Exp.5

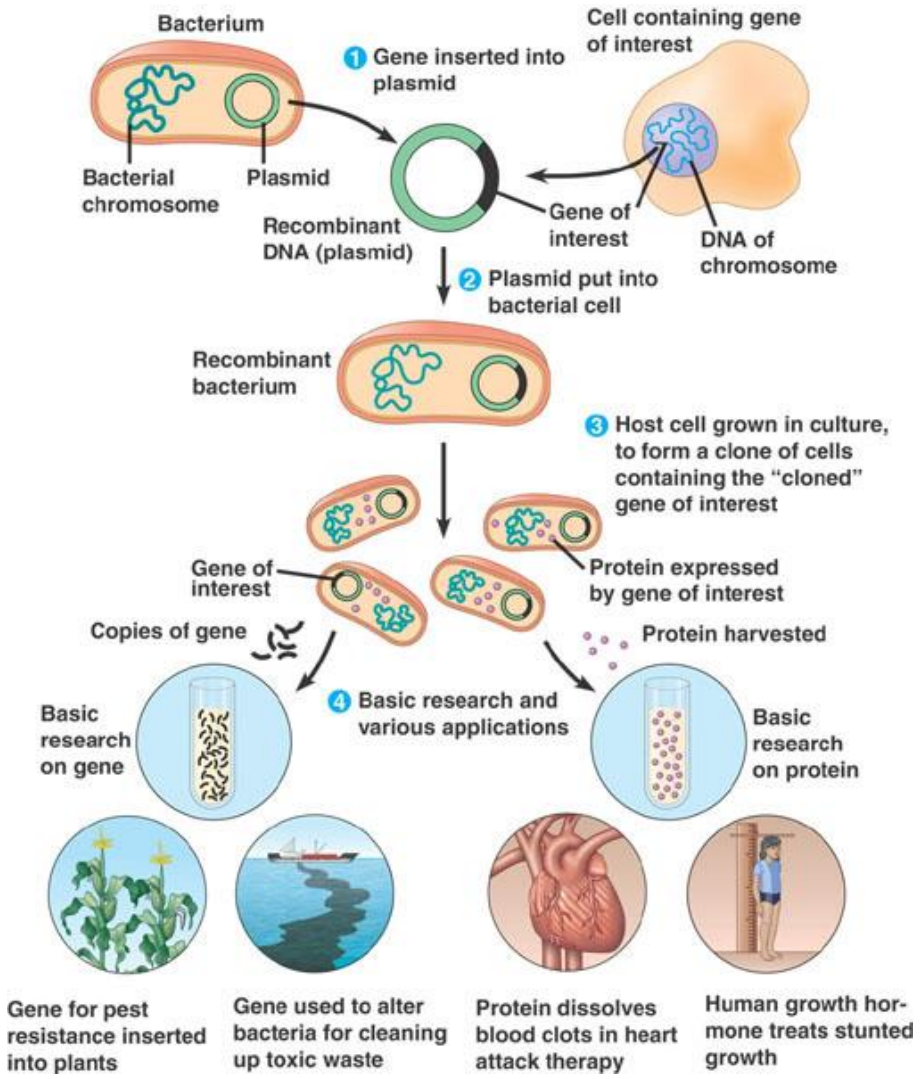
Polymerase Chain Reaction (PCR)

KAIST CHEMISTRY

Biological Engineering and Cloning

Biological engineering, or **bioengineering/bio-engineering**, is the application of principles of biology and the tools of engineering to create usable, tangible, economically viable products.

