

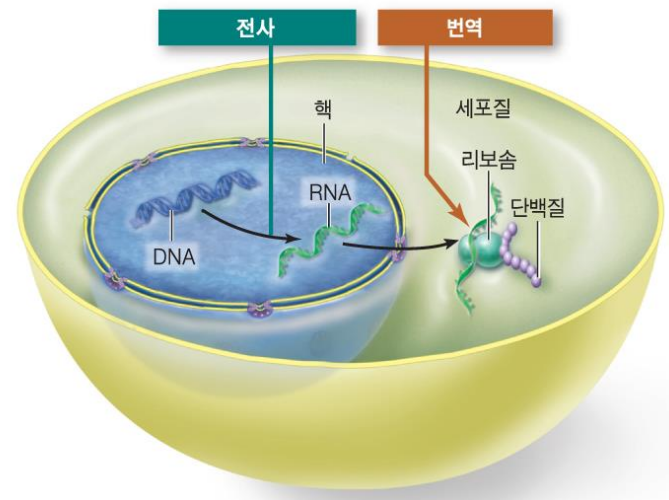
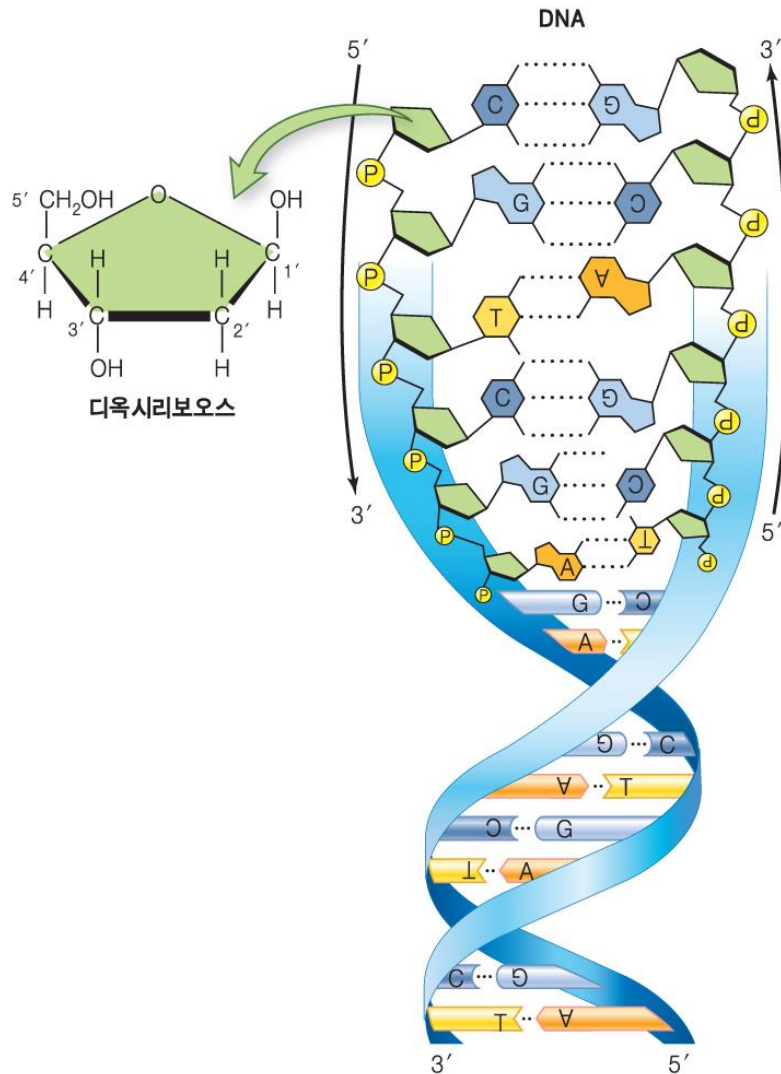
일반생물학

