Restriction enzymes - overview and protocols
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Restriction enzyme digestion became a routine method of molecular biology 2 decades ago. Till now researches use restriction enzymes for cloning, analysis of genomic sequences and DNA methylation. Here I give a short overview on the usage of restriction enzymes and some tips from my practical experience.

Suppliers of restriction enzymes

At the moment there are many different companies that supply a wide variety of restriction enzymes. I have an experience with Roche, NEB, Fermentas and Gibco. All enzymes I tried are of sufficient quality; therefore my final decision is based on unit price of the enzymes. At the moment most of enzymes we use are from MBI Fermentas. Some rare are from NEB. It is important to note, that NEB buffer system is compatible with that of Fermentas.

Digestion of DNA with a single enzyme

If you want to digest plasmid DNA with one enzyme than you usual protocol will look like this:

DNA	up to 1 µg
Buffer (10x)	2 µl
Enzyme (10U/µl)	1 µl
H2O	to 20 µl

To prepare this reaction you first pipet together DNA, buffer and water, vortex the mixture and add the enzyme. Mix by pipetting and incubate at least 40 min at the temperature optimal for the enzyme (remember, that most, but not all enzymes work at 37°C).

If you want to digest genomic DNA, your assay will look somewhat different. Two things are important. 1) usually you have to digest more DNA, therefore you have to set your reaction in a bigger volume (5-10 µg in 100 µl final volume) and 2) you will need more time for complete digestion (it can be even 18 h). Typical reaction I used to digest human genomic DNA for Southern blot was

DNA	10µg
Buffer (10x)	10µl
Enzyme (10U/µl)	5µl
H2O	to 100 µl

I incubate such reaction overnight at optimal temperature. Note, that it is better to use an incubator instead of a heating block, since in the block you will have condensation of water on the lid of your tube and this will destroy the reaction. If DNA has to be digested for bisulphite treatment I incubate the samples for a shorter time, since usually I don't need a complete digestion in this case.

Digestion of DNA with 2 or more different enzymes

In the case of double or triple digestion you have 2 possibilities

Use simultaneous digestion

If you can find a buffer in which all enzymes have sufficient activity (usually not lower than 50%), you can set your digestion will all enzymes simultaneously. It is important that the total volume of enzymes you add to your reaction is not more than 1/10 of the total reaction volume. The reason for this is that some enzymes have star activity if the concentration of glycerol in the reaction exceeds 5%.

For plasmid DNA your digestion reaction will look like this

DNA	up to 1 µg
Buffer (10x)	2 µl
Enzyme 1 (10U/µl)	1 µl
Enzyme 2 (10U/µl)	1 µl
H2O	to 20 µl

Use sequential digestion

If you do not have a buffer in which all your enzymes function properly you will have to make a buffer exchange. This can be done in several different ways.

The most trivial way is to digest your DNA with one enzyme, then purify it with any kit for purification of DNA out of gel slice and set a reaction with the second enzyme. However in this case you have some losses of your DNA and as well you consume (probably unnecessary) purification reagents.

You can perform you double digestion sequentially using buffer adjustment. This is possible when your first enzyme (enzyme I) functions in a low salt buffer (buffer B from Fermentas, for example) and the second one (enzyme II) functions in a high salt buffer (buffer R or O from Fermentas). In this case you first set a reaction with enzyme I in a smallest possible volume.

DNA	1µl
Buffer B	1µl
Enzyme I	1µl
H2O	7µl

You perform the digestion for 1 h and then inactivate your enzyme (check in the table, usually 65°C for 10-15 min). Then you set a second step reaction as follows

Reaction 1	10µl
Buffer R	5µl
Enzyme II	1µl
H2O	34 µl

Perform your digestion for 1 h and analyze the result on a gel.

Below is the table that shows you what happens in your reaction tube. As you can see, there is some increase in concentrations in final reaction, but this does not influence the activity of the enzyme.

Component	*buffer B*	*buffer R*	*buffer R+1/5B*
Tris-HCl	10 mM	10 mM	12 mM
$MgCl_2$	10 mM	10 mM	12 mM
BSA	0.1 mg/ml	0.1 mg/ml	0.12 mg/ml
KCl	-	100 mM	100 mM

This way of buffer adjustment is possible for different buffer combinations. It usually functions fine and saves time and materials. The only disadvantage is the big volume after the second reaction.