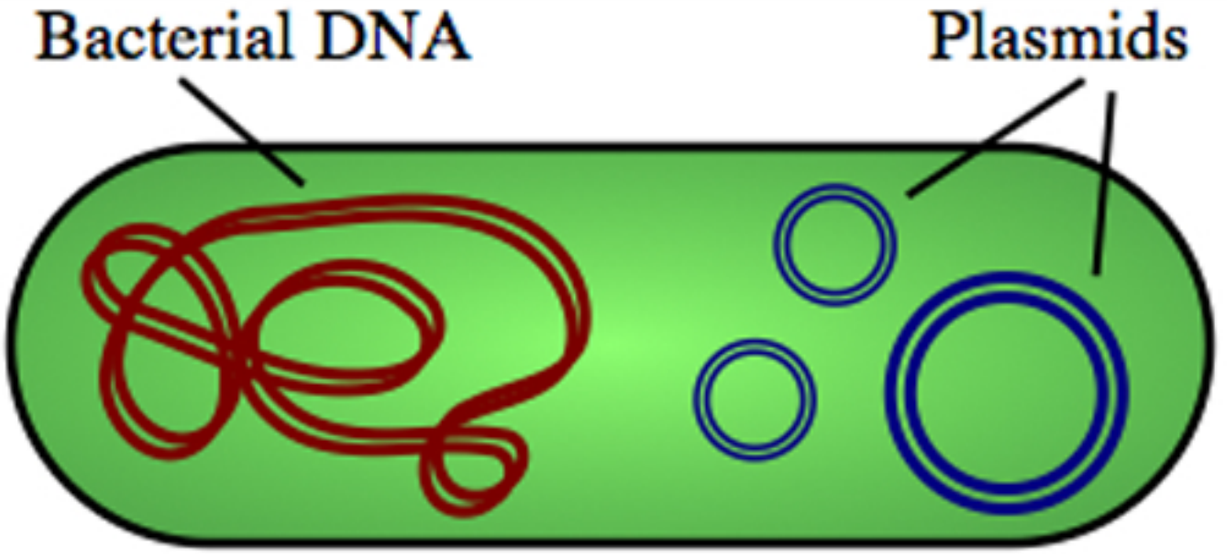
Module 14

Genomics Laboratory

Session Slides with Notes

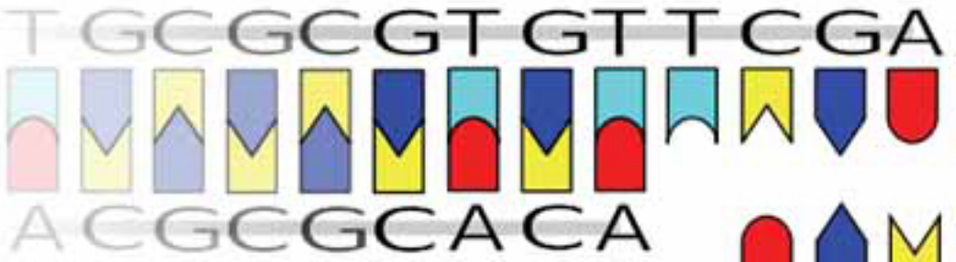
DNA Lab



Cohen ad Boyer

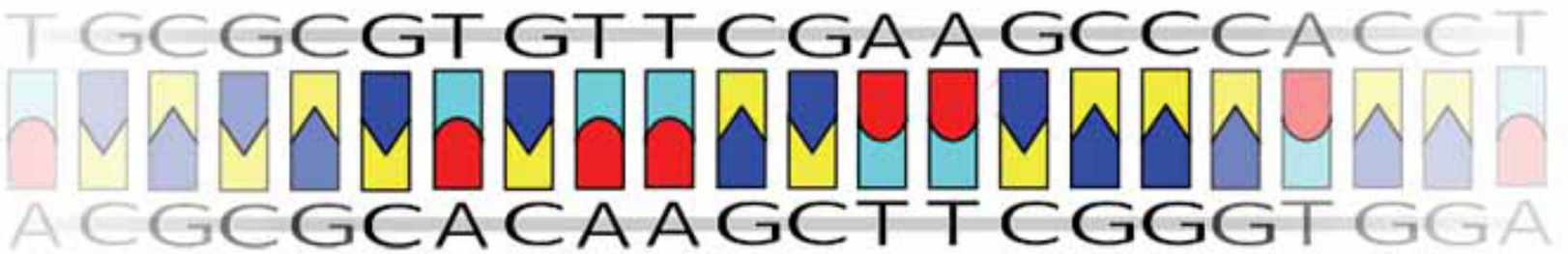
Transformed bacteria with recombinant plasmid.

Using restriction enzyme - (restriction endonuclease)

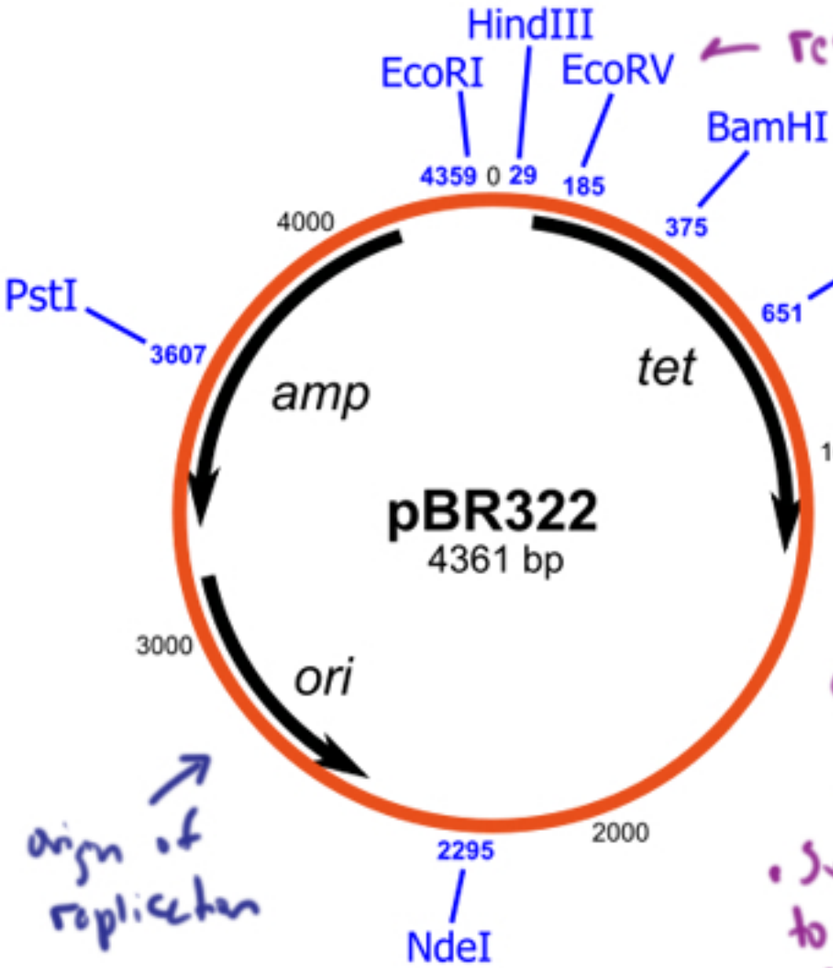


- 2 fold palindromic symmetry
- sticky ends are identical

sticky end



Any DNA cleaved by same restriction enzyme may ligated.

