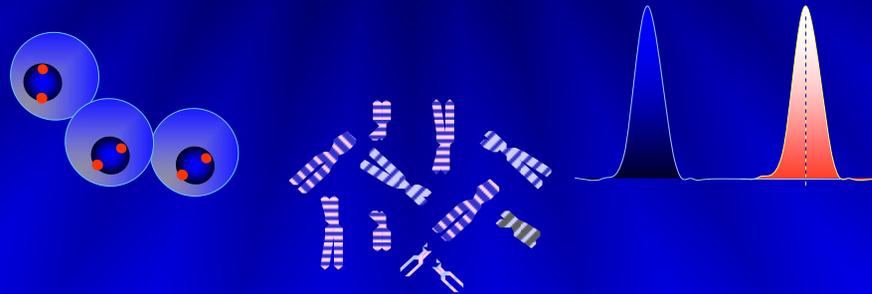
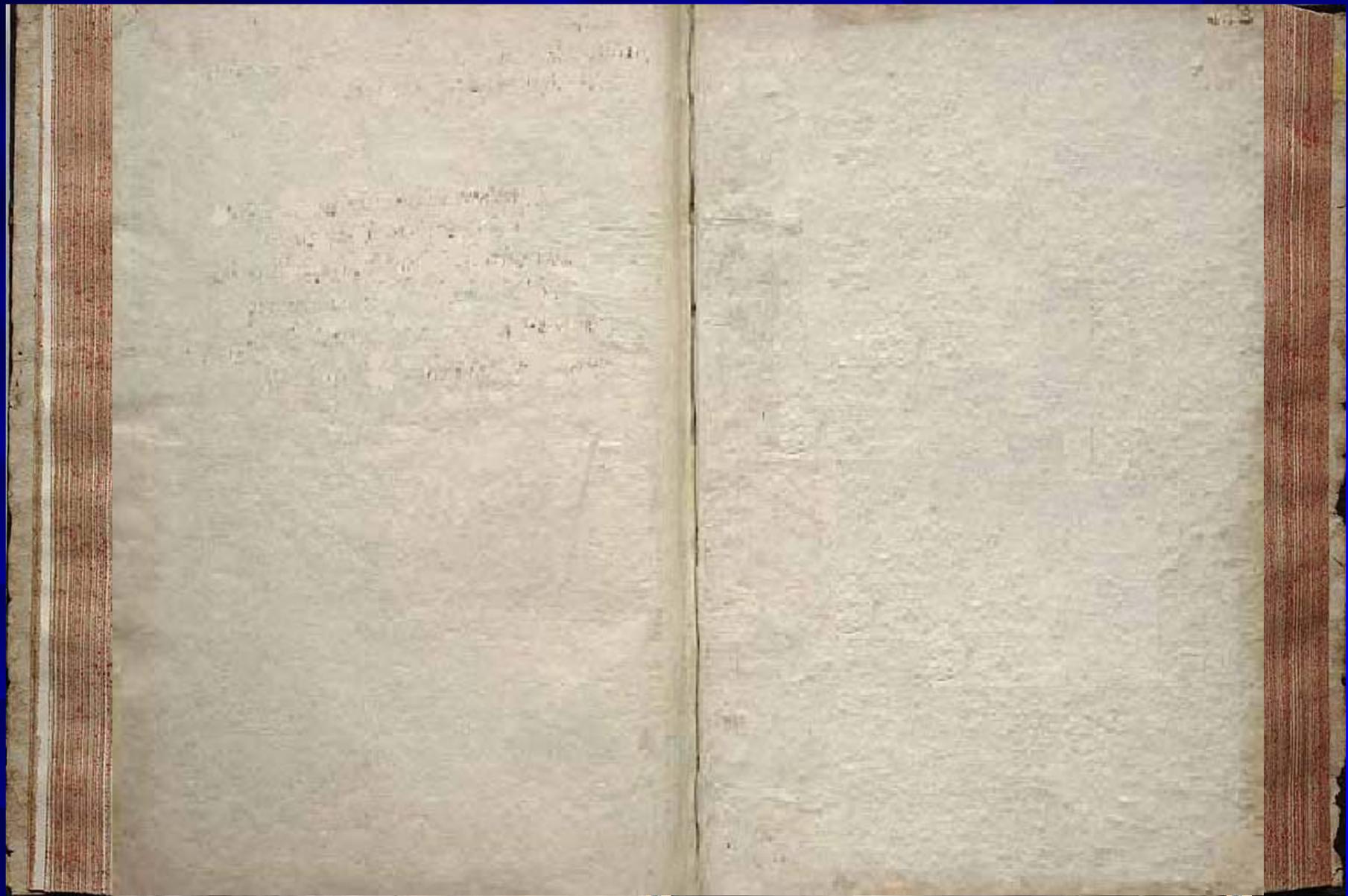


