พันธุวิศวกรรมและการโคลนยีน
DNA fingerprinting
Gel electrophoresis
DNA sequencing

มรรษภูมิ เดชเจริญ
matsapume.d@psu.ac.th
หลักสูตรชีววิทยา

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Polymerase Chain Reaction (PCR)

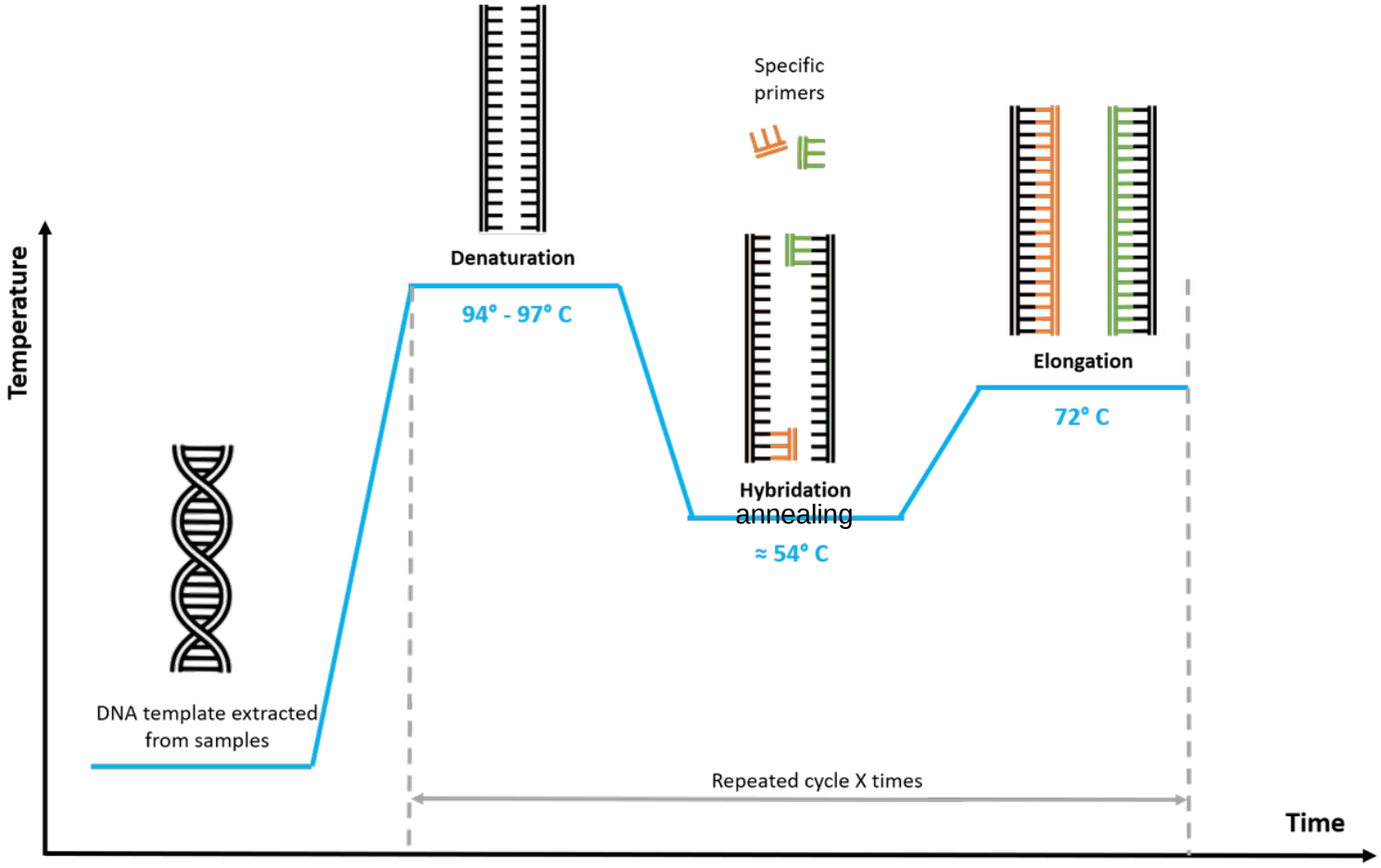
- Template DNA
- DNA polymerase
- Primers (forward and reverse)
- Deoxynucleoside triphosphates (dNTPs: dATP, dCTP, dGTP, and dTTP)
- Required cofactor for activity of DNA polymerases: Mg^{2+}
- Buffer: suitable chemical environment for activity of DNA polymerase

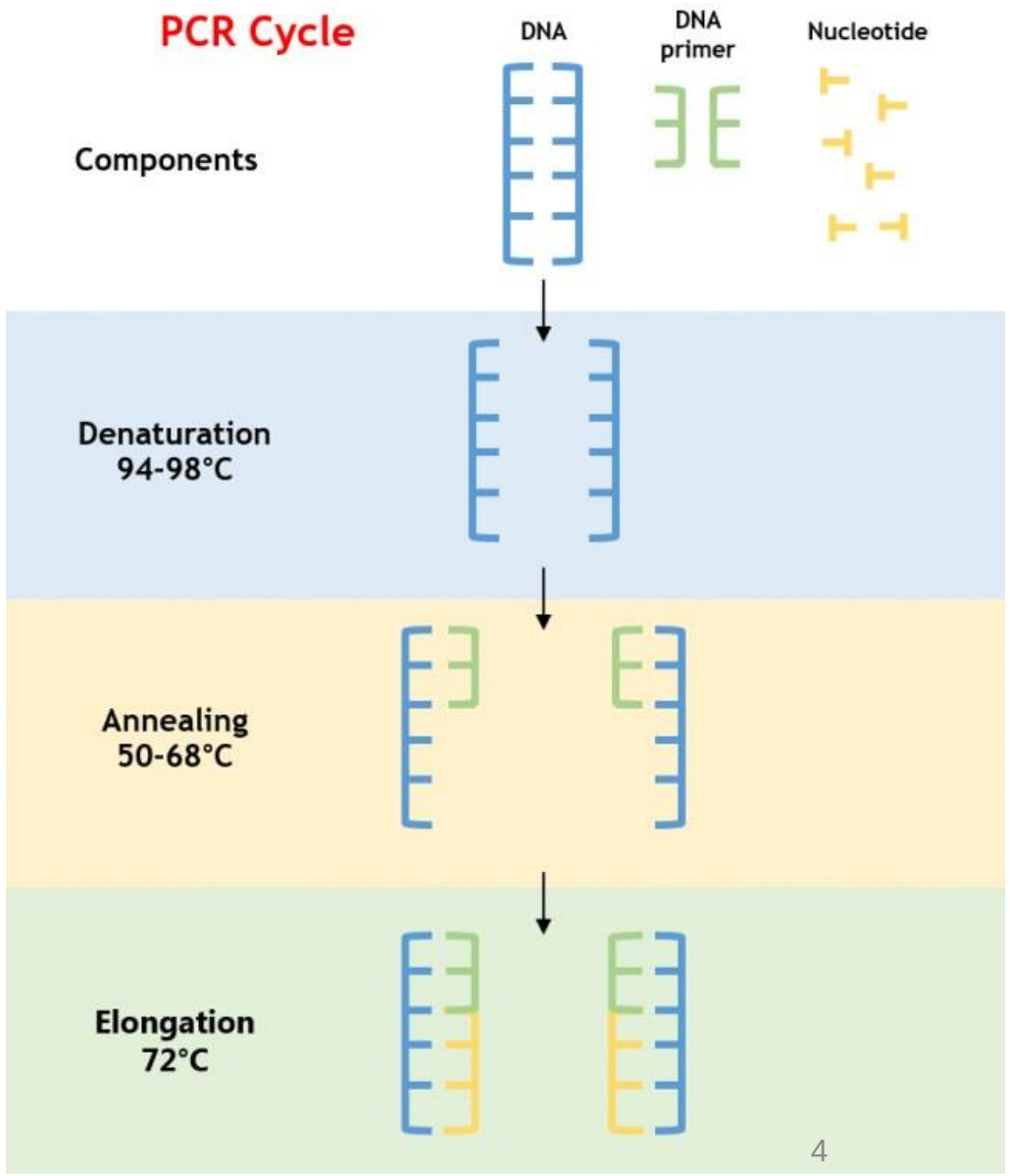
Polymerase Chain Reaction (PCR)

PCR thermocycler

Polymerase Chain Reaction (PCR)