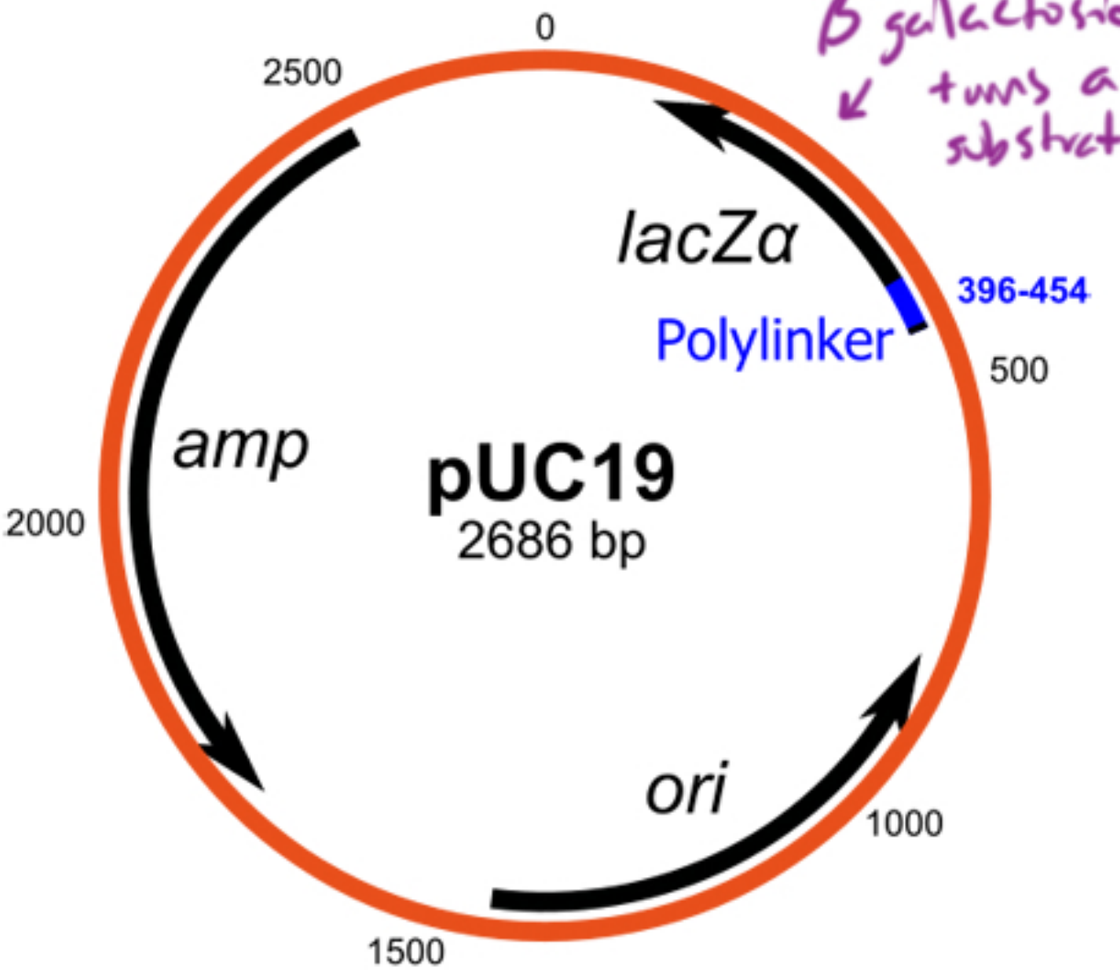


← restriction sites

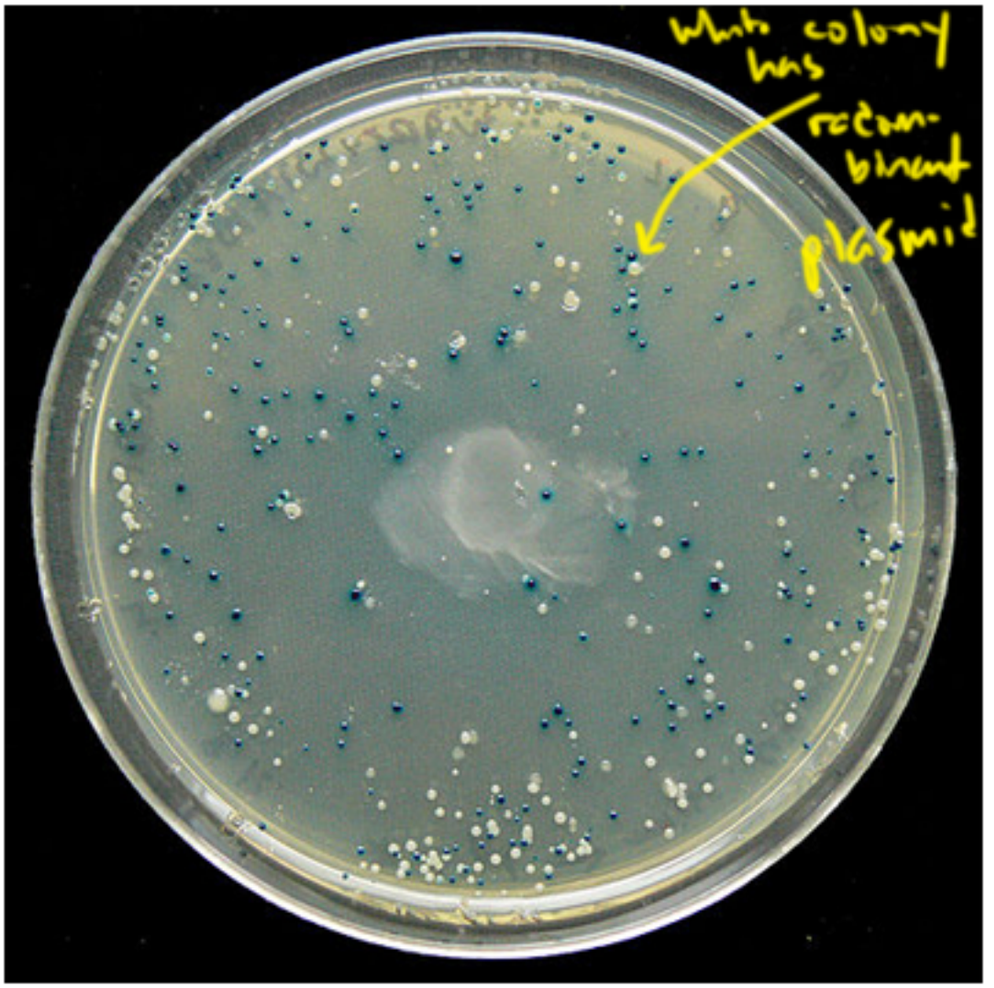
origin of replication

amp & tet antibiotic resistance genes for screening

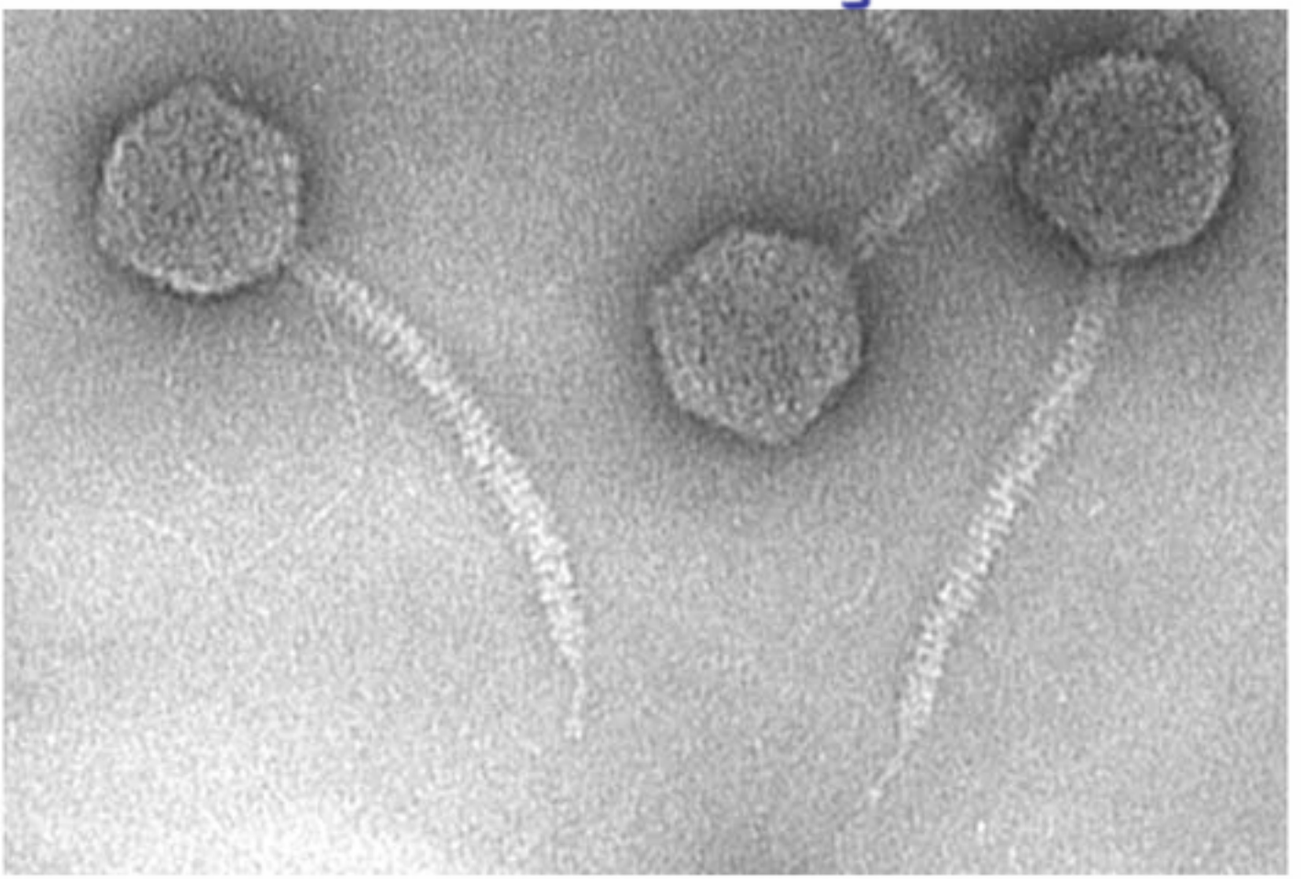
- Suppose we used BamHI to form recombinant plasmids and transform bacteria.
- Is a colony ampicillin resistant? It received plasmid
- Is tetracycline resistance missing? The plasmid was recombinant.



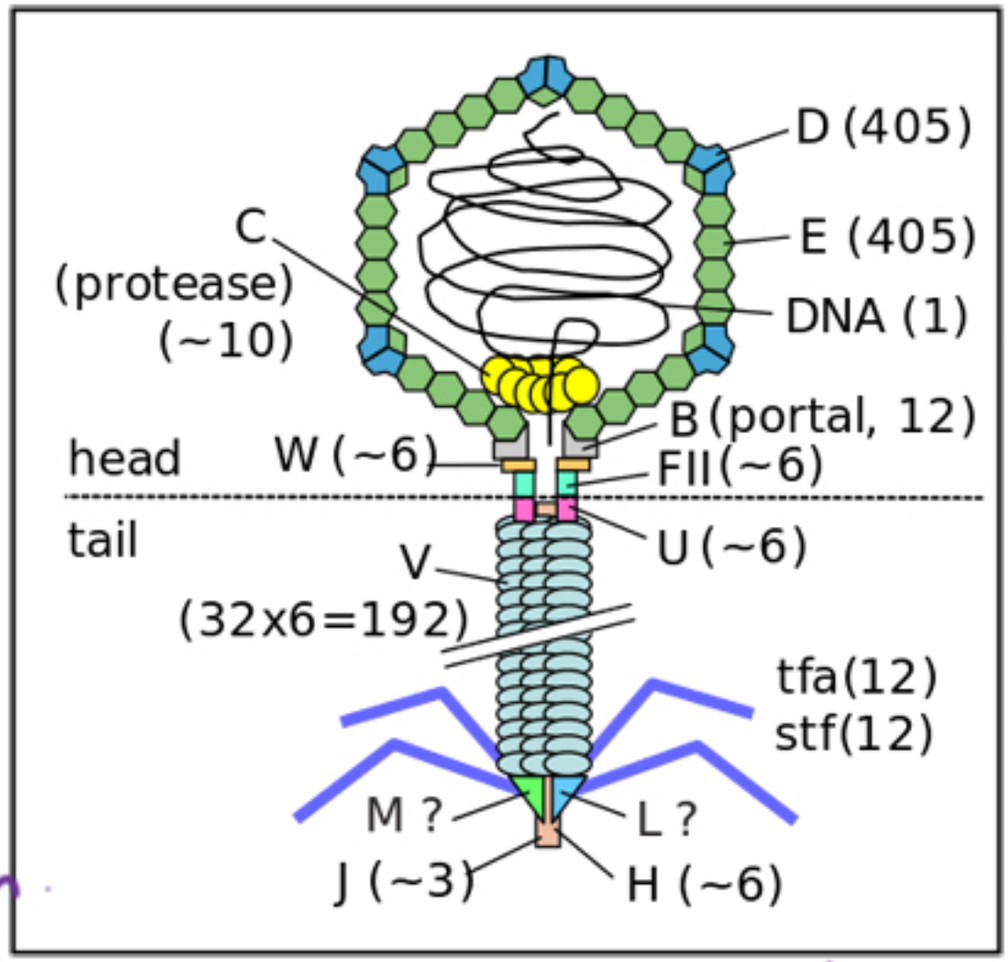
β galactosidase
↙ turns a special substrate blue


white colony has recombinant plasmid

Lambda Phage



λ Phage



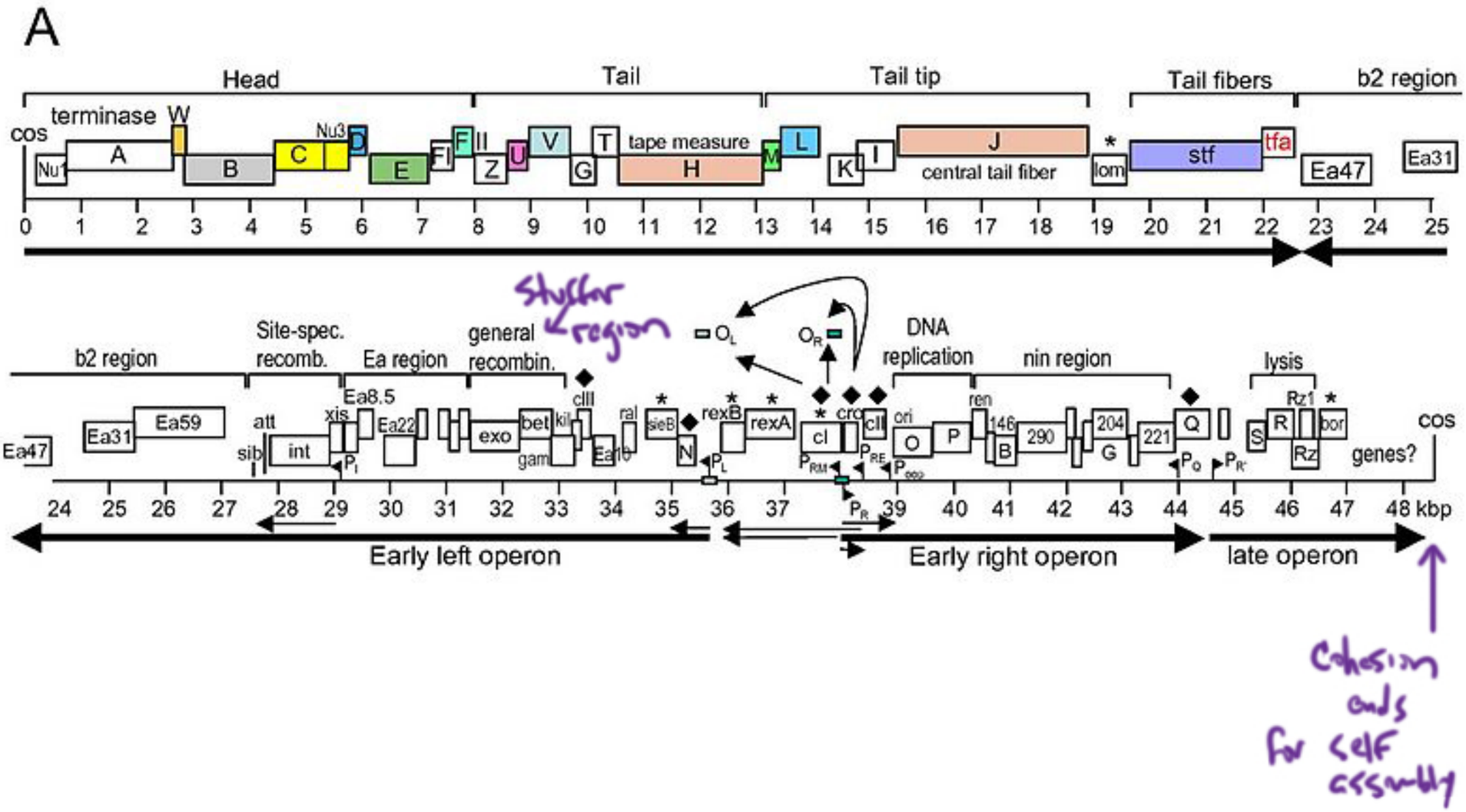
λ phage can accomodate several tens of thousands of BP DNA in its stuffer region.

