PCR Experimental Success Guidelines

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Hints, Help & Troubleshooting

DNA Amplification/Fingerprinting Experiments

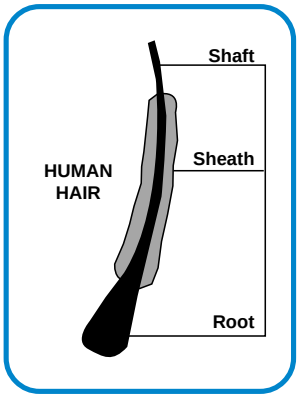
EDVOTEK experiments which involve the extraction and amplification of DNA for fingerprinting are extremely relevant, exciting and stimulating classroom laboratory activities. These experiments have been performed successfully in many classrooms across the country, but do require careful execution because of the small volumes used. The following guidelines offer some important suggestions, reminders and hints for maximizing success.

DNA Extraction and Sample Preparation

CELL PREPARATION:

1. **Sufficient Cells:** It is critical that there are sufficient cells to obtain enough DNA that will yield positive DNA fingerprinting results. Cell sources include human, plant, drosophila and bacterial cells. Remember - without enough cells, there will not be enough DNA template for the PCR reaction.

2. **Human (Self) DNA Fingerprinting:** Cells obtained from human sources such as cheek cells need to be harvested with caution. Aerosol can result and cross-contamination among students can be a health hazard. Hair follicles do not pose the same problem and yield adequate DNA required for the PCR reaction.



3. **Hair Cells:** At least four (4) hair follicles are needed. The **preferred** source is hair from eyebrows. Use only hairs containing a sheath, a barrel-shaped structure (often white in color) encircling the shaft near the base of the hair (see figure at left). Centrifuge the hair follicles to the bottom of the microcentrifuge tube to ensure direct contact with the reagents used in subsequent steps.

4. **Cheek Cells:** A white pellet must be visible after centrifuging the cell suspension obtained from cheek cell swabbing. If necessary, repeat the centrifugation to obtain a visible pellet. After removal of the supernatant, suspend the pellet in the chelating agent by repeated vortexing and pipetting up and down.

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DNA Extraction and Sample Preparation, continued

5. **Chelating Agent:** Chelex removes Mg (required by DNA-degrading nucleases and DNA polymerases). The small beads must be suspended in the buffer prior to delivery to the cells (i.e., mix the chelex just before you transfer it to the tube containing the cells.

6. **Boiling:** The boiling step for 10 minutes is required to obtain cell lysis. Boiling will not degrade the DNA and nucleases will NOT degrade DNA in the absence of Mg.

7. **Centrifugation:** Centrifuge the cell suspension carefully after cooling. If the pellet loosens, repeat this step. The supernatant should be clear, not cloudy, and the pellet should be solid at the bottom of the tube. Repeat centrifuation for a longer period of time, if necessary.

8. **DNA Transfer:** Transfer the DNA to a new microcentrifuge tube very carefully. It is the step prior to the PCR reaction. If any chelex beads (as few as one or two) are transferred, they can easily trap the Mg required by the *Taq* DNA polymerase as a cofactor for catalysis. As an additional precaution, centrifuge the supernatant a second time.

   Remember: Any carry-over of chelex to the PCR reaction will not yield results.

The PCR Reaction

9. **Add Primers and DNA to the PCR Reaction Bead:** Add the primer mixture (forward and reverse primers) and the cell DNA (supernatant) as specified in the experimental procedures to the microcentrifuge tube containing the PCR reaction bead. Make sure that the bead (which contains the *Taq* DNA polymerase, the 4XdTPs, Mg and the PCR reaction buffer) is completely dissolved. Do a quick spin in a microcentrifuge to bring the entire sample to the bottom of the tube. Prepare the control reaction similarly.

10. **The Thermal cycler:** The thermal cycler must be programmed for the correct cycle sequence. It is critical that the temperatures and the time for each of the cycles are accurate.

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The PCR Reaction, continued

11. **Oil or Wax:** For certain thermal cyclers which do not have a top heating plate, it is necessary to overlay the reaction in the microcentrifuge tubes with oil or wax to prevent evaporation.

12. **Manual Water Bath PCR:** Three water baths can be used as an alternative to using a thermal cycler for PCR. Samples require oil or wax layers. This method requires extra care and patience and results are more variable than when using a thermal cycler.

Gel Preparation and Staining

13. **Concentrated agarose:** Gels of higher concentration (> 0.8%) require special attention when dissolving or re-melting. Make sure that the solution is completely clear of "clumps" or glassy granules. Distorted electrophoresis DNA band patterns will result if the gel is not properly prepared.

14. **Electrophoretic separation:** The tracking dye should travel at least 6 cm from the wells for adequate separation before staining.

15. **Staining:** Staining of higher concentration gels (> 0.8%) require additional care to obtain clear, visible results.
    - After staining (15 to 30 min.) with Instastain® Ethidium Bromide or liquid ethidium bromide, examine the results using a UV (300nm) transilluminator. Repeat the staining as required.
    - Gels stained with Instastain® Methylene Blue or liquid methylene blue stain may fade with time. Re-stain the gel to visualize the DNA bands.

16. **DNA 200 bp markers:** After staining the agarose gel, the DNA 200 bp markers should be visible after staining. If there are visible bands in the markers and control lanes, but bands in the sample lanes are faint or absent, it is possible that the DNA was not successfully extracted from the cells. If markers, control and DNA bands are all faint or absent, problems could potentially be due to improper preparation of the gel, absence of buffer in the gel, improper gel staining or a dysfunctional electrophoresis unit or power source.