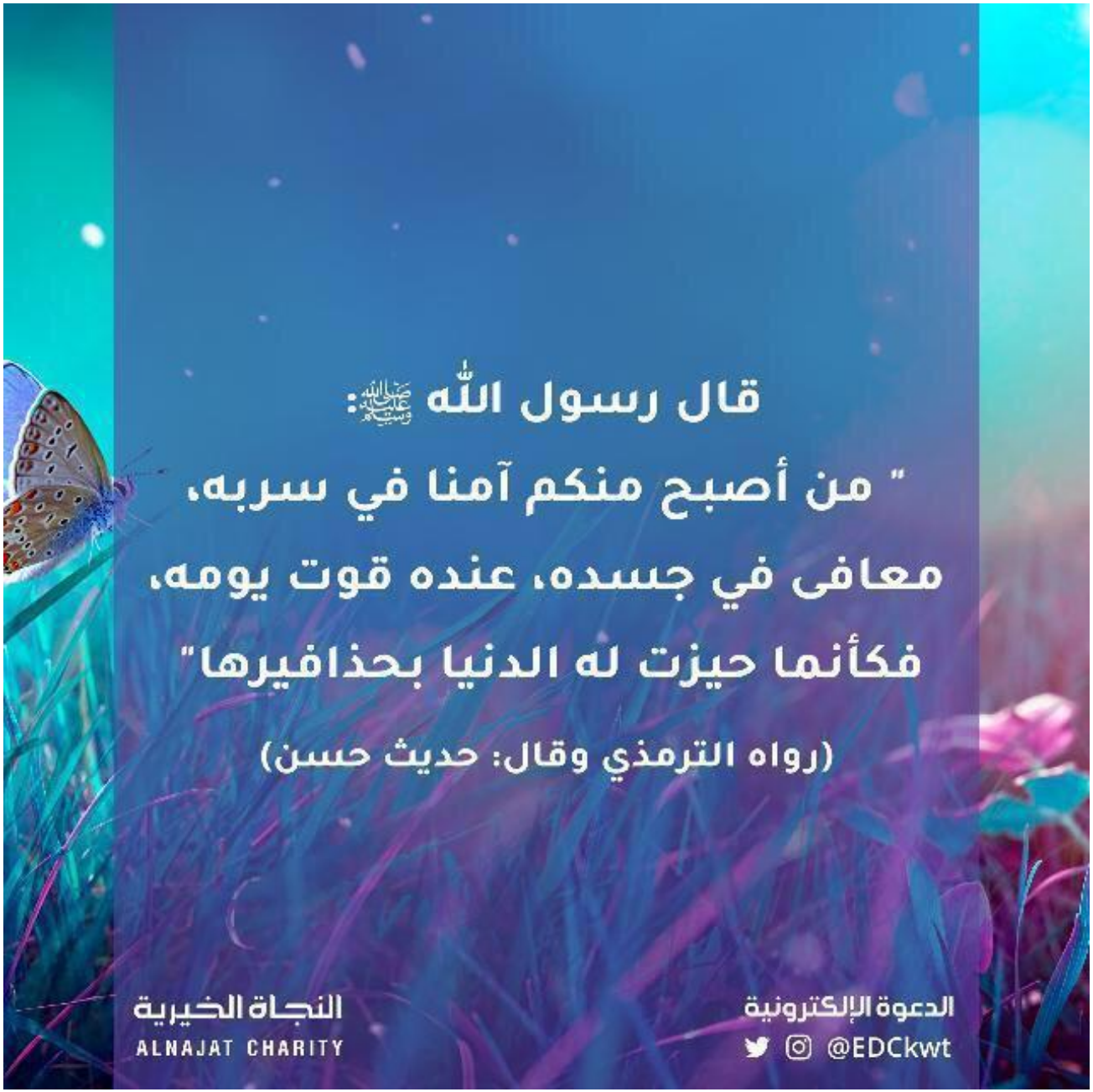

قال رسول الله ﷺ:
" من أصبح منكم آمنا في سربه،
معافى في جسده، عنده قوت يومه،
فكأنما حيزت له الدنيا بحذافيرها"
(رواه الترمذي وقال: حديث حسن)
النجاة الخيرية
ALNAJAT CHARITY
الدعوة الإلكترونية
@EDCkwt

STAGES:

1. The DNA to be sequenced is extracted from phage or E. coli for sequencing purpose.
2. A synthetic 5'-end-labeled oligodeoxynucleotide is used as the primer.

لازم يكون labelled

3. The template DNA is hybridized to the primer.

برتبط ال template dnaبال primer

4. The primer elongation is performed in four separate polymerization reaction mixtures. Each mixture contains

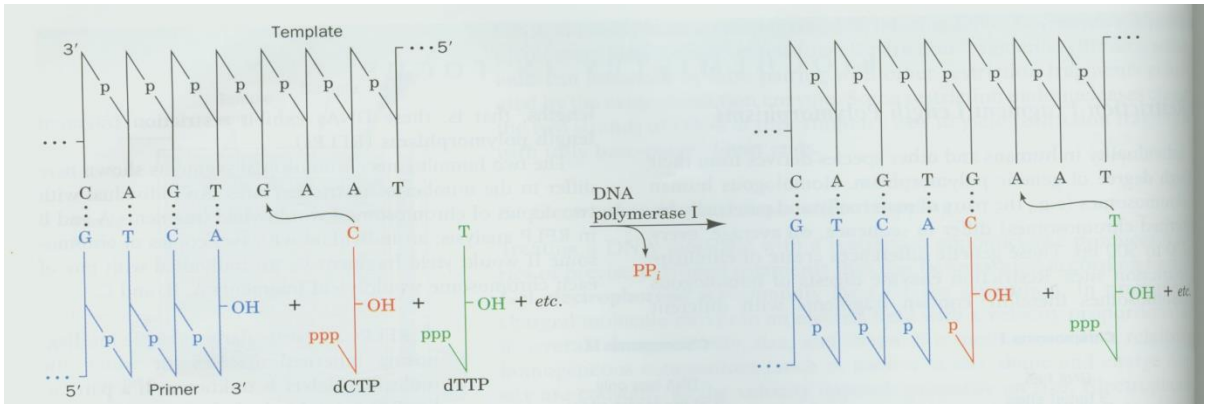
- 4 normal deoxynucleotides (dNTPs) in higher concentration and

بعدين بنحط ال dntpبكميات عالية والي هي بتعمل عمل النيوكليوتايد فبتحفز الاستنساخ عكس ال ddntpالي بتعمل termination

- a low concentration of the each of the 4 ddNTPs.