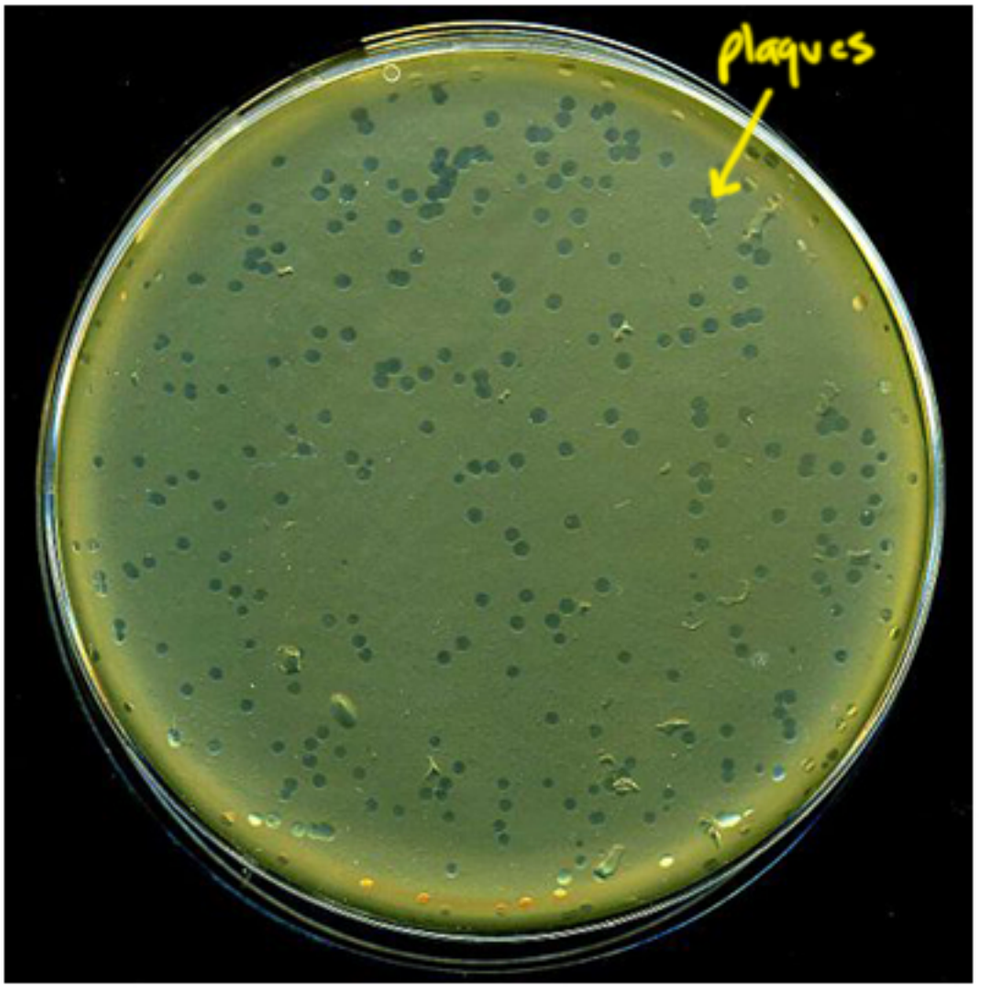
The virus will self assemble as a recombinant

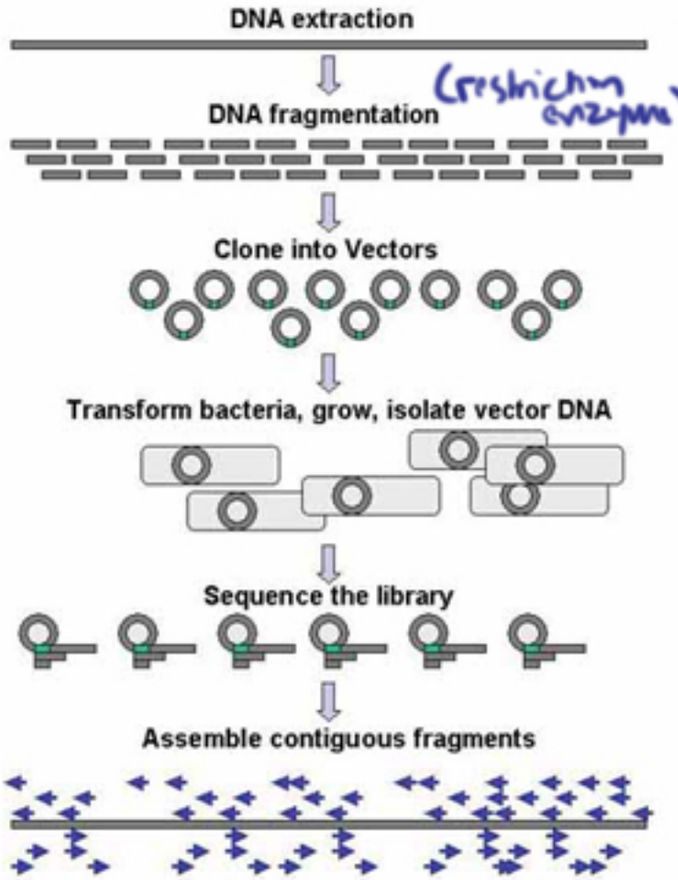
Important note - viral self-assembly with a phage sections of DNA called cohesion ends (cos)

Certain capsid proteins recognize and bind cohesion ends and nucleate self-assembly

Figure 1.


A
Head
Tail
Tail tip
Tail fibers
b2 region
cos
terminase
W
Nu1
A
B
Nu3
C
D
E
FI
FII
Z
U
V
G
T
tape measure
H
M
L
K
I
J
central tail fiber
*
lom
stf
tfa
Ea47
Ea31
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
b2 region
Site-spec. recomb.
Ea region
general recombin.
O_L
O_R
DNA replication
nin region
lysis
Ea8.5
cIII
xis
att
sib
int
P_I
Ea22
exo
bet
gam
kil
ral
Ea10
sieB
N
P_L
rexB
rexA
cro
cI
cII
P_{RM}
P_{RE}
P_{aQ2}
ori
O
P
ren
146
B
290
204
G
221
Q
P_Q
$P_{R'}$
S
R
Rz1
Rz
bor
genes?
cos
24 25 26 27 28 29 30 31 32 33 34 35 36 37 39 40 41 42 43 44 45 46 47 48 kbp
P_R
Early left operon
Early right operon
late operon
stuffer region
Cohesive ends for cell assembly


plaques

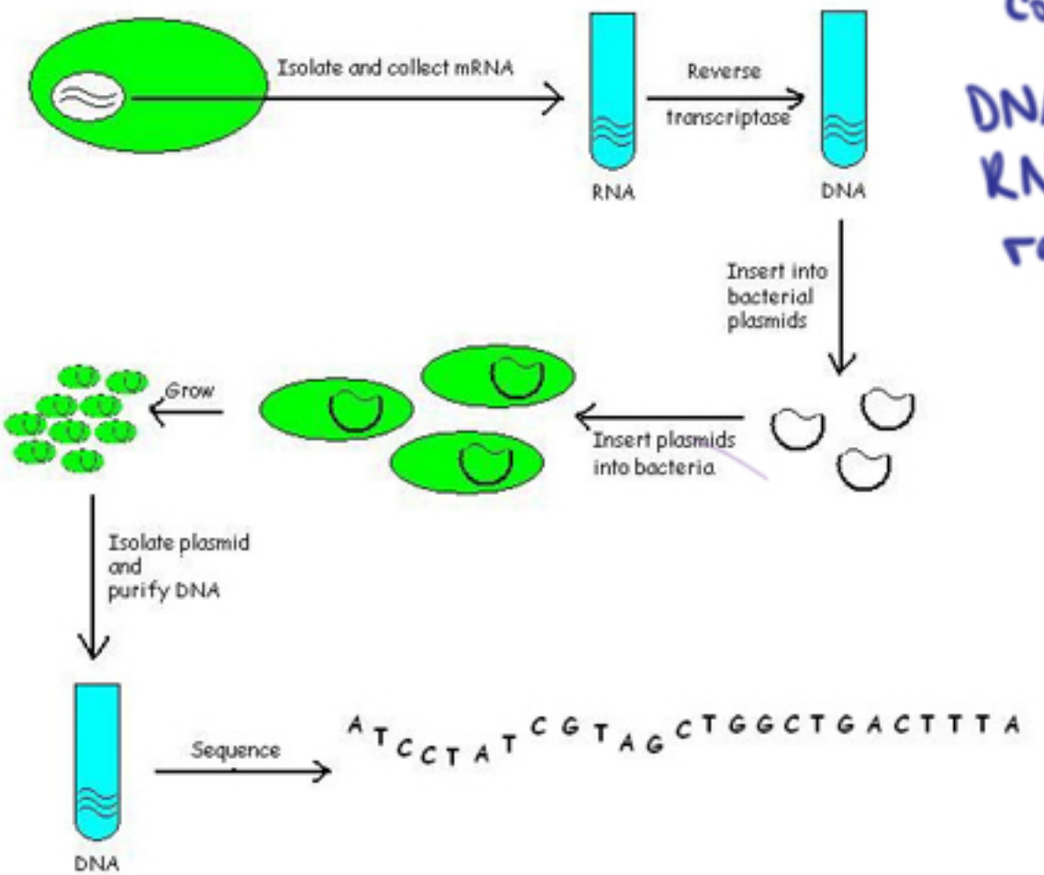


(restriction enzyme)

