7.13 Experimental Microbial Genetics

Fall 2008

PCR with the Platinum PCR SuperMix High Fidelity

How PCR works:

The polymerase chain reaction (PCR) is a rapid procedure for *in vitro* enzymatic amplification of a specific segment of DNA. The applications of PCR are numerous. They include direct cloning from genomic DNA or cDNA, *in vitro* mutagenesis and engineering of DNA, genetic fingerprinting, and prenatal diagnosis of genetic defects. The theoretical basis of PCR is outlined in Figure 1, below.

There are three nucleic acid segments: the segment of double stranded DNA to be amplified and two single stranded oligonucleotide primers flanking it. Additionally, there is a thermostable DNA polymerase, appropriate deoxyribonucleoside triphosphates (dNTPs), a buffer, and salts.

The reaction is performed in a thermocycling machine which can be programmed to repeat the cycle of denaturing, annealing and extension many times. The primers are added in vast excess compared to the DNA to be amplified. They hybridize to opposite strands of the DNA (one to the top strand and one to the bottom strand) with their 3' ends facing each other so that synthesis by DNA polymerase (which goes 5' to 3') extends across the segment between them.

In the next cycle each one of the new products can hybridize to the primers and act as a template. The amount of product doubles with every subsequent cycle of synthesis so that 30 cycles should result in a 270 million fold amplification of the exact desired fragment of DNA.

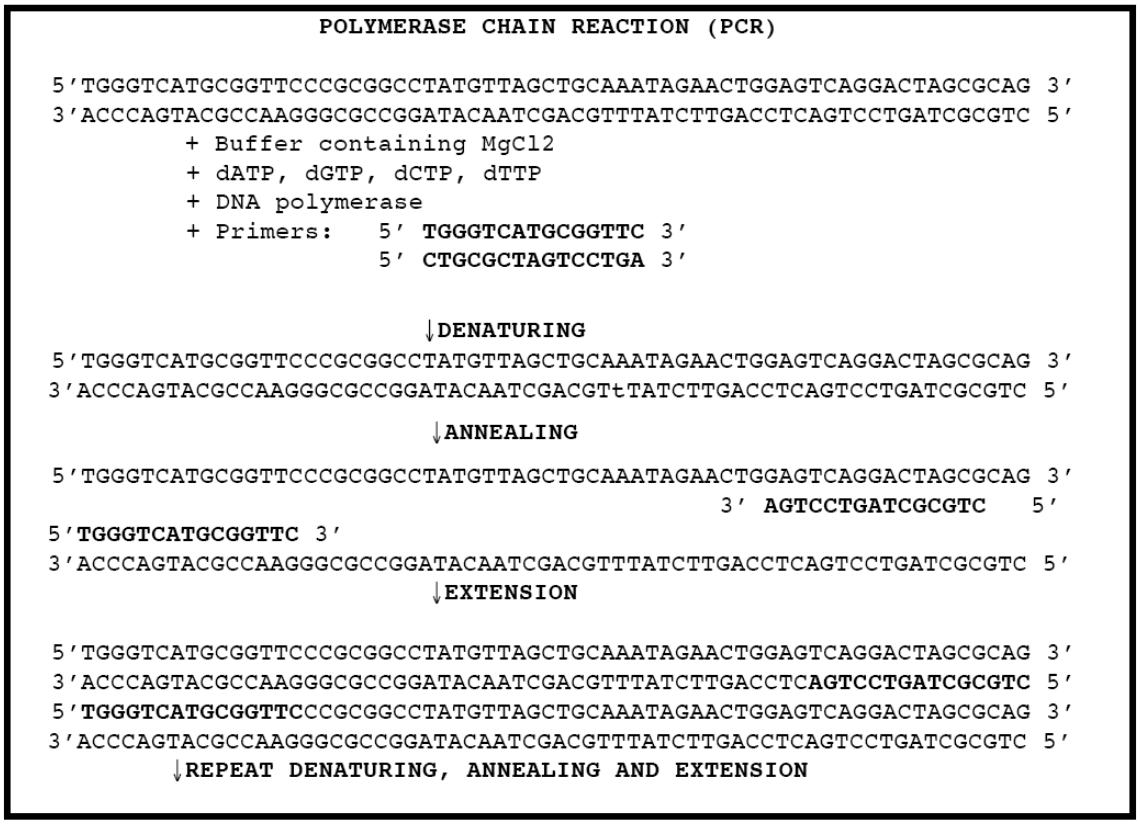

Figure 1. The polymerase chain reaction.

