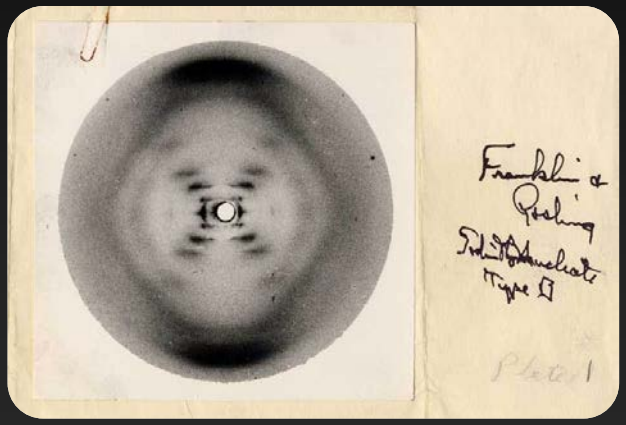
De l'hélice à l'électron.

Dr. P. Beckers
Service de Génétique
Laboratoire de Biologie Moléculaire

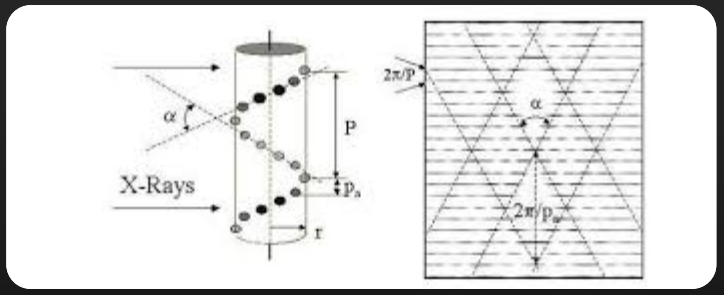
Une situation surréaliste…

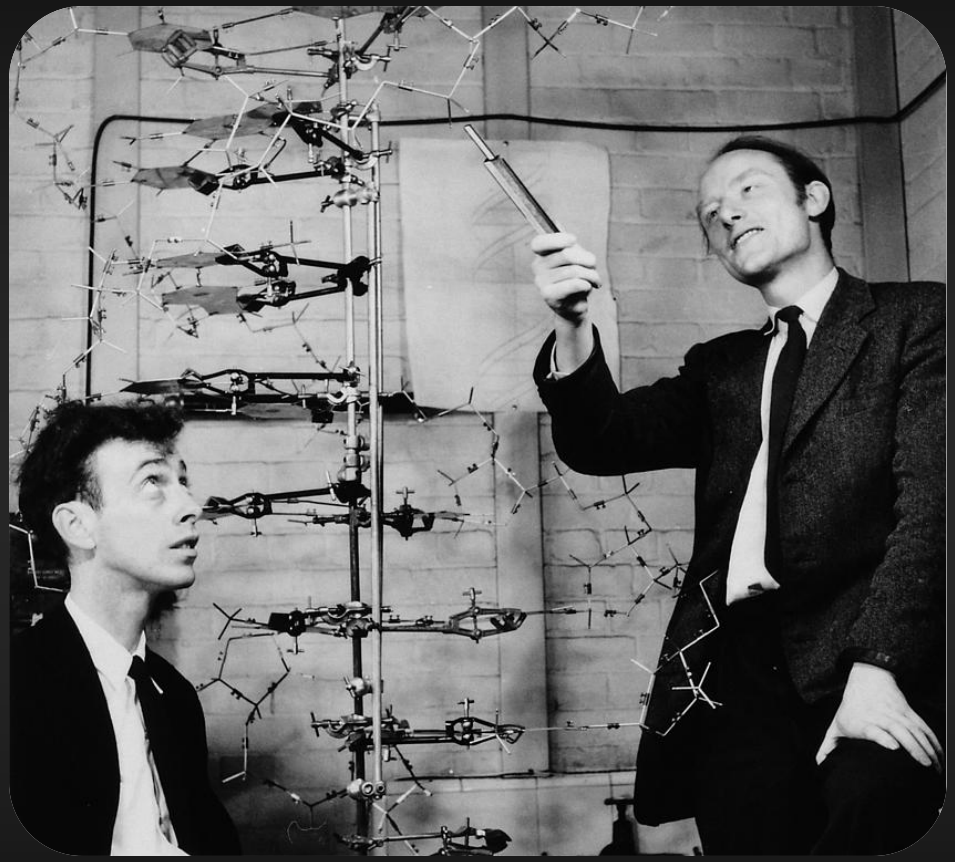
- Caryotypes
- FISH
- CGH
- MLPA
- Sanger
- NGS
- Nanopore

Watson et Crick - 1953



Maurice Wilkins et Rosalind Franklin





Au commencement…

Au sein de l'œuf, la matrice…

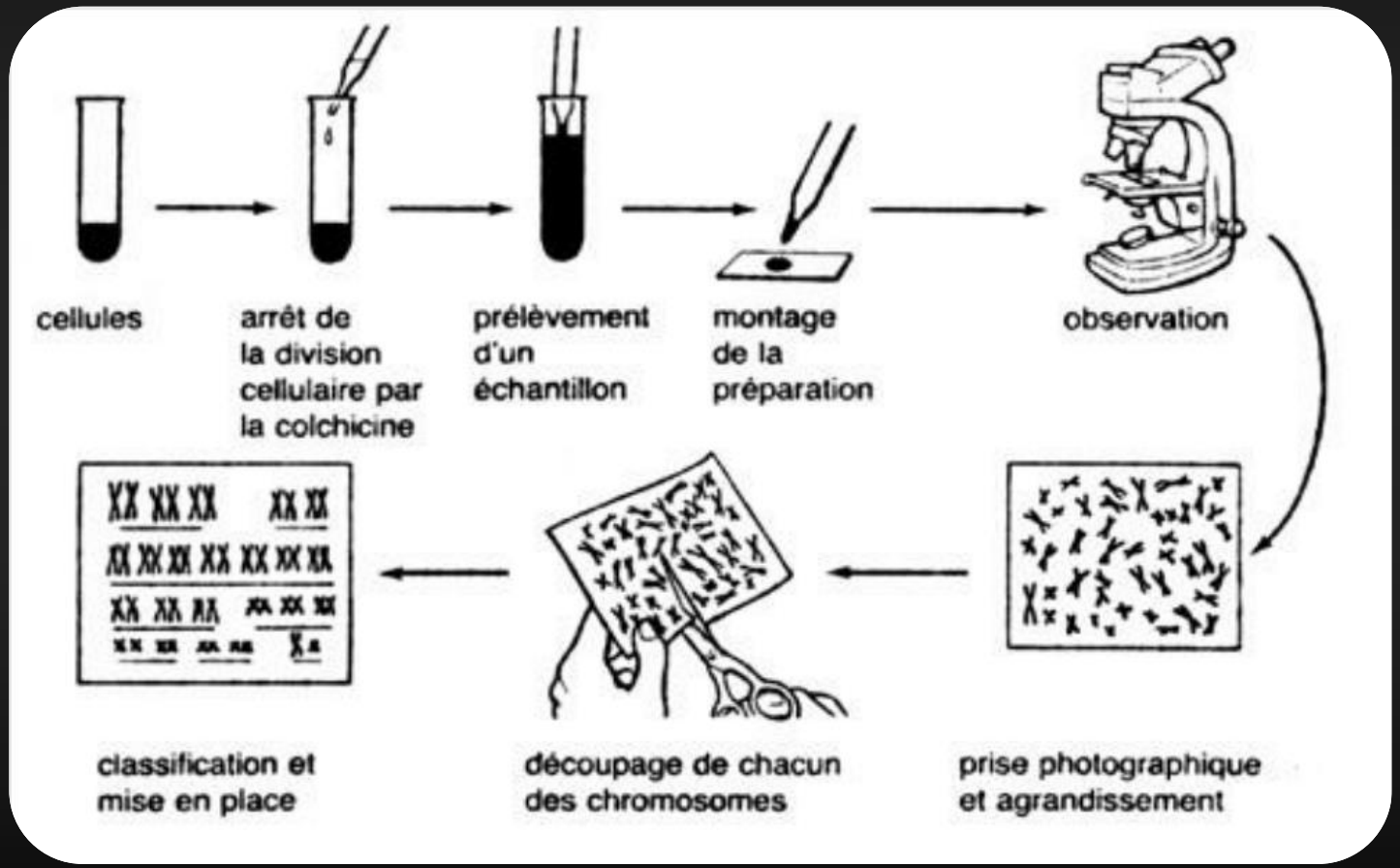
- L'ADN peut être extrait :
  - Des cellules nucléées (leucocytes)
  - Des cellules tumorales.
  - Attention : un patient ayant reçu une greffe de moëlle, nécessité le prélèvement d'autres cellules somatiques que les leucocytes…