ليش بنضيف بكميات قليلة لانه بدنا يتكون strandsكثيرة لل dnaوبالصدفة يتوقف ويصيرله termination

5. There is initiation of DNA synthesis by adding enzyme DNA polymerase since the enzyme cannot distinguish between the normal nucleotides and their analogues.



Action of DNA polymerase I

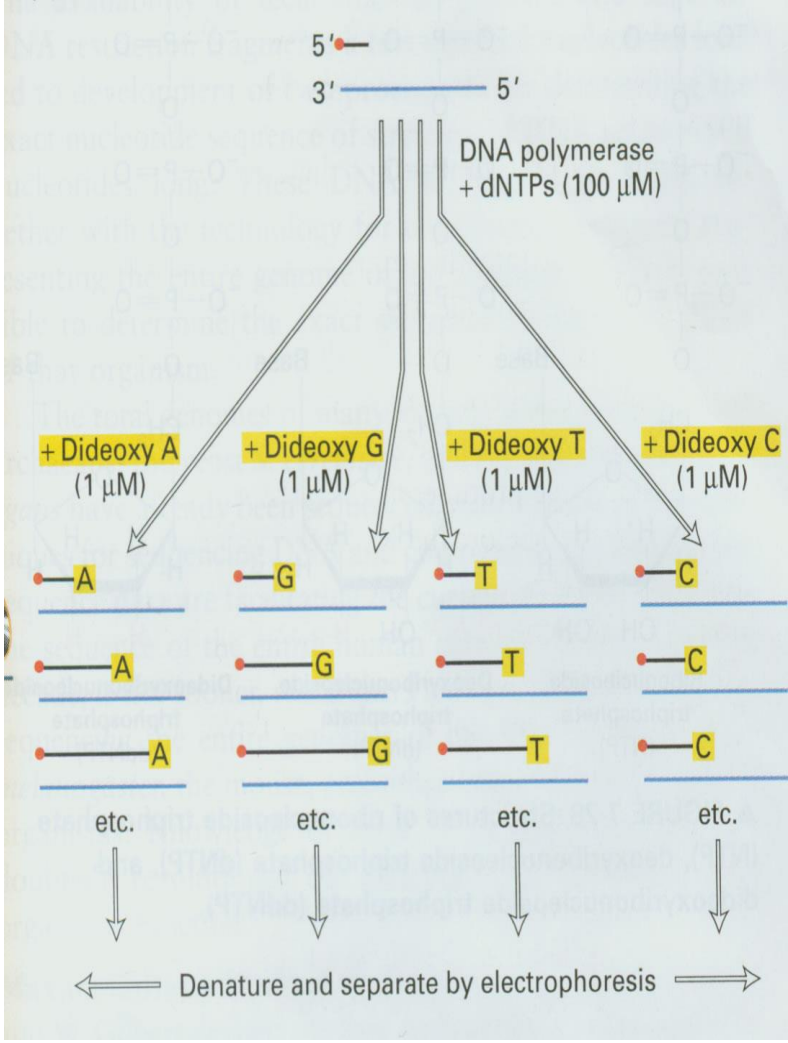
6. The strand synthesis continues until a ddNTP is added. The chain elongation ceases on the incorporation of a ddNTP because it lacks a 3’-OH group which prevents addition of the next nucleotide.

بتضل هاي العملية النسخ شغالة لحد ما ييجي ال ddntp ويوقفها طب ليش بتتوقف ؟ !لانه على ال 3primeفي ال ddntpما فيه عليها ohفما بتقدر تربط بالنيوكليوتايد الي بيجي بعدها فبتوقف

7. There is a result of mixture of terminated fragments, all of different lengths.

8. Denature DNA fragments.

9. Each of the four mixtures are run together on a polyacrylamide gel for electrophoresis.



Sanger method

10. The separated fragments are

then visualized by autography.