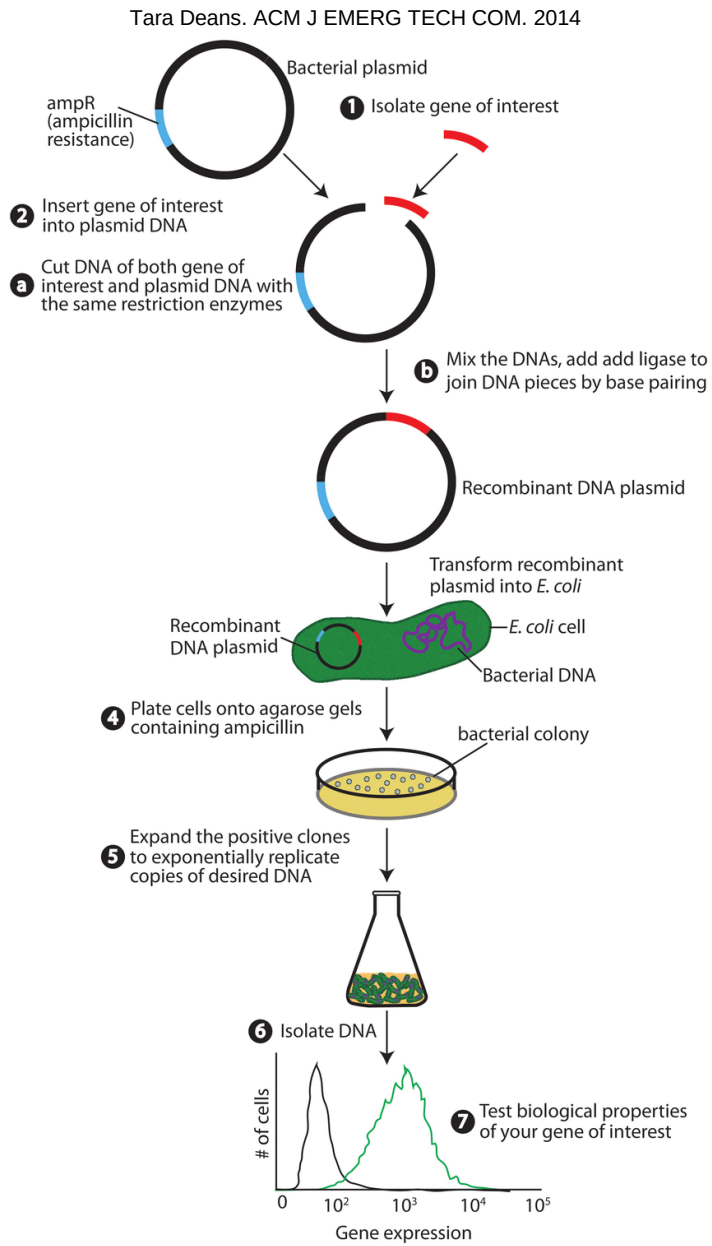
Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature, many organisms produce clones through asexual reproduction. Cloning in biotechnology refers to the process of creating clones of organisms or copies of cells or DNA fragments (molecular cloning).



KAIST CHEMISTRY

Recombinant DNA

Genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species. Such DNA becomes part of the host's genetic makeup and is replicated.



