TECHNIQUES OF MOLECULAR BIOLOGY:

GENE EXPRESSION

Instructor: Dr. M. A. Srour

Course: Molecular Biology (BIOL 333)

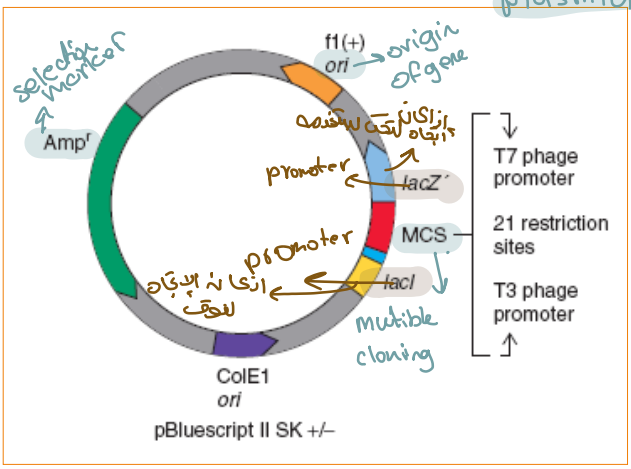
Textbook:
Watson J, et al. (2014). Molecular Biology of the Gene, 7th ed. Chap 7

Gene expression

- Why would we want to express a gene?
1. To study gene structure & expression
2. To make a large quantity of the gene's product, either for investigative purposes or for profit
- If the goal is to use bacteria to produce an eukaryotic gene> use of cDNA works better
- If an eukaryotic cell is used > cDNA usually is preferred
- Applications of gene expression: recombinant protein production (Biotechnology)

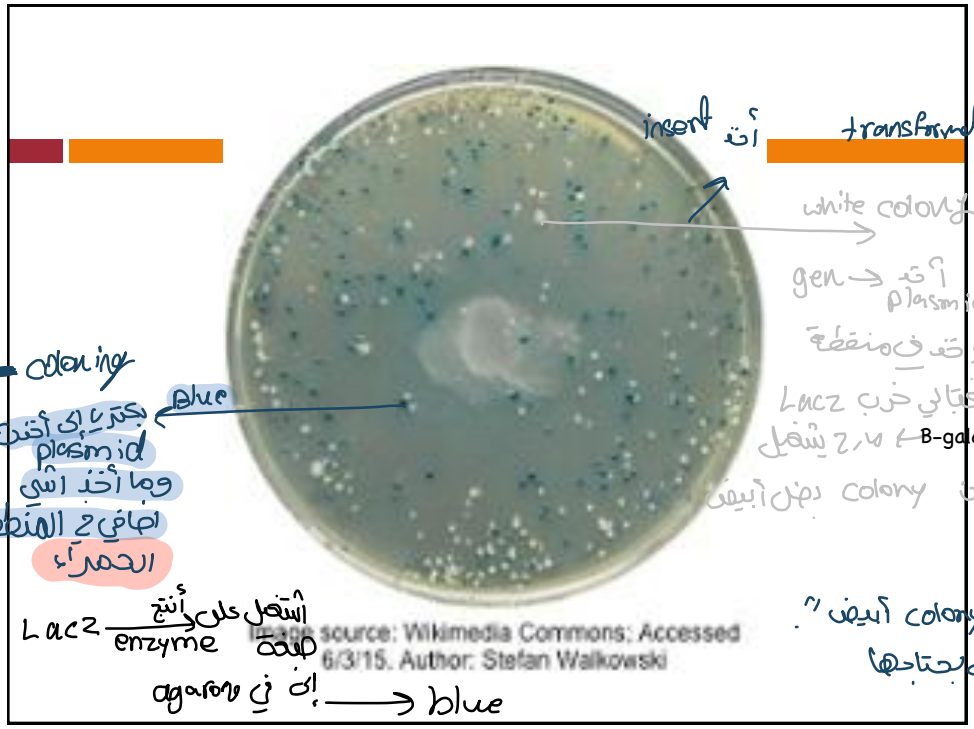
Prokaryotic Expression vectors

- Ampicillin resistance gene
- Origin of replication (ori)
- Phage f1 origin of replication
- MCS contains 21 unique restriction sites



The pBluescript vector

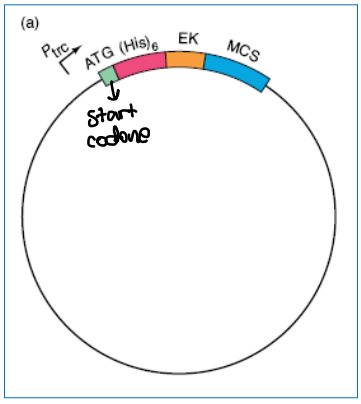
- MCS is situated between two phage RNA polymerase promoters (T7 and T3)
- MCS is embedded in an *E. coli lacZ´gene (blue), so the* uncut plasmid will produce the β-galactosidase N-terminal fragment when an inducer such as isopropylthiogalactoside (IPTG) is added to counteract the repressor made by the *lacI gene (yellow). Thus, clones* bearing the uncut vector will turn blue when the indicator X-gal is added.
- By contrast, clones bearing recombinant plasmids with inserts in the MCS will have an interrupted *lacZ´gene, so no functional* β-galactosidase is made. Thus, these clones remain white.


