DNA Digestion

1. make mixture of DNA digestion reaction (total of ~10 ul):
   - a. Desired DNA(e.g. from Miniprep) 1 ul
   - b. 10X reaction buffer (dependent on enzymes) 1 ul
   - c. enzyme 1 (e.g. BamHI) 0.5 ul
   - d. enzyme 2 (e.g. EcoRI) 0.5 ul
   - e. H2O 1 ul
   - f. 100X BSA (Optional) 0.1 ul
2. incubate the mixture in 37oC incubator for 2-3 hr.
3. During the waiting time, prepare 1X TAE buffer (dilute the 50X TAE stock solution with H2O).
4. make 50 ml 1% agarose gel solution for 1 gel tray (i.e. 0.5 agarose in 50 ml 1X TAE buffer).
5. Microwave about 40 sec-1min until boiling and agarose completely dissolved. (Caution! avoid the over-spill).
6. add 2.5 ul of EB and swirl to dissolve.
7. cool down a bit and pull the solution the gel tray of DNA electrophoresis system.
8. Place desired combs in the gel solution to create the wells.
9. when the digestion reaction is ready, take out reaction mixture, add 6X DNA loading buffer into the mixture and dilute to 1X (e.g. add 2 ul of 6X loading buffer to 10 ul reaction mixture).
10. load the reaction mixture in the gel wells (Don’t forget the DNA ladder lane).