Typical Restriction Digest of Genomic DNA

For a total reaction volume of 40 µl[1]

1. 10 µg genomic DNA
2. Enzyme - use <u>20-30 total units</u> (keep final glycerol concentration at 5% or less)[2]
3. 10x reaction buffer (supplemented with 10X BSA) – usually supplied by the enzyme vendor.
4. RNAse – use 1 µl of 10 µg/µl stock
5. Spermidine – use 0.4 µl of 1 M stock (10 mM final)

Make a mix sufficient for the total number of samples (plus 10%). For example, if you have 27 samples make a mix for 30[3]:

<u>example</u>

Eco RI	2µl	x30	=	60 µl	(20 units/µl)
10 X Enzyme Buffer	4µl	x30	=	120 µl	
RNAse	1µl	x30	=	30 µl	
Spermidine	0.4µl	x30	=	12 µl	

Mix, place 7.4 µl in each tube bearing genomic DNA plus TE (volume of 32.6 µl)[4]

Finger flick to mix. *DO NOT VORTEX.*

Pulse spin in microcentrifuge to collect.

Incubate 37^{0}C 6 hrs to overnight.[5,6]

Notes:

[1] dependent upon well size of gel comb.
[2] enzymes typically supplied in 50% glyceol
[3] to allow for pipetting wastage
[4] use genomic tips to pipette the DNA
[5] temperature dependent upon endonuclease
[6] the use of an incubator oven for the digestion reactions is preferable to use of a water bath to avoid condensation on the inner lids of the reaction tubes from altering the reaction concentrations

Entered by HKS from DD's notebook 3/24/99