13주차-11장. DNA기술

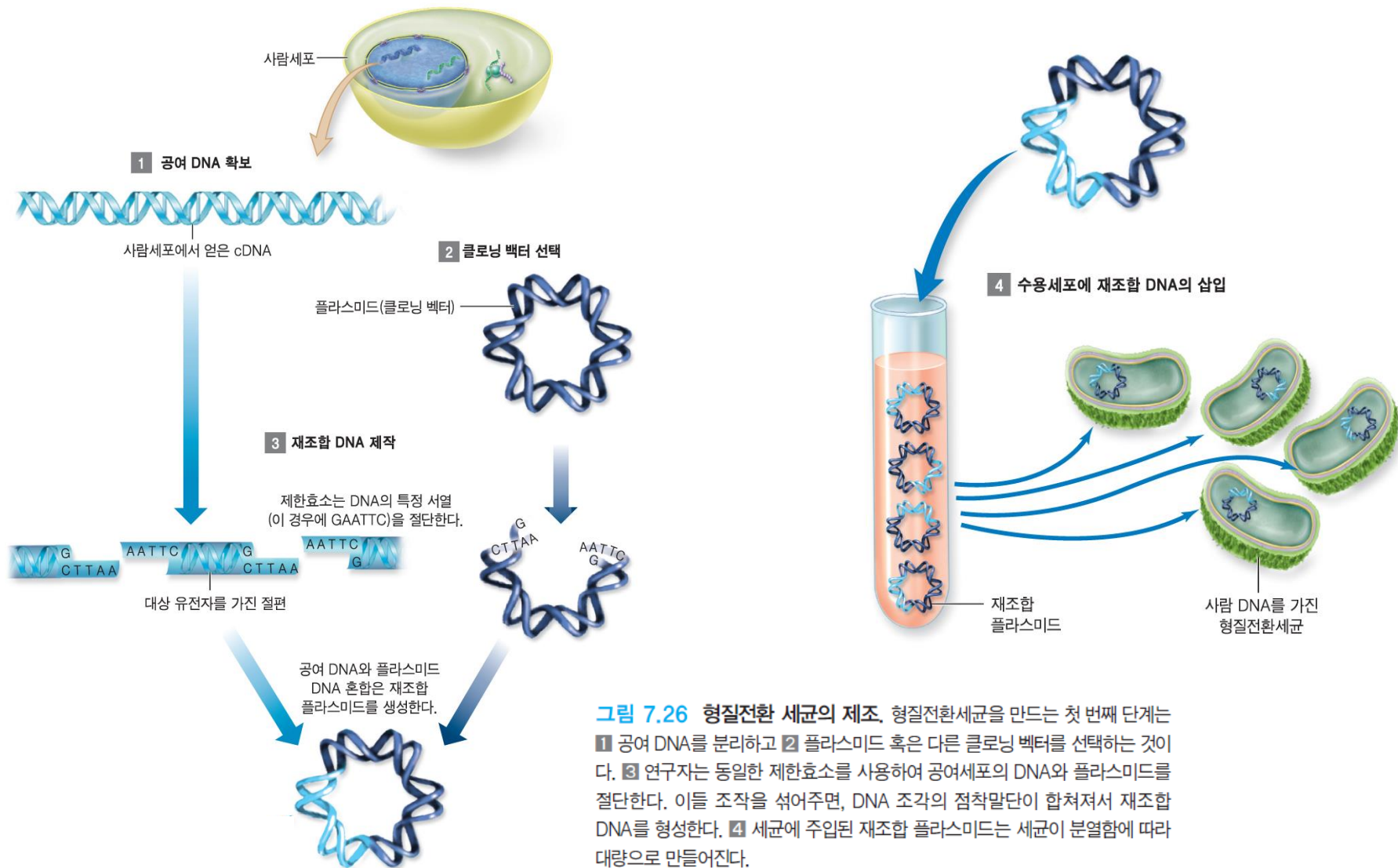
중부대학교 애완동물학부
정 태 호



Central Dogma (중심이론)



유전자 조작을 통한 형질전환



유전자 조작을 통한 형질전환

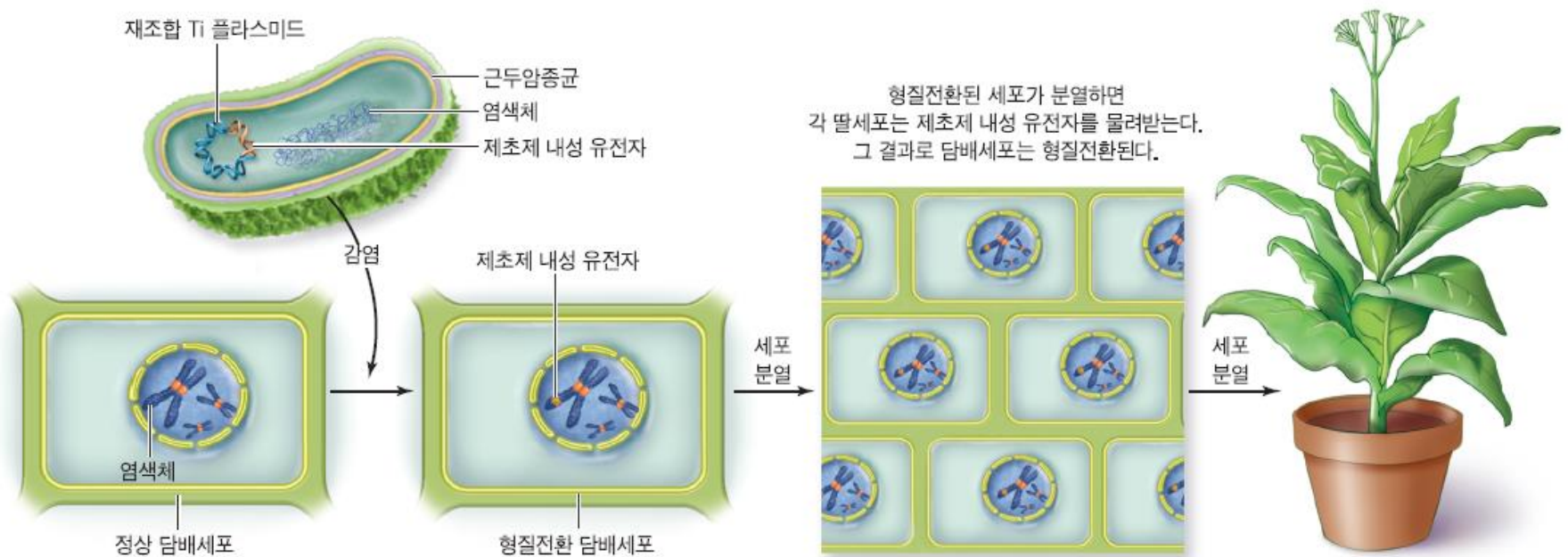
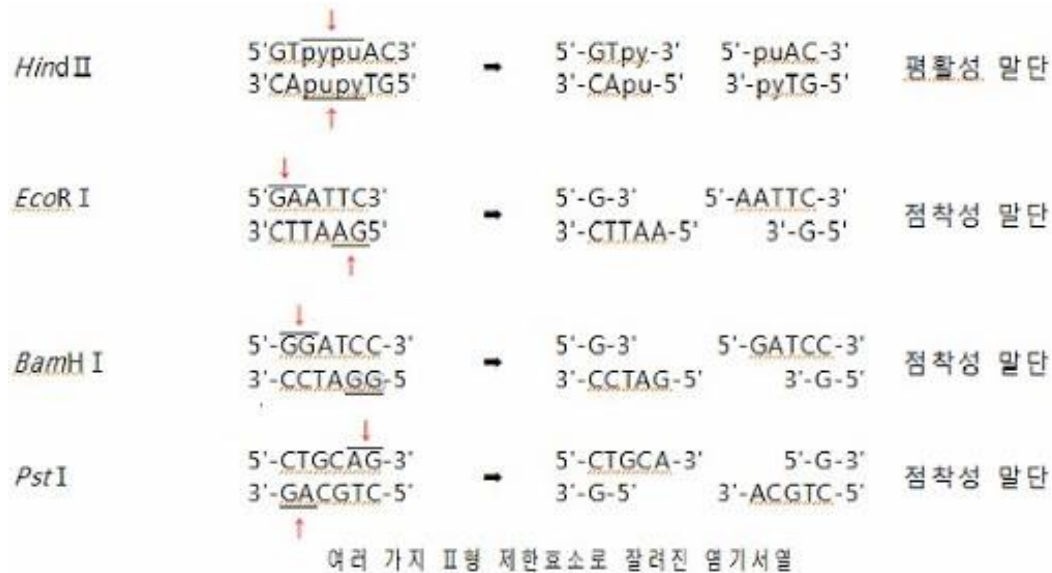


