TECHNIQUES IN MOLECULAR BIOLOGY:

DNA HYBRIDIZATION & CLONING

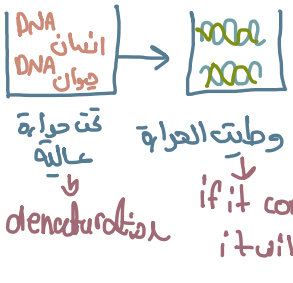
Course: Molecular Biology (BIOL333)

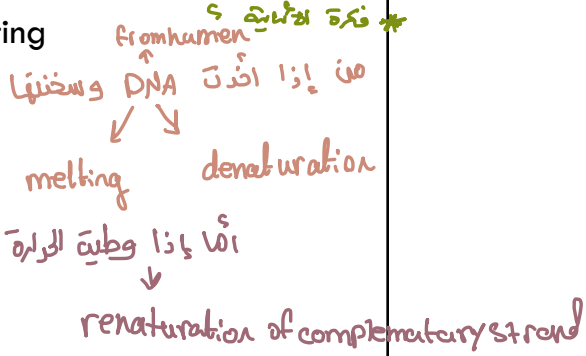
Instructor: Dr. Mahmoud A. Srour

Textbook:
Watson J, et al. (2014). Molecular Biology of the Gene, 7th ed. Chap 7

DNA hybridization

- Hybridization: the technique wherein renatured DNA is formed from separate single-stranded samples
- Applications> nucleic acid blotting
  - Southern blot hybridization
  - Northern blot hybridization





Southern Blot → DNA Analysis

- Described by Dr. Edward Southern (1975)
- Used to identify particular DNA fragment
- **Method**
- Digest and electrophorese DNA on agarose gel
- dsDNA in gel is denatured using alkali (NaOH)
- Transfer from gel to positively charged membrane > "imprint" or "blot"
- Immobilize the DNA to membrane by UV-cross linking
- Detect with a labeled probe (complementary to a sequence within the gene of interest)> hybridization
- When X-ray film is exposed to hybridized membrane > autoradiogram

Northern Blot → RNA Analysis

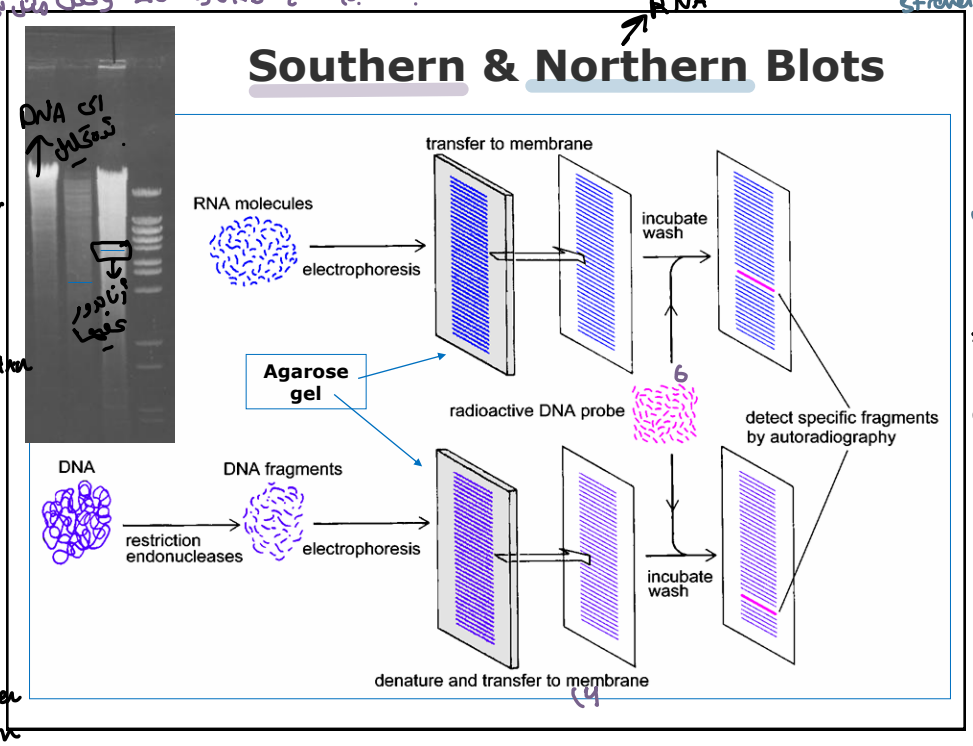
- Used to identify particular RNA fragment
- RNA are short (typically <5 kb) are not digested
- Method is similar to Southern blot
- Applications: Study gene expression or quantify the mRNA level of a specific gene
- Can study one gene at a time

weston blot → proten analysis

• DNA molecules large → fragment DNA
agarose gel ... 20-50kb

• RNA molecules small less than 5kb
RE ... already → small → single strand

Southern & Northern Blots



1) DNA ... RE
2) after Agarose gel
3) NaOH alkaline solution → because it double strand
→ denaturation → single strand
4) Nitrosilose membren
5) UV light ... to croslinking between DNA and membrene
↳ DNA → covelently crosslink

1. electrophoresis
2. Nitrosilate membren
3. add probe
4. hypredisation
5. autoradiography

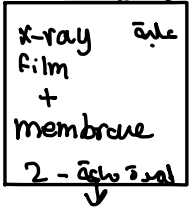
xprob → DNA because more stable

6. add probe ⇒ single strand complementary → hypredisation
200-800

7. take the membrane → autoradiography ← "xray-film"

DNA hybridization (animation)

- https://highered.mheducation.com/sites/9834092339/student_view0/chapter17/dna_probe__dna_hybridization_.html
- https://www.youtube.com/watch?v=yyLDwe_HXU0



