Quick Guide for Analysis of Precut Lambda DNA Kit

Lesson 1 Sample Preparation

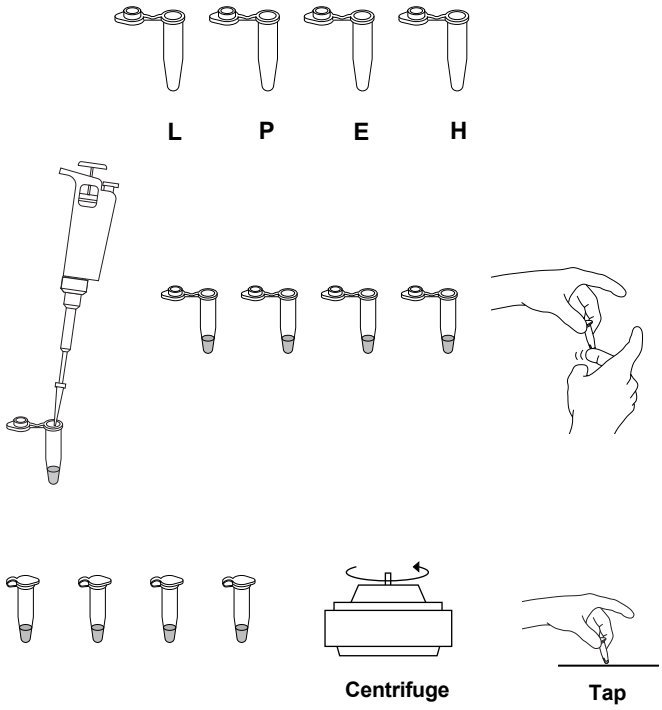
1. Obtain one of each colored micro test tube for each team and label each as follows:

   yellow, **L** = lambda DNA
   violet, **P** = *Pst*I lambda digest
   green, **E** = *Eco*RI lamgda digest
   orange, **H** = *Hind*III lambda digest

2. Using a fresh tip for each sample, pipet 10 µl of DNA sample from each stock tube and transfer to the corresponding colored micro test tube.
3. Add 2 µl of sample loading dye to each tube. Mix the contents by flicking the tube with your finger.
4. *Optional*: Heat the DNA samples at 65°C for 5 minutes.
5. Pulse-spin the tubes in the centrifuge to bring all of the liquid to the bottom or tap them gently on the benchtop.
6. You have two options:

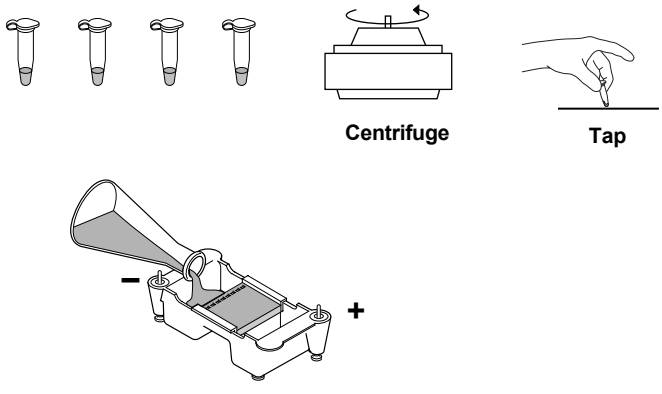
   Option one: Put the DNA samples into the refrigerator and run the agarose gel during the next class.

   Option two: Run the agarose gel the same day. Proceed directly to step 3 below.



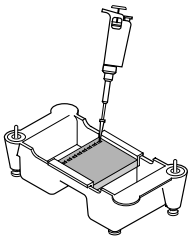
Lesson 2 Agarose Gel Electrophoresis

1. Remove the DNA samples from the refrigerator (if applicable).
2. Pulse-spin the tubes in the centrifuge to bring all of the liquid to the bottom or tap them gently on the benchtop.
3. Remove the agarose gel from the refrigerator (if applicable), remove the plastic wrap, and place the gel in the electrophoresis chamber. Fill the electrophoresis chamber and cover the gel with approximately 275 ml of 1 x buffer.
4. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.

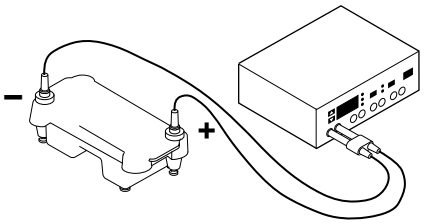


5. Load 10 µl of each sample into separate wells in the gel chamber in the following order:

   Lane 1: **L** (yellow tube)
   Lane 2: **P** (violet tube)
   Lane 3: **E** (green tube)
   Lane 4: **H** (orange tube)

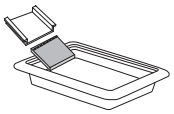


6. Place the lid on the electrophoresis chamber carefully. Connect the electrical leads into the power supply, red to red and black to black.
7. Turn on the power and run the gel at 100 V for 30 minutes.



Visualization of DNA Fragments

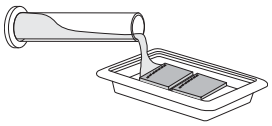
1. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.



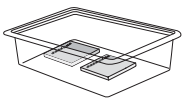
2. You have two options for staining your gel:

   Option one: Quick staining (requires 12–15 minutes)

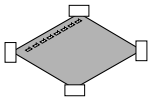
   a. Add 120 ml of 100x Fast Blast stain into a staining tray (2 gels per tray).



   b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.



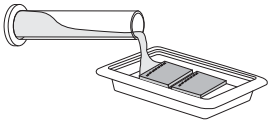
   c. Transfer the gels into a large washing container and rinse with warm (40–55°) tap water for approximately 10 seconds.
   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.
   e. Record results.
   f. Trim away any unloaded lanes.



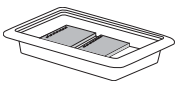
   g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.

   Option two: Overnight staining

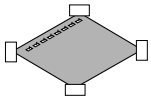
   a. Add 120 ml of 1x Fast Blast DNA stain to the staining tray (2 gels per tray).



   b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.



   c. Pour off the water into a waste beaker.
   d. Record results.
   e. Trim away any unloaded lanes.
   f. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.



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