insert أخذ
transformation
white colony
gen → أخذ
plasmid
Lacz
B-galactosidase
cloning
blue
Lacz → enzyme
agarose
blue
Image source: Wikimedia Commons; Accessed 6/3/15. Author: Stefan Walkowski
Blue-white selection

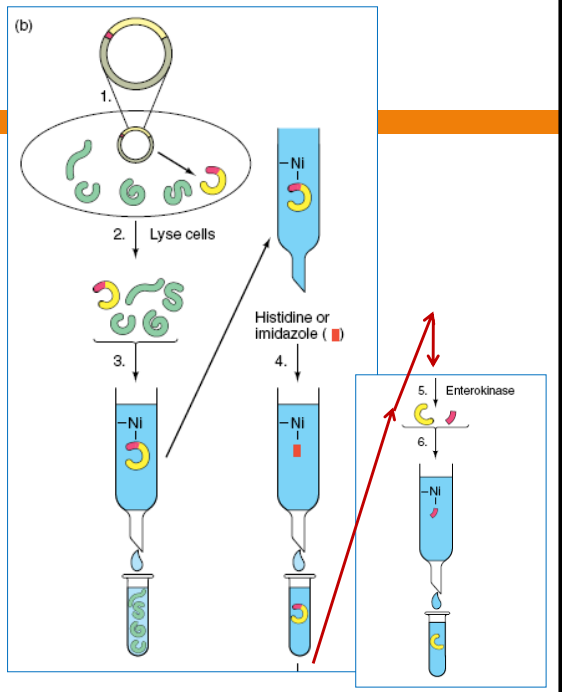
Expression systems

- Produce recombinant proteins, e.g, therapeutic proteins like GH, G-CSF, insulin
- Eukaryotes vs. prokaryotes
- Prokaryotes: lac promoter- induce with IPTG (lactose analog) > inducible promoter
- 6-His tag (epitope Tag) at C- or N-terminus for purification: bind to matrix with chelated nickel and release with low pH

Prokaryotic expression vectors that produce fusion proteins



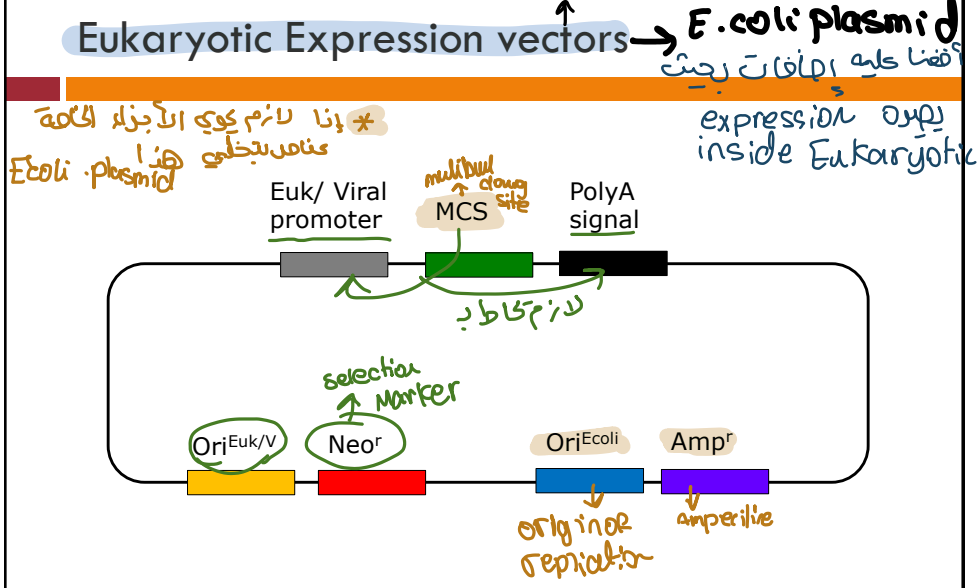
EK: a recognition site for the proteolytic Enzyme enterokinase



Using an oligohistidine (6xHis) expression vector

1. The gene of interest (yellow) is inserted into the vector in frame with the 6xHis (red), > Transform host cells > Cells produce the fusion protein (red and yellow), plus other proteins (green).
2. Lyse the cells, releasing the mixture of proteins.
3. Pour the cell lysate through a nickel affinity chromatography column, which binds the fusion protein but not the other proteins.
4. Release the fusion protein from the column with histidine or with imidazole, a histidine analogue, which competes with 6xHis for binding to the nickel
5. Cleave the fusion protein with Enterokinase (EK)
6. Pass the cleaved protein through the nickel column once more to separate 6xHis from the desired protein

Eukaryotic Expression vectors → E.coli plasmid



Basic elements of an Eukaryotic Expression vector

- **Euk/Viral promoter:** eukaryotic or viral promoter, inserted upstream of MCS, e.g HCMV promoter
- **MCS:** multiple cloning site, used to insert the gene or cDNA of interest
- **PolyA signal:** termination signal for transcription
- **Ori euk/Viral:** eukaryotic or viral ori, allows the plasmid to replicate inside eukaryotic cells ((Optional))
- **NeoR:** neomycine resistance gene, allows selection of plasmid inside eukaryotic cells
- **Ori E. coli & AmpR:** allows replication of plasmid inside E coli cells and selection, respectively

