Exploring Native Microbiota Lab Manual

BIO398, Winter 2024

Lab 5 : DNA Extraction

Overview:

Last week, you took a small microbial sample (filter or other material) and placed it in lysis buffer. The goal of this week's lab is to break open all of the cells in that sample to release the DNA inside. This will serve as the template for your subsequent PCR reactions. We will use a variety of means to try and fully lyse the cells that are in your sample.

As this is the first time I am running this lab, your patience and help troubleshooting will be appreciated. However, things should go more smoothly compared to last week since this is a procedure that I am very familiar with but some of the equipment in the lab I will be using for the first time.

One reminder: cleanliness is important. You should wash your hands when you come in, wear gloves when handling your samples, and avoid touching your skin, clothes, phone, etc with your gloved hands. Otherwise you may contaminate your samples with microbes found around you and skew your results. Also, please do not take directly from stock solutions - ask for an aliquot from me (this is to avoid any contamination of the stocks).

Goals for Today's Lab:

1. To lyse our microbial samples with a combination of physical, enzymatic, and chemical means. This will produce what is often referred to as a "lysate" which is a complex mixture of biomolecules including your DNA.
2. 400 µL of this lysate should be then transferred to a new centrifuge tube in preparation for precipitating your DNA. Depending on your sample matrix, you may require a brief centrifugation to remove particles prior to this transfer. The leftover lysate should be stored in the -80°C freezer in case your subsequent steps fail [**minimum goal**].
3. [**stretch goal**] If you are able to come in tomorrow afternoon (Friday), you can leave your samples to incubate overnight with proteinase K to get a more complete extraction.
4. Then on Friday, you can begin the process of precipitation, centrifuge your samples and purify the DNA. This will allow us more time to prep for PCR, so would be ideal, but even if I have a few brave volunteers that would be great to troubleshoot.

Safety notes:

- Physical hazards: high-speed centrifugation has the potential to cause injury if tubes are not balanced. It is therefore important to make sure the **volumes you are transferring are**

accurate. ***If you are still uncomfortable with pipetting, ask me or a fellow classmate to make sure you are doing it right.***

- The chemicals we are using do not produce any harmful vapours, but it is very important to wear gloves and avoid the potential for any ingestion of substances used today. Some are toxic (EDTA), whereas others are carcinogenic if ingested (linear acrylamide). So long as you do not ingest anything accidentally, you will be safe.

DNA Extraction Procedure:

1. Take out the 6 tubes you want to extract from and set them on the countertop to thaw in a tube rack. Any backup samples should be placed back in the -80°C freezer.
2. When the tubes are warm enough to handle comfortably (do not need to be fully thawed), add a small amount of ***combusted*** beads (~0.25g) to your tubes. ***The instructor will do this step.***
3. When the liquid in your tubes has completely thawed, place them in the shaker vortexer and vortex at maximum speed for one minute. ***Make sure your tubes are closed tightly. Centrifuge tubes can go right-side up, whereas cryotubes only fit in upside-down.***
4. Add 30 µL of lysozyme solution (1 mg/mL; prepared by instructor) to your solution. I will provide small aliquots for each 1-2 students. You can use the same tip for multiple samples if it does not come into contact with the solution - otherwise, change the tip to avoid cross-contamination. ***Make sure to ask if you are unsure about pipetting.***
5. Invert to mix and incubate your sample at 37°C for 30 minutes.
6. Add 180 µL of 10% SDS solution. I will do this, since this solution is quite viscous and easy to spill. Wipe the outside of the tube if it spills. Close tightly.
7. Place tubes in the shaker vortexer and vortex at maximum speed for two minutes. ***Make sure your tubes are closed tightly. Centrifuge tubes can go right-side up, whereas cryotubes only fit in upside-down.***
8. **[branch point; choice depends on whether you are available to come in next day**]. Add 1µL of proteinase K solution (20 mg/mL; prepared by instructor). Invert to mix and incubate at 37°C according to times found in the following two scenarios:
    1. I cannot come in Friday afternoon and want to finish as much as possible today - **follow branch 1**
    2. I can come in on Friday afternoon - **follow branch 2**

