Biology 324 - Laboratory 1 - Restriction Digest Tables

To set up a restriction digestion, you must mix precise amounts of various components to get the correct amount of DNA, enzyme, buffer and other reagents. The most effective method of determining how much of what to add is to set up a restriction digest table. This handout gives you a set of hypothetical restriction digests that you should work before coming to lab.

During the lab period, you will set up a real restriction digest using the same methodology. The specifics of the digest will be given during the lab. If you can work through these exercises the exercises for the lab will be a piece of cake.

Stock Solutions for Restriction Digests

10X RE buffer - provides sodium ions and a Tris buffer which keeps the pH at the right level

1 mg/ml BSA - to keep the enzyme stable during the digestion

1 mg/ml DNA - substrate for restriction enzyme

Restriction enzyme- @ 10 U/µl

(a unit of restriction enzyme activity is the amount of enzyme that cuts 1 µg of DNA in 1 hour at 37 degrees)

To figure out how much enzyme to add, you should *add 5X the minimal amount of enzyme needed to cut the DNA in the digest.*

For example, if you were cutting 1 µg of DNA, you would want to add 5 units of enzyme. Since the enzyme is at a concentration of 10 U/µl, you want to add 0.5 µl of enzyme. (5 U divided by 10 U/µl = 0.5 µl)

Restriction digest Example #1

Eco R1 digest of 1 µg of DNA in a final volume of 50 µl

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X	10	50 µl	5 µl
BSA	1 mg/ml	0.1 mg/ml	10	50 µl	5 µl

		final amount	volume
DNA	2 µg/µl	1 µg	0.5 µl
Eco R1	10 U/µl	5 U	0.5 µl
water			50-11 =39 µl

If you know the *concentration of the stock solution* and the *concentration of the final solution*, you can use the dilution factor system to figure out the amount of stock solution to add.
This works for the 10X buffer and the BSA.
Stock solution of BSA = 1 mg/ml,
the final concentration is 0.1 mg/ml,
the **dilution factor** = 1 mg/ml divided by 0.1 mg/ml = **10**.
The amount of stock solution needed = the final volume wanted (50 µl) divided by the dilution factor (10)= 5 µl.

If you know the *concentration of the stock solution* and the *final amount of the component*, then you divide the *amount of the component* by the *concentration of the stock solution*.
For example, if you want 1 µg of DNA and you have a stock solution of 2 µg/µl, you should divide 1 µg by 2 µg/µl which yields 0.5 µl.
This approach also works in figuring out the amount of enzyme needed. (Remember you want 5X the minimal amount required to digest DNA in 1 hour at 37 degrees).

Finally, you want to figure out how much water to add to get a final volume of 50 µl. The total amount of stuff you added is
11 µl. So you need 39 µl of water to get a final volume of 50 µl.

Restriction Digest Table #2 :

You want to digest 5 µg of DNA with Eco R1 in a final volume of 100 µl .
First figure out the dilution factors and volume of stock solution needed. Then calculate the volume of DNA and Eco R1 needed. (Don't use the dilution factor formula since you're not comparing concentrations). Finally, figure out how much water you need.

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X			
BSA	1 mg/ml	0.1 mg/ml			

		final amount	volume
DNA	2 µg/µl	5 µg	
Eco R1	10 U/µl		
water			

Restriction Digest Table #3

You want to digest 15 µg of DNA in a final volume 250 µl. Please note that the concentrations of BSA, DNA and Eco R1 are different.

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X			
BSA	2 mg/ml	0.1 mg/ml			

		final amount	volume
DNA	5 µg/µl	15 µg	
Eco R1	20 U/µl		
water			

Restriction digest table #4

You want to digest 15 µg of DNA in a final volume of 150 µl.

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X			
BSA	1 mg/ml	100 µg/ml			

		final amount	volume
DNA	500 ng/µl	15 µg	
Eco R1	10 U/µl		
water			

Answers: Restriction Digest Table 2

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X	10	100 µl	10
BSA	1 mg/ml	0.1 mg/ml	10		10

		final amount	volume
DNA	2 µg/µl	5 µg	2.5
Eco R1	10 U/µl	25	2.5
water			75

Restriction Digest Table 3 answers

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X	10	250 µl	25
BSA	2 mg/ml	0.1 mg/ml	20		12.5

		final amount	volume
DNA	5 µg/µl	15 µg	3
Eco R1	20 U/µl	75	3.75
water			~~204.5~~

Restriction Digest Table 4 answers:

Component	stock conc	final conc	dilution factor	final volume	volume of stock soln
RE buffer	10X	1X	10	150 µl	15
BSA	1 mg/ml	100 µg/ml	10		15

		final amount	volume
DNA	500 ng/µl	15 µg	30
Eco R1	10 U/µl	75	7.5
water			82.5