DETECCION DE MUTACIONES EN PATOLOGIAS DE ORIGEN GENETICO

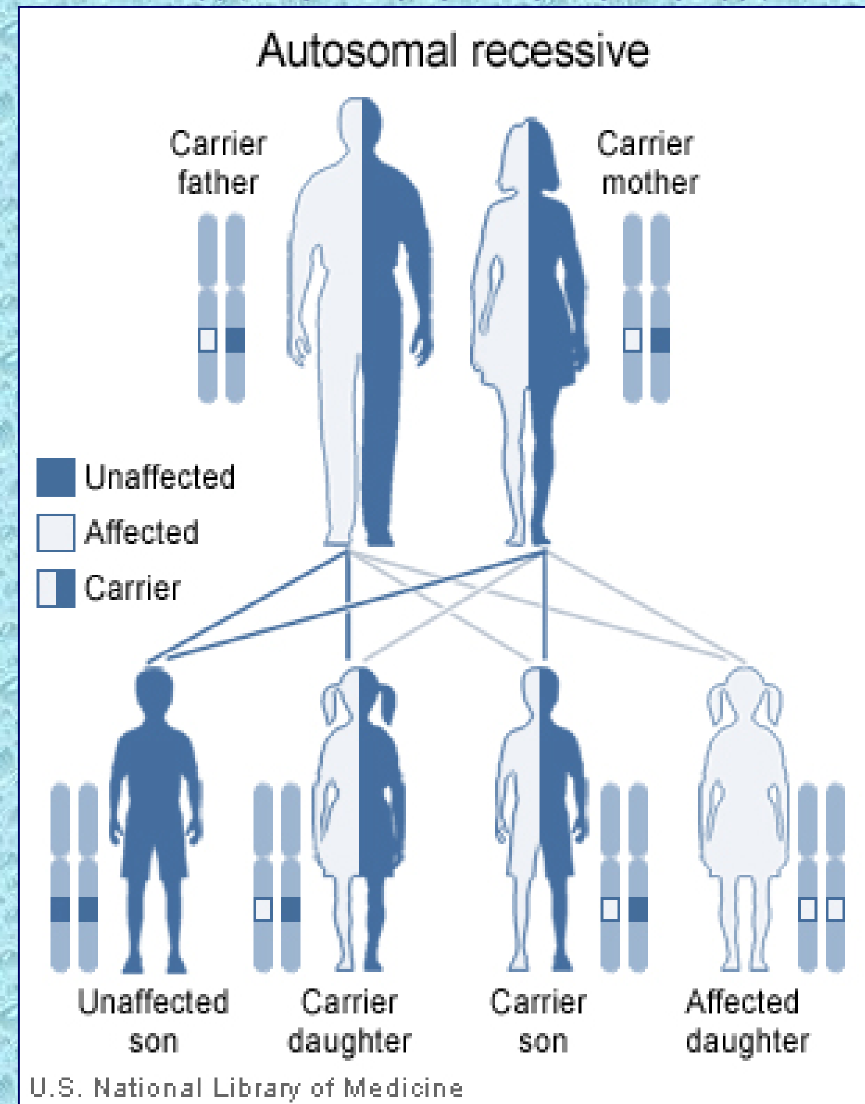
Fibrosis Quística

-Enfermedad genética severa mas frecuente en la población caucásica (1/2500 nacidos)

-Mutación en el GEN CFTR (regulador de la conductancia transmembrana de la fibrosis quística). Mut. mas frecuente ΔF508 (66%).

-Defecto o ausencia de una glicoproteína de membrana.

Patrón de herencia:



Cómo hacemos la Mix??????

PCR-ALELO ESPECIFICA

H_2O-PCR

Buffer PCR

$MgCl_2$

dNTPs

PRIMER REVERSE

Taq polimerasa

PRIMER

FOWARD N

PRIMER

FOWARD M o Δ

ADN

PCR-ALELO ESPECIFICA

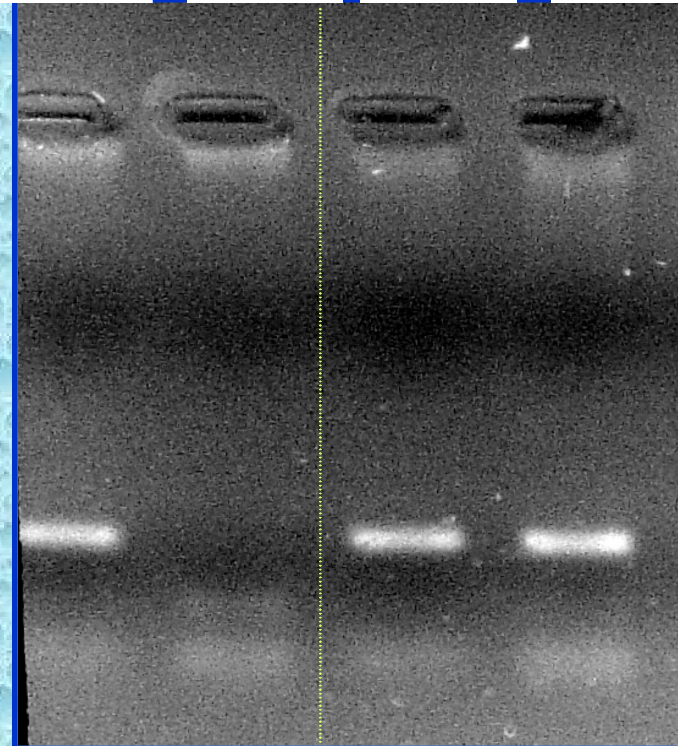
FQ: Mutacion en gen CFTR,
Mas frecuente ΔF508 (66%)