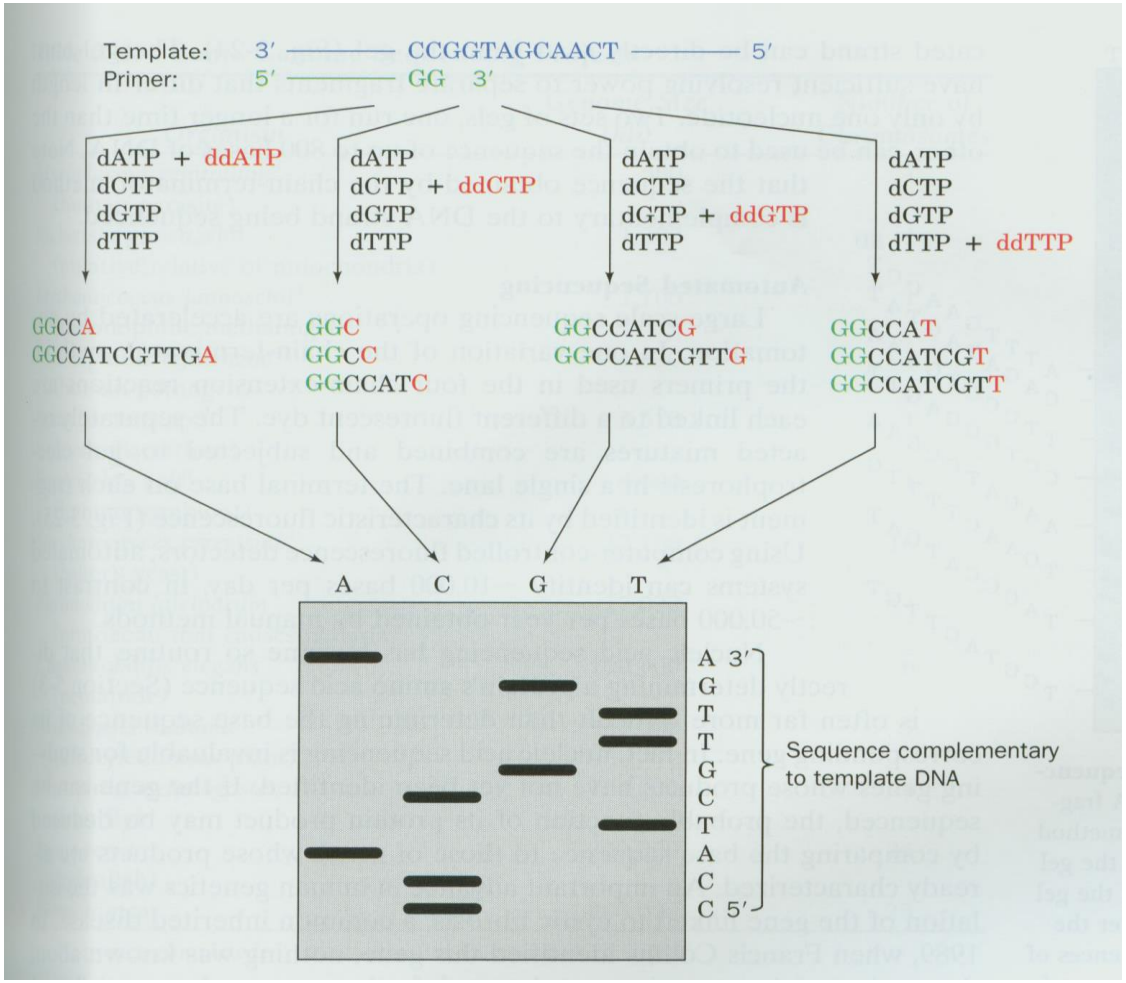
بالیزر او بال x ray

11. From the position of the bands of the resulting autoradiogram, the sequence of the original DNA template strand can be read directly.

بنقرأها من تحت لفوق من ال 5 prime بنبلش

وبالمناسبة لدكتورة بتحكي على القرائة سؤال بالامتحان بس الي مش شاطر بس بقدرش يحله فلا تكونوا مش شاطرين

وللمرة ال ١٠٠احنا ما بنستخدم agaros بهاي الحالات لانه قطع ال dna كثير صغيرة



Chain termination method

ADVANTAGES AND DISADVANTAGES

- Most popular method.

  حتى التطورات الي صارت صارت على هاي الطريقة زي ال capillary electroforesis

- Simpler and quicker allowing large output. Within an hour the primer-annealing and sequencing reactions can be completed.

  قرائتها والتعامل معها اسهل يعني لكل نيوكليوتايد بكون عنا لون معين

- Yielding of poor results owing to secondary structure in the DNA as sometimes DNA polymerases terminate chain elongation prematurely.

  في اجزاء ممكن ما يصيرلها sequencing

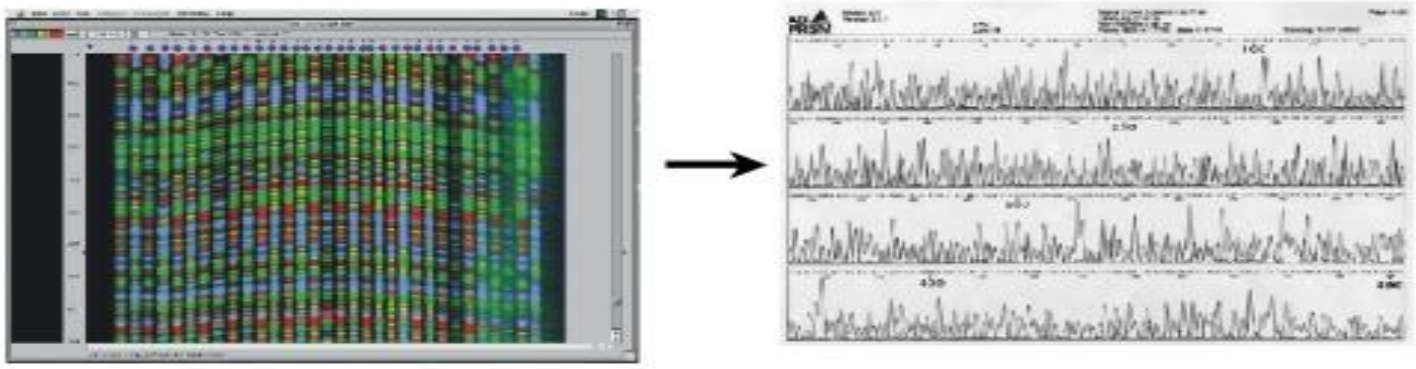
- The sequence is obtained not from the original DNA molecule but from an enzymatic copy. So, there is a chance of incorporation of wrong bases.

- لانه احنا بنستنسخ ال dnaوبنطلع على النخة الي بنطلعها ما بنعمل sequencingلل dna نفسه

IMPROVED APPROACHES AND AUTOMATED DNA SEQUENCING

- Updated version of Sanger method
  - صارت الامور كلها automatedعكس الطرق الثانية
- Fluorescence detection with lasers
- Cycle sequencing
- Shotgun sequencing

Automated procedure for DNA sequencing



A computer read-out of the gel generates a "false color" image where each color corresponds to a base. Then the intensities are translated into peaks that represent the sequence.

CYCLE SEQUENCING

- There are two basic differences between cycle sequencing and PCR amplification:
  - The presence of only one primer in the cycle-sequencing reaction used to prime synthesis of one strand of the DNA
  - بنستخدم فيها كمية اقل من الdna
  - The presence of dideoxynucleotide triphosphates in the sequencing reactions that create the base-specific terminations required.
- The result of the temperature cycling is linear amplification of the sequencing product leading to an increase in the signal generated during the sequencing reaction when compared with standard sequencing protocols.