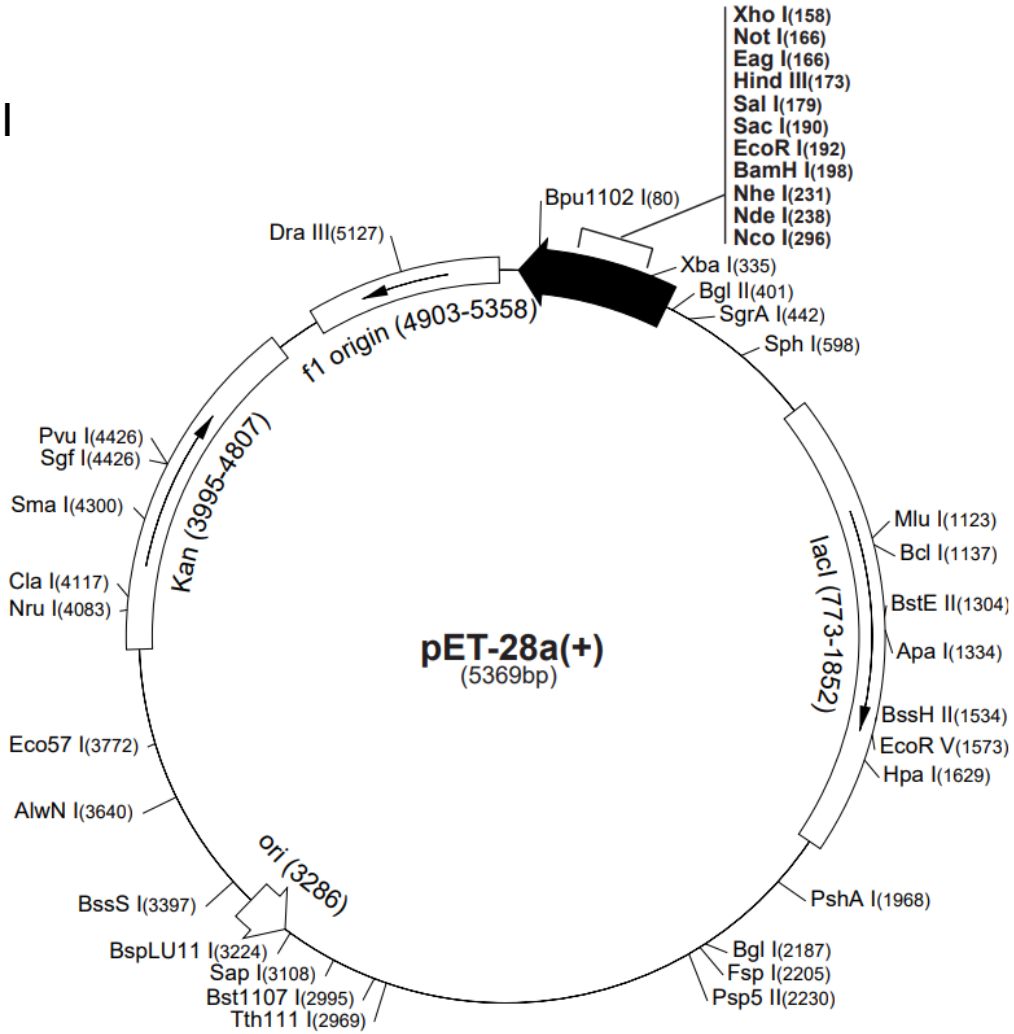
KAIST CHEMISTRY

Target Plasmid

Vector : pET 28a

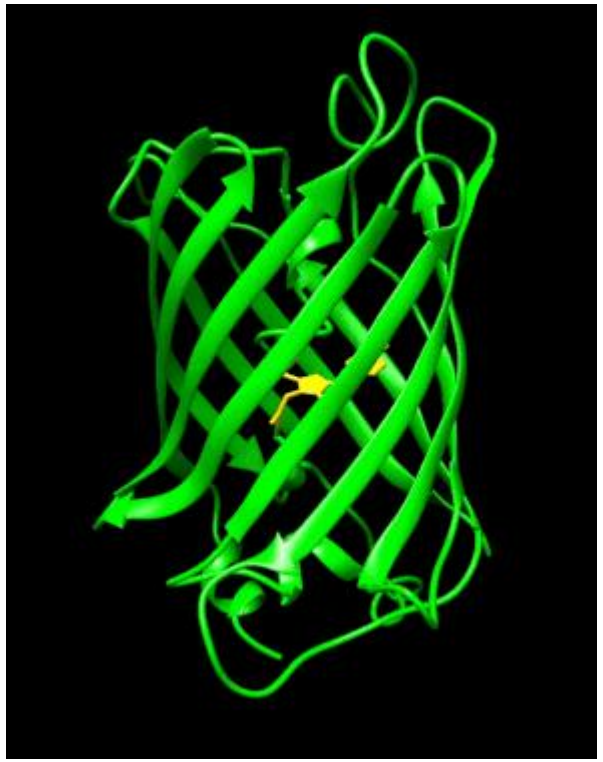
Enzyme site to use : Nde I, Xho I

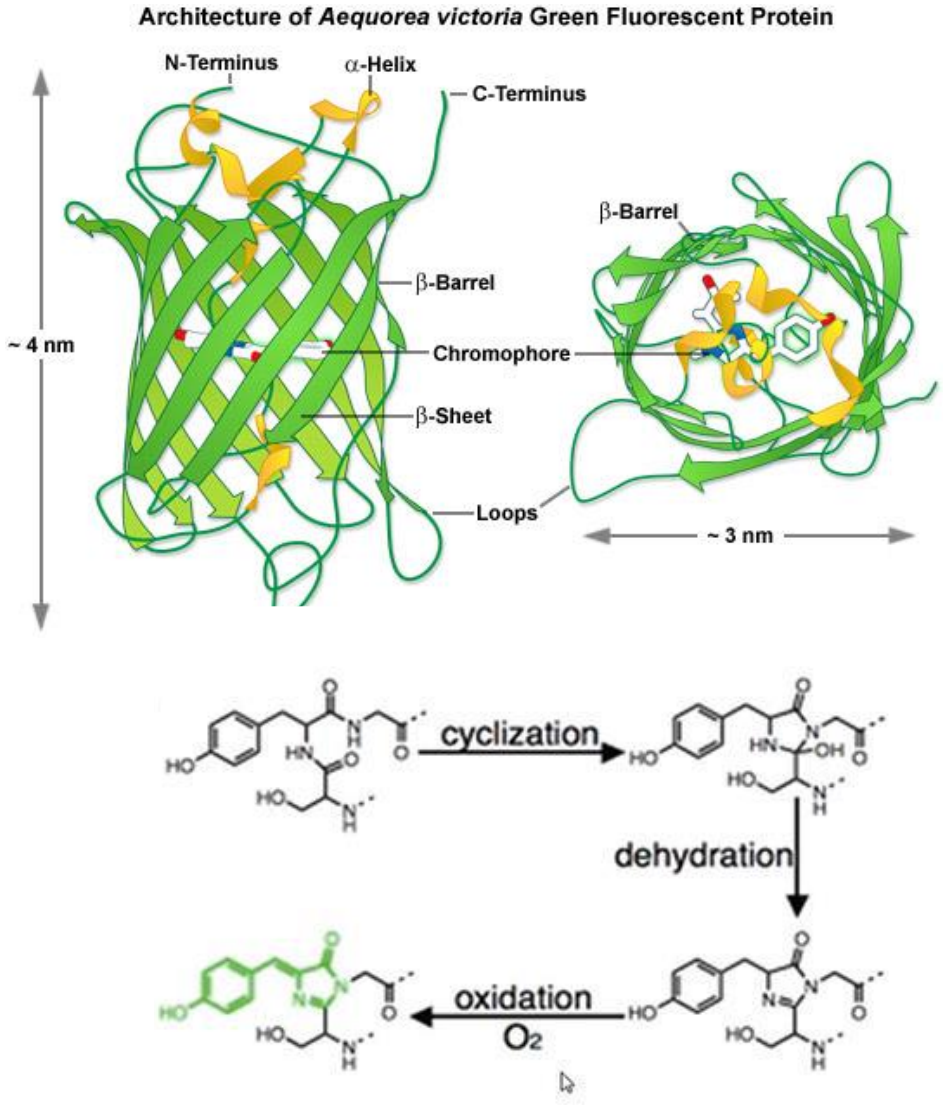
Antibiotics : Kanamycin



Template DNA information

Green fluorescent protein





Template DNA information

Green fluorescent protein(GFP)

DNA sequence(750bp)

ATGGGCAGCAGC**CATCATCATCATCATCAC**AGCAGCGGCCTGGTGCCGCGCGGCAGCCATATG**AAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAAT**
TAGATGGTGATGTTAATGGGCACGAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCAACAATCGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCT
GTTCCATGGCCAACACTTGTCACTACTCTGACTTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGT
TATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAAATACAAGACGCGTGCTGTAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTAC
TGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAATACAACTTTAACTCACACGATGTATACATCACGGCAGACAAACAAGAAAATGGAATCAAAGCTGAA
TTCACTGTTCGCCACAACGTTGAAGATGGCTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCT
GTCGACACAAACTGTTCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCACGAGTACGTAAACGCTGCTGGGATTACA TGA

Start codon
Histidine tag
GFP
Stop codon

Primer

Forward primer (Nde I)

5’ actg**CATATG**aaaggagaagaactttcactggagt 3’

