the Column Preparation Solution to each pre-assembled GenElute™ Miniprep Binding Column—centrifuge at 12,000 ×*g* for 1 minute. Discard the eluate.

- **Note-** The Column Preparation Solution maximizes DNA binding to the membrane resulting in more consistent yields.

10 **5. Prepare for Binding-** Add 200 µL of ethanol (95–100%) to the lysate and mix thoroughly by vortexing for 5–10 seconds.

(A homogeneous mixture is essential!)

11 **6. Load Lysate-** Transfer the entire contents of the tube into the binding column. Use a wide-bore pipette tip to reduce the shearing of the DNA when transferring the contents into the column. Centrifuge at .≥ 6500 × g for 1 minute. Discard the collection tube containing the eluate and place the column in a new 2 mL collection tube.

12 **7. First Wash-** Add 500 µL of Wash Solution 1 (W0263) to the column and centrifuge for 1 minute at ≥ 6500 × g. Discard the collection tube containing the eluate and place the column in a new 2 mL collection tube.

13 **8. Second Wash-** Add 500 µL of Wash Solution to the column and centrifuge for 3 minutes at maximum speed (12,000–16,000 × g) to dry the column. **The column must be free of ethanol before eluting the DNA.** Centrifuge the column for an additional 1 minute at maximum speed if residual ethanol is seen. You may empty and re-use the collection tube if you need this additional centrifugation step. Finally, discard the eluate collection tube and place the column in a new 2 mL collection tube.

(Important Reminder: Verify that ethanol has been added to the bottle of Wash Solution Concentrate.)

14 **9. Elute DNA-** Pipette 200 µL of the Elution Solution (B6803) directly onto the center of the column; centrifuge for 1 minute at ≥ 6500 × g to elute the DNA. **To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.**

- **Optional-** A second elution can be collected by repeating this step with an additional 200 µL of Elution Solution and eluting it into a new 2 mL collection tube or the same 2 mL collection tube used for the first eluate. When performing a second elution, the yield can be improved by 20–50%. The eluate contains pure genomic DNA. For short-term storage of the DNA, 2–8 °C is recommended. For longer-term storage, –20 °C is recommended. Avoid freezing and thawing, which causes breaks in the DNA strand. The Elution Solution will help stabilize the DNA at these temperatures.

15 **10. NanoDrop-** Using a NanoDrop 1000 spectrophotometer, place a 200 µL drop of RNase-free water in the spot for the sample to run a blank measurement. Then, clean it with a Kim wipe and place a 200 µL drop of sample, close the sampling arm, and start recording. Run three trials for each sample cleaning in between each sample with RNase-free water and running a blank. Save data.