

# MIRACLE Academy

قال تعالى (يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

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# Transformation and gel electrophoresis

تعني كيف ندخل الـ DNA لداخل الخلايا



Transformation

# DNA transformation

- DNA can be exchanged among bacteria by three methods:
- **Transformation**: the most popular techniques of molecular genetics that first discovered in bacteria. Transformation works best when the donor and recipient cells are very closely related.
- **Transfection** is process by which foreign DNA is introduced into a cell by a virus or viral vector and is used mainly for mammalian cells
- **Conjugation**: introduction of gene strand through pilus



في عنا عدة طرق لإدخال ال DNA لداخل الخلية :

1 ال **Transformation** : و هي أكثر طريقة مستخدمة لإدخال البلازميد بالبكتيريا ، ومثل ما قلنا ممكن ندخله للبكتيريا أو نعمل Purification و Lysis للخلية ونحصل ال DNA تبعنا.

2 ال **Transinfection** : هو عن طريق إستخدام Viral vector ، مثل ما حكينا سابقاً عن ال Bacteriophages ، و هي ال Vector بندخلهم على Mamalian cells.

3 ال **Conjugation** : هي عن طريق ال Pilus بنعمل Introducing of gene strand.

# Transformation

- Chemical transformation, cell are made competent (able to take up exogenous DNA) by treatment with divalent cations such as calcium chloride, which make the bacterial cell wall more permeable to DNA.
- Heat shock is used to temporarily form pores in the cell membrane, allowing transfer of the exogenous DNA into the cell.
- Electroporation, a short electrical pulse is used to make the bacterial cell temporarily permeable.
- Particle bombardment, is typically used for the transformation of plant cells. Gold or tungsten particles are coated with the DNA construct and physically forced into the cell by gene gun.



← عشان نبدأ بعملية ال Transformation ، لازم يكون أول شي ال Cell wall تاغ الخلية ضعيف عشان يقدر ياخذ ال DNA.

● فعشان يكون ضعيف بنروح بنحضر شي اسمه ال Competent cells ، وهي عبارة عن خلايا معالجة بـ Divalent cations مثل Calcium chloride.

← أول طريقة لعملية ال Transformation هي ال Heat shock ، فممكن الواحد ياخذ الخلايا خلينا الها ال Cell wall ضعيف و نحطها مع ال DNA و بنحطهم بال Ice لمدة 20 دقيقة ثم بنحطهم على 42 درجة لمدة دقيقة و نرجعها عال Ice مرة ثانية ، فهي الدقيقة اللي عرّضنا الخلية و ال DNA فيها لـ Heat shock بتخلي ال Pores تفتح و ال DNA يدخل لجوا الخلية.

← ثاني طريقة هي ال Electrophoration ، عبارة عن إسقاطات كهربائية صغيرة ( Short electrical pulse ) بتخلي ال Pores تفتح و تصير Permeable أكثر و يدخل ال DNA.

← آخر طريقة هي بيستخدموها لل Plant cells اللي هي ال Particle bombardment ، عبارة عن شي بنسميه Gene gun و يكون في Gold or tungsten particle بغلفوها بال DNA اللي بدنا ندخله للخلية و بعدين بعملوا Shooting of DNA+gold particle to the cell.



# Competent cells

موضح بالاسلايد الجاي

- Prepare a small, overnight culture of the bacteria in LB broth. Grow at 37°C without shaking.
- Use 1.0 mL of the overnight culture to inoculate 100 mL of fresh LB broth. This culture is grown with rapid shaking at 37°C until it reaches an OD600 of 0.3-0.4. Transfer the culture to sterile plastic centrifuge tubes. Cool on ice for 10 min.
- Centrifuge at 5000 g for 10 min at 4°C using a refrigerated centrifuge.
- Pour off the supernatant and resuspend cells in 50 mL of cold 0.1M CaCl<sub>2</sub>. Leave on ice for at least 20 min.
- Centrifuge again as before and resuspend the cells in 20 mL of cold 0.1M CaCl<sub>2</sub>. Transfer the suspensions to sterile Eppendorf tubes as 0.1ml aliquots and store at -80 degrees C.



● مثل ما قلنا إنه ال Competent cells هي خلايا ال Cell wall تاعها ضعيف، فما بنخليها تعيش بال Culture تبعها فبالتالي بنروح بنعمل التالي:

1 بناخد Small overnight culture من البكتيريا بتكون بال LB broth .

2 ثم بنعمل الها Growing at 37°C بدون Shaking وهي تعتبر Culture صغيرة يعني يا دوب 5ml، فنروح بناخد منها 1ml لكل 100ml of fresh LB broth .

3 ال 1ml اللي اخذناها هي بنروح بنعملها inoculation من ال Overnight Culture و بنحطها بهاد ال LB broth الجديد وعلى 37 درجة بس هسا مع Shaking عشان نكثرها و بدنا يوصل ال Absorption تبعها بين 0.3 و 0.4 على OD600 .

4 رح يصير في Growth للبكتيريا، و بننقلهم على 2Centrifuge tube و بنعملهم Cooling in ice لمدة دقيقتين ثم Centrifugation على 5000g لمدة 10 دقائق على درجة حرارة 4 مئوية و بهي المرحلة رح يكون كلشي عنا مبرد، يعني زي ما شفتوا يا بال ice يا بالطرد المركزي على درجة حرارة 4 مئوية.

5 ال Supernatant ما بدنا ياه، لكن بدنا الخلايا فنروح بنعمل Resuspension بـ 50ml of cold 0.1M CaCl<sub>2</sub> .

6 بنتركه بال Ice لمدة 20 دقيقة، ثم بنرجع مرة ثانية نعمل ال Centrifugation و كمان مرة بـ 20ml of CaCl<sub>2</sub> هالمرة كمية أقل ( يعني زي كانه عملنا washing و تخلصنا من أي LB medium عالقة من قبل).

7 ننقلهم لـ Eppendorf tubes معقمة و كلهم بال Ice، و بنحط 100µL يعني 0.1ml من ال Baterial aliquots بال CaCl<sub>2</sub> و بنخزنهم على - 80 درجة مئوية و أي شي باخذه من الفريزر بزبطش ارجعه .

# DNA transformation protocol

موضح بالاسلايد الجاي

- Thaw all reagents completely on ice.
- Add 1  $\mu\text{L}$  of ligation reaction to thawed competent cells.
- Gently mix by tapping tube of competent cells.
- Incubate reaction on ice for 30 minutes.
- Heat shock the competent cell mixture by incubation for 30 to 60 seconds in a 42°C heating block.
- Incubate tubes on ice for another 10 minutes.
- Add 500  $\mu\text{L}$  of LB media and incubate at 37°C with shaking at 250 rpm.
- Warm selection plates to 37°C and spread 50  $\mu\text{L}$  of transformed cells on selection plates.
- Incubate plates at 30°C overnight



## نبدأ الآن بعملية ال Transformation :

← ال Ligation reaction يكون عنا جاهزين و كذلك ال Competent cells جاهزين حَضْرناهم فوق على درجة حرارة 8-.

