Biospin – 유전체R
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Chapter 2
DNA Techniques

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1. DNA 분리기술

1) DNA 분리용 시료의 선택
2) 항응고제의 선택
3) DNA분리의 기본원리
4) DNA정량 및 정도 관리
5) DNA 시료의 전기영동 분리
6) Souther blot 분석

2. 재조합 DNA 클로닝 기술

1) DNA조작효소
   - ① DNA복제효소
   - ② Nuclease
   - ③ DNA결합효소
   - ④ DNA 말단 조작효소
   - ⑤ 역전사효소
2) DNA클로닝벡터
3) DNA클로닝 기술

3. PCR 증폭기술

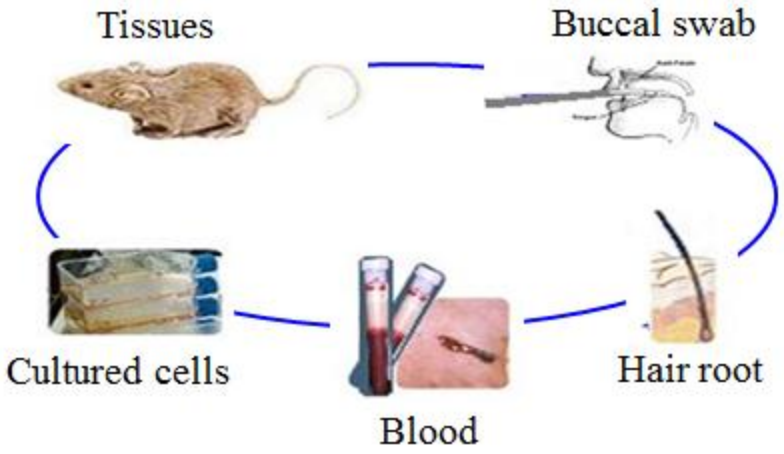
1) PCR의 원리
2) Reverse Transcriptase(RT)-PCR 기술
3) Real-time PCR 기술
4) Digital PCR 기술

1. DNA 분리기술

DNA 분리용 시료의 선택

- genomic DNA 분리용 세포 - 조직, 모근, 구강상피세포, 배양된 세포 및 혈액.
- 많은 양의 DNA 필요 시
  - 사람의 혈액에 존재하는 B세포를 Epstein-Barr virus(EBV)로 감염시켜 불멸화를 유도 lymphoblastoid cell line(LCLs)을 만들어 사용.
  - 단점 : 세포주 만드는 과정 또는 세포 배양 과정에서 일부 세포주에서 유전체 변이가 발생하는 문제점.
  - 하지만 대규모 유전체 연구에 사용된 시료는 대부분 EBV-transformed B cell 사용.
- 하지만!!! 최근에는
  - whole-genome amplification기술의 발전과 array-based DNA genotyping 분석 기술의 발전으로 **적은 양의 유전체 시료로도 많은 종류의 유전체 분석이 가능**하게 되었다.
  - 사람의 혈액으로 부터 바로 DNA를 분리하는 방법을 많이 사용.

Workflow: DNA Preparation to Applications



Manually or DNA isolation kit

Genomic DNA isolation

Applications:
- Cloning
- Sequencing
- Genotyping

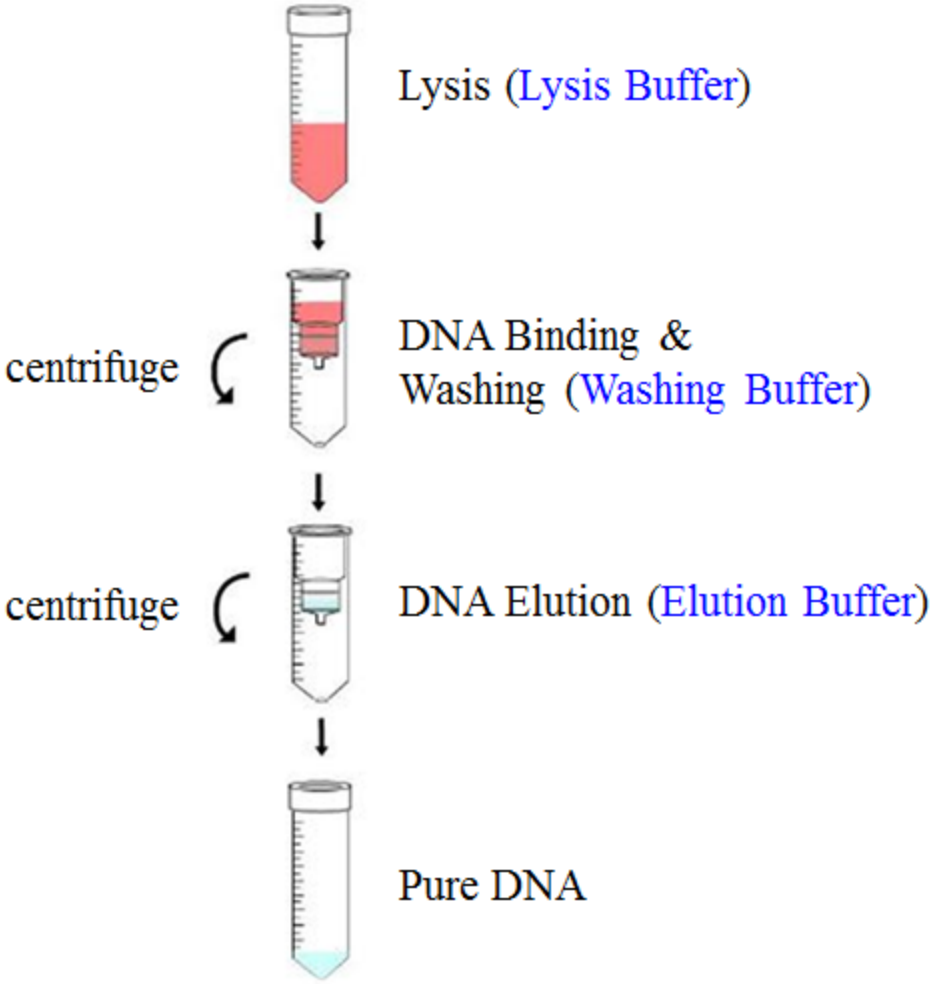
항응고제의 선택

- EDTA
  - DNA분리용 혈액 채취시 사용하는 항응고제
  - DNase 저해기능
- ~~heparin~~
  - DNA분리과정에서 DNA와 결합
  - PCR증폭에 사용되는 Taq pol을 저해
- 상온 수송, 채취한지 3일 이상 되면 DNA가 분해된다

DNA분리의 기본 원리

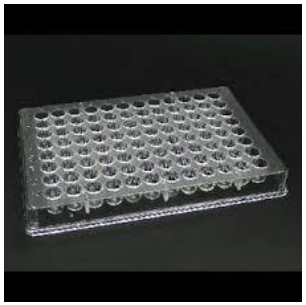
- DNA분리과정
  - 세포 파쇄(Cell lysis)
  - lysis
  - 단백질 제거
  - DNA 수집
- from 혈액
  - 적혈구 세포 제거 (핵x) 백혈구 세포만을 분리
  - 분리된 백혈구 세포 - 세포막을 파괴
  - 단백질 제거
  - 용액속에 존재하는 DNA를 에탄올로 침전
- 다양한 KIT
  - cell lysis : 세포를 lysis buffer로 파괴.
  - 컬럼을 통과 시켜 컬럼에 DNA결합시킨다.
  - 다른 물질들을 씻어낸다.
  - 컬럼에 붙어있던 DNA를 TE buffer로 씻어내어 순수한 DNA만을 분리
  - 분리된 DNA -70도씨에 보관

DNA Isolation Using Commercial Kit



DNA 정량 및 정도 관리

- DNA 정량(quantification)
  - UV spectrophotometer
  - nano drop
  - Qubit



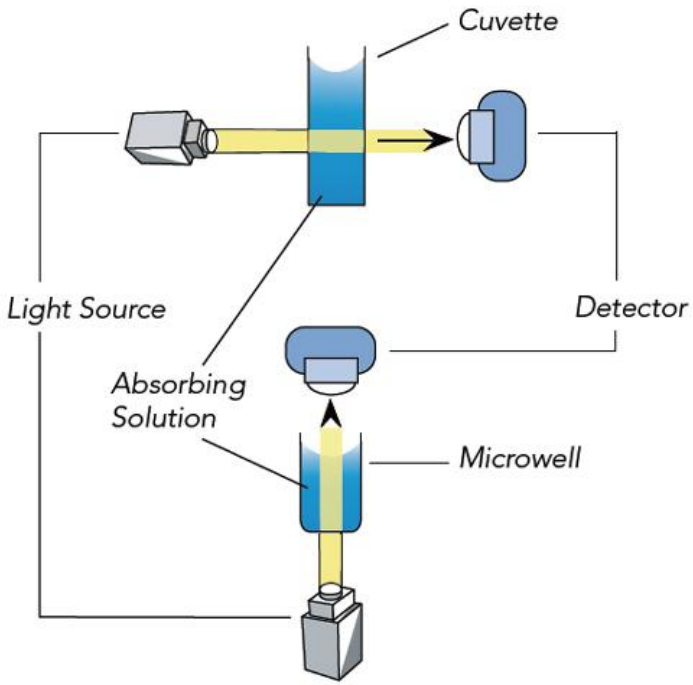
DNA Quantitation
Using a Spectrophotometer
https://www.youtube.com/watch?v=qw2ZaUXgWHU

- 1 A260 = 50 ug/ml , A260 = 0.1~1.0
- A260/A280

  = 1.8~2.0 ; 양질의 DNA

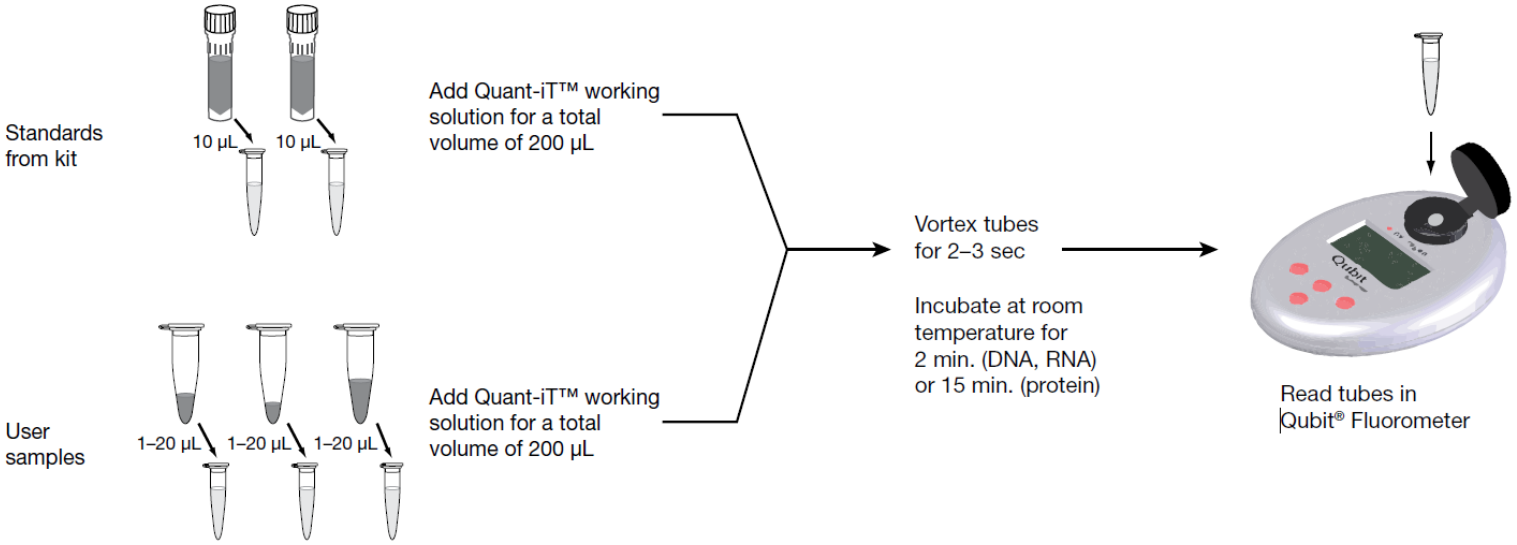
  < 1.6 단백질 오염 (재침전 필요)

  >2.0 RNA오염



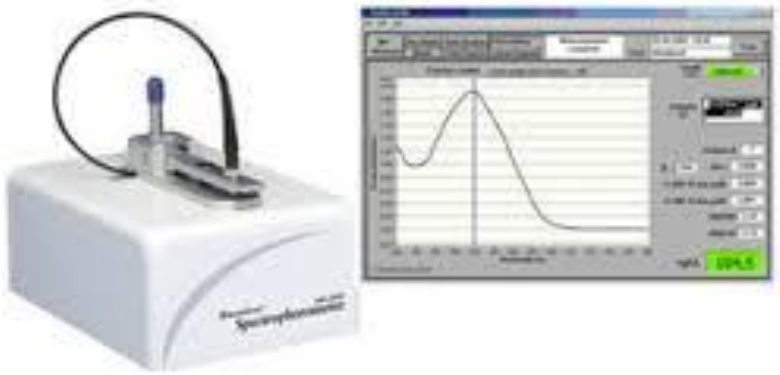
Qubit (invitrogen)

qubit 1.0
https://www.youtube.com/watch?v=RRKZN--7jqg
qubit 2.0
https://www.youtube.com/watch?v=bSSIO2fqEN8
qubit 3.0
https://www.youtube.com/watch?v=ymny7xABaa4



nanodrop

https://www.youtube.com/watch?v=FiGZnNs2xXY



DNA Quantity and Quality

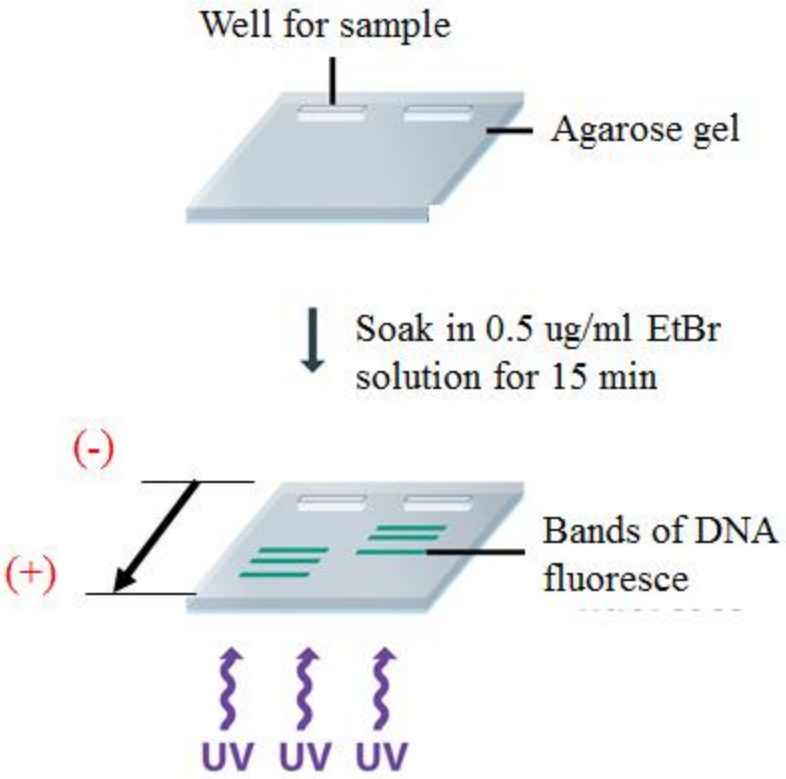
- **Measured by UV spectroscopy**:
  - NanoDrop ND-1000 spectrophotometer (NonoDrop technologies, USA)
    - *particularly good in case of the limited amount of DNA available
    - *it requires 1 ul of samples for measurement

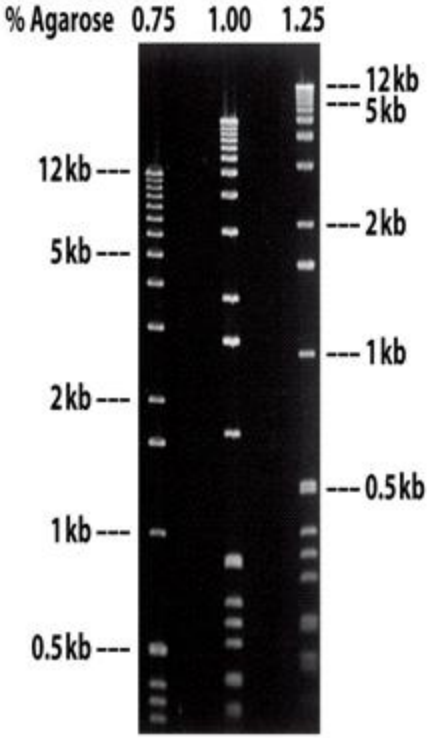
- **Quantification of DNA amount:**

  1 A_{260} = 50 μg/ml DNA (range of A_{260} for accurate DNA quantification= 0.1~1.0)

- 260/280 ratio = 1.8 ~ 2.0 → good quality DNA
- 260/280 ratio < 1.6 → protein contamination (should be re-precipitated)
- 260/280 ratio > 2.0 → RNA contamination

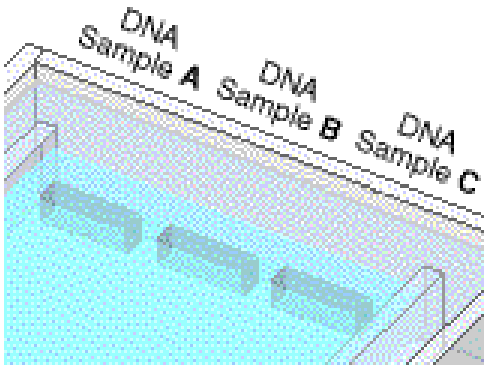
Agarose Gel Electrophoresis



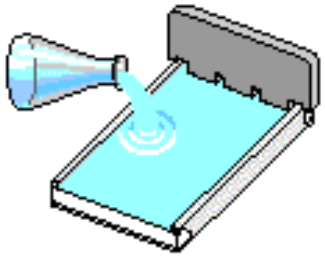


Agarose concentration:	high → good for separation of small DNA fragment	→ slow migration
	low → good for separation of large DNA fragment	→ fast migration

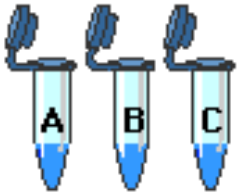
전기영동



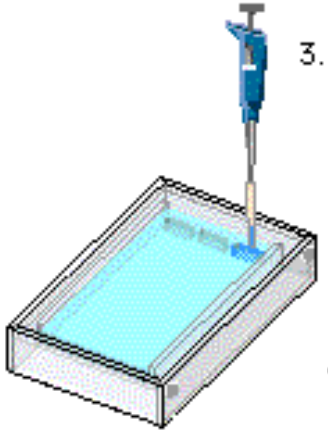
1. Make gel.



