

Quick DNA Isolation from *P. fluorescens*

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Introduction

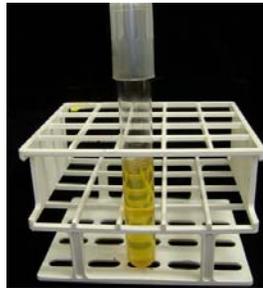
A number of procedures have been developed for DNA isolation from a variety of sources. The procedure presented here is an extension of a KOH lysis protocol used to differentiate Gram-negative from Gram-positive bacteria. Gram-negative bacteria will lyse in 3% KOH, whereas Gram-positive will not (Schumann & Jasalavich, 2001). *Pseudomonas fluorescens* was chosen for student work because it is a harmless soil bacterium, unlike its pathogenic cousin *Pseudomonas aeruginosa*. However, this procedure will likely work for several different Gram-negative bacteria. Since DNA is negatively charged and glass rods carry a net positive charge, a glass rod was used to spool the thread-like DNA out of solution. The isolated DNA was then re-hydrated and subjected to DNA-specific enzymes, the effects of which were visualized using DNA gel electrophoresis.

Protocol for DNA Isolation

Inoculate 13 mL of nutrient broth (Oxoid #2) in 2 culture tubes (per student pair) with *Pseudomonas fluorescens* (Carolina) and grow either 2 days shaking at 26°C or a minimum of 4 days at room temperature.

- A. Wear gloves and pour 12 mL into a sterile Falcon Tube (use gradations on side of tube as a guide). Spin 5 minutes at 3000 rpm to pellet the cells.
- B. Decant supernatant back into the culture tube, leaving the pellet behind and repeat with the second tube to increase pellet size (culture tube will be autoclaved).
- C. Add 1 mL of 3% KOH to the pellet (use a sterile disposable capillary pipet with volume gradations on it). To lyse the cells, stir and gently aspirate up & down for 30 seconds using this pipet, discard pipet into autoclave waste bag.
- D. To clarify this lysate, place tube into boiling water bath for 3 minutes (make sure cap is loose to allow air escape). Place on ice for 3 minutes.
- E. Use a new disposable pipet to slowly add 1 mL ice cold 100% ethanol down the tube wall, this will create an interface between the 2 layers of liquid.
- F. Prepare a sterile 1.5 mL microcentrifuge tube with 60 µL of sterile water.
- G. Insert a 6 inch glass Pasteur Pipet (previously autoclaved) so the fine tip touches the bottom of the tube. Hold your index finger at the top of the tube to rest the pipet against the wall and spin it in one direction with your other hand. Spin it 100 times to spool DNA onto the rod.
- H. Carefully lift the spooled DNA out of the tube, place it into the microcentrifuge tube and close the lid onto the glass area just behind the DNA. Break off the tip of the pipet by snapping it away from you (you may wish to wear goggles and do this over a sink or large beaker). The DNA and shattered glass will fall into the liquid at the bottom of the tube. Dispose of the remainder of the pipet into a glass waste container.
- I. Gently grind with a plastic hand-held pestle (Kontes) to break up the glass into fine pieces. Do not use motorized grinders, to avoid shearing of the DNA. Pestles may be rinsed and re-used several times.
- J. Let sit for 5 minutes at room temperature.
- K. Spin in a microcentrifuge at 13,000 rpm for 1 minute.
- L. Transfer 50 µL of the supernatant to a new 0.5 mL tube labeled with your initials. This sample may be placed in the freezer or used for enzyme digests.
- M. Enzyme digests typically use 10 – 17 µL of the DNA prep to create a total reaction volume of 20 µL. This volume works well for subsequent DNA electrophoresis.

Results and Observations



P. fluorescens culture



Pellet in Falcon Tube



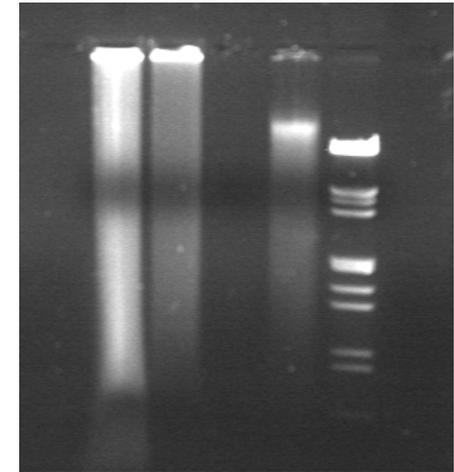
Boiling Falcon Tubes



Spooling DNA onto glass pipet



Hand-held pestle for grinding glass



Lanes 1 2 3 4 5

- 1 = Hae III cut DNA
- 2 = EcoRI cut DNA
- 3 = DNase treatment (10 mg/mL)
- 4 = Uncut *P. fluorescens* DNA (10 µL)
- 5 = EcoRI/Hind III cut lambda DNA

Literature Cited

Schumann, G.L. and C.A. Jasalavich. 2001. DNA the Easy Way. The Plant Health Instructor. DOI: 10.1094/PHI-K-2001-0222-01

Acknowledgements

Special thanks to Marc Breton '12 for helping to optimize this protocol and compile photographs. Thanks also to Dr. Gerald Kreider for his invaluable assistance.