Production of eukaryotic proteins

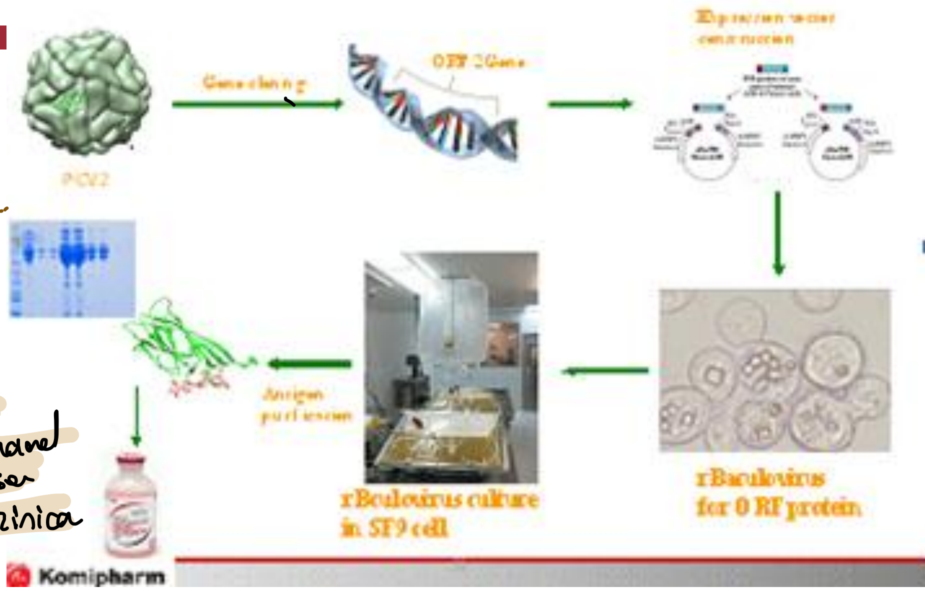
- Advantages of eukaryotic systems over prokaryotic systems:
- Eukaryotic proteins made in eukaryotic cells tend to be properly folded & not aggregated into insoluble inclusion bodies
- Eukaryotic proteins made in eukaryotic cells are modified in a eukaryotic manner

"vacsin" هو أخذ ت
* protein → على سطح فيروس
surface antigens
↓
وأعطيته للشخص

"heptailes B virus" صحة

1.
DNA → بعث عن الجين مسؤول عن أنتاج "surface antigen"
↓
cloning in plasmid
↓
expression
in prokaryotic
كثير من قطعة منه
↓
purification
↓
vacsin
↓
شخص نوخذه و يصير عنده مناعة
* Recompenent DNA technology

PCV2 vaccine development



2.
covid-19
↓
vacsin → nucleic acid
3
↙ ↘
RNA DNA
Sputnic
Jonsonand Jonson
astrazinica
→ "gene" → that produce surface antigen "S protein" → put it on expression vector "virus vector" → infiction of cell → مجرد ما دخل الفيروس بيطلع virus → plasmid → in cell "expression of proteins" → the cell recognation "بصير عنده مناعة"

* Recompent protens technology

RNA → بنتج MRNA → تزنه هش و fragin
↓
Stablization
↓
نجعله على Nano partical
↓
نحقنه في muscles
↓
جزء دخل جوا الخلايا و جزء لا
→ MRNA جيهز
↓
expression in cell
↓
produce protein
↓
نتعرف عليه ونعمل مناعة

→ protein → بواسطة Recombinet DNA
مكانه علاج لمرض hemophlya type A

Production of recombinant proteins

- Obtain cDNA
- Insert cDNA into plasmid/ expression vector
- Transfect/transform plasmid into appropriate host cells
- Downstream processing

Production of Eukaryotic proteins in ***E. coli*** from plasmid vectors containing **lac promoter**.

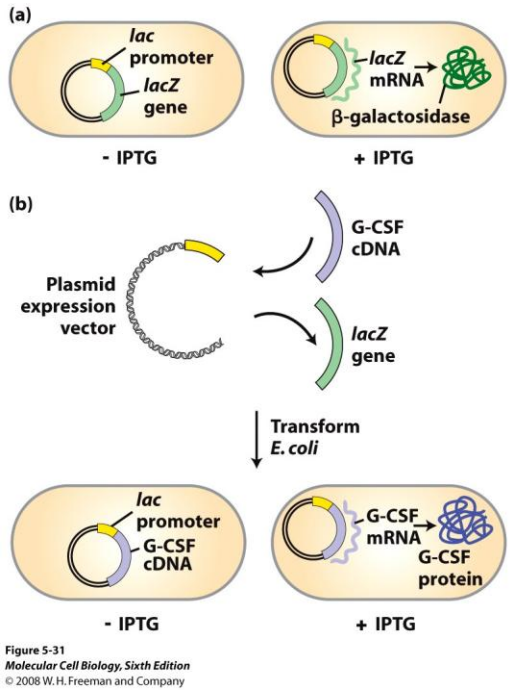


Figure 5-31
Molecular Cell Biology, Sixth Edition

* ملاحظة على Slaid 11 :

