

## SEED 2009 Pre-Lab, Day 2

**Part I.** Go to the New England Biolabs (NEB) website (<http://www.neb.com>). From the homepage, click on “Products” and then “Restriction Endonucleases.” Find the four enzymes that we are going to use in this class (EcoRI, PstI, SpeI, and XbaI).

- For each enzyme: list the recognition sequence, describe (draw) the DNA after the cut has been made (overhang), and describe the inactivation conditions.
- How is a unit of restriction endonuclease activity defined?

**Part II.** The following problems will give you more practice with everyday (!) calculations that are performed in molecular biology, biochemistry, etc. labs. **Pay very careful attention to the given units!** (This is true for this worksheet and the lab alike.) You should be beginning to feel very comfortable making these conversions and working these types of problems. If not, let us know and we can help you (i.e. discuss your challenges, work more problems, etc.).

**If a protocol asked for...**

**you would “put in”...**

0.5 mL water

\_\_\_\_\_  $\mu\text{L}$  water

50  $\mu\text{L}$  of 1X buffer

\_\_\_\_\_  $\mu\text{L}$  5X buffer

50 ng DNA

\_\_\_\_\_  $\mu\text{L}$  0.1  $\mu\text{g}/\mu\text{l}$  DNA

1X “Final Concentration” of buffer  
(50 $\mu\text{L}$  total reaction volume)

\_\_\_\_\_  $\mu\text{L}$  10X buffer

+

\_\_\_\_\_  $\mu\text{L}$  water

25  $\mu\text{M}$  dNTP

\_\_\_\_\_  $\mu\text{L}$  100 $\mu\text{M}$  dNTP

What is a dNTP?