For use with CarolinaBLU™ stain:

Tube	BamHI–HindIII restriction enzyme mixture	Restriction Buffer–RNase	Suspect 1 DNA	Suspect 2 DNA	Evidence A or B	H_2O
S1	3 µL	3 µL	10 µL			2 µL
S2	3 µL	3 µL		10 µL		2 µL
EA or EB	3 µL	3 µL			10 µL	2 µL

3. Mix reagents by pipetting gently up and down.

4. Incubate all of the reaction tubes for 1 hour at 37 °C.

NOTE: Your instructor will freeze your completed restriction digests at -20 °C until the next lab period.

III. Electrophorese Digests

Reagents:

- Restriction digests from Part II, on ice
- 10x loading dye, 10 μL

Supplies and Equipment

- Gel electrophoresis chamber with agarose gel in gel tray, power supply
- 1-20 μL Micropipette and pipet tips

Load the Gel

1. Use a micropipette to add 2 μL of 10× loading dye to a reaction tube. Use the pipet tip and gently pipet up and down a couple of times to mix the 10× loading dye with the digested DNA. Use a new pipet tip and repeat for each digest.

2. Use a micropipette to load the contents of each reaction tube (20 μL total) into a separate well in the gel. Use a fresh pipet tip for each reaction tube and write down the order in which the samples are loaded.

NOTE: Be careful not to punch the tip of the pipet through the bottom or side of the well.

While loading,

- steady the pipet over the well using two hands. You may wish to place one or both elbows on the lab bench to steady your hands.
- be careful to expel any air in the pipet tip end before loading the gel. If an air bubble forms a cap over the well, the sample will flow into the buffer around the edges of the well.