* Liposomes :- بعمله في Lab أجيب phospolipid byliyer

↓

بقطعه لقطع صغيرة

↓

Emulation

↓

membrane جزء من

↓

بقل كرة

↓

زي الخلية ولكنها فاضية من داخلها fluid

* بقدر بوحد Liposome وأكاديها بحيث تحل plasmid inside it

↓ then

added to cell

↓

بضيف عليها مواد في بيئة معدة

فبقل Liposome ←

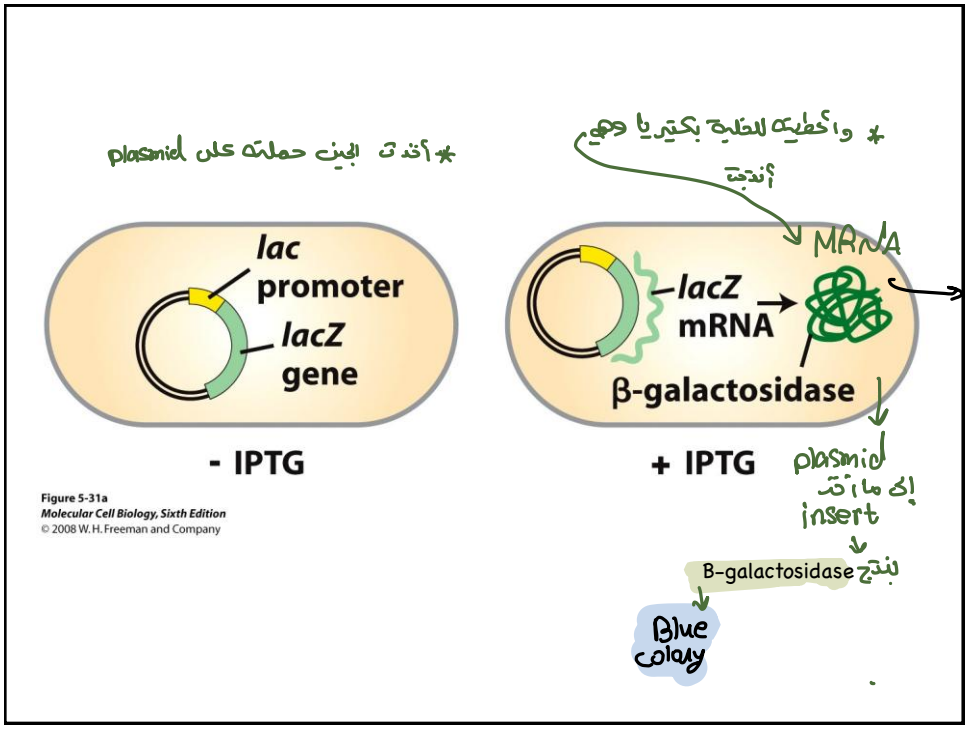
fuges with plasma membrane

↓

Liposome بتاي محتوى بندمج داخل الخلية

↓

هي ايش استفدنا؟

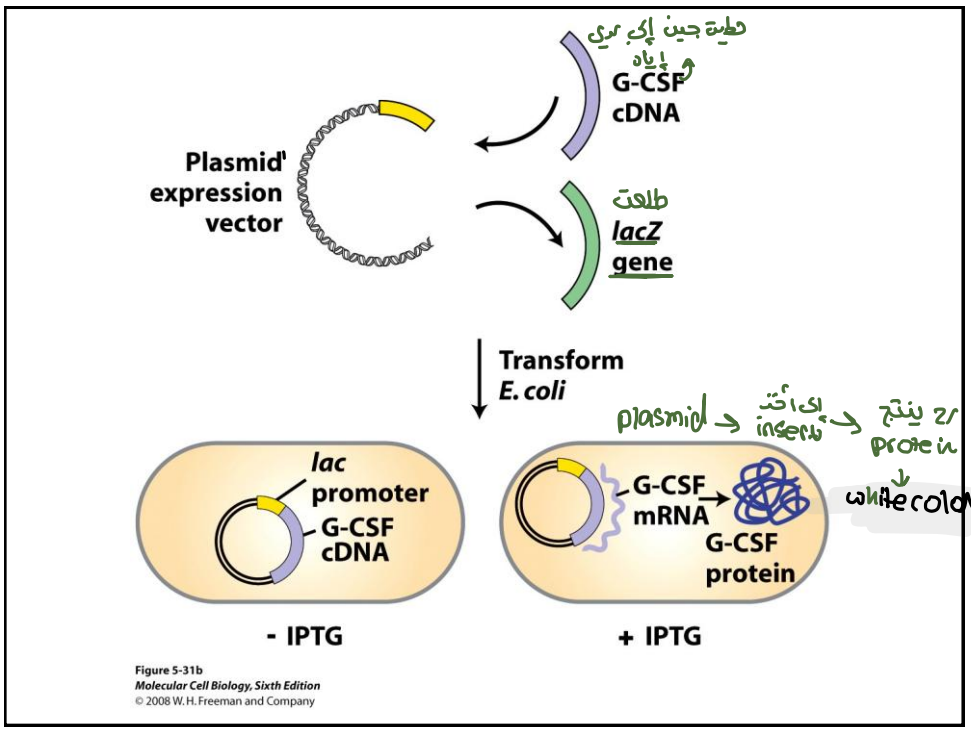


* أخذت البين حملته على plasmid

* وأعطيته للخلية بكتيريا وهي أنتجت

إن أخذ → protein خطوة إلى تحت

plasmid إلى ما أخذ insert → بنتج B-galactosidase → Blue colony



plasmid → إلى أخذت insert → بنتج protein → white colony

