This protocol is a consensus protocol between protocols on NEB and OpenWetware.
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∅∅∅∅∅ **Using Restriction Enzymes**

Restriction enzymes are enzyme capable of cutting DNA at specific locations. Often used by bacteria as a defense against viral genes, they are readily useful in biology and genetic engineering as tools for modifying custom DNA sequences.

Stock enzymes must be kept at very cool temperatures or they will denature. It is imperative the enzyme stocks are kept in ice when removed from the freezer.

We currently use NEB Buffers. These buffers vary greatly in their ability to support enzymes, especially with multiple digests. The following table demonstrates buffers to be used with standard BioBrick enzymes:

	NEB 1	NEB2	NEB3	NEB4
EcoRI	1.0	1.0	1.0	1.0
XbaI	0.0	1.0	0.75	1.0
SpeI	0.75	1.0	0.25	1.0
PstI	0.75	0.75	1.0	0.5

This protocol will vary depending on the nature of the digest. Two procedures, one for single digests and one for double, will follow; single digests are useful for linearizing plasmids or other circular DNA for use in gels (as well as some assembly protocols), where double will be useful when assembling BioBricks. This reaction mixture will produce 50µL total DNA; if the DNA to be used is dilute, *double it to 100*µL! Do not double enzyme concentration!

Compounds.
DNA solution
Restriction enzyme(s)
Buffer
diH2O
BSA solution

Materials
10µL, 50µL pipettes
PCR tubes
ICE

You will also need access to a:
PCR machine
Vortexer

External protocols:
DNA Purification (PCR/Digest)

Procedure (single digest)

1. Add **33.5µL diH2O** to a **PCR tube** with a **50µL pipette.**

2. Vortex the NEBuffer. Add **5µL NEBuffer** to the PCR tube with a Vortex the BSA solution. Mix by pipetting up and down about 3-5 times.

3. Vortex the BSA solution. Add **0.5µL BSA** to the PCR tube with a **10µL pipette**.

4. Add **10µL DNA solution** to the PCR tube with a **10µL pipette**.
   ⇒ *If your solution is dilute, add 20*µL *and double the other volumes except for enzyme.*

5. Add **1µL Enzyme**.
   ⇒ *Carefully touch the tip if your pipette to the surface of the glycerol (don't plunge the pipette in!) and withdraw the volume; wait a second for the liquid to fully flow into the pipette, then add to your reaction mixture.*

6. Incubate at **37°C** for **2-3 hours**, then heat inactivate at **80° C** for **15 minutes.**
   Store at **4°C**.

Procedure (double digest)

1. Add **32.5µL diH2O** to a **PCR tube** with a **50µL pipette.**

2. Vortex the NEBuffer. Add **5µL NEBuffer** to the PCR tube with a Vortex the BSA solution. Mix by pipetting up and down about 3-5 times.

3. Vortex the BSA solution. Add **0.5µL BSA** to the PCR tube with a **10µL pipette**.

4. Add **10µL DNA solution** to the PCR tube with a **10µL pipette**..
   ⇒ *If your solution is dilute, add 20*µL *and double the other volumes except for enzyme.*

5. Add **1µL of *both enzymes***. The total volume added at this step should equal 2µL.

6. Incubate at **37°C** for **2-3 hours**, then heat inactivate at **80° C** for **15 minutes.**
   Store at **4°C**.

⇒ Proceed to the *DNA Purification (PCR/Digest)* protocol.