5´............ATC TT 3´ **Primer N**
..........TAG AA ....3´

5´............ATT GG 3´ **Primer Δ**

.........TAA CC.............3´

TTT/C:F
GG_:G



N/N N/Δ

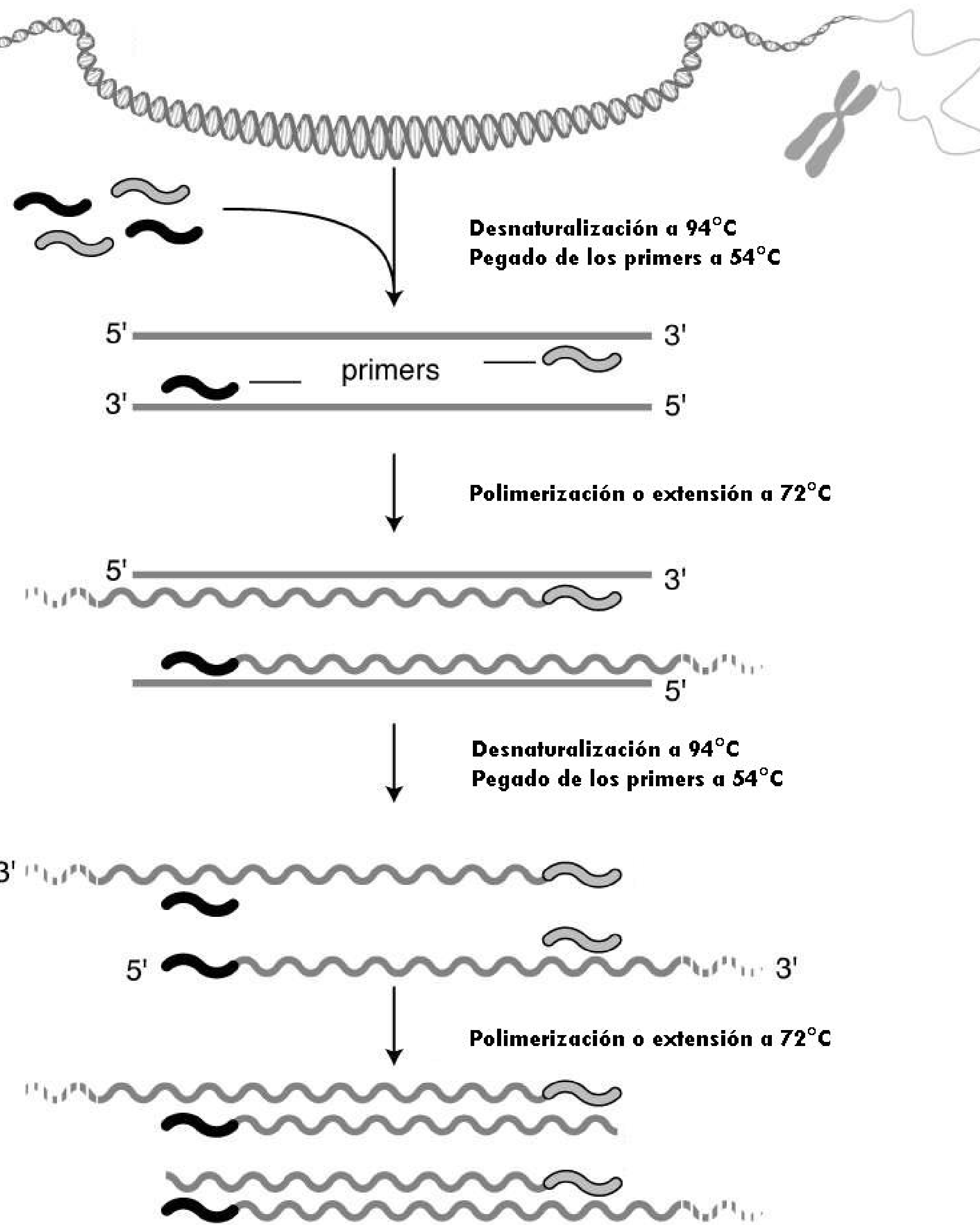
Pcr alelo específica!!!!!

Con este ensayo puedo saber

Si el paciente tiene x mutación (voy a buscar una mutación determinada)

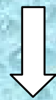
NO puedo saber qué otra mutación tiene (no es una estrategia de búsqueda de mutaciones)

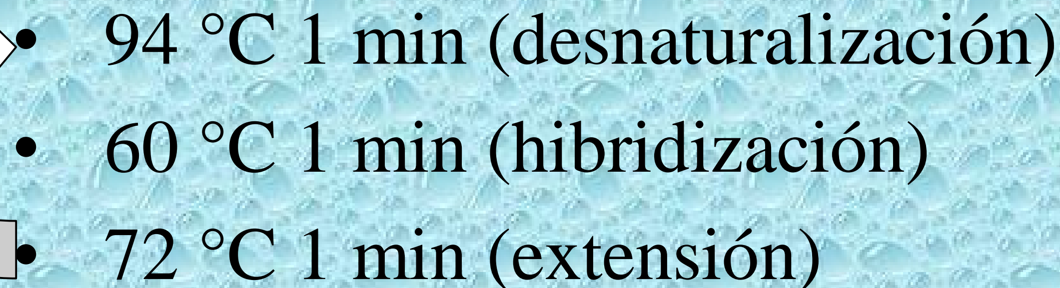
Polymerase Chain Reaction (PCR)



Programa de PCR

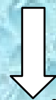
- 94°C 3 min (desnaturalización inicial del templado)





- 94 °C 1 min (desnaturalización)
- 60 °C 1 min (hibridización)
- 72 °C 1 min (extensión)

30 ciclos



- 72°C 3 min (extensión final de productos incompletos)

Master Mix:

	ul
-Buffer 10X	2,5
-MgCl2 50mM	1,25
-dNTP 10mM	0,5
-Primer Rev 10uM	2,5
-H_2O (Vol f 25ul)	13,125
-Taq polim (5U/ul)	0,125
Subtotal	20