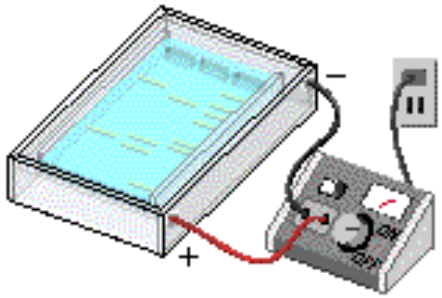
2. Obtain prepared DNA samples.



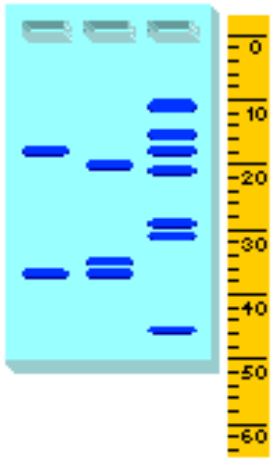
3. Load samples into gel.



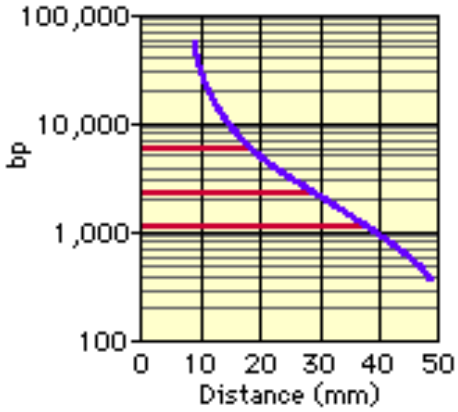
4. Separate fragments by electrophoresis.



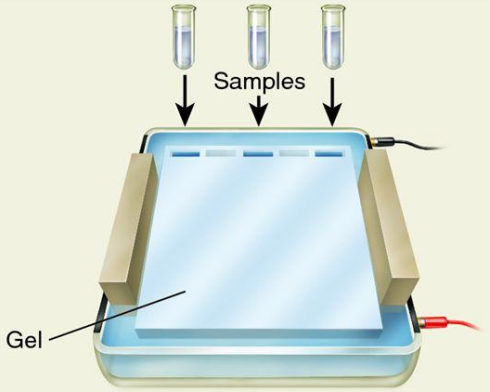
5. Stain DNA fragments and measure distances.



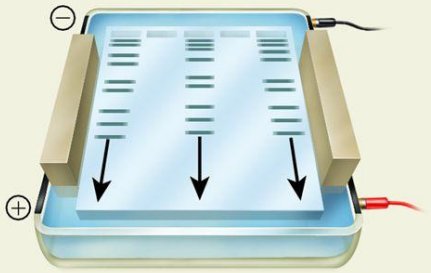
6. Prepare a standard curve. Determine fragment sizes.



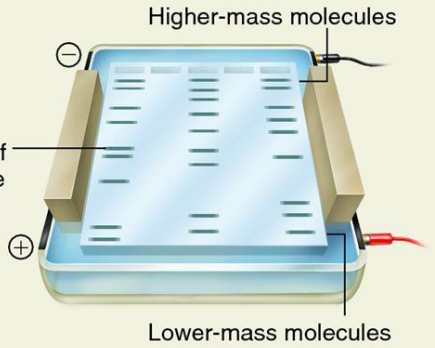
1 Load samples of DNA fragments into wells at the top of the gel.

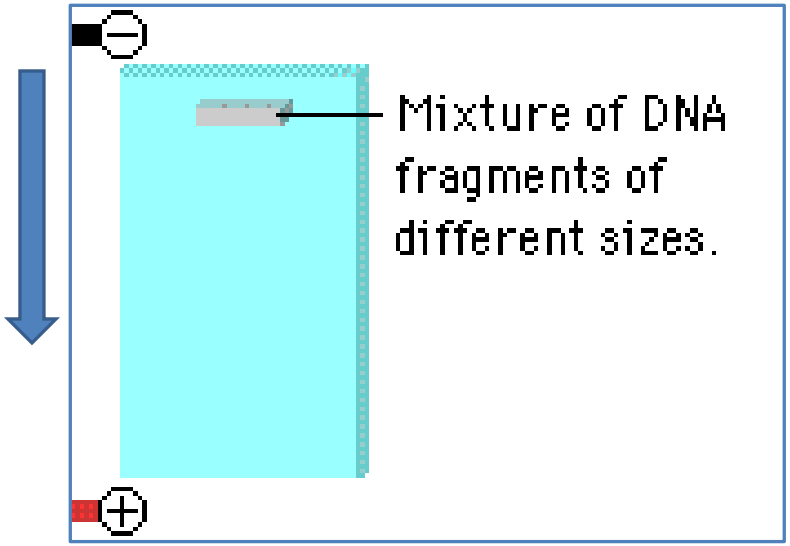


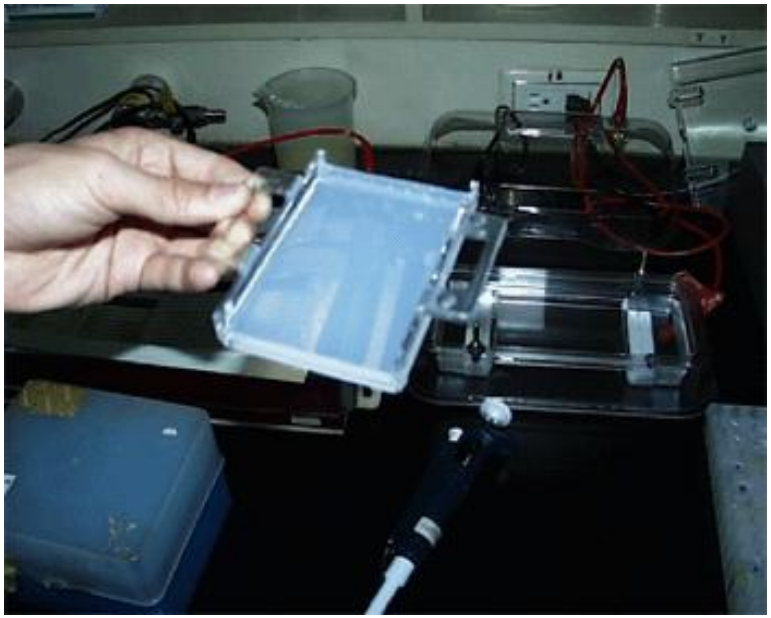
2 Apply an electric field.



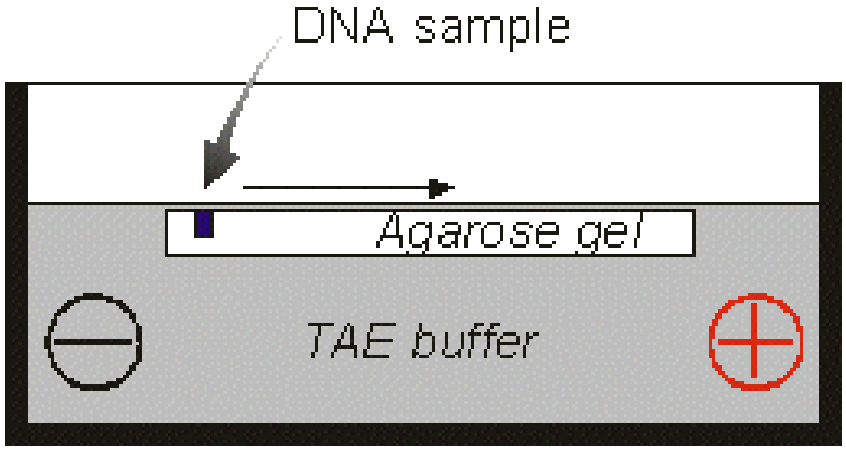
3 Wait additional time.



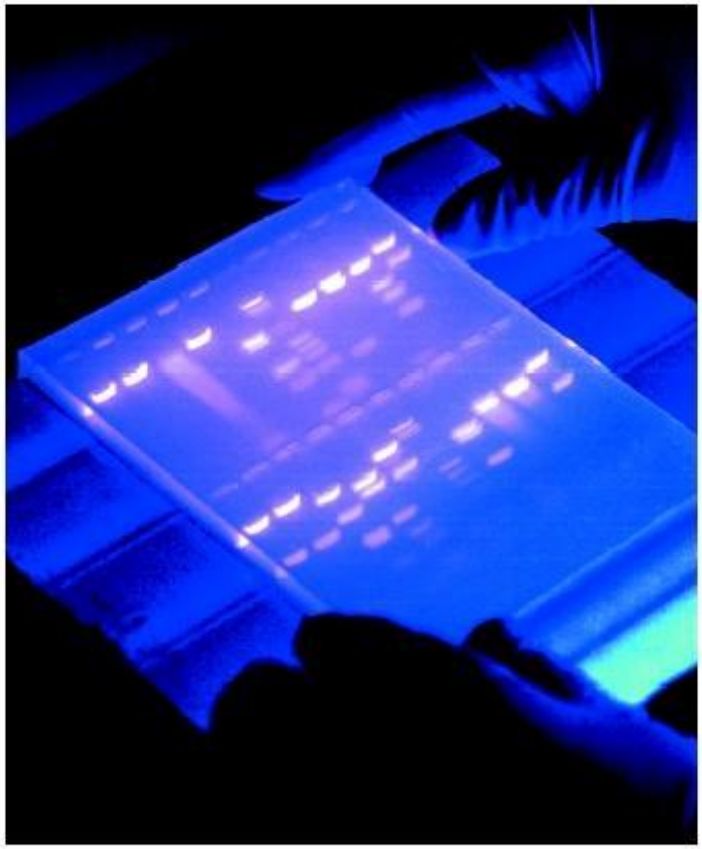


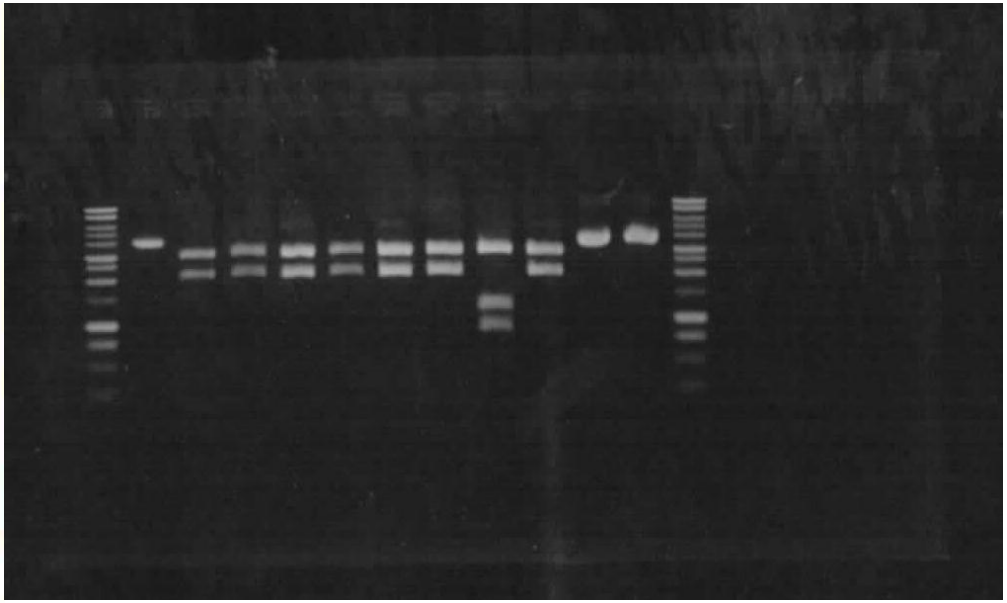


Agarose gel을 이용한 DNA의 전기영동



Gel electrophoresis

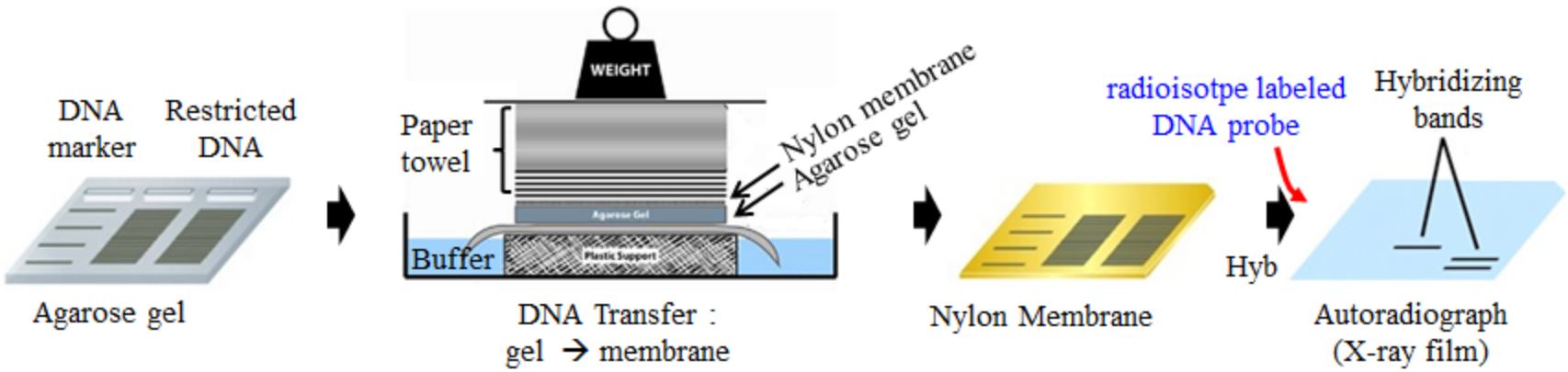




EtBr Stain & UV light

http://learn.genetics.utah.edu/content/labs/pcr/

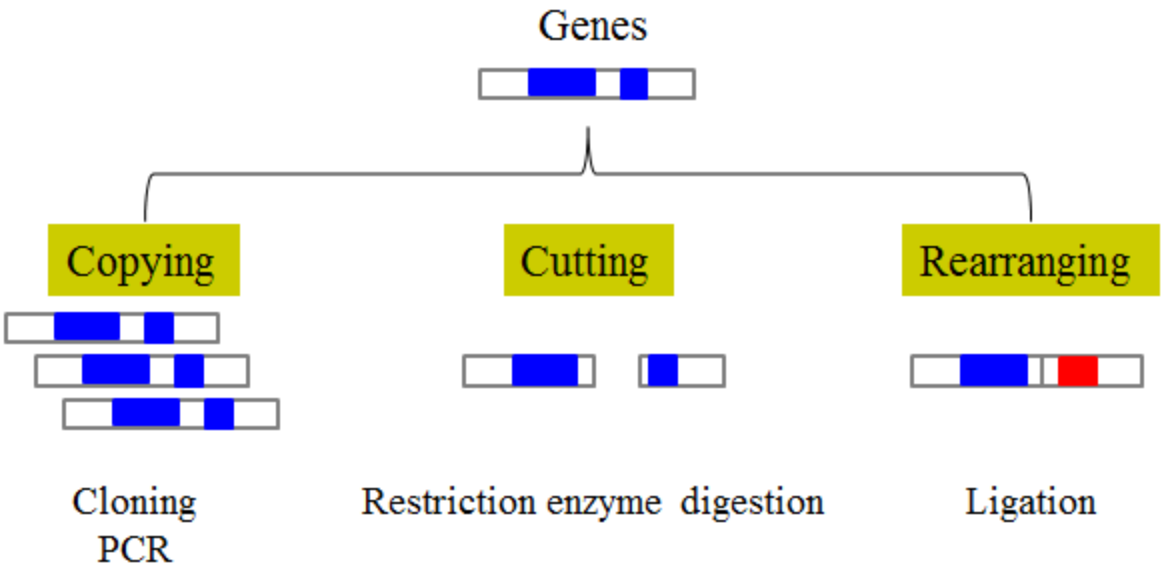
Southern Hybridization/Blotting



Southern blot	DNA detected by labeled DNA
Northern blot	mRNA detected by labeled DNA
Western blot	Protein detected by antibody (Ab)