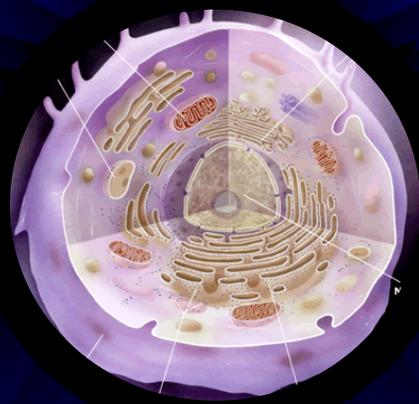


QF-PCR

Un Nouveau Concept Pour Le Diagnostic Prénatal







6 pg d 'ADN \Rightarrow 170 cm

24 molécules

3 Milliards de pb

46 Mille gènes

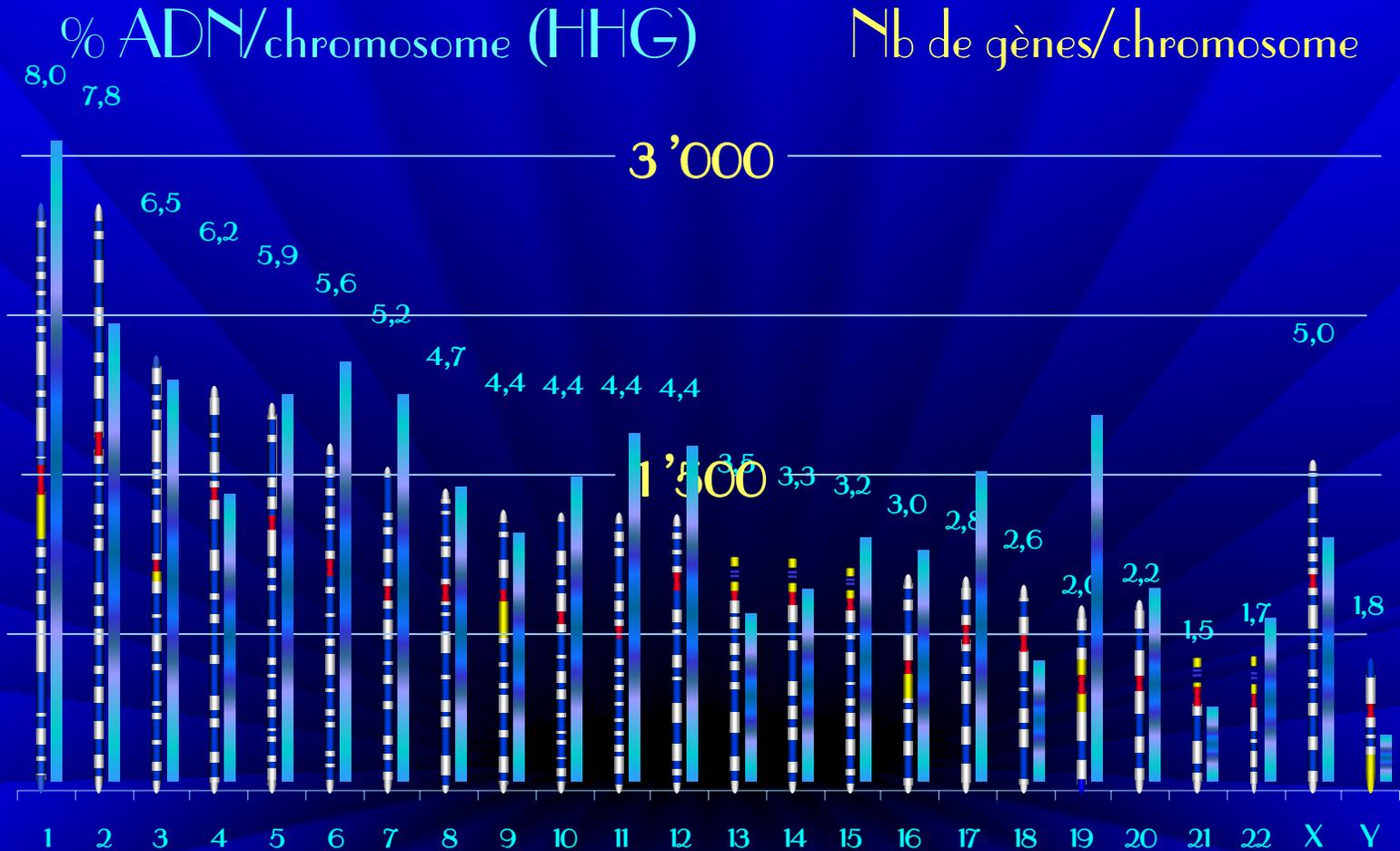
3%

97%

Junk DNA (robaccia)

15% répétitif

L'ADN : 24 chromosomes, 31'400 gènes connus, 3'000 Mb



Anomalies cytogénétiques

N = 9'060

Numériques

Fonctionnelles

Structurales

Aneuploidies

Translocations

Trisomies

Inversions

Monosomies

UPD

Duplications (Micro)

Mosaïques

Imprinting

Délétions (Micro)