CYCLE SEQUENCING

- Cycling the sequencing reactions results in several advantages

(1) The amount of template necessary for the sequencing reaction is greatly reduced

(2) because smaller amounts of template are added, fewer impurities are introduced, meaning less template preparation is required; and

(3) The high temperature at which the sequencing reactions are run and the multiple heat-denaturation steps allow double- stranded templates such as plasmids, cosmids, X DNA, and PCR products to be sequenced reliably without a separate denaturation step

بتختلف كمان عن ال pcrانها بتستخدم ال ddntp for terminationوكمان بتوخذ وقت اقل بكثير لانه الكمية الي بدي اياها اساسا قليلة بس اني افحصها

SHOTGUN SEQUENCING

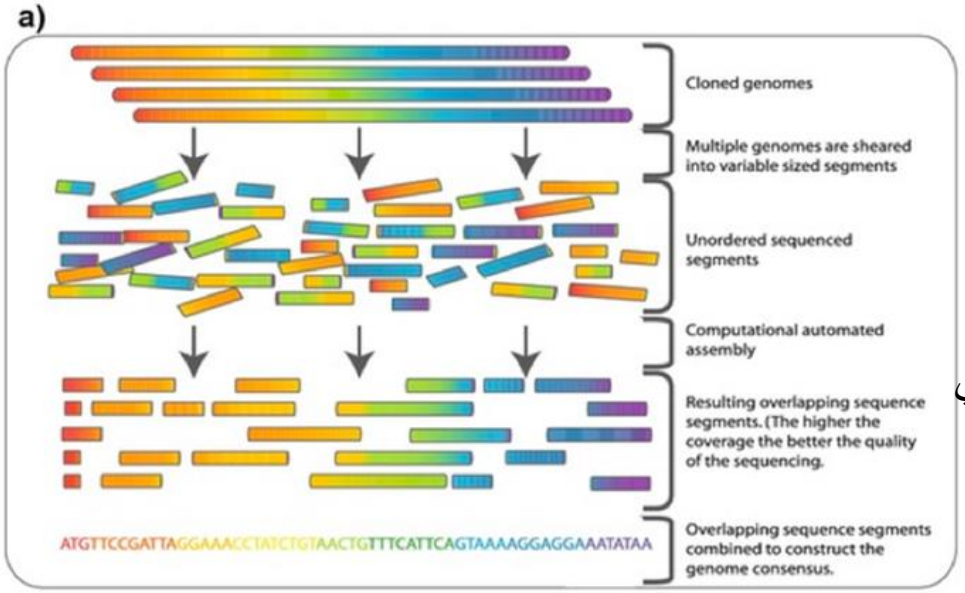
- is a method used for sequencing long DNA strands

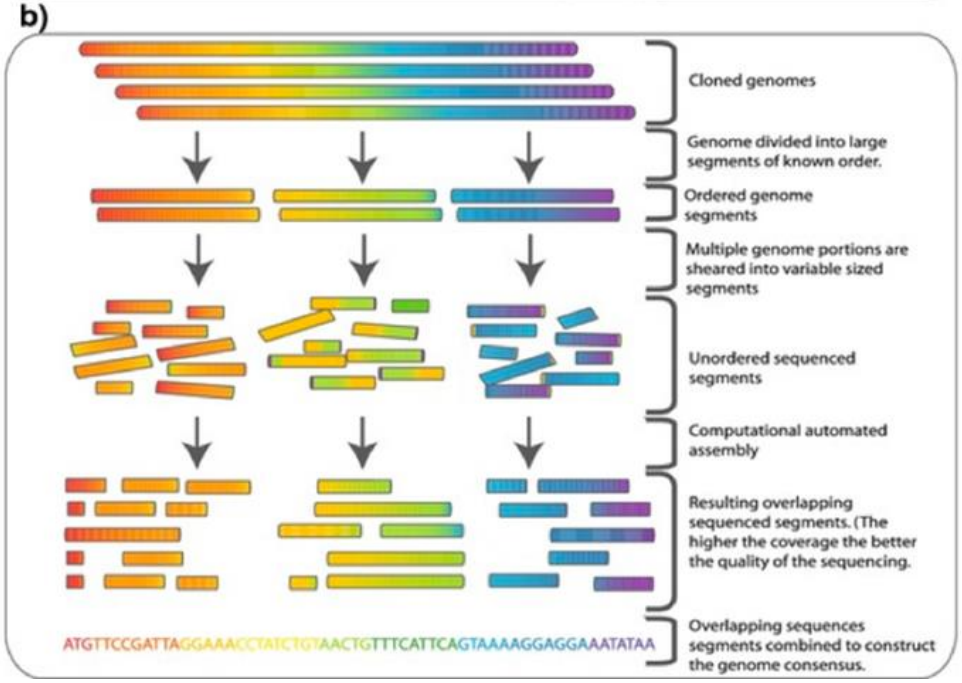
يعني بحدود ال٦٠٠٠_٧٠٠٠bp

ساعتها بنروح بنقطع ال dnaوبعمله digestionلاكثر من قطعة وبعدين بنعمل sequencingللقطع

- DNA is broken up randomly into numerous small segments, which are sequenced using the chain termination method to obtain *reads*.
- Multiple overlapping reads for the target DNA are obtained by performing several rounds of this fragmentation and sequencing.
- Computer programs then use the overlapping ends of different reads to assemble them into a continuous sequence
- بعدين بنساوي شغلة اسمها alimentبطابقها ببرامج كمبيوتر مع ال dna الاصلية