Southern Blots

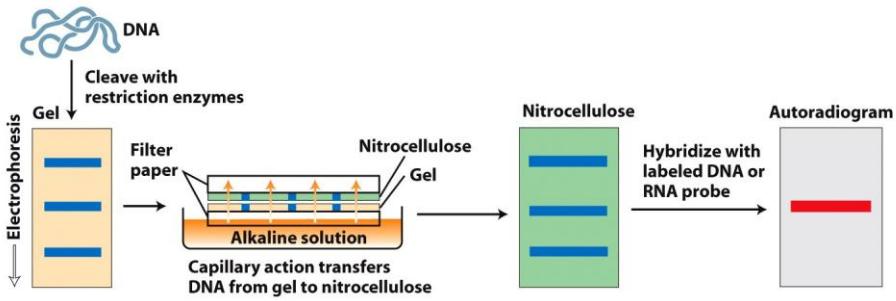


Figure 5-26
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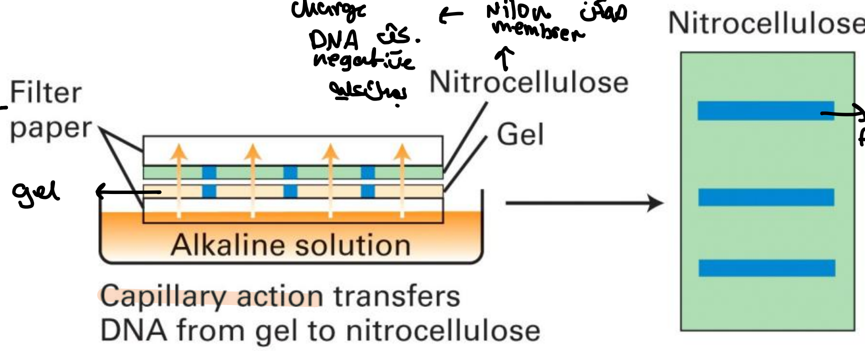


positive charge ← "Nilon" membren ممكن
DNA كث. negative بيمسك عليه

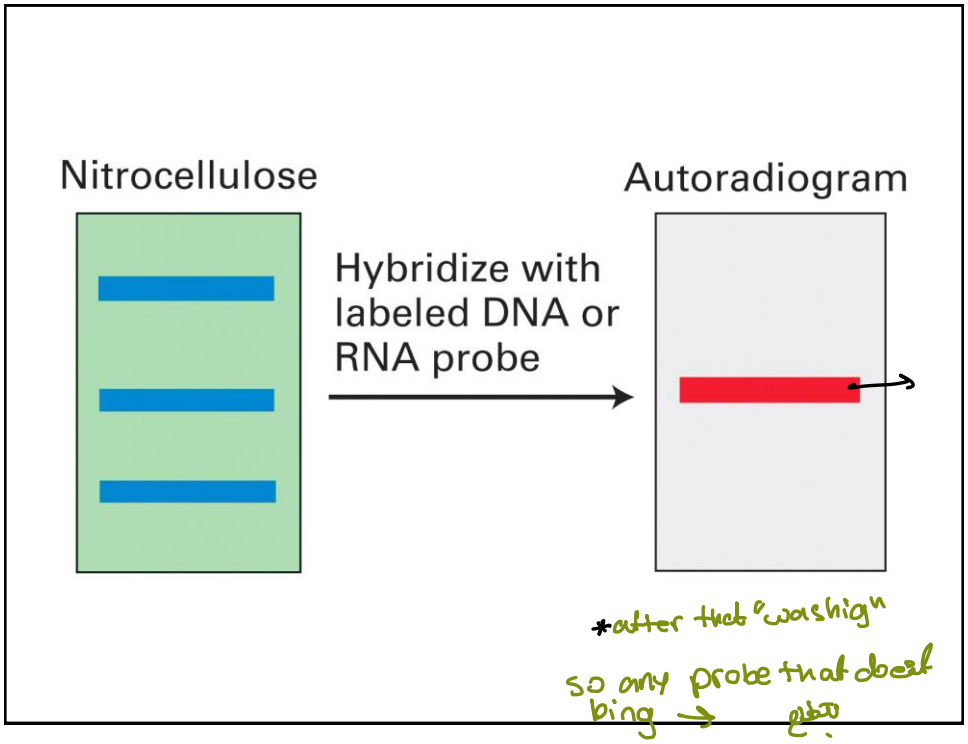
1) عشان تسحب المحلول "membren" خلال gel

gel

→ DNA fragment.

وبتالي من خلال "buffer" by capillary action
2) Buffer جزيئات تستطع تدخل 2) DNA molecules تتحرك هاي
↓
gel يتنقل من
↓
membran → تقوبجزتر فبعلق فيها

2) after 14-18 hr 3) UV
لفصلهم عن بعض
لمه ← طبقة الاكل عن agarose gel

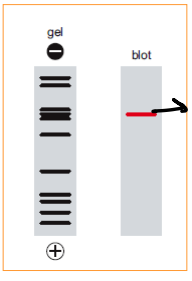


*after the washing
so only probe that doesn't bind → يطلع

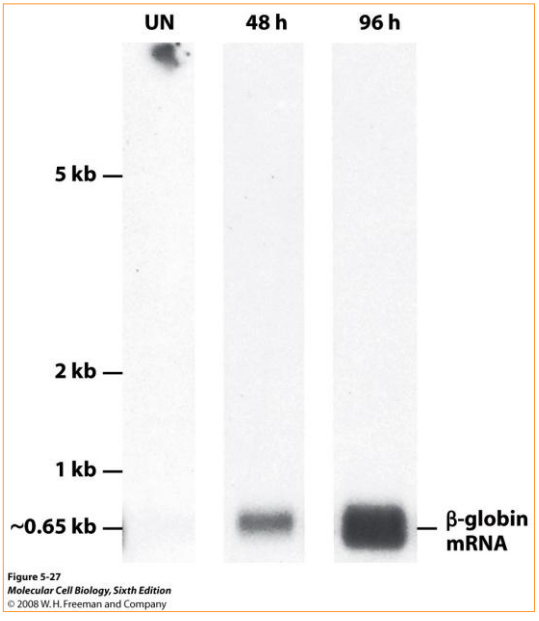
يمكن ارتباط probe positive

Northern blotting
> autoradiogram

Southern blot > autoradiogram



ال نبحث عنها



Nucleic acid Probes

- Previously cloned genes
- Synthetic oligonucleotides or DNA/RNA fragment (complementary to part or all of target sequence)
- Radioactive labeled (^{32}P-dNTP)
- Nonradioactive labeled
  - DiG-dUTP
  - Biotin-dUTP