Cada grupo:

-Primer F (N o Δ)	2,5
-DNA paciente	2,5
Vol total	25

2 Tubos/Gr:

	#Gr N	#Gr Δ
-Master Mix 20ul	+	+
-Primer F 2,5ul	N	Δ
-DNA 2,5 ul	+	+

Gr 1 a 4 DNA L
Gr 5 a 8 DNA H
Gr 9 a 12 DNA F

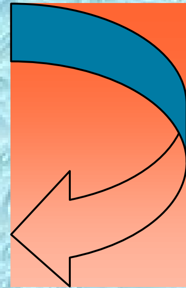
Master Mix: un grupo:

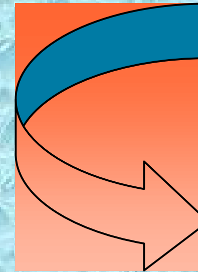
	ul	X30 ul
-Buffer 10X	2,5	75
-MgCl2 50mM	1,25	37,5
-dNTP 10mM	0,5	15
-Primer Rev 10uM	2,5	75
-H2O (Vol f 25ul)	13,125	394
-Taq polim (5U/ul)	0,125	3,75
Subtotal	20	600

Cada grupo:

-Primer F (N o Δ)	2,5
-DNA paciente	2,5
Vol total	25

Las mutaciones son cambios en la secuencia de ADN que pueden o no conferir un cambio fenotípico.

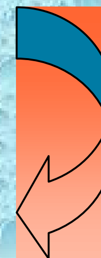


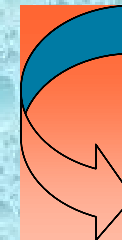


POLIMORFISMO

Variaciones normales de la sec de ADN >1% de la poblacion.