Branch 1 (finish in one day)

1. Incubate your sample with proteinase K for 30 minutes.
2. After 30 minutes, remove your sample from the oven.

3. Centrifuging as necessary to remove particles (~3000xg for 1 min), carefully remove 400µL of your lysate and transfer to a clean, 15 mL centrifuge tube. ***Make sure tubes are labelled and that you use a fresh tip each time to avoid cross-contamination***.
4. Add 700 µL of high EDTA TE buffer ($T_{10}E_1$) to each centrifuge tube and mix with the 400 µL of lysate.
5. Freeze these samples in the -20°C freezer (in a whirlpack bag or rack) until you return next Thursday (steps 6-end of Branch 2).
6. If you haven't already, get 6 Nalgene or Oakridge centrifuge tubes. Clean them according to the Fuhrman lab procedure (leaving them sitting in 5% acid) [**end-point for branch 1**].

Branch 2 (continue on Friday)

1. Incubate your sample with proteinase K overnight.
2. If you haven't already, get 6 Nalgene or Oakridge centrifuge tubes. Clean them according to the Fuhrman lab procedure (leaving them sitting in 5% acid overnight) [**you can now leave for the day**].
3. When you return the next day, remove your sample from the oven.
4. Centrifuging as necessary to remove particles (~3000xg for 1 min), carefully remove 400µL of your lysate and transfer to a clean, OakRidge or Nalgene centrifuge tube. ***Make sure tubes are labelled and that you use a fresh tip each time to avoid cross-contamination.***
5. Add 700 µL of high EDTA TE buffer ($T_{10}E_1$) to each centrifuge tube and mix with the 400 µL of lysate.
6. Add 5 µL of linear acrylamide to each sample with a fresh tip. Immerse the tip into the solution to make sure it all gets in. The solution may get cloudy at this point - this is OK (it is the DNA precipitating). [**If you are re-entering the procedure from branch 1 here, then you must transfer your lysate + TE mixture to the OakRidge/Nalgene tubes that you have cleaned**]
7. Add 350 µL of 3M Sodium Acetate to each tube. You can most likely use the same tip, just avoid touching the side of tubes to avoid cross-contamination.
8. Add 3.5 mL of 100% ethanol to each tube. The instructor will most likely do this step.
9. Invert to mix, and place in the -20°C freezer for 1 hour.
10. Centrifuge samples for 20,000xg for 10 minutes at 4°C. ***Do not operate the centrifuge without supervision!*** Note which direction the tubes are facing, as this will help you know where your pellet is.
11. Carefully pour off the supernatant into a beaker that is labelled for extraction waste. Pour in a direction that minimizes the potential resuspension of your DNA pellet.

12. Wash with 500 µL of ice-cold ethanol to remove contaminating substances. Centrifuge samples for 20,000xg for 10 minutes at 4°C. ***Do not operate the centrifuge without supervision!*** Again note which direction the tubes are facing, as this will help you know where your pellet is.
13. Draw off as much of the ethanol as possible with a P1000 pipette tip. Air dry your pellet with caps off on the benchtop for 30 minutes. Try not to get any dust or anything in your tube. There is a hack with upside-down tubes we can try to minimize contamination (remind me if I forget). Or you can lay your tubes sideways on a large kimwipe (probably easier).
14. After 30 minutes, check to make sure there is no more liquid in your tube - it should be completely dry. If not, dry for another 10 minutes. Do not overdry or your DNA will become difficult to resuspend.
15. Resuspend your DNA with 50 µL of Low-EDTA TE ($T_{10}E_{0.1}$). Use aliquots provided by the instructor. You should roll the droplet around to try and pick up as much DNA as possible from the area you expect the pellet to be in. You can also vortex gently. It's OK if you don't see anything - there will be DNA there in most circumstances even if you can't see it.
16. Transfer this liquid containing your DNA into a previously UV'd centrifuge tube, and store at -80°C until quantification and PCR.