DNA Library

shotgun sequencing

Formation of a cDNA Library

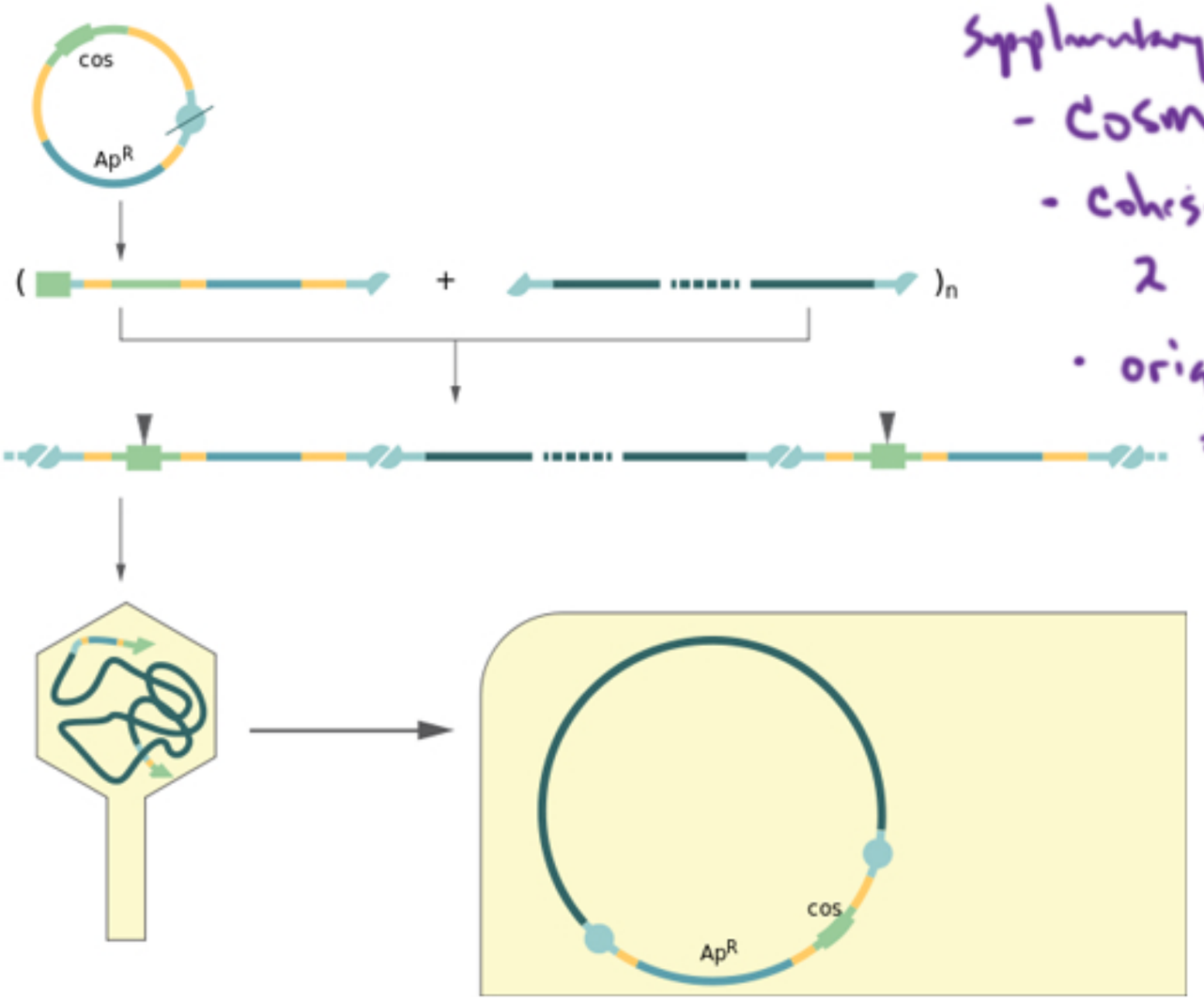


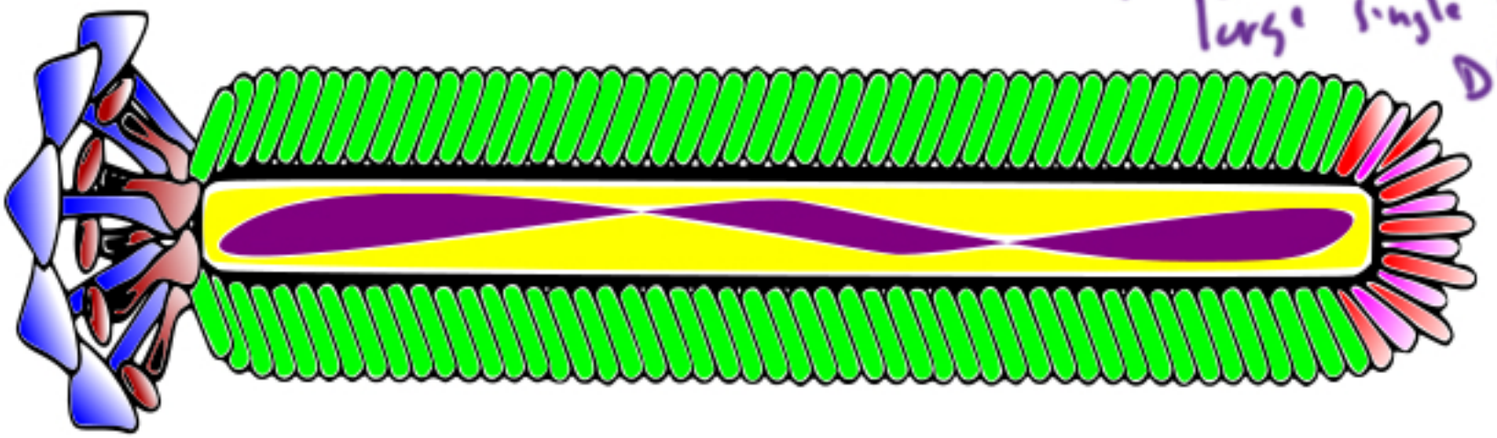
cDNA - Complimentary DNA

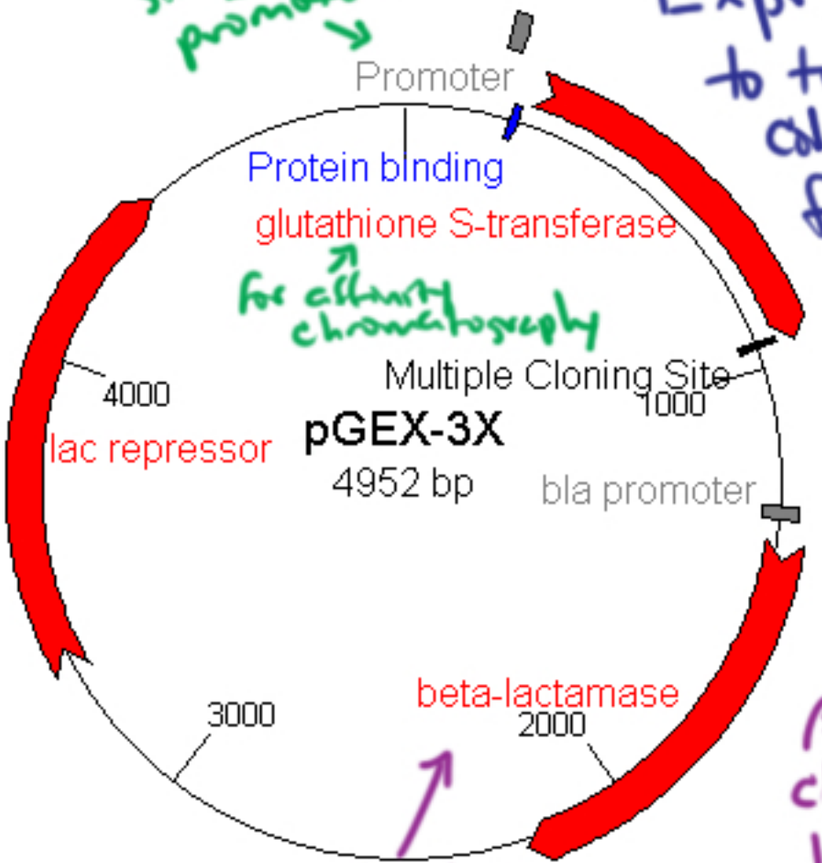
DNA found from RNA using reverse transcriptase

Supplementary

- Cosmid
  - Cohesion ends of λ phage
  - origin of replication of a plasmid.




phagemid -
large single stranded
DNA



strong promoter

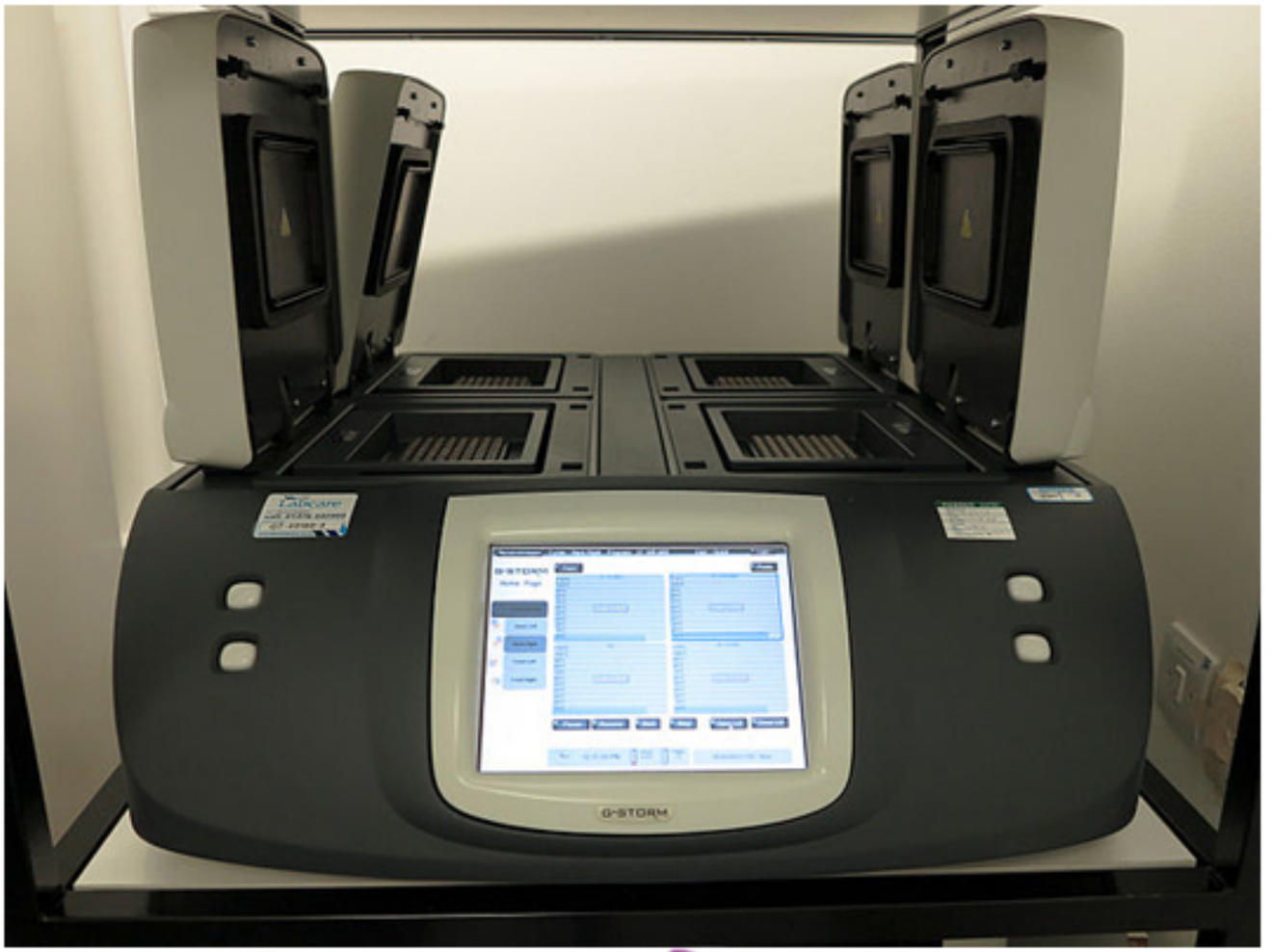
Expression vector to turn a bacterial colony into a protein factory