SHOTGUN SEQUENCING

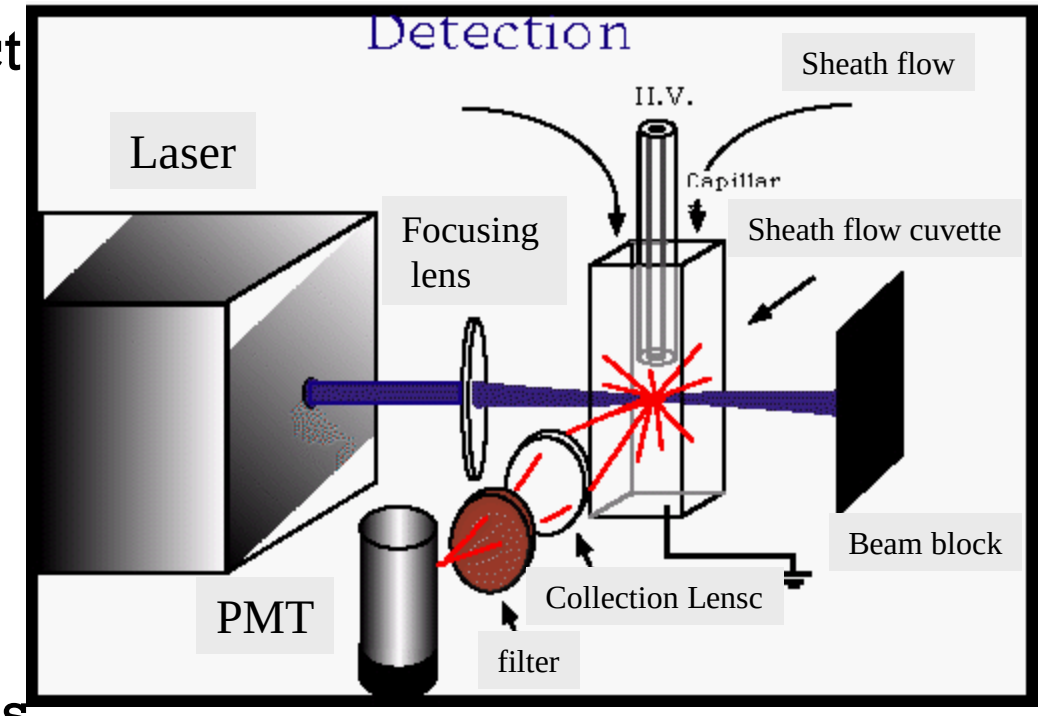




هون بنشوف عملية alimentكيف الكمبيوتر بطابق قطع ال dnaمع ال dnaالاصلية قال يعني لونه فالح وبعرف يطابق كان عرف يقرأه وهو طويل وبلاها هالغلبة

High-throughput sequencing: Capillary electrophoresis

لولاها كان ما قدروا يعملوا sequencing للـ human genome بسرعة كبيرة



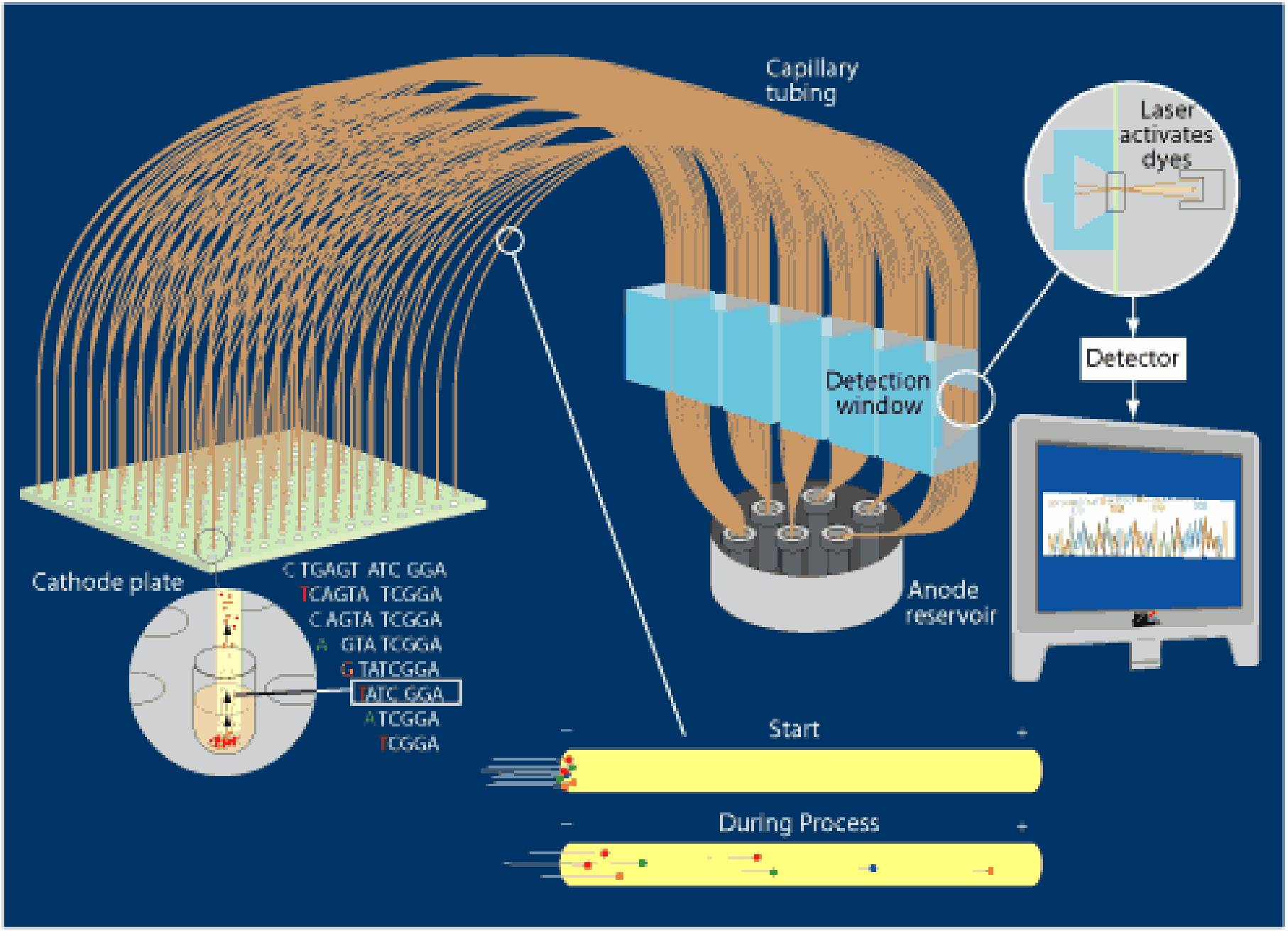
The human genome project has spurred an effort to develop faster, higher throughput, and less expensive technologies for DNA sequencing. Capillary electrophoresis (CE) separation has many advantages over slab gel separations. CE separations are faster and are capable of producing greater resolution. CE instruments can use tens and even hundreds of capillaries simultaneously. The figure show a simple CE setup where the fluorescently-labeled DNA is detected as it exits the capillary.