Steps





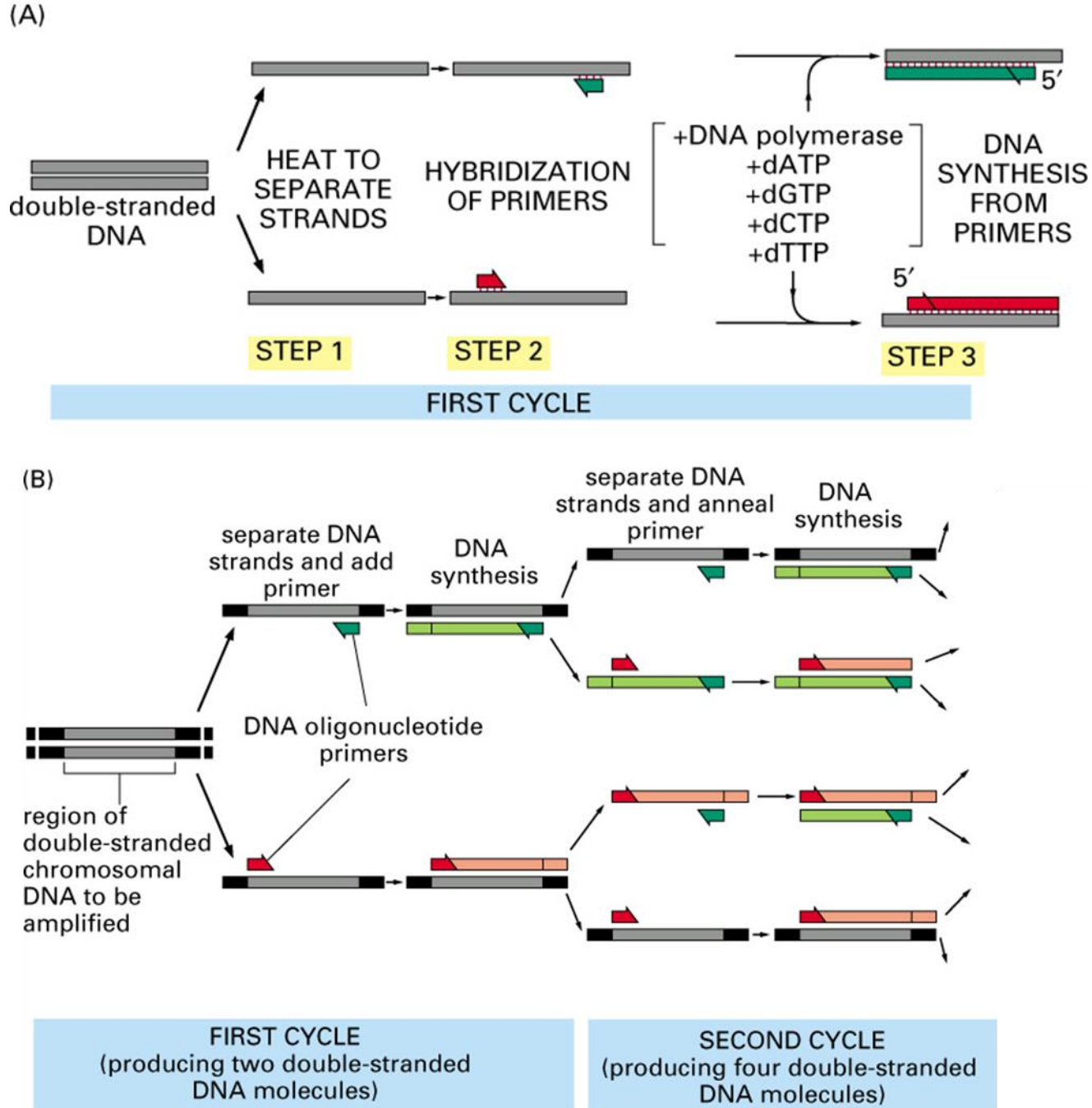
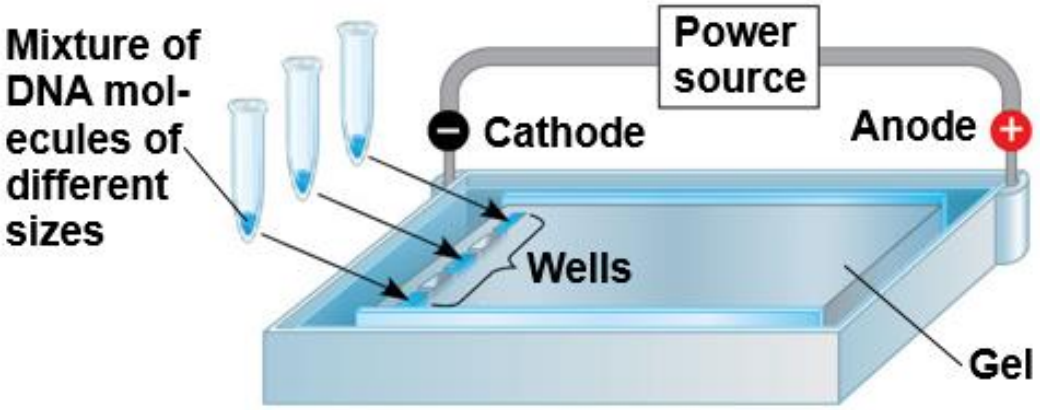
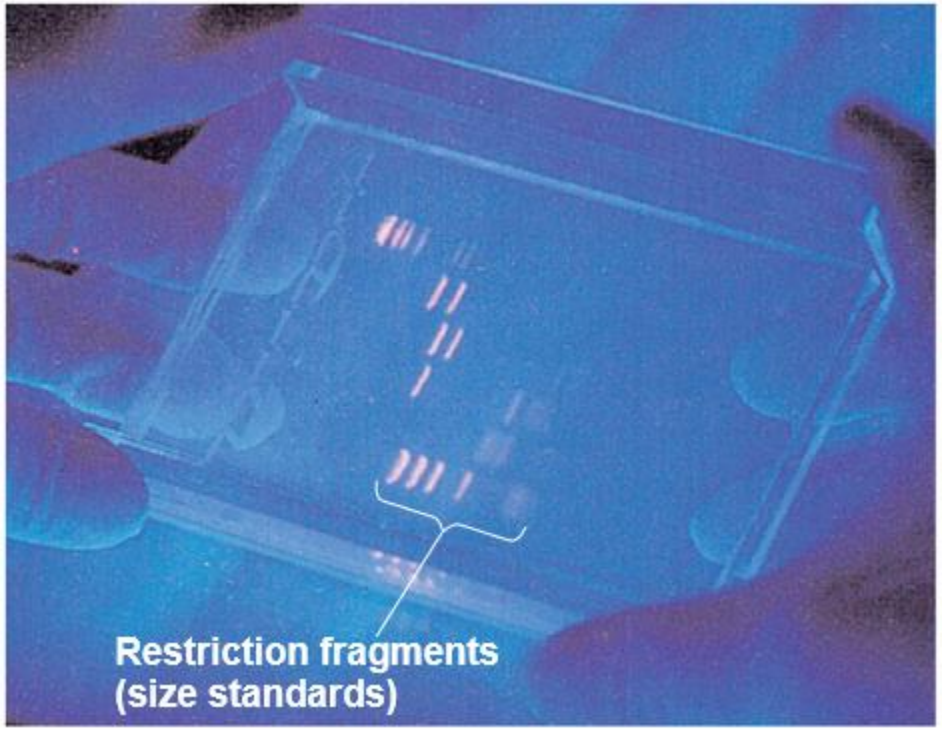