for affinity chromatography

can control rate with lactose levels

β lactams - class of antibiotics like penicillin

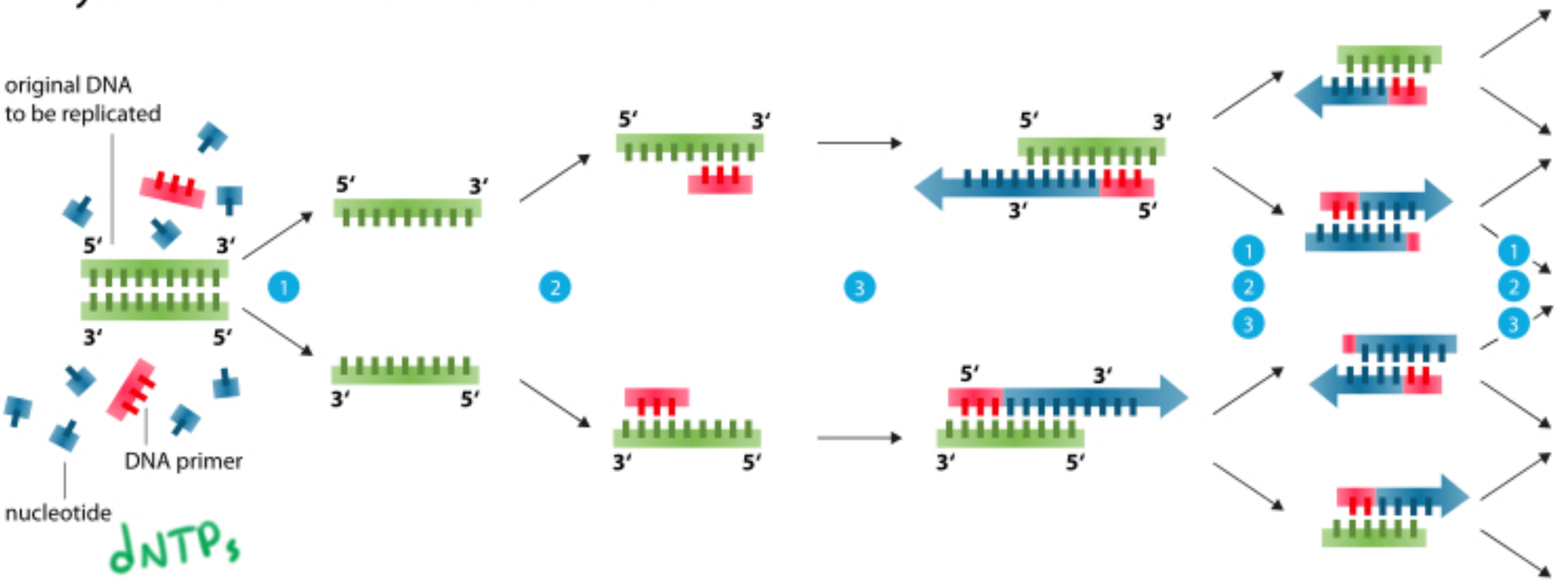
antibiotic resistance for screening



- denature
- anneal primers
- elongate

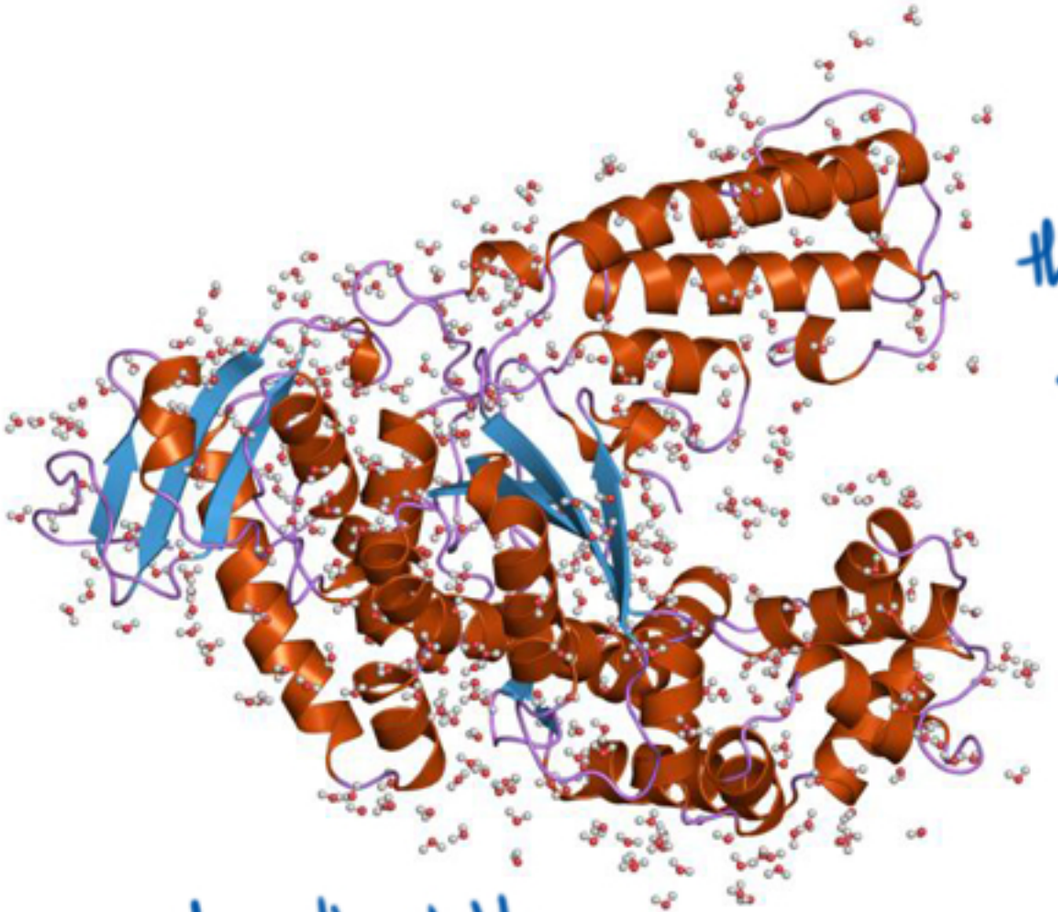
thermal cycler for PCR
(polymerase chain reaction)
to turn a small amount of DNA
into a large amount

Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

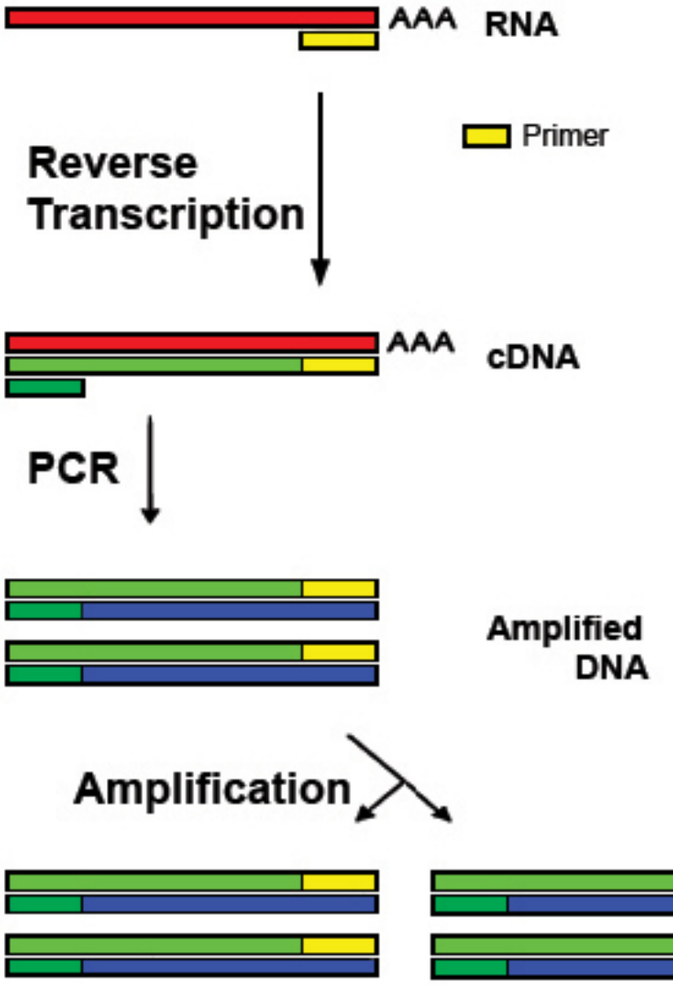
Taq polymerase



Taq thermoaquaticus

- bacteria discovered in oceanic hydrothermal vents

thermally stable DNA polymerase



RT PCR

↑

reverse transcriptase

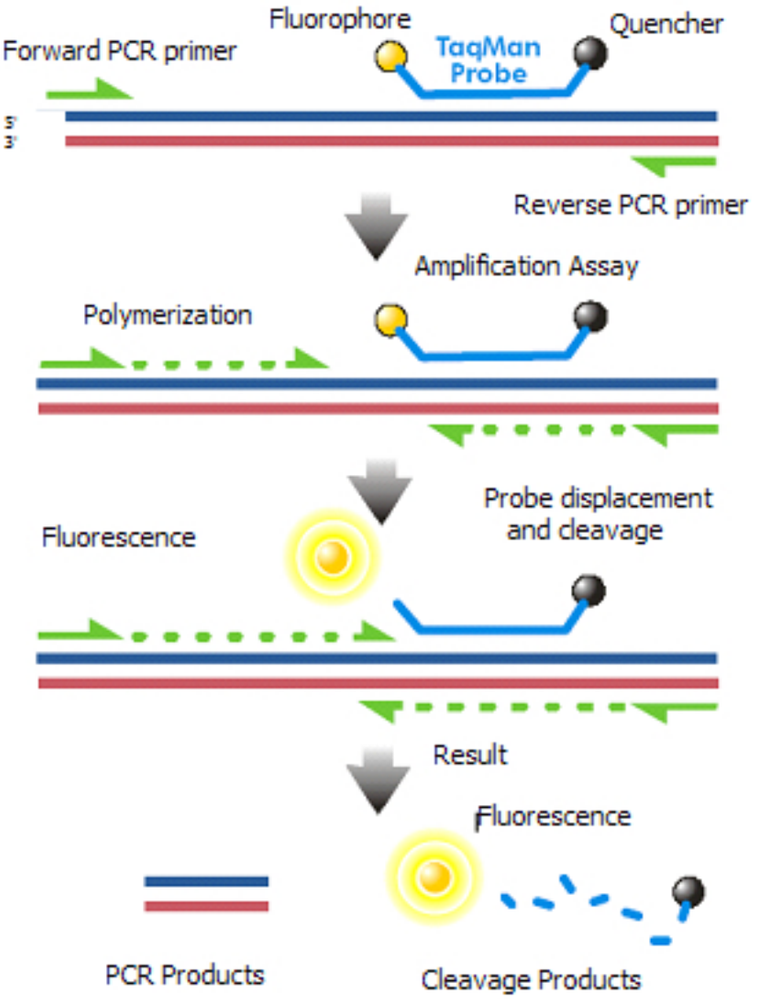
(RT is not 'real time')