Nucleic acid Probes

- **The two basic methods for labeling probes are:**
- End labeling using T4 polynucleotide kinase:
  - adds the γ-phosphate from ^{32}P-ATP to the 5'-OH group
- Incorporation of labeled nucleotides into probe via PCR:
  - labeled nucleotides are usually nucleotides modified with either a radioactive atoms or fluorescent moiety.
  - Radioactive nucleotides typically have ^{32}P incorporated in the α–phosphate of one of the 4 dNTPs

*in two condition biotin, Dig → after adding antidig or streptavidin → "washing" after that we add the substrate to enzyme ← as result may appear a color yellow or blue ⇒ there is reaction and probe exist. صحيح

"membrane colorless"

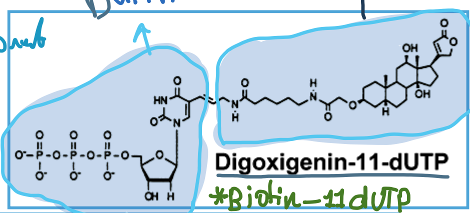
ليش استخدمنا ال dig على رغم من أنها معقدة؟ لأنها تبين لها antibody وصعب أعطل بالعادة

Labeled DNA Probes

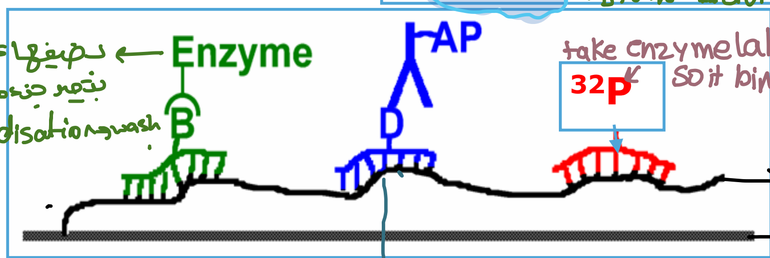
- Enzyme-Linked Probes
  - Biotin / streptavidin
  - Digoxigenin → chemical compound
    UTP/antibody
  - Radioactive ^{32}P → phosphate
    half life "short" so easy to get rid of it

DUTP nucleotide

*Dig-dUTP
إذا أعطينا لإنزيم "polymrase" في PCR so it will used instead of "U" or "T" in DNA condition so it will be part of DNA



*Biotin-11dUTP



1) PCR بنوظفه ← Enzyme
نجيب DNA جديد
2) hypredisation → wash
3- add Streptavidin
bind to antibody
may bind to enzyme "AP"

take enzyme labled probe so it bind to complementary regoin

→ single stranded DNA molecule
→ nitroselulose membrane ← covalenty bond

add antidig "antibody"
because we can't see the antibody we make a change on it we bind it to ← ال fragment في DNA or RNA molecules on membrane

↑ أو في Biotin
Alkalin phosphatase

Factors Affecting Hybridization

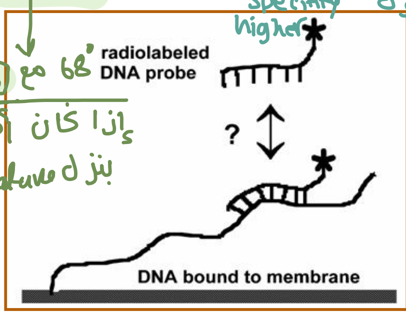
→ probe with Lable ← being or DNA with RNA bound molecules

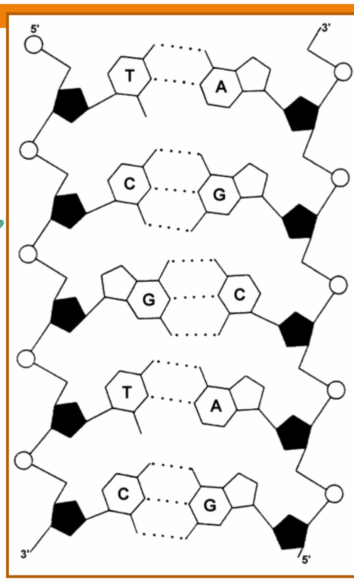
- Temperature → usually 68° above that probe بعد عمل ترابط template
- Ionic strength → spicifity high
- Probe length → 200-800 kb
- Probe mismatch → إذا كثرت علاقات فيها ليس في ال probe specifity أعلى كان كل ما قلت higher
- % GC

as high tem hybridization specifity ↑
على حرارة عالية بتعلق ال bio complementary إذا حرارة واطية بتعلق أحسن

probe أخذت وأعطيت فيه نقطة G بدل specific Strand في A حطيت ذهبت ارتباطه بنجاوزه أنقص temperature

50% مع 68°
إذا كان أقل من 50% بنزل temperature





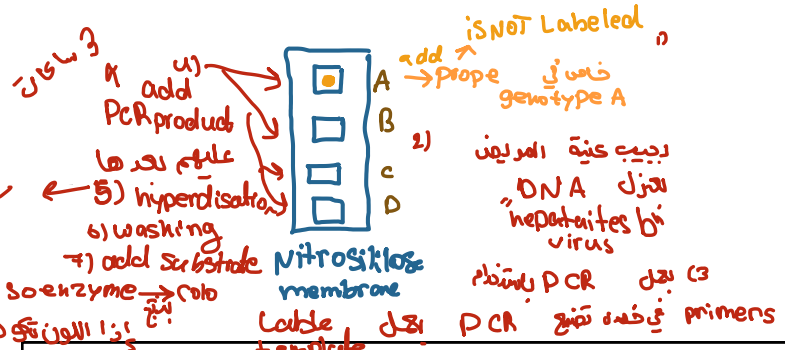
Other forms of hybridization

Dot/Slot Blot

- DNA samples are spotted directly on the membrane and No electrophoresis is needed

