



Isolating & Fingerprinting DNA



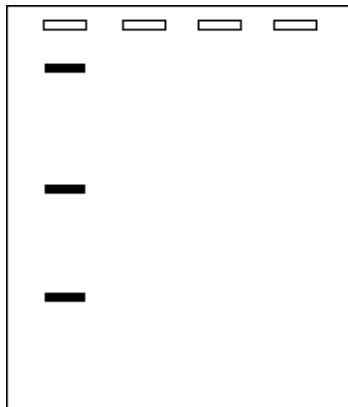
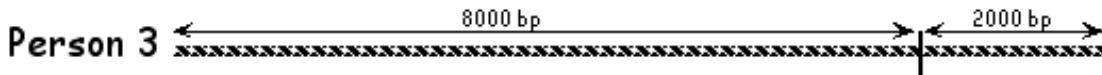
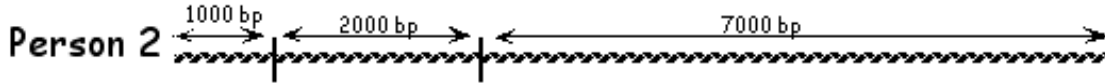
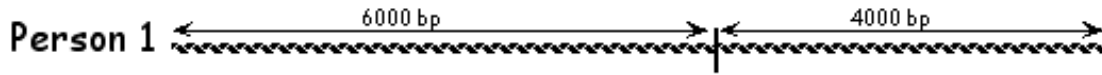
Background	<p><u>Our Research Project - What We Are Cloning and Why</u> We hope to identify new hot spring bacteria that cannot be grown on lab media To study these organisms, we extract DNA from hot springs with unknown bacteria We clone a specific identification gene (the 16S gene) from the hot spring DNA We place each hot spring gene into E. coli, our cloning factory Then we fingerprint and DNA sequence each hot spring clone</p>																								
Objectives	<p>Each student will isolate DNA from clones which originated with samples collected from Fairy Spring in Yellowstone National Park. Students will use restriction enzymes to cut and then “fingerprint” their clones, and learn about what their results mean.</p>																								
Materials	<table border="0"> <tr> <td>Pipetteman</td> <td>Blue dye</td> <td>Microfuge tubes</td> </tr> <tr> <td>Culture tubes</td> <td>Microcentrifuge</td> <td>WASTE containers</td> </tr> <tr> <td>STET</td> <td>Water bath</td> <td>Lysozyme</td> </tr> <tr> <td>Floating racks</td> <td>Toothpicks</td> <td>Sodium acetate</td> </tr> <tr> <td>Isopropanol</td> <td>Paper towels</td> <td>Chem.-wipes</td> </tr> <tr> <td>Tube racks</td> <td>TE/RNase</td> <td>Ice & ice bucket</td> </tr> <tr> <td>Cocktail mix</td> <td>Incubator</td> <td>Freezer</td> </tr> <tr> <td>Agarose gel</td> <td>Loading dye</td> <td>Ladder markers</td> </tr> </table>	Pipetteman	Blue dye	Microfuge tubes	Culture tubes	Microcentrifuge	WASTE containers	STET	Water bath	Lysozyme	Floating racks	Toothpicks	Sodium acetate	Isopropanol	Paper towels	Chem.-wipes	Tube racks	TE/RNase	Ice & ice bucket	Cocktail mix	Incubator	Freezer	Agarose gel	Loading dye	Ladder markers
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<u>Procedure</u> Pipetteman Practice	<p>Pipetteman are expensive (\$250 apiece) and misuse will result in reactions not working, expensive reagents being wasted, and costly repair labor. When operating a pipetteman, <i>ALWAYS BE GENTLE, SLOW, AND DELIBERATE.</i></p> <p><u>Understand Setting a Pipetteman:</u></p> <table border="1" data-bbox="493 1129 1451 1234"> <thead> <tr> <th></th> <th>Range</th> <th>3-Digit Shaft Reads</th> <th>Example</th> </tr> </thead> <tbody> <tr> <td>P20</td> <td>0.2-20 ul</td> <td>00.0</td> <td>5 ul, setting = 050 (05.0)</td> </tr> <tr> <td>P100/200</td> <td>2-100/200 ul</td> <td>000</td> <td>5 ul, setting = 005 (005)</td> </tr> <tr> <td>P1000</td> <td>20-1000 ul</td> <td>X10 for actual amount</td> <td>500 ul, setting = 050 (50X10)</td> </tr> </tbody> </table> <p>Before you begin, feel and understand the two settings on the thumb press:</p> <ol style="list-style-type: none"> First/Less Pressure: accurately withdraws the set amount Second/More Pressure: completely expels sample during transfer. <p>Unless we tell you otherwise, ALWAYS change tips between solutions.</p> <p>Press and hold to the first point BEFORE you withdraw.</p> <p>Watch everything. Place tip JUST at surface of samples and withdraw slowly.</p> <p>To expel, LOOK into the recipient tube as you expel to the BOTTOM. Press slowly to the second point. Slowly release pressure AFTER tip is removed from the tube.</p> <p>We will watch you measure 10 ul and 1 ul for practice before proceeding. There are tubes of blue dye at your desk, practice using the pipetteman with these.</p>		Range	3-Digit Shaft Reads	Example	P20	0.2-20 ul	00.0	5 ul, setting = 050 (05.0)	P100/200	2-100/200 ul	000	5 ul, setting = 005 (005)	P1000	20-1000 ul	X10 for actual amount	500 ul, setting = 050 (50X10)								
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<p>Procedure DNA Isolation</p>	<p>Label tops of 2 X 2ul microfuge tubes with clone #'s you have been given. Carefully pour 2 ml of culture into each respective microfuge tube. Pellet cells by spinning at max speed in your microcentrifuge for 2 minutes. Gently pour the supernatant (liquid on top of the pellet) into a WASTE container. The pellet will not move after it has been spun down, but STILL BE CAREFUL! Add 0.35ml of STET. Gently pipette up and down to resuspend material.</p> <p><i>The next few steps need to be organized and discussed before doing them. Make sure each person at your table has a job and knows how to do it. The procedures here need to be done quickly and efficiently for the best product results. Suggestion: one person should add down the line, another cap, another vortex, and the fourth organize the floating rack.</i></p> <p>Add 40 ul lysozyme, vortex 2 seconds and place samples in circular floating racks. Place samples in boiling bath at same time for EXACTLY 60 seconds. IMMEDIATELY following boiling, spin at max speed 10 minutes. Immediately remove "snot-like" pellet with non-sterile toothpick – discard. Precipitate DNA by adding 40 ul 3M salt then 0.42 ml alcohol – invert/shake. Freeze 30 minutes in the -80 C freezer to fully precipitate DNA. After freezing, remove the tubes from the freezer. Spin for 10 minutes at max. Remove the tubes from the centrifuge, carefully pour supernatant into WASTE. Place tubes upside down on a paper towel on your lab table 20-30 minutes. Place the tubes back into the tube rack upright. Add 80 ul of TE/RNase to each tube and pipette to resuspend the pellet.</p> <hr/> <p><i>Teachers: need to pre-prepare restriction enzyme cocktail OR have students mix and run, which will add at least another hour to total time.</i></p>
<p>Procedure Agarose Gels & Fingerprinting</p>	<p>Collect your samples making sure you have the correct clone #'s. Place 2 ul of loading dye at the top of each tube. Tap down so your sample is blue. I will do a quick demonstration load of the standard markers. Load your samples, setting the pipetteman to 15 ul; run gels at 100 volts. Make ABSOLUTELY CERTAIN you draw EXACTLY what you loaded where.</p> <p><i>When the gels are done running, they need to be stained with ethidium bromide, a very toxic chemical. Students WILL NOT be handling the gels when they are in the stain. During the staining, students will be working on the DNA Fingerprinting problems given to them. After staining is complete, I will place the gels under the UV light and take pictures for students to take home with them.</i></p>