1. Cytogénétique
2. Génétique Moléculaire

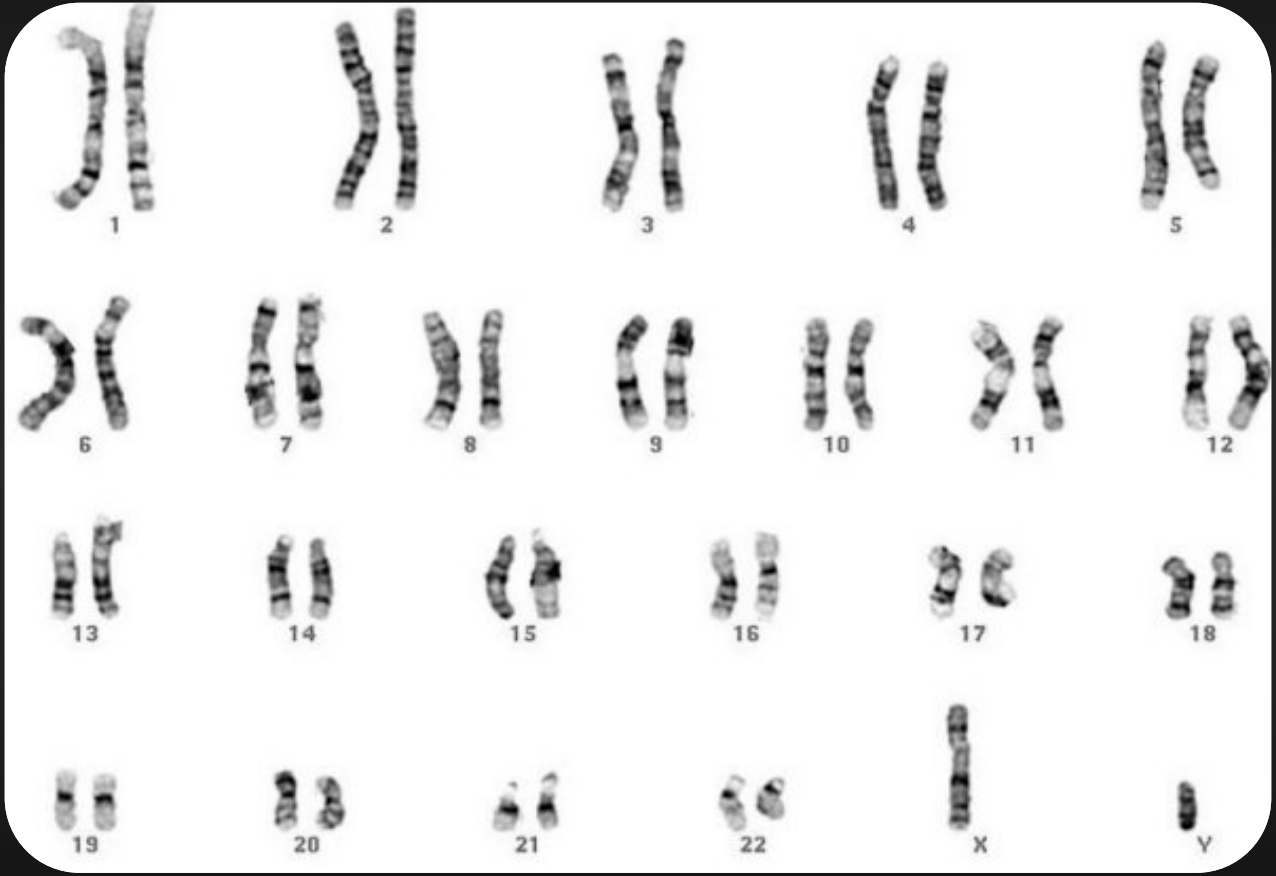
1. Cytogénétique

- Cytogénétique **conventionnelle** = Etude des chromosomes de la cellule en métaphase, par le :
  - Caryotype
- Cytogénétique **moléculaire** = Etude de fragments chromosomiques avec des sondes moléculaires fluorescentes.
  - FISH *(Fluorescence In Situ Hybridization)*
- Cytogénétique **moléculaire** = Etude de fragments chromosomiques par comparaison avec une séquence de référence.
  - CGH *(Comparative Genomic Hybridization)*

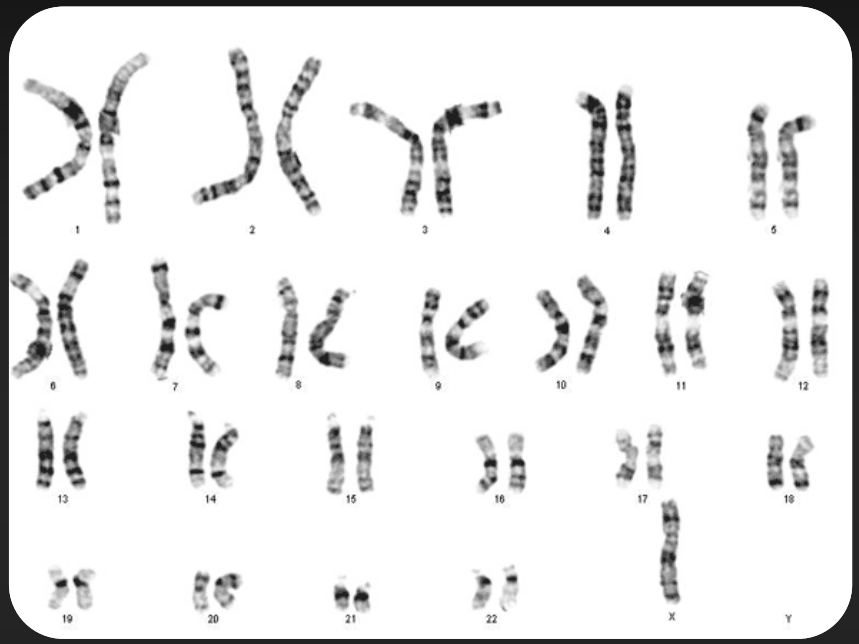
Caryotype


cellules
arrêt de la division cellulaire par la colchicine
prélèvement d'un échantillon
montage de la préparation
observation
prise photographique et agrandissement
découpage de chacun des chromosomes
classification et mise en place

