SEQUENTIAL DIGESTS
(FOR WHEN NOTHING ELSE WILL DO)

IMPORTANT NOTE
Use this protocol only as a last resort if you need to digest a plasmid using two enzymes that refuse to cut in the same buffer. For example, trying to perform a clean double digest with HpaI (100% in Buffer 4 and 10% in Buffer 3) and BglII (10% in Buffer 4 and 100% in Buffer 3) is almost impossible in any of the NEB Buffers.

EQUIPMENT REQUIRED
Incubator set to the appropriate temperature for your enzymes
1.5 mL microcentrifuge tubes
Ice bucket to keep the enzymes cool

REAGENTS REQUIRED
A. First Digest
- Appropriate 10X Buffer (100% activity for your first enzyme)
- Plasmid DNA
- First enzyme
- 100X BSA

B. DNA Precipitation
- 10M NH_4OAc (in water)
- Isopropanol
- 70% Ethanol
- Tris/T.E./H_2O

C. Second Digest
- Appropriate 10X Buffer (100% activity for your second enzyme)
- Plasmid DNA from [A.]
- Second enzyme
- 100X BSA

A. FIRST DIGEST

1 Setup a restriction digest on ice as follows (add reagents in the order given):
- 43.5 μL DNA (midi-prep. DNA is best)
- 5 μL 10X Buffer
- 0.5 μL BSA
- 1 μL First enzyme

2 Incubate at the appropriate temperature for 2 hours to ensure complete digestion.

B. DNA Precipitation

1 After digestion precipitate the DNA by adding the following (in the order given):
- 12.5 μL 10M NH_4OAc
- 62.5 μL Isopropanol

2 Mix well and incubate on ice for 10 minutes.

3 Pellet the DNA by spinning it down for 15 minutes at 15k rpm.

4 Wash the pellet (usually it will be invisible so just trust that it is there) with 70% ethanol.

5 Dry at 50 - 60 C for a minute or two.

6 Suspend the pellet in 25.7μL of Tris/T.E./H_2O

C. Second Digest

1 Setup a restriction digest on ice as follows (add reagents in the order given):
- 25.7 μL DNA (from the precipitation in [B.])
- 3 μL 10X Buffer
- 0.3 μL BSA
- 1 μL Second enzyme

2 Incubate at the appropriate temperature for 2 hours to ensure complete digestion.

3 Your DNA should now be completely digested!

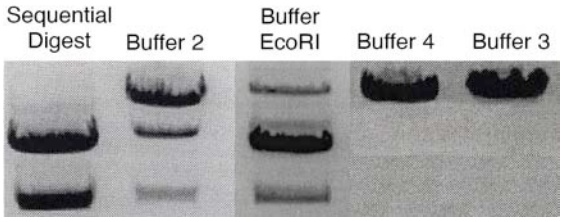

Figure 1. Example digest composite image using HpaI/BglII showing the results of a sequential digest (BglII then HpaI), both enzymes in Buffer 2 or Buffer EcoRI (partial digests) and in Buffers 4/3 (single cut). All digestions were preformed over a 4 hour period.