2. 재조합 DNA 클로닝 기술

Recombinant DNA Technology

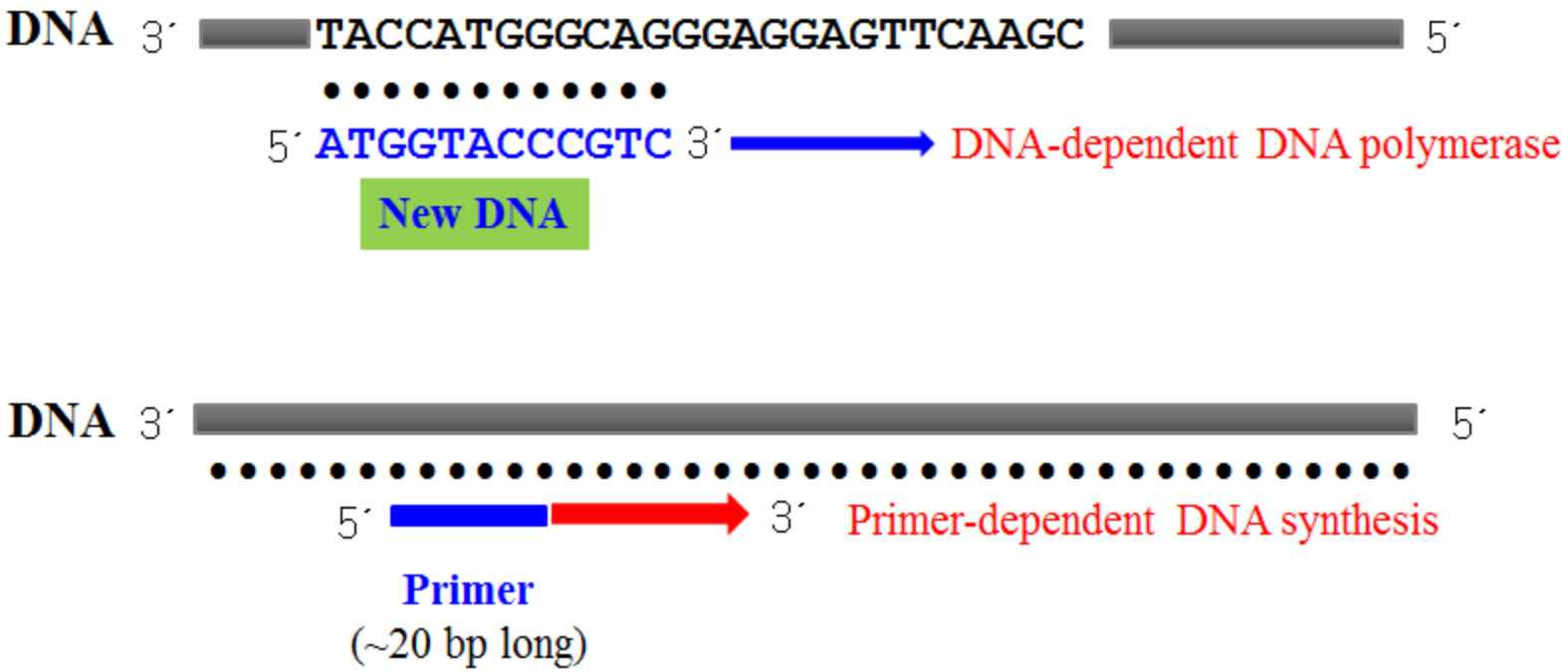


DNA조작효소

1. DNA복제효소
2. Nuclease
3. DNA결합효소
4. DNA 말단 조작효소
5. 역전사효소

1. DNA복제효소

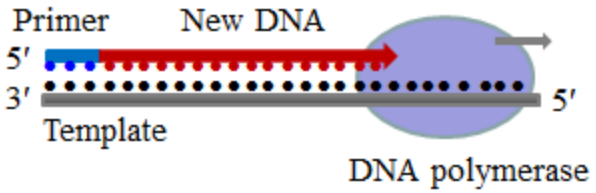
DNA- and Primer-dependent DNA Synthesis



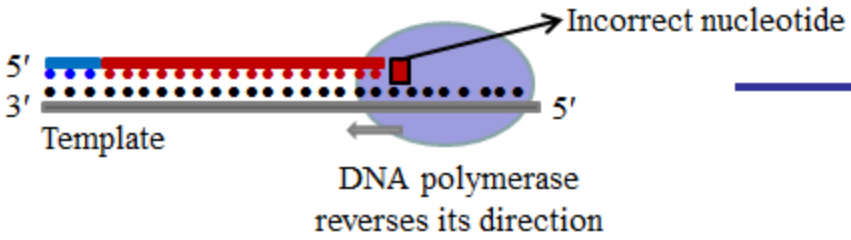
- DNA synthesis requires a primer (No DNA synthesis without primer)
- The primers determines which part of a DNA molecule is copied

Multifunctional Activity of DNA Polymerase

(A) 5′ → 3′ DNA synthesis

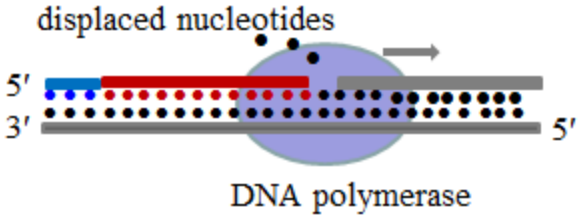


(B) 3′ → 5′ exonuclease activity



proofreading activity (pfu enzyme)

(C) 5′ → 3′ exonuclease activity



Less common (in some DNA polymerases)

DNA Polymerases

Types of DNA Polymerase	Description	Main Use
DNA polymerase I	Unmodified *E. coli* DNA polymerase	DNA labeling
Klenow polymerase	Modified *E. coli* DNA polymerase I	DNA labeling
Sequenase	Modified phage T7 DNA polymerase I	DNA sequencing
Taq polymerase	*Thermus aquaticus* DNA polymerase I	PCR
Reverse transcriptase	RNA-dependent DNA polymerase (from retroviruses)	cDNA synthesis

E coli DNA polymerase= polymerase activity
5'→ 3' exonuclease activity
3'→ 5' exonuclease activity

Klenow polymerase= polymerase activity
3'→5' exonuclease activity
Lack of 5'→3' exonuclease activity: good for sequencing/labeling

Optimum Rx temperature for all DNA polymerase except Taq: 37 °C (72 °C for Taq)
RTase (reverse transcriptase): RNA-dependent DNA polymerase (retroviruses such as HIV)

2. Nuclease

Nucleases

Nuclease	Description	Main Use
Restriction endonucleases	Sequence-specific DNA Endonucleases	Many applications
Nuclease S1	Endonuclease specific for **single-stranded** DNA and RNA	Transcript mapping
Deoxyribonuclease 1	Endocnuclease specific for **double-stranded** DNA and RNA	Nuclease footprinting

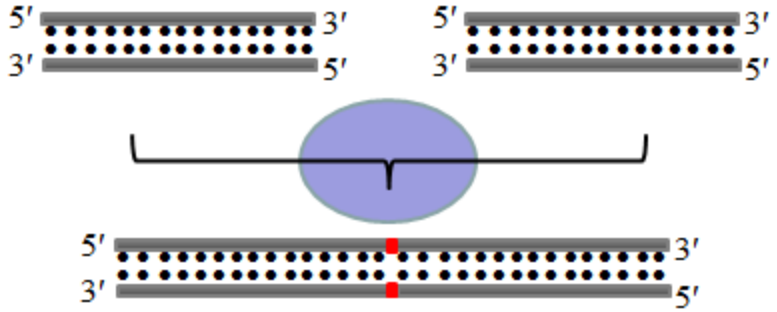
Restriction Enzymes

Enzyme	Recognition sequence	Type of ends	End sequence
AluI	5′-AGCT-3′ 3′-TCGA-5′	Blunt end	5′-AG CT-3′ 3′-TC GA-5′
EcoRI	5′-GAATTC-3′ 3′-CTTAAG-5′	Sticky end (5′ overhang)	5′-G AATTC-3′ 3′-CTTAA G-5′
PstI	5′-CTGCAG-3′ 3′-GACGTC-5′	Sticky end (3′ overhang)	5′-CTGCA G-3′ 3′-G ACGTC-5′

- >2500 type II enzymes have been isolated
- >300 are available for use in the lab.

Most recognition sequence have inverted symmetry (= ***Palindromes***); when read in the 5′ → 3′ direction, the sequence is the same in both strands.

DNA Ligase



Two bonds synthesized by DNA ligase

3. DNA 결합효소
4. DNA 말단 조작 효소
역전사효소

(2) 클로닝 벡터

Cloning Vectors

1. Plasmid vector
2. Lambda phage vector
3. Bacteriophage vector
4. BACs (bacterial artificial chromosome)
5. YACs (yeast artificial chromosome)

+

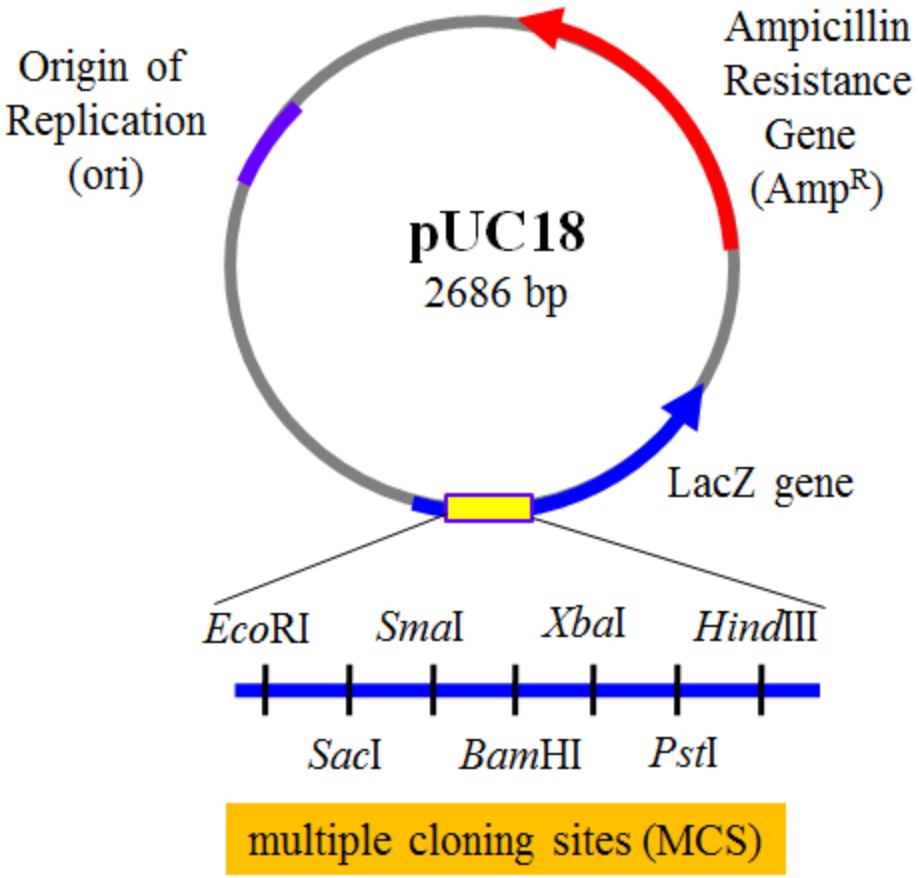
6. Expression vector (for cDNA expression cloning)

1~5: depending on insert size

Bacterial Cloning Systems

Vector type	Cloning capacity (kb)	Examples	Year of appearance	Mode of introduction to E. coli host cell	Mode of growth on agar plate	No. of copies per cell
Plasmids	0.1-12	pBR322, pUC	1977	transformation/ electroporation	colonies	50-100
M13	0.1-3.5	M13mp18	1981	transfection	plaques	N/A
Bacteriophage λ	10-20	EMBL, FIX	1983	packaging and infection	plaques	N/A
Cosmid	35-45	pWE15, Lawrist	1981	packaging and infection	colonies	5-50
Fosmid	35-45	pFOS1	1992	packaging and infection	colonies	1-2
P1	30-90	pAd10SacBI I	1990	packaging and infection	colonies	1-2
PAC	30-300	pCYPAC2	1994	electroporation	colonies	1-2
BAC	30-300	pBeloBAC1 1	1992	electroporation	colonies	1-2

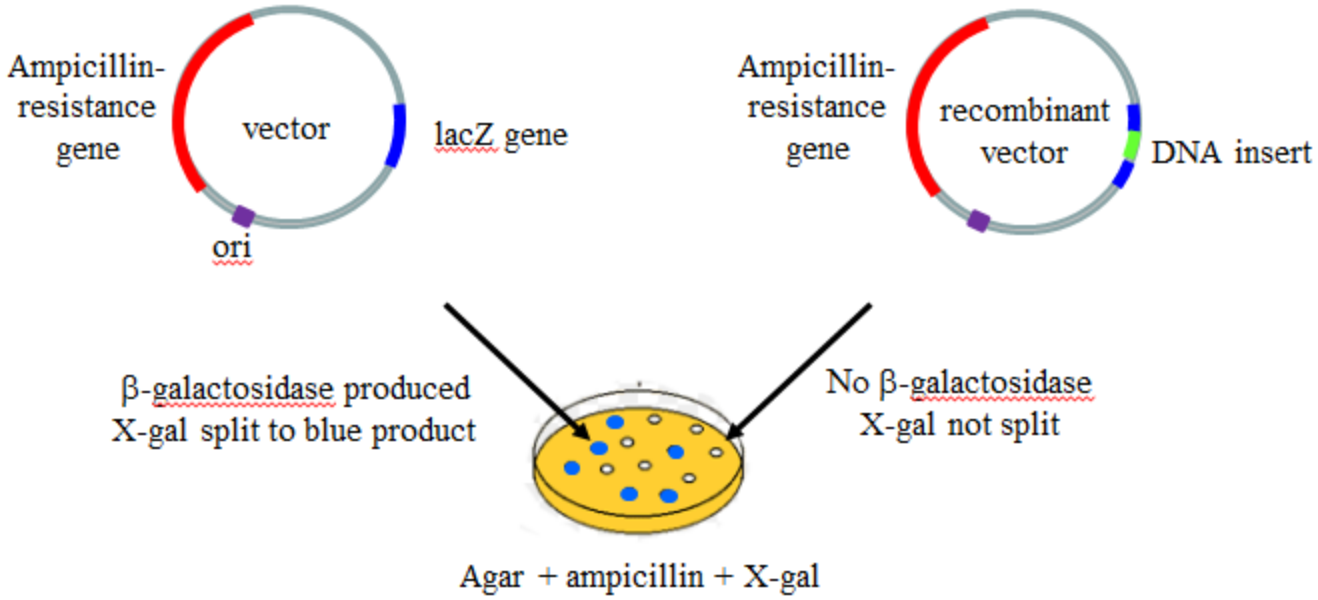
Cloning Vector: pUC18 Plasmid Vector



Cloning vector:

1. origin of replication
2. antibiotics (Amp) resistance gene (=β-lactamase)
3. lacZ gene (= β-galactosidase)
4. Multiple cloning site (MCS) at lacZ gene

Recombinant Selection: LacZ & Antibiotics



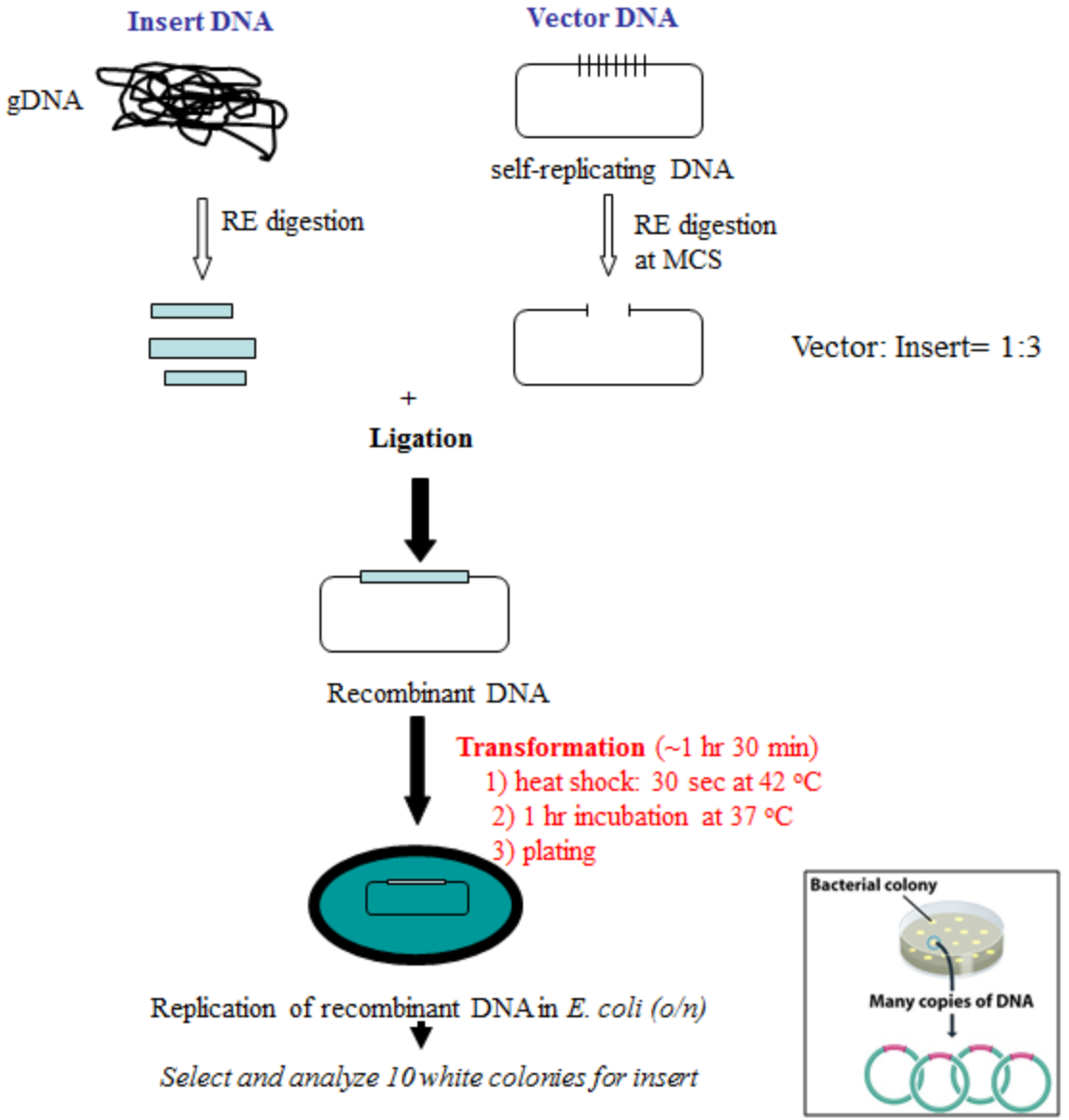
Blue colony:	non-recombinant
White colony:	recombinant

