

# Example of properly submitted protocol for NEB Course Support application

In this example, the teacher correctly highlighted the areas where specific enzyme volumes are cited (in red, in this case). A summary of each product is included (showing total volume or number of units required per experiment, as well as the total amount needed). This information can then be used to calculate the number of vials to order.

Clearly indicate required reagents within protocol

## PROTOCOL FOR DNA DIGESTION

A. Label three microcentrifuge tubes:

1. BamHI-HF<sup>®</sup>
2. EcoRI-HF
3. BamHI-HF + EcoRI-HF

*Tubes 1 and 2 are single digests and Tube 3 will be a double digest.*

B. Assemble reagents in the following order:

For Single Digests using BamHI-HF or EcoRI-HF:

- 23.5 microliters dH<sub>2</sub>O (From microfuge tube not squirt bottle)
- 3 microliters NEB CutSmart Buffer (use 10X stock as provided)
- **2.5 microliters of pUC19 DNA**

For Double Digest using BamHI-HF and EcoRI-HF:

- 20 microliters dH<sub>2</sub>O (From microfuge tube not squirt bottle)
- 3 microliters NEB CutSmart<sup>®</sup> Buffer (use 10X stock as provided)
- **5 microliters of Lambda DNA**

C. Bring your tube to the instructor bench where:

- to tube EcoRI-HF you add **1 microliter of EcoRI-HF**
- to tube BamHI-HF you add **1 microliter of BamHI-HF**
- to tube EcoRI-HF + BamHI-HF you add **1 microliter of EcoRI-HF AND 1 microliter of BamHI-HF**

*Total Volume in each tube should be: 30 microliters*

D. Flick the tubes a few times (don't vortex). Spin down tube for 5 seconds in the micro-centrifuge also called a microfuge.

E. Incubate at 37°C for 15 minutes (if the water bath is lower than 36°C, then extend incubation time).

F. Add **5 microliters of Purple Loading Buffer** to each of your three digests.

G. Load 15 microliters on your agarose gel. In a separate tube you will find the 1kb DNA Ladder (markers). Take **10 microliters of 1kb DNA Ladder** out of the stock tube and load it directly onto your gel in one lane.

## SUMMARY OF PRODUCT REQUIREMENTS

Multiply **Volume Required per Experiment** by the **Number of Experiments**

Volume supplied in each vial from NEB

Compare **NEB Vial Volume** to **Total Volume Required**

NEB PRODUCT	NUMBER OF EXPERIMENTS	VOLUME REQUIRED		VOLUME SUPPLIED PER VIAL	QUANTITY OF VIALS REQUIRED
		PER EXPERIMENT	TOTAL		
BamHI-HF	100	2 µl	200 µl	0.5 ml	1
EcoRI-HF	"	2 µl	200 µl	0.5 ml	1
pUC19	"	2.5 µl	250 µl	50 µl	5
Lambda DNA	"	5 µl	500 µl	0.5 ml	1
Gel Loading Dye, Purple (6X), no SDS	"	5 µl	500 µl	4 ml	1
Quick-Load <sup>®</sup> Purple 1 kb DNA Ladder	"	10 µl	1 ml	1.25 ml	1

Please indicate these numbers on the NEB order form