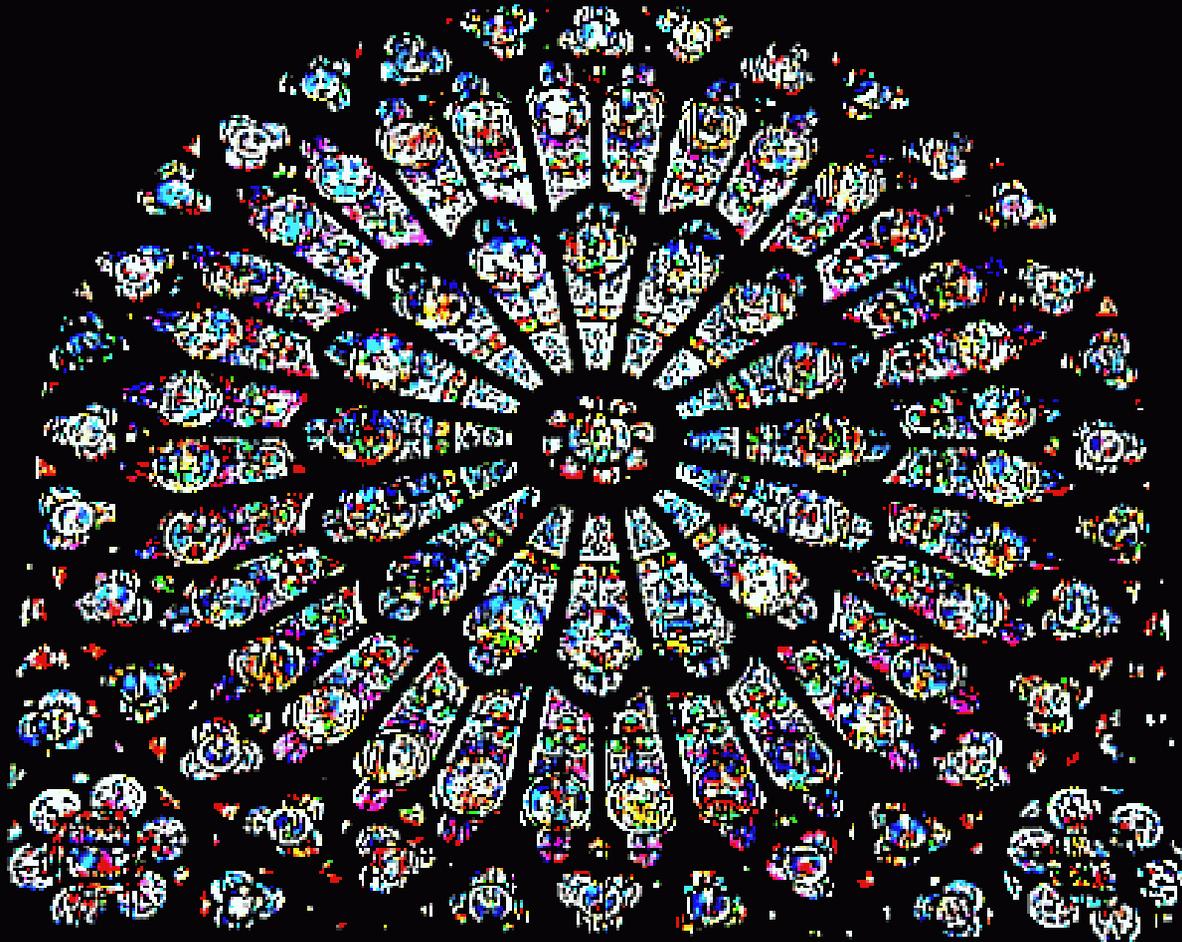
Téломères

μ déldup-UPD-Sites fragiles

N = 2'336

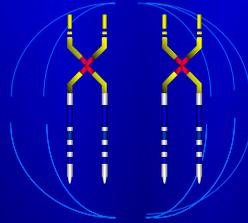


Origine des anomalies génétiques



In vitro

Origine des Anomalies Cytogénétiques (%)



Erreurs
méiotiques

8

(Méiose I>II)

