

**University of Southampton iGEM 2009 Protocol:**

DNA Double Digestion:  
(NEB Restriction Enzymes)

Double Digestion Protocol:<sup>[1]</sup>

**20  $\mu$ L total reaction volume:**

(e.g. higher concentration DNA sample - insert)

Component	Amount/ $\mu$ L
NEBuffer 1-4	2
BSA (optional)	0.2
DNA	x
Restriction enzyme 1	1
Restriction enzyme 2	1

**50  $\mu$ L total reaction volume:**

(e.g. lower concentration DNA sample - plasmid)

Component	Amount/ $\mu$ L
NEBuffer 1-4	5
BSA (optional)	0.5
DNA	x
H <sub>2</sub> O	Make up to 48 $\mu$ L
Restriction enzyme 1	1
Restriction enzyme 2	1

**NB.** The ratio of restriction enzyme may be altered from 1:1 if one enzyme has a lower activity in the NEBuffer of choice.

**NB.** The quantity of DNA used depends on the concentration of the DNA, which is deciphered using the Nano-drop.

1. Add the components in the order in which they are written.
2. Briefly centrifuge the micro-centrifuge tubes then incubate the reactions at 37 °C for 2 hours.
3. An analysis gel can be performed using 1  $\mu$ L reaction solution to probe if the digestion has gone to completion.
4. If the DNA fragments are to be blunt-ended post-digestion, the restriction enzymes should be heat-inactivated at 65 °C (restriction enzyme dependent)
5. Purify the DNA fragment by gel purification (using the Sigma GenElute Gel Extraction and Purification kit).



6. Samples can be stored at -20 °C until further use.

[1] NEB Restriction enzyme product technical reference.