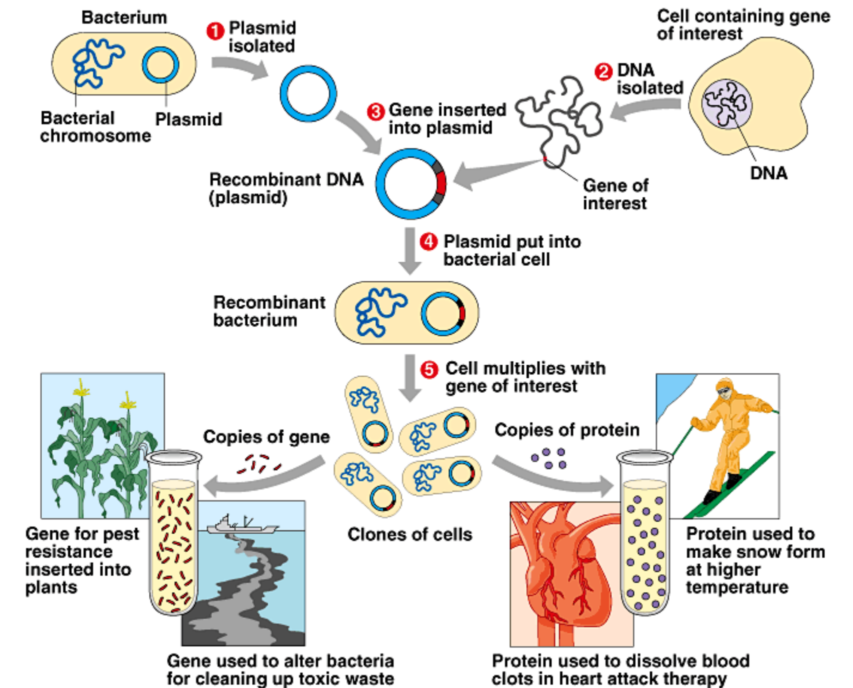
Exp.5. Polymerase Chain Reaction (PCR)

2018 Fall Chemistry Major Laboratory III (CH353)

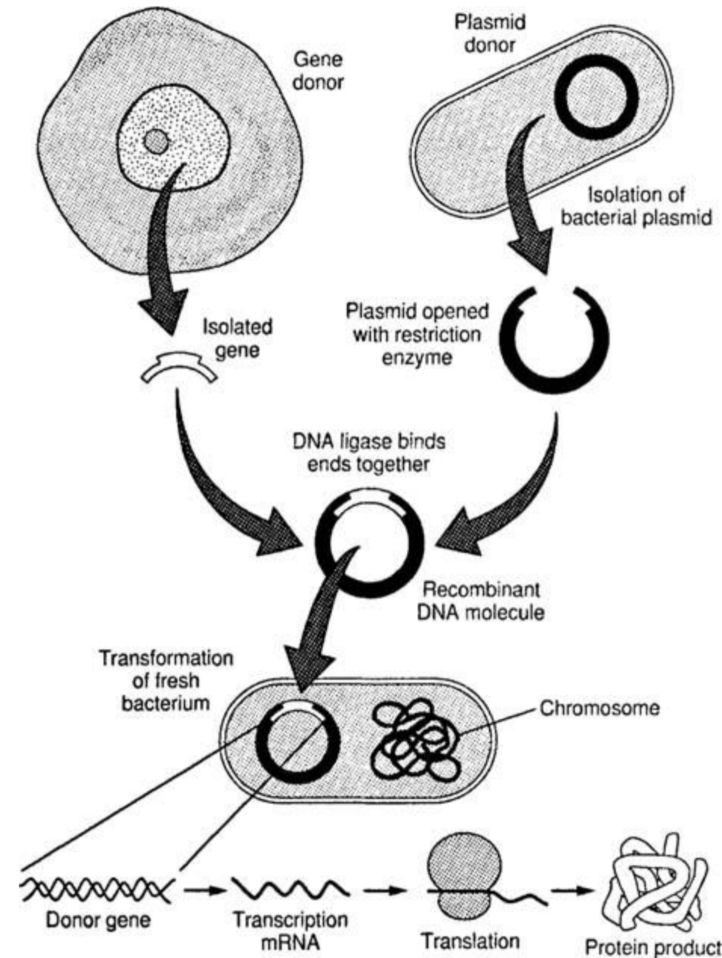
Biological Engineering and Cloning

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).

