Restriction Digest Protocol
iGEM Protocol

estimated time: 30 min. active, 50 min. incubation
The following protocol assumes you'll be doing restriction digests for 3A assembly, therefore we refer to your digests as:

a. Part A (The 1st part in the future composite part)
b. Part B (The 2nd part in the future composite part)
c. Linearized plasmid backbone (The destination plasmid backbone for your composite part)

If you are simply doing a restriction digest for quality control, you can use the protocol below.

Materials

1. Ice and bucket/container
2. (1) 8-tube strip, or (3) 0.6ml thin-walled tubes
3. Part A (Purified DNA, > 16ng/ul)
4. Part B (Purified DNA, > 16ng/ul)
5. Linearized plasmid backbone (25ng/ul)
6. dH2O
7. NEB Buffer 2
8. BSA
9. Restriction Enzymes: EcoRI, SpeI, XbaI, PstI, DpnI
10. Thermal cycler

Notes:

1. You should keep all materials on ice.
2. At iGEM HQ we use restriction enzymes from New England Biolabs

Procedure

1. Keep all enzymes and buffers used on ice.
2. Thaw NEB Buffer 2 and BSA in room temperature water. Mix by shaking the tubes, and flick/spin them to collect the liquid at the bottom of the tube.
3. Add 250ng of DNA to the appropriately labelled tube. Add distilled water to the tubes for a total volume of 16ul in each tube.

Calculation example (with 25ng/ul as DNA sample concentration):
250ng ÷ 25ng/ul = 10ul of DNA sample
16ul (total volume) – 10ul (DNA sample) = 6ul of distilled water

1. Pipet 2.5ul of NEB Buffer 2 to each tube.
2. Pipet 0.5ul of BSA to each tube.
3. In the Part A tube: Add 0.5ul of EcoRI, and 0.5ul of SpeI.
4. In the Part B tube: Add 0.5ul of XbaI, and 0.5ul of PstI.
5. In the pSB1C3 tube: Add 0.5ul of EcoRI, 0.5ul of PstI, and 0.5ul of Dpn1.
6. The total volume in each tube should be approximately 20ul. Mix well by pipetting slowly up and down. Spin the samples briefly to collect all of the mixture to the bottom of the tube.
7. Incubate the restriction digests at 37°C for 30 minutes, then 80°C for 20 minutes. We use a thermal cycler with a heated lid.
8. (Optional, but recommended) Run a portion of the digest on a gel (8ul, 100ng), to check that both plasmid backbone and part length are accurate.
9. Use ~2ul of the digest (25ng of DNA) for ligations.

	Part A	**Part B**	**linearized plasmid backbone**
DNA	250ng	250ng	250ng (10ul @ 25ng/ul)

dH2O	adjust to 16ul	adjust to 16ul	6ul
NEB Buffer 2	2.5ul	2.5ul	2.5ul
BSA	0.5ul	0.5ul	0.5ul
Enzyme 1	0.5ul EcoRI	0.5ul XbaI	0.5ul EcoRI
Enzyme 2	0.5ul SpeI	0.5ul PstI	0.5ul Pst1
Enzyme 3			0.5ul DpnI

Single Reaction

1. Add 250ng of DNA to be digested, and adjust with dH20 for a total volume of 16ul.
2. Add 2.5ul of NEBuffer 2.
3. Add 0.5ul of BSA.
4. Add 0.5ul of EcoRI.
5. Add 0.5ul of PstI.
6. There should be a total volume of 20ul. Mix well and spin down briefly.
7. Incubate the restriction digest at 37C for 30min, and then 80C for 20min to heat kill the enzymes. We incubate in a thermal cycler with a heated lid
8. Run a portion of the digest on a gel (8ul, 100ng), to check that both plasmid backbone and part length are accurate.