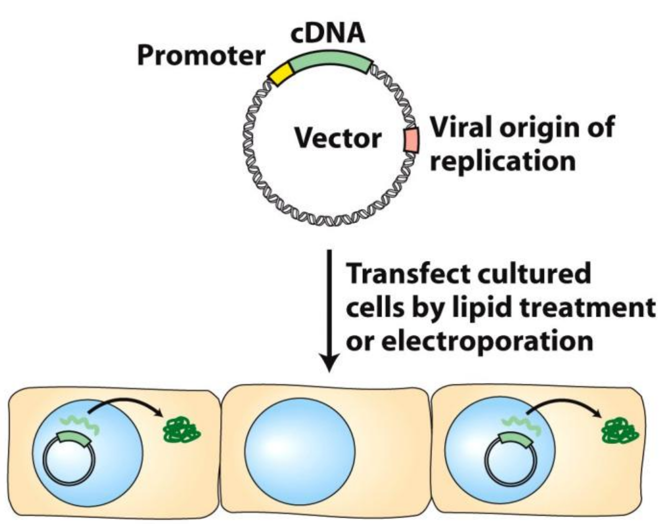
* من خطة :- مثل كل بروتينات بقدر أنتجها داخل ناس E. عشان هيرع

في ناس Eukaryotic cell → transfection

Transfection of animal cells

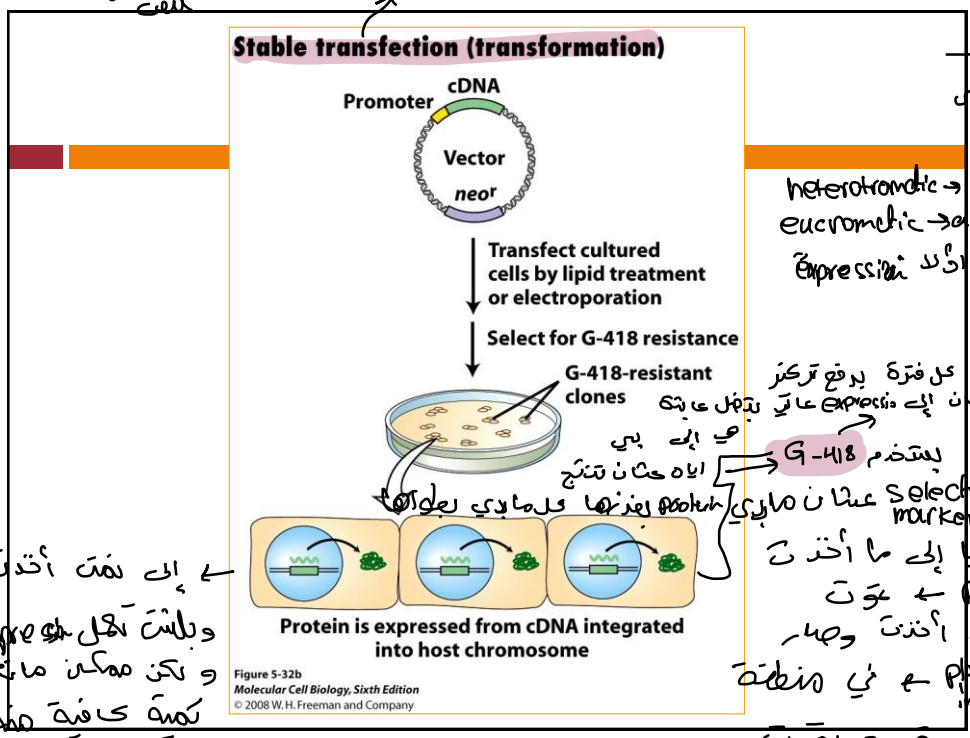
- Method: Calcium phosphate; liposomes; electroporation; or by direct physical methods like microinjection using fine glass pippettes or firing metallic microparticles coated with DNA using "gene gun".
- Transient transfection: short-term expression from strong promoter, plasmid lost during cell division
- Stable transfection: vector integrates into host chromosome; cells selected using selectable markers like neo^r (neomycin phosphotransferase; select with Geneticin or G-418)

