



النادي
MC
الطبي

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لا تنسونا من دعائكم بالتوفيق

← بعض الطفرات harmless وعلیم ضالینت →

↓

The severity of mutation depends on the function of protein being affected . Some mutations can be harmless like **pentosuria** and **fructosuria** (Appearance of pentose and fructose sugars in urine due to defect in their metabolisms, respectively).

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↓ طفرات تانية harmful

Others can be **harmful** due to decreased formation of an important structural protein like **collagen** or **receptor protein** like **LDL receptor** or some **enzymatic defect in metabolism** that causes one of the following metabolic changes:

* بعض الاضطرابات ضارة **harmful** ، والتي يمكن ان تراها على **metabolic pathways**

1. Decrease in rate of product formation:

Deficiency of **glucose 6-phosphatase** liver enzyme in glycogen catabolic pathway leads to reduced formation of glucose from glucose-6-phosphate. The genetic disease is called **Gierke's glycogen storage disease** in which **liver** abnormally accumulates glycogen without being degraded.

اسماء تانية زيادتين
Von Gierke's Disease
Glycogen storage disease type 1

2. Decrease in rate of substrate removal:

زيادة تركيز ال substrate بسبب قلة عملية ازالته
 نقص انزيم phenyl alanine hydroxylase
 له الحيف المسؤول عن انتاجه خارجيه لفترة ونظن
 يطرح هاد الانزيم
 جين هاد الانزيم مش يعمل ؟
 تحول ال phenyl alanine الي Tyrosine
 لما يطرح هاد الانزيم رح تتركب ال substrate (phenyl alanine)
 ولما تتركب بقوله لهاد الحيف اسمهم phenyl ketons
 والاسم تاني (Deaminated products of phenyl alanine)
 ويخرج بصيغتهم
 Urine في ال excretion
 واسم المرض phenylketonuria

3. Altered feedback control:

Deficiency of **21-hydroxylase enzyme** causes reduced formation of cortisol which **stops** the feedback control mechanism and leads to increase secretion of adrenocorticotrophic hormone (ACTH) in a disease called **Congenital adrenal hyperplasia**.

اسم المرض
 ال ACTH ال adrenal gland وتكونه مافي تخليها كافي
 بقله ال cortisol بنصير سير hyperplasia بتكثر عدد الخلايا
 □ adrenocorticotrophic hormone (ACTH) is produced & secreted by the anterior pituitary gland. Its principal effects are increased production & release of cortisol by the cortex of the adrenal gland.





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مثال مع أمراض سائدة

• **Example:**



-**Familial hypercholesterolemia:**

• Monogenic disorder, autosomal dominant.

• LDL receptor gene mutations → LDL في الدم يتركز بالساعة LDL receptor الجين الذي تأثر كان يعمل

• Very high plasma cholesterol and LDL-C levels.

• Premature CHD (teenage years).

• Lipid deposits at eyelids, tendons, hand, cornea.

• Heterozygotes: also symptomatic, develop CHD at the age of 20s-50s.

نتيجة طفرة جين واحد فقط

مورث





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X-linked dominant
بصبيوا ال male و ال
Female و بصبروا
rare

- The x-linked dominant disorder is rare, which shows the clinical symptoms in the heterozygous female or in a male with single copy of the mutated allele.

بصبي ال ال واحد من طرفين

❖ **X-linked hypophosphatemia (XLH) or X-linked dominant hypophosphatemic rickets or X-linked vitamin D resistant rickets**: a form of rickets, this disease occurs due to an excess excretion of phosphates from the body, which results in bones being unable to properly calcified and having short stature.