Description of the Platinum PCR SuperMix High Fidelity:

Platinum® PCR SuperMix High Fidelity provides qualified reagents for high fidelity amplification of DNA templates by polymerase chain reaction (PCR). It is effective over a large range of target sizes—up to 15 kb genomic DNA. The mixture contains anti-*Taq* DNA polymerase antibody, Mg++, deoxyribonucleotide triphosphates, recombinant *Taq* DNA polymerase, and *Pyrococcus* species *GB-D* thermostable polymerase. Anti-*Taq* DNA polymerase antibody inhibits polymerase activity, providing an automatic "hot start" and permitting room temperature set-up (polymerase activity is restored after a denaturation step in PCR cycling at 94°C). Antibody mediated hot starts improve PCR specificity and yield. *Pyrococcus* species *GB-D* polymerase possesses a proofreading ability by virtue of its 3' to 5' exonuclease activity (3). Mixture of the proofreading enzyme with *Taq* DNA polymerase increases fidelity approximately six times over that of *Taq* DNA polymerase alone.

Platinum® PCR SuperMix High Fidelity is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions.

Platinum® PCR SuperMix High Fidelity = 22 U/ml complexed recombinant *Taq* DNA polymerase, *Pyrococcus* species *GB-D* thermostable polymerase, and Platinum® *Taq* Antibody; 66 mM Tris-SO4 (pH 8.9); 19.8 mM (NH4)2SO4; 2.4 mM $MgSO_4$; 220 µM dNTPs; and stabilizers.

Storage Conditions:

After thawing, Platinum® PCR SuperMix High Fidelity may be stored at 4°C for 3 months or -20°C for 1 year. Storage at 4°C avoids the necessity of thawing the mix before assembling the reaction. There is no detectable decrease in enzyme activity or performance after storage for 3 months at 4°C, or after 15 freeze-thaw cycles.

Recommendations and Guidelines:

• Because PCR is a powerful technique capable of amplifying trace amounts of DNA, take all appropriate precautions to avoid cross-contamination.
• Reactions may be assembled either at room temperature or on ice. **NOTE: this is not true for all PCR polymerases!!!** Always read up on the particular polymerase you are using because some lose activity if care is not taken to prepare the reactions on ice!!!
• For multiple reactions, you can prepare a master mix of Platinum® PCR SuperMix High Fidelity and the component(s) common to all reactions.

PCR Protocol:

1. Add the following components in any order to each PCR tube (smallest tubes oyu have or the strip tubes):
    a. 45 µl Platinum® PCR SuperMix High Fidelity
    b. Primer solution (200 nM final concentration of each is recommended)*
    c. Template DNA solution (1–200 ng genomic DNA)*

*A standard 50-µl PCR reaction includes a combined primer and template volume of 5 µl; we have observed no decrease in product yield if the amount of primer and template solution is between 1 µl and 15 µl.

2. Mix contents of tubes, centrifuge briefly to collect liquid at bottom of tube and cover with mineral or silicone oil if necessary.

3. Cap tubes and load in thermal cycler.

4. Incubate tubes at 94°C for 30 s to 2 min to completely denature the template and activate the enzyme.

5. Design the appropriate parameters for your reaction and program them into the thermocycling machine according to the manufacturer's instructions. You can use the cycling parameters below as a starting point from which to perform your PCR amplification:

1. Heat denature 1 min. at 95°C
2. Denature 94°C for 15-30 s
3. Anneal 55°C for 15-30 s
4. Extend 68°C for 1 min per kb
5. Repeat steps 2-4 for 25-35 cycles
6. 10 min. at 72°C.
7. 4°C for infinite time (to hold until you come and get it)

6. Check your PCR reaction on an agarose gel to check if the PCR reaction worked, if the amplified product is the right size and if the product is clean (no other amplification products).

7. Clean up your insert by using either the QIAquick Gel Extraction (if you will be cloning your PCR product) or the QIAquick PCR purification kit (if you will be sequencing your PCR product)

Tips:

• If the PCR efficiency is not optimal, repeat the reaction with different primer concentrations from 100 to 500 nM, in 100 nM increments.
• For longer genomic DNA targets (>15 kb), we recommend adding 1–1.5 U of Platinum® *Taq* DNA Polymerase (Cat. no. 10966-018) to the reaction mix and increasing the extension time as specified (1 min per kb).
• At higher volumes of primer and template, the MgSO4 concentration in the reaction will drop to suboptimal levels and yield will decrease. For combined primer-template volumes of >15 µl (in solution with 45 µl of Platinum® PCR SuperMix High Fidelity), we recommend adjusting the final MgSO4 concentration in the reaction to 2.2 mM.