MUTACION





GRANDES

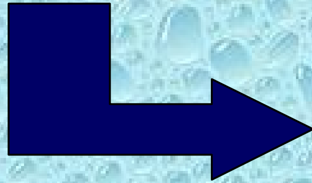
$2\text{-}250 \times 10^{6}$ pb

PEQUEÑAS

1- 50000 pb

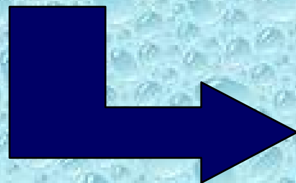
MUTACIONES

M
A
C
R
O



ESTRUCTURALES Y NUMERICAS

M
I
C
R
O



DELECIONES

INSERCIONES

CAMBIO DE SENTIDO

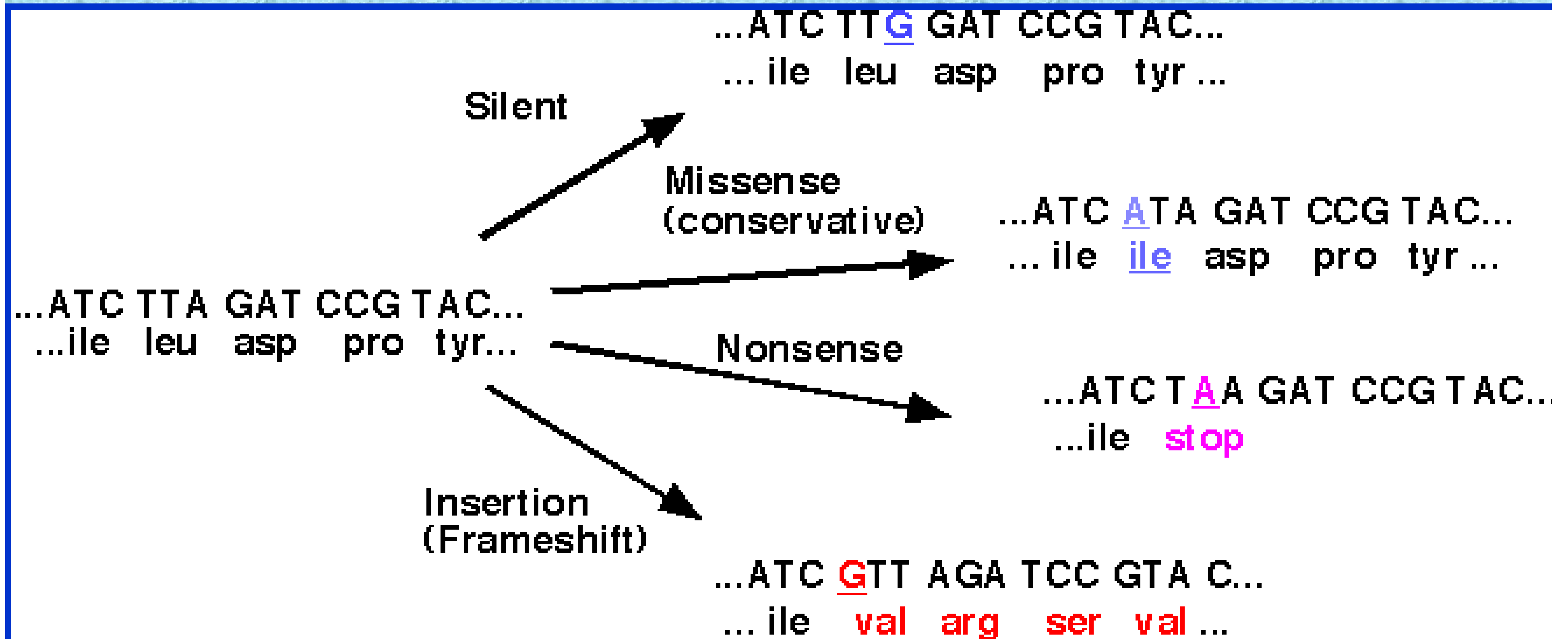
STOP

SPLICING

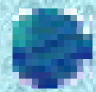
CAMBIO MARCO DE LECTURA

DINAMICAS

Clases mutaciones

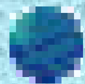


ESTRATEGIAS DE BUSQUEDAS DE MUTACIONES



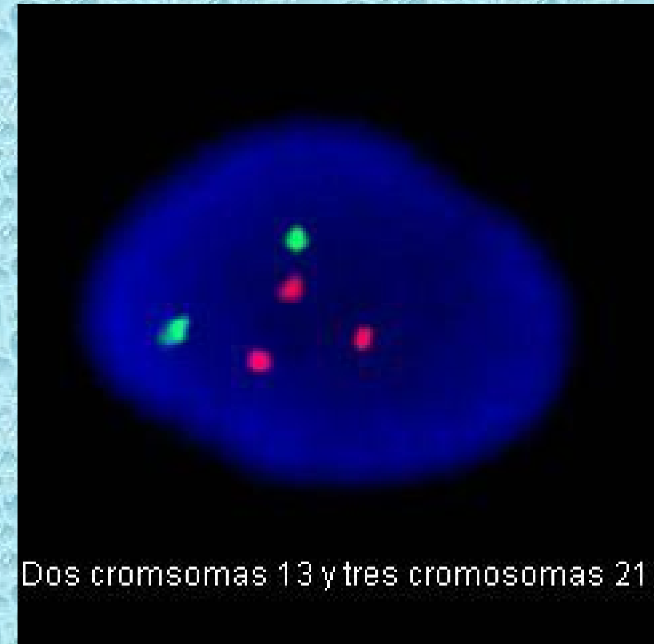
GRANDES VS PEQUEÑAS

CONOCIDAS VS DESCONOCIDAS



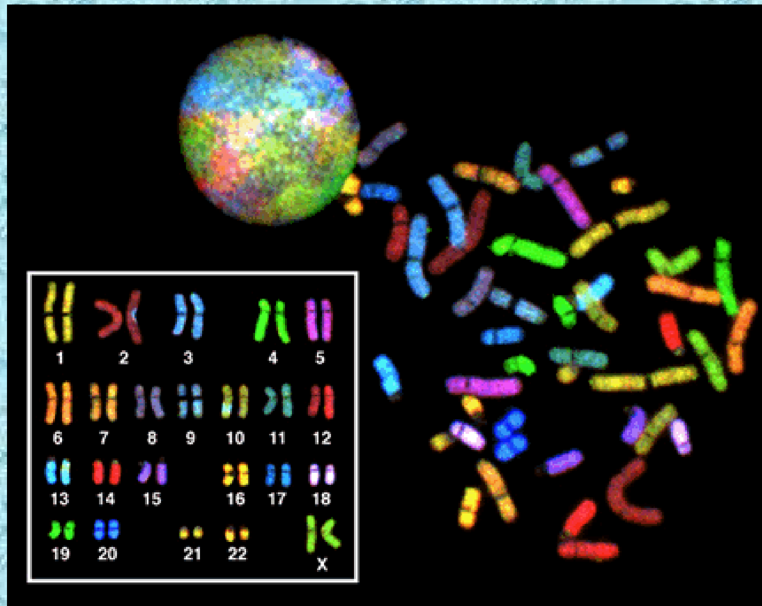
ESTATICAS VS DINAMICAS

MUTACIONES GRANDES



BANDEO G