Transient transfection



Protein is expressed from cDNA in plasmid DNA

Figure 5-32a
Molecular Cell Biology, Sixth Edition

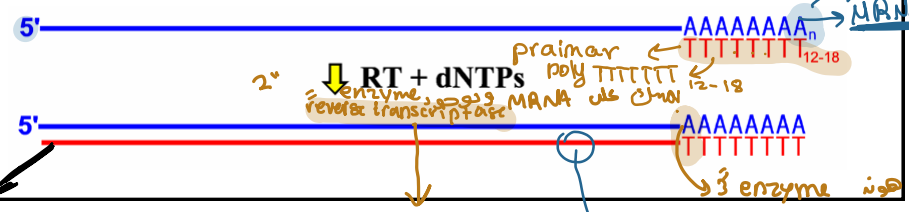


Genomic & cDNA libraries

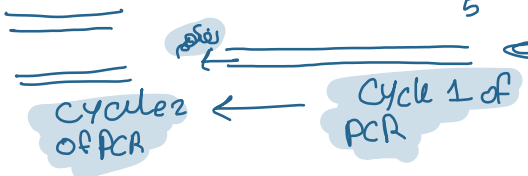
- **Genomic library:** a collection of DNA molecules each cloned into a vector molecule (e.g, λ-clones) representing all DNA sequences in the genome of an organism
- **cDAN library:** a collection of cDNA molecules each cloned into a vector molecule (e.g, λ-cDNA clones) representing all the mRNAs expressed in a cell type (tissue-type specific)
- **cDNA:** DNA molecule copied from an mRNA by reverse transcription

Preparation of cDNA

1. Preparation of mRNA (or total RNA) from target tissue
2. Synthesis of DNA-RNA hybrid (first strand) using
   - Reverse transcriptase (RNA dependent DNA Polymerase)
   - Oligo-dT primer or random priming (hexamers) or gene-specific primers
   - RNAse inhibitor
3. Synthesis of second DNA strand & amplification by conventional PCR using a pair of gene-specific primers



- http://www.sumanasinc.com/webcontent/animations/content/dnalibrary.html • Genomic DNA library:
- cDNA library:
- https://www.youtube.com/watch?v=qMcdzn6tLPE



Synthesis of cDNA

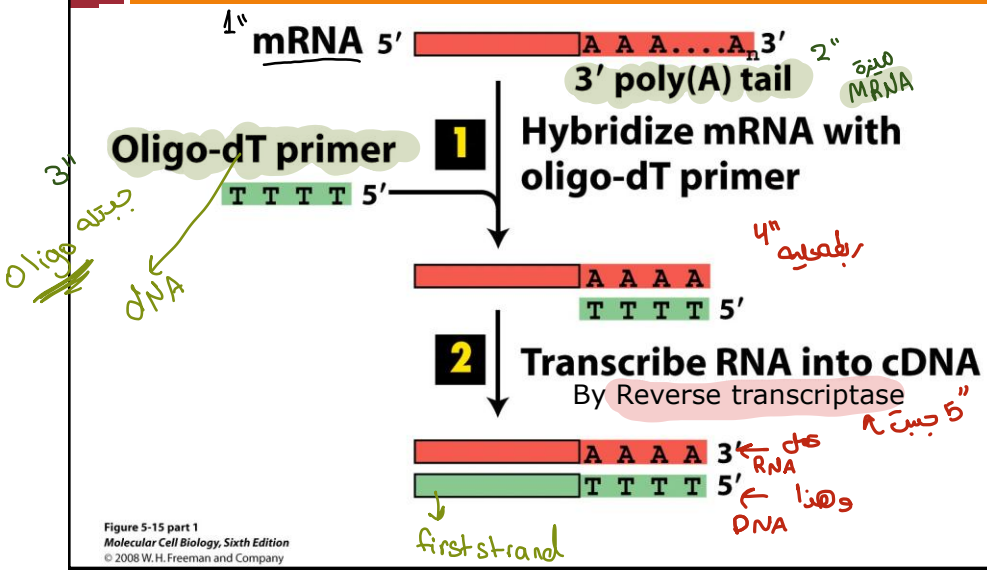


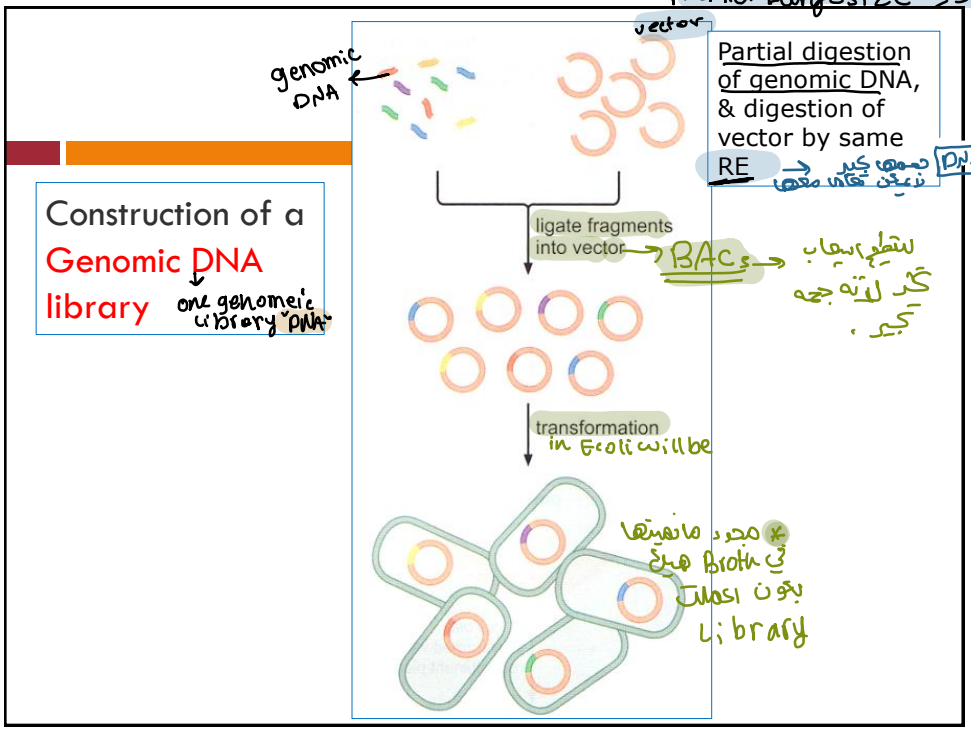
Figure 5-15 part 1
Molecular Cell Biology, Sixth Edition

