

# *Dpn I Digestion of the Template DNA*

Revised on October 17, 2011

## *Introduction*

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The *Dpn I* endonuclease (target sequence: 5'-Gm6ATC-3') is specific for methylated and hemimethylated DNA and is used to digest the parental DNA template in PCR or mutagenesis reactions. DNA isolated from almost all *E. coli* strains is dam methylated and therefore susceptible to digestion.

## *Materials*

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- *Dpn I* (New England BioLabs, R0176S)

## *Procedure*

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1. Add 0.5  $\mu$ l of the *Dpn I* restriction enzyme (20 U/ $\mu$ l) directly to each amplification reaction (PCR or mutagenesis reactions).  
**Note:** One unit is defined as the amount of enzyme required to digest 1  $\mu$ g of pBR322 DNA (dam methylated) in 1 hour at 37°C in a total reaction volume of 50  $\mu$ l.
2. Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Spin down the reaction mixtures in a microcentrifuge and immediately incubate each reaction at 37°C for 1 hour to digest the parental supercoiled dsDNA.
3. **Optional:** Heat-inactivate *Dpn I* for 20 minutes at 80°C.