

Homework #4

1. 50 µl Reaction:

1 µg DNA
1x Buffer
1x BSA
0.5 U EcoR I (U=units)

Reaction Mixture:

0.2 µl (5 mg/ml DNA)
5.0 µl (10x Buffer)
0.5 µl (100x BSA)
0.5 µl (1 U/µl EcoR I)
43.8 µl H₂O

50.0 µl total volume

DNA

$$1 \cancel{\mu\text{g}} \times \frac{1 \cancel{\mu\text{l}}}{5 \cancel{\mu\text{g}}} = 0.2 \mu\text{l of } 5 \mu\text{g}/\mu\text{l DNA}$$

Buffer

$$\begin{aligned} C_1 V_1 &= C_2 V_2 \\ (10x) \times V_1 &= (1x) \times 50 \mu\text{l} \\ V_1 &= \frac{(1x) \times 50 \mu\text{l}}{(10x)} \\ &= 5 \mu\text{l of } 10x \text{ Buffer} \end{aligned}$$

BSA

$$\begin{aligned} C_1 V_1 &= C_2 V_2 \\ (100x) \times V_1 &= (1x) \times 50 \mu\text{l} \\ V_1 &= \frac{(1x) \times 50 \mu\text{l}}{(100x)} \\ &= 0.5 \mu\text{l of } 100x \text{ BSA} \end{aligned}$$

EcoR I Enzyme

$$0.5 \cancel{U} \times \frac{1 \cancel{\mu\text{l}}}{1 \cancel{U}} = 0.5 \mu\text{l of } 0.5 \text{ U}/\mu\text{l EcoR I}$$

2. 100 ml of 1% Agarose, 1x TAE

$$\text{Agarose } 1\% = 1 \text{ g}/100 \text{ ml} = 1 \text{ g of Agarose}$$

TAE

$$\begin{aligned} C_1 V_1 &= C_2 V_2 \\ (50x) \times V_1 &= (1x) \times 100 \text{ ml} \\ V_1 &= \frac{(1x) \times 100 \text{ ml}}{(50x)} \\ &= 2 \text{ ml of } 50x \text{ TAE} \end{aligned}$$

Combine 2.0 ml of 50x TAE and 1 g of Agarose. Once dissolved, dilute to 100 ml with H₂O.

3. Calculate and describe how you would make 20 ml of 10 mg/ml BSA (bovine serum albumen). BSA is a protein that is commonly incorporated into biochemistry experiments. It is sold as a dry powder.

$$20 \text{ mL} \times 10 \text{ mg/mL BSA} \Rightarrow 200 \text{ mg of BSA is required}$$

Place the 200 mg of BSA in a graduated cylinder and add 10 mL of deionized water and ensure that the solid is dissolved. Then add deionized water until the level is at the required 20 mL level.