RESTRICTION ENDONUCLEASE DIGESTION

Mapping of miniscreen DNAs

Do 10 ul reactions containing 2 ul of miniscreen DNA where 20 ul DNA is the yield from 1.5 ml saturated cells by standard lab miniscreen protocol. (See alkaline lysis miniprep method).

Choose digest such that you can use one or two enzymes, preferably of compatible salt optima (see NEB or MBI catalogue), of high concentration (see label on enzyme tube), and known to cleave miniscreen DNA well (ask around).

Important considerations in choosing sites include size of relevant fragments (preferably greater than 400 base pairs (bp)), and number of fragments generated (preferably few sites so that digest will go to completion and not generate a confusing pattern of partial and irrelevant fragments).

Where a new site has been created, one strategy is to demonstrate that the parent plasmid is linearized while the desired construction results in a large 500 - 1500 bp drop out. The reverse where a site has been destroyed. If comparison is being made between size of the restriction fragment of the parent and of the construct choose sites to maximize contrast, e.g. if difference is less than 100 bps, fragments being compared should not be greater than 500 bp since the sizing accuracy using the 1 kb ladder as markers is poor above this.

Including a control digest of parent to contrast with miniscreens is a good idea.

For example, if mapping 12 miniscreens and with 1 control digest of parent plasmid (total 13 samples):

1. Label tubes 1 - 13, put on ice.
2. Prepare a "master mix" containing everything except DNA (i.e. includes salts, buffer, divalent ions, BSA and enzymes). If a double digest is being done with comparable salt optima and temp optima, mix both enzymes together in the master mix. If incompatible, the digests must be done sequentially and I usually start with the lower temp or lower salt enzyme, adding the other subsequently, as will be described. By making a master mix you can deliver all components except DNA in a single pipetting, after vortexing the master mix and spinning down for 1 second. The master mix also makes it convenient to dish out miniscule amounts of enzyme since you measure out not .2 ul of enzyme, but rather the amount for (in this case) 14 tubes = 2.8 ul, and you dispense a larger volume to each tube since all components are mixed together (except the DNA which is the variable).
3. Dispense master mix to all tubes on ice first (you can use the same pipette tip since all the tubes are empty at that point and you are not going into the enzyme stock again. Absolutely never reuse a pipette tip to get enzyme or buffer, etc. from the stock tube, even if you don't think it touched any other components -- if you are wrong, everyone is screwed.

   To continue our example:

14 x 1 ul CB 10 X (10-fold concentrate of buffer salts; this is usually provided with the enzyme by the company that sells it. Most enzymes in our lab are from either NEB or MBI Fermentas. Always check with the catalogue first before planning a digest. Both catalogues have convenient charts showing the percentage activity of each enzyme in each buffer. Some enzymes require special "unique" buffers.)

14 x 1 ul 10 X BSA (check the catalogue to see if the enzyme you are using requires BSA)

14 X 0.2 ul enzyme(s) (volume may vary from 0.05 ul per reaction to 1 ul maximum, typically 0.2 ul enzyme per reaction, depending on enzyme concentration, shooting for approx 1 unit per digest. Especially in the case of double digests, remember that enzyme -- sold in 50% glycerol -- must not make up more than 10% of the total reaction volume or it may inhibit the enzyme or alter its specificity.

81.2 ul H2O

Total master mix volume is now approx. 109.2 ul, enough to dispense the correct 8 ul to all 13 tubes on ice.

4. Now add 2 ul dissolved, vortexed and spun down miniscreen DNA changing tips after each addition and mixing the DNA directly into the 8 ul droplet of master mix in each tube.
5. For the control digest (tube 13) use 0.25 ul of 1 mg/ml stock plasmid DNA and make up the rest of the volume by adding 1.75 ul sterile distilled water.
6. Place tubes to incubate at desired temp. For 37øC incubations, digest for 2 hrs, for 50 to 60øC digestions, stop after 1 hr since the reaction volume has just about evaporated at that point, anyway. For this reason it is usually a good idea to spin down high temperature digests every 15 minutes.
7. If a second digest at different salt or temp is to be done, don't forget to add either the additional enzyme or combination of salt and enzyme and continue the incubation at the appropriate temp. You can transfer the tubes to ice while you are getting your second "master mix" ready. This addition of salt and/or enzyme should be done in less than 1 ul to keep close to the correct total volume, and the tube should be spun down for one second in the cold, vortexed and put to incubate. If only enzyme is being added, 0.2 ul is too small to be practical for a pipette, so use either the P2 pipette or simply take a very small amount at the end of the yellow tip using the pipette person.
8. When finally through with digests, add 5-10 ul of DNA loading buffer.
9. Load 10 ul of sample onto gel. 1% agarose in TAE is good for large fragments and linearization. 110 volts constant voltage. Remember to use 1 KB markers.(aliquots of these can be found in the DNA freezer in the "buffers and markers" box.)
10. Run until lower blue (bromophenol blue) is at least half way to 3/4 of the way down the gel. Dial down voltage, turn off power, cut out your piece of gel, (wear gloves), and place it in the ethidium bromide solution for at least 15 min. Visualize bands on transilluminator while wearing safety glasses. Take picture if desired, and dispose of gel

immediately in the garbage can near the transilluminator.