Restriction digestions

Restriction digestions was done using the following basic setup adopted from http://nebcloner.neb.com/#!/redigest.

Incubation time, temperature, and buffer used varied depending on the restriction enzymes used as suggested by NEB.

Protocol

1. For each 10 uL reaction the following was mixed:

Component	10 uL reaction
10x NEB buffer	1.0 µL
DNA	4.0 µL
Restriction enzyme 1	0.25 µL
Restriction enzyme 2 or H_2O	0.25 µL
Nuclease-free water	4.5 µL

2. Subsequently samples were incubated as suggested (generally 1 hour at 37 degrees).

Verification

Verification was done using gel electrophoresis.