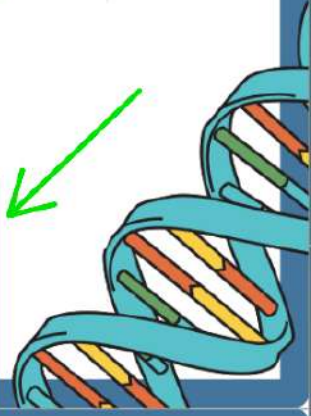
مثال عليه ← الكساح
واله ع أسوأ

Autosomal hypophosphatemic rickets

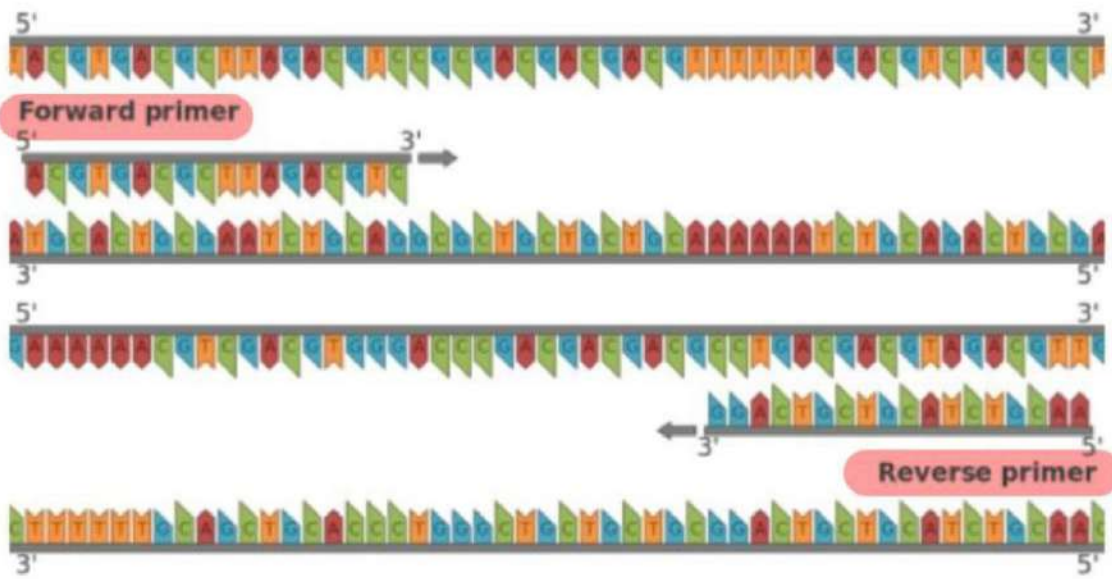
د اسم رايح (نقص بروتين بيس هوش نقص المشيب)

نتيجة لنقصه الفوسفات
وليس الكالسيوم
ف علاج مش ال D

Gene mutations in PHEX prevent it from correctly regulating fibroblast growth factor 23. The resulting overactivity of FGF-23 ↓ reduces phosphate reabsorption by the kidneys, leading to hypophosphatemia and the related features of hereditary hypophosphatemic rickets.

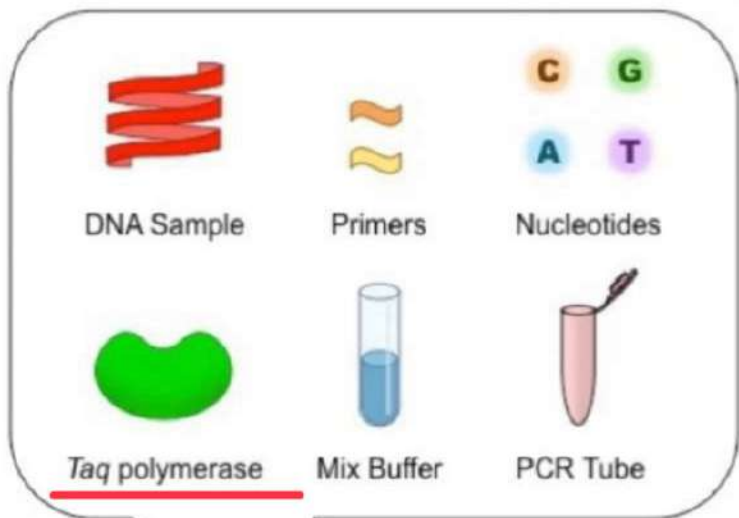


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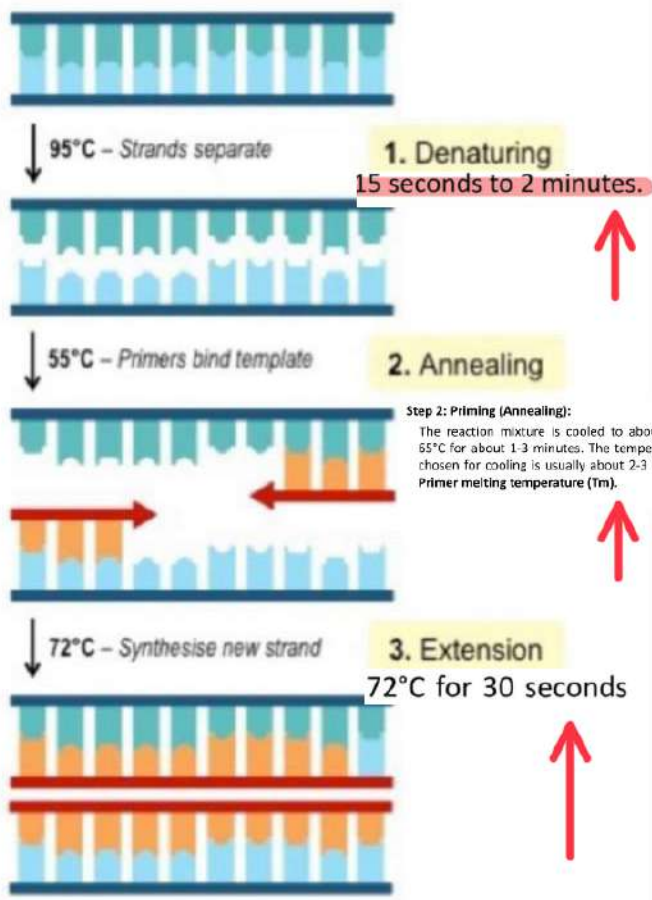


•Forward primers anneal to the antisense strand of the double-stranded DNA, which runs from 3' to 5' direction, whereas reverse primers anneal to the sense strand of the double-stranded DNA, which runs from 5' to 3' direction.