1 بناخذ 1μL من ال Ligation reaction و نضيفه على ال Competent cells ونخبط بالأصبع على Tube tap

2 بنعملهم Incubation بال Ice لمدة نص ساعة.

3 هسا مرحلة Heat shock، بنعرّضهم لحرارة 42 درجة مئوية لمدة دقيقة كاملة بعدين بنرجّعهم عال Ice لمدة 10 دقائق.

4 بنضيف 500μL من LB medium ثم بنعملهم Shaking على ال Shaker لمدة ساعة بدرجة حرارة 37 مئوية على 250rpm

5 بناخذ عينة و بنحطها على ال Plate أو ممكن نحضّر هي ال Culture من أجل Overnight culturing ، المهم انه هاد ال Plate بنعمل ال Incubation على درجة حرارة 30° لطول الليل.

وهيك بنكون حَضْرنا ال Clones اللي بدنا ياهم

# Trouble shooting in transformation

## ① • Few or no colony transformants

Cause	Solution
<u>Wrong antibiotic was used or antibiotic concentration was too high</u>	<ul style="list-style-type: none"> <li>Ensure the correct antibiotic was applied to plates.</li> <li>Use only concentration recommended by competent cell or antibiotic manufacturer.</li> </ul>
<u>Competent cell viability is low</u>	<ul style="list-style-type: none"> <li>Thaw competent cells on ice and use immediately.</li> <li>Check expiration date of cells. → صلاحيتها شهرين لـ 3 شهور مش أكثر</li> <li>Do not re-freeze cells. → طالما طلعت من الفريزر بترجعش عليه</li> <li>Do not vortex cells - gently tap to mix. → ما بنخلط بقوة و إنما بس بنضرب بأصابعنا على ال Vial من برا</li> </ul>
<u>DNA insert encodes protein that is toxic to cells</u>	<ul style="list-style-type: none"> <li>Use a lower incubation temperature (25–30°C).</li> <li>Use a cell strain and vector designed for tightly controlled transcription. → نستخدم خلايا ال Translation of protein الها ضعيف أو قليل</li> </ul>
<u>Heat-shock incubation too long</u>	<ul style="list-style-type: none"> <li>Reduce incubation time from 45 to 25 seconds.</li> </ul>
<u>Construct is too big</u>	<ul style="list-style-type: none"> <li>Use electroporation for vectors over 10 kb.</li> </ul>
<u>Too much ligation mixture was used for the transformation</u>	<ul style="list-style-type: none"> <li>Ligation reaction components can inhibit transformation. Dilute ligation reaction with TE buffer (up to 5 times).</li> </ul>



Aliquot معناها إنه كل 2ml بنحطهم بـ Vial و نحطهم بالفريزر فكل مرة بس بطلع الكمية الفعلية اللي بحتاجها و فش داعي اطلع كل الكمية و اذوبها ع نتفة 2ml بدي ياهم

# Trouble shooting in transformation

## ② No Plasmid in colony transformants •

Antibiotic concentration too low	Use antibiotic concentration recommended by manufacturer. → عشان نتخلص من الخلايا اللي ما أخذت البلازميد تاينا
Antibiotic is degraded	<p>• Aliquot working volumes of antibiotic and avoid freeze-thaw cycles. لما نطلعاه من الفريزر ونعمل Thawing يعني بذوب و نرجعه عالفريزر و تضل تتكرر هالحركة</p> <p>• Add antibiotic to liquid plate media after sufficient cooling.</p>
<p>بنكون حطينا ال Antibiotic قبل ليبرد ال Agar فبيخرب من الحرارة أو خضع ل Freezing cycle كثيرة</p>	

## ③ No insert in colony transformants plasmids •

Vector re-ligation	<p>• Vector insert ratio not optimal. Use a vector:insert molar ratio from 1:1 to 1:10. Use a DNA concentration of 1-10 µg/ml.</p> <p>• Dephosphorylate DNA with phosphatase to prevent re-ligation</p>

# Trouble shooting in transformation

- ④ Sequencing of transformants plasmid reveals wrong plasmid sequence

<u>DNA insert encodes protein that is toxic to cells</u>	<ul style="list-style-type: none"><li>• Use a lower incubation temperature (25–30°C).</li><li>• Use a cell strain and vector designed for tightly controlled transcription.</li></ul>
<u>Mutations introduced by initial PCR</u>	<ul style="list-style-type: none"><li>• Use a high-fidelity polymerase.</li></ul>
<u>Inconclusive sequencing artifacts</u>	Repeat sequencing reaction. ↴ يا بنعيد ال Sequencing أو نعيد قراءته من الكمبيوتر بطريقة صحيحة



