Protocol

Digestion of PCR Products

This protocol is for the Digestion of PCR Products

The most convenient option for digestion of PCR-amplified DNA is the addition of a restriction enzyme directly to the reaction tube after completion of PCR. The majority of restriction enzymes are active in PCR buffers.

However, digestion of PCR products in the amplification mixture is often inefficient. Therefore, PCR reaction mixture should not make more than ⅓ volume of digestion reaction mixture to avoid inhibitory effects.

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.

Note

- 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.
- If the restriction enzyme requires special additives (e.g., SAM), reduce the amount of water appropriately.
- If cleavage of the PCR product is inefficient purify the PCR products with the GeneJET™ PCR Purification Kit (#K0702) prior to digestion.
- After digestion, gel-purify the PCR product with the GeneJET™ Gel Extraction Kit (#K0692) or Silica Bead DNA Gel Extraction Kit (#K0513) to remove short DNA fragments which compete with the insert in a ligation reaction.

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