**Restriction Enzyme Digestion**

- Add reagents in following order:
  
  \[ x \, \text{uL} \, \text{dH}_2\text{O} \text{ (enough to bring reaction volume to 50 uL)} \]
  
  \[ y \, \text{uL of DNA (depends on concentration)} \]
  
  \[ 5 \, \text{uL of appropriate 10X buffer} ^1 \]
  
  \[ 5 \, \text{uL of 10X BSA (even if not required)} ^1 \]
  
  \[ z \, \text{uL Restriction Enzyme(s) (keep on ice!)} ^2, ^3 \]

- Tap the reaction tube gently several times to mix
- Pulse spin the reaction to the bottom of the tube
- Incubate the reaction at 37°C ^4

**NOTES:**

1= Many NEB enzymes now work in the new buffer system called CutSmart. CutSmart is basically NEB Buffer #4 and BSA combined (10X solution). Before using CutSmart, ensure your enzyme's compatibility on [www.neb.com](http://www.neb.com)

2= Restriction enzyme activity is measured in “units.” One unit is defined as the amount of the enzyme required to digest 1 ug of DNA in 60 minutes. 10-fold overdigestion is recommended. In our lab, use 10 units of enzyme for DNA amounts of 1 ug or less. Add 10 units for each additional 0.1-1 ug of DNA being digested (e.g. for 3.5 ug of DNA, use 40 units of enzyme)

3= The volume of restriction enzyme used in a reaction should not exceed 10% of the total reaction volume to reduce star activity

4= For standard diagnostic digestions, a 1-2 hour digestion should be sufficient. For digestions whose product will be used for subsequent cloning steps, an overnight (16 hour) digestion is recommended