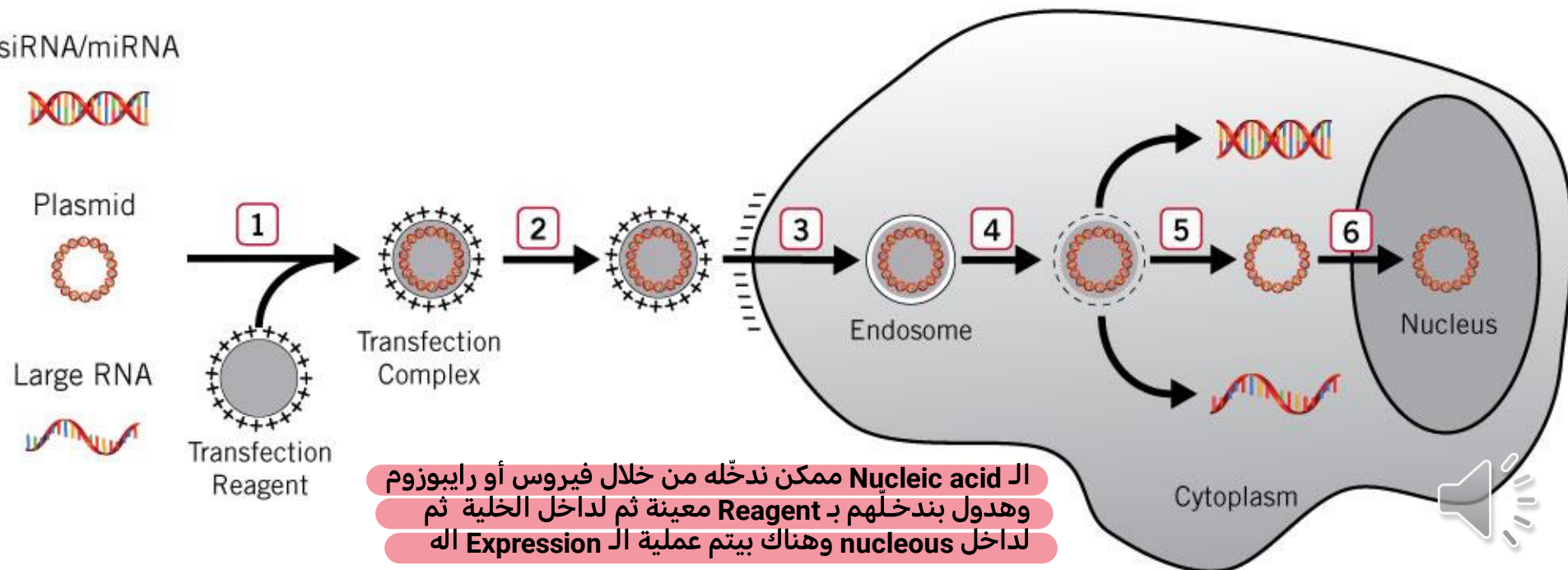
# Electroporation

- An instrument called an electroporator produces a brief electrical shock that introduces DNA into the cells without killing them
- Advantages
  - Rapid
  - Requires fewer cells
  - Can be used to introduce DNA into other cell types
  - More efficient process (More efficient than heat shock)



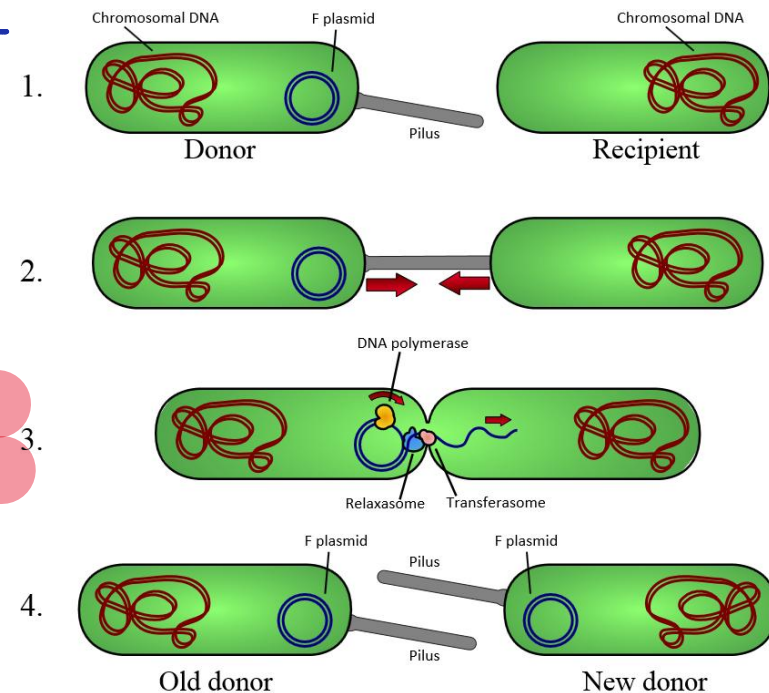
# Transfection

- **Transfection** is the way to deliver exogenous nucleic acids such as DNA, RNA or oligonucleotides into cells.
- These nucleic acids can be transported by polymeric or lipidic transfection reagents that facilitate their cellular uptake



# Conjugation

- Donor cell produces pilus.
- Pilus attaches to recipient cell and brings the two cells together.
- The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
- Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor



هون يكون ال Doner cell بينتج Pilus ، البلازميد المسؤول عن Formation of pilus ممكن إنه ينقل المادة الوراثية لخلية ثانية غير قادرة على إنتاج ال Pilus.

بروح ال Pilus بيرتبط مع الخلية الثانية، والبلازميد الموجود بينفصل ال DNA تبعه ل 2 Strands و وحدة منهم بتروح من خلال ال pilus تنتقل للخلية الثانية

الخلية اللي اجتها هي ال Strand بتروح بتنسخ عنها النسخة الثانية من البلازميد أي بتنسخ Complementary strand و بصير عندها ال DNA اللي بيعمل Expression of pilus و بيصير عندها قدرة تنتج Pilus.

# Gel electrophoresis

- Electrophoresis is a standard method used to separate, identify and purify nucleic acids
- Agarose or polyacrylamide gels can be used as both gels are porous in nature

SDS Page

بيفصلوا القطع حسب الحجم



# ① Agarose gel electrophoresis (AGE)

## Polysaccharide

الطحالب  
البحرية

- Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool.
- For most applications, only a single-component agarose is needed and no polymerization catalysts are required.
- Agarose gels are simple and rapid to prepare. They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation but a relatively low resolving power, since the bands formed in the gels tend to be fuzzy and spread apart. This is a result of pore size and cannot be largely controlled.



بنحط ال Agar بـ Buffer و بنسخّنه و نغليه و بعدها بنعمله Cooling و بنصبه.

← فعملية تحضيره Very simple & rapid و بنستخدم بس one components، لا بنحتاج نستخدم Initiater أو Accelerater.

● هو الأفضل لعمليات فصل ال DNA اللي بكونوا Large size و moderate size.

● مع ال Small DNA بتكون ال Resolving power مشكلة حيث الفصل بكون مش كثير منيح الهم فبنلاقي ال Band واسعة شوي بنسميها Fuzzy band فعملية فصل ال Bands بكون فيها صعوبة

**Fuzzy**



# Advantages and disadvantages

- **Advantages**

- Nontoxic gel medium
- Gels are quick and easy to cast → سهل يتحضر و سهل ينصب
- Good for separating large DNA molecules
- Can recover samples by melting the gel, digesting with enzyme agarose or treating with chaotropic salts

- **Disadvantages**

- High cost of agarose
- Fuzzy bands
- Poor separation of low molecular weight samples



# Agarose concentration (Agarose Conc.)

أي تركيز بالضبط نستخدم يعتمد على الـ DNA اللي بدنا نفصله

- Agarose gels are normally in the range of 0.2% to 3%.
- If the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended

Concentration of agarose (%)		DNA size range (bp)
0.2		5000-40000
0.4		5000-30000
0.6		3000-10000
0.8	كلما قل حجم الـ DNA كلما كان Conc of agarose أكبر	1000-7000
1		500-5000
1.5	لذلك التركيز العالي مع القطع الصغيرة، لأنه لو استخدمناه مع القطع الكبيرة رح يعيق حركتها	300-3000
2		200-1500
3		100-1000



## ② Polyacrylamide gel electrophoresis (PAGE)

- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N'-methylenebisacrylamide.
- The reaction is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and N,N,N',N'-tetramethylethylenediamine (TEMED) as the catalyst.