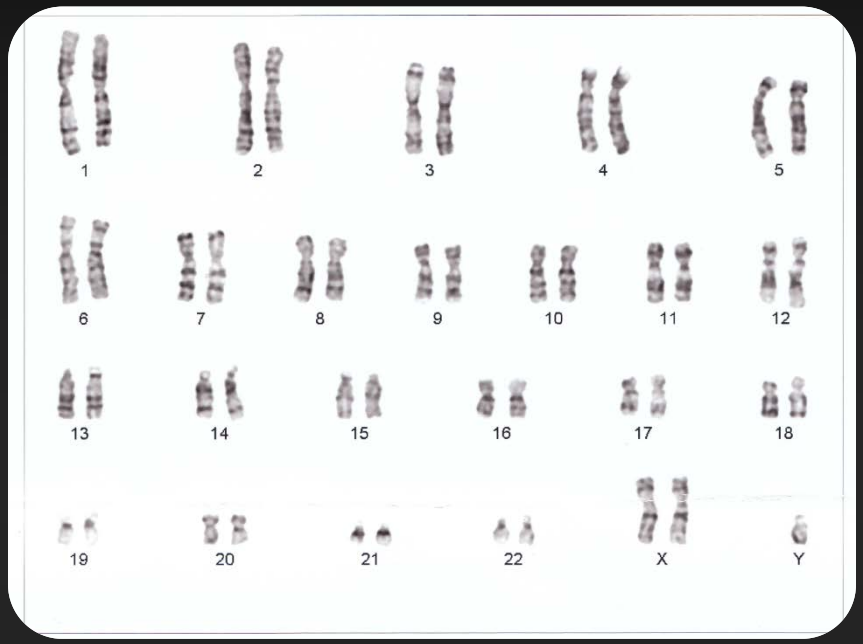
Caryotype



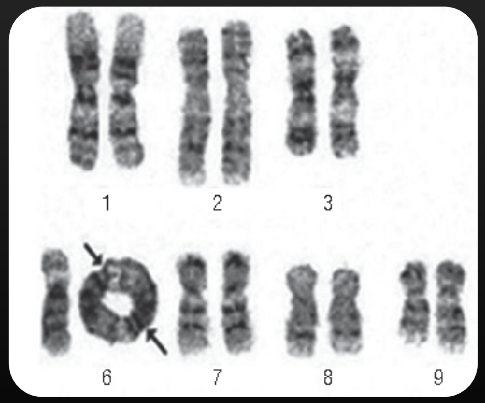
Caryotype



Turner

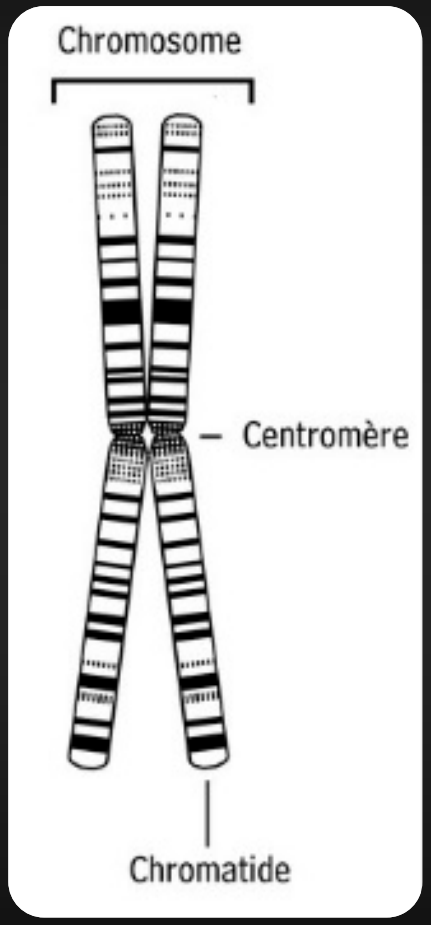


Klinfelter

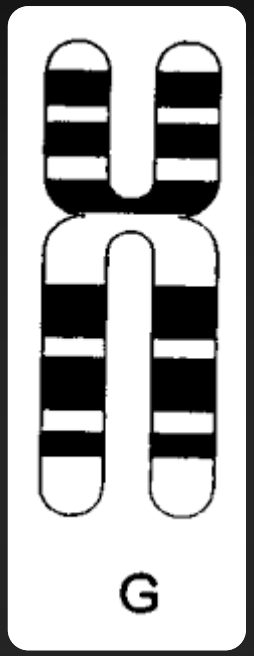


O Ring

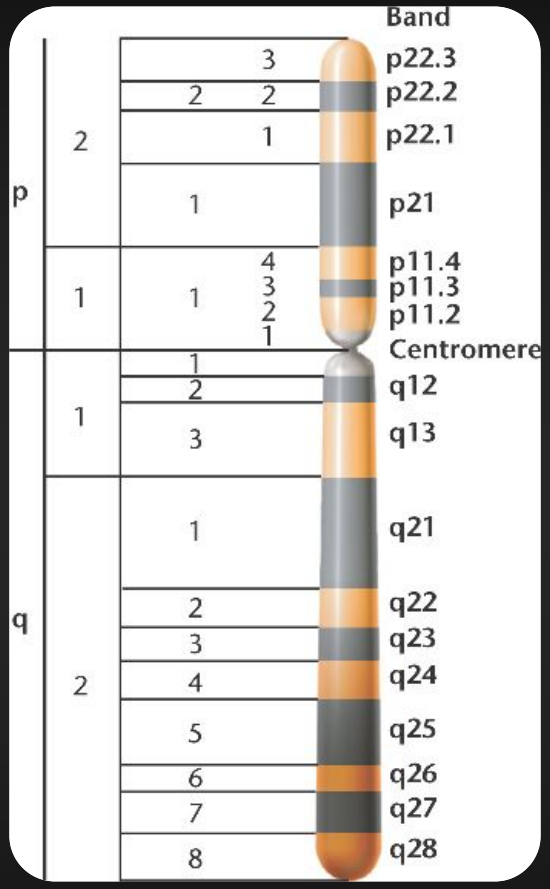
Caryotype

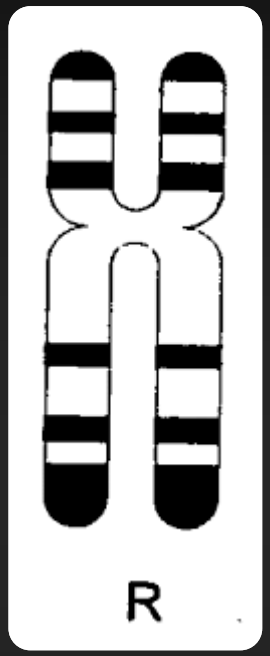


Caryotype



Bandes G
Extrémités claires
ADN riche en paires A-T

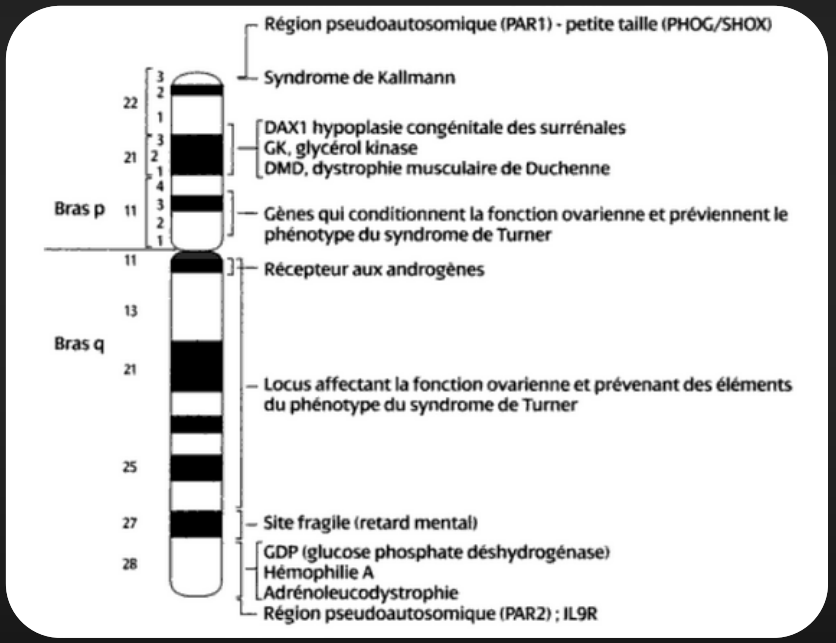




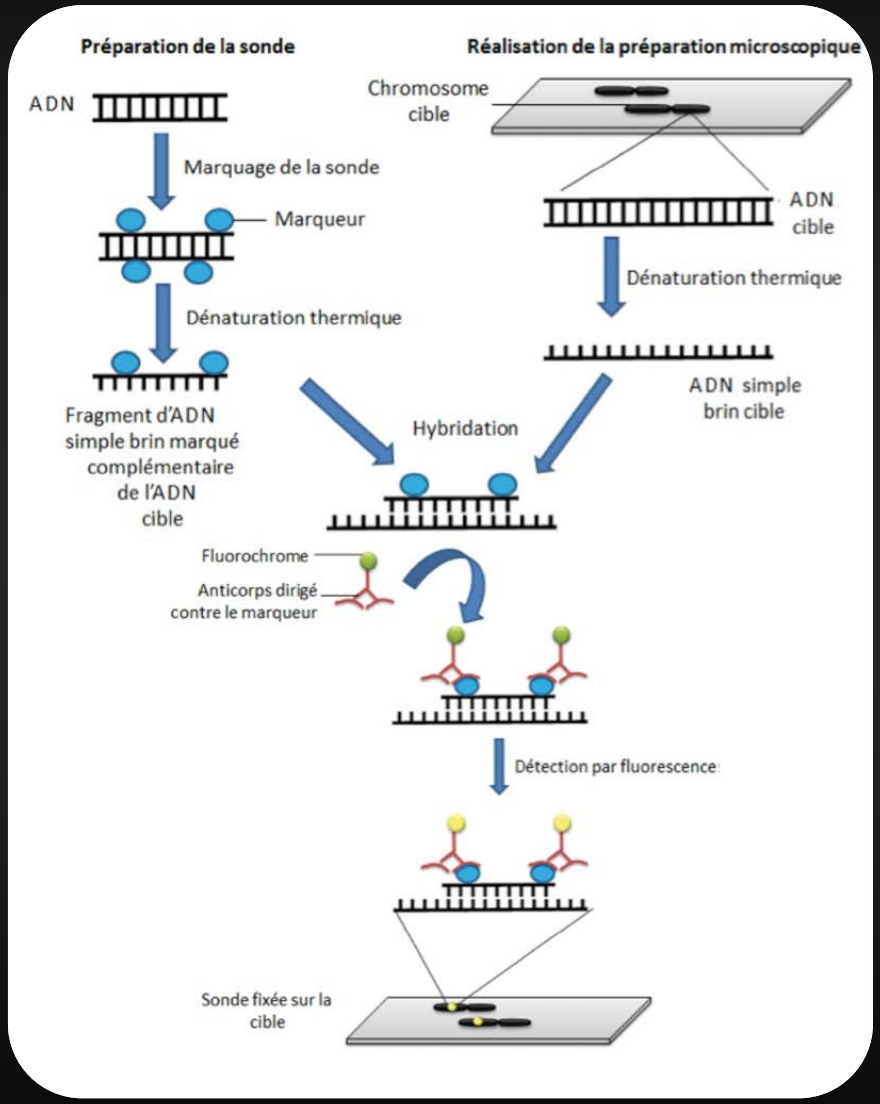
Bandes R (Reverse)
Extrémités foncées
ADN riche en paires G-C

