

Restriction:

Digest plasmid backbone:

Backbone used- pSB1C3.

1. Mix the reaction components at room temperature. Total volume of 20uL .
 - 2 uL 10X FastDigest or 10X FastDigest Green Buffer
 - 1 uL **EcoRI**
 - 1 uL **PstI**
 - 200 ng of DNA - 8uL
 - complete to 20uL with nuclease-free water(#R0581) - 8 uL
2. Mix Gently and Spin down.
3. Incubate at 37⁰C for 15 min
4. Inactivate enzyme by heating for 5 min at 80⁰C.

if the FastDigest Green buffer was used in the reaction load an aliquot directly to a gel.

Digest DNA parts:

1. Make different eppendorfs for each sample digested.
2. Mix the reaction components at room temperature. Total volume of 20uL .
 - 2 uL 10X FastDigest or 10X FastDigest Green Buffer
 - uL **EcoRI**
 - uL **PstI**
 - 300 ng of DNA – 3 uL of 100 ng/uL
 - complete to 20uL with nuclease-free water(#R0581) - 13 uL
3. Mix Gently and Spin down.
4. Incubate at 37⁰C for 15 min.
5. Inactivate enzyme by heating for 5 min at 80⁰C.

if the FastDigest Green buffer was used in the reaction load an aliquot directly to a gel.