ال SDS page و Native page في منه Polyacrylamide ←

ال SDS page هي المستخدمة أكثر. ✓

يتم خلط مادتين هي Acrylamid و Bisaclyramide وحدة بتعمل Polymerization و وحدة بتعمل Cross linking. ←

المادة هي Very toxic لأنه مبدؤها قائم على ال Free radicals و نحتاج لإضافة ال Ammonium persulfate ك Initiator  
لل Polymerization ، ونضيف TEMED هاد ك Catalyst of Polymerization process ←

# Polyacrylamide gel electrophoresis (PAGE)

- Although the gels are more difficult to prepare and handle, involving a longer time for preparation than agarose gels, they have major advantages over agarose gels. They have **a greater resolving power, can accommodate larger quantities of DNA** without significant loss in resolution and the DNA recovered from polyacrylamide gels is extremely **pure**
- The **pore size** of the polyacrylamide gels can be altered in an easy and controllable fashion by **changing the concentrations** of the two monomers.
- Polyacrylamide is a **neurotoxin** (when unpolymerized), but with proper laboratory care it is no more dangerous than various commonly used chemicals



← ال Polyacrylamide اصعب أكثر بالتحضير من Agarose gel بس فوائده أكثر من Agarose gel من ناحية Greater resolving power فعملية الفصل أوضح و أحسن و كمان بنقدر نستخدم كميات أكبر من ال DNA و كمان ال DNA اللي بنفصله بإستخدامه بكون Extremely pure DNA.

← نستطيع التحكم بال Pore size عن طريق إنه نغير ال Conc of acrylamid & Conc of Bisaclyramide.

● المشكلة اللي فيه هو إنه وهو على شكل Monomer بكون Neurotoxic و هي مشكلة مش بسيطة لإنه بكون سام للأعصاب فلازم الواحد يكون لابس Gloves وهو بشتغل.

# Advantages and disadvantages

- **Advantages**

- Chemically stable cross-linked gel
- Sharp bands → ال Bands بتكون واضحة
- Good for separation of low molecular weight fragments

- **Disadvantages**

- Toxic monomers
- Gels are tedious to prepare and often leak
- Need new gel for each experiment

خلال عملية التحضير يكون بطيء جداً تحضيره يعني لدرجة أكثر من ساعة و أكثر من طبقة

يتم تحضيره لمرة وحدة بس، مش زي Agarose نحضره و نضل ناخذ من الكمية اللي بدنا ياها أكثر من مرة



# Polyacrylamide concentration

- With increasing the concentration of monomer in the gel, the pore size decreases in a nearly linear relationship.
- Researchers have settled on Concentration values of 5% (19:1 acrylamide/bisacrylamide) for most forms of denaturing DNA and RNA electrophoresis, and 3.3% (29:1) for most proteins, native DNA and RNA gels.

Acrylamide/Bis Ratio	Gel %	Native DNA/RNA (bp)	Denatured DNA/RNA (bp)
19:1	4	100-1500	70-500
	6	60-600	40-400
	8	40-500	20-200
	10	30-300	15-150
	12	20-150	10-100
	5	200-2000	70-800
29:1	6	80-800	50-500
	8	60-400	30-300
	10	50-300	20-200
	12	40-200	15-150
	20	<40	<40



← لما نكون بدنا نفصل Denatured RNA أو Denatured DNA معناها نستخدم نسبة 19:1 اللي هي يعني تركيز 5%.

← لو بدنا نفصل Protein سواء Native or Denatured ، أو Native DNA أو Native RNA يعني مش معمول الـ Denaturation ، معناها نستخدم 3.3% يعني نسبة 29:1.

# Electrophoretic buffer systems

- Effective separation of nucleic acids by agarose or polyacrylamide depends upon the **effective maintenance of pH within the matrix**. Therefore, buffers are an integral part of any electrophoresis technique.
- The electrophoretic mobility of DNA is affected by the **composition and ionic strength** (salt content) of the buffer. Without salt, electrical conductance is minimal and DNA barely moves. In a buffer of high ionic strength, electrical conductance is very efficient and a significant amount of heat is generated.
- Different categories of buffer systems for electrophoresis:
  - dissociating and non-dissociating
  - continuous and discontinuous.



لو فش Salt content أبدا معناها توصيل الكهرباء رح يكون ضعيف فإنتقال ال DNA و مشيته عالجل رح تكون ضعيفة كثير.

بينما لو Salt content كبير معناها توصيل عالي أي High electrical conductance فوقتها ممكن يولد حرارة ممكن تعمل Melting of the gel.

# Dissociating and non-dissociating buffer systems

- Separation on the basis of molecular weight requires the inclusion of denaturing agents, which unfold the DNA or RNA strands and remove the influence of shape on their mobility.
- The most commonly *dissociating buffer systems* used include **urea and formamide** as DNA denaturants.
- Denatured DNA migrates through these gels at a rate that is almost completely dependent on its base composition and sequence.
- Denaturing or dissociating buffer systems for proteins include the use of **sodium dodecyl sulfate (SDS)**. In the SDS-PAGE system, proteins are heat- denatured with SDS before electrophoresis so that the charge-density of all proteins is made roughly equal with net negative charge



← ال Buffer بس نضيفه إما إنه بعمل Denaturation أو بعملش Denaturation.

← ال Dissociating buffer مثل ال Urea و Formamide.

● بنستخدمه للـ DNA أو RNA بهدف ال Denaturation وينفصل حسب ال Mwt فبالتالي بمشي عالجل حسب Mwt.

← لما ال Dissociating buffer يكون Sodium dodecyl sulfate هاد بنضاف لعينة ال Proteins ثم بنعمل Heat Denaturation بنسخن على حرارة 95 لمدة 5 دقائق بعدين بنمشيه على Gel electrophoresis.

● ال SDS بعمل Denaturation و بيغلّف البروتين بـ negative charge اللي هو نفسه SDS. فبالتالي ينفصل حسب ال Mwt مش حسب Size or Shape.

# Dissociating and non-dissociating buffer systems

- When samples are electrophoresed, proteins separate according to mass alone, with very little effect from compositional differences.

نضيف SDS على DNA فقط بهدف Enhance resolution power of band

- DNA molecules are negatively charged; therefore the addition of SDS in the gel preparations is only with the aim of enhancing the resolution power of the bands

بالتالي بضل شكل ال DNA ك Rodlike

- In the absence of denaturants, double stranded DNA (dsDNA), like a PCR product, retains its double helical structure, which gives it a rodlike form as it migrates through a gel.