Applications of cDNA

- cDNA can be used for:
  - Cloning
  - DNA sequencing
  - Generation of cDNA library
  - Analysis of gene expression

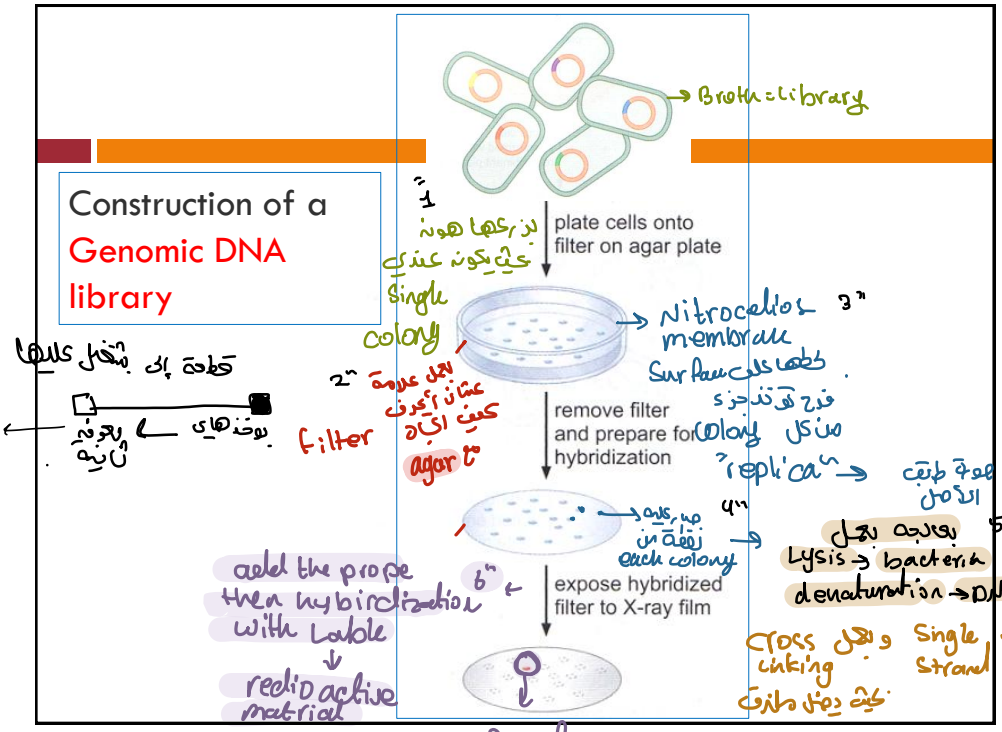
Plasmid Largesize → 50–300 kb

Construction of a Genomic DNA library



one genomic library "DNA"

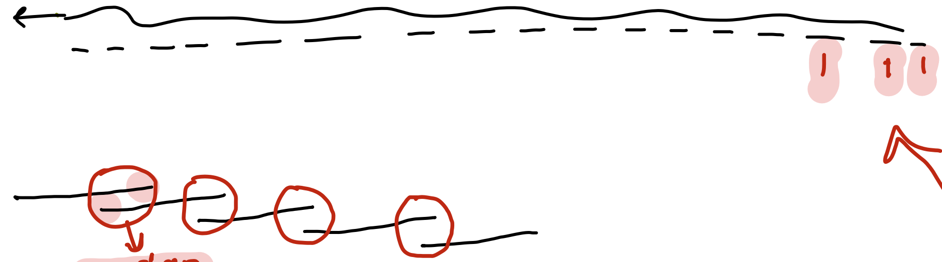
Construction of a Genomic DNA library



Sequenceing

complate digestion

partial digestion



overlap ← بساعد لمعرفة

ترتيب fragment ال genome

باتي بعمل Sequencing أمشي بالترتيب في cloning بس des.

بين عادي

* لو بدي أرجع أرتبهم بقدر مش زي complate

why we choose partial digestion??

