FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM
Department of Food Science, Cornell University

MQIP
Milk Quality Improvement Program

Title: Preparing cell lysate

Table of Content

1. INTRODUCTION 2

1.1 Purpose 2

1.2 Scope 2

1.3 Safety 2

2. MATERIALS 3

2.1. Enzymatic Lysis 3

2.2. Microwave Lysis 3

2.3. Heat Lysis 3

2.4. PowerSoil Kit-based DNA Extraction 4

3. PROCEDURES 5

3.1. Enzymatic Lysis 5

3.2. Microwave Lysis (dry colony) 6

3.3. Heat Lysis (dirty lysates) 7

3.4. PowerSoil Kit-based DNA Extraction 7

4. TROUBLESHOOTING 11

1. INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth the standardized protocols for the lysis of bacterial, yeast and mold cells to recover DNA for PCR. The protocols within include:

- **Enzymatic Lysis:** A high throughput method of producing high quality DNA lysates for PCR from bacterial cultures. Longer procedure but produces reliable results.
- **Microwave Lysis** (dry colony): For producing DNA lysates for PCR from bacterial cultures. Very fast but unreliable.
- **Heat Lysis** (dirty lysates): For producing DNA lysates for PCR from bacterial cultures. Faster method than enzymatic lysis only requiring streaked plates rather than overnight cultures.
- **PowerSoil Kit-based DNA Extraction**: For producing high quality genomic DNA from yeast and mold cultures for PCR or Whole Genome Sequencing.

1.2 Scope

This SOP applies to the Food Safety and MQIP Labs.

1.3 Safety

The thermocycler lid gets hot enough to cause severe burns; use appropriate caution when using the thermocycler.

Be sure to properly counterbalance the bucket centrifuge to avoid catastrophic failure of the machine.

If isolating DNA from BSL-2 organisms (e.g. *Listeria*), appropriate protective measures need to be taken. All BSL-2 waste needs to be treated accordingly.

Wear gloves for the entire procedure. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. Solution C5 in the Powersoil kit contains ethanol. It is flammable and should be kept away from open flames or sparks.

2. MATERIALS

2.1. Enzymatic Lysis

- Overnight cultures of isolates in BHI or other medium
- 96-well PCR plate(s) / 0.2ml strip tubes and strip tube rack
- Adhesive 96-well plate covers, foil or plastic
- Sterile reagent reservoirs
- 5X Colorless GoTaq Reaction Buffer
- Sterile ddH_2O
- Lysozyme, lyophilized
- Proteinase K, lyophilized
- Single-channel P1000 micropipette
- Multichannel P300 micropipette
- Multichannel P10 micropipette
- Thermocycler

2.2. Microwave Lysis

- Isolated cultures on agar media
- Autoclave sterilized toothpicks
- Autoclave sterilized 0.2ml strip tubes
- Strip tube rack
- Microwave

2.3. Heat Lysis

- Isolated cultures on agar media
- Autoclave sterilized toothpicks
- 96-well PCR plate(s) / 0.2ml strip tubes and strip tube rack
- Adhesive 96-well plate covers, foil or plastic
- Sterile reagent reservoirs
- Sterile ddH2O
- Single-channel P1000 micropipette
- Thermocycler

2.4. PowerSoil Kit-based DNA Extraction

- Isolated cultures of yeast or mold on agar media
- MO BIO Laboratories, Inc. PowerSoil DNA Isolation Kit, 100 Purifications Catalog # 12888-100

3. PROCEDURES

3.1. Enzymatic Lysis

The following procedure uses 96-well plates, but the same procedure can be used with 0.2ml strip tubes in a PCR tube rack

This protocol can be used with all bacterial types and can be used in high throughput scenarios.