Fluorescent in situ hybridization (FISH)

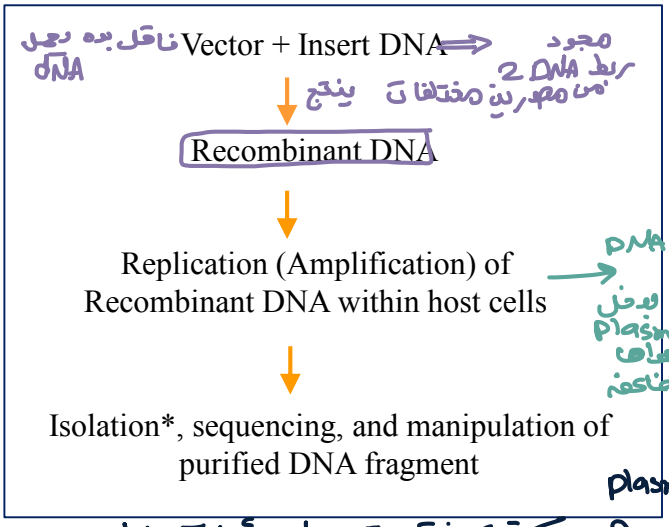
- combine with microscopy to localize cells expressing gene



DNA Cloning

- DNA cloning: the ability to construct recombinant DNA molecules and maintain them in cells
- A variety of techniques, often referred to as *recombinant DNA technology* are used in DNA cloning
- Isolation of a large amount of a single pure DNA molecule facilitates analysis of that DNA molecule.

DNA Cloning



Definition of terms used in cloning

- **Recombinant DNA:** any DNA molecule composed of sequences derived from different sources
- **Vector:** autonomously replicating genetic element used to carry an insert DNA (or cDNA fragment) into host cell for the purpose of gene cloning
- The most common host used is *E. coli* (genetically modified)
- **cDNA:** DNA molecule copied from an mRNA by reverse transcription

Recombinant vectors:

- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- ***E. coli* plasmids**
- Phage lambda
- Cosmids (phage lamda + plasmid)

- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Bacterial plasmids

- Occur naturally in bacteria (plasmids also present in single-cell eukaryotes, e.g, yeast)
- Circular dsDNA
- Autonomous replication
- Extra-chromosomal elements
- 1-200 kb size range
- Present in multiple copies per cell
- Transmitted during conjugation
- Exist in parasitic or symbiotic relationship with host cell (e.g, Antibiotic resistance).

Class activity?

- Why bacterial genomes and plasmids exist in a circular configuration??
  - Protection from degradation

"تسمية بلازميد" ← "pClass333" بنط "Small p" بعدها بنط تو بدي تطعليه بتعرف الاسم

Engineered / Recombinant plasmids

modification ← plasmid أخذ → جاهز لـ cloning

as larger ashard towork with →

عشان هيك بشعاب الأجزاء بك مش مفيدة إلي وهي مفيدة لبكتريا مع الأخذ بالاعتبار أنه في أجزاء مهمة لعملية as cloning vector

- **Basic elements of recombinant plasmids:**
- Small in size >1.2-3 kb → عشان أقدر أخد كلية قطع DNA بدي ايه صغير
- Origin of replication, specific DNA sequences of 50-100bp> (Ori) ← مكان إلي بيبدأ عليه DNA machinary
- Selection marker (antibiotic resistance gene)
- Polylinker/ multiple cloning site

plasmid universal cloning

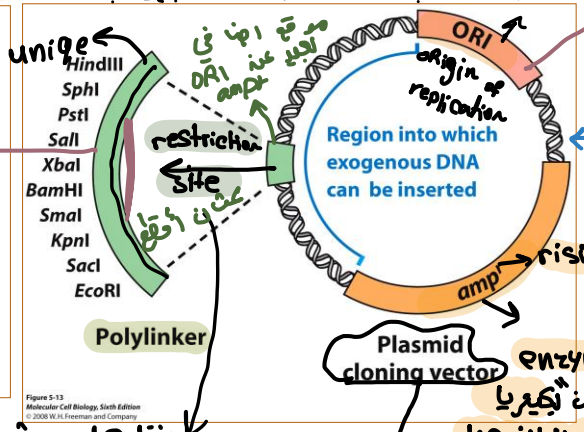
- Most vectors are derived from pUC or pBR322 → modification صار فيها
- Cloning capacity ~ up to 10kb (also up to 20kb or larger is possible, but is difficult) ← سعة

fake E.coli

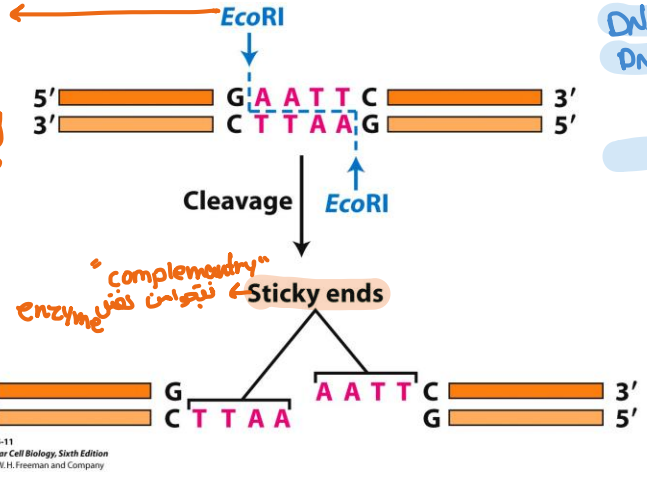
#PUC 18/19 → الرقمين إلي رح نشتغل معهم في المختبر

Basic components of an *E. coli* "cloning plasmid" (Vector DNA)

- Basic elements of a cloning vector:
  1. Origin of replication
  2. Selectable marker, usually an antibiotic resistance gene
  3. Polylinker, with one or more unique sites for REs



Cleavage of DNA by EcoRI



Ligation of restriction fragments with complementary sticky ends.