89

Total

97

Erreurs mitotiques

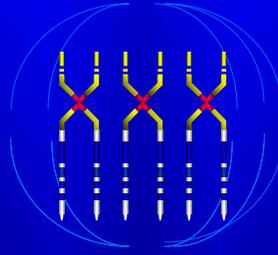


(Mosaïcisme, UPD) 3

Total

100





Non-disjonction



Total

3

Méiose I

66

69

5

Méiose II

23

28

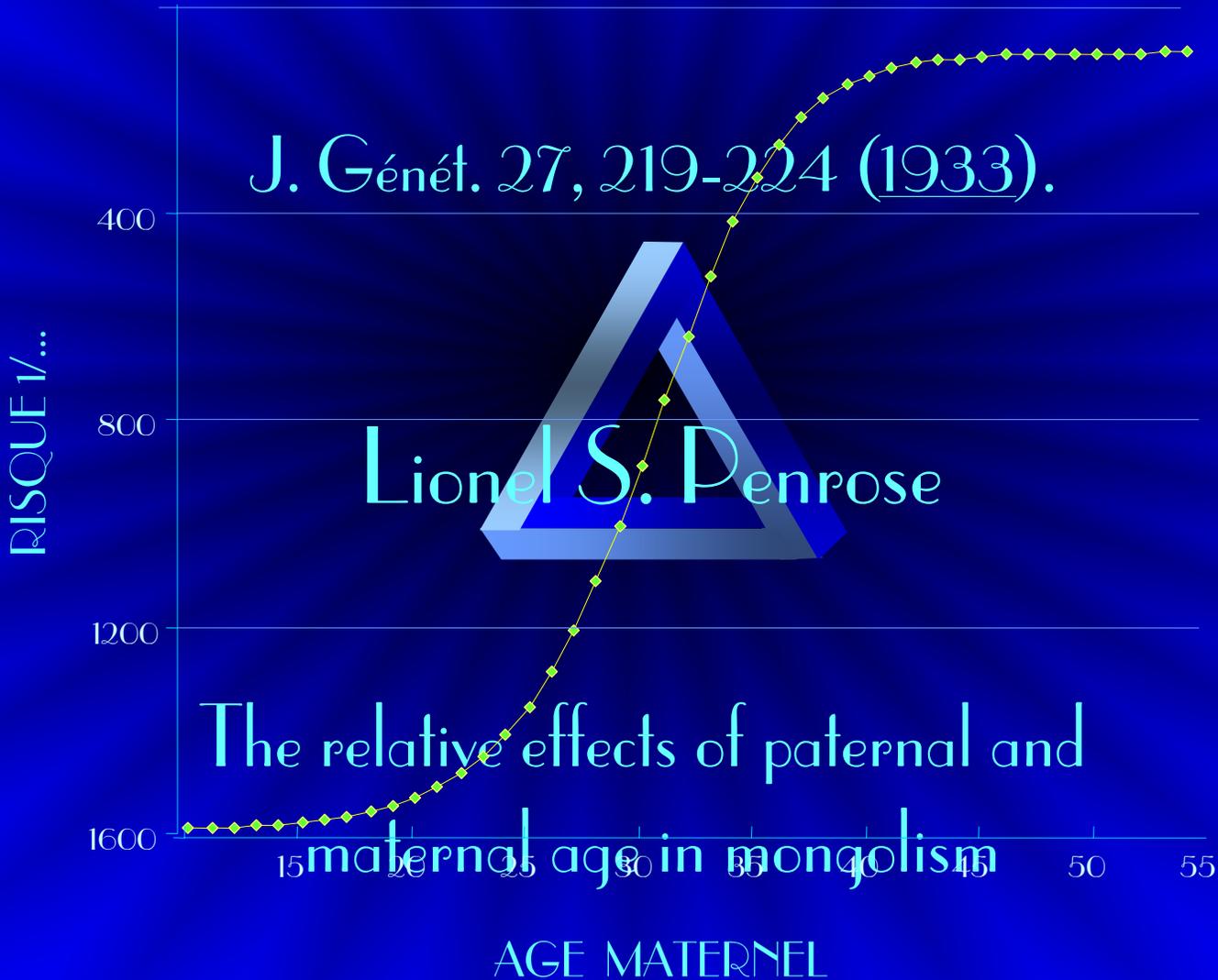


Total méiose 8

89

97

Facteurs de risques