Sieving matrix for CE

•To separate DNA fragments of different sizes the capillary needs to be filled with sieving matrix, such as **linear polyacrylamide -neuro toxic when its in monomer phase-** (acrylamide polymerized without bis-acrylamide).

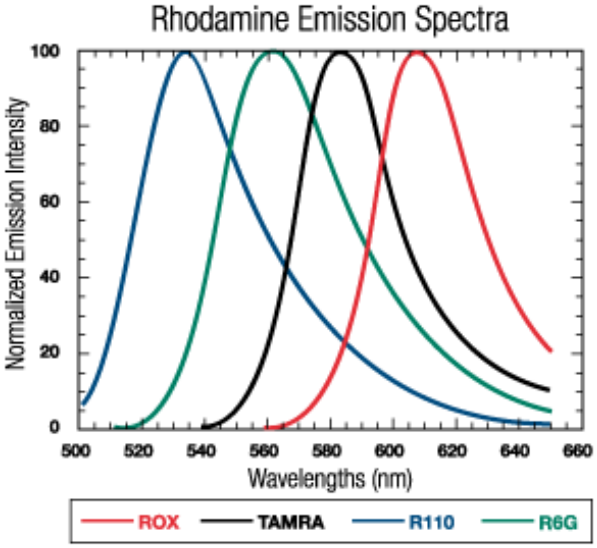
•This material is not rigid like a cross-linked gel but looks much like glycerol. With a little bit of effort it can be pumped in and out of the capillaries.

•To simulate the separation characteristics of an agarose gel one can use **hydroxyethylcellulose**. It is not much more viscous than water and can easily be pumped into the capillaries. and less toxic than others

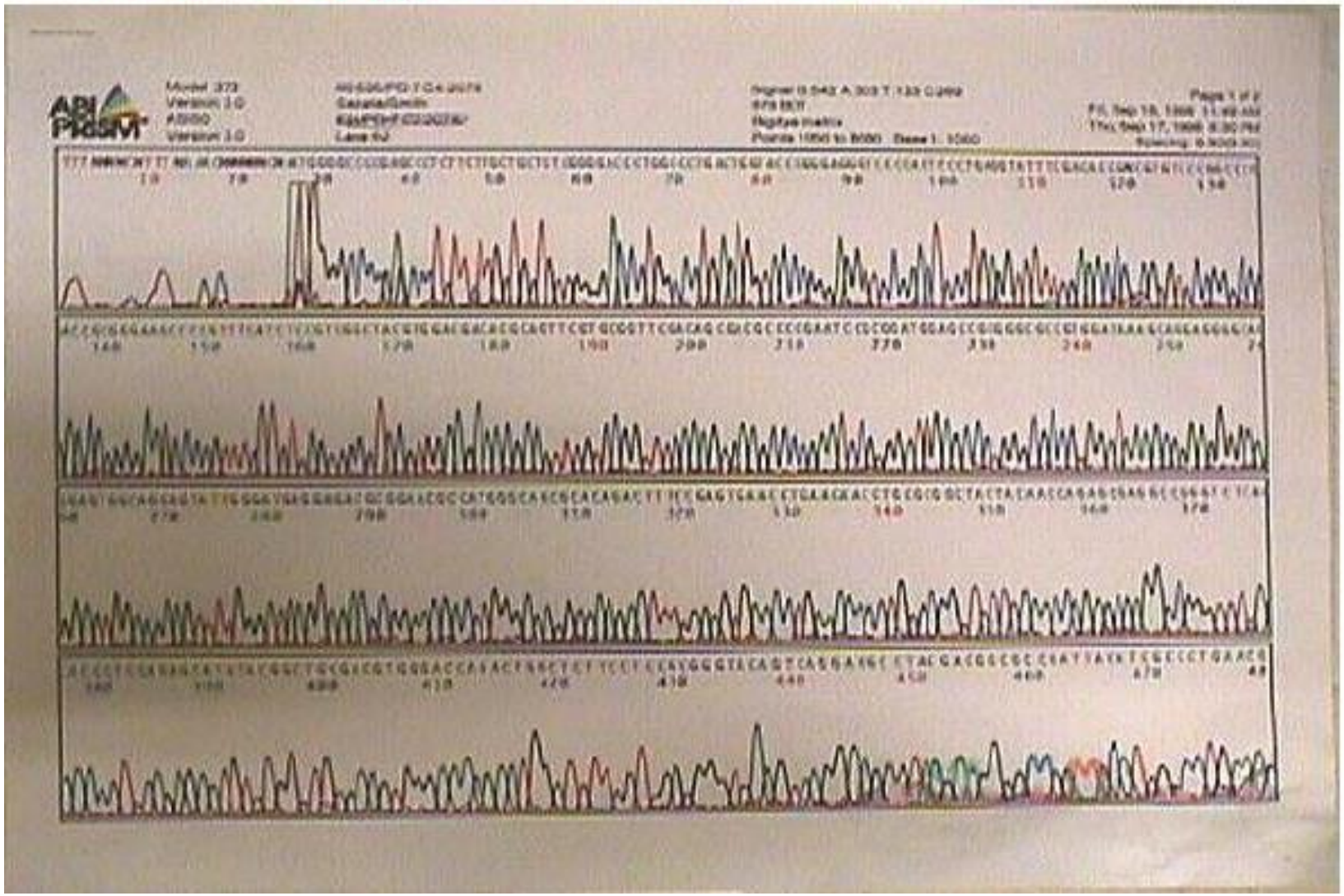

Capillary tubing
Laser activates dyes
Detector
Detection window
Anode reservoir
Cathode plate
C TGAGT ATC GGA
TCAGTA TCGGA
C AGTA TCGGA
A GTA TCGGA
G TATCGGA
TATC GGA
A TCGGA
TCGGA
Start
During Process

Fluorescent end labeling of DNA

هسة مبدأها بنحط ال dnaالي بدنا نعمله sequencingفي تيوب وبنضيفله
ال flourecent dyeو ال ddntpولكل وحدة من ال ddntpبكون flourecently labelledمثلا ال ddgtpبكون لونها احمر وال ddatpبكون لونها اخضر وهكذاوبكون فيه detecterبميز اللون والطول لقطعة ال dnaوبترتبوا من الاقصر للاطول وبطلع معنا على شكل beaksزي الموجودات بالالسلايد الي ورا ومش احنا الي بنقرأهم بقرأهم الكمبيوتر لحاله



DNA SEQUENCING OUTPUT



What is the function of the sequenced gene?