Figure 8–39 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

Gel Electrophoresis

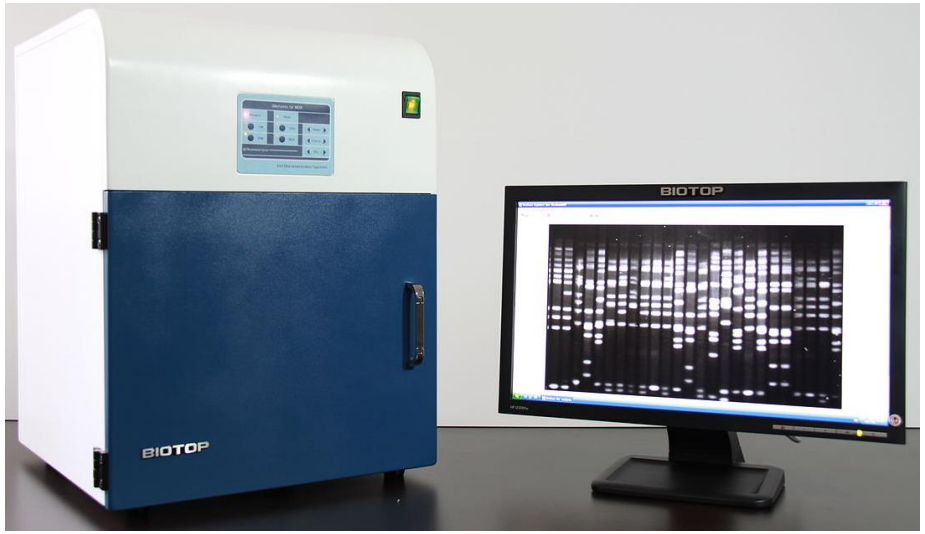


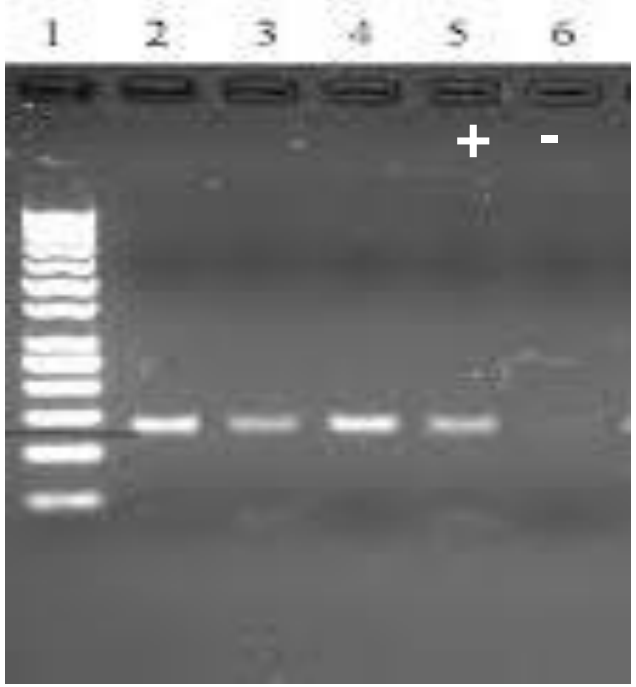
a) Negatively charged DNA molecules will move toward the positive electrode.



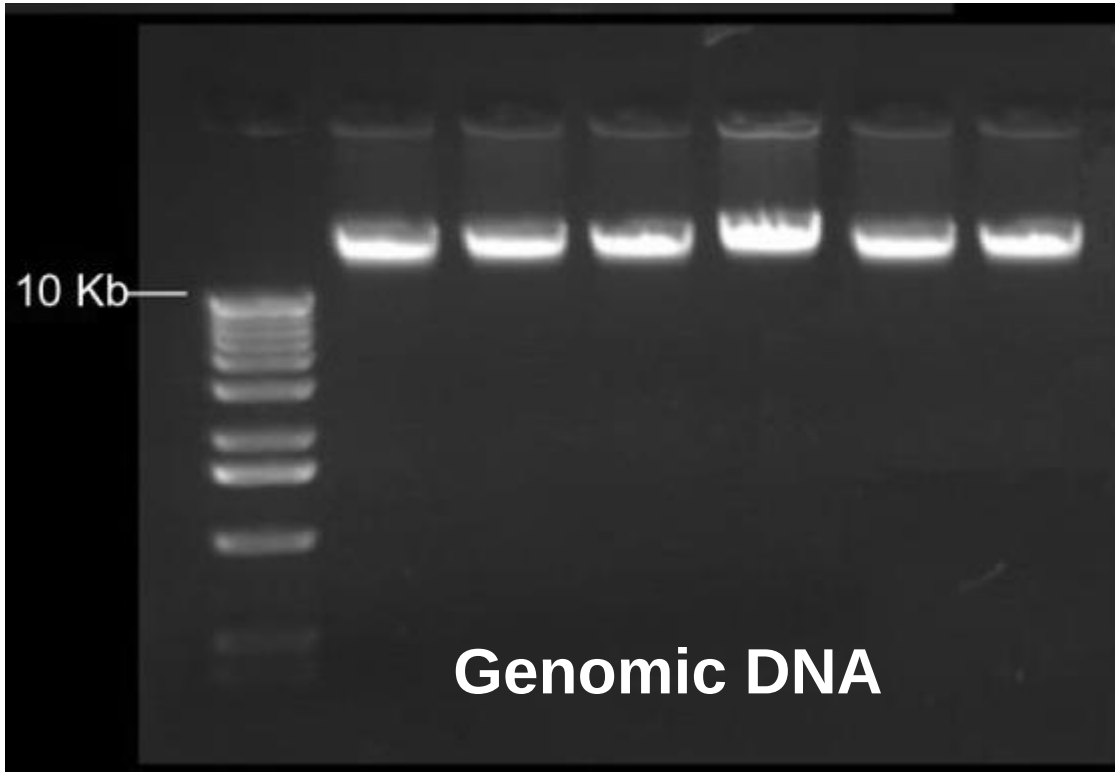
b) Shorter molecules are slowed down less than longer ones, so they move faster through the gel.

