DYS SEE

PCR-Kapa Taq.

Protocols

Polymerase Chain Reaction Protocol with Kapa Taq DNA Polymerase

1. Set up a 25μL PCR reaction (Keep all your reagents on ice):

   - 1μL Template DNA (10 ng-500 ng)
   - 2.5μl 10X Taq buffer with $MgCl_2$
   - 0.5μl dNTP mix (10 mM each nt)
   - 0.8μl Forward Primer (10μM stock)
   - 0.8μl Reverse Primer (10μM stock)
   - 0.1μl Taq DNA Polymerase (5 units/μl)
   - 19.3μl Sterile dH_2O (variable)

   Note: DMSO is added in some reactions to increase the specificity of GC rich templates, at 5% of total volume (1.25 μl). In that case, ddH2O is less.

2. Place reaction tubes in PCR machine.
3. Set annealing temperature 5°C below the primer melting temperature (Tm). Alternatively, use NEB's Tm Calculator to determine the optimal temperature.
4. Set extension step at 1 minute per kilobase of product depending on whether you are using a polymerase with proofreading capabilities.

   Note: See manufacturer's instructions for specific instructions about extension time and temperatures.

5. Initial Denaturation for 3 minutes at 95°C.
6. Denature for 30 seconds at 95°C.
7. Anneal primers for 30 seconds at 55°C (or 5°C below Tm).
8. Extend DNA for 2 minutes at 72°C.
9. Repeat steps 2-4 for 34 cycles.
10. Final Extension for 2 minutes at 72°C.
11. Run 10μL on a gel to check size and concentration of PCR product.

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