

## PCR Optimization: dNTP

- ▶ The concentrations of dNTPs used in a reaction are determined by the affinity of the enzyme for substrate (the  $K_m$  of the enzyme).
- ▶ dNTPs on the order of 1 mM final concentration are generally appropriate (1 mM total, 250  $\mu$ M each).
- ▶ Concentration of dNTPs decreases as the reaction proceeds.
- ▶ Excessive dNTP concentration reduced polymerase accuracy and requires higher  $Mg^{2+}$  in the reaction mix.