- During the electrophoresis of native molecules in a *non-dissociating buffer system*, separation takes place at a rate approximately inversely proportion to the log10 of their size

هي لما يكون بدي يضل بدي ال Molecule تضل Native و ما يصير لها Denaturation، وما بتكون المسافة بين القطع المفصولة متساوية



# Continuous and discontinuous buffer systems

الفائدة إنه بنقدر كمية أكبر من  
ال Sample و نفصلها بالطريقة

- In the *continuous buffer systems* the identity and concentration of the buffer components are the same in both the gel and the tank. Although continuous buffer systems are easy to prepare and give adequate resolution for some applications, bands tend to be broader and resolution consequently poorer in these gels.
- These buffer systems are used for most forms of DNA-AGE, which commonly contain EDTA (pH 8.0) and Tris-acetate (TAE) or ② Tris-borate (TBE) at a concentration of 50mM (pH 7.5-7.8).
- TAE is less expensive, but not as stable as TBE. In addition, TAE gives better resolution of DNA bands in short electrophoretic separations and is often used when subsequent DNA isolation is desired. TBE is used for PAGE of smaller molecular weight DNA (MW<2000) and AGE of longer DNA where high resolution is not essential.

# Continuous and discontinuous buffer systems

- *Discontinuous (multiphasic) systems* employ different buffers for tank and gel, and often two different buffers within the gel.
- Discontinuous systems concentrate or “stack” the samples into a very narrow zone prior to separation, which results in improved band sharpness and resolution. The gel is divided into an upper “stacking” gel of low percentage of acrylamide and low pH (6.8) and a separating gel with a pH of 8.8 and much smaller pores (higher percentage of acrylamide).
  - ①
  - ②
- The stacking gel prevents any high-molecular-weight DNA present in the sample from clogging the pores at the top of the running gel before low molecular-weight DNA has entered. Both, the stacking and the separating gels, contain only chloride as the mobile anion, while the tank buffer contains glycine as its anion, at a pH of 8.8. The major advantage of the discontinuous buffer system over continuous buffer system is that this gel system can tolerate larger sample volumes.

● ال Continuous buffer system هو نفس ال Buffer يكون موجود بالجل و بالتانك وهو الأكثر إستخداما بحالة ال Agarose عشان فصل و بيعطي Resolution كافي.

← لازم يكون حجم ال Band كبير عشان يعطي Resolutions منيح ،بينما لو حجمهم صغير ممكن يكونوا Broad و ال Resolution مش كثير واضح.

1 ال Tris acetate EDTA على pH تساوي 8، أرخص بس مش Stable مثل Tris borate و إله Resolution أفضل، و تستخدم للـ High Mwt ولما نكون رح نستخدم ال Agarose لفصل DNA.

2 او Tris borate EDTA و ال pH بين 7.5 & 7.8، و تستخدم للـ Low Mwt و تستخدم لما نكون رح نستخدم Polyacrylamide لفصل ال DNA

● ال Discontinuous system هذا المستخدم أكثر بالـ Polyacrylamide gel و ال Buffer المستخدم بالجل مش نفسه بالتانك، و حتى بالجل بنلاقي فيه 2 buffers.

← هذا ال System مكوّن من :

1 ال Stacking gel بيكون من فوق و هاد بيرتب العينة يعني بيعمل فصل مبدئي حسب الحجم عشان ما تروح القطع الكبيرة تعلق و تحجز فوقها الصغيرة، بيكون فيه Low conc of acrylamid و بتكون pH=6.8.

2 ال Separating gel بيكون من تحت وهو اللي بي فصل البروتينات و ال DNA ، بتكون ال pH=8.8 و ال Conc of Polyacrylamide أعلى فبالتالي ال Pore size بيكونوا أصغر

ال Buffers كلهم Tris سواء اللي بالتانك أو بالجل ، اللي بالجل بتكون ال pH of Tris buffer معدلة لـ 6.8 عن طريق استخدام HCL لكن بالتانك بنلاقي في Glycine فالـ pH تساوي 8.8.

# Loading buffer

هذا ال Buffer ما بنستخدمه  
مع الجل مثل اللي قبل و إنما  
مع ال Sample

- This is the buffer to be added to the DNA fragment that will be electrophoresed. This buffer contains **glycerol or sucrose** to increase the density of the DNA solutions; otherwise, the samples would dissolve in running buffer tank and not sink into the gel pocket.

العينة بتضل بال Whells تأعت ال Electrophoresis buffer فما بصير لها Sink

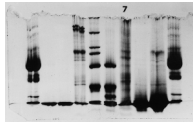
- The gel loading buffer also contains dyes that facilitate observation of the sample during gel loading and electrophoresis, such as **bromophenol blue or xylene cyanol**.

Because these molecules are small, they migrate quickly through the gel during electrophoresis.

الصبغتين ما بتفاعلوا لا مع Protein ولا مع DNA ولا بتصبغهم وإنما الها Mwt قليل فبتكون تركض قدام البروتينات و DNA و تبتلنا وقع شي من الجل او لا فبس نشوفها وصلت للأخر بنروح بنوقف الكهربي

- The components and concentrations of the 6X loading dye usually used are: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol; or 0.25% bromophenol blue, 50 mM EDTA, 0.4% sucrose.

# Voltage/current applied



- The higher the voltage/current, the faster the DNA migrates. If the voltage is too high, **band streaking**, especially for DNA  $\geq 12-15\text{kb}$ , may result. Moreover, high voltage causes a tremendously **increase** in buffer **temperature and current** in very short time. *This lead to gel melting*
- The high amount of the heat and current built up in the process leads to the melting of the gel, **DNA bands smiling**, **decrease of DNA bands resolution** and **fuse blowout**. it is highly recommended not exceed 5-8 V/cm and 75 mA for standard size gels or 100 mA for minigels.
- When the voltage is too low, the **mobility of small ( $\leq 1\text{kb}$ ) DNA is reduced** and **band broadening** will occur due to dispersion and diffusion.



# Visualizing the DNA

- After the electrophoresis has been completed there are different methods that may be used to make the separated DNA species in the gel visible to the human eye.

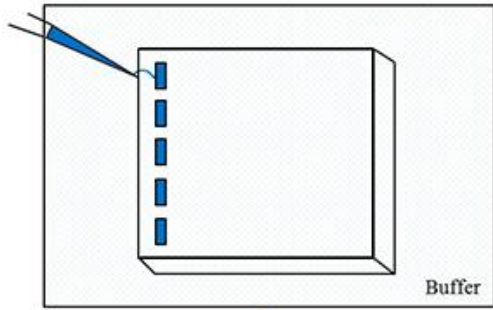
## 1. Ethidium bromide staining (EBS)

→ Colorless

- The localization of DNA within the agarose gel can be determined directly by staining with low concentrations of intercalating fluorescent ethidium bromide dye under UV light. The dye can be included in both, the running buffer tank and the gel, the gel alone, or the gel can be stained after DNA separation.
- For a permanent record, mostly instant photos are taken from the gels in a dark room. إضافة EPS إما مباشرة عالج أو Buffer tank أو بنضيفها على مي و و نخط نص الجل فيها و نرجع نطلع الجل و بنمشي عليه ال DNA
- Note that ethidium bromide is a potent mutagen and moderately toxic after an acute exposure. Therefore, handle it with caution.