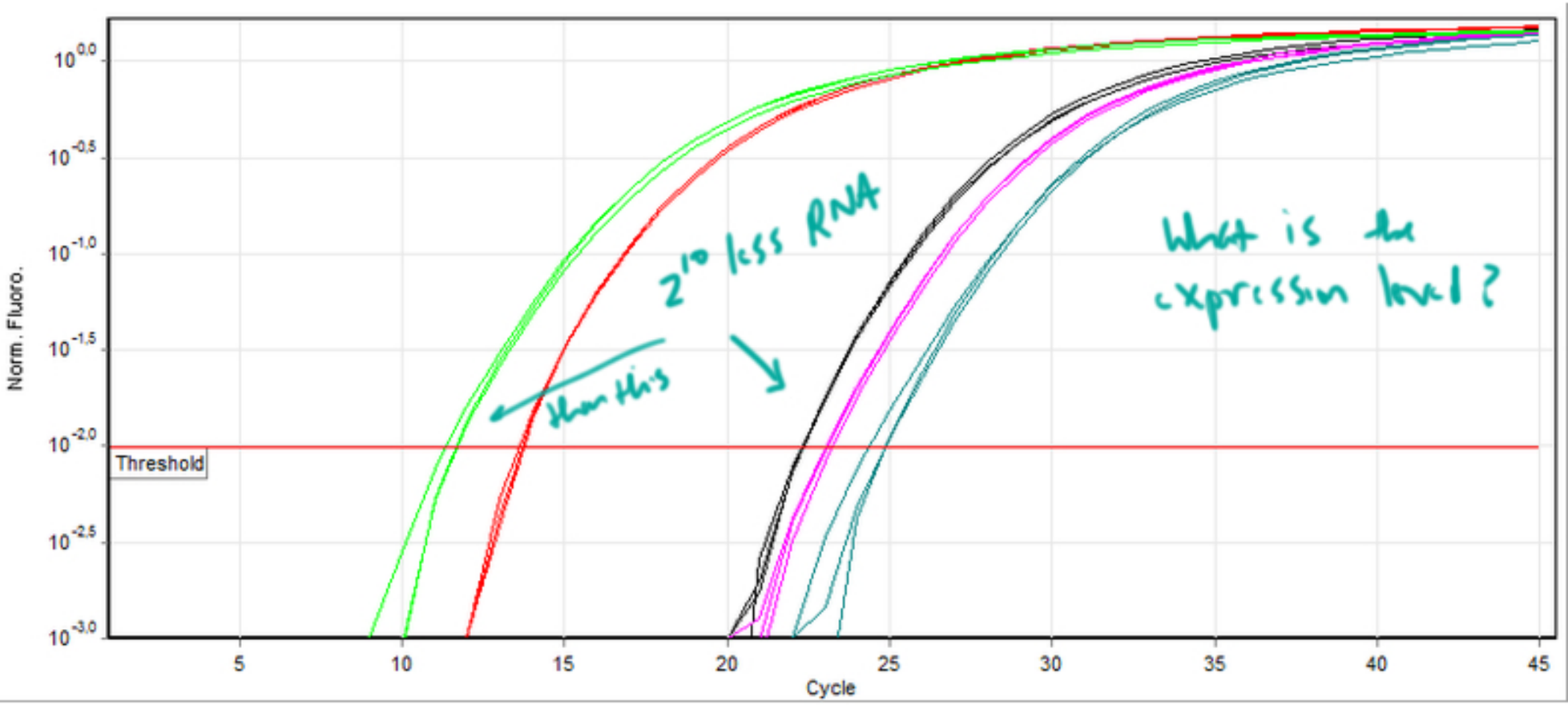
Real Time PCR

"Quantitative PCR"

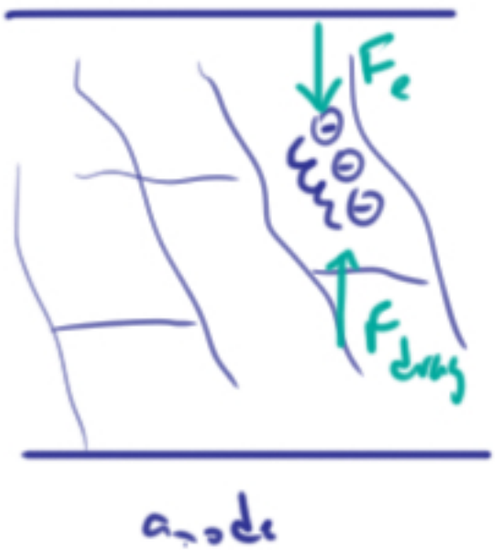
How many cycles does it take to achieve threshold of detection?

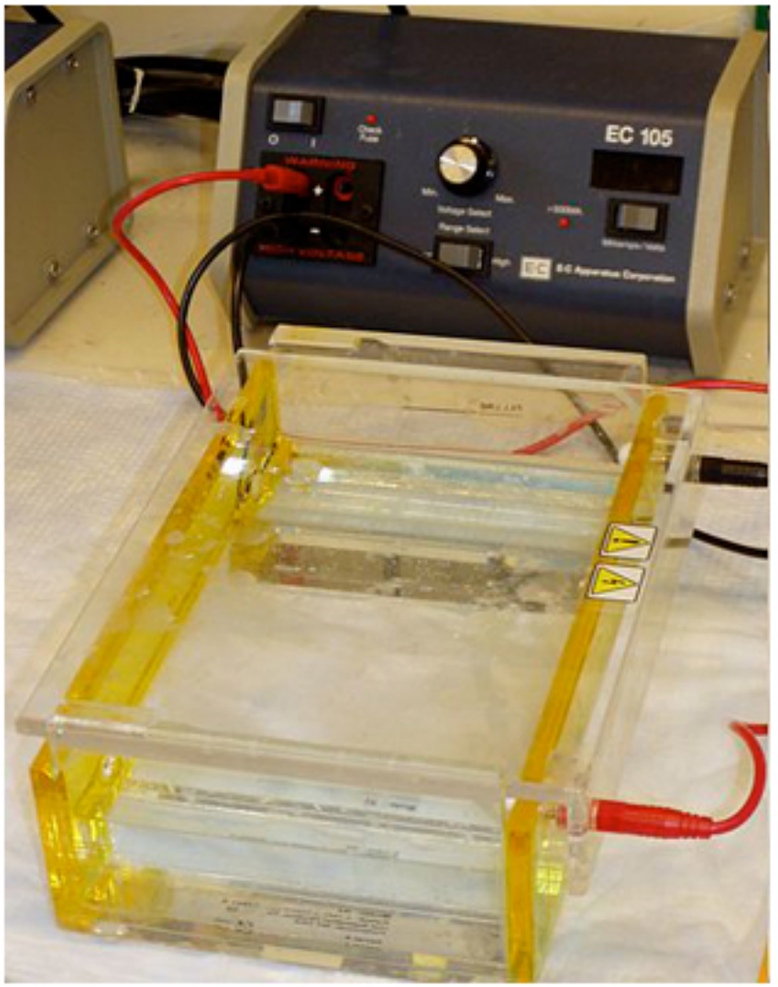
This depends on how much you started with.





Data from Real Time RT PCR





DNA ad RNA electrophoresis.

PAGE - polyacrylamide gel electrophoresis

or

agarose (for bigger stands)

Denaturing (PAGE) urea - H_2NCNH_2 (O)

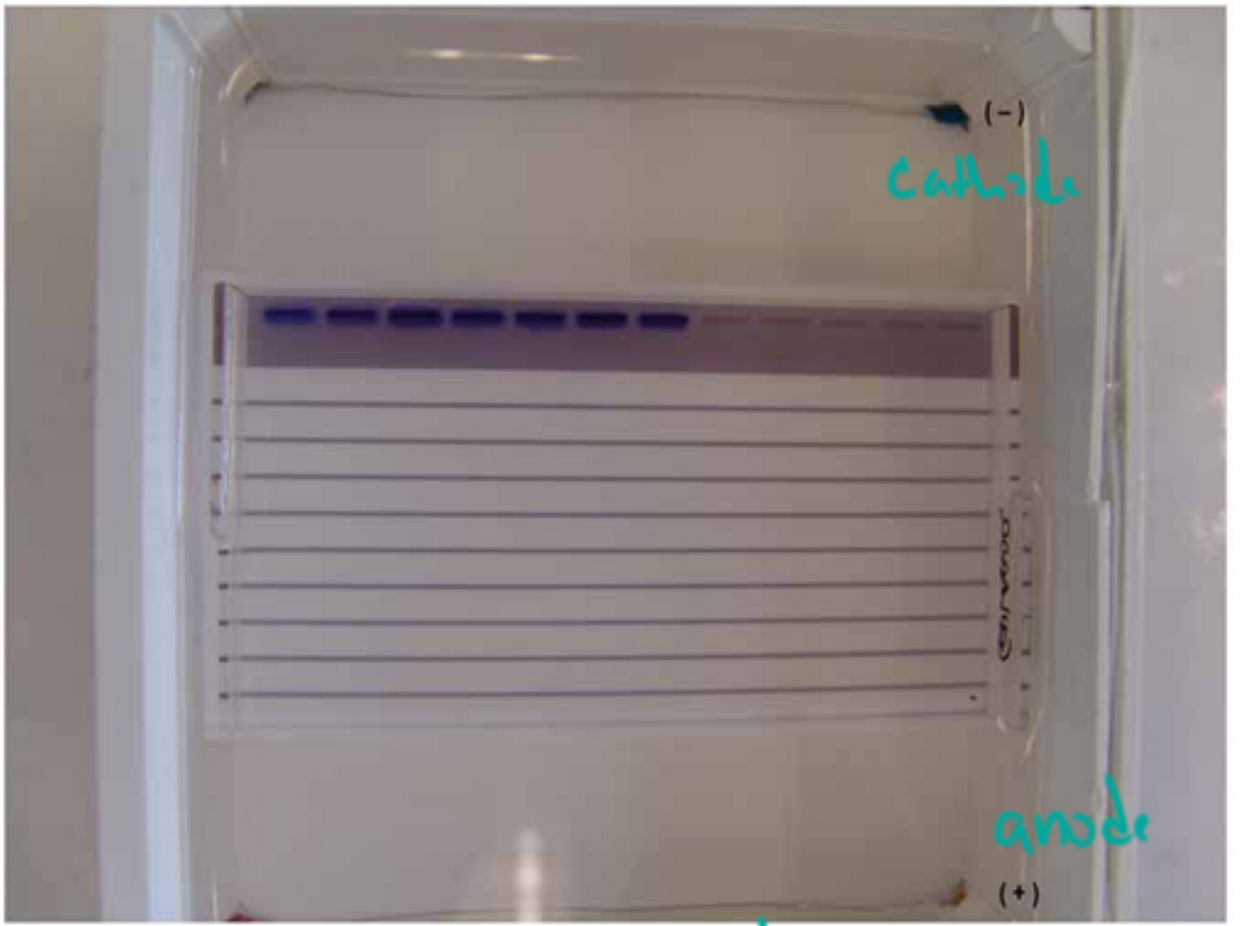
or

Native (double stand)