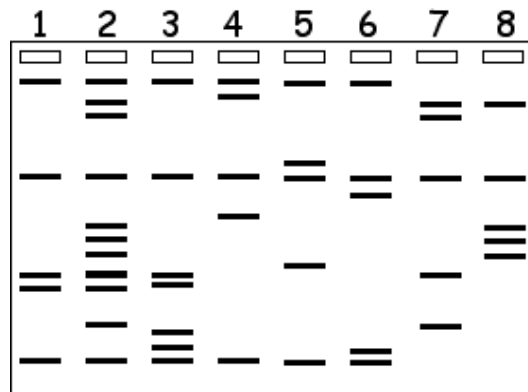
DNA Fingerprinting Problems

Complete As A Team While Gels Are Running

(1) You are studying DNA from 3 different people. The restriction enzyme you are using cuts at the vertical lines. The sizes of the fragments are shown. Your Task: draw the DNA bands on the gel below (Person 1 in lane 2; person 2 in lane 3; person 3 in lane 4). Lane 1 was loaded with DNA of known sizes: 8000 base pairs, 4000 bp, and 2000 bp. Can you tell them apart using this enzyme?



2. "Murder Crime Scene Analysis" - Forensic Science can use DNA fingerprinting to analyze, compare, and often identify individuals based on small amounts of DNA from scene evidence like blood, hair, skin cells, etc. The first lane is the victim's pure DNA. The second lane is from a mixture of blood at the crime scene. Lanes 3-8 are suspects.



3. "Medical Genetic Testing" - Genetic counselors can use DNA Fingerprinting to diagnose genetic diseases. In this example, we will consider the gene for sickle cell anemia, a disease that debilitates red blood cells. Mutations in the normal gene result in different forms of the disease - and in different

cutting patterns with restriction enzymes. Consider the following gel: the first lanes are "controls" that show you the patterns for "normal" (N), "mild" (M), and "fatal" (F) anemia. Using this information, play genetic counselor by helping this family determine which forms of anemia mom, dad, and all the kids have. If you have had some genetics, try to assign a "genotype" to each individual.