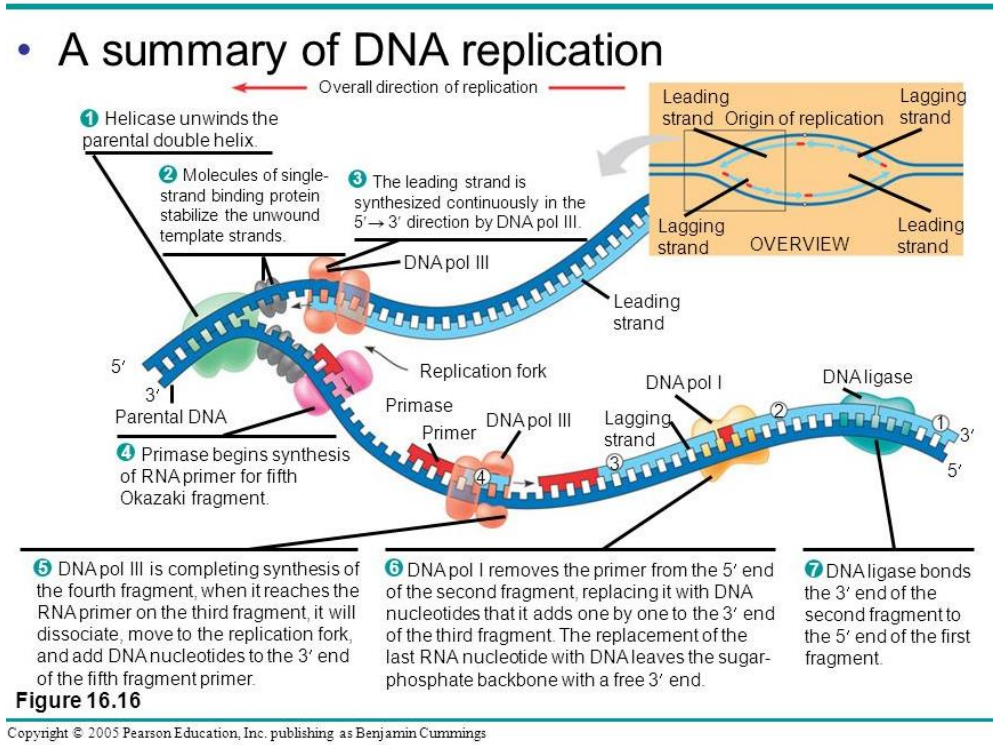
Reverse primer (Xho I)

5’ actg**CTCGAG**tcatgtaatcccagcagc 3’

KAIST CHEMISTRY

Principles of PCR

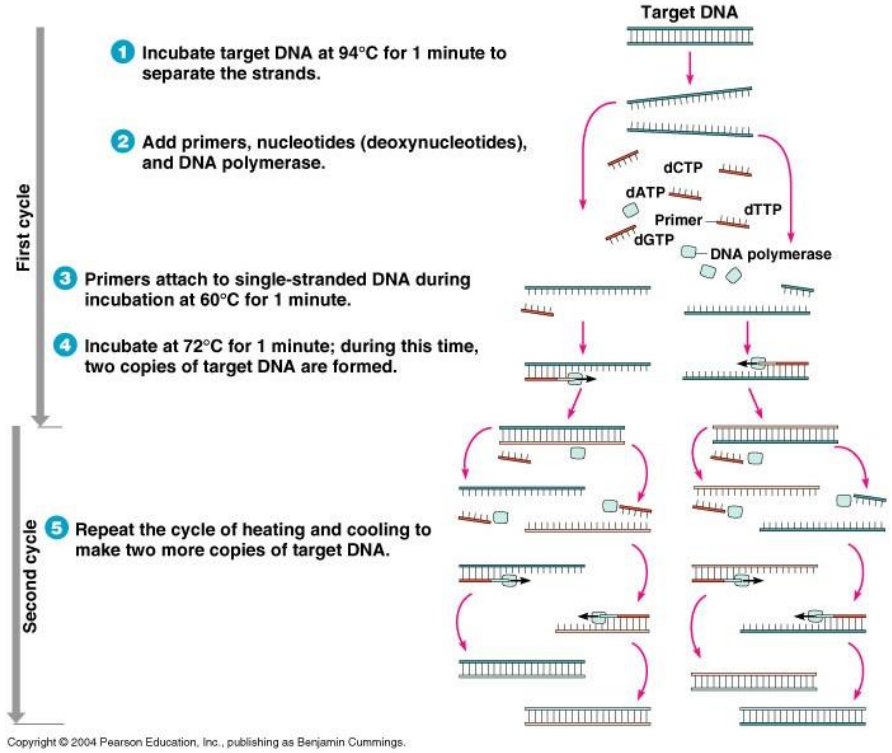
Organisms



Essential components of DAN replication

1. Short complementary oligomer synthesized by **Primase**
2. **DNA polymerase**
3. **Helicase** for unwinding double stranded DNA
4. **Nucleotides**