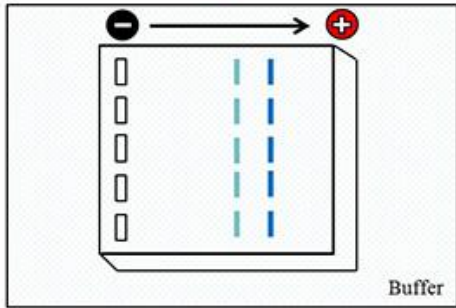
# Ethidium bromide staining

Fluorescent ethidium bromide dye هي الصبغة بترتبط مع الـ DNA و بتعطي UV light بتضوي و بعدها بنصّورها بالكاميرا فلما نحط على



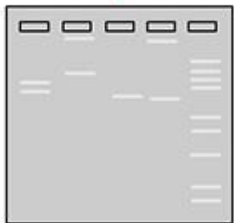
DNA/RNA samples and marker loaded in the horizontal gel electrophoresis system

حطوا العينات عالجل



Direction of migration of DNA/RNA samples in horizontal gel electrophoresis system

بّلش يعملهم Run و هي الصبغة الزرقا ماشية قدام القطع لنشوف الـ Run وين وصلت



Agarose gel after ethidium bromide staining

عال Agarose ما بنشوف الصبغة ولا أي Band بتكون بالأصل مو واضحة ولكن تحت UV بتكون الـ Bands ضاوية



Under UV

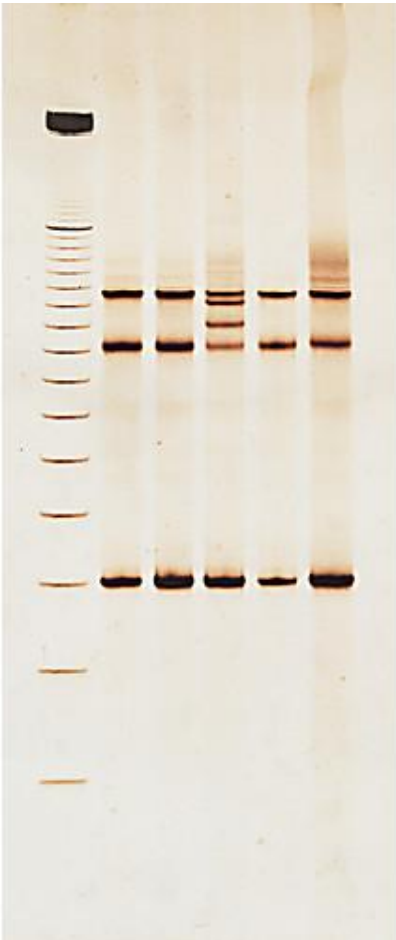


# Silver staining (SS)

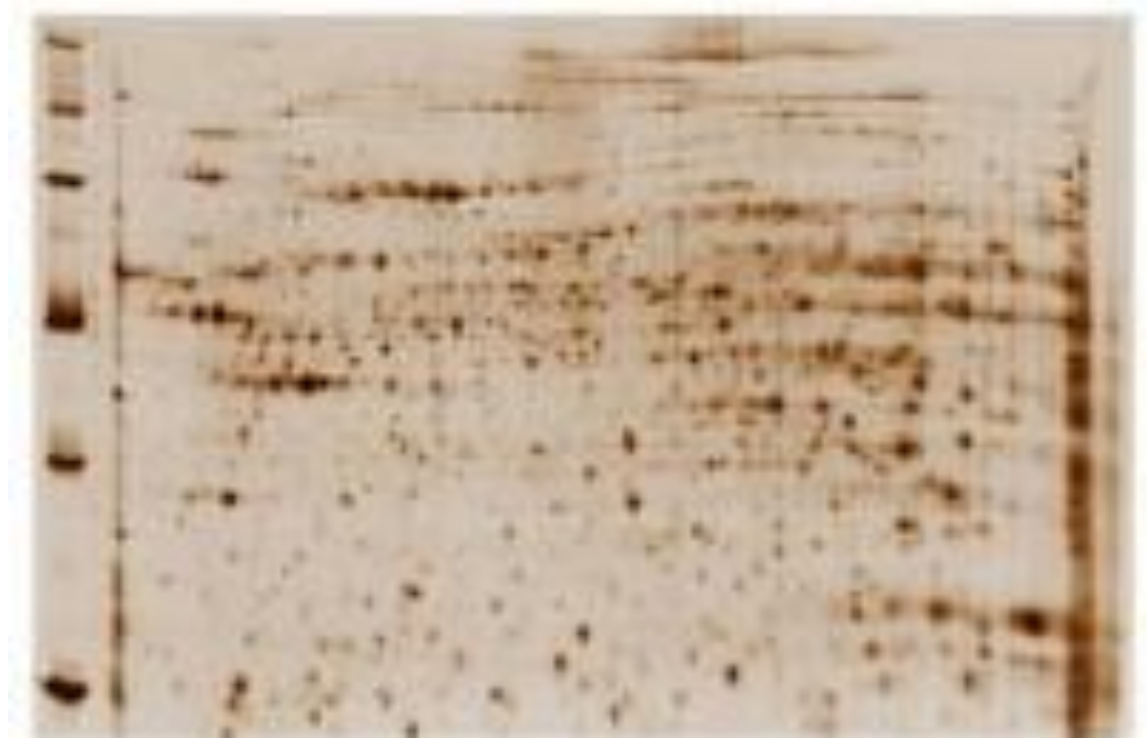
هي لل DNA & proteins  
عكس EPS اللي كانت بس  
لل DNA

- Silver staining is a highly sensitive method for the visualization of nucleic acid and protein bands after electrophoretic separation on polyacrylamide gels.
- Nucleic acids and proteins bind silver ions, which can be reduced to insoluble silver metal granules. Sufficient silver deposition is visible as a dark brown band on the gel. Silver staining protocols include many steps:
  - i) fixation to get rid of interfering compounds,
  - ii) silver impregnation with either a silver nitrate solution or a silver-ammonia complex solution,
  - iii) rinses and development to build up the silver metal image, and
  - iv) stop and rinse to end development prior to excessive background formation and to remove excess silver ion

