

Protocol: Restriction Digestion

The Idea

A restriction digest is used to cut DNA at specific sequences to leave “sticky” ends. If two pieces of DNA have complementary “sticky” ends, they can be joined together to form a longer piece of DNA via *ligation*. The pSB4A5 plasmid will be cut with restriction endonucleases EcoRI and PstI.

Materials

- * DNA (to be digested)
- * Restriction enzymes (EcoR I, Spe I, Xba I or Pst I) from NEB
- * NEB2 buffer
- * BSA (bovine serum albumin)
- * Deionized, sterile H₂O

Digest Mixture

- * 5 μ L NEB2 buffer
- * 42.5 μ L DNA (mini-prepped pSB4A5 DNA).
- * 0.5 μ L 100X BSA
- * 1 μ L EcoRI
- * 1 μ L PstI

Procedure

1. Add appropriate amount of 4A5 Plasmid DNA to sterile 1.7ml microcentrifuge tube
2. Add restriction enzyme buffer to the tube.
3. Add BSA to the tube.
Vortex BSA before pipetting to ensure that it is well-mixed.

4. Add 1 μ L of each enzyme.

Important note: the enzymes are extremely temperature-sensitive! They should be kept on ice at ALL times when not in use. Vortex enzyme before pipetting to ensure that it is well-mixed. Also, the enzyme is in some percentage of glycerol which tends to stick to the sides of your tip. To ensure you add only 1 μ L, just touch your tip to the surface of the liquid when pipetting.

5. Mix and vortex the reaction mixture one final time.
6. Quickly spin reaction mixture (about 5s) to collect all liquid in the bottom
7. Incubate at appropriate temperature (37C for our purposes).