Gel documentation system

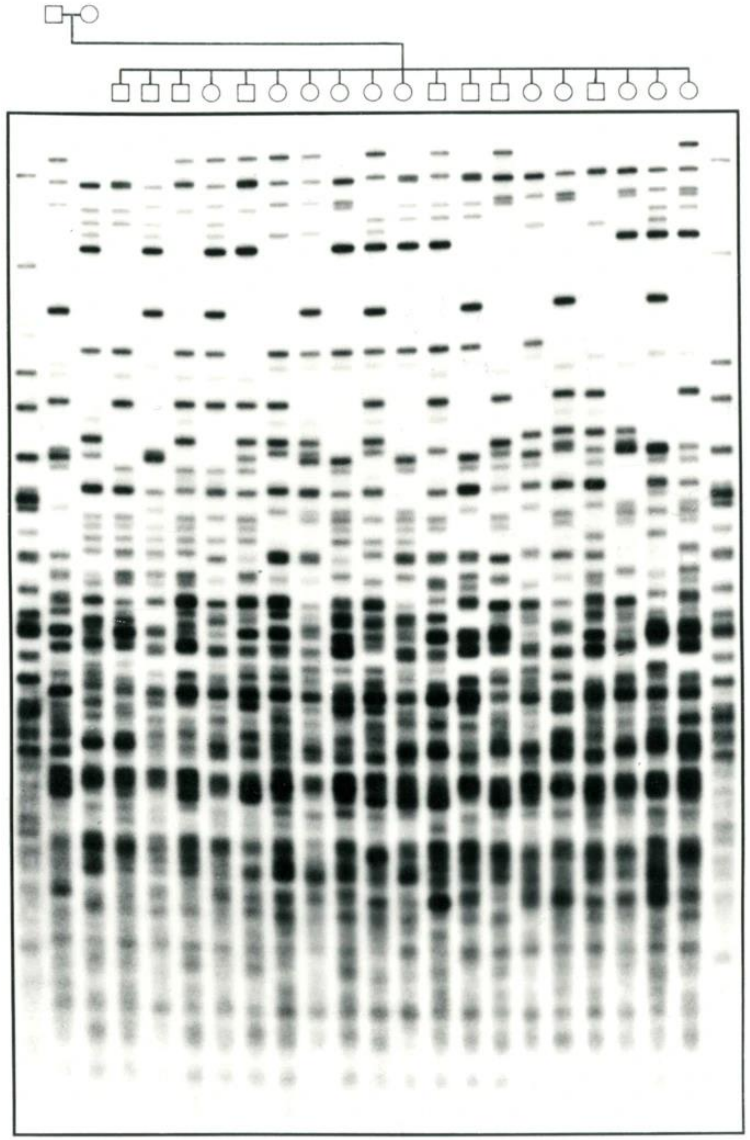


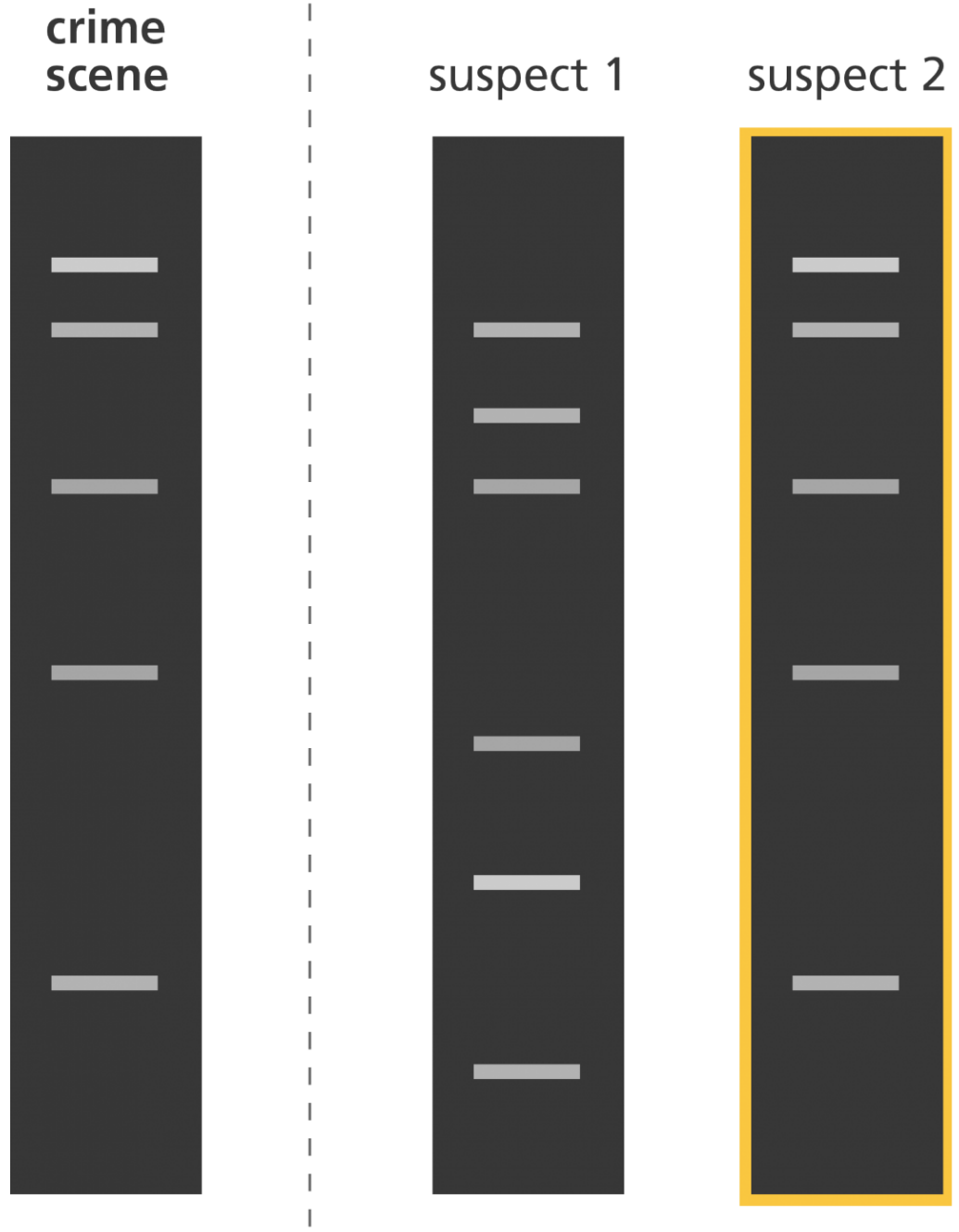


PCR product: Amplicon

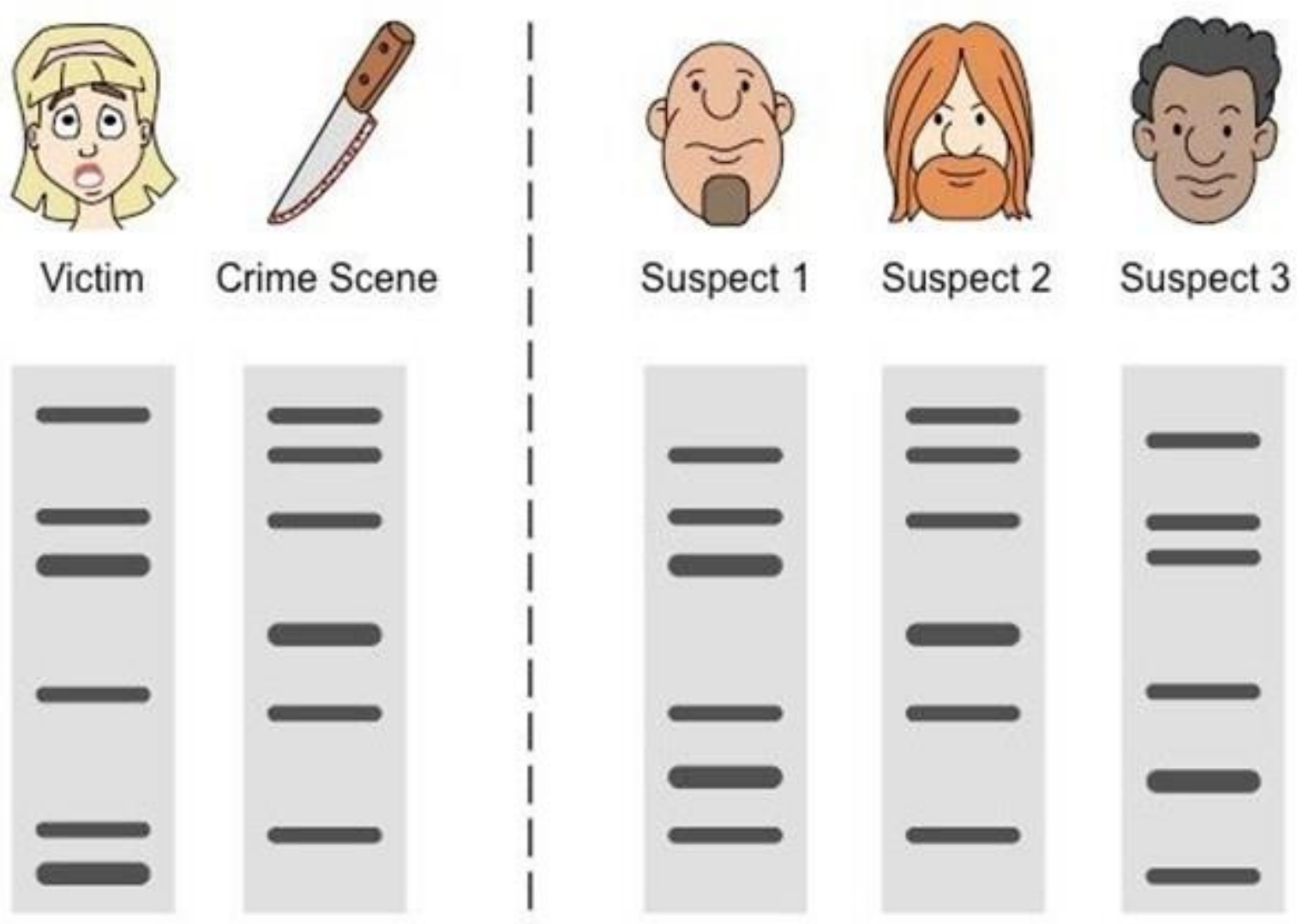


DNA fingerprinting





DNA fingerprinting

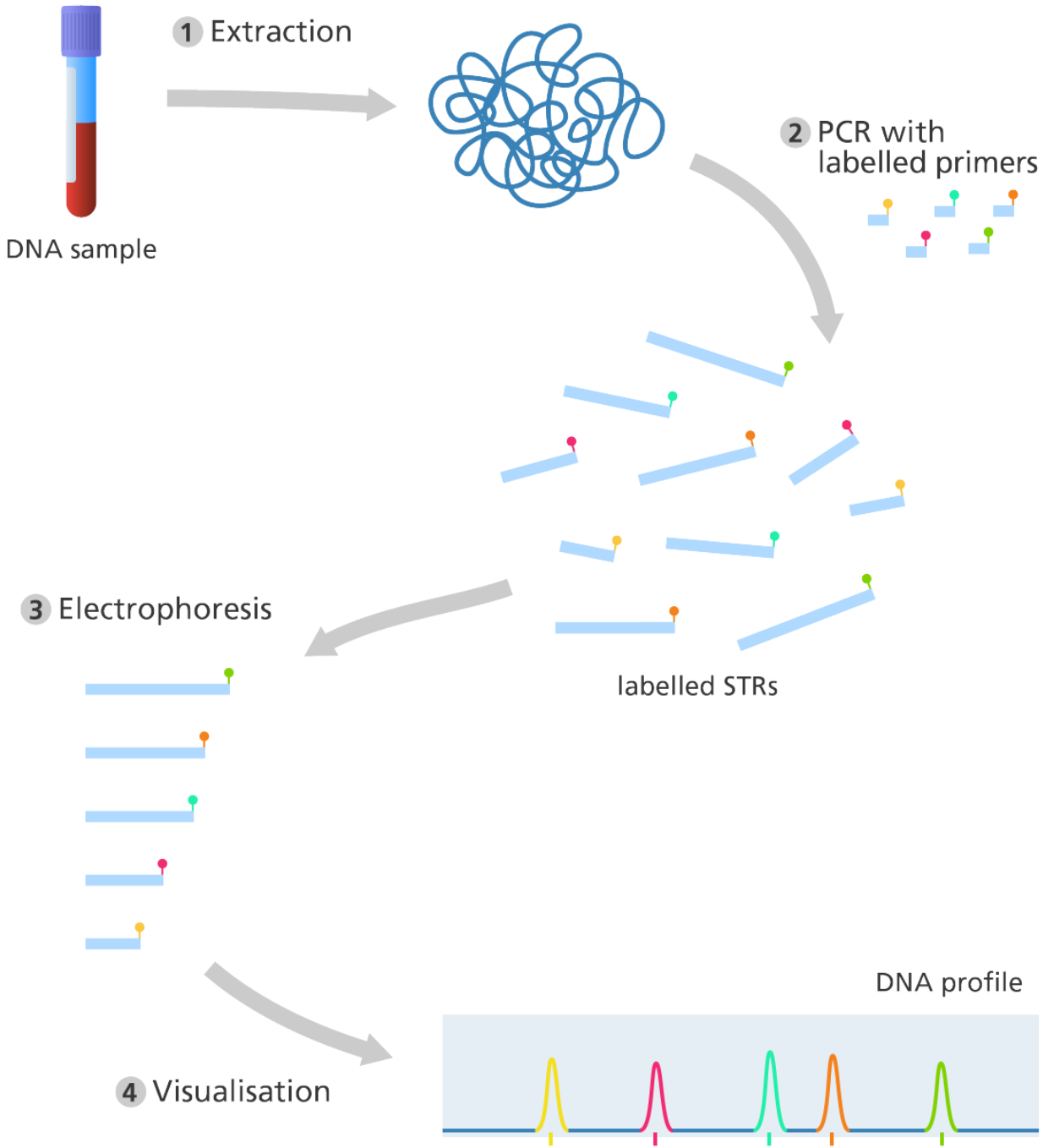


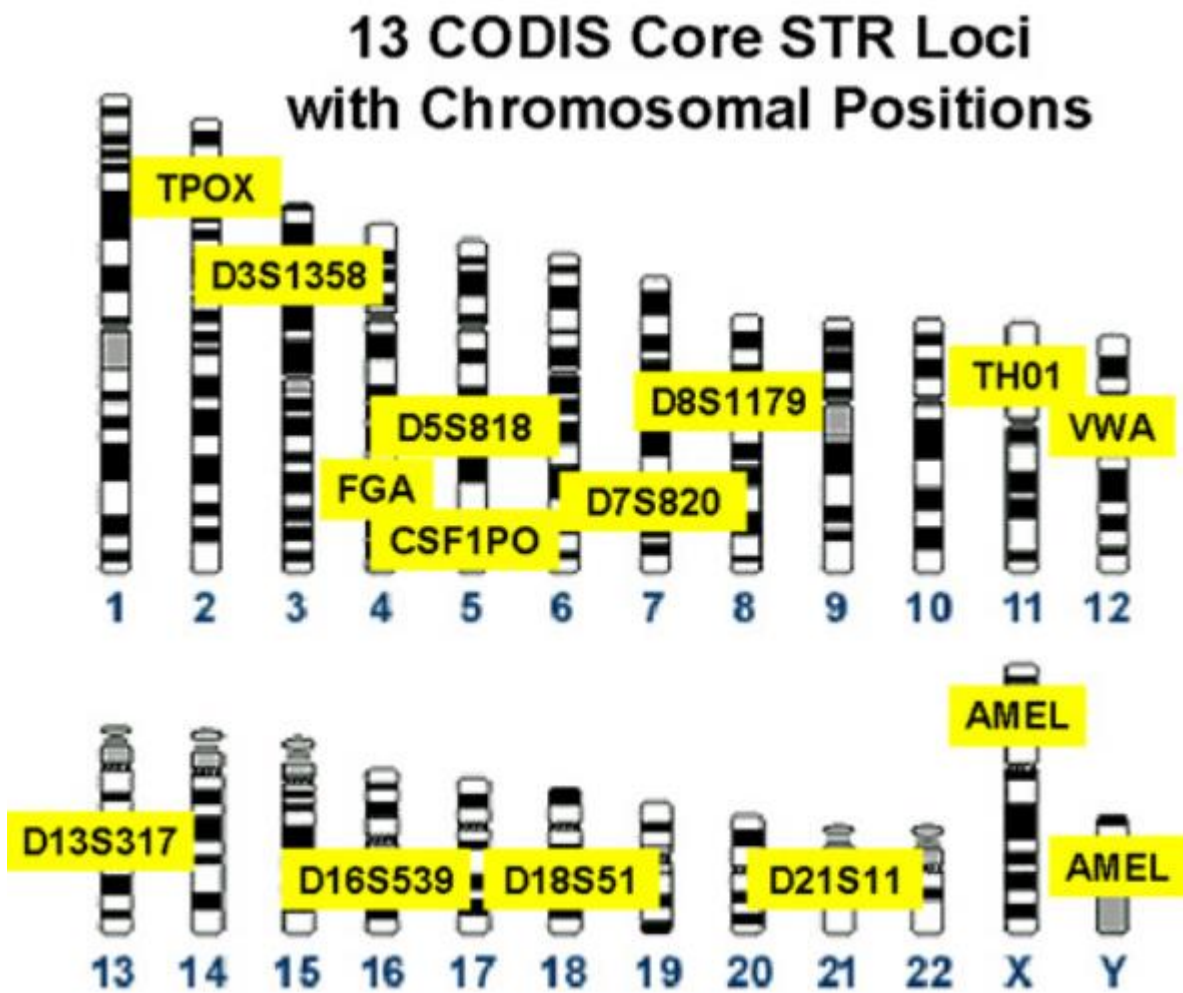
DNA fingerprinting

DNA repeats

- ATATATATATATATATATATATAT, $(AT)_{11}$
- GCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA, $(GCA)_{12}$
- TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
- GTCAGTCAGTCAGTCAGCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGT CAGTCA