Début Méiose

Stop Méiose

Follicules

Reprise Méiose



La patience : longue attente en prophase de M1

GP2

FŒTUS → Mitoses

NAISSANCE →

PUBERTÉ →

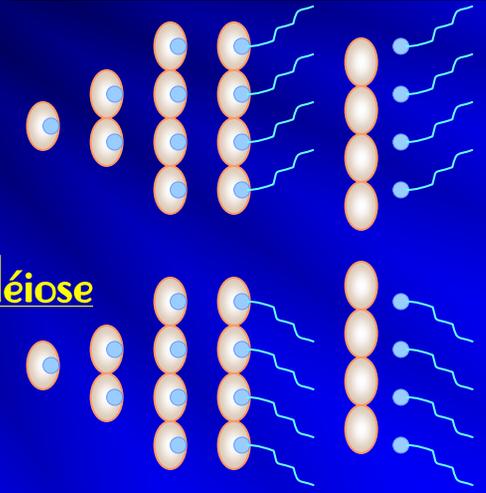
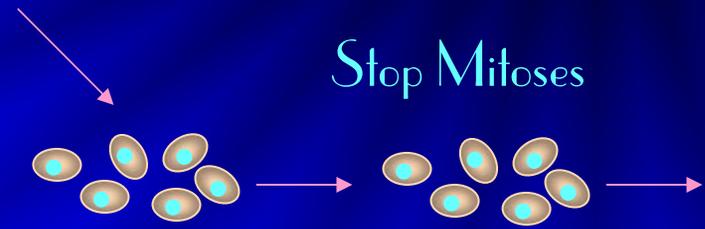
Fin Méiose

La frénésie!