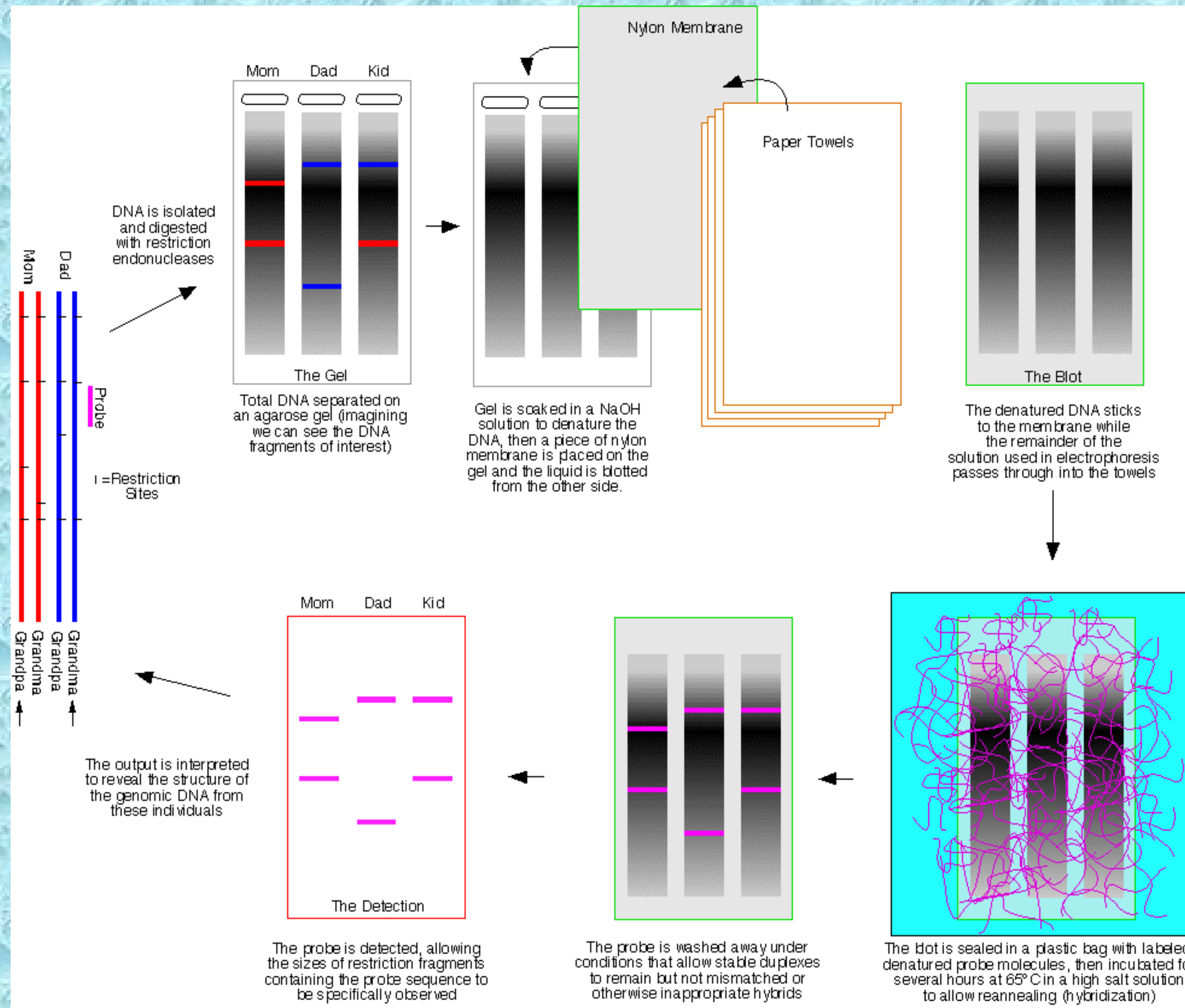
FISH



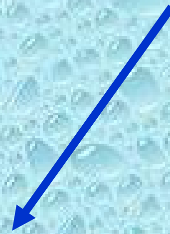
CITOGENETICA

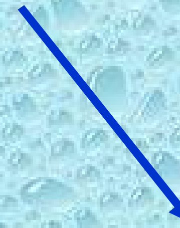
PINTADO CROMOSOMICO

Southern blot



Detección de mutaciones de tamaño pequeño.

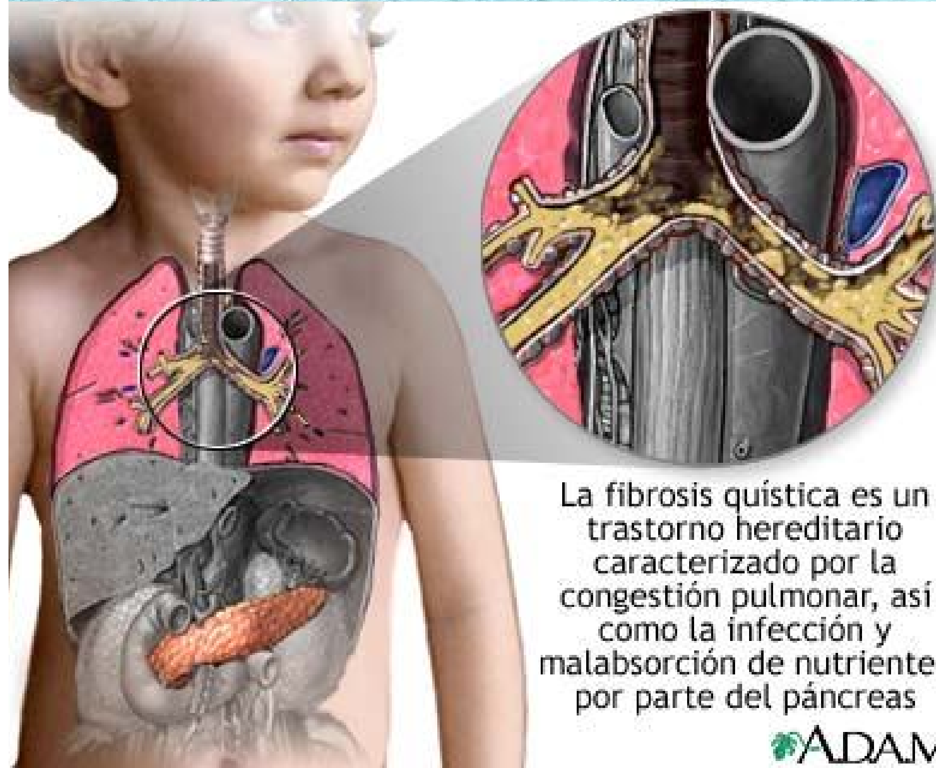




Conocidas **Desconocidas**

Fibrosis Quística

También es conocida como fibrosis quística pancreática.



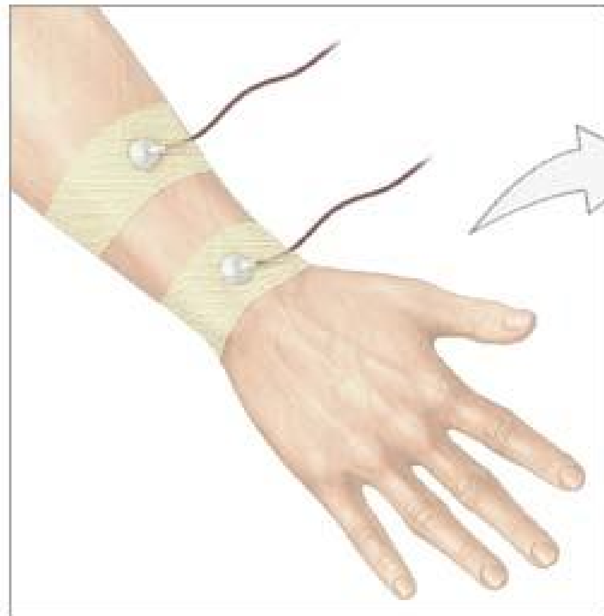
-Enfermedad genética severa mas frecuente en la población caucásica (1/2500 nacidos)

-Insuficiencia pancreatica e instestinal. Daño Pulmonar.

-Mutación en el GEN CFTR (reg. de la conductancia transmembrana de la fibrosis quística)

-Defecto o ausencia de la glicoproteína de membrana CFTR.