# Silver staining



For DNA



For protein



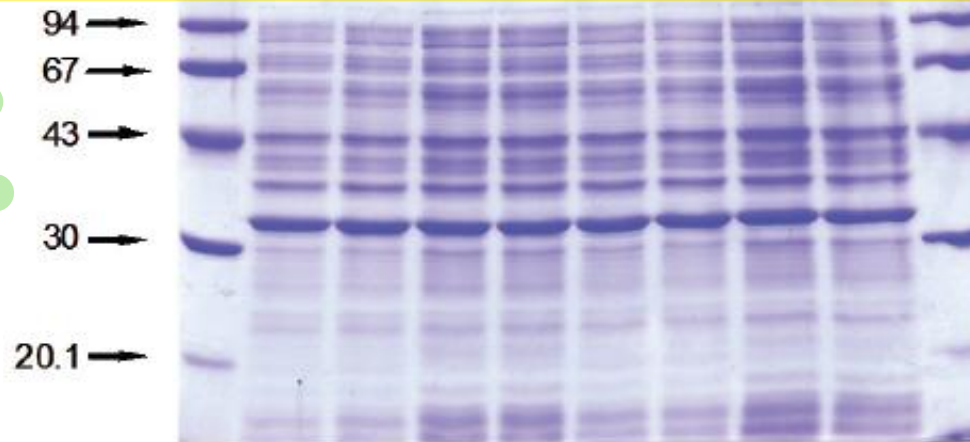
# Coomassie staining

هي فقط للبروتينات

This dye used for detecting & quantitative measurements زر

- The Coomassie blue staining allows detecting up to 0.2 to 0.6  $\mu\text{g}$  of protein, and is quantitative (linear) up to 15 to 20  $\mu\text{g}$ .
- It is often used in methanol-acetic acid solutions and is discolored in isopropanol-acetic acid solutions

بنروح بناخد الجل بعد ما تنفصل القطع عليه و بنحطه بـ Coomassie blue و هي الصبغة اصلا بتكون مع Methanol و Acetic acid ، ثم نترك الجل لحد ما يتلون باللون الأزرق ، و الـ Bands ما بتكون مبينة و واضحة فبنروح بنعمل Discoloration