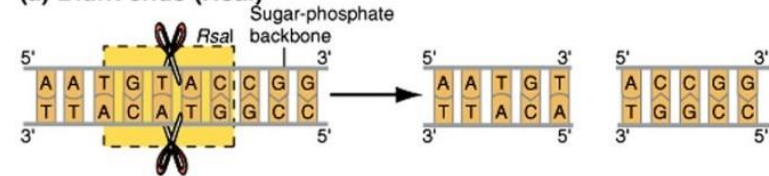
그림 7.27 형질전환식물 제조. 유전적으로 변형된 근두암종균(*Agrobacterium*) 세포는 제초제에 내성을 부여하는 유전자를 암호화하는 재조합 Ti 플라스미드를 가지고 있다. 이 세균을 담배식물 세포에 주입하여 식물세포의 DNA 속에 Ti 플라스미드를 삽입한다. 형질전환 식물세포는 모든 세포에 살충제 내성 유전자를 발현하는 담배식물로 자란다.

Restriction Enzyme

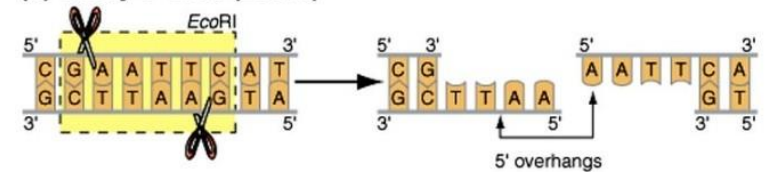


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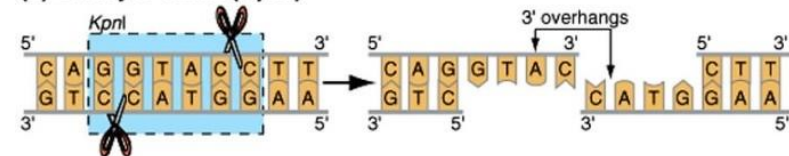
(a) Blunt ends (*Rsa*I)



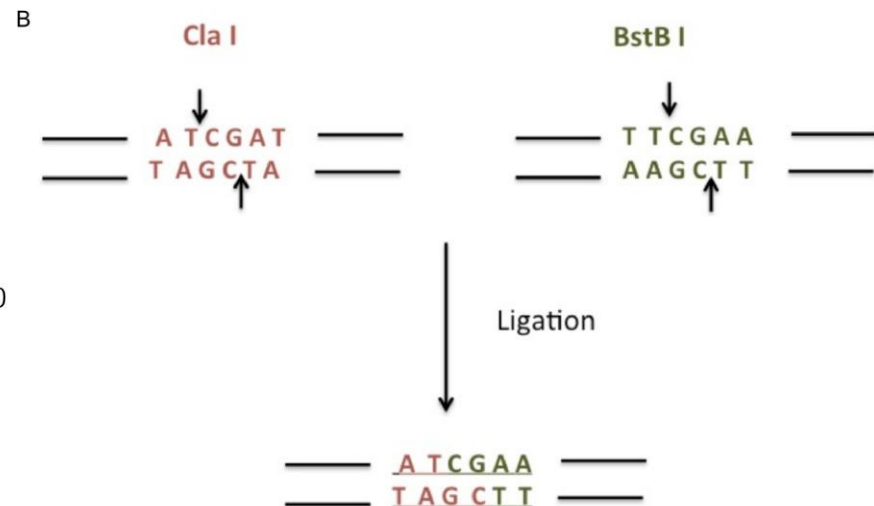
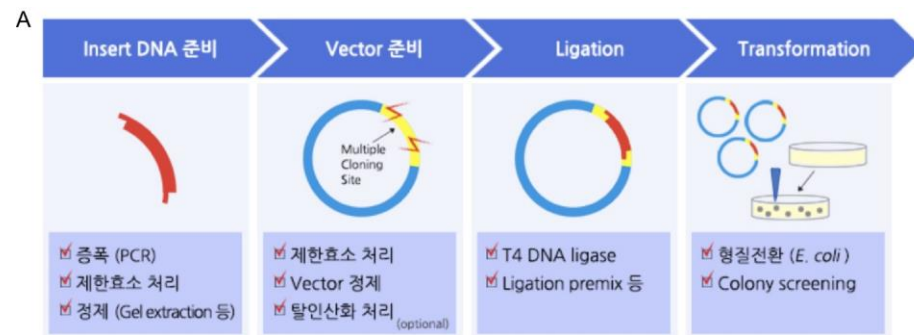
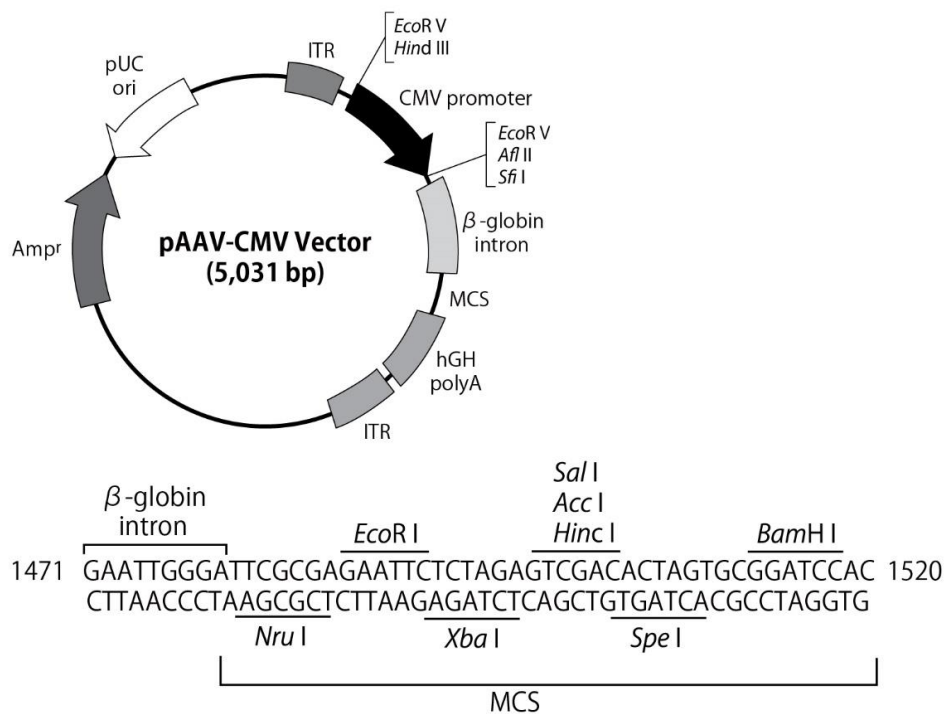
(b) Sticky 5' ends (*Eco*RI)