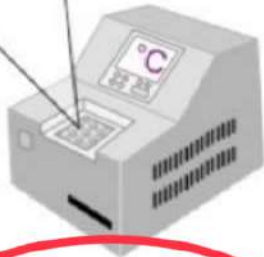
PCR Components



PCR Process (ONE Cycle)



Step 2: Priming (Annealing):
The reaction mixture is cooled to about 50-55°C for about 1-3 minutes. The temperature chosen for cooling is usually about 2-3 below Primer melting temperature (T_m).



Thermal Cycler
(Tempcycler)

PCR Cycle

Presence of HIV RNA in blood can be detected as early as 4 weeks after infection.

انقل

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There are three types of HIV tests: antibody tests, antigen/antibody tests, and nucleic acid tests (NAT).

بالتوصيفه كانه البروتين اللي بلزق بالنهاية اللاصقة
probe (a single-stranded sequence of DNA or RNA of variable length used to search for its complementary sequence and can be radioactively or fluorescently labeled to allows its binding to be visualized).

لو انت عندك sequence من ال DNA موجود وسط آلاف ال sequences من ال DNA فكيف
 اعمل pick up اله ؟
 بروح بصنعله probe و يفضل يكون مشع كدا (radioactive) بحيث اول ما يمسك كدا يعطيني
 signal و انا بقدر to detect ال sequence بتاعي

1. اول اشئ هو ال Restriction endonucleases و همئ عبارة عن انزيمات بستخدمها فئ قطع الجين مشان اعمل اله isolation و الصقه على البلازميد
2. ثاني اشئ اسمه ال vectors او vehicle DNA و همئ عبارة عن وسيلة نقل بنقل فيها ال human gene لل hosts و احد الأمثلة عليها هو البلازميد
3. ثالثا ال human gene او passenger DNA بعمل اله لصق على البلازميد
4. و آخر اشئ هو living cell سواء كانت نباتية ، بكتيرية او حيوانية مشان اذرع جواتها (و طبعا كل هذا لازم يكون داخل وسط مناسب)

Tools of recombinant DNA technology

1 Restriction endonucleases

2 Vectors or vehicle DNA

3 Passenger DNA (foreign DNA)

4 Hosts: they include Bacterial-animal or plant cells 4

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كربعا التصيقه بتمر من خلال
DNA Ligase و الئ بحتاج طاقة ATP

Restriction sites

شروط

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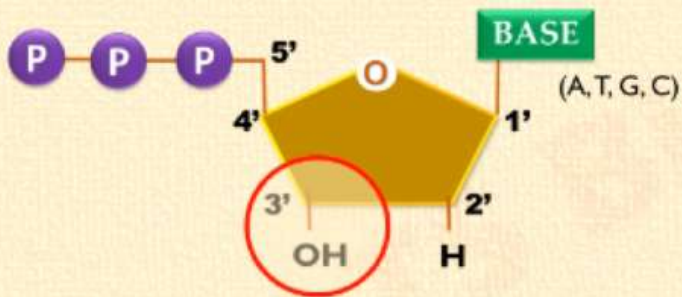
1. They are sequences of four, six, eight or rarely more bases with palindrome arrangement.
2. Palindrome in Greek means "to run backwards". It is similar to a word that reads backwards or forwards similarly e.g. madam. These are also called inverted repeat sequences which means the nucleotide sequence in 5`to 3` direction is the same in both strands.

Preparation of Chimeric DNA Molecules

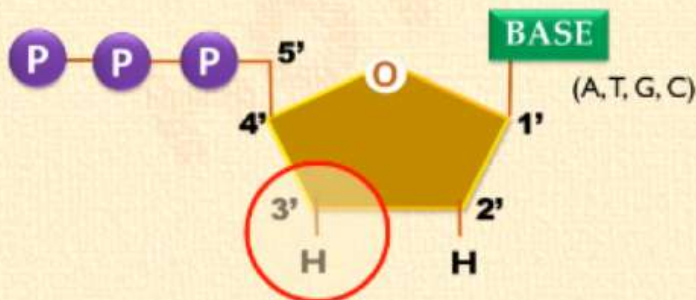
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- Chimera is the Greek mythological monster with a lion's head, goat's body and serpent's tail.
- A vector carrying a foreign DNA is called Chimeric DNA or Hybrid DNA or Recombinant DNA.