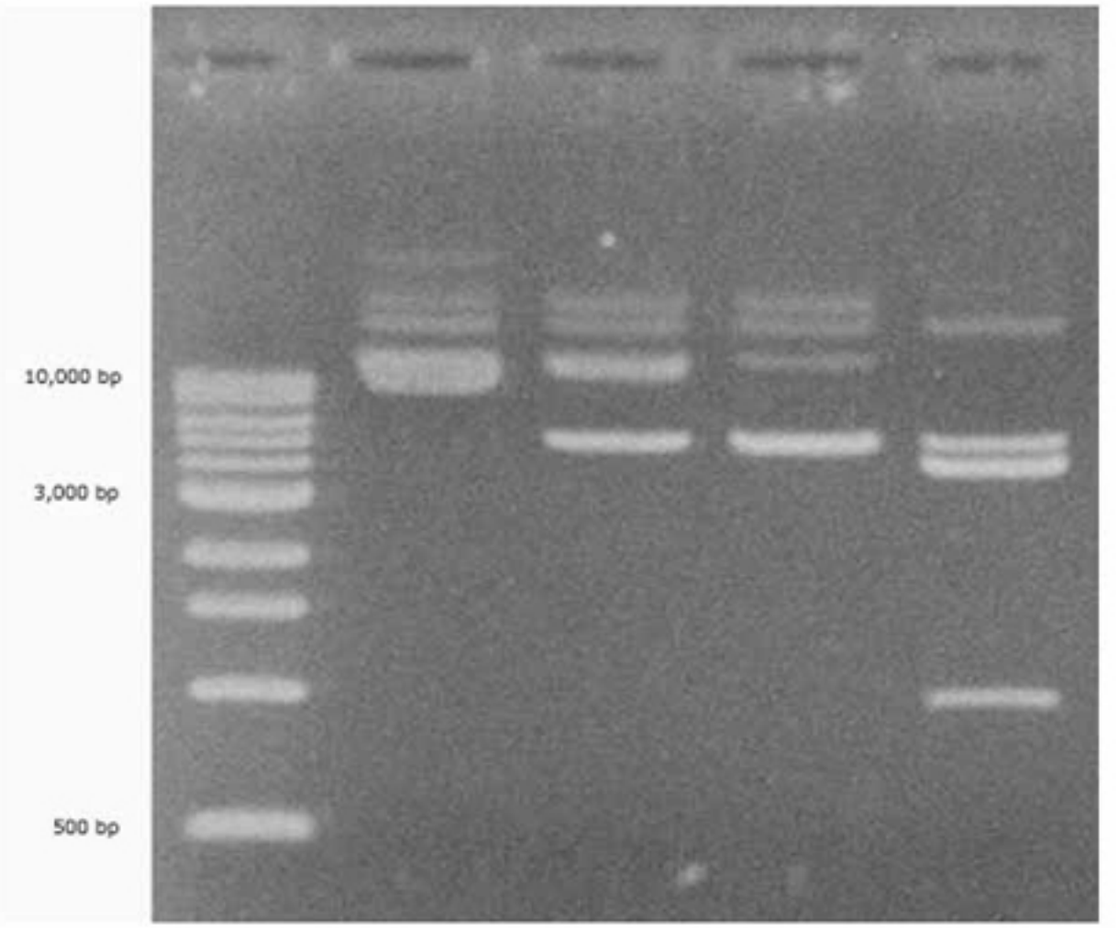
Post Electrophoresis - staining or autoradiography (^{32}P labeled DNA) photographic visualizing of radionuclids

-Blotting - Southern DNA

Northern RNA



agarose gel



agarose gel stained with ethidium bromide

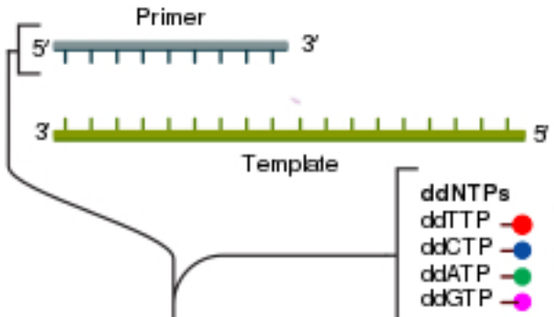
Sanger Sequencing

① **Reaction mixture**

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

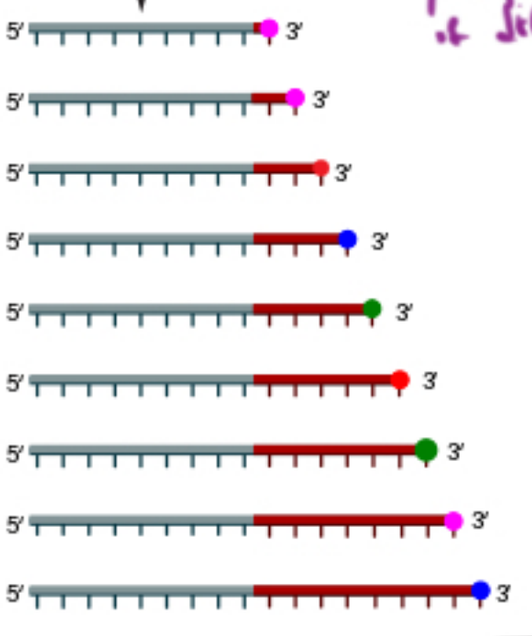
the regular nucleoside triphosphates

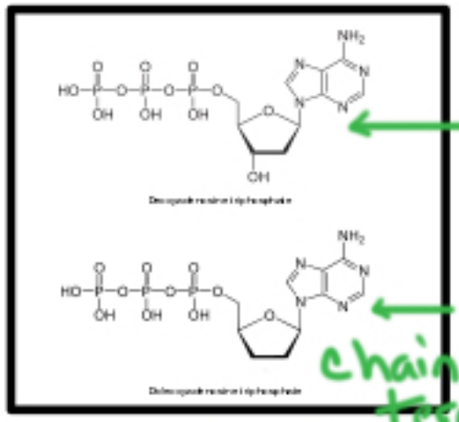
plus a small amount of these dye terminators



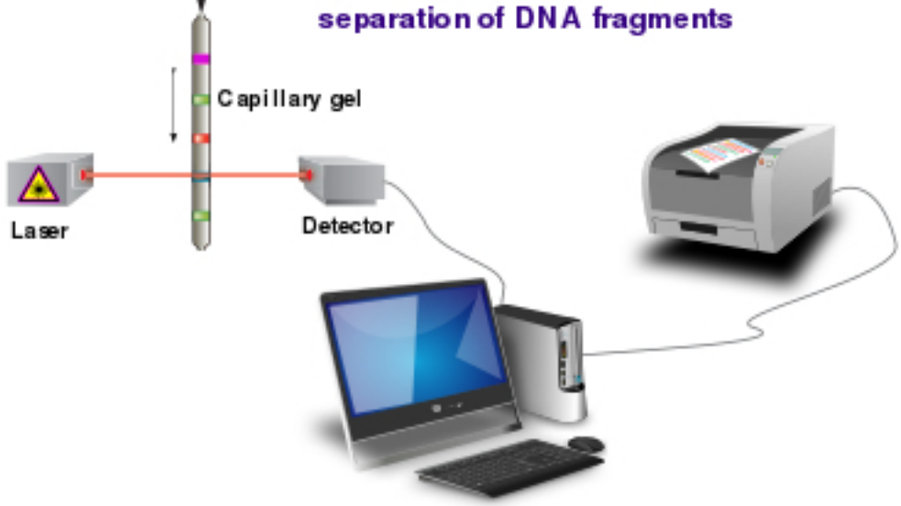
② **Primer elongation and chain termination**

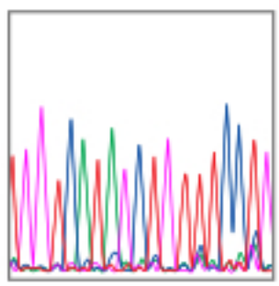
you get fragments of different length





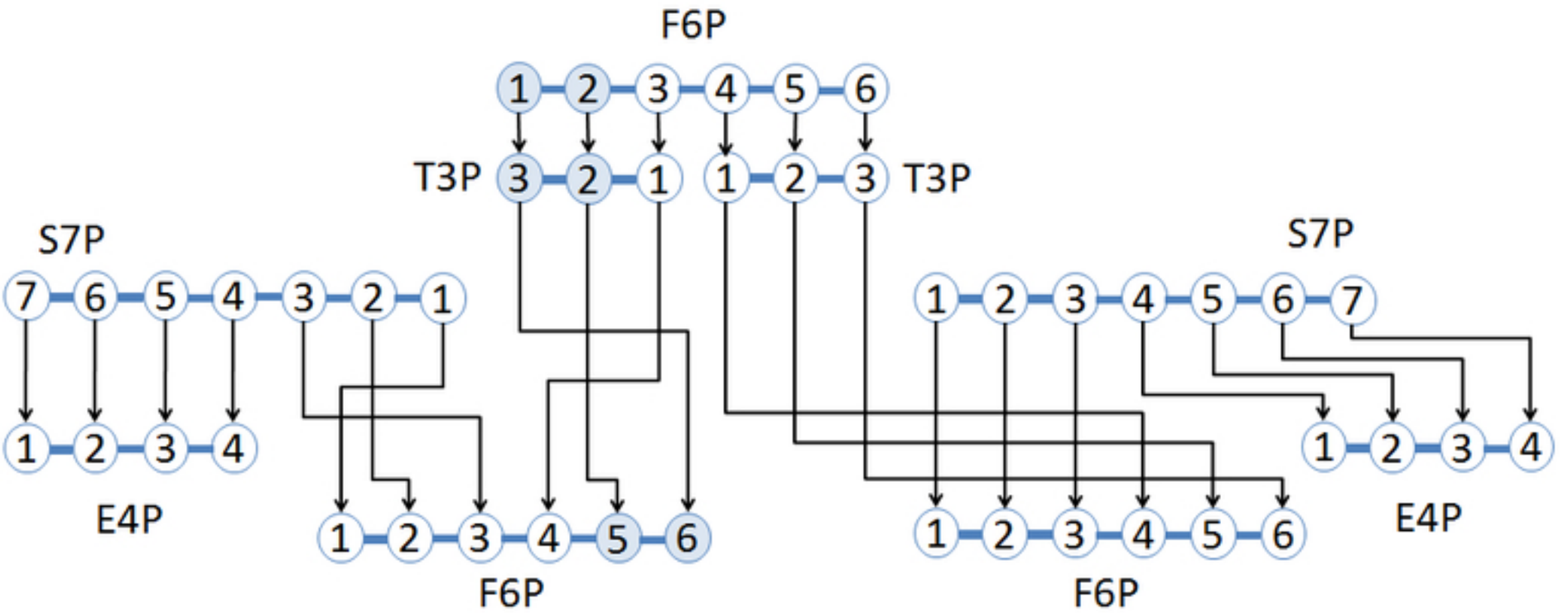
③ **Capillary gel electrophoresis separation of DNA fragments**