Ligation of sticky ends is more efficient than blunt ends.

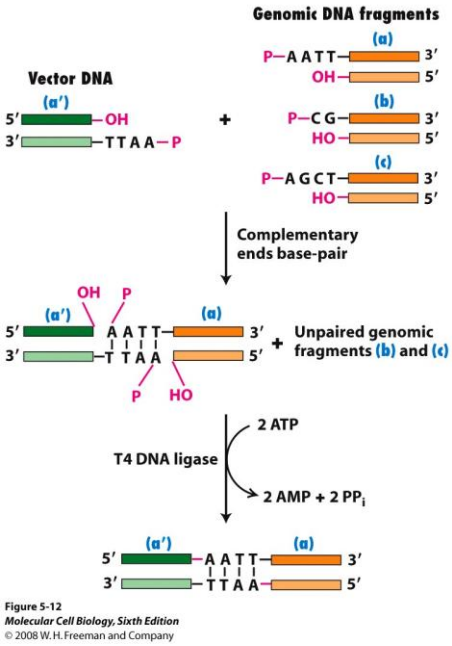


Figure 5-12
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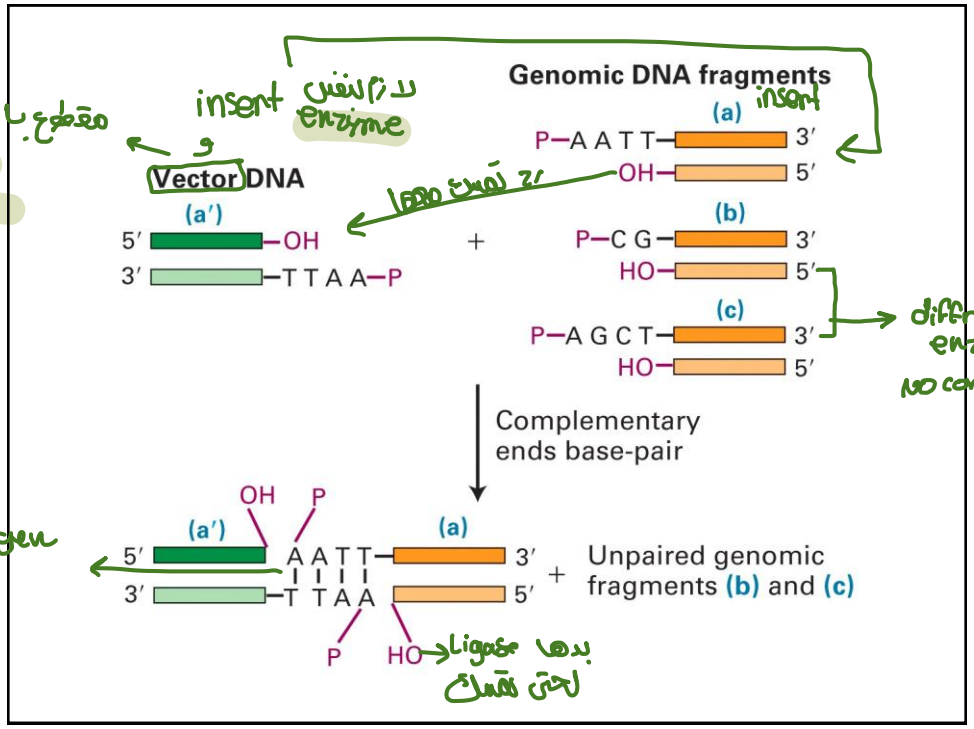
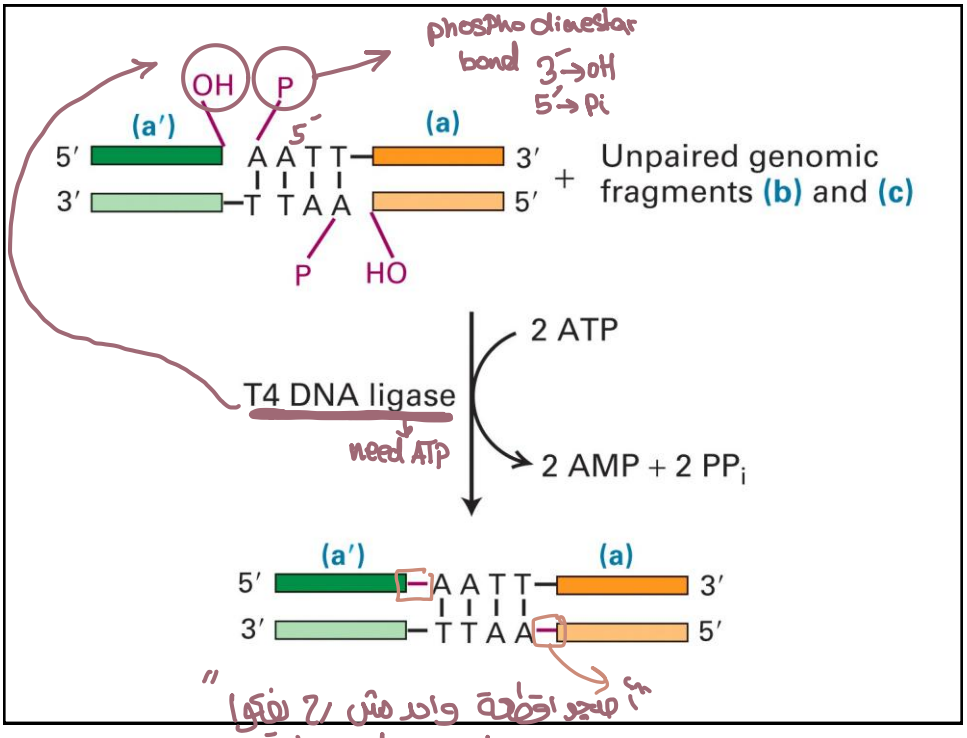
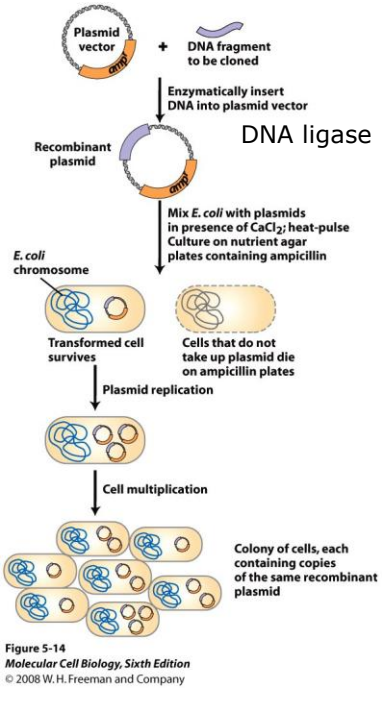