- Minisatellites
- Microsatellites


1 Extraction
DNA sample
2 PCR with labelled primers
labelled STRs
3 Electrophoresis
4 Visualisation
DNA profile


13 CODIS Core STR Loci with Chromosomal Positions
TPOX
D3S1358
D5S818
FGA
CSF1PO
D7S820
D8S1179
TH01
VWA
D13S317
D16S539
D18S51
D21S11
AMEL
AMEL
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 X Y

TPOX

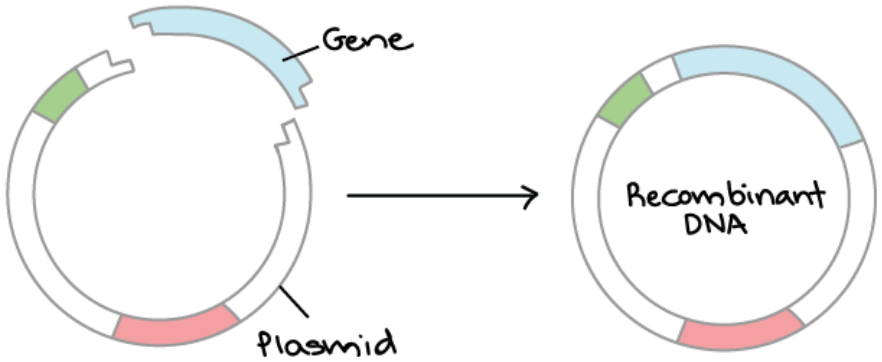
Other Names	Chromosomal Location
hTPO, TPO UniSTS: 240638	**2p25.3**; intron 10 of human thyroid peroxidase gene Chr 2; 1.472 Mb (May 2004, NCBI build 35)