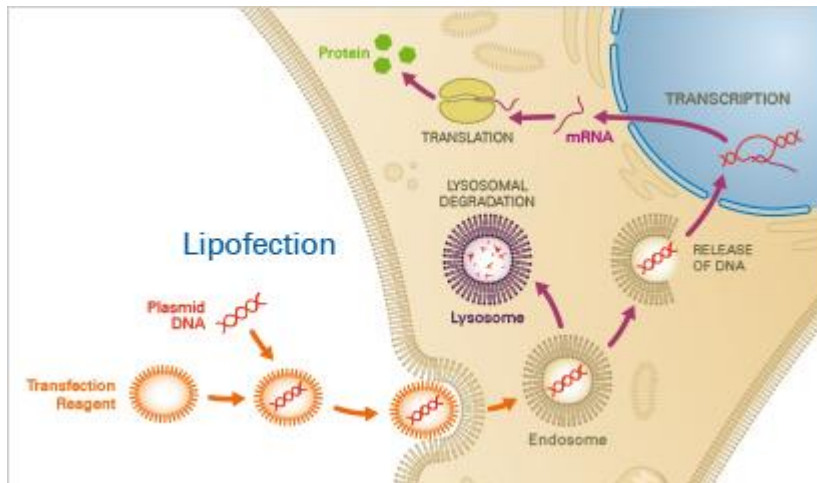
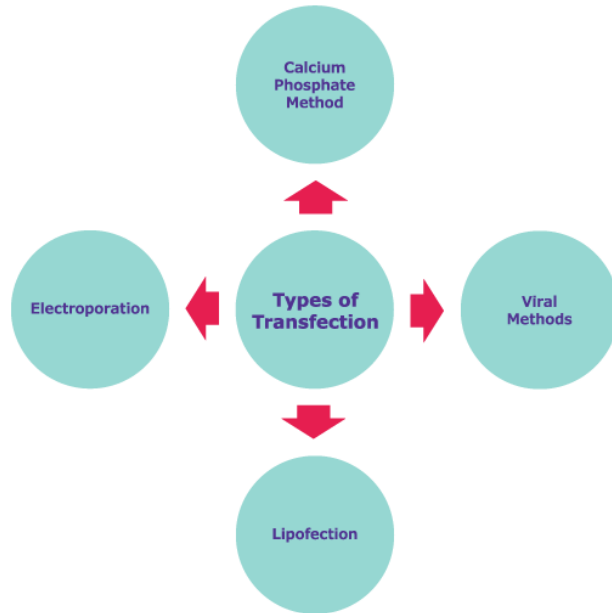
(c) Sticky 3' ends (*Kpn*I)



