Protocol : Digestion Protocol

1) Set up the reaction mixture as below

Miniprep DNA	500 ng
Restriction Enzyme 1	1.0 µl
Restriction Enzyme 2	1.0 µl
10X NEBuffer (1, 2, 3, 4)	5.0 µl
100X BSA (bovine serum albumin)	0.5 µl
H_2O	to 50.0 µl

Concentration of DNA in the Mini prep samples will be measured using Nano drop technology.
Calculation shows the volume of miniprep sample:
Nano drop reading for each miniprep sample (Yng) = 1 µl
So 500 ng = (1 µl /Yng) x 500ng

2) Incubate the restriction digest reaction at 37°C for 15 minutes and then heat inactivate at 80°C for 20 minutes.

Note: Enzyme should be the last component added to the mixture.

Before setting up digestion measure the DNA concentration in your mini-prep or PCR purification using NanoDrop spectrophotometer in Barclays lab (upstairs where we visualise gels)

Source: http://www.neb.com/nebecomm/products/protocol445.asp