DNA cloning in a plasmid vector permits amplification of a DNA fragment



Plasmid preparation ←

(2)

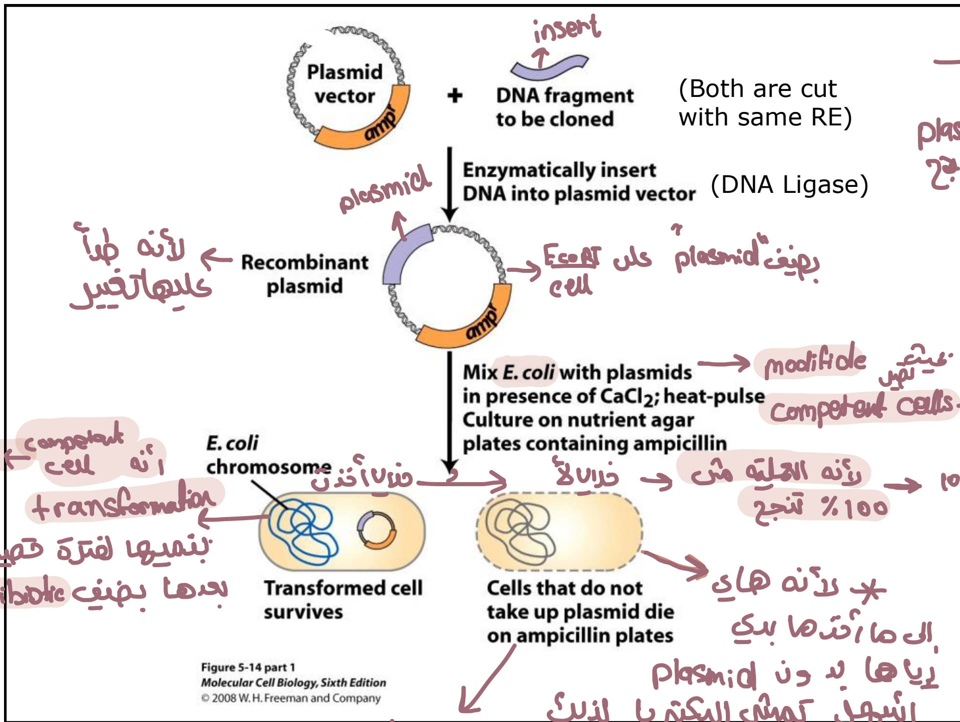

Plasmid vector
amp^r
insert
DNA fragment to be cloned
(Both are cut with same RE)
Enzymatically insert DNA into plasmid vector
(DNA Ligase)
plasmid
Recombinant plasmid
لأنه فيها عليها تغيير
بيضيف "plasmid" للـ Ecoli cell
Mix *E. coli* with plasmids in presence of $CaCl_2$; heat-pulse Culture on nutrient agar plates containing ampicillin
modified عشان تصير competent cells
يعني عندها القدرة تُوخذ foreign DNA
E. coli chromosome
take the plasmid
لأنه competent cell
transformation
بتميها لفترة قصيرة
بعدها بنضيف Antibiotic
خلايا أخذت
خلايا
لأنه العملية مش 100% تنجح
100 cell bacteria
30 → plasmid take
70 → X
Transformed cell survives
Cells that do not take up plasmid die on ampicillin plates
لأنه هاي الما أخذت بكيريا بدون plasmid
الأسهل تمشي البكتيريا لذلك

Figure 5-14 part 1
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هاي antibiotic تقتلها أما الثانية بتعيش

إذا ما اعملت selection وما أضفت ampicillin
بلاقي بكتيريا تاني يوم أنه اختفت
إلي مع plasmid وهي الي مطلوبة