Caryotype

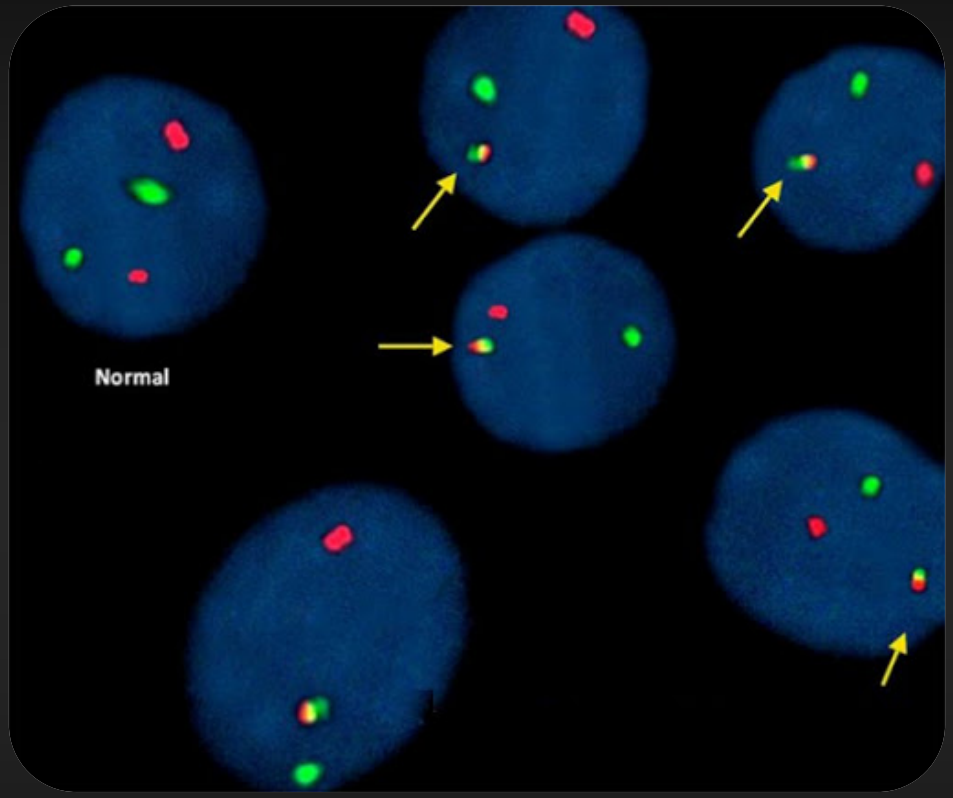
Exemple Chromosome X



Fluorescence In Situ Hybridization (FISH)

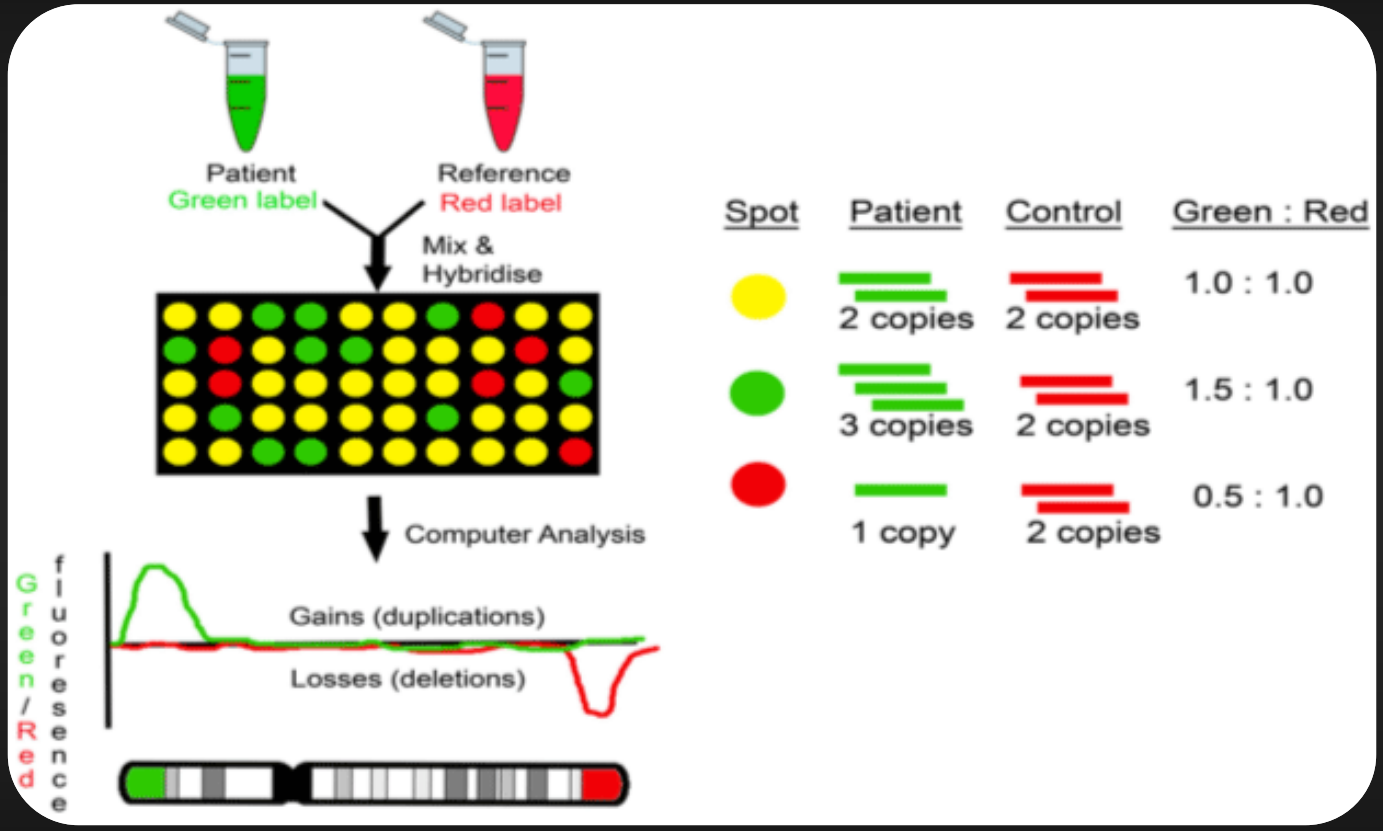


Fluorescence In Situ Hybridization (FISH)

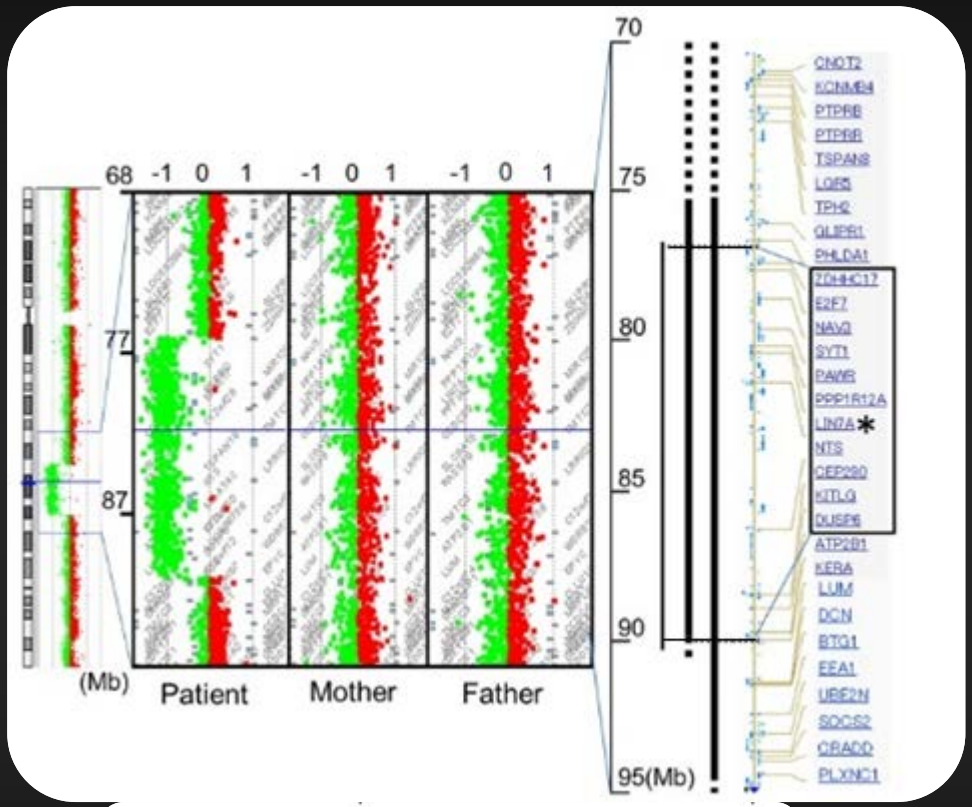


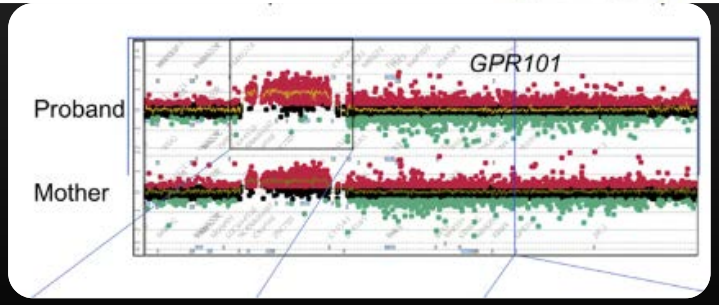
Translocation (7;12)

array-Comparative Genomic Hybridization (aCGH)



array-Comparative Genomic Hybridization (aCGH)