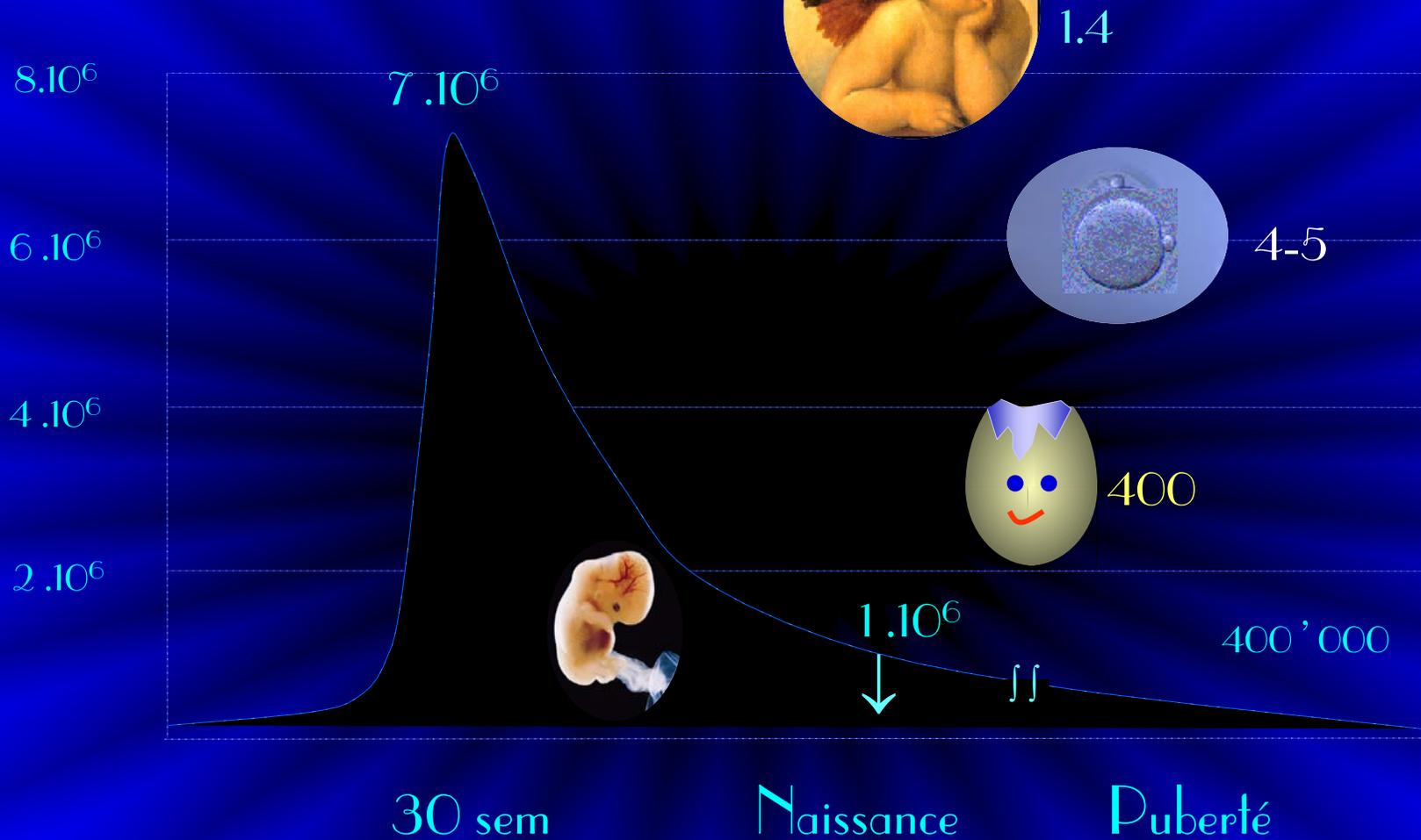
Mitoses

Stop Mitoses

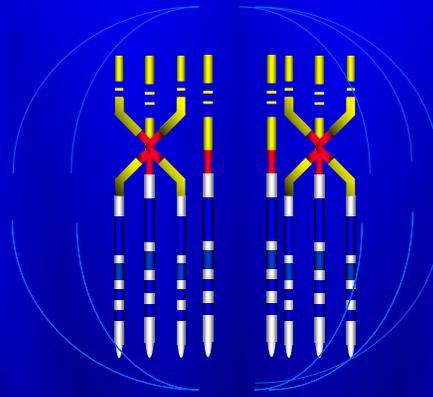
Début Méiose



Histoire d'O...vocytes