resistant → selection على
so it named selection marker

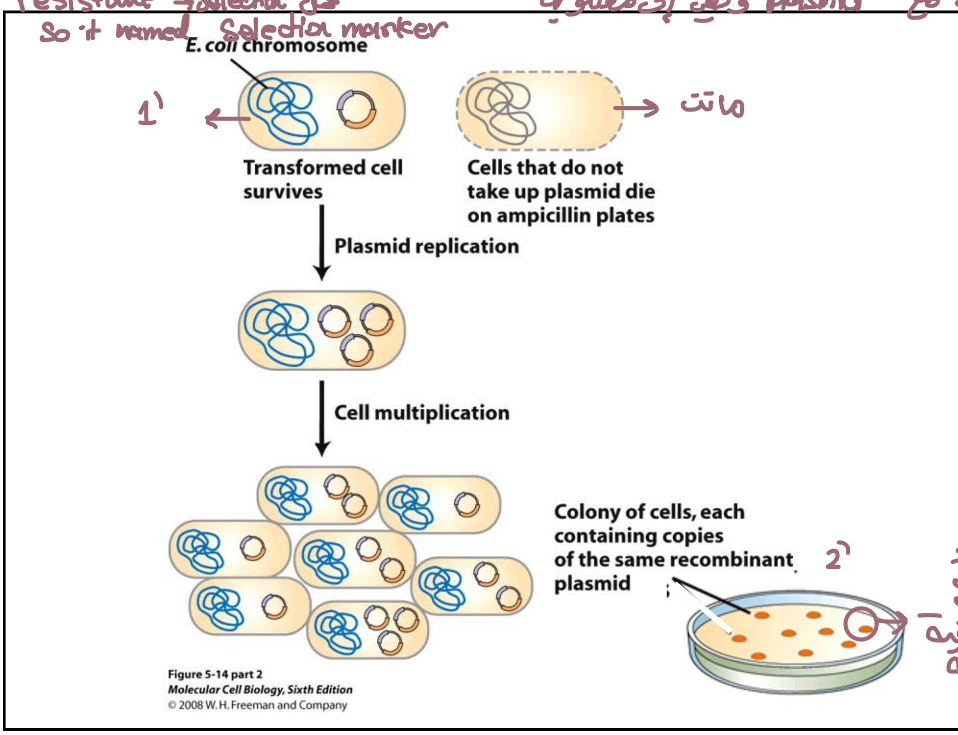
E. coli chromosome
1
Transformed cell survives
Cells that do not take up plasmid die on ampicillin plates
ماتت
Plasmid replication
Cell multiplication
Colony of cells, each containing copies of the same recombinant plasmid
2
بوخذ colony
بحضر منه هون plasmid
حصلت على كمية كافية من plasmid ← بعزله

Figure 5-14 part 2
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هدف من عملية تخزين plasmid

* take bacteria → add glycerol put in the frige -70°
بتالي صارت بكتيريا موجودة بلا زمن جاهزة اي وقت بدي اياه بطوله
فش داعي اعمل cloning مرة ثانية وهي مفرزة
او احطها في agar plate بيجمع على
مصدر دائم لـ plasmid

1. بوخذ bacterial cell وبنميها في مختبر لما تنمو الـ growth curve
log phase

washing بوخذها هي هون بغسلها
Ice cold cacl₂ →
Competent بعمل عدة مرات بتخلي بكتيريا تصير
وبحط معها كالسيوم كلورايد وبفرزها
بتخلي بعض Cation calsium بيمسك في السطح
plasmid — charge
Ca + بيمسك فيه وبدخله
Ca cell ← competent
بوخذها وبضيف عليها plasmid
وبضيف عليها ثلج بعدها بنقلها من ثلج و بحطها
على 42° ← 90 sec.
بعدها برجعها على ثلج "heat shock" بتعمل توبيخ في الخلية
"بالسطح وبلازميد له جزء منه بدخل" →

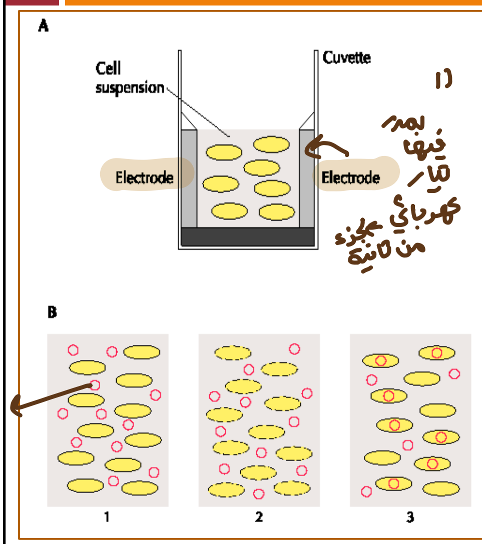
Genetic Transformation of Prokaryotes

- Transformation: the process by which a host organism can take up DNA from its environment
- **Two methods are commonly used:**
  1. Chemical transformation
     - Usually involves $CaCl_2$ and heat shock
  2. Electroporation ← effincy أعلى
     - Electric field mediated membrane permeabilization
     - 10-100 times more efficient that chemical approach

after heat shock بنقلها → "broth" → نغطيها اعادة احياء بدون antibiotic تعمل healing على 37° فيه
بعدها بحطها antibiotic بعدها فترة 2 تر تاج ← بتصلح الجدار و plasmid
ما بوخذ وقت يصنع resebint enzyme

Electroporation →

بحضر بكتيريا ولكن "shock" "electrical"



1) بمرر فيها تيار كهربائي بجزء من ثانية
2) نقوب صغيرة فيها بتالي تسمح complex + that on surface to enter the cell
3) بعدها تستعيد الجدار

- Cells suspended in DNA solution in cuvette between two electrodes
- High voltage electric field pulses administered → 400 volt → 0.01 second
  لو استمر 0.01 ثانية كان البكتيريا بتنفجر
- DNA migrates through HVEF induced openings in cells

Recombinant vectors:

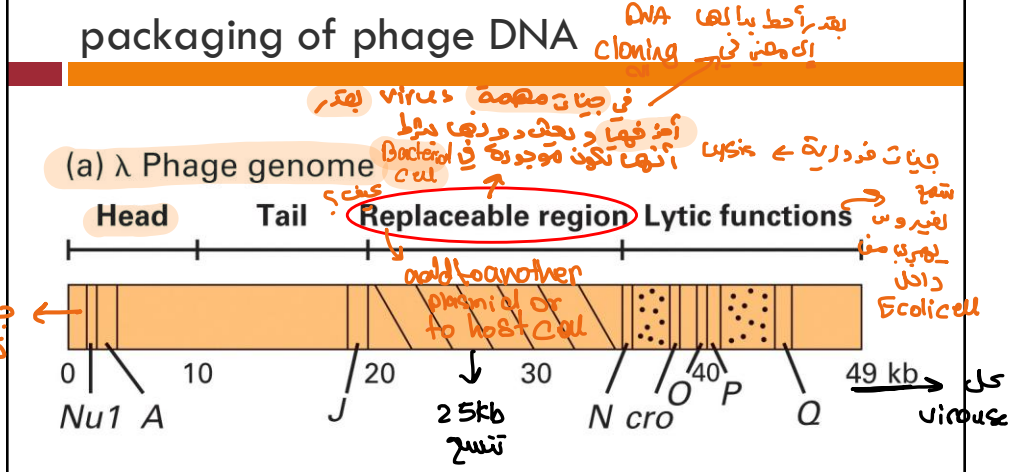
- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- *E. coli* plasmids
- **Phage lambda**
- Cosmids (phage lamda + plasmid)

- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Phage Lambda (λ phage)

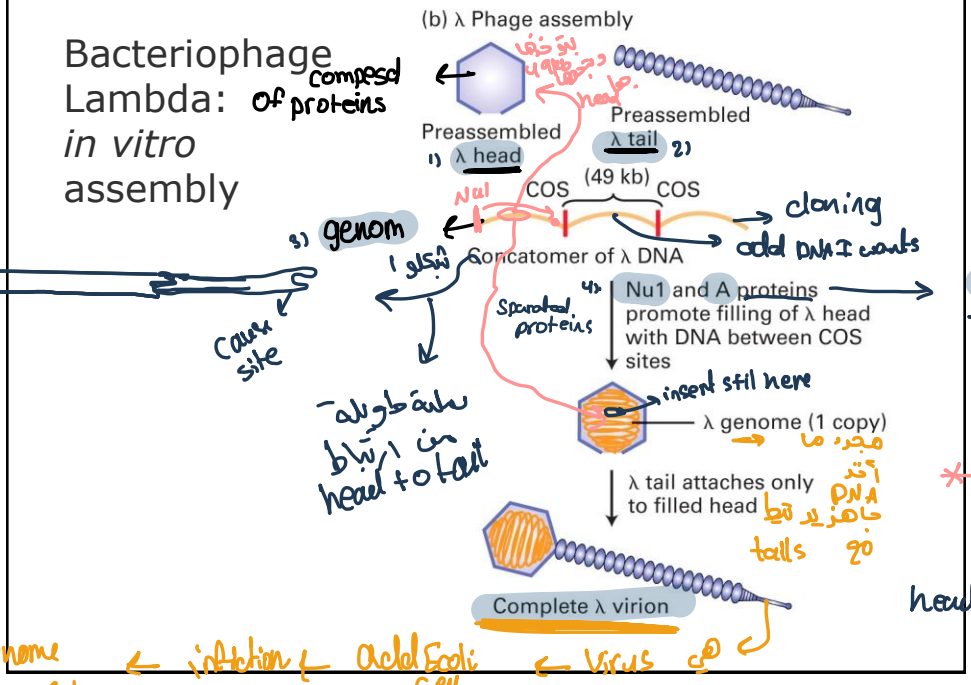
- Infection of E. coli by λ phage is 1000-fold more efficient than plasmid transformation
- Many more clones of phage can be grown on a single plate
- Infection and lysis of cells> ~100 phages /cell>plaques
- Can be assembled *in vitro*
- Cloning capacity ~ 25 kb

Bacteriophage Lambda genome and packaging of phage DNA



Some regions of the genome can be replaced by exogenous DNA (diagonal lines) or deleted (dotted) without affecting the ability of phage to infect host cells and assemble new virions.

Bacteriophage Lambda: *in vitro* assembly



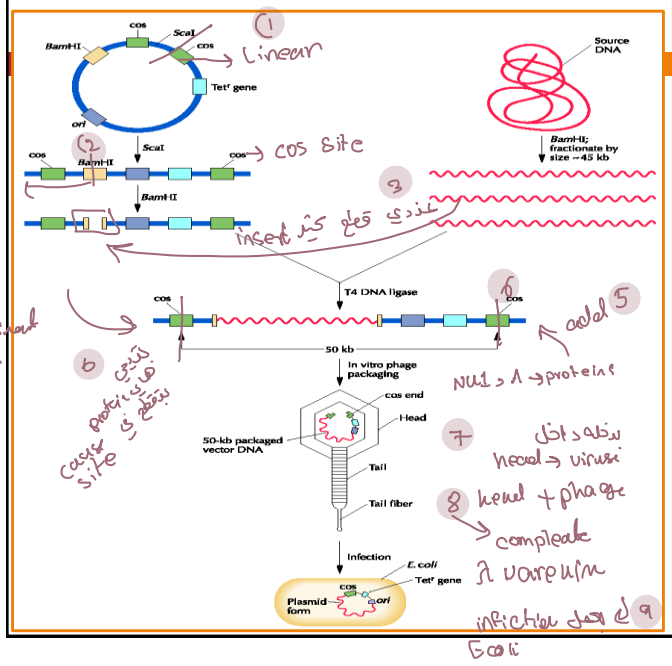
Recombinant vectors:

- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- *E. coli* plasmids
- Phage lambda
- **Cosmids (phage lamda + plasmid)**
- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Cosmids

- Combine the properties of plasmids and λ phage
- Cloning capacity 35-45 kb
- Common cosmids: pLFR-5 (6kb): a plasmid with 2 cos sites
- Cosmids based on P1 phage (115 kb) can carry up to 85kb insert

Cosmid Cloning System



λ-cos sites inserted into a small plasmid

Target DNA ligated between two cosmid DNA molecules

Recombinant DNA packaged and *E. coli* Infected as before

Can clone DNAs up to 45 kb

END