Sanger DNA sequencing technique makes use of **modified deoxynucleotides** known as **DIDEOXYNUCLEOTIDES**.



Deoxynucleotide (dNTP)



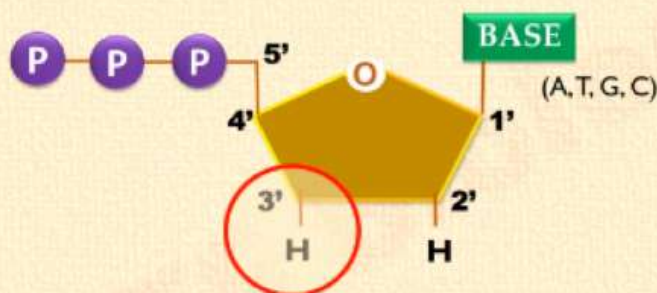
Dideoxynucleotide (ddNTP)

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اللهم اعني على ذكرك وشكرك وحسن عبادتك

When a **ddNTP** is added in a DNA synthesis reaction.....

DNA synthesis will **TERMINATE** with the incorporation of ddNTP.



ddNTPs are also known as **chain terminating nucleotides**.

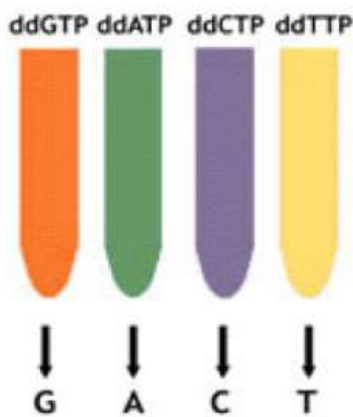
Sanger Sequencing is also known as **chain termination method** or **dideoxy DNA sequencing**.

لانه رح يوقف ال synthesis

Chain termination method (sanger dideoxy method)

The sample is divided into four reaction tubes and a small amount of one of the four **dideoxynucleoside triphosphate (ddNTP)** is added to each tube.

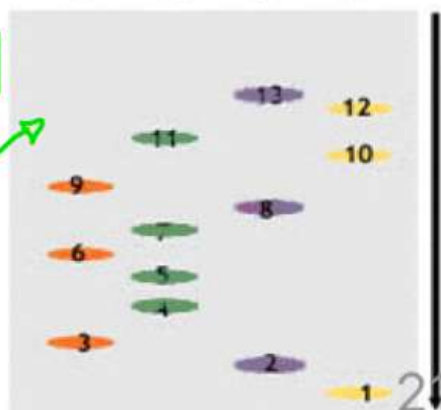
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Add ddGTP, ddATP, ddCTP, ddTTP, one to each of four tubes containing target DNA. Load each onto a separate lane on a gel.

polyacrylamide gel

الاهلام اللي بمشي فيه القطر



Largest

To sequence, read the order of bases from the smallest to the largest.

TCGAAGACGTATC

Smallest

3. How the Genes are Introduced?

كيف نعدل الجينات التي بدنا نعدلها للمريض

In vivo strategy,

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اما بهاي الطريقة ما بناخذ ال cells برا الجسم ، إنما بناخذ ال vector مباشرة على ال cells في حالة ال cystic fibrosis
بأثر على ال respiratory tract cells فنبروح بندخل ال cystic fibrosis gene لل Respiratory tract directly عن طريق ال
inhalation or other specific methods و بتدخل هاد ال vector لل cells

Ex vivo strategy

هون بناخذ ال patient cells و بنزرعها
باللاب و بندخلها ال new genes الي رح
تعمل expression و تطلع normal products
و بعدها بناخذ هاي ال modified cells و
بنرجعها على patient

Gene
therapy

The Vectors

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retroviruses

يتم تعديله عشان ما يسبب امراض
لكن له سلبيات:

insertional mutagenesis

uncontrolled cell division
(cancer) can occur.

adenovirus:

ال Adenovirus مشكلته انه ال expression تبعه يكون transient

يعني لفترة قصيرة عشان هيك بنعطي

هدول الناس dose تانية بس المشكلة انه بعض الناس

لما نعطيهم جرعة تانية بصيرلهم immune reaction

و ممكن يوصل لل death

بتعطي مع ال (Plasmid)

Plasmid Liposome Complex:

It is a non-viral vector system

The advantages

can carry human gene of big size,
do not replicate and evoke
only very weak immune responses.

The disadvantage

غالباً بتكسر جوا ال host cell
بالتالي ال efficiency تاعته قليلة