**Enzyme Digestion**

Digest the plasmid DNA for the two parts with the appropriate enzymes for the desired ligation.

2.1 If you are digesting with EcoRI and PstI to verify the size of an insert, use 12 μL of miniprep DNA, or at least 400 ng. If digesting to make a vector or an insert, the amount of DNA to digest depends how much DNA you need for your ligation.

2.2 Mix the DNA with 2 μL of the appropriate buffer (see Table 1) in a 500-μL microfuge tube. Add
1 μL of each enzyme (total enzyme volume cannot exceed 10% of reaction volume). Increase the volume
to 20 μL with dH2O.Salt conditions for double digestion. Useful enzyme combinations for digesting
BioBrick parts with their optimal buffer and what the digestion produces. All five reactions are optimal at 37° C

Table 1

| Restriction enzymes | | Buffer | Product |
|---|---|---|---|
| EcoRI | XbaI | Low | Front vector |
| EcoRI | SpeI | Low | Front insert |
| SpeI | PstI | Medium | Back vector |
| XbaI | PstI | Low | Back insert |
| EcoRI | PstI | Buffer H | Whole insert |

2.3 For size verification, incubate the reaction at 37° C for at least half an hour. For inserts and vectors, incubate the reaction for at least 3 h. For maximum digestion, incubate the reaction overnight.