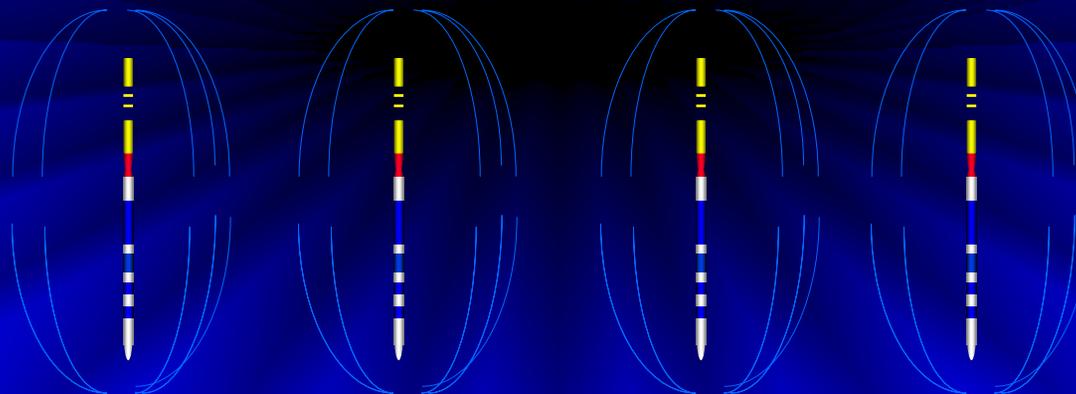
LA MÉIOSE
Expliquer
sans comprendre



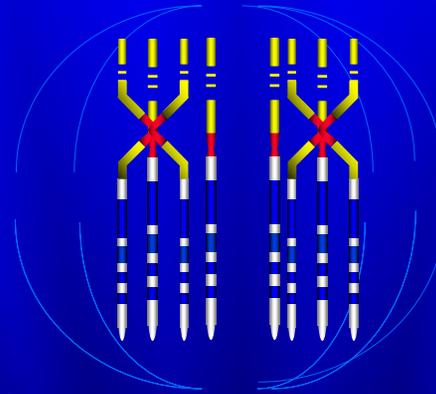
Réplication



