Restriction analysis of plasmid DNA

The following protocol is suitable for restriction analysis of a small amount of plasmid DNA. Adjust the scale accordingly in order to digest a large amount of DNA (e.g., digest 1 μg of plasmid DNA with 2 – 3 units of enzyme in order to purify a restriction fragment for ligation).

1. Mix the following 10-μL reaction in an eppi tube:
   0.25 μg plasmid DNA[1]
   1 μL 10X Reaction Buffer[2]
   1 μL 10X BSA[3]
   sterile water
   0.5 units[4] of Restriction Enzyme[5]
2. Vortex briefly, then pulse-spin at full speed for 5 seconds.
3. Incubate at 37˚C for 1 – 2 hours.
4. Analyze the entire volume by agarose gel electrophoresis.

*If many samples are to be analyzed with the same enzyme(s), prepare a "master mix" containing all of the reagents except the DNA, and add aliquots of this mix to an aliquot of each DNA sample. To account for volume loss, the master mix should contain enough reagents for all of the samples to be analyzed plus an additional 2 – 3 samples.

For example, if 18 minipreps prepared using the alkaline lysis method are to be analyzed using BamHI:

reagent:	**for 1 sample:**	**master mix for 20 samples:**
sterile water	6.75 μL	135 μL
10X Reaction Buffer	1 μL	20 μL
10X BSA	1 μL	20 μL
BamHI (20 U/μL)[5]	0.25 μL	5 μL
total volume:	9 μL	180 μL

Vortex master mix briefly, then pulse-spin at full speed for 5 seconds. Dispense 9 μL of the master mix to eppi tubes in which 1 μL of each sample has been aliquoted.

Notes:

[1]If the DNA was prepared using a CsCl gradient or using an EppendorfQiagen kit, calculate its concentration by measuring the A_{260}. If the DNA was prepared using the alkaline lysis method, 0.25 μg is usually equivalent to 1 μL of DNA harvested from 1.5 mL of overnight bacterial culture that was resuspended in 50 μL of TE.

[2]Supplied by the manufacturer of the restriction enzyme.

[3]The manufacturer of the restriction enzyme usually supplies a 100X stock of bovine serum albumin (BSA), but it is more useful to prepare a 10X stock by diluting the 100X stock using sterile water. Store at -20˚C.

[4]One unit is defined as the amount of restriction enzyme required to digest 1 μg of DNA in 1 hour. Unless partial digests are desired, always add 2-3X more enzyme than is necessary in order to ensure complete digestions. Because most restriction enzymes are supplied at concentrations that are 5 – 20 units per μL, it is often convenient to add 0.25 – 0.5 μL of enzyme per 0.25 μg of DNA. However, the precise amount of enzyme necessary for digestion can be calculated as follows:

$$\frac{[\#\text{ of sites cut by enzyme in your plasmid}]}{[\text{size of reference DNA (e.g., lambda is 48 kb)}]} \text{ divided by: } \frac{[\#\text{ of sites cut by enzyme in reference DNA}]}{[\text{size of your plasmid}]}$$

Solving this equation provides you with the number of units of enzyme needed per μg of your plasmid DNA. To ensure complete digestion, multiply this number by 3.

[5]Add the restriction enzyme last.

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