3.1.1. Prepare the reagents in appropriately-sized sterile tubes and store on crushed ice:

1X PCR Buffer:

Reagent	1 reaction	100 reactions
Sterile ddH_2O	76 µL	7.6 mL
5X Colorless GoTaq Buffer	19 µL	1.9 mL

Lysozyme Solution (50 mg/mL):

Reagent	1 reaction	100 reactions
Sterile ddH_2O	6 µL	600 µL
Lysozyme, lyophilized	0.3 mg	30 mg

Proteinase K Solution (10 mg/mL):

Reagent	1 reaction	100 reactions
Sterile ddH_2O	4 µL	400 µL
Proteinase K, lyophilized	0.04 mg	4 mg

3.1.2. Prepare a template of the 96-well plate indicating the well in which each unique isolate will be placed.

3.1.3. Pipet 250 µL of each overnight culture into the designated well of the 96-well plate. Cover the plate with an adhesive cover.

3.1.4. Centrifuge the plate in the bucket centrifuge for 10 minutes at 4,000 rpm and at 4°C. If centrifuging only one plate, make sure to use a counterbalance plate containing 250µL per well.

3.1.5. Remove the plate from the centrifuge, carefully remove the cover, and use the P300 multichannel pipette to carefully remove the supernatant from each

well. Be sure not to disturb the cell pellets, and change tips between every row of wells. It is acceptable for a small amount of supernatant to remain.

3.1.6. Pour the 1X PCR buffer solution into a sterile reservoir, and use the P300 multichannel pipette to add 95 µL per well. Pipette up and down vigorously and use the pipette tip to physically break up the pellet in each well until homogeneous solutions form. Change pipette tips between every row of wells.

3.1.7. Pipette the lysozyme solution into a sterile reservoir, and use the P10 multichannel pipette to add 6 µL per well. Pipette up and down to mix. Change pipette tips between every row of wells. Cover the plate with an adhesive cover and allow the plate to sit at room temperature for 15 minutes.

3.1.8. Pipette the proteinase K solution into a sterile reservoir, and use the P10 multichannel pipette to add 4 µL per well. Pipette up and down to mix. Change pipette tips between every row of wells. Cover the plate with an adhesive cover.

3.1.9. Move the plate to a thermocycler, place two heating pads on top of the plate, and close the lid. Heat in the thermocycler using the following conditions:

Parameter	Temperature (ºC)	Time (min)
Incubate	58	60
Incubate	95	10
Store	4	hold

3.1.10. Stop the thermocycler when the program has completed and remove the plate. Lysates can be stored at 4°C for up to one week or at -20°C for up to 6 months.

3.1.11. Before removing the cover from the 96-well plate containing the lysates, centrifuge at 4,000 rpm for 3 minutes to prevent contamination. The lysates are quite concentrated, and it may be beneficial to dilute them 1:10 before proceeding with PCR.

3.2. Microwave Lysis (dry colony)

Taq, Vent, and PFU polymerases work with this method, and both touchdown and hotstart work as normal with this method

3.2.1 Select colonies to be used for PCR template one by one with a sterile toothpick.

3.2.2 Touch the tips to the bottom of a PCR reaction tube.
3.2.3 If you need a record of the colonies you've selected, as in screening for transformants, then after you've touched the tip to the colony and then touched the tip to the tube, gently streak the tip along a plate containing media of whatever type is necessary for your experiment.
3.2.4 Please note: having a huge glob of bacteria in the tube will not help facilitate a clean PCR. You should put enough of the colony into the tube such that you just see where you've scraped.
3.2.5 Microwave the tubes on high for 1 minute.
3.2.6 Add whatever master mix you desire to the tubes directly after microwaving is finished.
3.2.7 Proceed with your PCR set-up as usual.