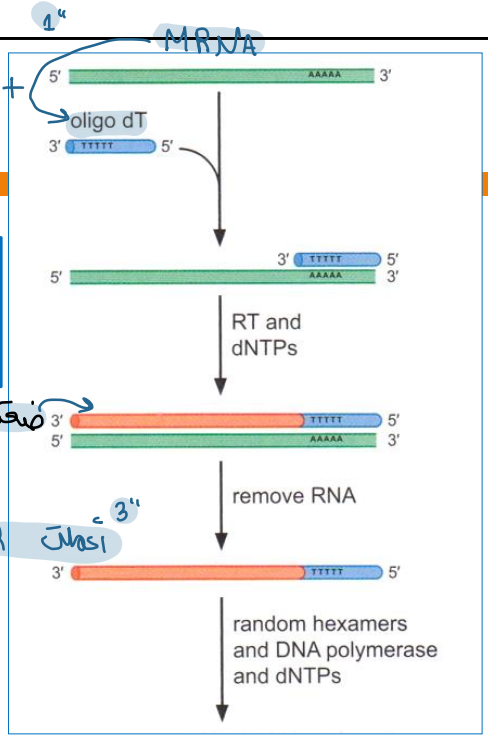
لو بدي أعمل Sequencing لـ 1، 2 أختار واحد من Vector وبلش أعمل Sequencing

إذا عملت complate (1، 2، بعرف مين 1، 2، 3 لأنه ما في إشي مشترك بينهم

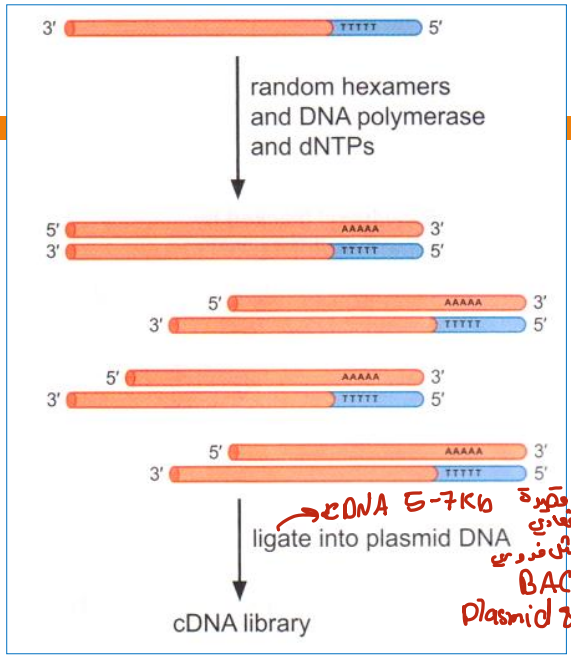
أما partial لما أعمل cloning لوحدة بعرف باقي بوخذ طرف fragment

وبستخدمها كـ "probe" عشان أدور على طرف ثاني .

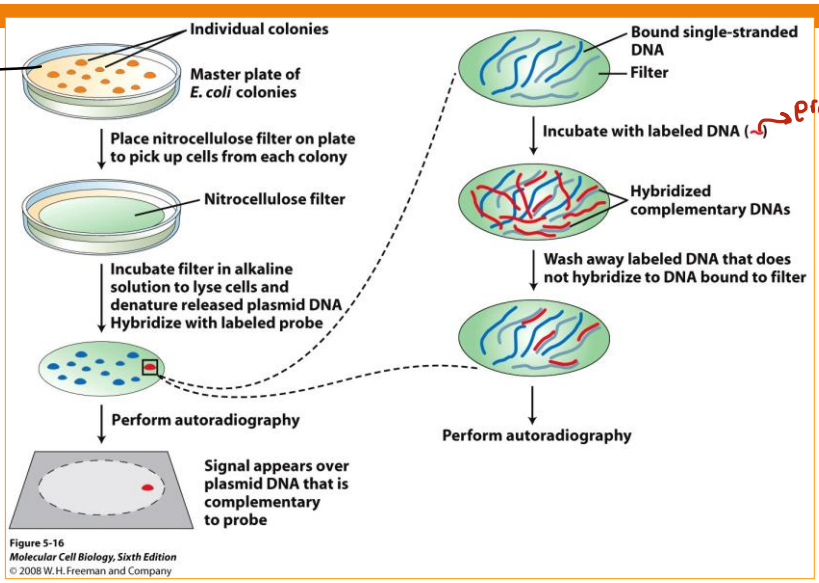
Construction of a cDNA library



Construction of a cDNA library



Screening of a cDNA library by ***Hybridization*** to a labeled oligonucleotide **probe**



From protein to DNA; or from DNA to protein?

- Synthesis of a probe based on aa sequence

6 6 1 2 1 3 2 4 1 6

Arg-Leu-Met-Glu-Trp-Ile-Cys-Pro-Met-Leu

How many different 17-mers are needed?

5aa+first 2 bases of 6th aa,

Met-Glu-Trp-Ile-Cys (15 nts)> 1X2X1X3X2=12 probes;

Plus first 2 bases of Pro (CC; CCU/A/C/G)

عندي هنا ، واحد

6 6 1 2 1 3 2 4 1 6 → the Number of genetic code

Arg-Leu-Met-Glu-Trp-Ile-Cys-Pro-Met-Leu

"17 mers" كيف بحصلها 3

متتالية amino acid خمسة ، ستة.

everyamino acid have genetic codone *

↓

3 nucletide

* we need 17mers → 5,5 aminoacid

مين أفضل 6 أخذهم

5 + first 2 bases of 6th aa

ليش أخذت لهذا ترتيب؟

إذا ابني أعمل probe في

أكثر من احتمال بدي أجرب

كلهم

فعدد الاحتمالات

$1 \times 2 \times 1 \times 3 \times 2 = 12$ احتمال

↓

12 probe محتمل

+ coden تبع protein pro

بطلع 48 هنا كثير

فعشان هيك أخذت أول

2 nucletide