Sizes of Human Genomic Libraries Prepared in Different Types of Cloning Vector

Type of vector	Insert size (kb)	No. of clones	
		$P = 95\%$	$P = 99\%$
Cosmid	40	240,000	370,000
BAC	300	32,000	50,000
YAC	600	16,000	24,500

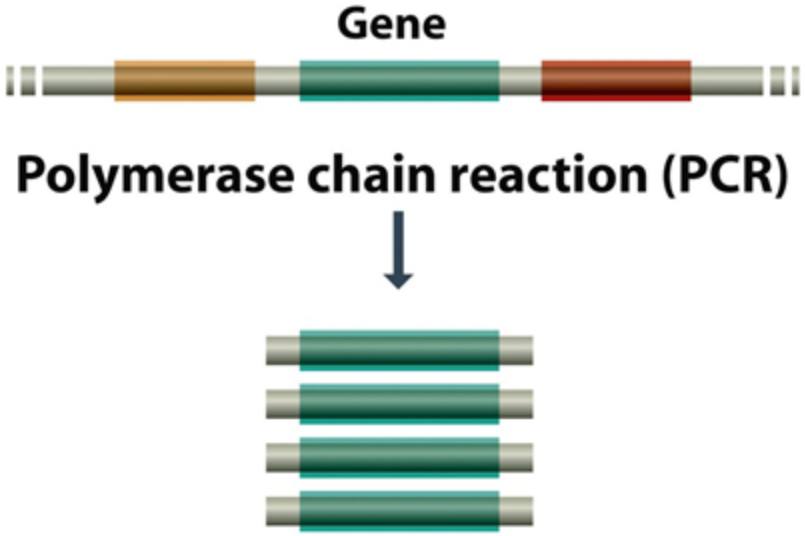
(3) 클로닝 기술

Experimental Outline of DNA Cloning



PCR 증폭기술

Polymerase Chain Reaction (PCR)



- Principle of PCR
- Discovery of Taq Enzyme
- Development of Hot start PCR
- Many applications of PCR:
  - RT-PCR
  - RACE
  - DD RT-PCR, etc

http://www.ncbi.nlm.nih.gov/genbank/

Primer Design

예제) Genbank의 p53 유전자 서열

```
   1 gtctagagcc accgtccagg gagcaggtag ctgctgggct ccggggacac tttgcgttcg
  61 ggctgggagc gtgctttcca cgacggtgac acgcttccct ggattggcag ccagactgcc
 121 ttccgggtca ctgccatgga ggagccgcag tcagatccta gcgtcgagcc ccctctgagt
 181 caggaaacat tttcagacct atggaaacta cttcctgaaa acaacgttct gtccccctg
 241 ccgtcccaag caatggatga tttgatgctg tccccggacg atattgaaca atggttcact
 301 gaagacccag gtccagatga agctcccaga atgccagagg ctgctccccc cgtggcccct
 361 gcaccagcag ctcctacacc ggcggccccc gcaccagccc cctcctggcc cctgtcatct
 421 tctgtccctt cccagaaaac ctaccagggc agctacggtt tccgtctggg cttcttgcat
 481 tctgggacag ccaagtctgt gacttgcacg tactcccctg ccctcaacaa gatgttttgc
 541 caactggcca agacctgccc tgtgcagctg tgggttgatt ccacacccgc gcccggcacc
 601 cgcgtccgcg ccatggccat ctacaagcag tcacagcaca tgacggaggt tgtgaggcgc
 661 tgcccccacc atgagcgctg ctcagatagc gatggtctgg cccctcctca gcatcttatc
 721 cgagtggaag gaaatttgcg tgtggagtat ttggatgaca gaaacacttt tcgacatagt
 781 gtggtggtgc cctatgagcc gcctgaggtt ggctctgact gtaccaccat ccactacaac
 841 tacatgtgta acagttcctg catgggcggc atgaaccgga ggcccatcct caccatcatc
 901 acactggaag actccagtgg taatctactg ggacggaaca gctttgaggt gcgtgtttgt
 961 gcctgtcctg ggagagaccg gcgcacagag gaagagaatc tccgcaagaa aggggagcct
1021 caccacgagc tgcccccagg gagcactaag cgagcactgc ccaacaacac cagctcctct
1081 ccccagccaa agaagaaacc actggatgga gaatatttca cccttcagat ccgtgggcgt
1141 gagcgcttcg agatgttccg agagctgaat gaggccttgg aactcaagga tgcccaggct
1201 gggaaggagc cagggggggag cagggctcac tccagccacc tgaagtccaa aaagggtcag
1261 tctacctccc gccataaaaa actcatgttc aagacagaag ggcctgactc agactga
```

Primer Design

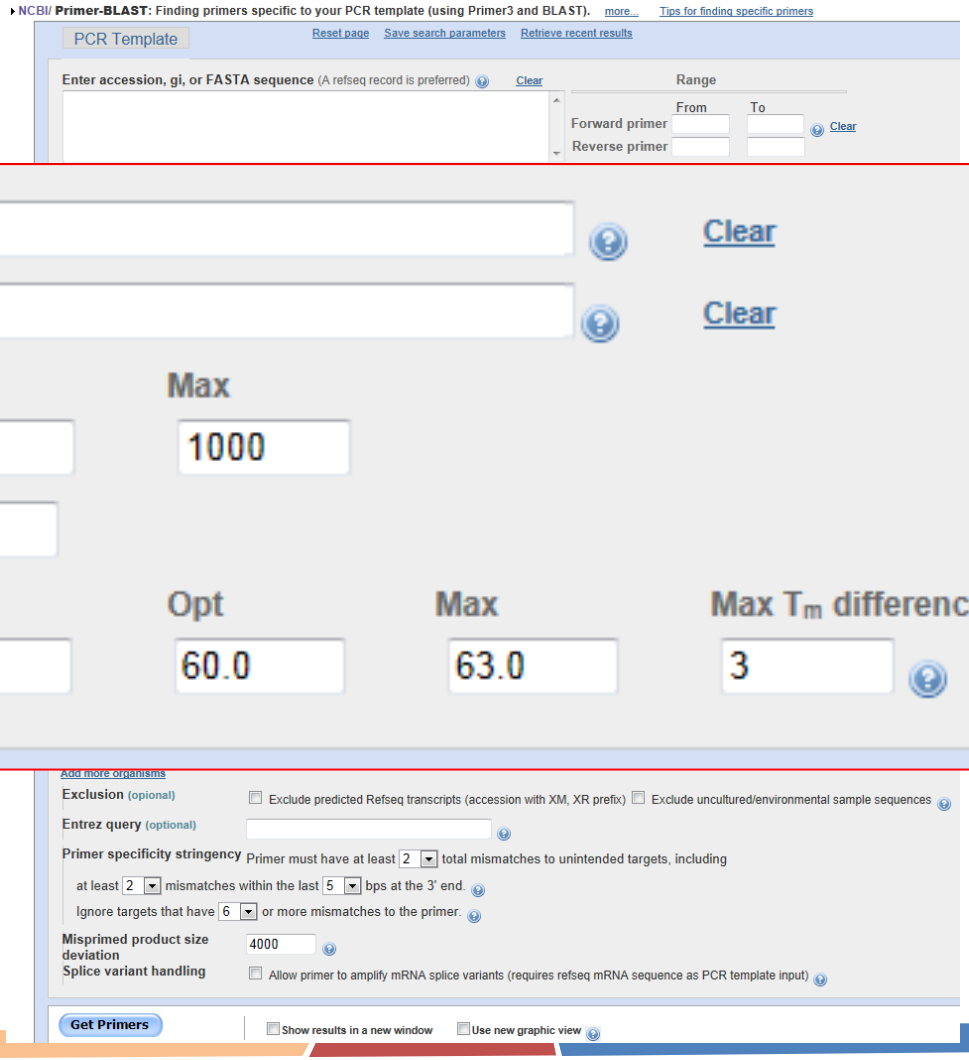
- 길이 20-25mer
- Tm (melting temp.
- GC content
- Hair pin 구조 유무
- Self dimer 형성 유

Length	Usually about 20 nt for target sequences in complex genomic DNA; can be much less if target DNA is less complex
Base composition	Substantial tandem repeats of one or more nucleotides to be avoided. Overall %GC plus length to be chosen so that the T_m of each oligonucleotide (*Table 5.2*) should be equal or nearly identical
Secondary structure	Avoid sequences prone to secondary structure which could form hairpins etc. (see *Figure 1.7A*)
3′ end	Base complementarity of the two bases at the extreme 3′ end of the two primers to be avoided. Otherwise primer dimers can result, reducing amplification efficiency