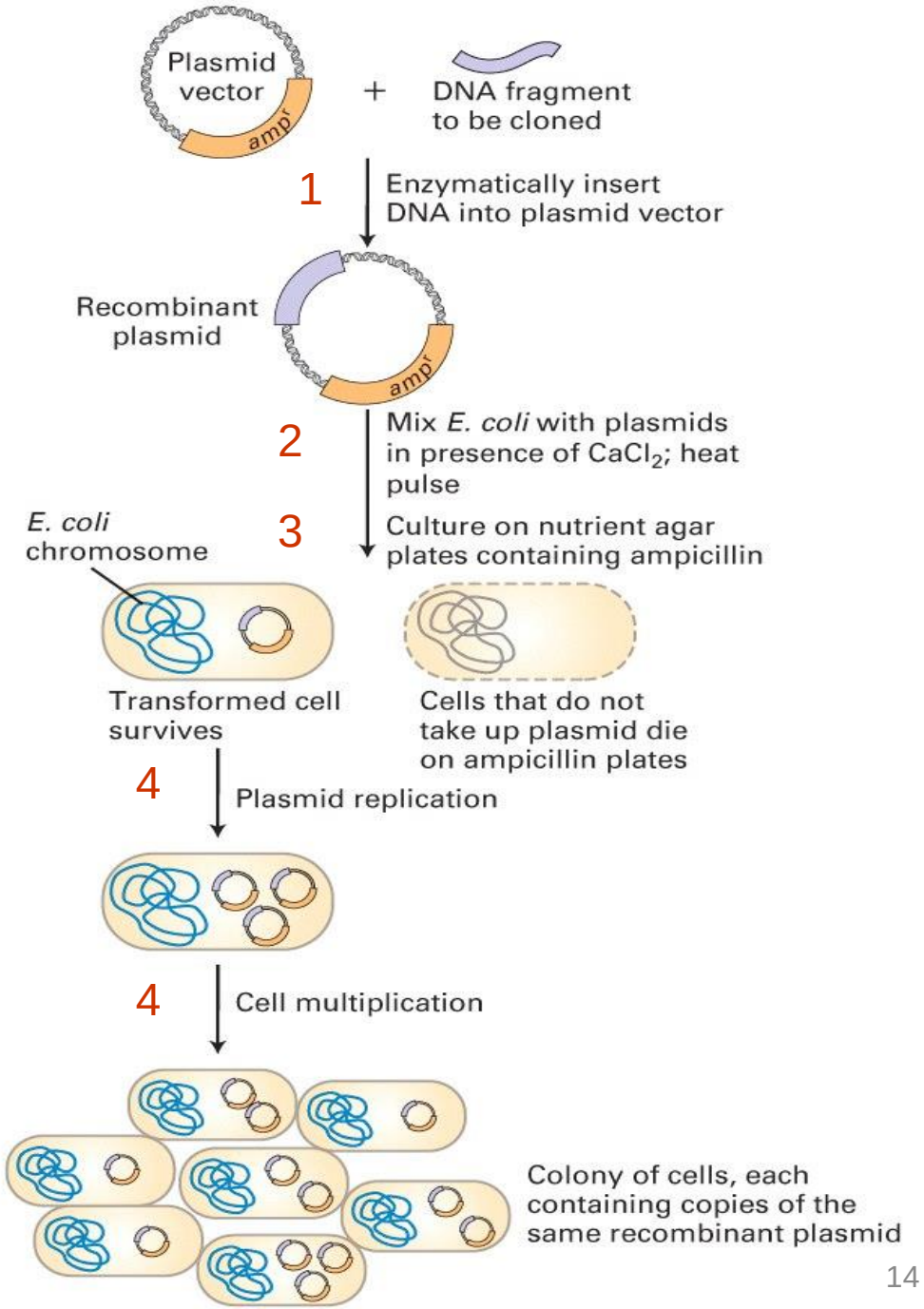
FBI CODIS Core STR Loci:

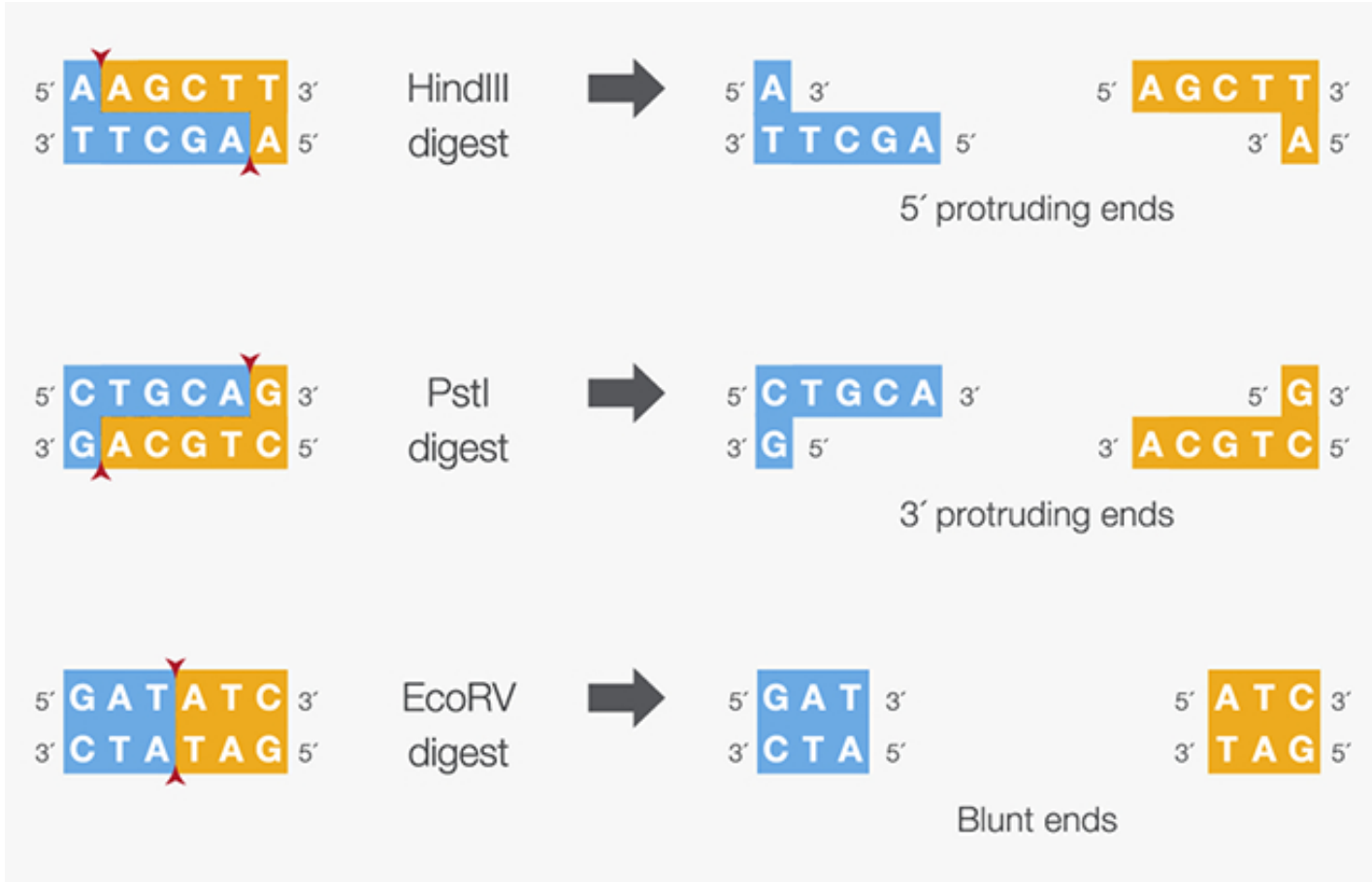
ENFSI
EUROPEAN NETWORK OF FORENSIC SCIENCE INSTITUTES