En résumé…

Caryotype
Full Génome
Basse résolution
5 à 10 Mb

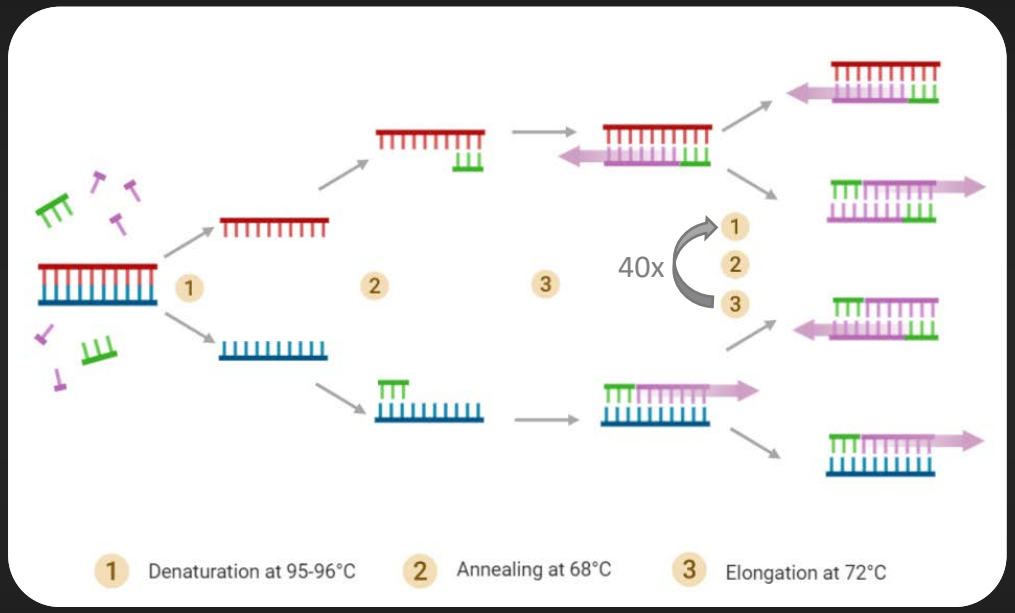
FISH
Site Spécifique
Meilleure résolution
100 à 300 kb

aCGH
Full Génome
Haute résolution
5 à 500 kb

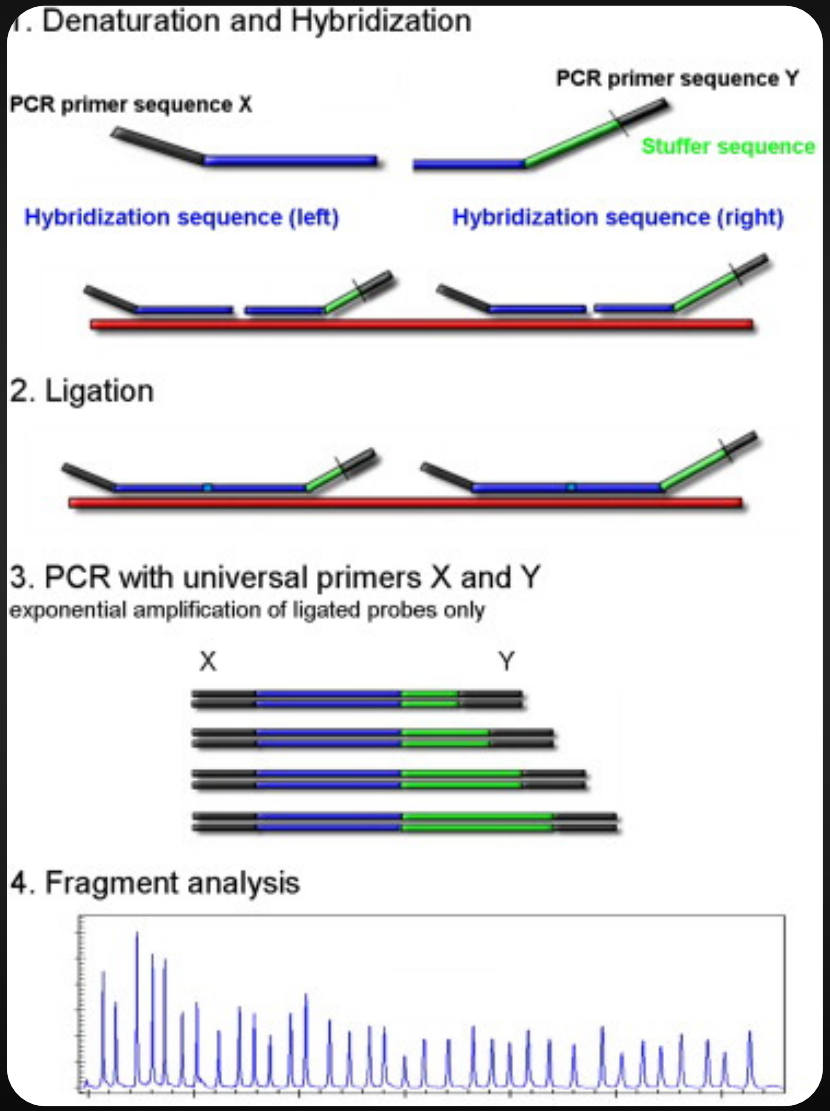
2. Génétique Moléculaire

- Génétique moléculaire = Etude de l'ADN, recherche de variants dans des gènes, après extraction de l'ADN des cellules nucléées
- MLPA
- Séquençage Sanger
- NGS
  - Nanopore

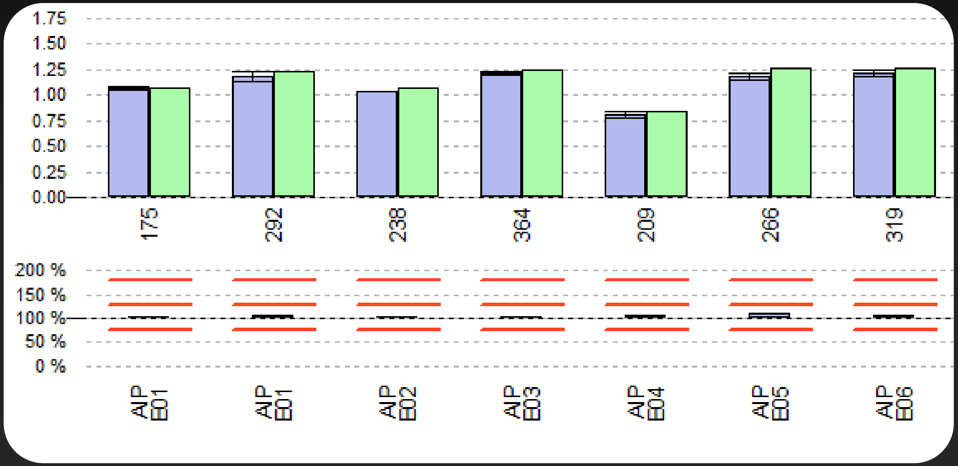
La Polymérase Chain Reaction (PCR)

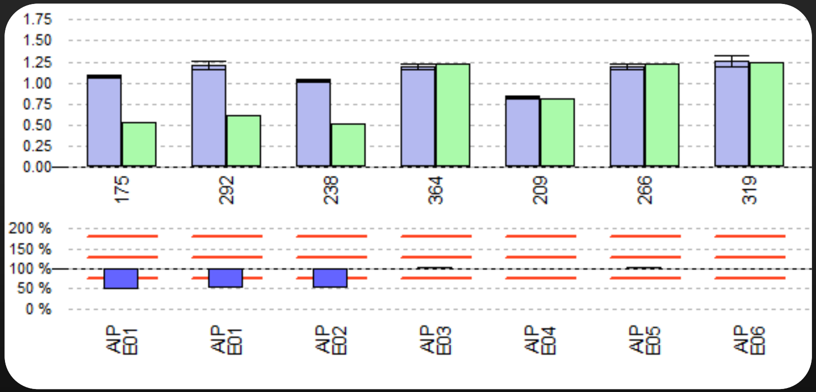


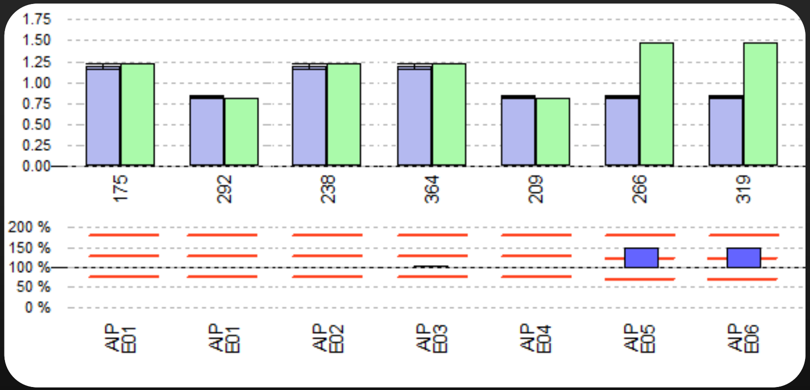
La multiplex ligation-dependent probe amplification (MLPA)



