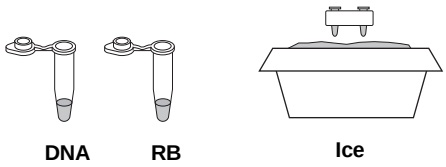
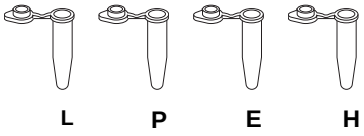
Quick Guide For Restriction Digestion and Analysis

Lesson 1 Restriction Digestion

1. Obtain micro test tubes that contain each enzyme stock solution, lambda DNA, and restriction buffer. Keep all the stock solutions on ice.



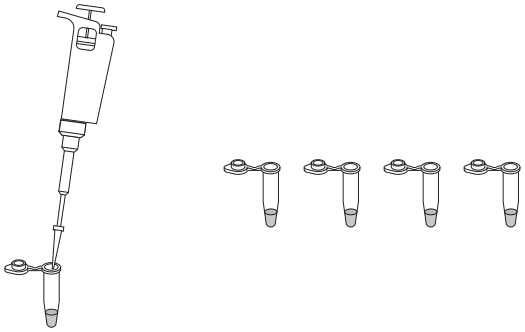
2. Obtain one of each colored micro test tubes and label them as follows:
   yellow tube: **L** (lambda DNA)
   violet tube: **P** (*Pst*I lambda digest)
   green tube: **E** (*Eco*RI lambda digest)
   orange tube: **H** (*Hind*III lambda digest)



Additional Tubes: P/E, P/H, H/E

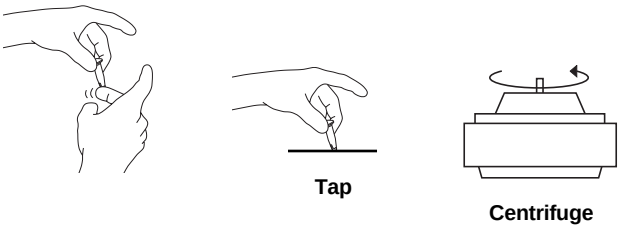
3. Using a fresh tip for each sample, pipet the reagents into each tube according to the table below:

tube	DNA	buffer	*Pst*I	*Eco*RI	*Hin*dIII
L	4 µl	6 µl	–	–	–
P	4 µl	5 µl	1 µl	–	–
E	4 µl	5 µl	–	1 µl	–
H	4 µl	5 µl	–	–	1 µl

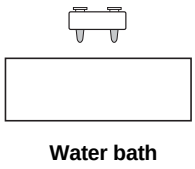


(Use same proportions for additional tubes, just add extra enzyme.)

4. Mix the components by gently flicking the tube with your finger and tapping gently on the table to collect liquid to the tube bottom. Pulse-spin the tubes in a centrifuge to collect all the liquid to the bottom, or tap them gently on the benchtop.



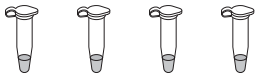
5. Place the tubes in the floating rack and incubate for 30 minutes at 37°C or overnight at room temperature.



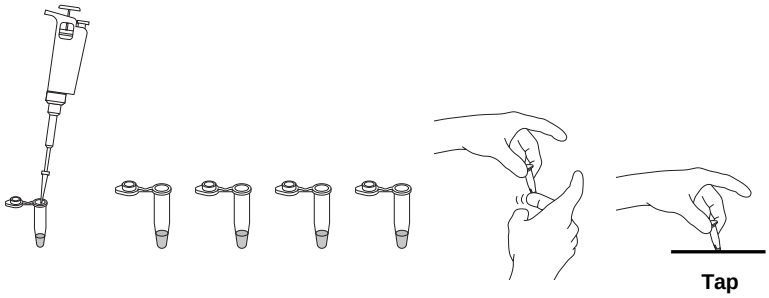
6. After the incubation, place the samples in the refrigerator until the next laboratory period, or proceed directly to step 2 of Lesson 2.

Lesson 2 Agarose Gel Electrophoresis

1. Remove the digested DNA samples from the refrigerator (if applicable).



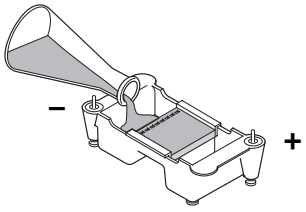
2. Add 2 µl of sample loading dye into each tube. Mix the contents by flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.



3. Obtain the DNA marker (M) from your teacher.

4. Optional: Heat your DNA samples at 65°C for 5 minutes and then put them on ice.

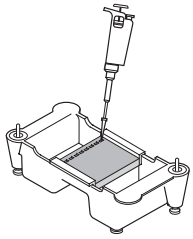
5. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap. Fill the electrophoresis chamber and cover the gel with 1x TAE buffer (about 275 ml of buffer).



6. 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.

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

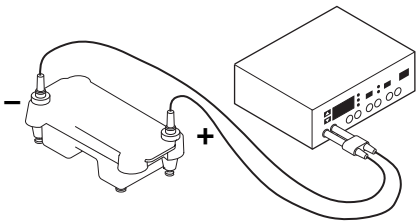
Lane	Sample
1	**M,** marker (clear tube)
2	**L**, uncut lambda DNA (yellow tube)
3	**P**, *Pst*I lambda digest (violet tube)
4	**E**, *Eco*RI lambda digest (green tube)
5	**H**, *Hin*dIII lambda digest (orange tube)



Don't forget to use:
Lane 6 for P/H
Lane 7 for P/E
Lane 8 for H/E

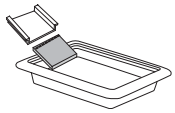
8. Carefully place the lid on the electrophoresis chamber. Connect the electrical leads into the power supply, red to red and black to black.

9. Turn on the power and run the gel at 100 V for 30 minutes.



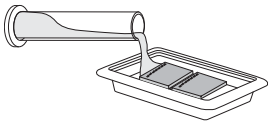
Visualization of DNA Fragments

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:

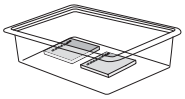
   Quick staining (requires 12–15 minutes)



   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.

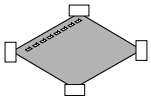
   c. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.



   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.

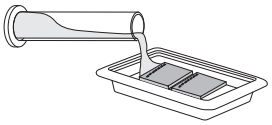
   e. Record results.

   f. Trim away any unloaded lanes.



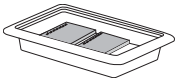
   g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.

   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.

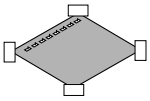


   c. Pour off the water into a waste beaker.

   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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