3.3. Heat Lysis (dirty lysates)

3.3.1. For each isolate to be lysed, pipet 100 µL of sterile ddH_2O into one well of the 96-well plate or one 0.2 µL PCR tube placed in a PCR rack.
3.3.2. Use a sterile toothpick to gently touch a single colony from an overnight plate of the culture to be lysed. A partial colony will usually provide a sufficient quantity of DNA for the PCR, though addition of too much template DNA may inhibit the PCR.
3.3.3. Insert the toothpick into the appropriate well or tube containing 100 µL of ddH2O and swirl the toothpick briefly to dislodge the cells.
3.3.4. Repeat steps 3.1.1-3.1.3 for each sample and for a positive and negative control.
3.3.5. Seal the PCR plate with a cover or close the lids on the PCR tubes.
3.3.6. Use a thermocycler to heat the plate or tubes containing the lysates at 95°C for 5 minutes, then cool to 4°C.
3.3.7. Lysates can be stored at 4°C for up to 1 week, or can be stored at -20°C for up to 6 months.
3.3.8. If storing lysates at -20°C, freeze-thaw cycles should be limited to avoid shearing of DNA.

3.4. PowerSoil Kit-based DNA Extraction

Requires the use of the MO BIO Laboratories, Inc. PowerSoil DNA Isolation Kit Catalog # 12888-100

3.4.1. Use a loopful (~10 µl) of yeast or mold from plate, scraping as much as possible off the plate to get this amount. Add this to the PowerBead Tubes.

3.4.2. Gently vortex to mix. This mixes the components in the PowerBead Tube and begins to disperse the sample in the PowerBead Solution.

3.4.3. Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until the precipitate has dissolved before use. Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

3.4.4. Add 60 µl of Solution C1 to the PowerBead Tube and invert several times.

3.4.5. Secure PowerBead Tubes horizontally using a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. This vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-4 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.

3.4.6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature. Be sure not to exceed 10,000 x g or tubes may break.

3.4.7. Transfer the supernatant to a clean 2 mL Collection Tube. You should expect between 400 to 500 µl of supernatant at this step. The exact recovered volume depends on the absorbency of your starting material and is not critical for the procedure to be effective.

3.4.8. Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes. Solution C2 contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

3.4.9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g

3.4.10. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 mL Collection Tube. The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields and quality, avoid transferring any of the pellet.

3.4.11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes. Solution C3 is a second reagent to precipitate additional non-DNA organic and inorganic material including humic cell acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

3.4.12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g

3.4.13. Transfer up to 750 µl of supernatant to a clean 2 mL Collection Tube. The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields and quality, avoid transferring any of the pellet.

3.4.14. Shake to mix Solution C4 before use. Add 1.2 mL of Solution C4 to the supernatant, being careful that the solution does not exceed the rim of the tube. Vortex for 5 seconds. Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may be still present at low levels, to the Spin Filters.

3.4.15. Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Note: a total of three loads for each sample processed are required. DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

3.4.16. Add 500 µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g . Solution C5 is an ethanol 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, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

3.4.17. Discard the flow through from the 2 mL Collection Tube. This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

3.4.18. Centrifuge at room temperature for 1 minute at 10,000 x g. This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications like PCR, restriction digests, and gel electrophoresis.

3.4.19. Carefully place Spin Filter in a clean 2 mL Collection Tube. Avoid splashing any Solution C5 onto the Spin Filter. Note: it is important to avoid any traces of the ethanol based wash solution.

3.4.20. Add 100 µl of Solution C6 to the center of the white filter membrane. Note: placing the Solution C6 (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 C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10mM Tris) which lacks salt.

3.4.21. Centrifuge at room temperature for 30 seconds at 10,000 x g.

3.4.22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

4. TROUBLESHOOTING

If lysates appear to be turbid following inoculation, lysates may be diluted prior to use as template in the PCR. Addition of too much template will inhibit the reaction by exhausting the dNTPs and primers added, before full length products can be generated. The template added to each reaction should appear clear, and should not have obvious evidence of bacterial debris.

For Powersoil protocol:

DNA floats out of well when loaded on a gel:

This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 19 of the procedure not to transfer liquid onto the bottom of the spin bilker basket.

Ethanol precipitation (described above in "Concentrating the DNA") is the best way to remove residual Solution C5

Concentrating the DNA

The final volume of eluted DNA will be 100 µl. The DNA may be concentrated by adding 4 µl of 5 M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 10,000 x *g* for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol by air drying. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.