Test del sudor



CLORURO:

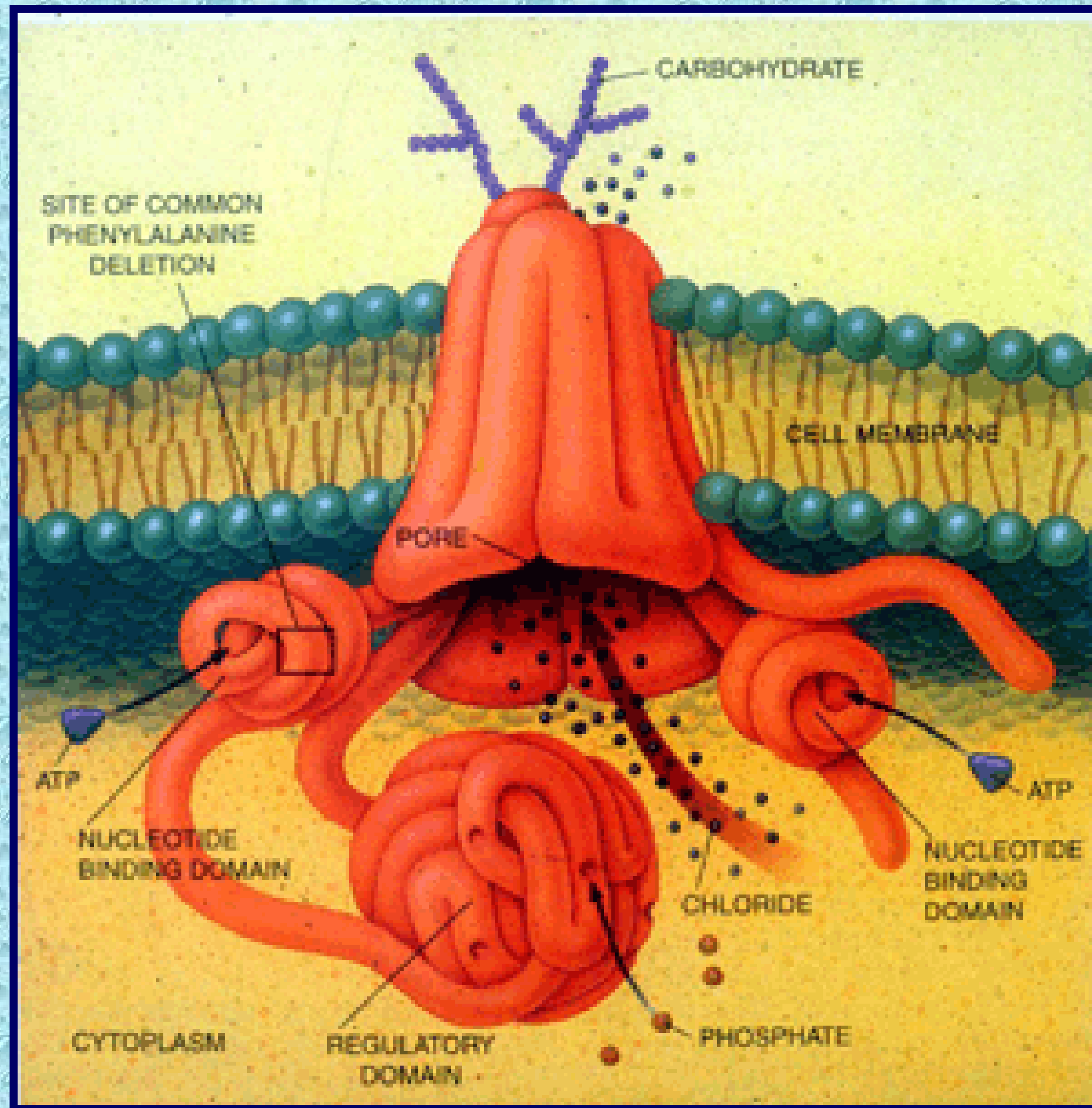
Normal <40mEq/L

Dudoso >40<60mEq/L

Patológico >60mEq/L

Tripsina Inmunoreactiva

Modelo del CFTR



Tipos de mutaciones del CFTR

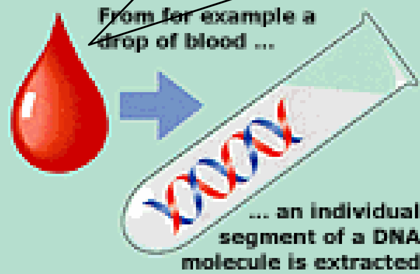
Defect Classification	Normal	I	II	III	IV	V
Defect Result		No synthesis	Block in Processing	Block in Regulation	Altered Conductance	Reduced Synthesis
Types of Mutation		Nonsense; Frameshift	Missense; Amino Acid Deletion (ΔF508)	Missense; Amino Acid Change (G551D)	Missense; Amino Acid Change (R117H) (R347P)	Missense; Amino Acid Change (A445E) Alternative Splicing
Potential Therapy		Gentamicin, Gene Transfer	Butyrates, Gene Transfer	Genistein, Gene Transfer	Milrinone, Gene Transfer	Gene Transfer

Illustration: Seward Hung

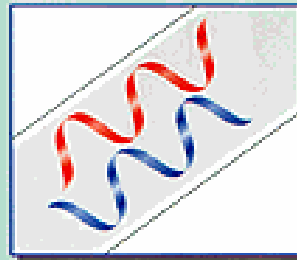
Figure 3. The 800 or so genetic mutations associated with cystic fibrosis have been divided into five broad classes based on their impact on the CFTR transporter molecule. An impressive number of corrective agents are in or approaching clinical trials; however, only gene transfer represents a potential cure. (Adapted from Zielenski and Tsui, 1995)

MUESTRA ADN INCÓGNITA

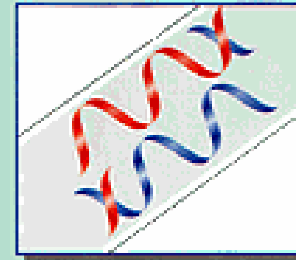
From for example a drop of blood ...