Restriction Enzyme



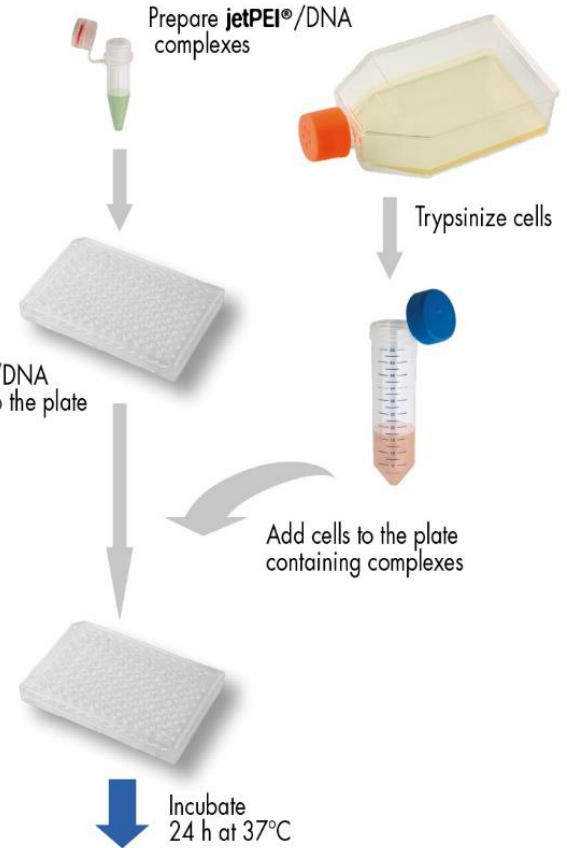
Transfection



jetPEI® Reverse HTS Transfection



DAY 1
Prepare
the cells
Transfect
and Plate

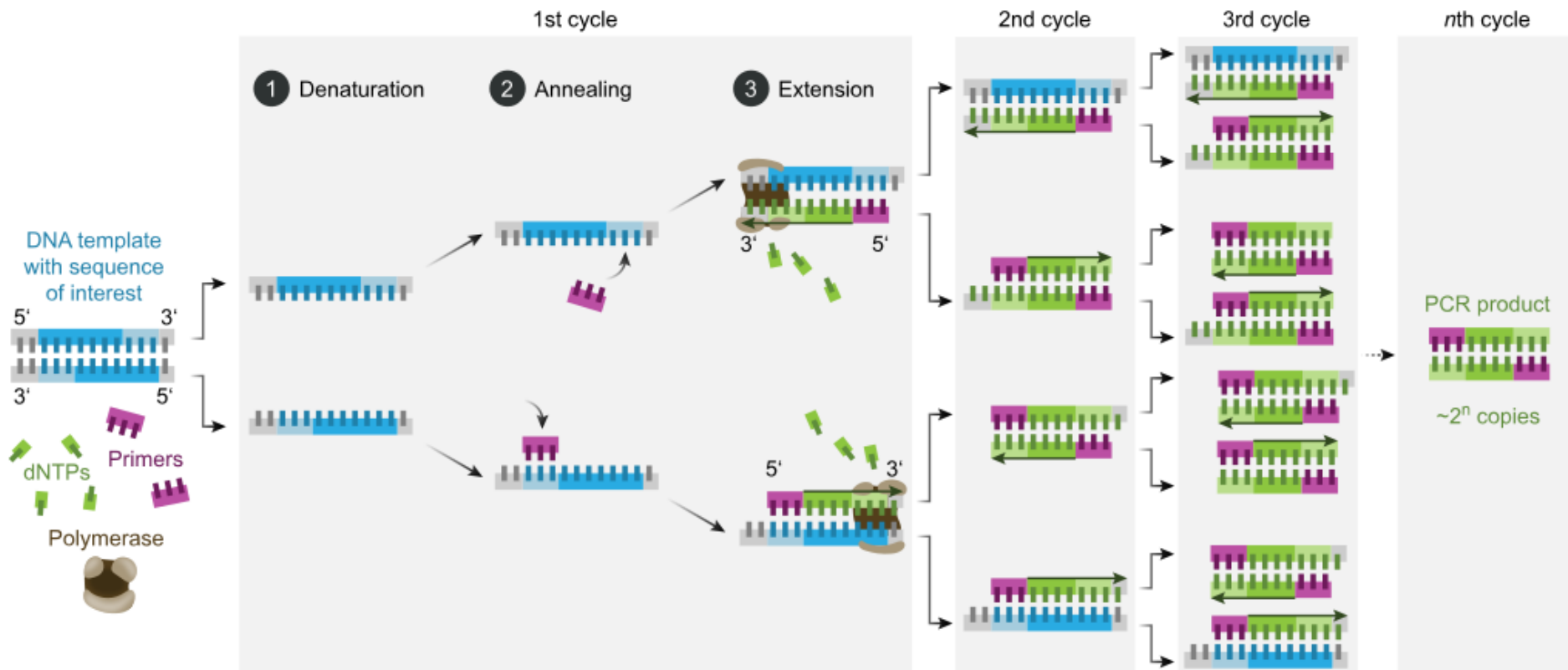


DAY 2
Assay

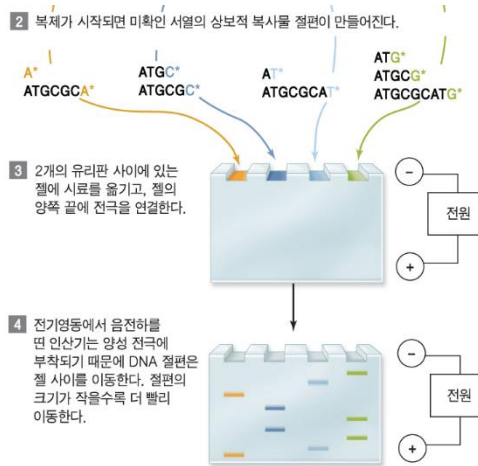
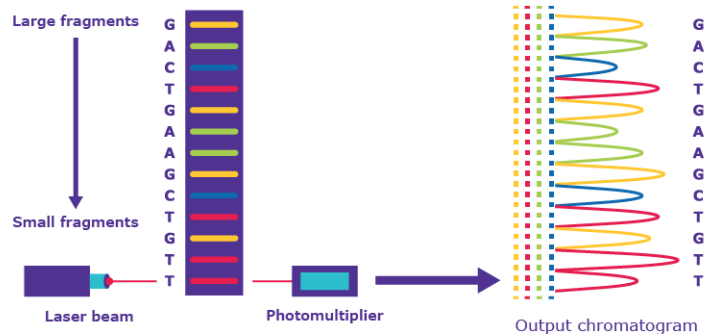
**Measure gene or
protein expression**

PCR

- <https://www.youtube.com/watch?v=iQsu3Kz9NYo>



3 Laser excitation & detection by sequencing machine



TGGAGAAAAATTCCCTTTTGAATTTATCTCCAAACAAAGTTAGGGGTGAATATTTATTTT
 150 160 170 180 190

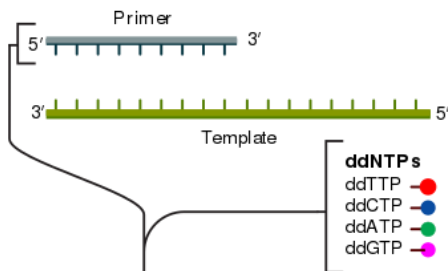
5 질편을 크기별로 읽어 원래 서열을 추론한다.

참보물로부터
원래 서열 추론

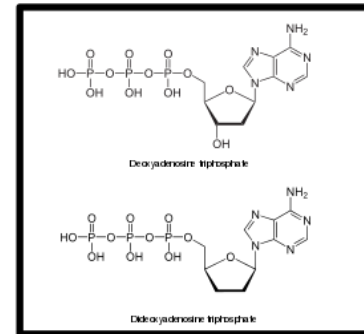
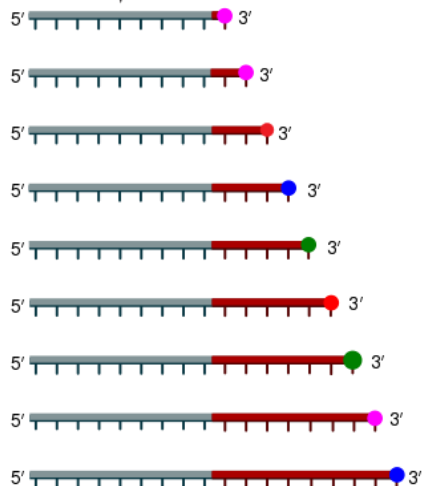
Sanger sequencing

