

LAB 8: RESTRICTION ENZYME DIGESTION OF
CRIME SCENE AND SUSPECT DNA SAMPLES

1. Each group should get 5 microcentrifuge tubes and label them:

Tube	Label	Content	Function
1	CS Control	Crime Scene DNA	Remain as uncut control
2	CS	Crime Scene DNA	Cut with enzyme
3	S1	Suspect #1 DNA	Cut with enzyme
4	S2	Suspect #2 DNA	Cut with enzyme
5	S3	Suspect #3 DNA	Cut with enzyme

2. Get all necessary reagents (DNA, restriction enzymes and restriction enzyme buffers), as directed by your teacher. Keep ALL the reagents ON ICE. Add the appropriate solutions as indicated below:
- Sample CS Control**
6 μ l 2X Restriction Enzyme buffer
2 μ l **CS Control DNA**
4 μ l sterile water
 - Sample CS**
6 μ l 2X Restriction Enzyme buffer
2 μ l **CS DNA**
4 μ l sterile water
 - Sample S1**
6 μ l 2X Restriction Enzyme buffer
2 μ l **S1 Suspect DNA**
4 μ l sterile water
 - Sample S2**
6 μ l 2X Restriction Enzyme buffer
2 μ l **S2 Suspect DNA**
4 μ l sterile water
 - Sample S3**
6 μ l 2X Restriction Enzyme buffer
2 μ l **S3 Suspect DNA**
4 μ l sterile water
3. After all three solutions have been added to each tube, close the caps tightly and mix by finger vortexing (that just means holding the top of the tube with the thumb and index finger of one hand while gently vibrating the bottom of the tube with the index finger of your other hand. Spin the samples in a microcentrifuge for 5 seconds. **NOTE!!!! Remember that the samples must be put in the centrifuge rotor in pairs so that the machine is balanced, otherwise it will shake and move and not spin correctly. What will you need to do in order to have a balanced set of tubes?**
4. Place your samples in a 37 °C water bath for a minimum of 30 minutes. Then leave the samples overnight at room temperature or store in a refrigerator.