Classical methods:

- mutate gene, characterize phenotype for clues to function (genetics)
  - بنصير نعرف هذا الجين شو بعمل بالجسم
  - زي العائلات الي بكون عندها امراض وراثية مثل السكري والسرطان بكون عندهم خلل جيني معين بنقدر نكتشفه عن طريق الsequencing
- purify protein product, characterize *in vitro* (biochemistry)
  - زي لما يكون عنا بروتين كميته كثير قليلة في الخلية ممكن نعمله sequencing برا الخلية ونعمل دراسات عليه ونشوف الfunction تبعته

What is the function of the sequenced gene?

Comparison to previously characterized genes:

- genes sequences that have high sequence similarity usually have similar functions

- if your gene has been previously characterized (using classical methods) by someone else, you want to know right away! (avoid duplication of labor)

- هسة كيف بدنا نعرف ال functionالجين ؟!

- هسة اذا جين معروف شو هو زي جين الانسولين بقارنه مع sequenceالجينات الموجودة عندي واي جين بتطابق معاه معناته نفس ال function

طيب ولو كان الجين جديد بنروح على السلايد الي بعده لنعرف

NCBI

NCBI home page -Go to www.ncbi.nlm.nih.gov for the following pages

اذا كان ال sequenceالي طلع معي جديد وبدخله على ال databaseبروح الجهاز بعملي بحث في كل اشي موجود عنا على الداتا بيس وبقارن بينهم واذا كان في جين مطابق معناته نفس ال functionواذا كان في جين قريب عليه باختلاف عدد قليل من النيوكليوتايد بكون نفس الجين غالبا بس صايرله mutationsفبصير اشوف شو تأثير الجين هذا مثلا لو كان جين انسولين بشوف الشخص هذا شو تأثير ال mutaionفي الجين عليه صابه سكري وراثي او صابه سكري مبكر وبروح بدخل ال mutaionهاي على الداتا بيس وهي حاطينلنكوا الرابط تبع الموقع في حال كنتو بتلعبوا بالجينات وانتو قاعدين بالصيدلية مستقبلا واكتشفتوا طفرة جينية تدخلوها على الdata base

Pubmed: search tool for literature--search by author, subject, title words, etc.

All databases: "a retrieval system for searching several linked databases"

BLAST: Basic Local Alignment Sequence Tool

OMIM: Online Mendelian Inheritance in Man

Books: many online textbooks available

Tax Browser: A taxonomic organization of organisms and their genomes

Structure: Clearinghouse for solved molecular structures

What does BLAST do?

1) Searches chosen sequence database and identifies sequences with similarity to test sequence

2) Ranks similar sequences by degree of homology (E value)

بطلع قديش نسبة التطابق زي مثلا لما نطابق الانسولين للانسان مع الفار بطلع التطابق لحد 80%

1) Illustrates alignment between test sequence and similar sequences

Alignment of sequences:

The principle: two homologous sequences derived from the same ancestral sequence will have at least some identical (similar) amino acid residues

Fraction of identical amino acids is called "percent identity"

Similar amino acids: some amino acids have similar physical/chemical properties, and more likely to substitute for each other-these give specific similarity scores in alignments

زي الانين والجلايسين راح تلاقيهم كثير similarفي كثير صفات زي انه الثنين

hydrophobicواليوسين والفالين كمان بتشابهوا اللايسين والهستيدين والارجينين كلهم

basic amino Acidفمرات بين كل جينبن بنلاقي احماض امينية مختلفة لاكنها similarبالوظيفة تبعتها

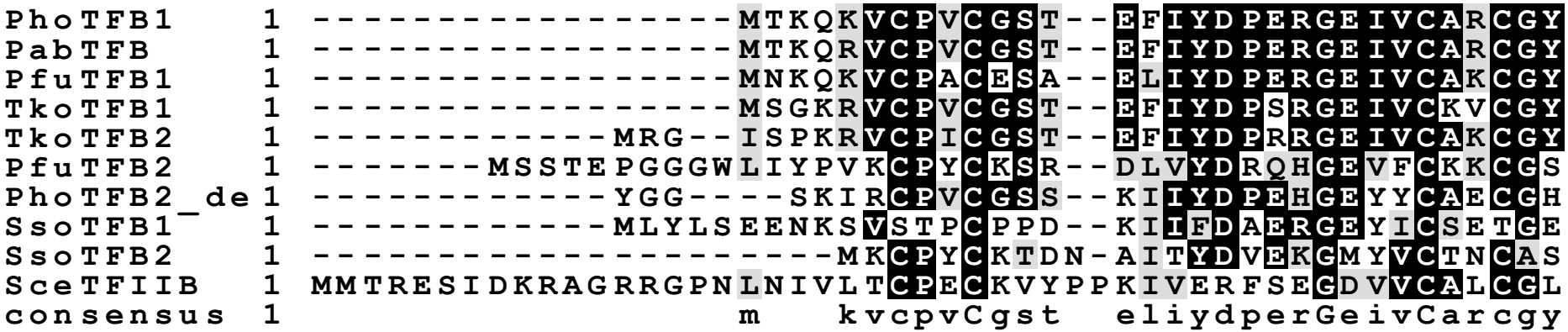
Gaps in similar/homologous sequences are rare, and are given penalty scores

penalty هاي لما يكون في امينو اسيد زيادة مش موجود بالجين الثاني بسميه

Homology of proteins

Homology: similarity of biological structure, physiology, and development based on genetic inheritance

Homologous proteins: statistically similar sequence, therefore similar functions (often, but not always)



Alignment of TFB and TFIIB sequences
TFB stands for archaeal transcription factor

Translating the DNA sequence

زي اسمك جاي

starting لازم يطون في prokariotic بتبلش القرأة من اي مكان عادي بينما في الeukariotic هسة في ال
codon زي ال AUG (methionine) بتبلش فيه قرائة الانسولين وبعد ما نخلص لازم نشيله طبعا مشان ما يأثر على البروتين

The order of amino acids in any protein is specified by the order of nucleotide bases in the DNA.

