Protocols: Restriction Digestion

Determine the order of the two parts you will be putting together; the one in front will be referred to as the insert, while the one behind will be referred to as the vector. Both the vector and the insert need to have their own separate tube, at least in the beginning.

Restriction Digest Protocol

In the Insert Tube...

- 600 ng of DNA (To figure out the volume, the calculation is 600 / concentration of plasmid. This gives you volume in µL).
- Water, so that the volume of both DNA and water in the tube is 35 µL total
- 4 µL of React 1 Buffer
- 0.5 µL of EcoR1
- 0.5 µL of Spe1
- 1 µL BSA

In the vector Tube...

- 250ng of DNA (To figure out the volume, the calculation is 250 / concentration of plasmid. This gives you volume in µL).
- Water, so that the volume of both DNA and water in the tube is 35 µL total
- 4 µL of React 2 Buffer
- 1µL BSA
- 0.5 µL of Xba1
- 0.5 µL of Pst1

Put both tubes into the 37°C water bath for one hour. After, place them into the 65°C heating block for 10 minutes. This deactivates any enzymes in the tube (which is ok, because by now they've done all they need to). Take the insert out, and put it in a -20°C freezer.