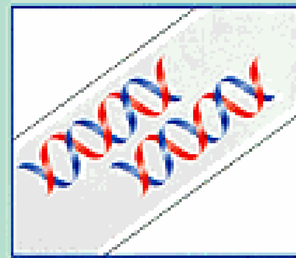
... an individual segment of a DNA molecule is extracted



By raising the temperature to about 90°C the strands are separated.

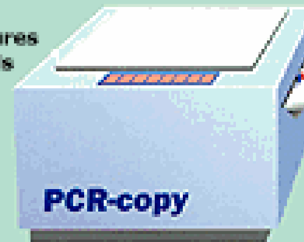


The temperature is lowered about 55°C and synthetic DNA framgents are added. These bind to the strands at the correct positions.



The temperature is now raised to about 70°C and the enzyme DNA polymerase which is added builds up two new complete copies of the DNA strands.

By cycling through the three temperatures the strands are separated and built up again.



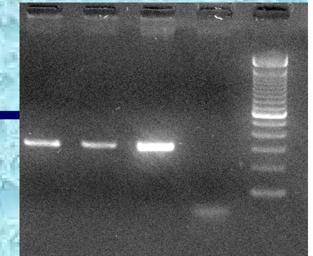
The whole process works like a copying machine.

Millions of copies an hour ...