① Reaction mixture

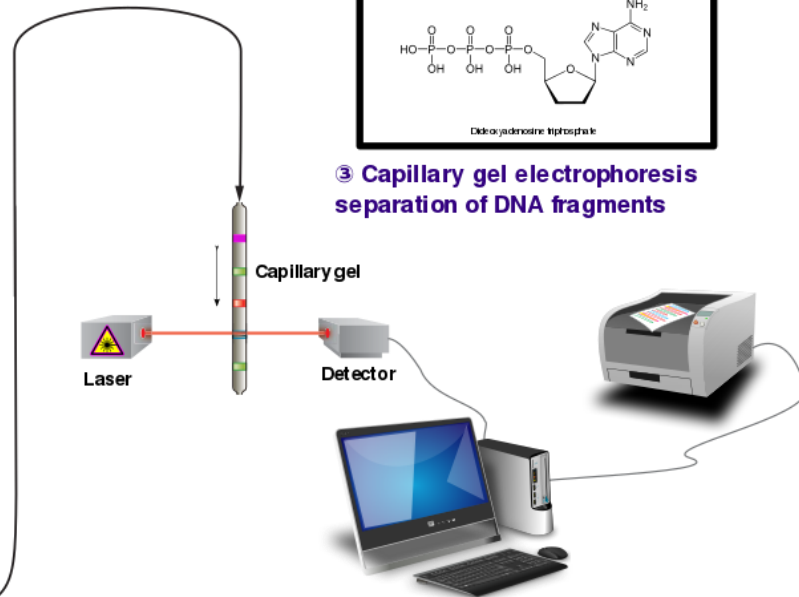
- ▶ Primer and DNA template ▶ DNA polymerase
- ▶ ddNTPs with flouorochromes ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)



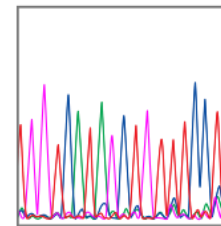
② Primer elongation and chain termination



③ Capillary gel electrophoresis separation of DNA fragments



④ Laser detection of flouorochromes and computational sequence analysis

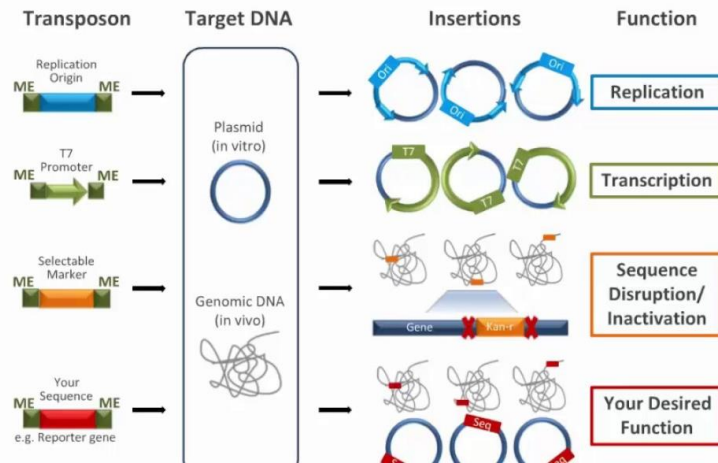


Chromatograph

Transposon & tandem repeat

Power of Transposomics

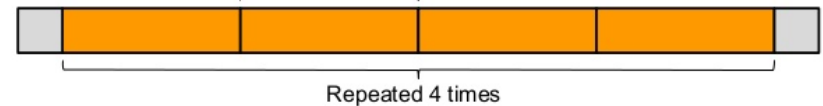
Transposons impart desired function to target DNA



Tandem Repeat elements

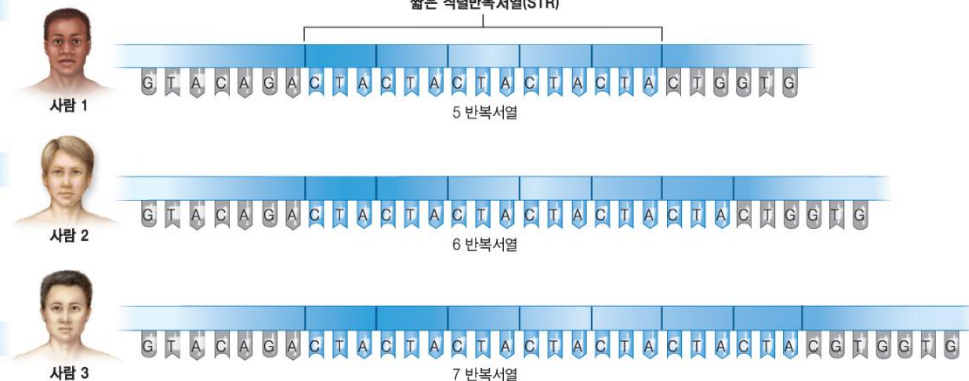
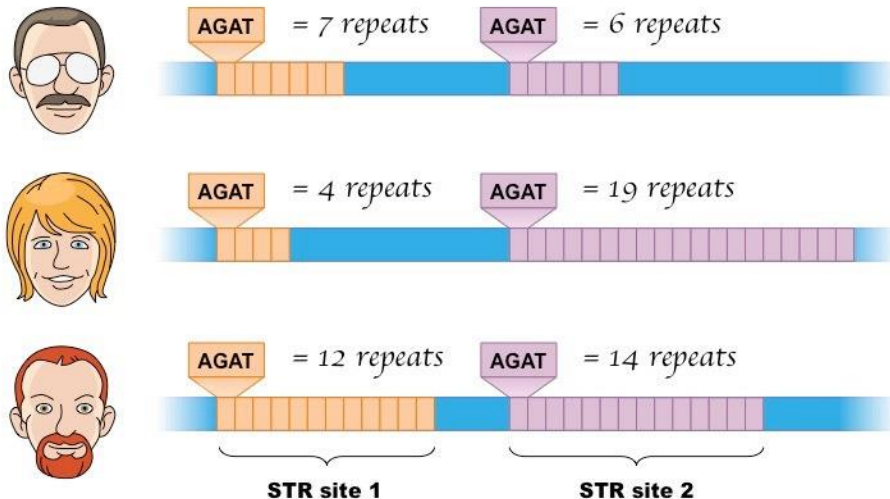
Minisatellite: Variable Number Tandem Repeats (VNTR)