http://www.ncbi.nlm.nih.gov/tools/primer-blast/

Primer-BLAST

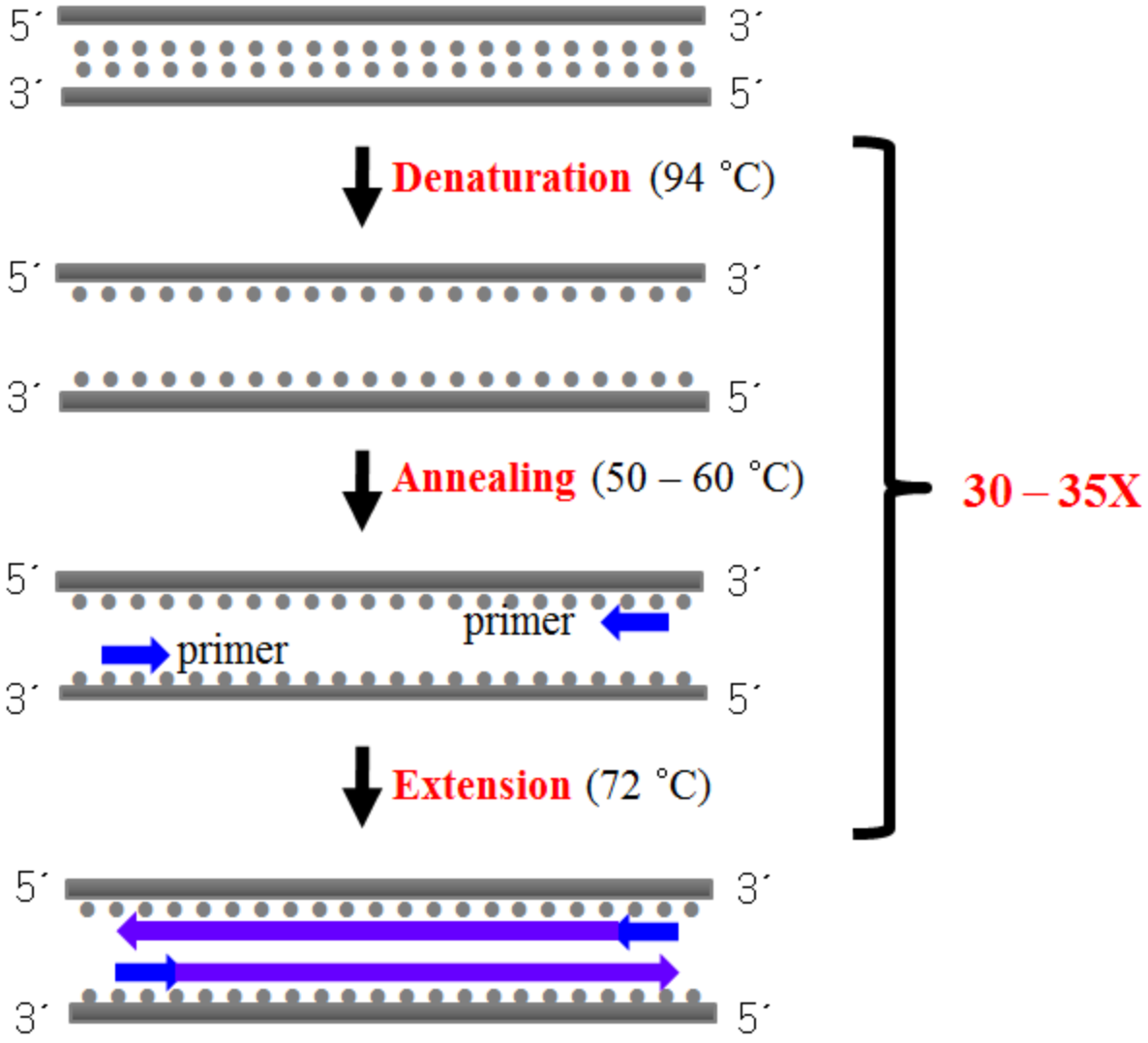
- Primer Design



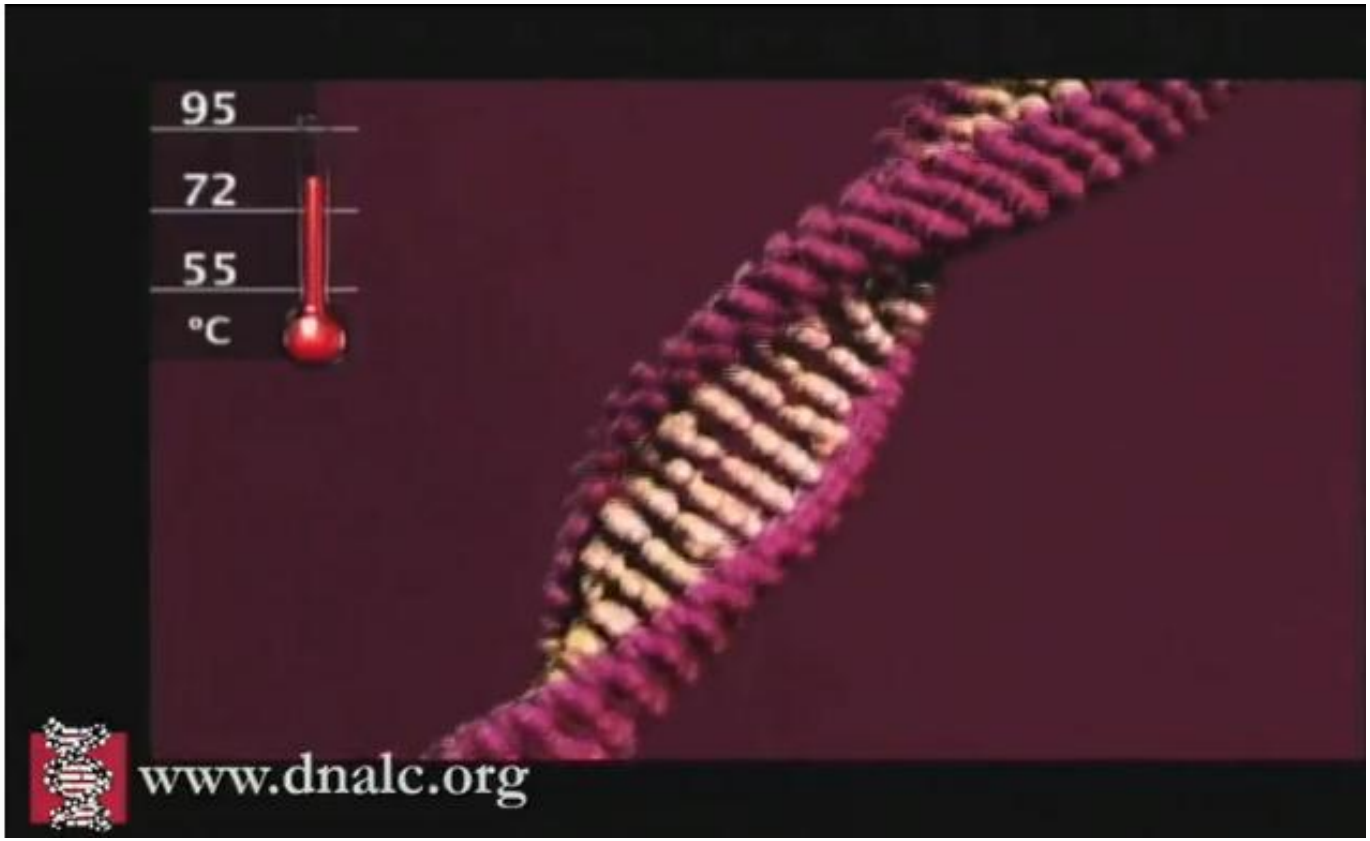
Primer 주문

- Oligo 주문 form

Workflow: DNA Preparation to Applications

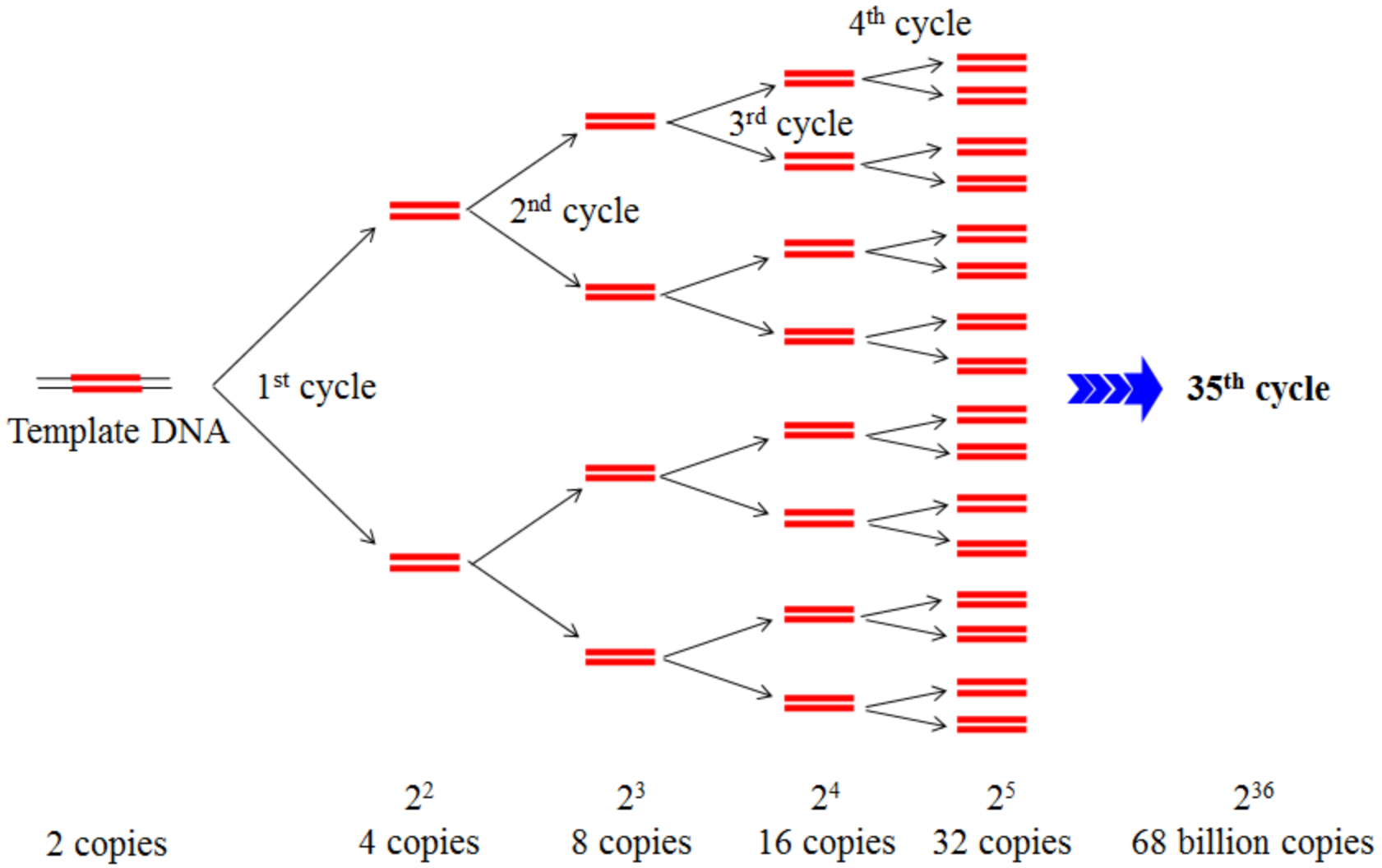


Polymerase Chain Reaction



http://www.youtube.com/watch?v=2KoLnIwoZKU

Exponential Amplification of PCR Product



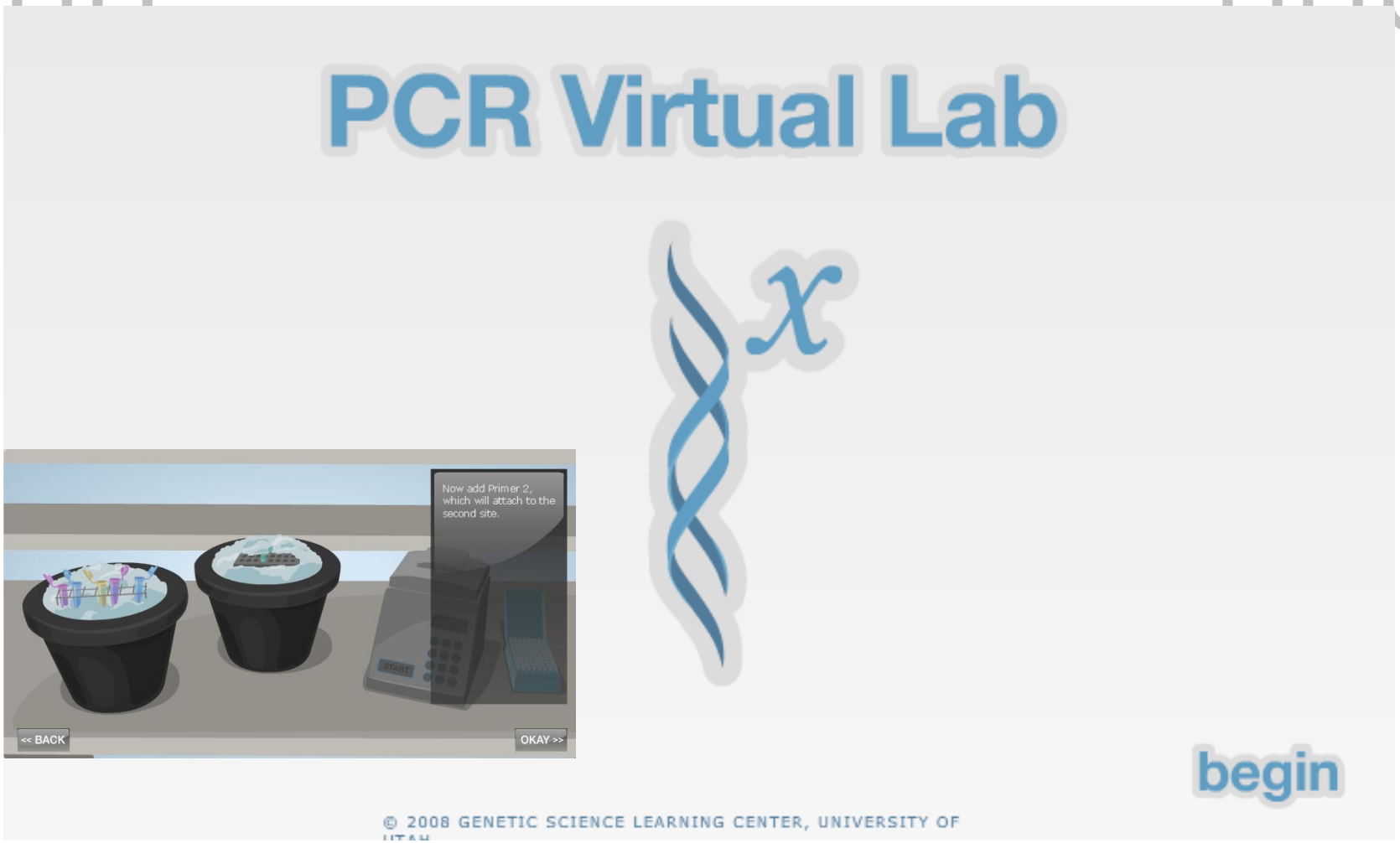
PCR Amplification Protocol

PCR Amplification Protocol

10X PCR buffer containing 15 mM $MgCl_2$	2 ul
dNTP (2.5 mM each)	0.8 ul
Forward PCR primer (2.5 uM)	1.2 ul
Reverse PCR primer (2.5 uM)	1.2 ul
template (genomic DNA; 20 ng/4ul)	**4 ul**
AmpliTaq Gold(5 unit/ul) (1 unit/20 ul Rx)	0.15 ul
dsH2O	10.8 ul
---	
final vol.	20 ul

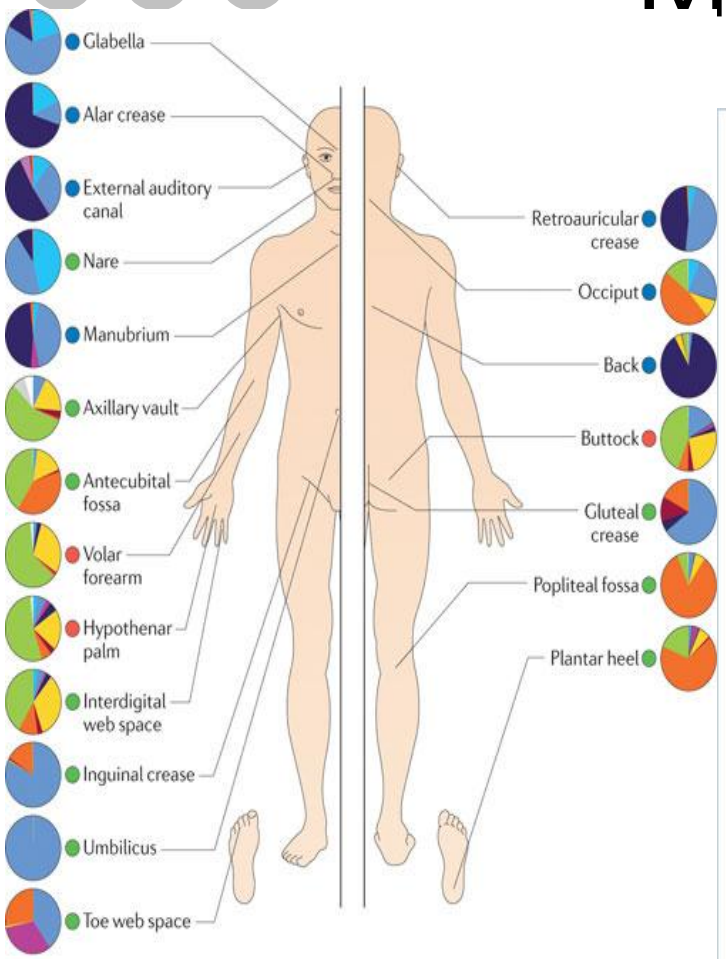
	94 °C --- 5 min	(1 cycle)
denaturation:	94 °C --- 45 sec	
annealing:	55 °C --- 30 sec	(35 cycles)
extension:	72 °C --- 1:30 sec	
	72 °C --- 10 min	(1 cycle)

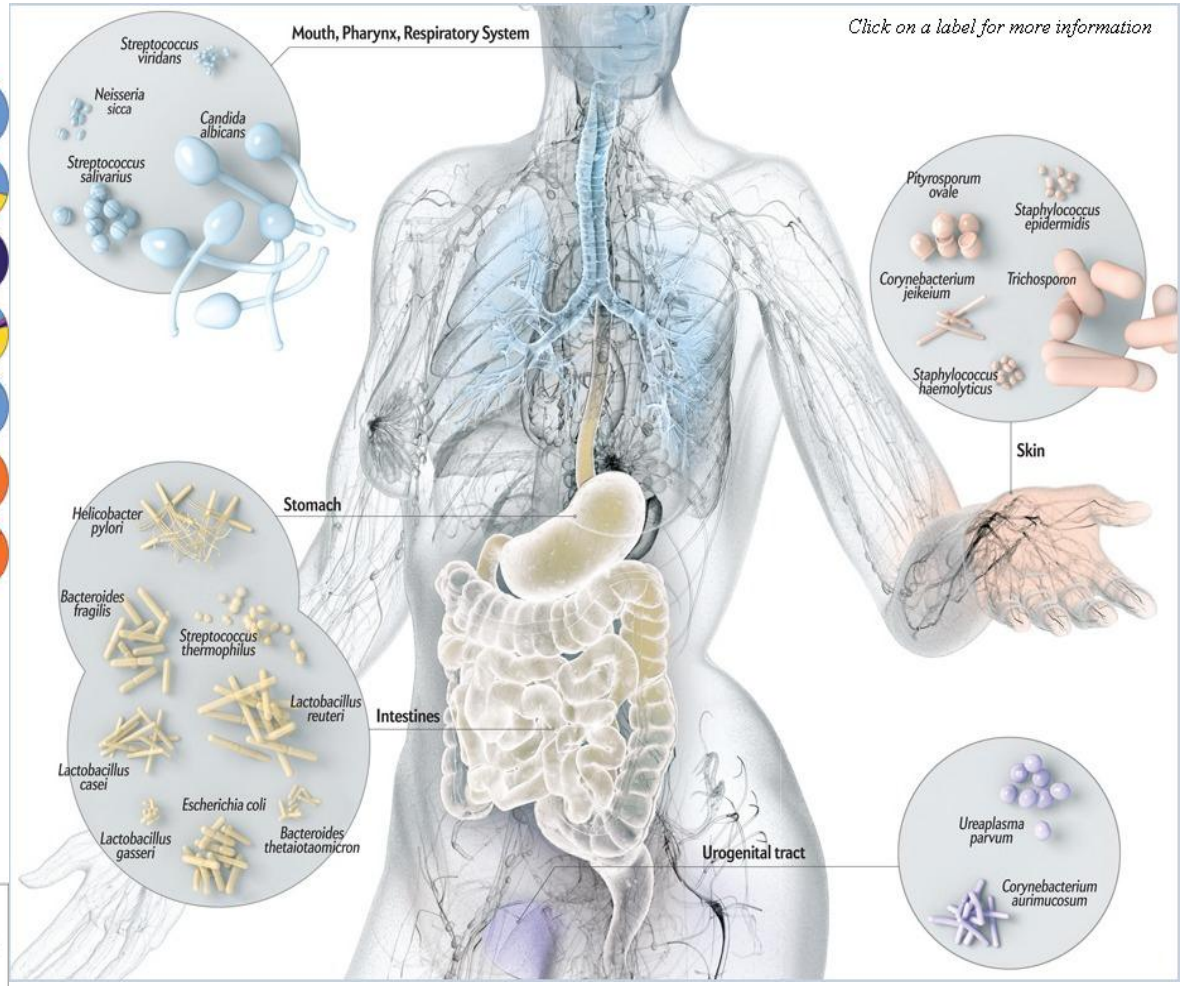

Fisherbrand
Redi-Tip
PCR
PCR



- http://learn.genetics.utah.edu/content/labs/pcr/

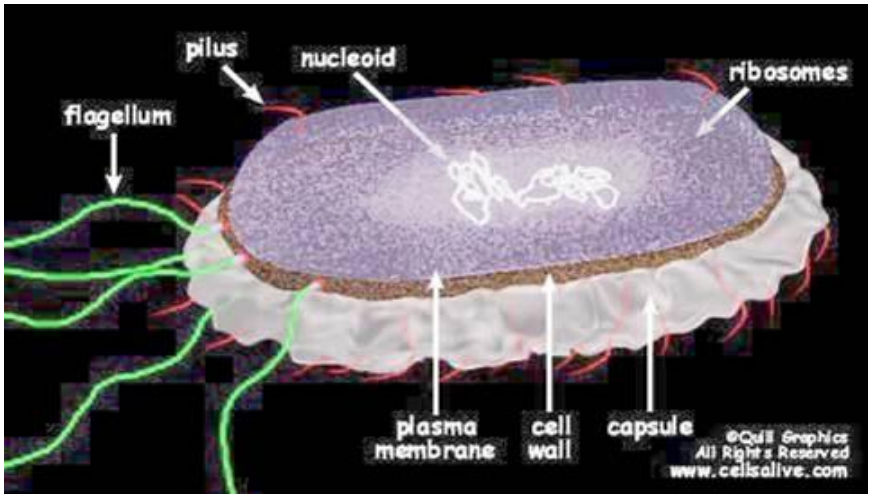
Microbiome

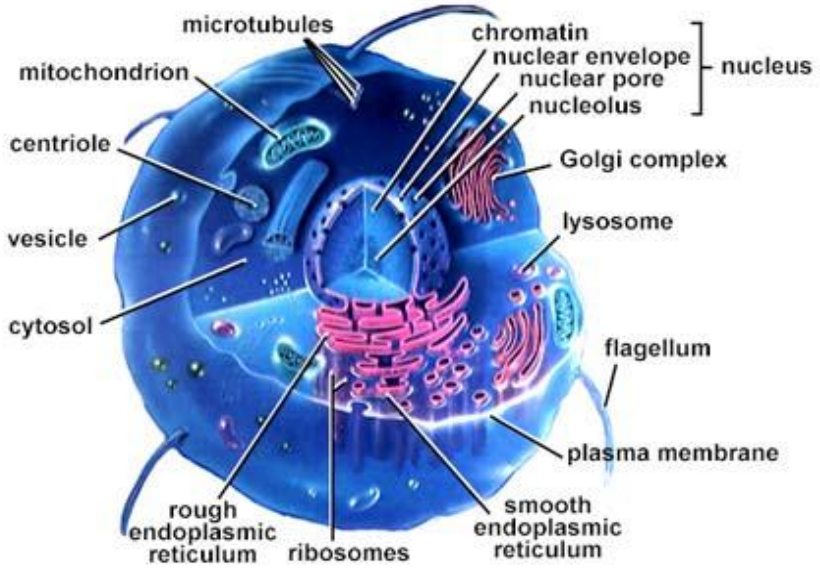

Glabella
Alar crease
External auditory canal
Nare
Manubrium
Axillary vault
Antecubital fossa
Volar forearm
Hypothenar palm
Interdigital web space
Inguinal crease
Umbilicus
Toe web space
Retroauricular crease
Occiput
Back
Buttock
Gluteal crease
Popliteal fossa
Plantar heel
Actinobacteria
Corynebacteriaceae
Propionibacteriaceae
Micrococciaceae
Other Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Other Firmicutes
Staphylococcaceae
Proteobacteria
Divisions contributing <1%
Unclassified
Sebaceous
Moist
Dry
Nature Reviews | Microbiology


Click on a label for more information
Mouth, Pharynx, Respiratory System
Streptococcus viridans
Neisseria sicca
Candida albicans
Streptococcus salivarius
Pityrosporum ovale
Staphylococcus epidermidis
Corynebacterium jeikeium
Trichosporon
Staphylococcus haemolyticus
Skin
Stomach
Helicobacter pylori
Bacteroides fragilis
Streptococcus thermophilus
Lactobacillus reuteri
Intestines
Lactobacillus casei
Escherichia coli
Lactobacillus gasseri
Bacteroides thetaiotaomicron
Urogenital tract
Ureaplasma parvum
Corynebacterium aurimucosum

