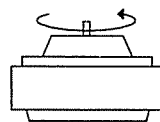


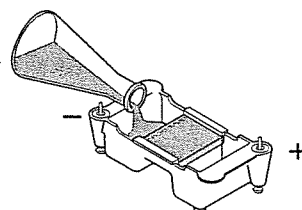
Lesson 3 Electrophoresis of Amplified PCR Samples and Staining of Agarose Gels

1. Obtain your PCR tube from the thermal cycler and place in the capless micro test tube. Pulse-spin the tube for ~3 seconds at 2,000 x g.
2. Add 10 μ l of PV92 XC loading dye into your PCR tube and mix gently.
3. Place an agarose gel in the electrophoresis apparatus. Check that the wells of the agarose gel are near the black (-) electrode and the base of the gel is near the red (+) electrode.

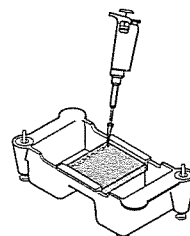


Centrifuge

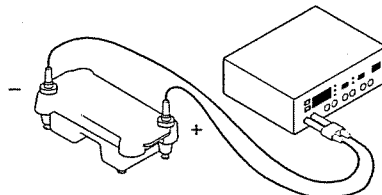
4. Fill the electrophoresis chamber and cover the gel with 1x TAE buffer. This will require ~275 ml of 1x buffer.
5. Using a clean tip for each sample, load the samples into 8 wells of the gel in the following order:



Lane	Sample	Load Volume
1	MMR (DNA standard)	10 μ l
2	Homozygous (+/+) control	10 μ l
3	Homozygous (-/-) control	10 μ l
4	Heterozygous (+/-) control	10 μ l
5	Student 1	20 μ l
6	Student 2	20 μ l
7	Student 3	20 μ l
8	Student 4	20 μ l

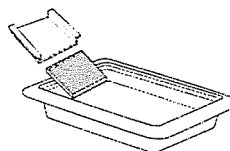


6. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.
7. Turn on the power supply and electrophorese your samples at 100 V for 30 minutes.



Staining of Agarose Gels

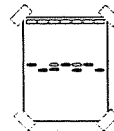
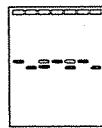
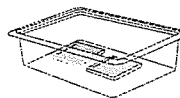
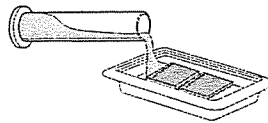
1. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, **the gel is very slippery**. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.



2. There are two protocols for staining your gel. Your instructor will inform you which one you will use.

Protocol 1: Quick staining (requires 12–15 minutes)

- Add 120 ml of 100x Fast Blast stain into your staining tray (2 gels per tray).
- Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use. Stain can be reused at least 7 times.
- Transfer the gels into a large washing container and rinse with warm (40–55°) tap water for approximately 10 seconds.
- Destain by washing **twice** in warm tap water for 5 minutes each with gentle shaking for best results.
- Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.
- With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion.
- Trim away any empty lanes of the gel with a knife or razor blade.
- To obtain a permanent record, air-dry the gel on gel support film. Tape the dried gel onto your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.



Protocol 2: Overnight staining

- Add 120 ml of 1x Fast Blast DNA stain to your staining tray (2 gels per tray).
- Let the gels stain overnight, with gentle shaking for best results. No destaining is required.
- The next day, pour off the stain into a waste beaker.
- Place the gel on a light background and record your result. With a permanent marker, trace the wells and band patterns onto a clear sheet of plastic or acetate sheet.
- With the help of your instructor, determine whether you are homozygous or heterozygous for the Alu insertion.
- Trim away any empty lanes of the gel with a knife or razor blade.
- To obtain a permanent record, air-dry the gel on gel support film. Tape the dried gel into your lab notebook. Avoid exposure of the stained gel to direct light, since it will cause the bands to fade.