Repeat unit size = hundreds base pairs



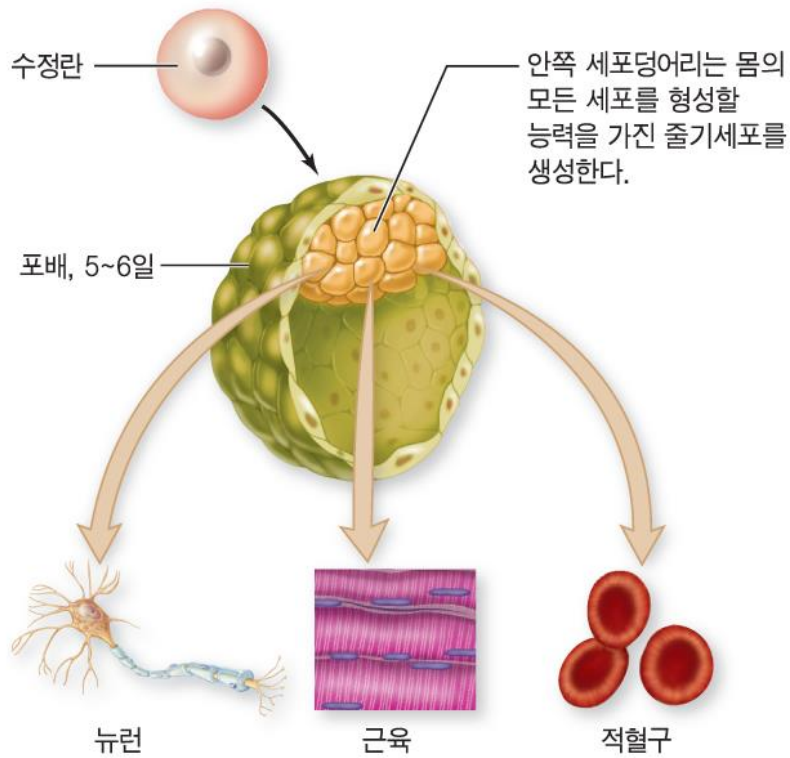
Microsatellite: Short Tandem Repeats (STR) – Simple Sequence Repeats (SSR)

