Protocol

Guidelines for Primer Design

This protocol is for the Guidelines for Primer Design

Use the REviewer™ primer design software or follow general recommendations for PCR primer design below:

- PCR primers are generally 15-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Prefer one or two G or C at the 3'-end of the primer, but avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of nonspecific priming.
- Avoid primer self-complementarities, complementarities between the primers and direct repeats in a primer to prevent hairpin formation and primer dimerization.
- Check for possible complementary sites between primers and template DNA.
- When designing degenerate primers, place at least 3 conservative nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) of the two primers should not exceed 5°C for conventional PCR.

Estimation of Primer Melting Temperature

- For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:
  $Tm = 4 (G + C) + 2 (A + T)$, where G, C, A, T – number of respective nucleotides in the primer.
- If the primer contains more than 25 nucleotides specialized computer programs e.g, REviewer™ are recommended to account for interactions of adjacent bases, effect of salt concentration, etc.
- For calculation of primer melting temperature only consider nucleotides homologous to the template.

Considerations for Subsequent Cloning of PCR Products

- When introducing restriction endonuclease sites into primers for subsequent digestion and cloning of the PCR product, refer to tables "Cleavage Efficiency Close to the Termini of PCR Fragments" or "Reaction Conditions for FastDigest® Restriction Enzymes" to determine the number of extra bases outside of the recognition sequence that are required for efficient cleavage.

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