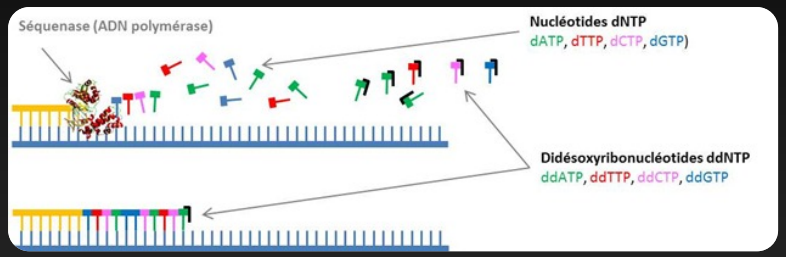
La multiplex ligation-dependent probe amplification (MLPA)

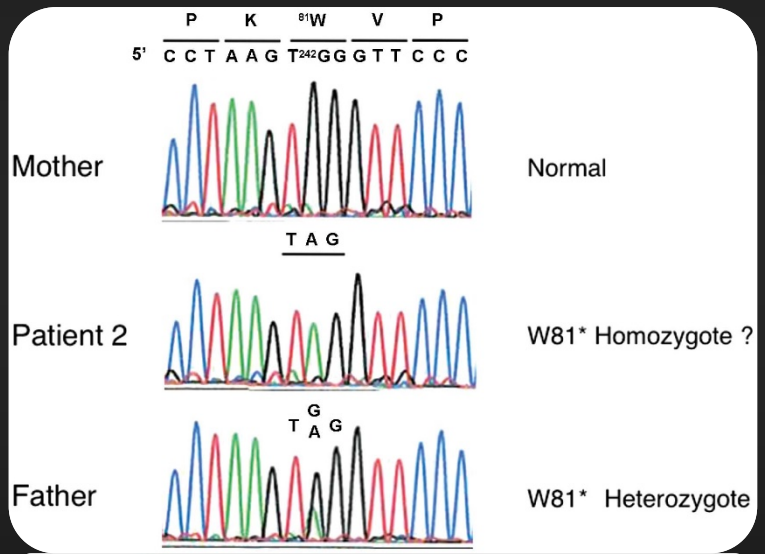


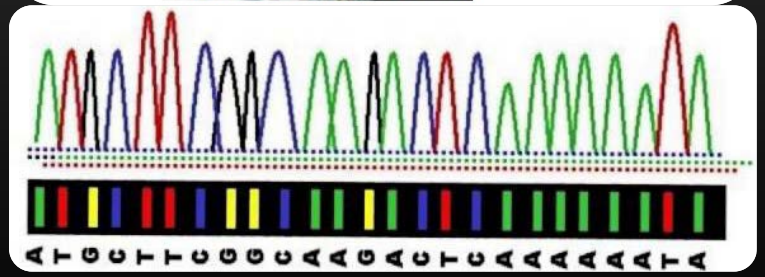


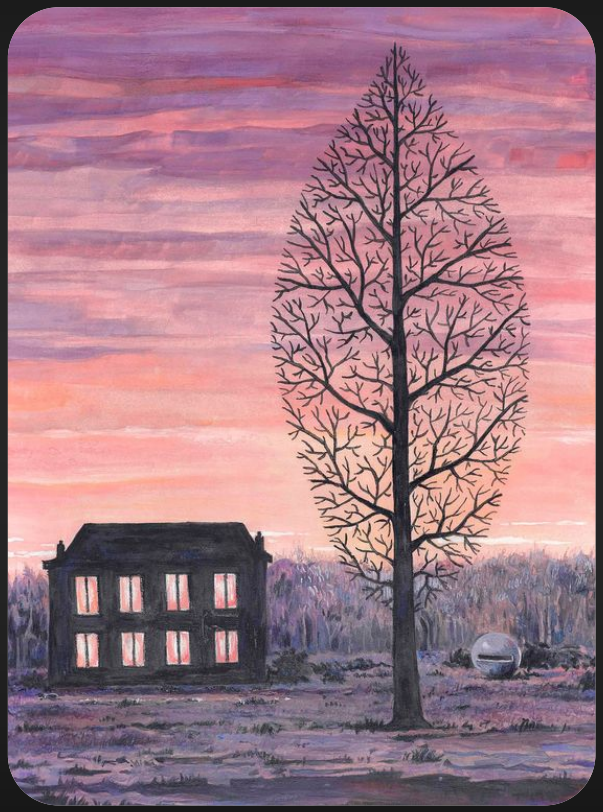


Séquençage Sanger

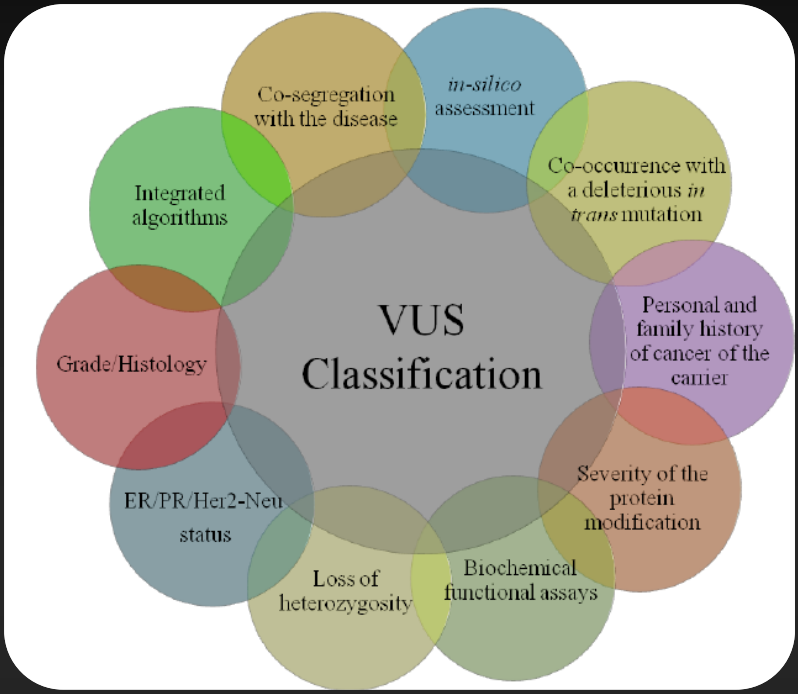






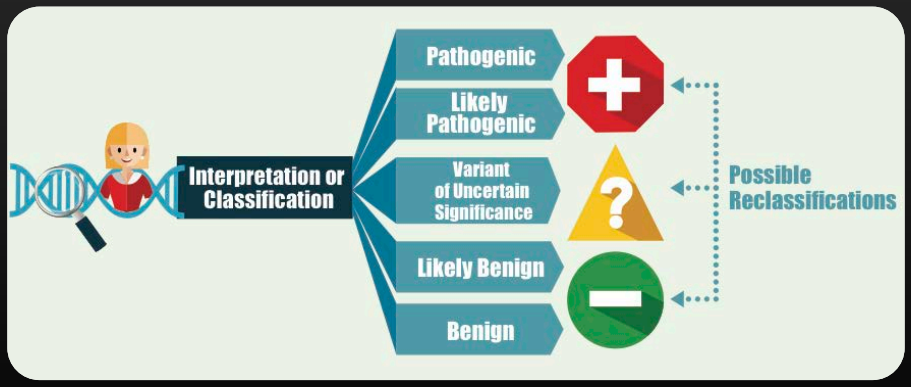


Interprétation des variants



Recommandations de l'American College of Medical Genetics (2015)