Each amino acid is coded by the particular sequence of three bases.

To convert a DNA sequence

First, find the starting codon. The starting codon is always the codon for the amino acid methionine. This codon is AUG in the RNA (or ATG in the DNA):

GCGCGGGUCCGGGCAUGAAGCUGGGCCGGGCCGUGC....

Met

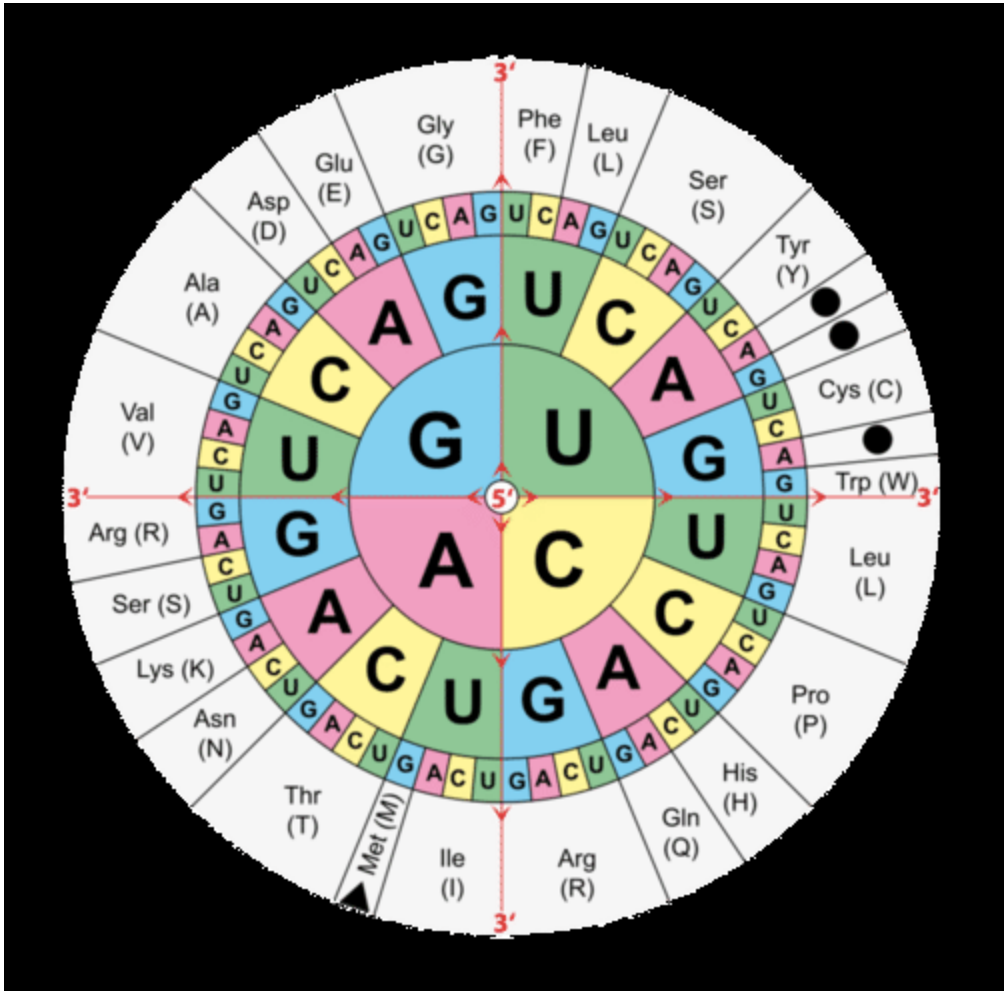
In this particular example the next codon is AAG. The first base (5'end) is A, so that selects the 3rd major row of the table. The second base (middle base) is A, so that selects the 3rd column of the table. The last base of the codon is G, selecting the last line in the block of four.

The codon table

5'-Base	Middle Base				3'-Base
	U(=T)	C	A	G	
U(=T)	Phe	Ser	Tyr	Cys	U(=T)
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U(=T)
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U(=T)
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U(=T)
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

طيب كيف بترجم القرائة بنبلش قرائة من ال 5-3طبعا كل nucleotideمع بعض وبنشوف وين بلتقي العامود الي على الشمال معا الصف الي فوق مع العامود الي على اليمين بعطينا الامينو اسيد الناتج مثلا CAUبنبل ش من العامود الي على المال بنشوف ال cوبنشوف ال A بالصف الي فوق وبنشوف الUبالعامود الي على اليمين بعطينا histidineالي عليها دائرة

The codon table



هاي الطريقة اسهل وهي الي بشتغلوا عليها بس مش راح تيجي بالامتحان مثلا CAUبنشوف ال C وبندور على الAتحتها وبندور على ال uتحت ال A بطلع معنا histidineالحاله

Translating the DNA sequence

وطبعا بنحط دايما اخر اشي stop codonsمشان ما يضل يترجم زي الUAA,UAG,UGA

This entry AAG in the table is Lysine (Lys).
Therefore the second amino acid is Lysine.

The first few residues, and their DNA sequence, are as follows (color coded to indicate the correct location in the codon table):

Met Lys Leu Gly Arg … …

AUG AAG CUG GGC CGG GCC GUG C..

This procedure is exactly what cells do when they synthesize proteins based on the mRNA sequence. The process of translation in cells occurs in a large complex called the ribosome.

HUMAN GENOME PROJECT (HGP)

HGP is a national effort to sequence and analyze the human genome which is a very complex system consisting of 50,000 to 100,000 genes. These genes are located on 23 base pairs of chromosome. The complete sequence was complete in 2005.

Some reasons for studying Human genome:

- Better medical practice طور اكتشاف الامراض وعلاجها
- High-quality diagnosis of diseases
- Understanding of evolution fully
  - فهم قصص التطور وانه الانسان اصله قرد والحكي هاض كله تبع الملحدين
- Improvement in biological research and forensic science
  - تحسن البحث الجنائي واكتشفوا قضايا كانت مغلقة من زمان
- Improvement in agriculture etc.

The latest research on HGP are

- Pulsed electrophoresis
- Fluorescence microscopy
- 2D gel electrophoresis
- gtc double-stranded subclone inserts