Chromatograph

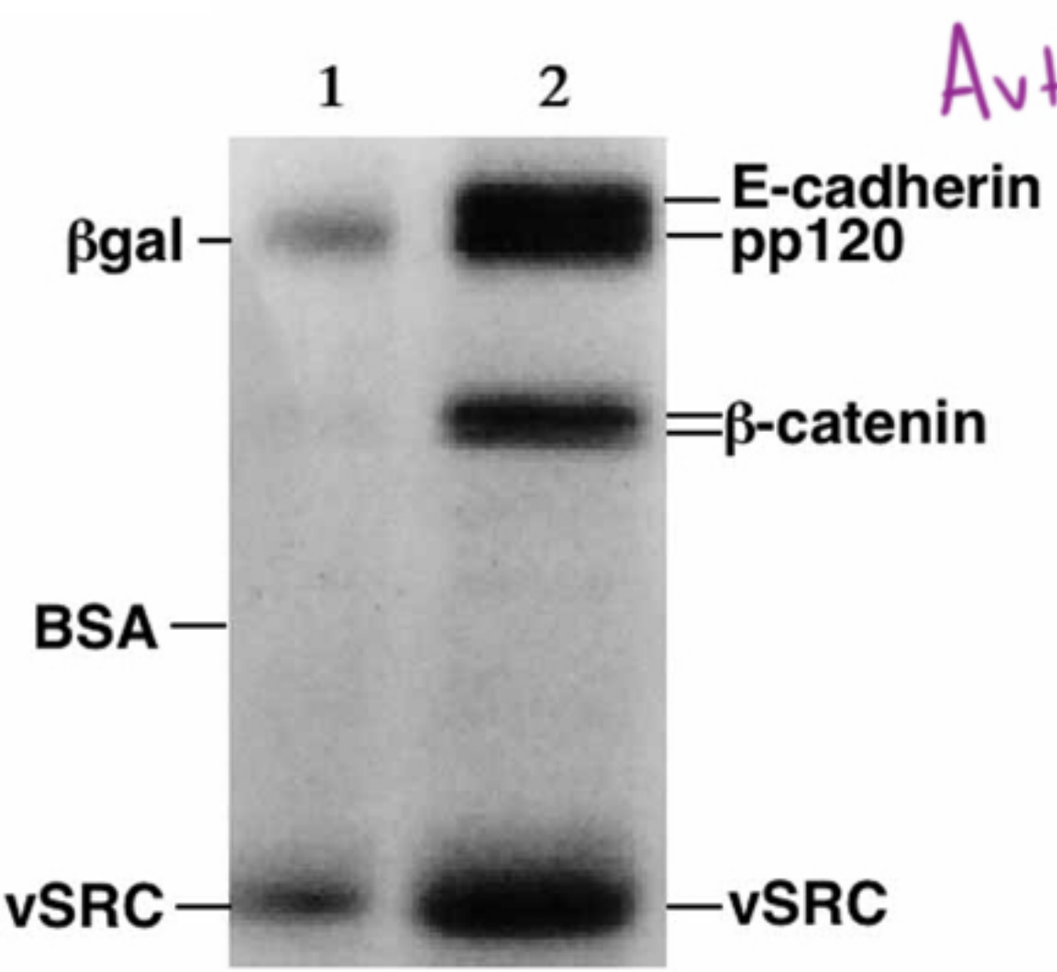
④ **Laser detection of flourochromes and computational sequence analysis**



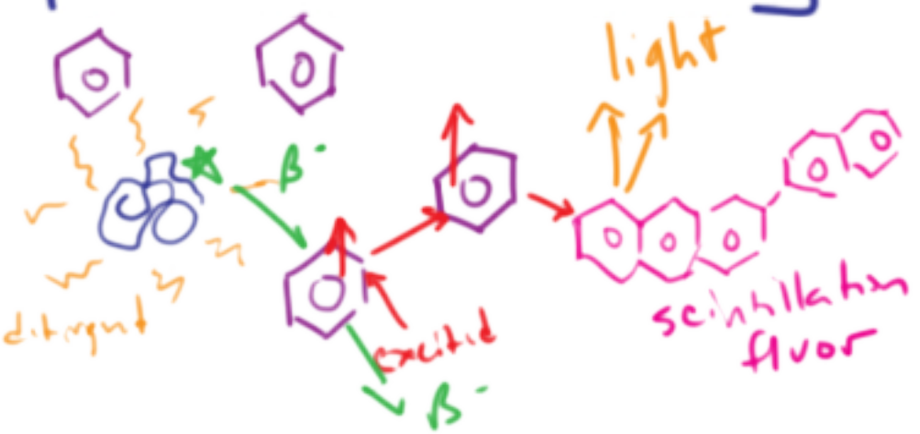
Radiolabeling

- β^- emitters
  ^3H, ^{14}C, ^{32}P, ^{35}S
- β^+ emitter ^{18}F
- Heavy
  ^2H, ^{13}C, ^{15}N, ^{18}O

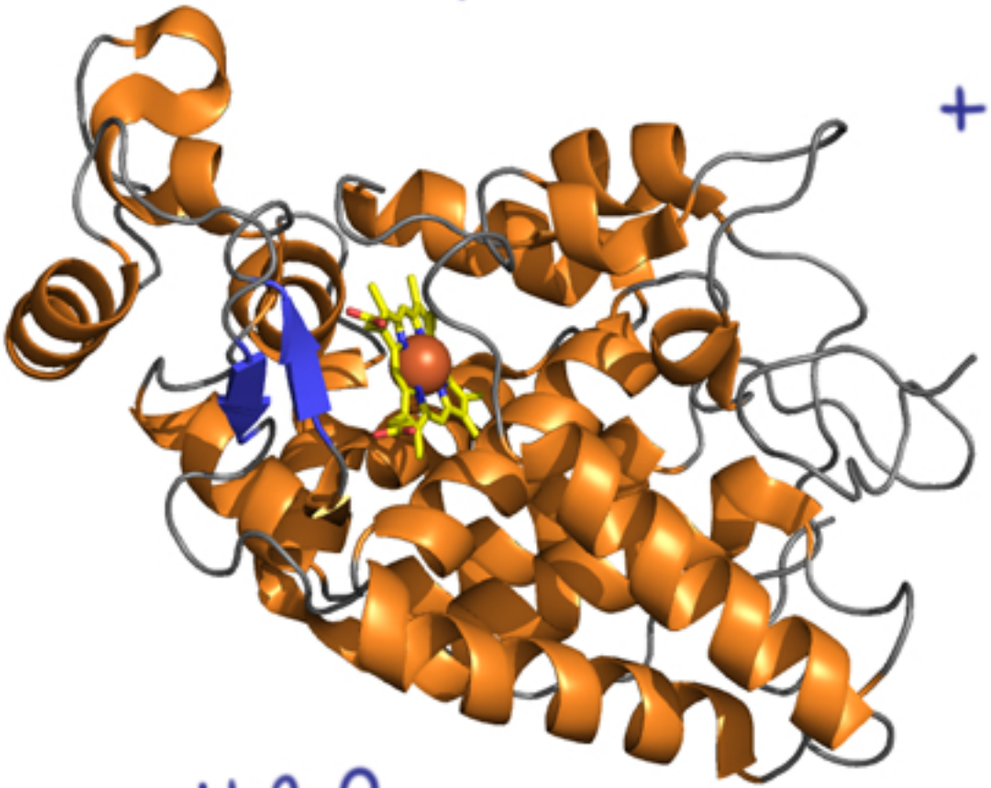
Autoradiography



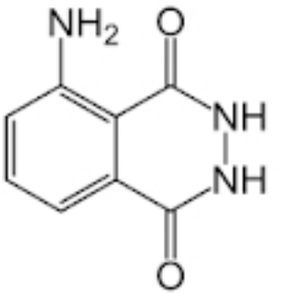
Liquid Scintillation Counting



Enzyme Label



+



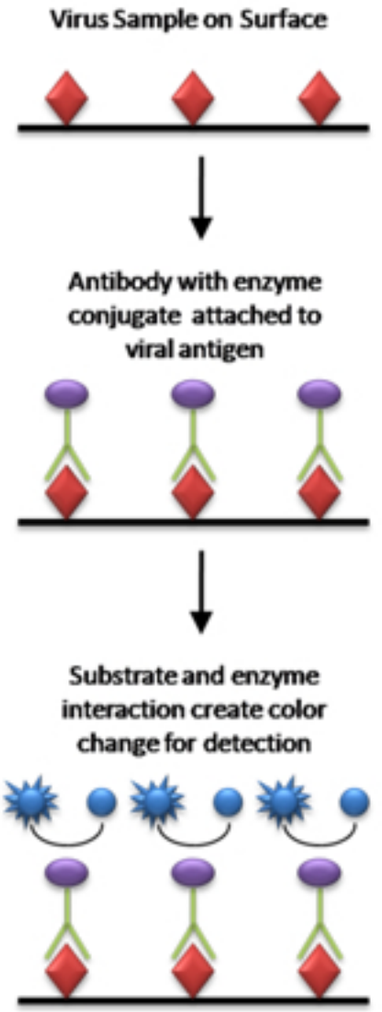
→ light

luminol

often on secondary antibody in Western blotting

HRP

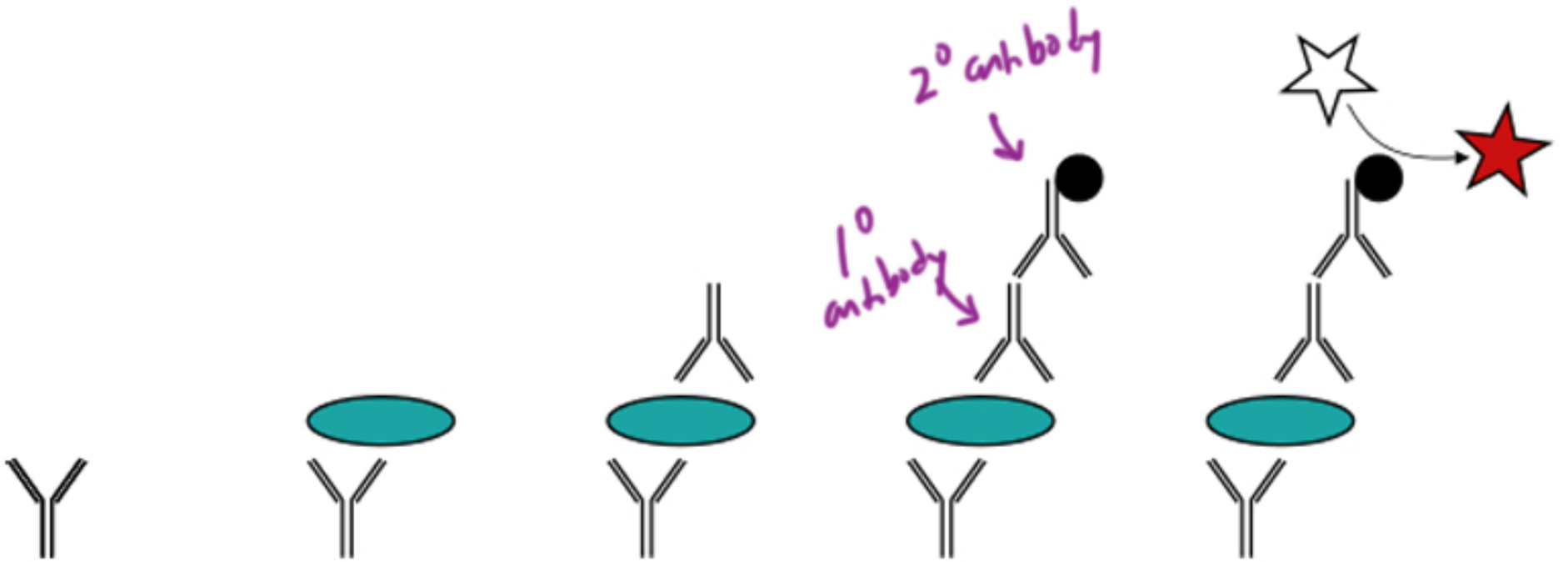
Horseradish Peroxidase



ELISA

enzyme linked immunosorbent assay

(1) (2) (3) (4) (5)



making monoclonal antibodies.

- harvest a B cell from rat thymus
- fuse this with multiple myeloma cell such as HLA (Henrietta Lacks)
- creates a hybridoma - leads to an antibody producing cell culture