Restriction Digestion

Aim:

- Digest DNA fragments

Timeframe:

- Preparation: 15 minutes
- Wait-time: 80 min
- Overall: 95 min

Materials:

- ddH2O
- Liquid DNA aliquot of plasmid of interest
- Appropriate restriction enzyme (eg EcoR1 and Pst1 for BioBrick Assembly, BsaI for Type IIs)
- NEB CutSmart buffer
- Pipette tips of appropriate volumes (1 ul, 10 ul, 100 ul)

Procedure:

1. Turn on water baths, one at 37°C and one at the heat inactivation temperature specified below.
2. Fill an ice box with ice.
3. Keep enzymes on ice when not in freezer.
4. In a sterile, labelled 0.5 ml eppendorf tube, combine the following:
    - 3-5 µl 10X Buffer depending on total volume
    - ddH2O up to total volume (reach a total volume 50 µl when combined with other components
    - 10 units (~0.5 µl) Restriction Enzyme(s)
    - Up to 1 µg DNA
5. Mix components gently by pipetting up and down.
6. Incubate at 37˚C for 60 minutes.
7. Incubate at heat inactivation temperature specified below for 20 mins to heat inactivate restriction enzyme.
8. Continue with agarose-gel electrophoresis to ensure digestion was successful.

Making a **Master Mix**: in one 1.5 ml eppendorf tube, combine 10X Buffer, Sterile Water and Restriction Enzymes for all reactions

Heat Inactivation Temperatures

- NEB BsaI HF v2: 80˚C
- NEB EcoRI: 65˚C

- NEB PstI: 80˚C
- NEB XbaI: 65˚C