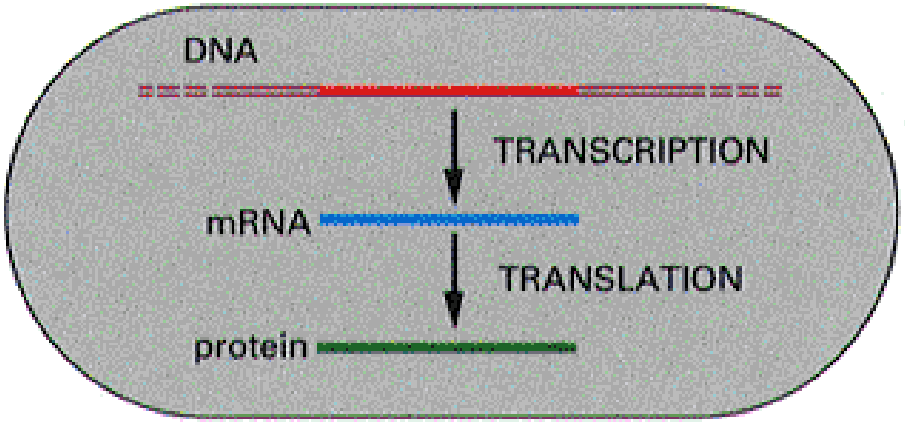
Types of Cells

- Prokaryote 원핵세포
  - cells with no nucleus or org anelles with membranes.
  - Bacteria
- Eukaryote 진핵세포
  - cells that contain a nucleus and organelles surrounded by a membrane.
  - protozoa, algae, fungi, plan ts, and animals, human

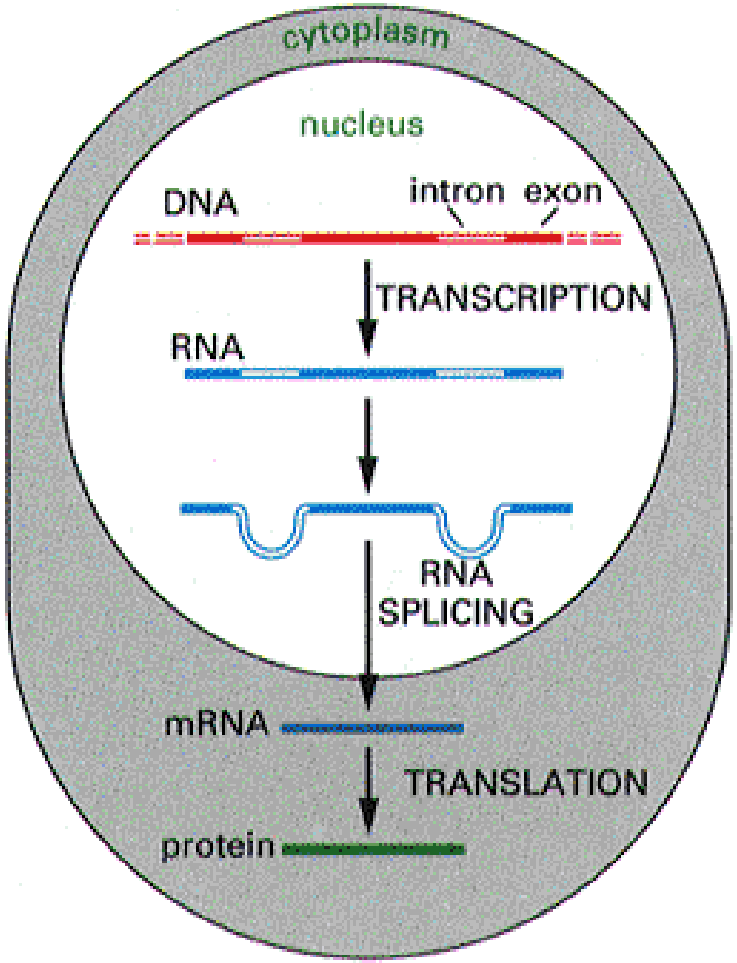


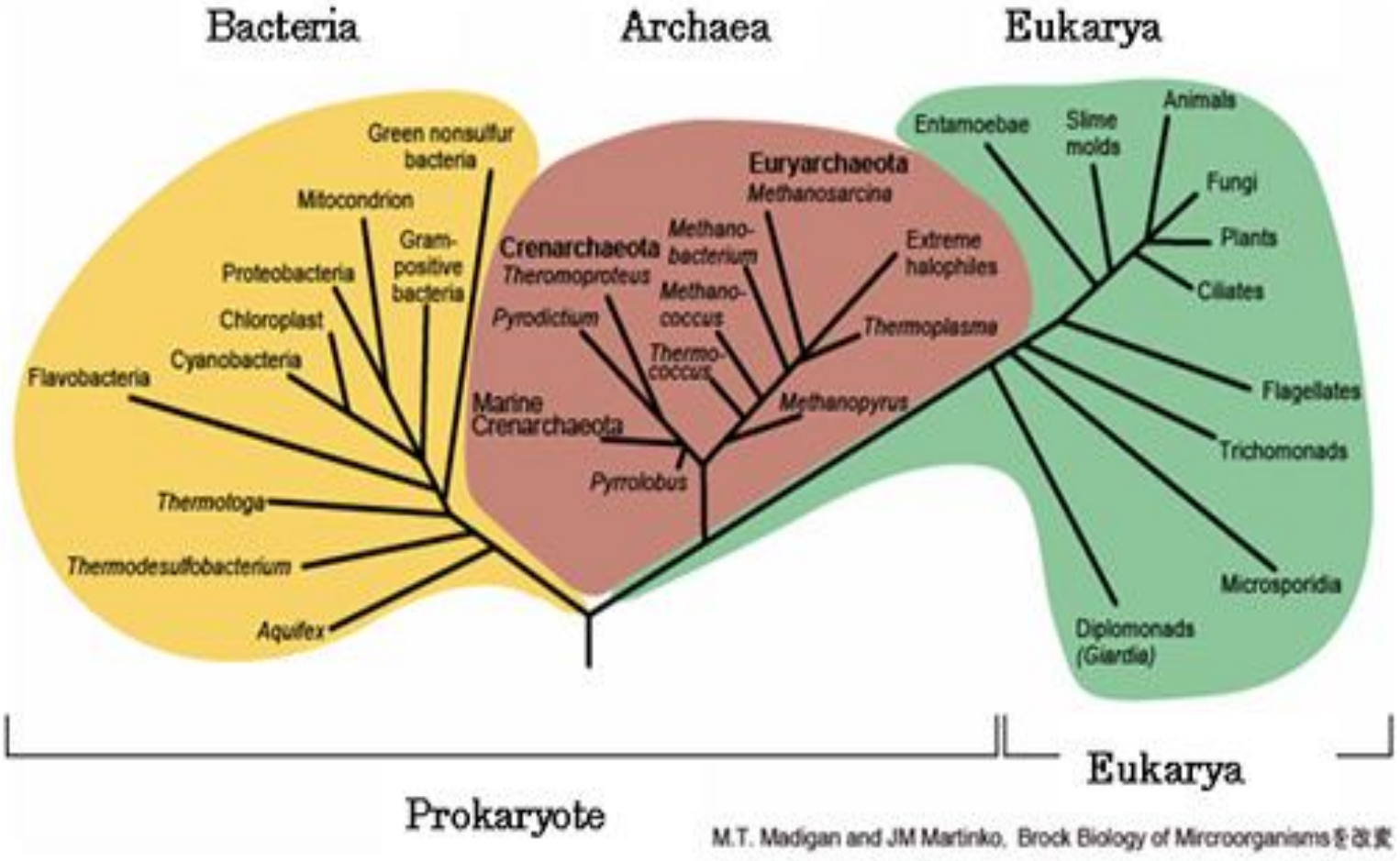


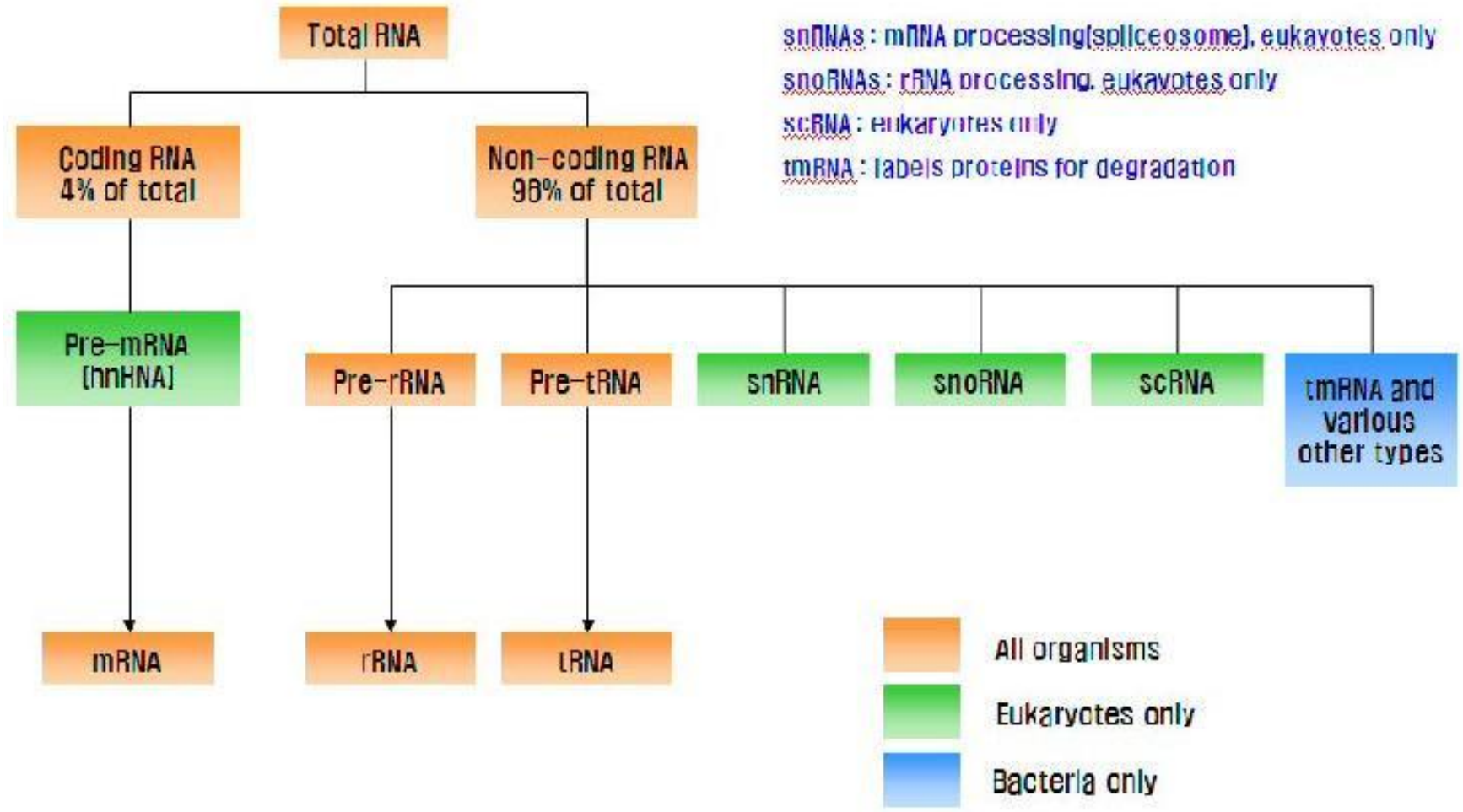
PROCARYOTES

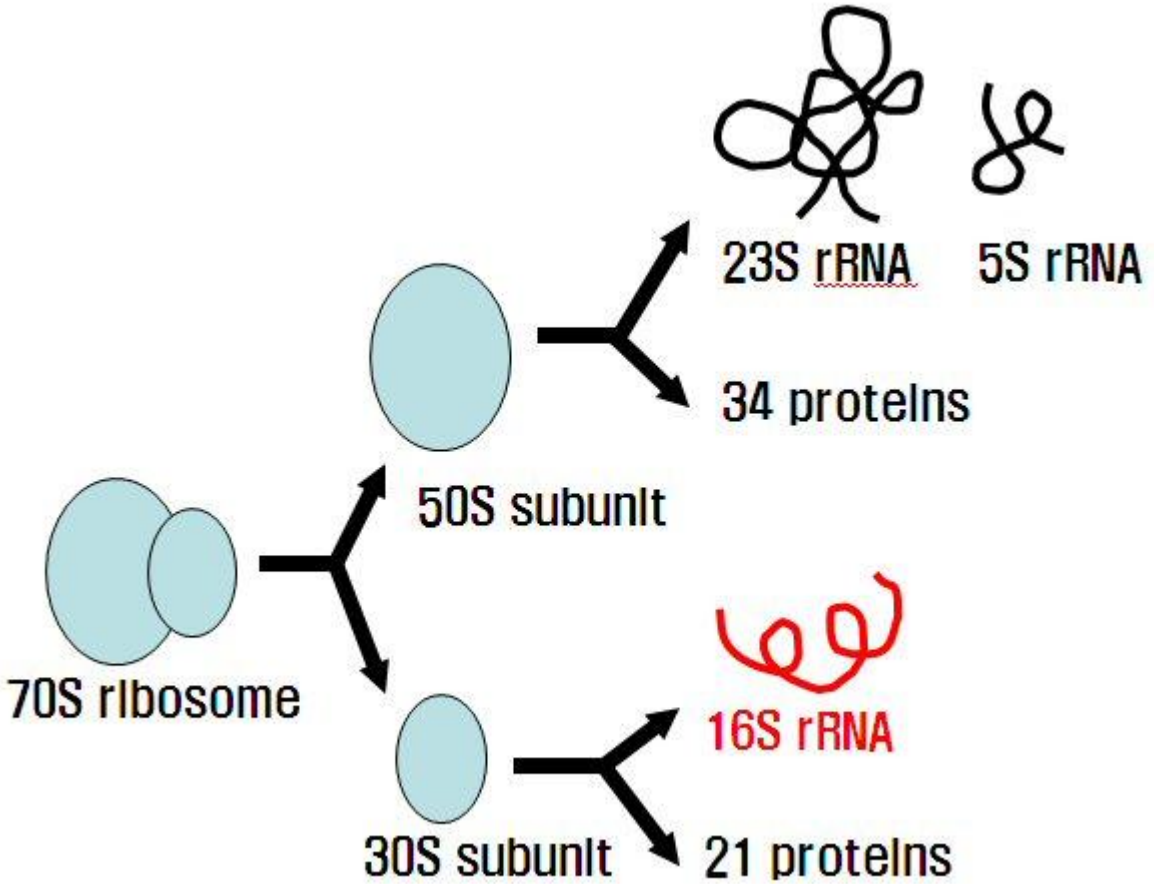


EUCARYOTES




Bacteria
Archaea
Eukarya
Green nonsulfur bacteria
Mitocondrion
Gram-positive bacteria
Proteobacteria
Chloroplast
Cyanobacteria
Flavobacteria
Thermotoga
Thermodesulfobacterium
Aquifex
Euryarchaeota
Methanosarcina
Crenarchaeota
Thermoproteus
Methano-bacterium
Methano-coccus
Thermo-coccus
Pyrodictium
Extreme halophiles
Thermoplasma
Methanopyrus
Marine Crenarchaeota
Pyrrolobus
Entamoebae
Slime molds
Animals
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia
Diplomonads (Giardia)
Eukarya
Prokaryote
M.T. Madigan and JM Martinko, Brock Biology of Mircroorganisms 修改

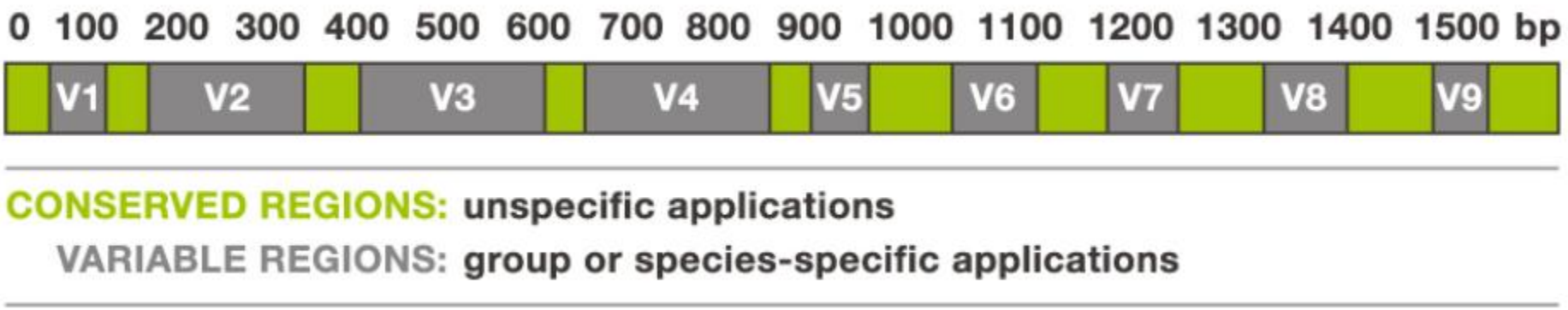

Total RNA
Coding RNA
4% of total
Non-coding RNA
96% of total
Pre-mRNA
(hnRNA)
Pre-rRNA
Pre-tRNA
snRNA
snoRNA
scRNA
tmRNA and
various
other types
mRNA
rRNA
tRNA
snRNAs : mRNA processing(spliceosome), eukayotes only
snoRNAs : rRNA processing, eukayotes only
scRNA : eukaryotes only
tmRNA : labels proteins for degradation
All organisms
Eukaryotes only
Bacteria only


23S rRNA
5S rRNA
34 proteins
50S subunit
70S ribosome
16S rRNA
30S subunit
21 proteins