لإزالة اللون نستخدم إما

ethanol مع Acetic acid ، أو

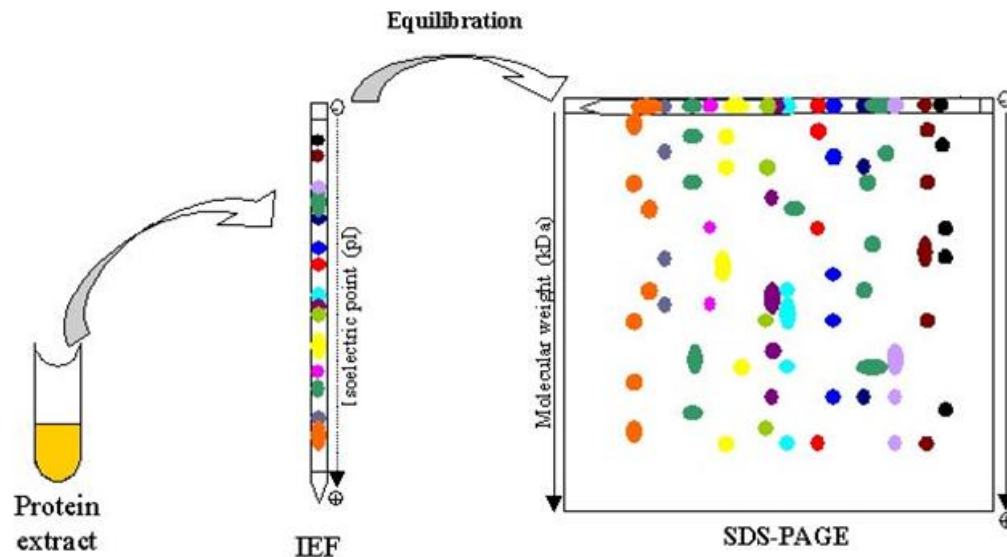
Isopropanol مع Acetic acid

1. SDS-PAGE.

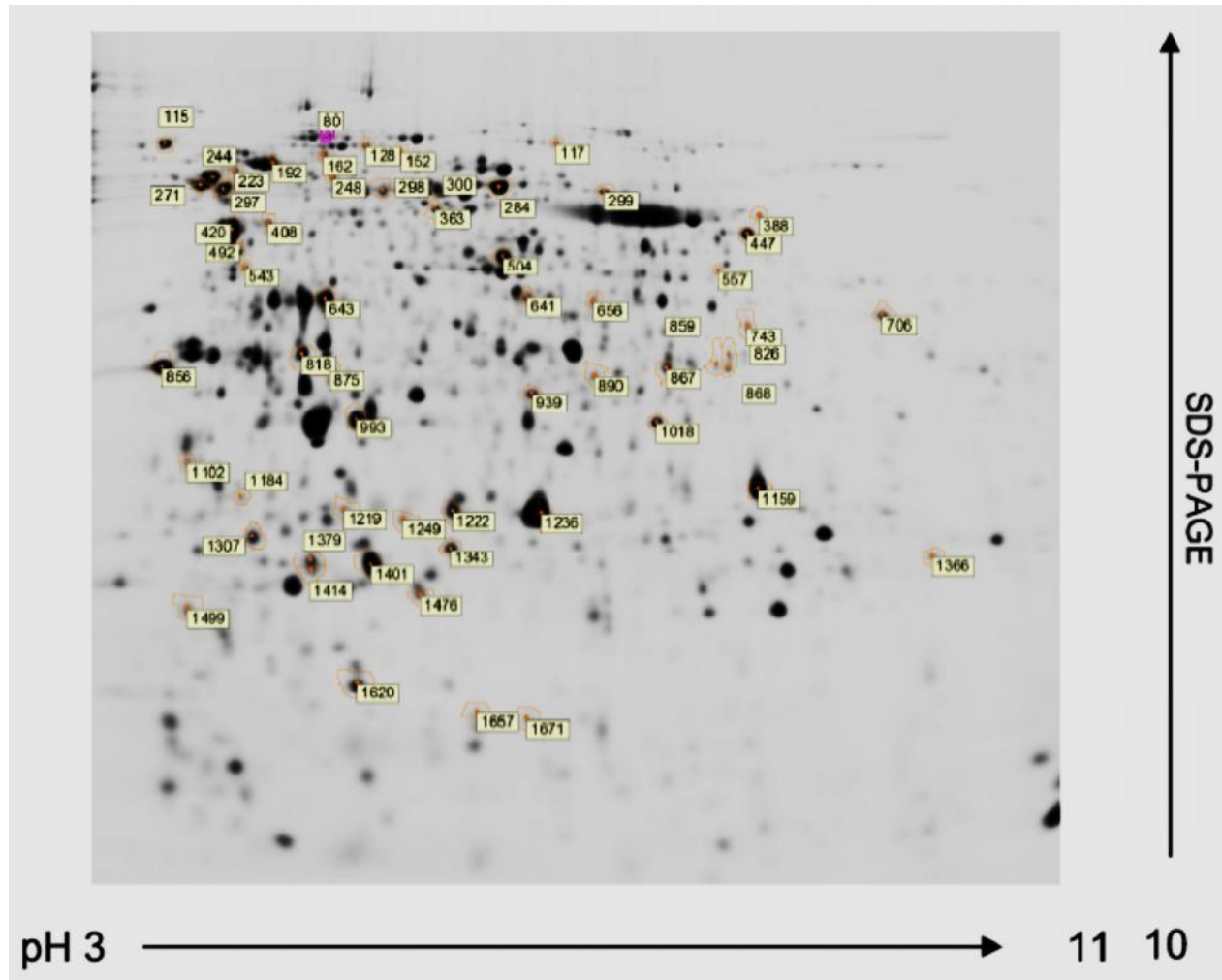


# Two-dimensional gel electrophoresis

- Two-dimensional gel electrophoresis (2-DE) is based on separating a mixture of proteins according to two molecular properties, one in each dimension.
- The most used is based on a first dimension separation by isoelectric focusing and second dimension according to molecular weight by SDS-PAGE.



# Two-dimensional gel electrophoresis organism protein fingerprinting





# Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE)

- A method that labels protein samples prior to 2-DE, enabling accurate analysis of differences in protein abundance between samples.
- The technology is based on the specific properties of fluorescent cyanine dyes that are spectrally resolvable and size- and charge-matched
- Identical proteins labeled with each of the three dyes (Cy2, Cy3 and Cy5) will migrate to the same position on a 2-DE gel. This ability to separate more than one sample on a single gel permits the inclusion of up to two samples and an internal standard (internal reference) in every gel.
- The internal standard is prepared by mixing together equal amounts of each sample in the experiment and including this mixture on each gel



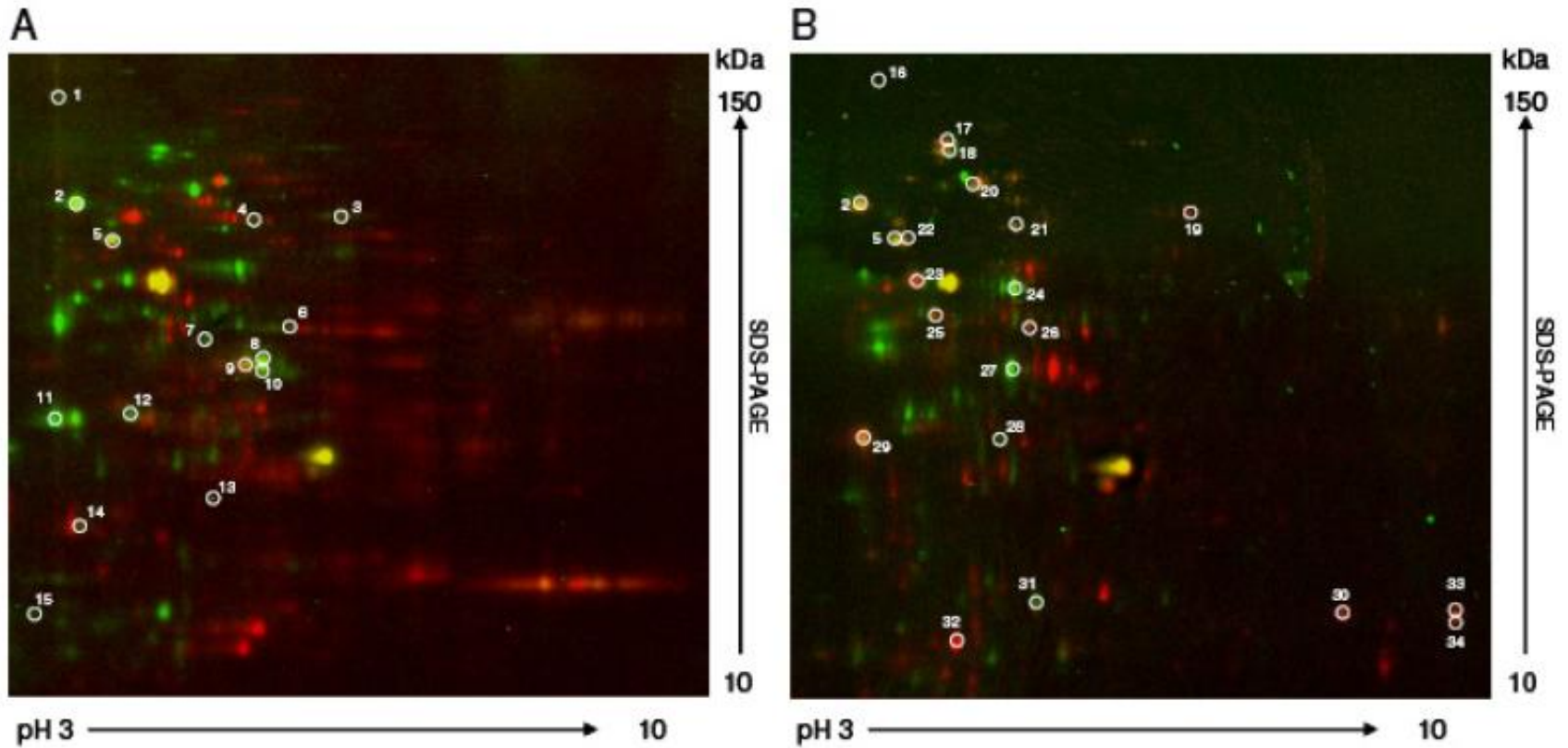
عملوا modification على 2dimensions method فطلع معهم D DIGE\_2.

بنضيف Fluorescent Cyanine dye على ال Sample فترتبط مع Specific protein موجود بال Sample ثم نمشيهم على Resolvable gel و بنفصلوا حسب ال Size & charge.

● بنقدر نستخدم أكثر من Sample بإستخدام هي الطريقة.

● هون بنستخدم كمان internal standard، يعني ك Reference بنرجعله، اللي هو عبارة عن خلط كميات متساوية من العينات بعديها بنحط على كل واحد من الجل لنتأكد إنه ال internal standard عم يعطيني الإشي الصح و ما عم تتداخل العينات ببعض.

# Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE)



كل Fluorescent dye لها لون ، فكل وحدة منهم مرتبطة مع بروتين معين و عم تعطي موقعه  
إذا كان صحيح بالضبط أو لا بالنسبة للـ MO هاد فهي عم تعطينا Identification of MO.



# Protein identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry

- Mass spectrometry is a technique to analyze with high accuracy the composition of different chemical elements and atomic isotopes splitting their atomic nuclei according to their mass-charge ratio ( $m/z$ ).
- It can be used to identify different chemical elements that form a compound or to determine the isotopic content of different elements in the same compound

