

Bi1X, Spring 2013

Week 4

- **Extraction of Oral Microbial DNA**
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Materials (per individual):

- Toothpicks
- Sterile Water
- Filter tips

Background:

Isolating bacteria that live in the mouth:

The human body is host to an astounding array of microbial organisms. Bacterial cells outnumber our own cells approximately 10:1. The relationship between humans and their individual *microbiomes*, or microbial communities living within our bodies, is still largely unknown. These populations may be diverse and interact in complicated ways. One way to begin studying the role of these microbes in our body is to identify the species living in each micro-environment. We can begin by narrowing our study to a single area such as the oral cavity. Species that live in our mouth are constantly subjected to a variety of insults including fluoride, toothbrushing, hot and cold temperatures, and even micro changes to oxygen levels. Additionally, microbes must face the body's immune system and predatory bacteriophages. To study the bacteria that thrive in this type of environment we must begin by extracting the DNA found in an oral sample. Once we have extracted and purified bacterial genomic DNA, we can use techniques from modern biology to identify the species of bacteria found in the mouths of various individuals.

Molecular Biology and DNA Extraction:

Molecular biology has evolved into a technical field that exploits the properties of cellular DNA and the machinery of associated macromolecules. You have already seen how DNA is cut using restriction enzymes and analyzed by size using gel electrophoresis. These are routine modern biological techniques used for studying the genetics of living organisms. Throughout the course you will be introduced to a variety of lab techniques including DNA extraction, amplification, and sequencing.

Today you will use a commercial kit, MoBio's PowerBiofilm, to break open the cells that live on the surface of your teeth and to isolate and purify the DNA from those cells.

Protocol:

The makers of PowerBiofilm did an excellent job describing their protocol including descriptions of why each step is performed. Therefore, I included the directions from their manual as the protocol we will use today. Since there are many intricate steps in the extraction process, it is necessary that we do the step-by-step process as a class. Your TAs will lead you through each step so that we can assure everyone successfully extracts DNA as we will continue to work with your purified DNA product over the next few weeks.

Protocol (from PowerBiofilm manual):



Detailed Protocol

Please wear gloves at all times

Warm Solution BF1 prior to use at 55°C for 5-10 minutes. Use Solution BF1 while still warm. Check Solution BF4 and warm at 55°C for 5-10 minutes if necessary. Solution BF4 can be used while still warm. Use only PowerBiofilm™ Bead Tubes with this kit.

1. Weigh out **0.05 to 0.20 g** of biofilm material and place it into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1 minute. Remove excess liquid using a pipette tip. For less saturated samples (ex. microbial mats) add directly to the PowerBiofilm™ Bead Tube (**For information on selecting the right amount of material to add, see Amount of Starting Material in the Hints and Troubleshooting Guide before continuing**).

Note: Use only PowerBiofilm™ Bead Tubes with this kit.

What's happening: Biofilm samples will vary in their moisture content. It is important to remove residual liquid to prevent dilution of the lysis components which could result in reduced DNA yield. Some biofilm samples, such as microbial mats may be added directly to the PowerBiofilm Bead Tube without an initial centrifugation step.

2. Resuspend the biofilm material in **350 µl of Solution BF1** and transfer to the PowerBiofilm™ Bead Tube. For less saturated samples, add **350 µl of Solution BF1** directly to the PowerBiofilm™ Bead Tube already containing the biofilm material.

Note: Solution BF1 must be warmed to dissolve precipitates prior to use. Solution BF1 should be used while still warm.

What's happening: Solution BF1 is a component of patented Inhibitor Removal Technology® (IRT). It is a strong lysing reagent that includes a detergent to help break cell walls and stabilizes and protects DNA from degradation. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution BF1 can be used while it is still warm.

3. Add **100 µl of Solution BF2**. Vortex briefly to mix.

What's happening: Solution BF2 contains a chaotropic agent that aids in lysis. BF2 also stabilizes and protects DNA integrity.

4. Incubate the PowerBiofilm™ Bead Tube at 65°C for 5 minutes.

What's happening: Lysis components are heat activated to aid in the breakdown of extracellular polymeric substances (EPS).

5. Bead beat the sample following one of the methods described below.

a) PowerLyzer™ 24 Homogenizer

- 1) Properly identify each PowerBiofilm™ Bead Tube on both the cap and on the side.

