Standard PCR Protocol

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[Abstract] This protocol describes basic steps of a PCR experiment using home-made Taq DNA polymerase. Some steps may vary with different DNA polymerase.

Materials and Reagents

1. Tris-HCl (Sigma-Aldrich)
2. KCl (EM SCIENCE)
3. $MgCl_2$ (EM SCIENCE)
4. Gelatin (Sigma-Aldrich)
5. Taq DNA polymerase (home-made)
6. dNTPs (New England Biolabs, catalog number: N0447L)
7. Template DNA (genomic, plasmid, cosmid, bacterial/yeast colony, etc.)
8. Primers

Equipment

1. Thermal cycler (MJ Research)

Procedure

A. Prepare DNA template:

Usually, for plasmid DNA, 1-10 ng; for genomic DNA, 50-100 ng per reaction is needed. Normally, DNA template does not need to be purified. However, both purity and the amount of template can strongly influence the outcome of the reaction.

B. Design primer:

Generally, primers used are 18-23 mer in length. Use Primer3 free online software (reference 1) to design primers.

C. Determine annealing temperature:

Melting temperature (Tm) of primers can be calculated by the following formula: Tm = [(#of A + T residues) x 2] + [(#of G + C residues) x 4] °C. Tm-5 °C is a good annealing temperature to start with. However, optimal annealing temperatures can only be determined experimentally

for a certain primer/template combination. Temperature gradient PCR is often a way to finalize an optimal annealing temperature.

D. Prepare 10x PCR reaction buffer, include:
100 mM Tris-HCl (pH 8.3)
500 mM KCl
15 mM $MgCl_2$
0.1% gelatin
Note: The $MgCl_2$ concentration is typically 10-15 mM. However, the optimum concentration needs to be determined experimentally. Mg^{2+} forms a soluble complex with dNTP's which facilitates dNTP incorporation, and stimulates polymerase activity. It also promotes and stabilizes primer and template interaction. Thus, Increasing the magnesium concentration has the same effect as lowering the annealing temperature. Too low Mg^{2+} leads to low yields (or no yield) and too much Mg^{2+} cause nonspecific products.

E. For a 100 µl reaction, add:
10x PCR buffer 10 µl
DNA template (5 ng μl^{-1}) 1 µl
Primer A (50 mM) 1 µl
Primer B (50 mM) 1 µl
dNTPs (2 mM) 10 µl
Taq (5 U μl^{-1}) 1 µl
Sterile ddH_2O 76 µl
Notes:
1. *For some PCR machines that do not have a heated lid, mineral oil needs to be added to each reaction to prevent evaporation of the sample.*
2. *Prepare a control reaction with no template DNA and an additional 10 µl of sterile water.*

F. A typical PCR program may be:
1. Initial denaturation, 4-8 min at 94-95 °C.
2. Denaturation, 15 sec at 94-95 °C.
3. Annealing, 15 sec at x °C (depends on Tm).
4. Extension, x sec (depends on product length, 1 min kb^{-1}) at 72 °C.
5. Return to step 2 for 30-35 additional cycles.
6. Final extension, 10 min at 72 °C.
7. Keep sample at 4 °C until loading.

References

1. http://frodo.wi.mit.edu/primer3/