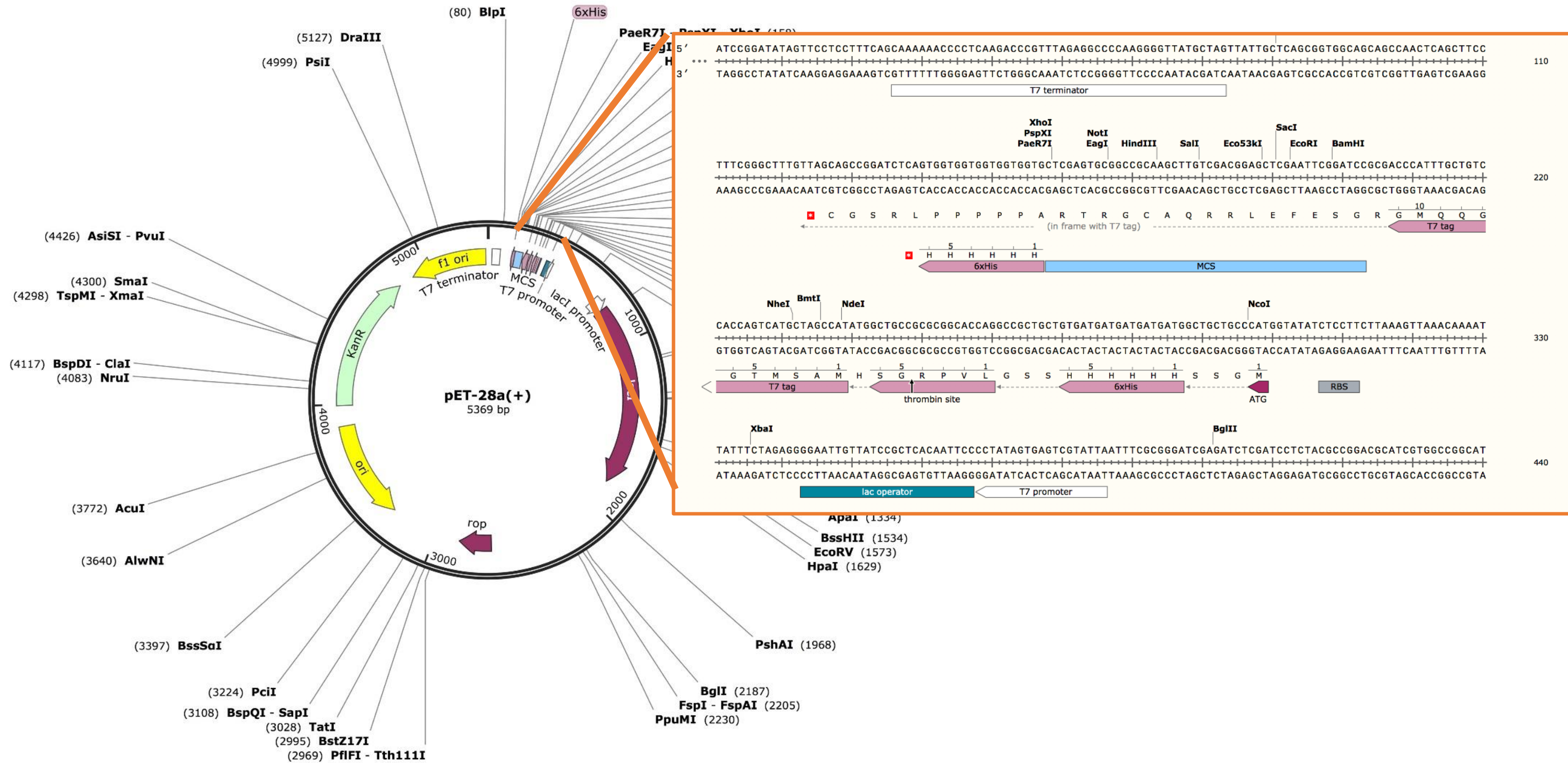


Recombinant DNA



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.

