3. Polymerase Chain Reaction (PCR) and DNA Purification

A. *Polymerase Chain Reaction (PCR)*

Polymerase Chain Reaction was first envisaged in 1984 by Kary Mullis. PCR allows the amplification of exceedingly small amount of DNA *in vitro*, without prior transfer into living cells. Because of PCR, "insufficient nucleic acid" is no longer a limitation in molecular biology research.

PCR requires a pair of short DNA fragment called primers, which is complementary to and flank the target DNA sequence to be amplified, in a series of DNA polymerase-catalyzed reactions. First, DNA containing the sequence to be amplified is heat-denatured and then annealed to the primers, which are present in excess (Step 1 and 2). Next, polymerase chain reaction is carried out from the primer 3' termini (Step 3). Then a second cycle of heat denaturation, primer annealing and extension of the annealed primers is carried out.

Using a thermo stable form of polymerase, for example, *Taq* polymerase, form *Thermus aquaticus*, a bacterium that live in high temperature (hot spring), and *Pfu* DNA polymerase, form *Pyrococcus furiosus*, avoids the need to add more polymerase at each cycle due to the enzyme is not inactivated at DNA-denaturing temperature. The extension products synthesized in a given cycle can serve as a temple in the next cycle, so the number of target DNA copies approximately doubles every cycle.

Here, you will learn the basic of PCR, the procedure to set up the PCR reactions, including the PCR reaction mixture and PCR thermo cycle. The PCR products will be finally characterized by electrophoresis in order to reveal the effect of annealing temperature on yield and quality of PCR product. Electrophoresis is also a purification step, to limit the side product and primer-primer dimer. Finally, the PCR product of interest will be collected form the gel for later cloning step.

The PCR condition should follow the manual recommended by the polymerase producer. In general, a PCR mixture contains components as follows:

1. DNA polymerase
2. DNA polymerase buffer
3. dNTPs (deoxynucleotide)
4. Forward and Reverse Primers

5. DNA template
6. DNase-free ddH2O

Typically, PCR include following steps:

1. Initial denaturation: it is only required for DNA polymerases that require heat activation by hot-start PCR). × 1 cycle.
2. Amplification (to produce the product) × 30 cycles
    1) Denaturation: it causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
    2) Annealing: let the primer anneal to template.
    3) Extension: DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs.
3. Final extension: it ensures that any single strand DNA produced is completely extended. × 1 cycle.

In each step, duration and temperature are different. The denaturation and extension temperature depends on the DNA polymerase used. Annealing temperature depends on the primers.

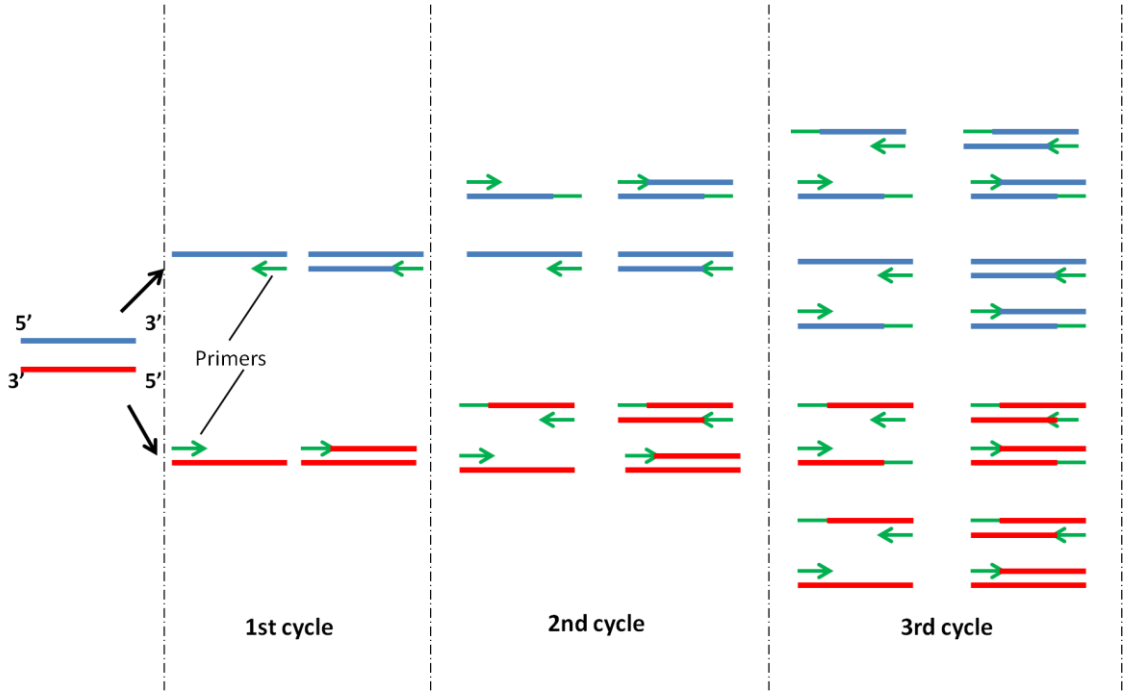


Figure 1 Three Cycles of the PCR

Materials

Phusion DNA polymerase

5X Phusion HF Buffer

10mM dNTPs

10uM Forward and Reverse Primers

DNA template (10 pg- 10 ng)

ddH2O (deionized, sterile, DNase-free)

PCR (200 µl) tubes

Thermocycler

Procedures

1. You have to calculate the volume of each component to be added and draft out thermal cycle details by filling in the following form.