Note: Due to the high energies of the PowerLyzer™ 24, the potential of marring the tops of the caps is possible, therefore, it is recommended to mark the side of the PowerBiofilm™ Bead Tube, as well as the cap, to ensure proper sample identification.



- 2) Place the PowerBiofilm™ Bead Tubes into the Tube Holder of the PowerLyzer™ 24. The Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize at 3200 RPM for 30 seconds.
- 3) Centrifuge the tube at 13,000 x g for 1 minute. Transfer the supernatant to a new 2 ml Collection Tube (provided).

Note: Expect approximately 325 - 400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

b) Vortex Adapter

- 1) Secure the PowerBiofilm™ Bead Tube horizontally to a MO BIO Vortex Adapter, Catalog# 13000-V1 and vortex at maximum speed for 10 minutes.

Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the time by 5 – 10 minutes.

- 2) Centrifuge the tube at 13,000 x g for 1 minute at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

Note: Expect approximately 400 - 450 µl of supernatant depending on sample material. . If the volume falls below this range, use less starting material.

What is happening: Dissolution of the biofilm matrix and lysis of microbial cells occurs using a combination of chemical (lysis buffers) and mechanical (bead beating) lysis conditions. The resulting cell debris is pelleted along the side of the tube while the DNA remains in the supernatant. This step is important for the removal of contaminating non-DNA organic and inorganic matter that may reduce the DNA purity and inhibit downstream applications.

6. Add **100 µl of Solution BF3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

Note: Use 200 µl of Solution BF3 if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. See “DNA Does Not Amplify...” in the Hints and Troubleshooting Guide before continuing.

What's happening: Solution BF3 is a component of patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, polyphenolics, polysaccharides and proteins. The system works by using changes in pH to precipitate insoluble large macromolecules. The nucleic acids do not precipitate and are cleared of inhibitors. It is important to remove contaminating organic and inorganic matter that may reduce the DNA purity and inhibit downstream DNA applications.

7. Centrifuge the tube at 13,000 x g for 1 minute at room temperature.
8. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).

Note: Expect approximately 375 - 450 µl in volume depending on sample material and bead beating method.

What's happening: The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yields and quality, avoid transferring any of the pellet.

9. Add **900 µl of Solution BF4** and vortex briefly to mix.

Note: Check Solution BF4 for precipitation prior to use. Warm if necessary. Solution BF4 can be used while still warm.

What's happening: Solution BF4 is a highly concentrated salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filter.

10. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x g for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

Note: A minimum of two loads for each sample processed are required. Depending on the sample and amount of BF3 used, up to three loads may be necessary.

What's happening: DNA is selectively bound to the silica membrane in the Spin Filter basket and the flow through containing non-DNA components is discarded.

11. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

What's happening: Due to the high concentration of salt in solution BF4, it is important to place the Spin Filter basket into a clean 2 ml Collection Tube to aid in the subsequent wash steps and improve DNA purity and yield.

12. Shake to mix Solution BF5 before use. Add **650 µl of Solution BF5** and centrifuge at 13,000 x g for 1 minute at room temperature.

What's happening: Solution BF5 is an alcohol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica membrane.

13. Discard the flow through and add **650 µl of Solution BF6** and centrifuge at 13,000 x g for 1 minute at room temperature.

What's happening: Solution BF6 ensures complete removal of Solution BF5 which will result in higher DNA purity and yield.

14. Discard the flow through and centrifuge again at 13,000 x g for 2 minutes to remove residual wash.

What's happening: The second spin removes residual Solution BF6. It is critical to remove all traces of wash solution because the ethanol in Solution BF6 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

15. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

16. Add **100 µl of Solution BF7** to the center of the white filter membrane.

Note: A reduction in yield will occur if less than 50 µl of Solution BF7 is used for elution. For the highest yields elute in the recommended 100 µl volume of Solution BF7.

What's happening: Placing Solution BF7 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution BF7 passes through the silica membrane, DNA



that was bound in the presence of high salt is selectively released by Solution BF7 (10 mM Tris) which lacks salt.

Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step. Solution BF7 contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of BF7 for elution of DNA from the Spin Filter.

17. Centrifuge at 13,000 x g for 1 minute.

18. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution BF7 contains no EDTA.

Thank you for choosing the PowerBiofilm™ DNA Isolation Kit!