Repeat unit size = 2 - 6 base pairs

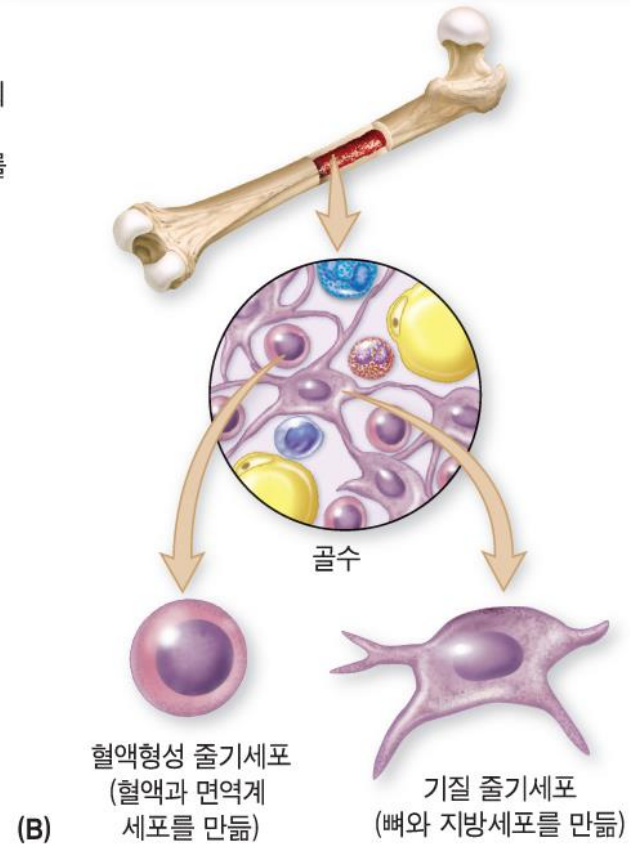


배아줄기세포와 성체줄기세포

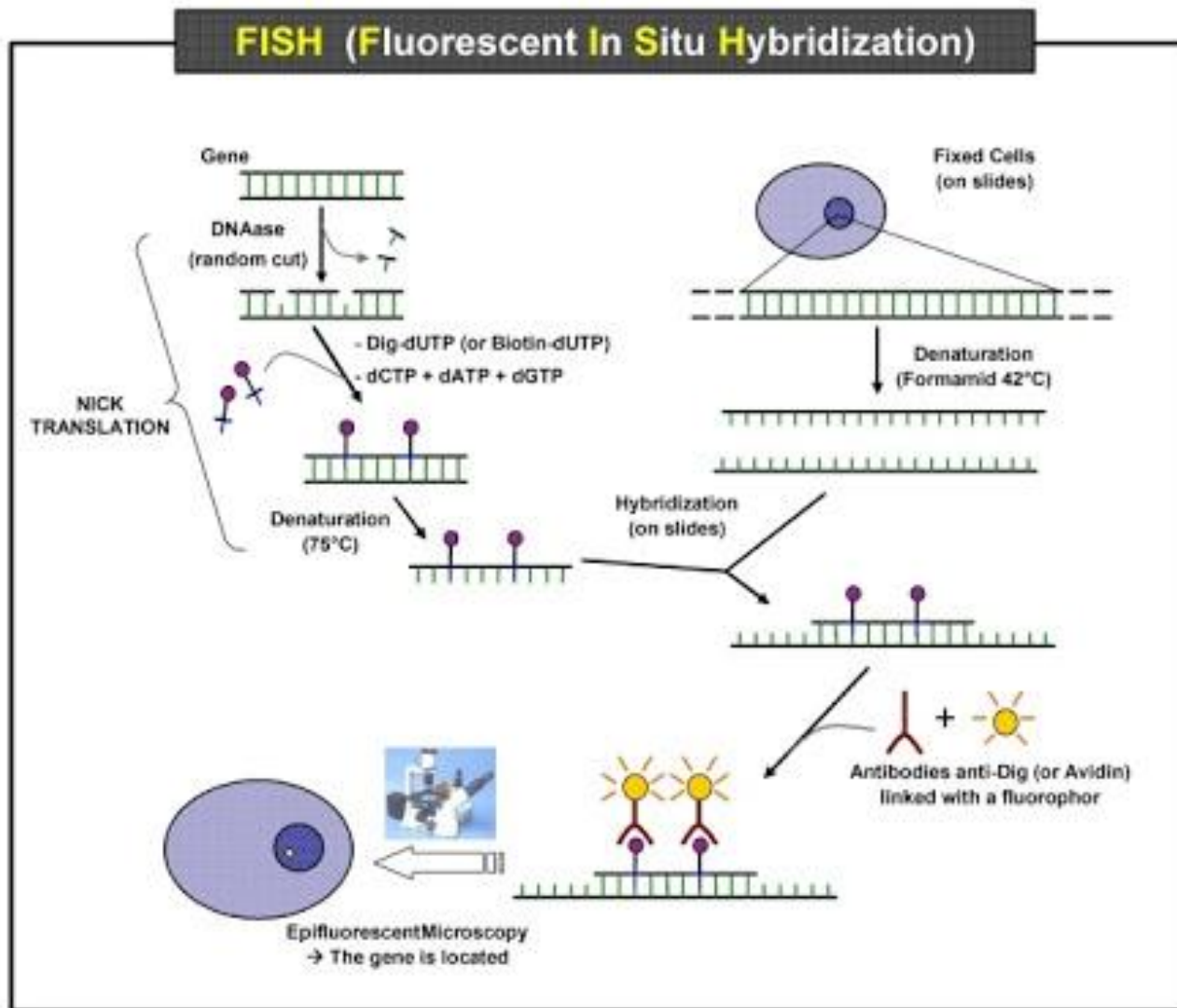
배아줄기세포



성체줄기세포



Fluorescence in situ hybridization (FISH)



Fluorescence in situ hybridization (FISH)

- 이수/배수체의 검출, microdeletion syndrome 및 aberration 등에 광범위하게 적용이 가능하여 백혈병, 림프종, 종양, 자폐증 및 기타 발달 증후군과 수많은
- 유전 질환에 임상적 의미
- Custom-labeled FISH probe는 각각의 유전자를 고유한 색의 형광으로 표지 하여 여러 가지의 이형을 동시에 검출

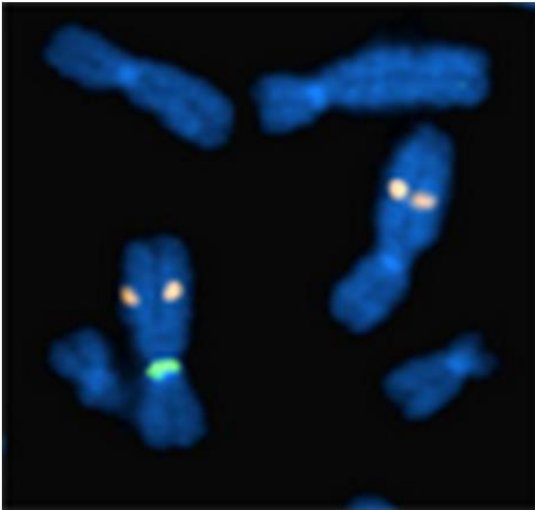


Fig.1 Empire Genomics has developed a 6q21 FISH Probe which can be used to detect 6q21 region aneusomy. The probe comes labeled in orange, and

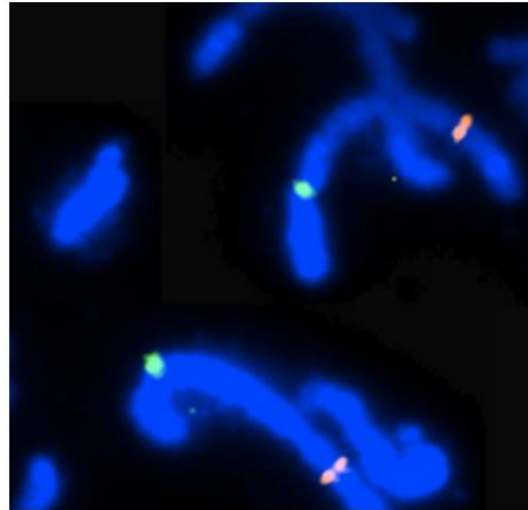


Fig.2 Empire Genomics has developed a 5p5q FISH Probe which can be used to detect 5p5q region aneusomy. The probe comes labeled in orange and

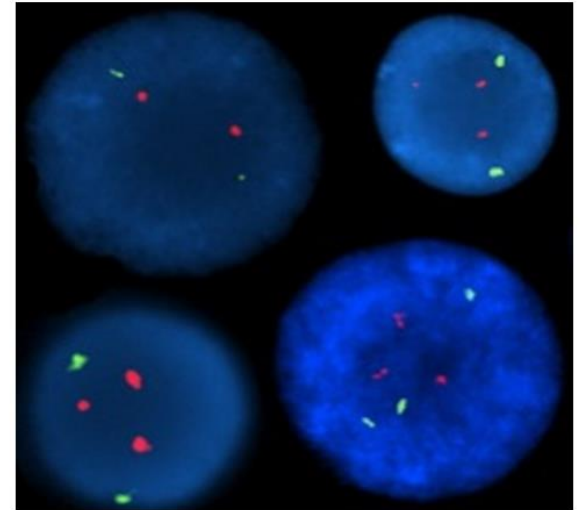


Fig.3 The 1p1q FISH Probe hybridizes to the 1p32.3 and 1q21.3 regions in normal cells. In cases of deletion in 1p32 region, the green signal is lost

Thank you!