CONCEPT: MARTIN EK, SVENSKA DAGBLADET

exón 3

309 pb

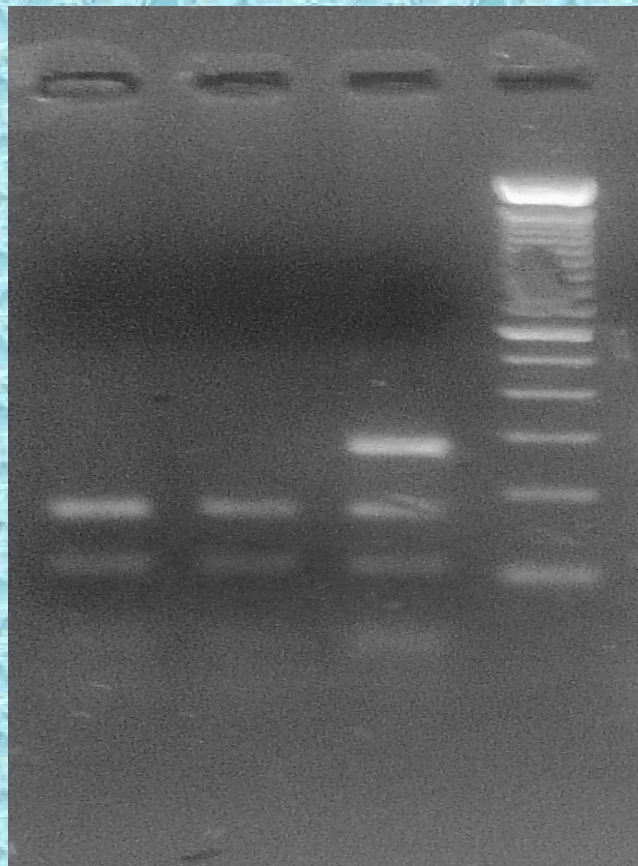


Hinf I

32 pb 105 pb 172 pb

277 pb

N/N N/N N/G85E

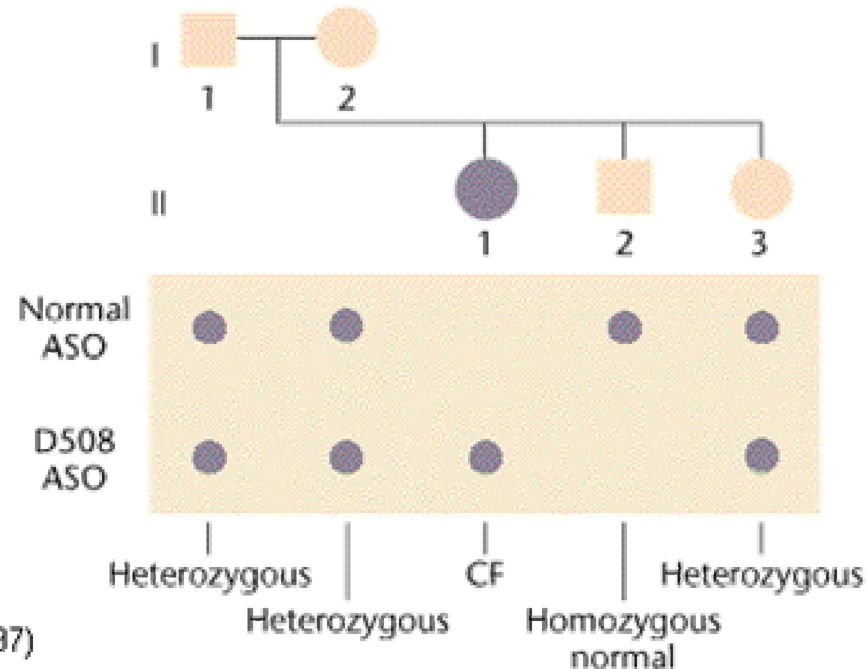


277 pb
172 pb
105 pb
32 pb

PCR-RFLP

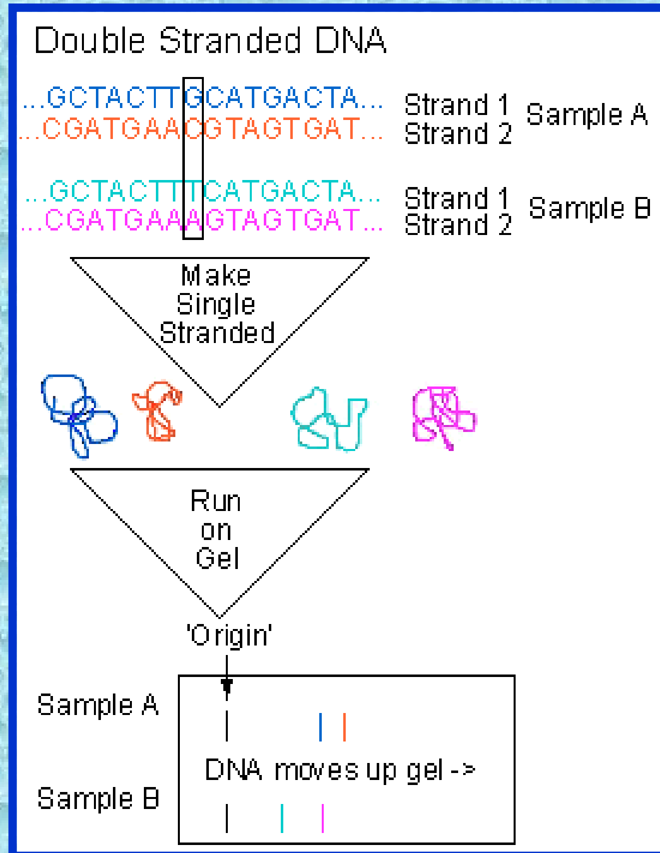
Restriction **F**ragments
Lenght **P**olimorphysm

PCR-ASO



Allele Specific Oligonucleotide

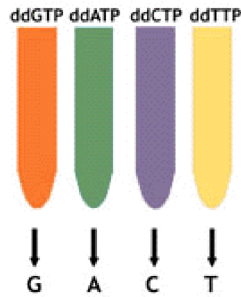
SSCP



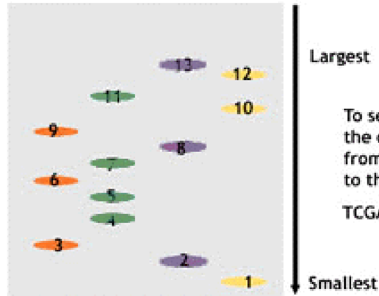
Single-Strand Conformation Polymorphism (SSCP) is the most widely used DNA screening method. SSCP detects single-base sequence changes by abnormal electrophoretic migration of one or both single strands on a nondenaturing polyacrylamide gel

The abnormal band found by SSCP is normally verified using sequencing.

Secuenciación

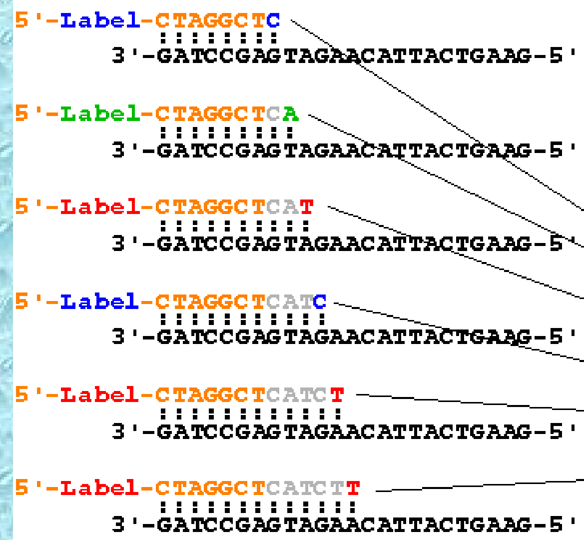


Add ddGTP, ddATP, ddCTP, ddTTP, one to each of four tubes containing target DNA. Load each onto a separate lane on a gel.

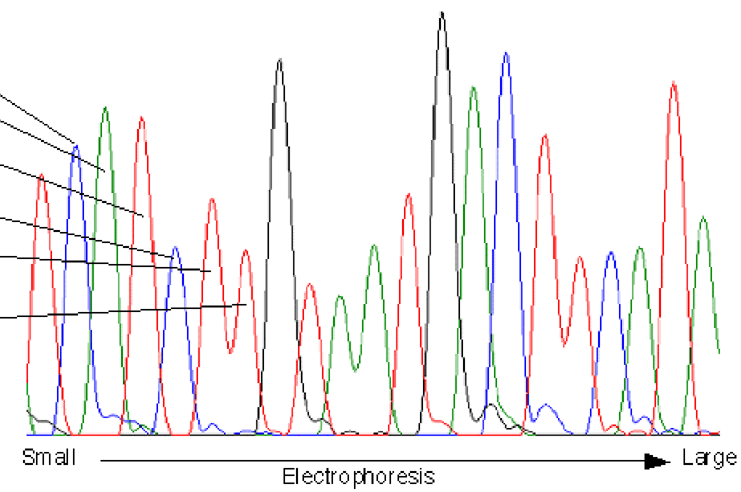


To sequence, read the order of bases from the smallest to the largest.

TCGAAGACGTATC



More typically now, sequencing reactions are denatured and the products are separated in a single gel lane or a single capillary tube. The products of the four reactions are labeled with a different fluorescent dye, and a single detector at the bottom of the apparatus detects the fluors as they emerge. The sequence can be read (automatically) from left to right.



Gel de agarosa corrido el sab 14/05 (1er. Turno)

M 1N 1Δ 2N 2D 3N 3Δ 4N 4Δ 5N 5Δ 6N 6Δ 7N 7Δ 8N 8Δ

M 9N 9Δ 10N 10Δ 11N11Δ Controles + C- M

Gel de agarosa corrido el sab 21/05 (2do. Turno)