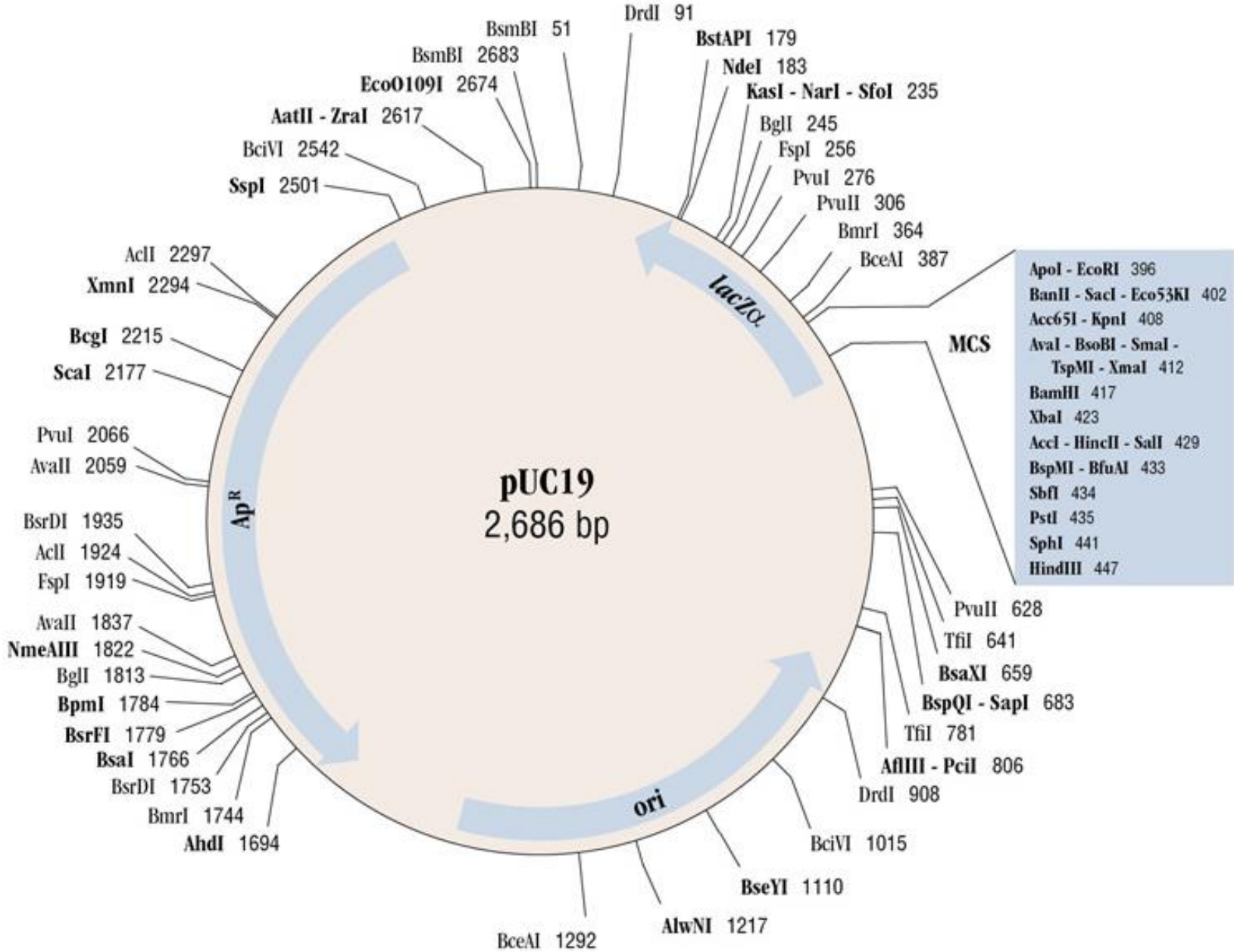
98 bp
102 bp
106 bp
110 bp
113 bp
114 bp
118 bp
122 bp
123 bp
125 bp
126 bp
130 bp
134 bp
135 bp
138 bp
142 bp
146 bp

DNA cloning

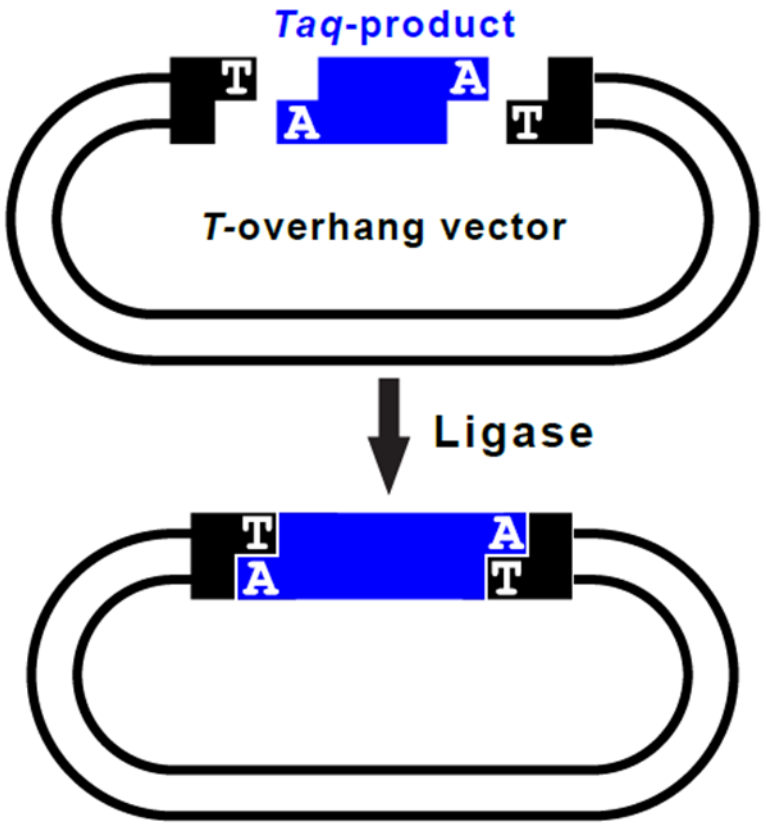

Gene
Plasmid
Recombinant DNA

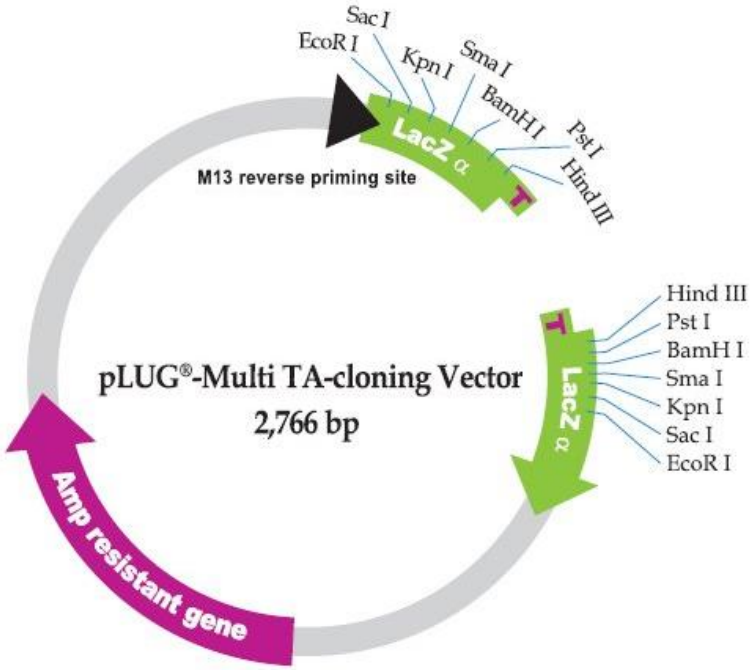

Plasmid vector
ampr
+
DNA fragment to be cloned
1
Enzymatically insert DNA into plasmid vector
Recombinant plasmid
ampr
2
Mix E. coli with plasmids in presence of CaCl2; heat pulse
3
Culture on nutrient agar plates containing ampicillin
E. coli chromosome
Transformed cell survives
Cells that do not take up plasmid die on ampicillin plates
4
Plasmid replication
4
Cell multiplication
Colony of cells, each containing copies of the same recombinant plasmid

