

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 50 μ L total DNA; if the DNA to be used is dilute, *double it to 100 μ L!*

Compounds.

DNA solution
Restriction enzyme(s)
Buffer
diH₂O
BSA solution

Materials

10 μ L, 50 μ L pipettes
PCR tubes
ICE

You will also need access to a:

PCR machine
Vortexer

External protocols:

PCR/Digestion purification

Procedure (single digest)

1. Add **33.5 μ L diH₂O** to a **PCR tube** with a **50 μ L pipette**.
2. Vortex the NEBuffer. Add **5 μ L 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 **0.5 μ L BSA** to the PCR tube with a **10 μ L pipette**.
4. Add **10 μ L DNA solution** to the PCR tube with a **10 μ L pipette**..
5. Add **1 μ L Enzyme**.
→ *Carefully touch the tip of 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 **32.5 μ L diH₂O** to a **PCR tube** with a **50 μ L pipette**.
2. Vortex the NEBuffer. Add **5 μ L 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 **0.5 μ L BSA** to the PCR tube with a **10 μ L pipette**.
4. Add **10 μ L DNA solution** to the PCR tube with a **10 μ L pipette**..
→ *If your solution is dilute, and 20 μ L and double the other volumes.*
5. Add **1 μ L of both enzymes**. The total volume added at this step should equal 2 μ L.
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 *PCR/Digestion purification* protocol.