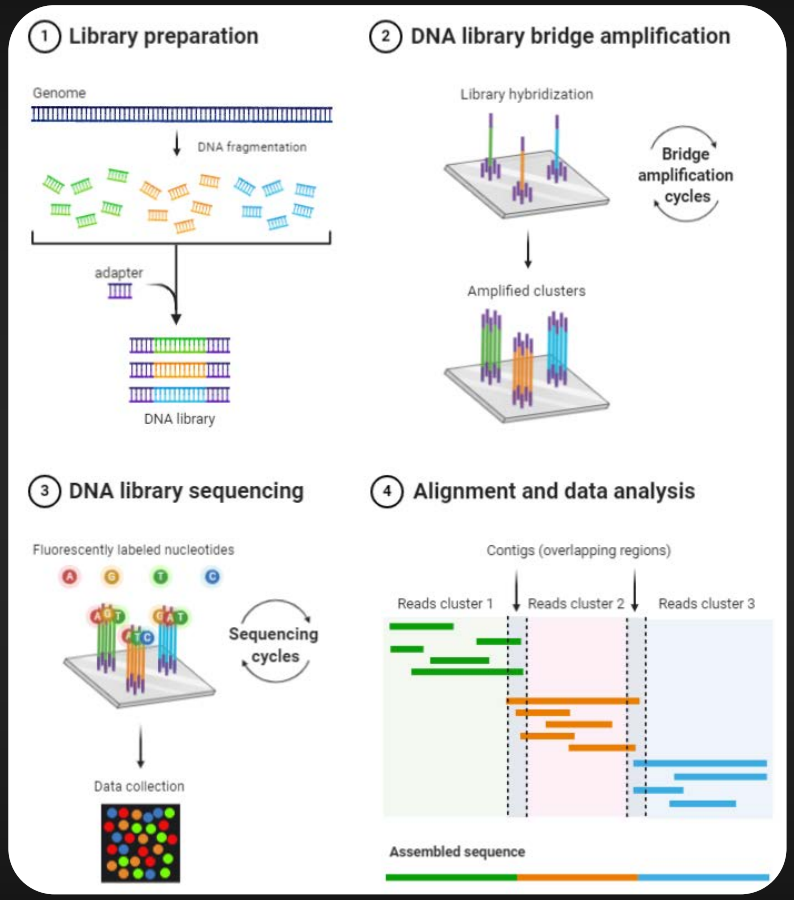
- Faisceau d'arguments
  - **En faveur de la pathogénicité**
    - PVS (Pathogenic Very Strong) argument très fort
    - PS (Pathogenic Strong) argument fort
    - PM (Pathogenic Moderate) argument moyen
    - PP (Pathogenic Poor or supporting) argument faible
  - **En faveur du caractère bénin**
    - BA (Benign stand Alone) argument suffisant
    - BS (Benign Strong) argument fort
    - BP (Benign Poor or supporting) argument faible

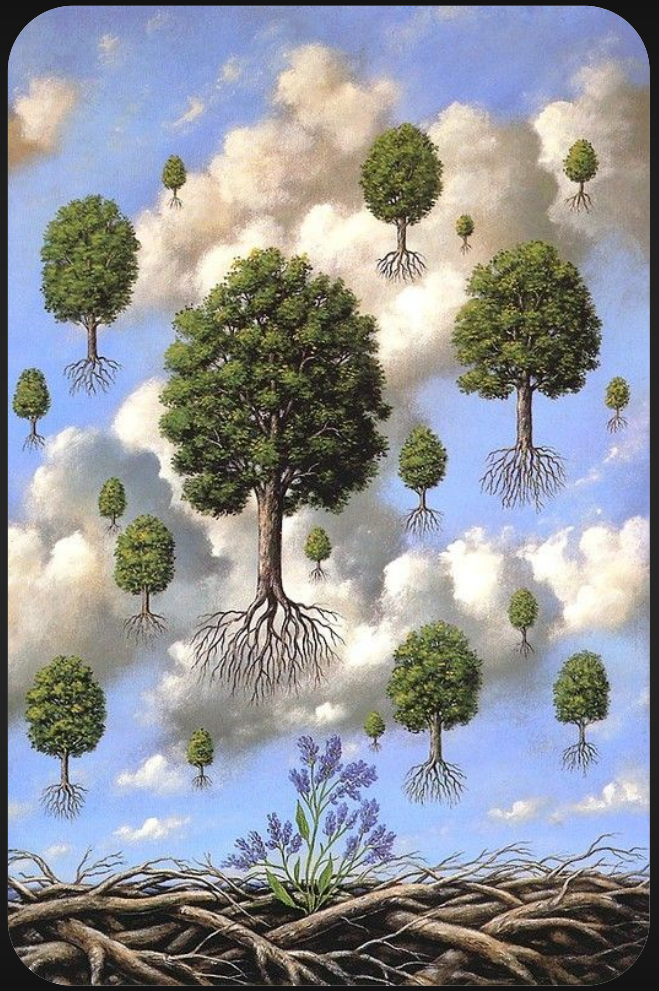


Seconde évaluation des variants

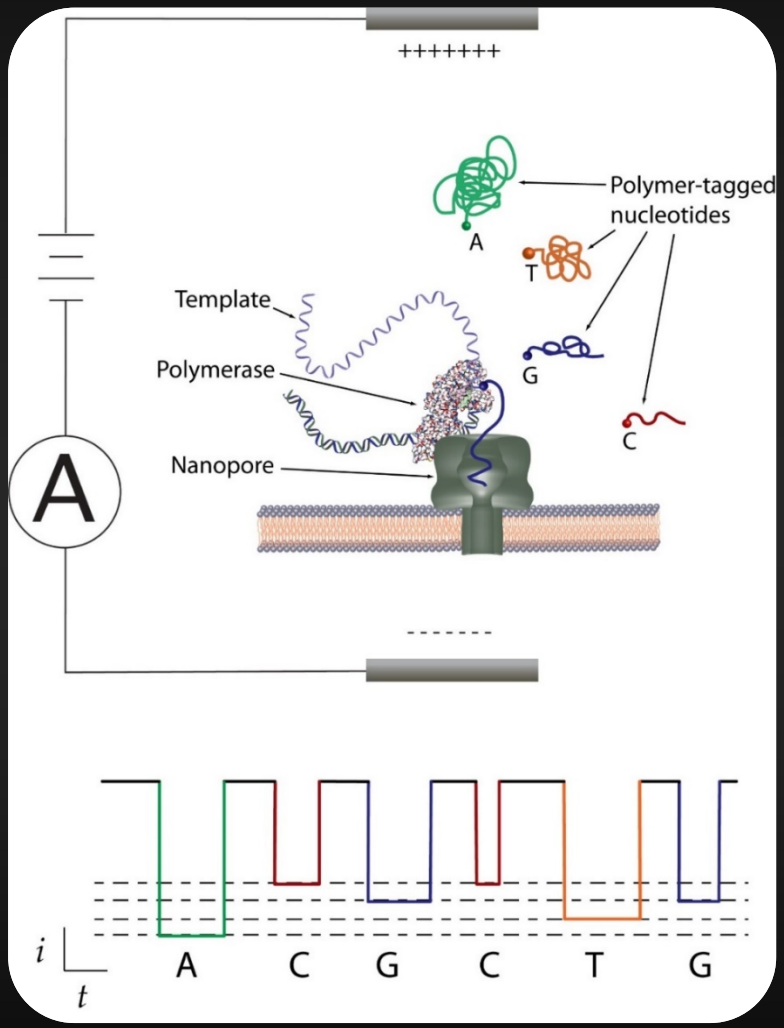
For variants with a prior score of:	n	when re-evaluated, variant score: Moved Down	Stayed Same	Moved Up	Odds (up)
(likely benign)	737	287 (38.9%)	444 (60.2%)	6 (0.8%)	.02
(VUS, but suggesting benign)	957	318 (33.2%)	619 (64.7%)	20 (2.1%)	.06
(VUS)	776	153 (19.7%)	518 (66.8%)	105 (13.5%)	.70
(VUS, but suggesting pathogenic)	427	22 (5.2%)	341 (79.8%)	64 (15.0%)	2.88
(likely pathogenic)	220	4 (1.8%)	186 (84.5%)	30 (13.6%)	7.56

Next Generation Sequencing (NGS)