Laboratory



Essential components of PCR

1. **Primer**, which is artificially synthesized short oligomer
2. **Heat-stable DNA polymerase (pfu/Taq)**
3. **PCR machine** for the cycle of heating and cooling step
4. **Nucleotides** and **buffer** for polymerase

KAIST CHEMISTRY

Principles of PCR

- Polymerase Chain Reaction(PCR)

  Technology that amplifies DNA by repeatedly replicating specific DNA sequence. The DNA amplification time is short and a large amount of DNA can be obtained from very small amount of DNA.

- Experiment term

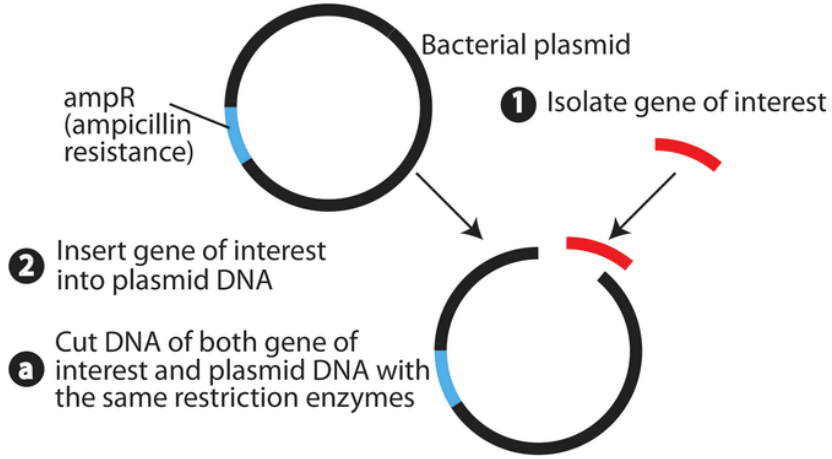
  Template DNA : Target DNA fragment to be amplified

  Primer : Short oligonucleotide that binds to the part to be amplified

  Pfu polymerase : Thermostable DNA polymerase. Low error rate

  dNTP : A nucleotide that becomes a material for synthesizing a gene

  Buffer : Contains various materials required for polymerase chain reaction



- PCR step

  Denaturation : Breaking hydrogen bonds in double helix. Double strand → Single strand

  Annealing : Binding primers to DNA

  Extension : Start synthesis of new DNA strands using dNTP

KAIST CHEMISTRY

Experimental process

1) Program as follows in PCR machine
2) Prepare reaction mixture as follows
3) Smooth pipetting, insert the PCR tube and run the program on the PCR machine.

Program setting

Cycle step	Temperature	Time	Cycle number
Initial denaturation	95℃	2min	1
Denaturation	95℃	30sec	25~35
Annealing	55℃	30sec	
Extension	72℃	30sec	
Final extension	72℃	5min	1
	4℃	hold	

Reaction mixture composition

Template	1㎕
Primer 1 (Forward)	1㎕
Primer 2 (Reverse)	1㎕
Pfu-DNA polymerase	1㎕
dNTP (10x)	2㎕
Buffer (10x)	2㎕
3rd distilled water	12㎕
Total volume	**20㎕**

** PCR is very sensitive at a extremely small quantity of DNA. So, all reagents should be stored as aliquots, and tip, tube and water for PCR should be sterilized.

KAIST CHEMISTRY

Pipette Usage

I. Use within a specific capacity range for each pipette
II. Do not apply more force than necessary
III. Works slowly when transferring solution
IV. Do not place pipette upside down or lay down while containing solution
V. Suction slowly from the top of the solution