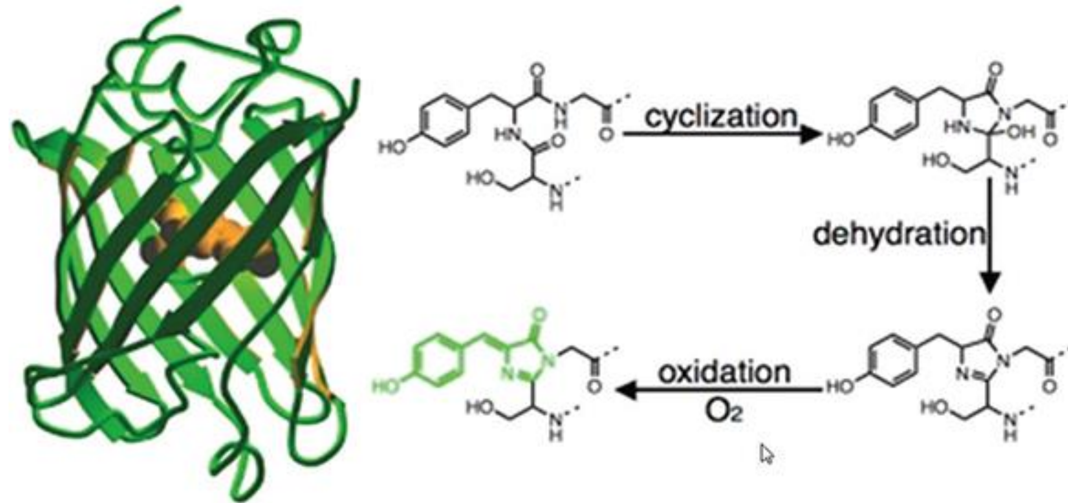
Target Plasmid, Vector: pET 28a



Snapgene , pET28a

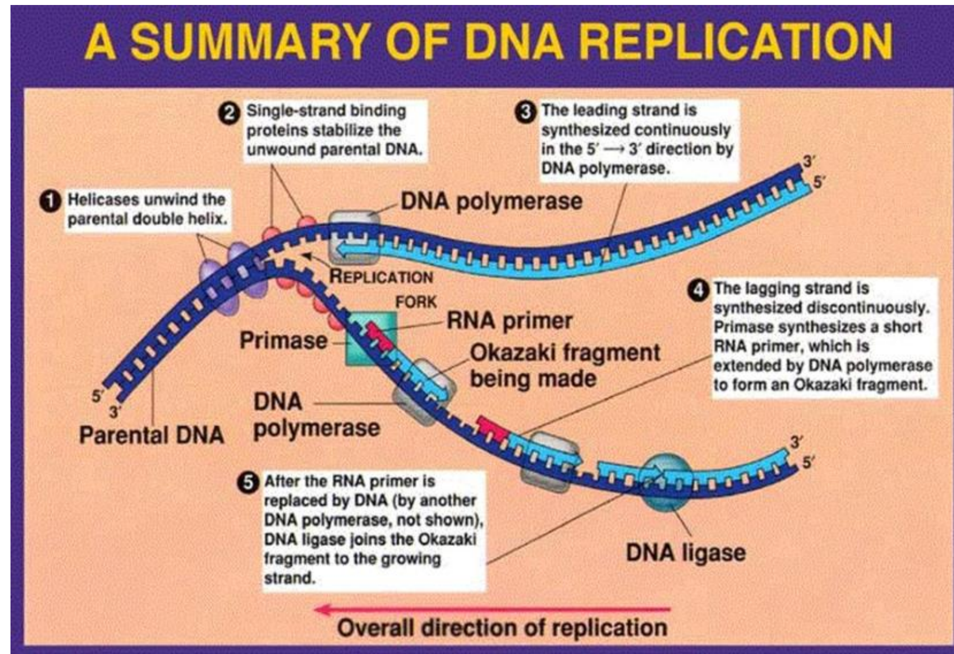
Target DNA of PCR: GFP

Green fluorescent protein (GFP)