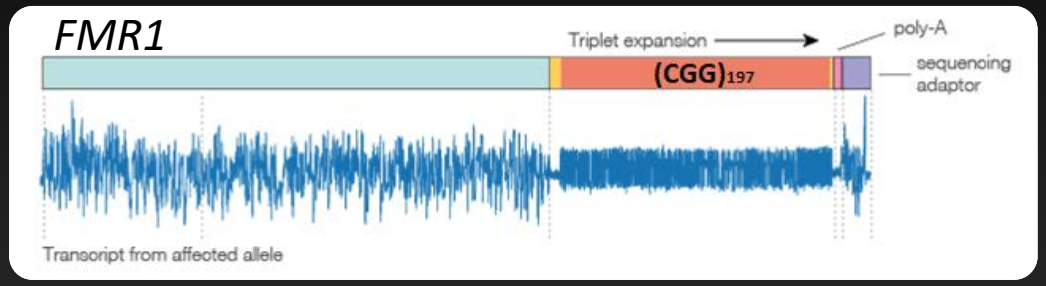


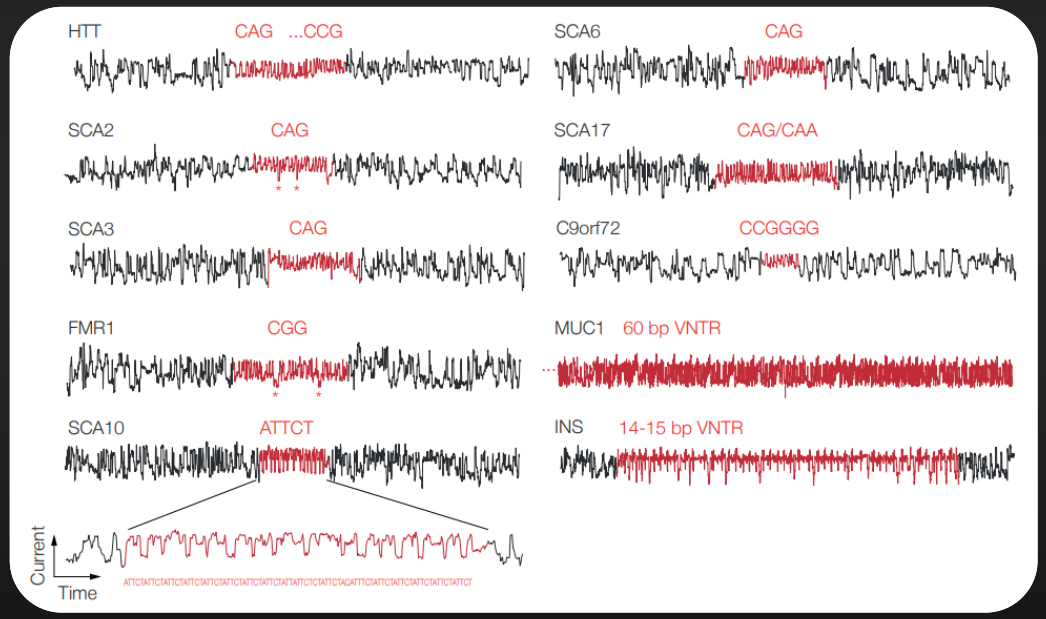


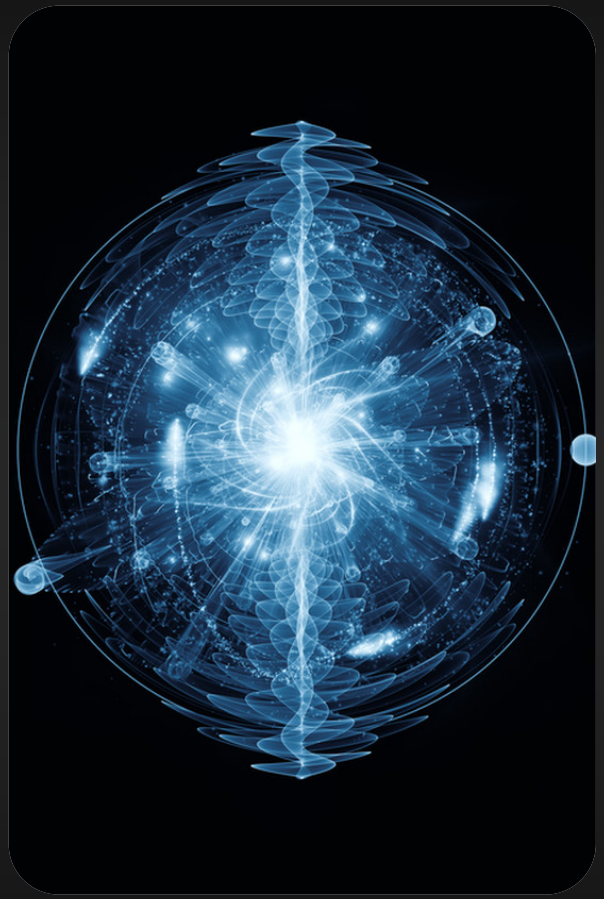
Nanopore


+++++++
Polymer-tagged
nucleotides
A
T
G
C
Template
Polymerase
Nanopore
A
----
i
t
A C G C T G

Résultats par nanopore





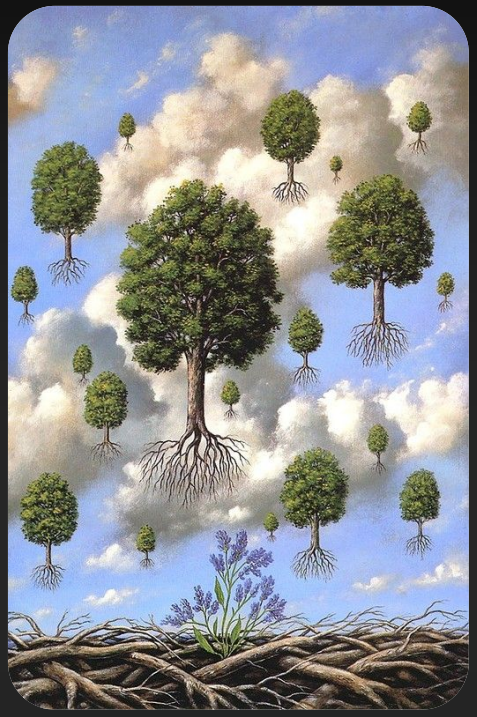


En résumé…

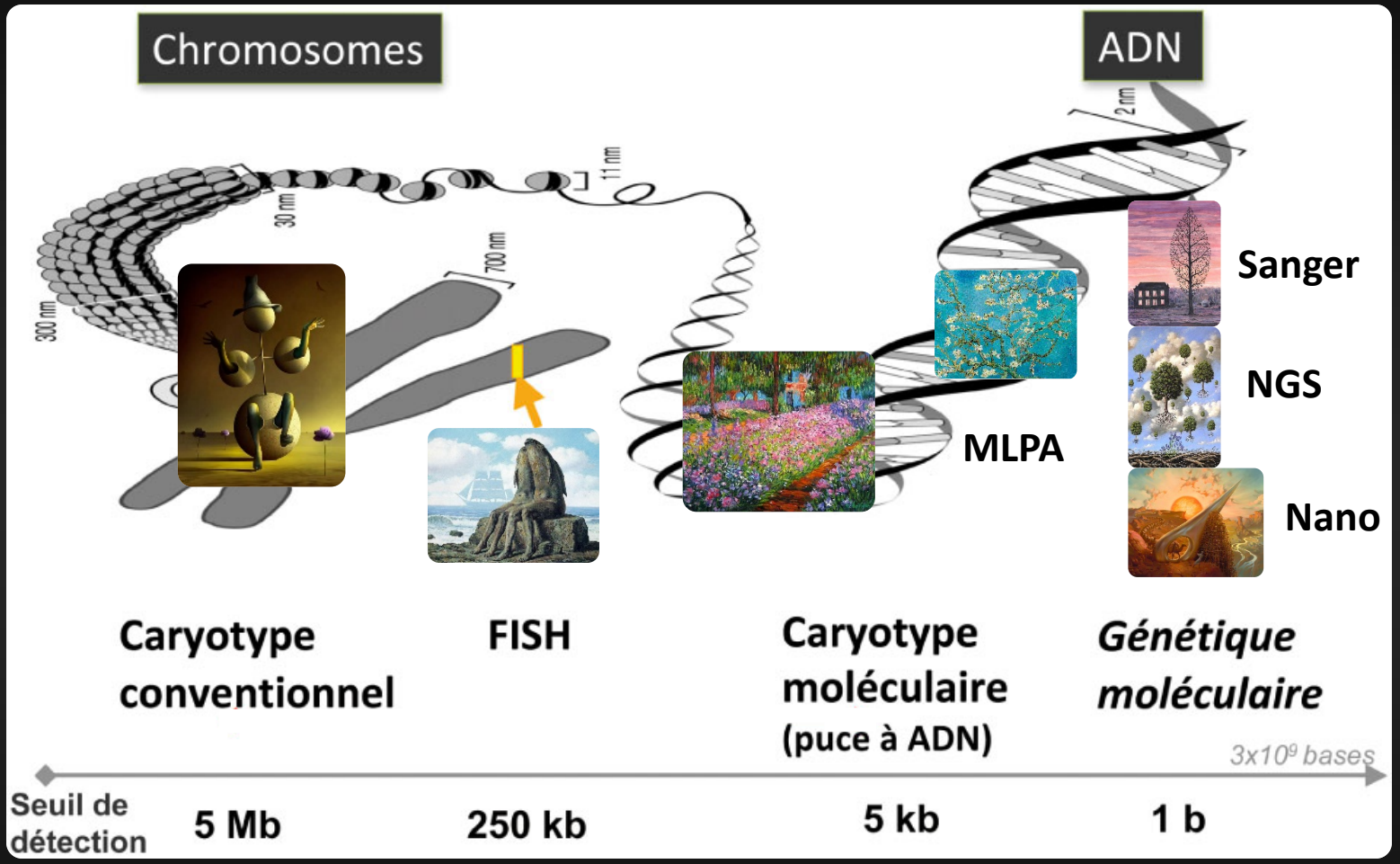
Sanger

MLPA

Nanopore



NGS


Chromosomes
ADN
300 nm
30 nm
700 nm
11 nm
2 nm
Sanger
NGS
Nano
MLPA
Caryotype conventionnel
FISH
Caryotype moléculaire (puce à ADN)
Génétique moléculaire
3x10⁹ bases
Seuil de détection
5 Mb
250 kb
5 kb
1 b

Remerciements

Merci à tous pour votre attention

« Les passions sont les vents qui enflent les voiles du navire ;
elles le submergent quelquefois, mais sans elles il ne pourrait voguer. »

Voltaire