PCR mixture	
Component	Volume (µl)
DNA polymerase	
DNA polymerase buffer	
dNTPs	
Forward Primer	
Reverse Primer	
DNA template	
DNase-free ddH_2O	
Total	

PCR thermal cycle			
	Temperature (℃)	Duration	Cycle
Initial denaturation			1
Denaturation			
Annealing			
Extension			

Final extension			1
Sample keeping	4	∞	1

2. Add the DNase-free ddH_2O in PCR tube.
3. Add the other Component in the tube. If the added volume is less, pipetting up and down is necessary.
4. Mix reagents in tubes by pipetting the solution up and down slowly.
5. Quick spin the PCR tube to ensure the mixture is in the bottom of the tube.
6. Put it in the thermocycler (PCR machine) and set the cycle information.
7. Start the cycle and wait till it is finished.
8. During the waiting time, the gel can be made for the characterization and purification.

(This video shows the procedures of setting up a PCR reaction)

B. *DNA Purification*

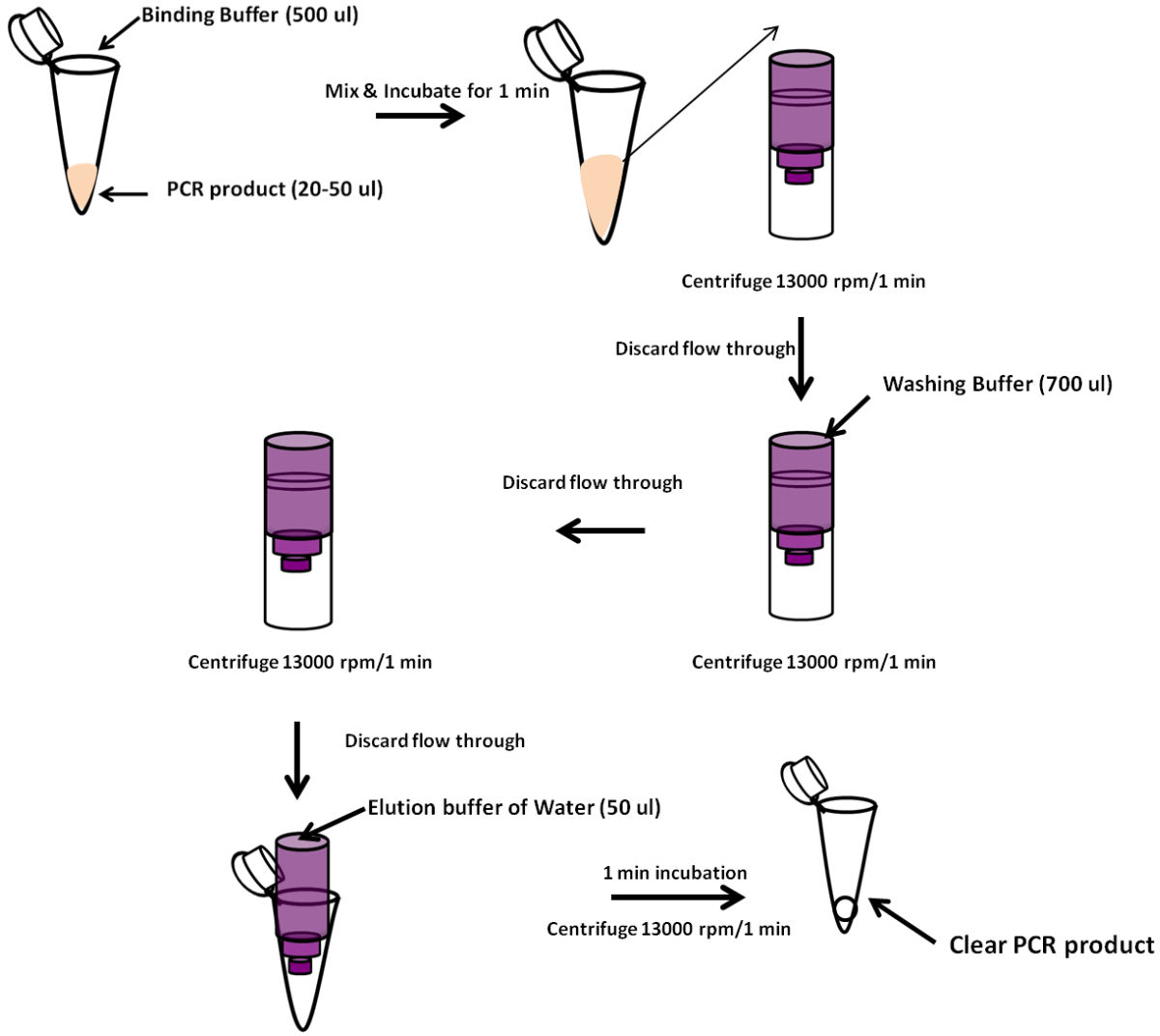
There are two methods, or kits, for DNA purification, PCR purification kit and Gel clean kit (purification from gel). The main difference is running the gel (electrophoresis) of not. The selection of these two kits is based on the purpose. If you want to remove excess nucleotides, enzymes and ions only, PCR purification kit is used. If you want to separate different pieces of DNA for target DNA selection, purification from gel is the only option.

Materials

Intron PCRquick-spin(TM) PCR Product Purification Kit

ddH_2O (deionized, sterile, DNase-free)

Procedures



(This video shows the procedures of DNA purification)

References

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eng.intronbio.com (2004). *PCR quick-spin(TM) PCR Product Purification Kit*. Retrieved January 15, 2016 from http://eng.intronbio.com/product/PCRquick-spin.htm