Protocol 1-3: Restriction Enzyme Digestion

General Protocol

1) Add the following reaction components in the order indicated:

PCR reaction mixture	10 µl (~0.1-0.5 µg of DNA)
Water, nuclease-free	16-17 µl
10X recommended buffer for restriction enzyme	2 µl
Restriction enzyme	1-2 µl (10-20 u)
Total volume	**30 µl**

2) Only 2 µl of 10X reaction buffer is required for unpurified PCR product in a 30 µl reaction volume.

3） Mix gently and spin down briefly.

4） Incubate at the optimal reaction temperature for 1-16 hours.

Tips

1) For cloning applications, purification of PCR products prior to digestion is necessary to remove the active thermophilic DNA polymerase present in the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the yield of ligation.
2) If the restriction enzyme requires special additives reduce the amount of water appropriately.

Reference

1) Fermentas:http://www.fermentas.com/en/products/all/conventional-restriction-enzymes/er027-ecori
2) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.