Principles of PCR

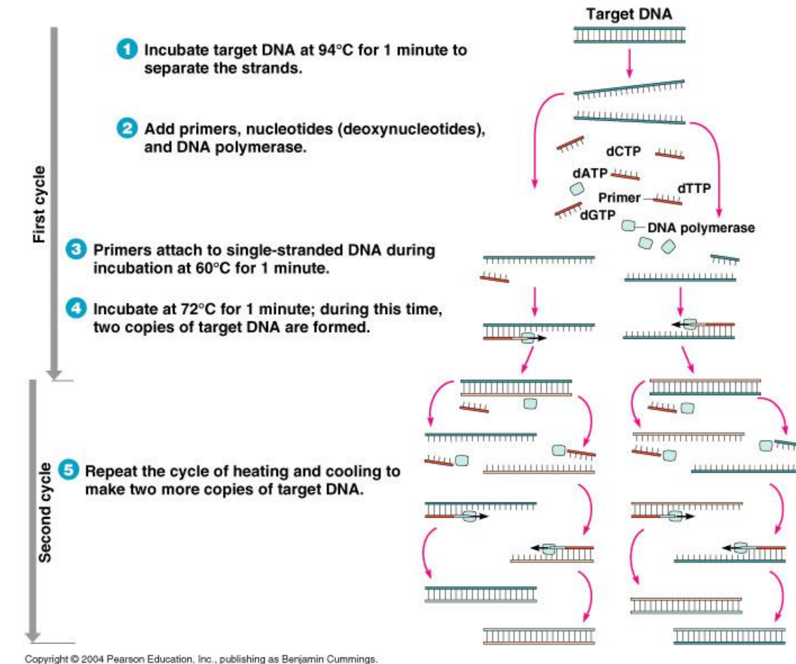
In organisms



Essential components of DNA replication

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

In laboratory



Essential components of PCR

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

Getting DNA Fragment of Target : GFP

- Green fluorescent protein (GFP)

- **GFP Sequence (**720 bp)
    - atggctagcaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagatggtgatgttaatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttacccttaaatttatttgcac
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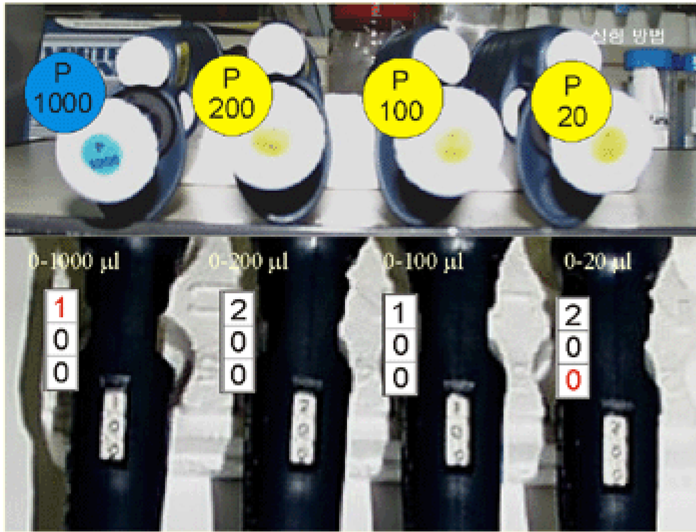
- Primer
    - Forward :GFP Nde1 for
        - 5′-caaCATATGatggctagcaaaggagaag-3′
    - Reverse :GFP Xho1 rev
        - 3′- ggatgaactctacaaataaCTCGAGaac -5′

          → 5′- gttCTCGAGttatttgtagagttcatcc -3′

Experiments on Today

1. PCR

파이펫 사용법



각 파이펫 별로 정해진 용량 범위 내에서 사용한다

누르다가 걸리는 곳까지가 정확한 용량이므로 꾹 눌러서 사용하지 않는다.

용액을 옮길 때 천천히 조작한다

용액이 담긴 상태로 파이펫을 거꾸로 세우거나 눕히지 않는다

용액의 위쪽부터 천천히 흡입하며 들어간다.

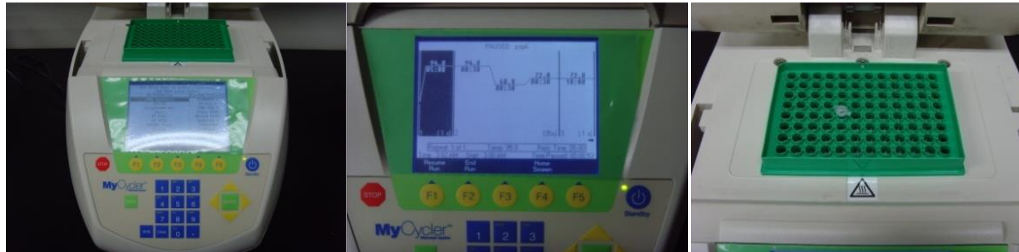
1) Experimental process: PCR

** 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.

1) Program as follows in PCR machine

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	

2) Prepare reaction mixture as follows



Template	1 ul
Primer 1 (forward)	1 ul
Primer 2 (Reverse)	1 ul
pfu-dna polymerase	1 ul
dNTP (10x)	2 ul
Buffer (10x)	2 ul
3rd distilled water	12 ul
Total Volume	20

3) Do pipetting gently and run the program of PCR machine, and put the PCR tube in