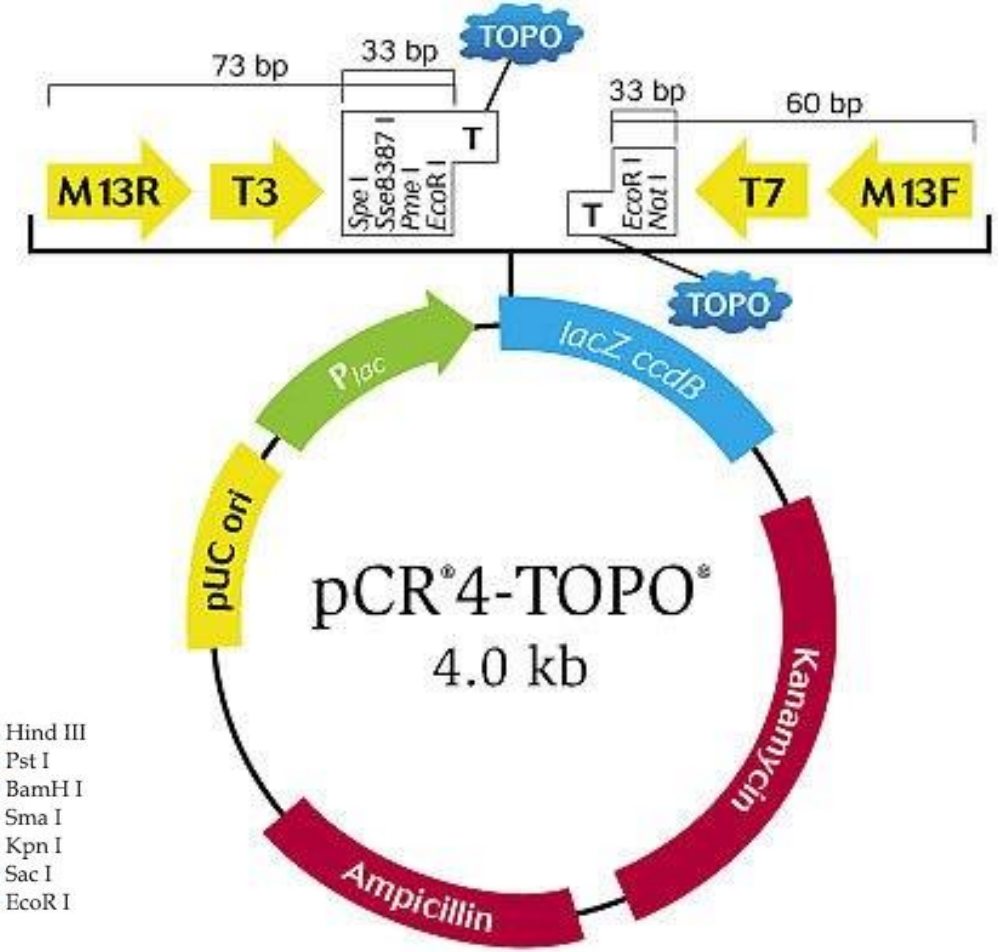

5′ A A G C T T 3′
3′ T T C G A A 5′
HindIII digest
5′ A 3′
3′ T T C G A 5′
5′ A G C T T 3′
3′ A 5′
5′ protruding ends
5′ C T G C A G 3′
3′ G A C G T C 5′
PstI digest
5′ C T G C A 3′
3′ G 5′
5′ G 3′
3′ A C G T C 5′
3′ protruding ends
5′ G A T A T C 3′
3′ C T A T A G 5′
EcoRV digest
5′ G A T 3′
3′ C T A 5′
5′ A T C 3′
3′ T A G 5′
Blunt ends


pUC19
2,686 bp
lacZα
ori
Ap^R
MCS
ApoI - EcoRI 396
BanII - SacI - Eco53KI 402
Acc65I - KpnI 408
AvaI - BsoBI - SmaI - TspMI - XmaI 412
BamHI 417
XbaI 423
AccI - HincII - SalI 429
BspMI - BfuAI 433
SbfI 434
PstI 435
SphI 441
HindIII 447
DrdI 91
BstAPI 179
NdeI 183
KasI - NarI - SfoI 235
BglI 245
FspI 256
PvuI 276
PvuII 306
BmrI 364
BceAI 387
PvuII 628
TfiI 641
BsaXI 659
BspQI - SapI 683
TfiI 781
AflIII - PciI 806
DrdI 908
BciVI 1015
BseYI 1110
AlwNI 1217
BceAI 1292
AhdI 1694
BmrI 1744
BsrDI 1753
BsaI 1766
BsrFI 1779
BpmI 1784
BglI 1813
NmeAIII 1822
AvaII 1837
FspI 1919
AclI 1924
BsrDI 1935
AvaII 2059
PvuI 2066
ScaI 2177
BcgI 2215
XmnI 2294
AclI 2297
SspI 2501
BciVI 2542
AatII - ZraI 2617
EcoO109I 2674
BsmBI 2683
BsmBI 51

DNA cloning


Taq-product
T
A
A
T
T-overhang vector
Ligase
T
A
A
T


Sac I
EcoR I
Kpn I
Sma I
BamH I
Pst I
Hind III
LacZ α
M13 reverse priming site
pLUG®-Multi TA-cloning Vector
2,766 bp
Amp resistant gene
Hind III
Pst I
BamH I
Sma I
Kpn I
Sac I
EcoR I


73 bp
33 bp
TOPO
33 bp
60 bp
M13R
T3
Spe I
Sse8387 I
Pme I
EcoR I
T
T
EcoR I
Not I
T7
M13F
TOPO
lacZ ccdB
P lac
pUC ori
pCR®4-TOPO®
4.0 kb
Kanamycin
Ampicillin

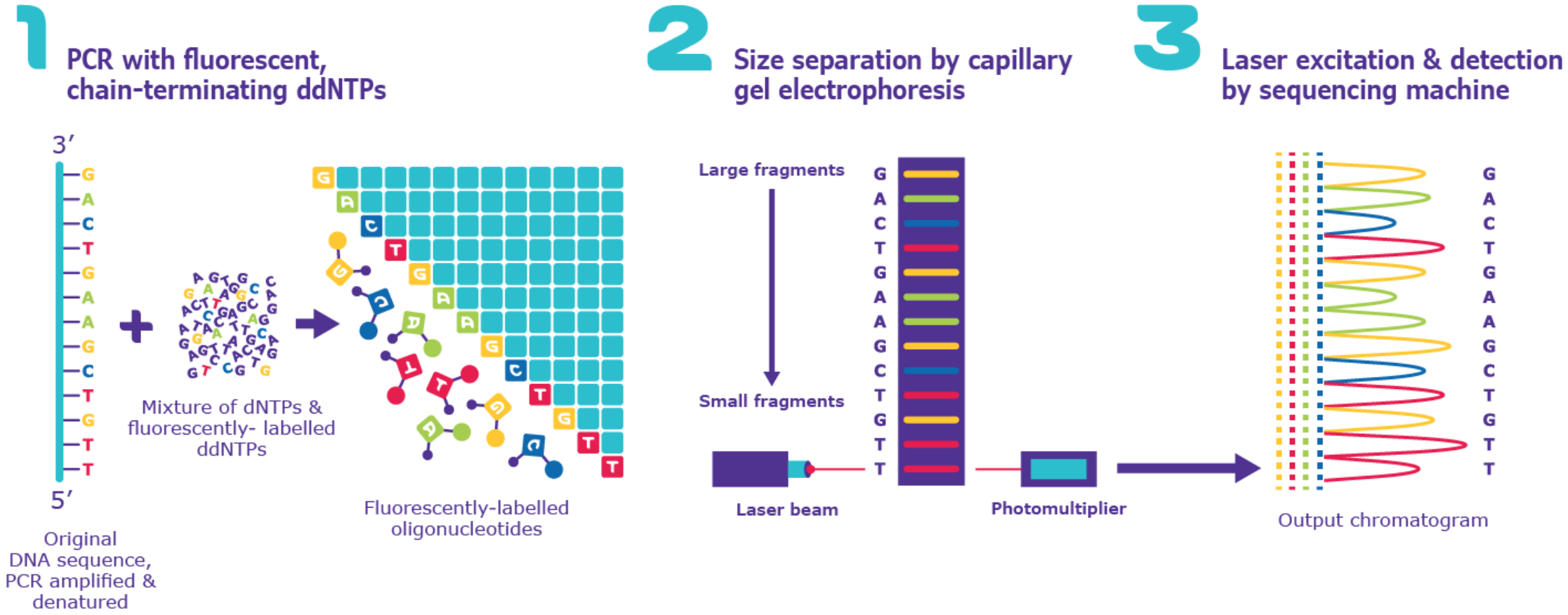
How do you sequence DNA?

- **Sanger** (**dideoxy**, enzymatic) – developed by Frederick Sanger and is still used today with little change to the basic method.

High-throughput DNA sequencing

- **Next-generation sequencing**

1. All 4 fluorescently-labeled ddNTPs are used in 1 reaction, each a different “color”
2. Fragments are separated in matrix-filled capillary tubes, 1 capillary per reaction
3. Laser detects fluorescence automatically as each fragment exits capillary
4. Computer software “calls bases” and processes sequence files



https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html

Sequencing chromatogram