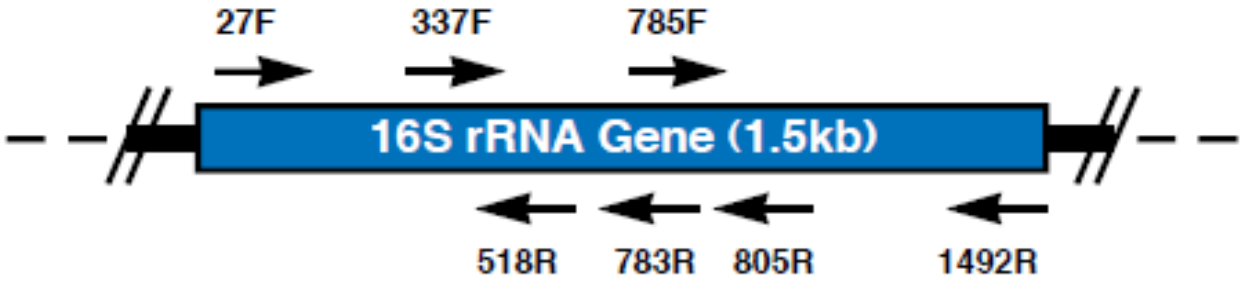
transcription ⇨

nucleotides	1542	76	2904	120

- 16S rRNA gene
  - illustrating the conserved (green) and variable



Bacteria, 유산균… 등

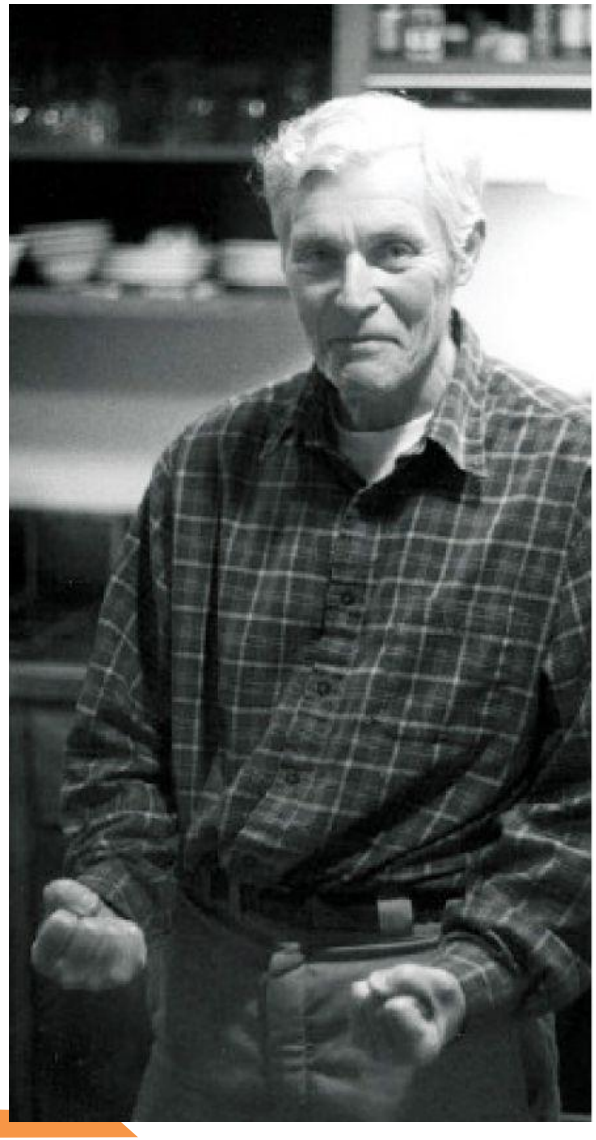


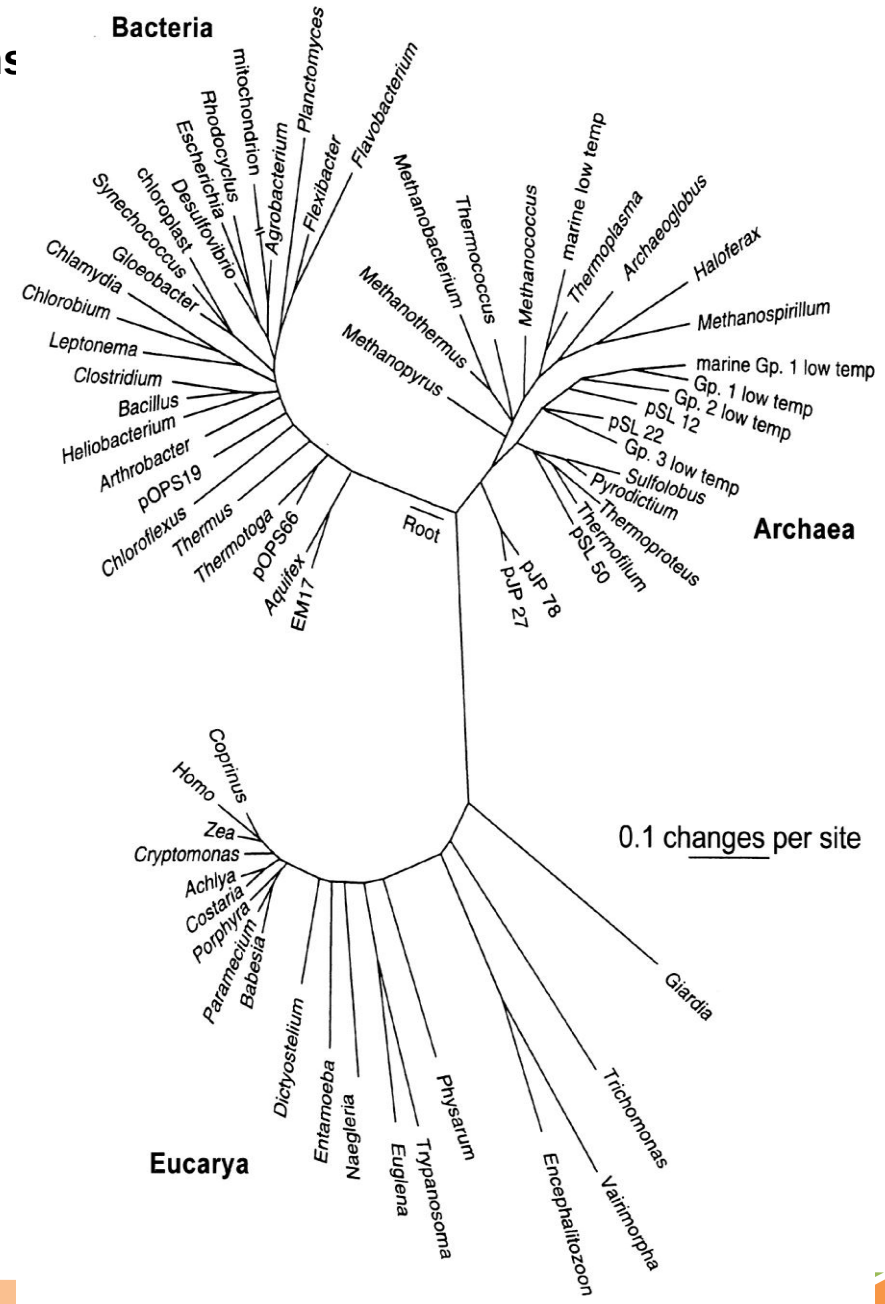
16S-27F : 5’ - AGAGTTTGATCCTGGCTCAG-3’
16S-1492R : 5’ - GGTTACCTTGTTACGACTT-3’

Fungi, Yeast 동정… 등

ITS1 : 5’ - TCCGTAGGTGAACCTGCGG - 3’
ITS4 : 5’ - TCCTCCGCTTATTGATATGC - 3’

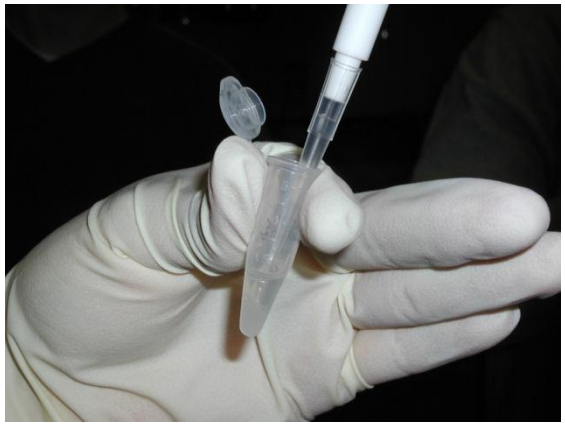
- **Universal phylogenetic tree based on the 16S rRNA gene sequence comparisons**





칼 우즈 - 1977년 16S 리보솜 RNA의 계통 분류를 통해 새로운 분류군인 고세균을 처음 정의

Jill E. Clarridge III Clin. Microbiol. Rev. 2004;17:840-862

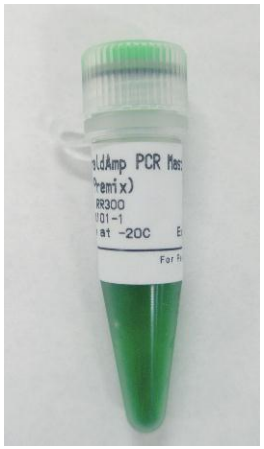


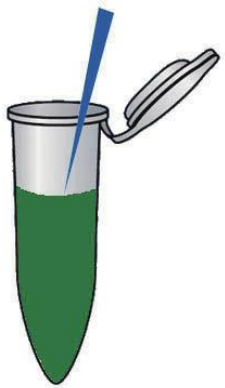
- *E. coli* (*Escherichia coli* DH5a)colony
- White tip을 이용 colony picking 하여
- 10ul의 autoclaved DDW에 희석
- Template로 1ul 사용

Colony PCR

- **Sample prep**
  - Autoclaved DDW 10ul에 picking 한 현탁한 다음 1ul를 template로 사용

- **EmeraldAmp GT PCR Master Mix**
  - Taq polymerase, dNTP, loading dye가 포함되어 있는 mixture 제품.
  - Template, primer 만 더 넣어주면 됨.

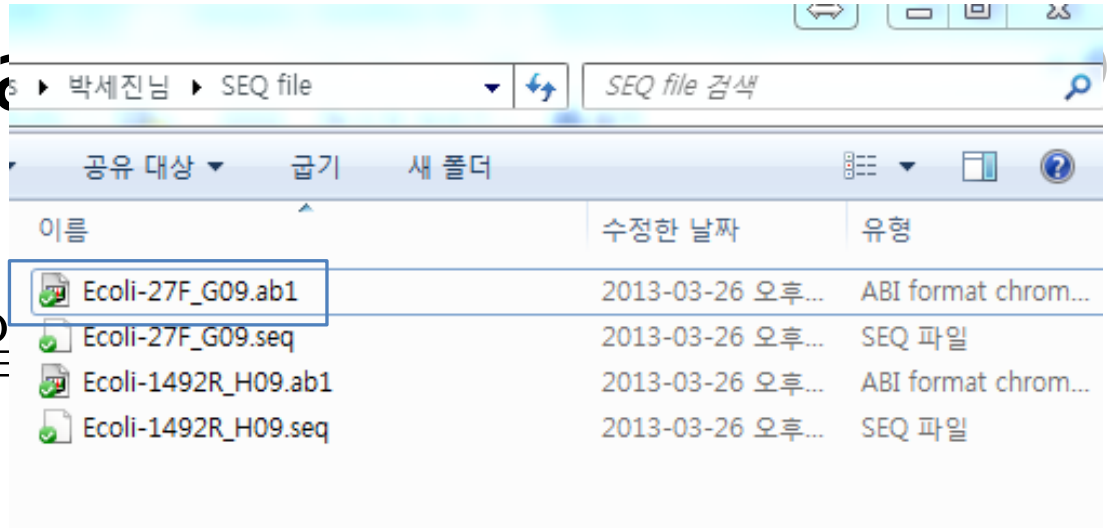


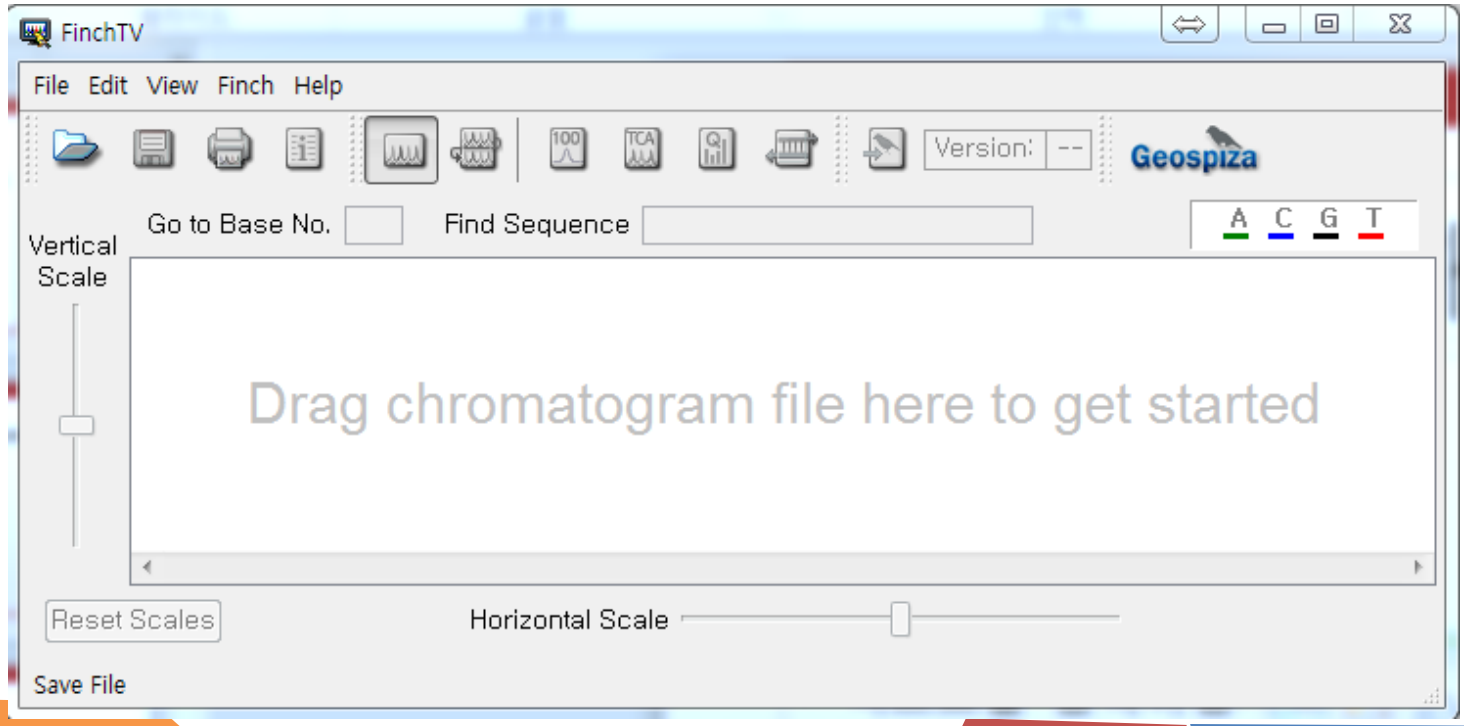



PCR product sequencing 후

- ab1, seq 파일 분석 - FinchTV, BIOEDIT
- Alignment
- Blast Search
- Phylogenetic Tree

- 확장자 ab1 파일
- ABI format






FinchTV - Ecoli-27F_G09.ab1
File Edit View Finch Help
Version:
Geospiza
Go to Base No.
Find Sequence
A C G T
Vertical Scale
Reset Scales
Horizontal Scale

- TGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGT
  GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
  ACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTG
  CCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTG
  GTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
  CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATG
  AAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATAC
  CTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
  GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGG
  TTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCA
  AGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGAT
  CTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGA
  AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
  CTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTG
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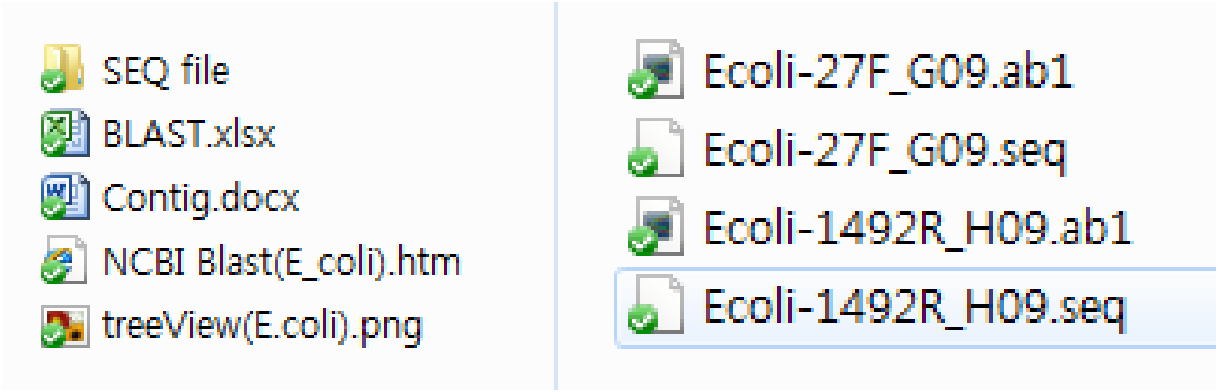
  GTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
  GTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAG
  GTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATG
  GCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAG
  TCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCA
  GAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGT
  GGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTACCAC

BLAST

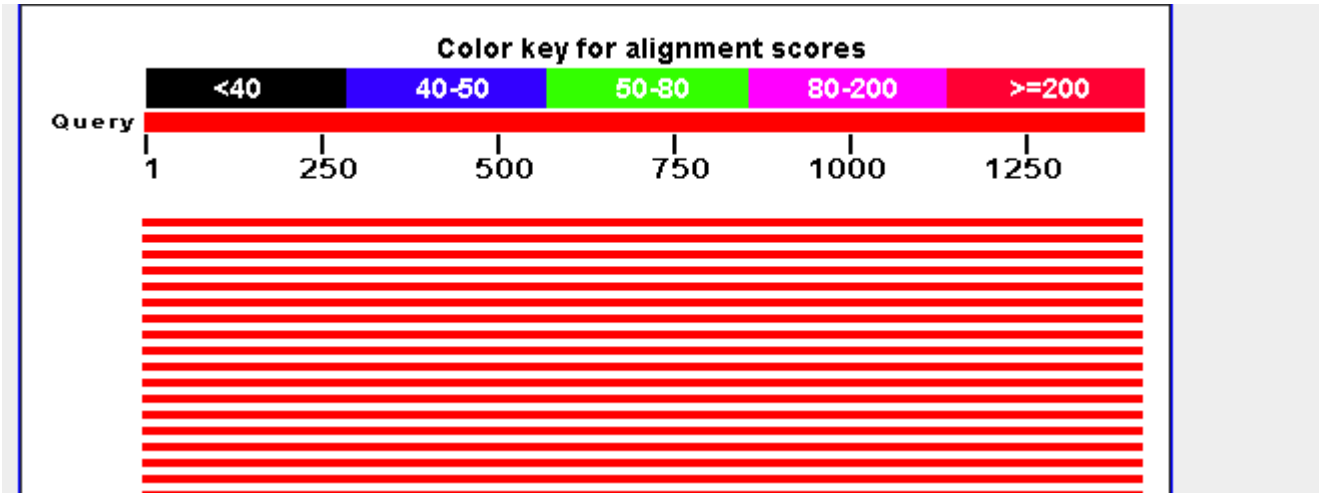
http://www.ncbi.nlm.nih.gov/blast

NCBI/ BLAST/ **blastn**

http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome



- Seq 파일, 또는
  Contig.docx 파일의 염기서열을 이용하여 상동성 분석



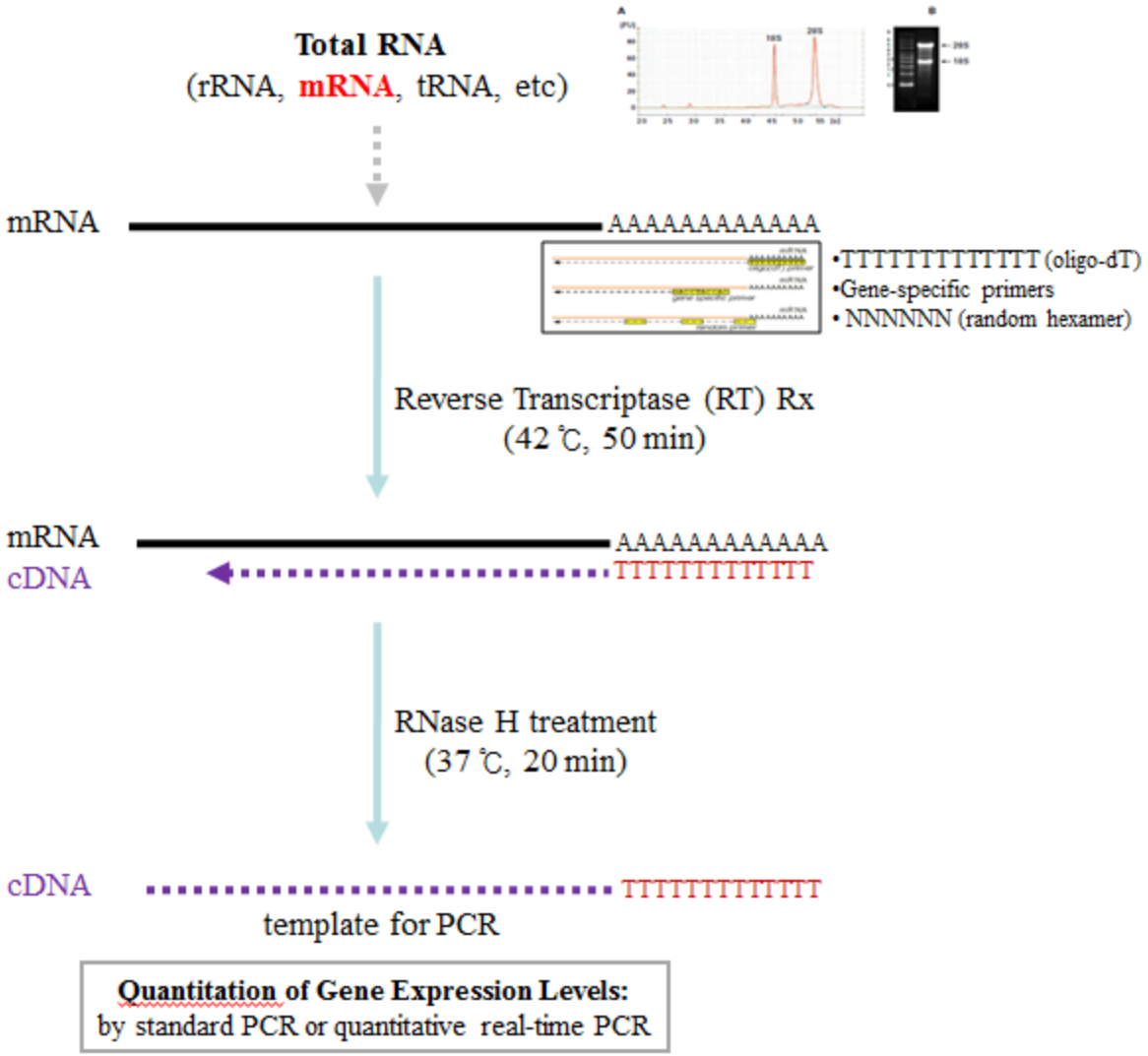
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Escherichia coli gene for 16S rRNA, partial sequence, strain: JCM 24009	2604	2604	100%	0.0	99%	AB548582.1
☐	Escherichia coli strain LW1655F+ 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	99%	AY616658.1
☐	Escherichia coli strain HM01 16S ribosomal RNA gene, partial sequence	2603	2603	100%	0.0	99%	JN811622.1
☐	Escherichia coli strain RW-29 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	99%	DQ182324.1
☐	Escherichia coli strain 2 16S ribosomal RNA gene, partial sequence	2601	2601	100%	0.0	99%	JQ907528.1
☐	Escherichia coli strain H1 16S ribosomal RNA gene, partial sequence	2601	2601	100%	0.0	99%	FJ949577.1
☐	Escherichia coli PMV-1 main chromosome, complete genome	2599	18140	100%	0.0	99%	HG428755.1
☐	Escherichia coli LY180, complete genome	2599	18051	100%	0.0	99%	CP006584.1

http://www.mbio.ncsu.edu/BioEdit/bioedit.html

- Contig align
- BlastN search
- Genetic Tree

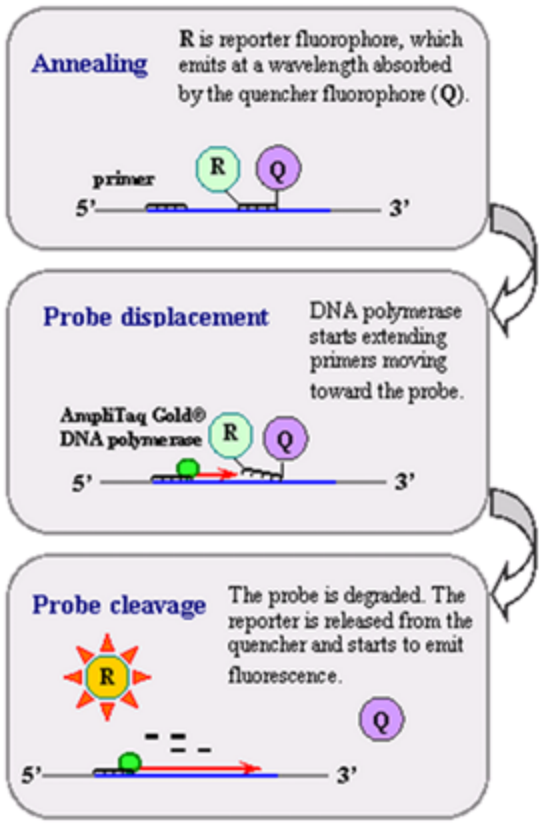
Reverse Transcriptase (RT) PCR 기술

Workflow of RT-PCR: RT Reaction for cDNA Synthesis from Total RNA

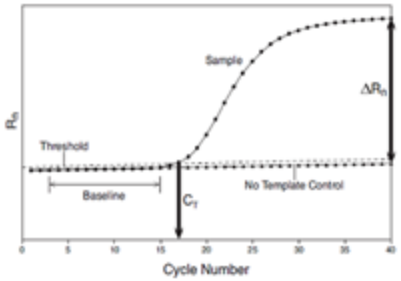


Real-time or Quantitative PCR (qPCR): Measuring Gene Expression Levels

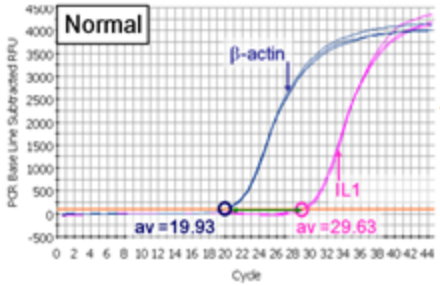
(A) TaqMan Assay



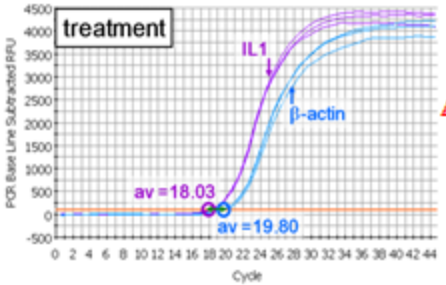
(B) Relative Quantitation: Ct-ΔCt-ΔΔCt



Ct =the cycle No. at which the fluorescence signal crosses the threshold.



ΔCt_0 = target − ref
= 9.70



ΔCt_1 = target − ref
= -1.7

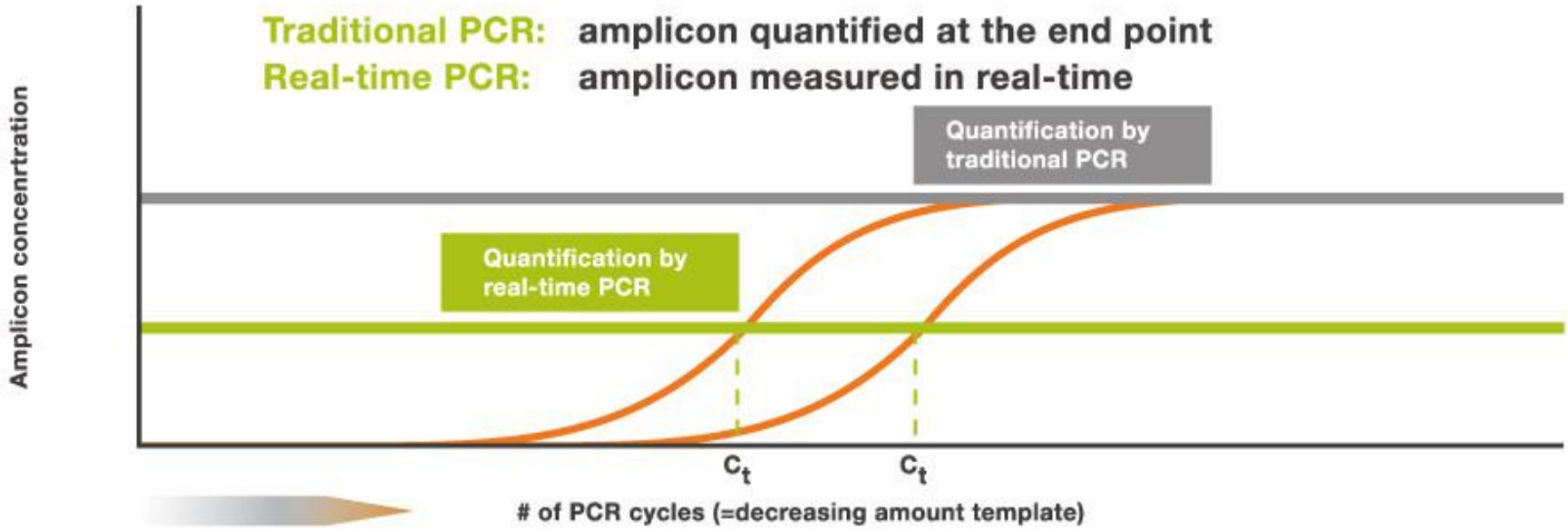
Difference (**ΔΔCt**)
= $\Delta Ct_1 - \Delta Ct_0$
= 9.70-(-1.7) = 11.40

RQ (relative quantitation)
=fold change = $2^{-\Delta\Delta Ct}$
= $2^{11.40}$ = 2702

- Ct is inversely correlated with starting copies (ie greater C_T = less starting copies).
- C_T depends on where the threshold is set.
- Same threshold must be set across samples for meaningful data comparison.

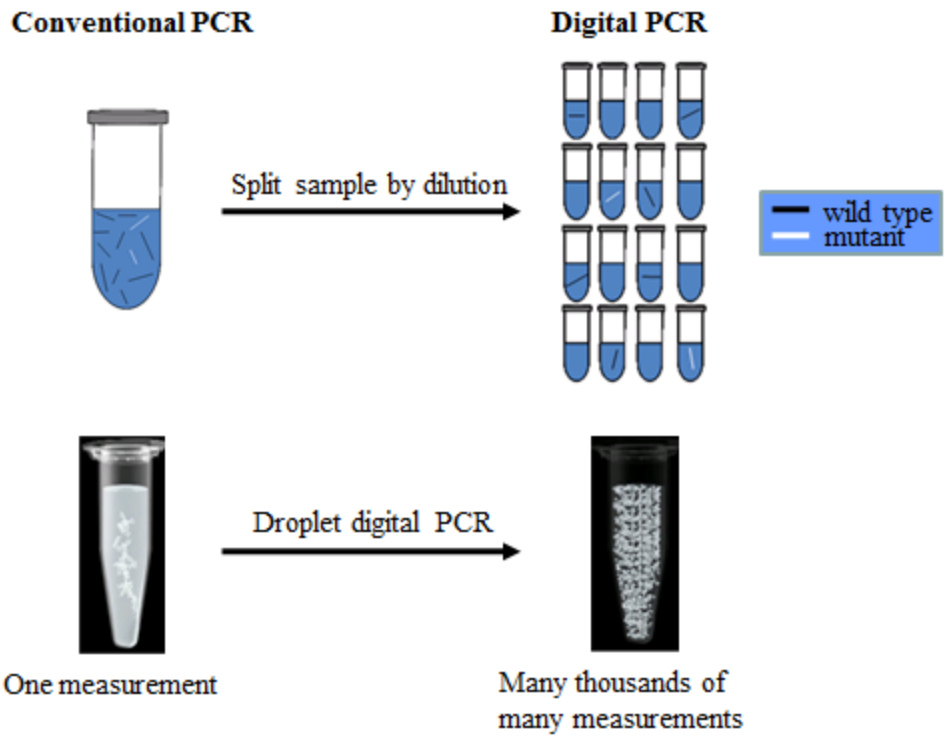
PCR vs Real-time PCR

- Quantitative real-time PCR



Principle of Digital PCR

Digital PCR (dPCR) is a refinement of conventional PCR methods that can be used to directly quantify and clonally amplify nucleic acids (including DNA, cDNA, methylated DNA, or RNA). The key difference between dPCR and traditional PCR lies in the method of measuring nucleic acids amounts, with the former being a more precise method than PCR. PCR carries out one reaction per single sample. dPCR also carries out a single reaction within a sample, however the sample is separated into a large number of partitions and the reaction is carried out in each partition individually. This separation allows a more reliable collection and sensitive measurement of nucleic acid amounts. The method has been demonstrated as useful for studying variations in gene sequences - such as copy number variants, point mutations, and it is routinely used for clonal amplification of samples for "next-generation sequencing."



- **RainDance (1,000,000 droplets)**
- **BioRad (20,000 droplets)**
- **Life Tech (20,000 chambers)**
- **Fluidigm**

Applications of Digital PCR

- **Copy number variation (CNV)** —1.2-fold (±10%) differences in gene copy numbers

- **Rare sequence detection** — as low as 1 in 100,000 (for example, a few tumor cells in a wild-type background)

- **Gene expression and miRNA analysis** — absolute quantification of expression levels, especially low-abundance miRNAs.

- **Single cell analysis** — the high degree (10–100-fold) of cell-cell variation in gene expression levels among homogeneous single-cell populations and stem cells drives the need for the analysis of single cells; digital PCR enables low copy number target quantification

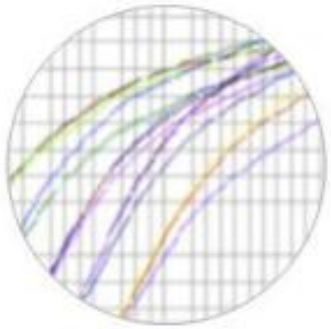
- **Pathogen detection** —detection and quantification of pathogens in samples quickly and precisely in very small amounts in biological samples.

- **Next-generation sequencing (NGS)** — quantification of NGS sample preparations to increase sequencing accuracy and reduce run repeats, and validation of sequencing results with absolute quantification

1st

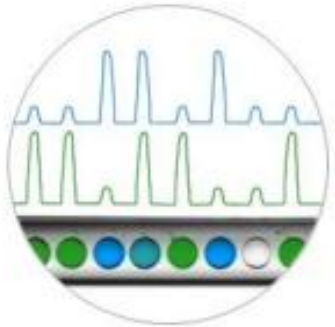
PCR
Qualitative

2nd



Real-time PCR
Relative Quantification

3rd



Digital PCR
Absolute Quantification