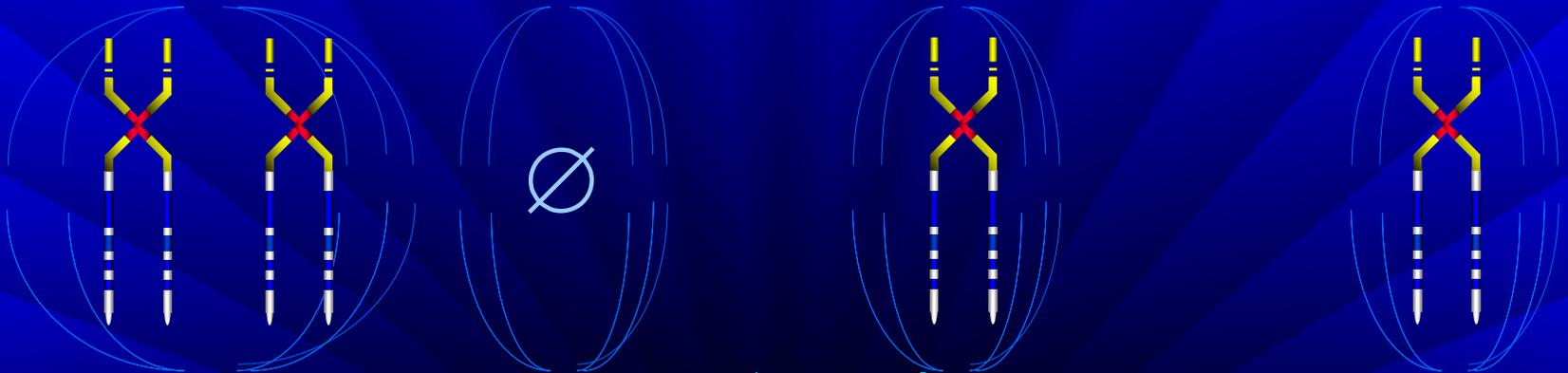
Exception



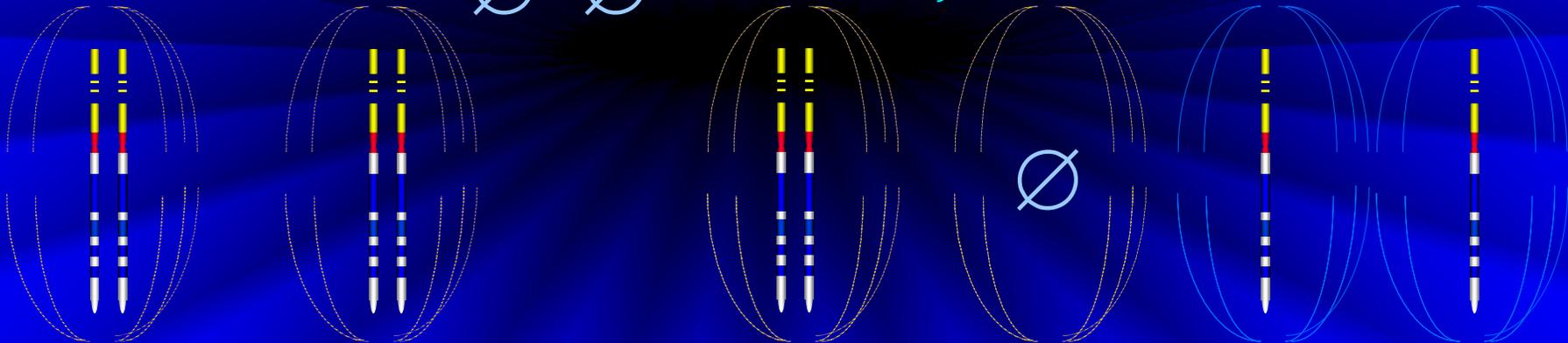
LA MÉIOSE



MI Non-disjonction



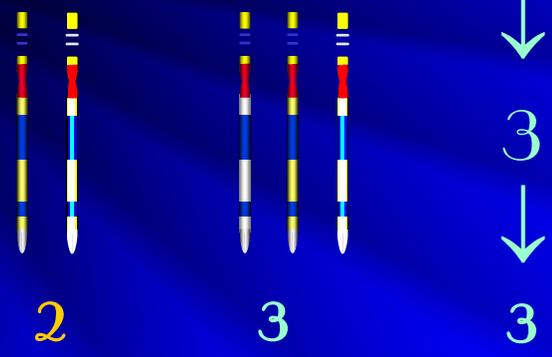
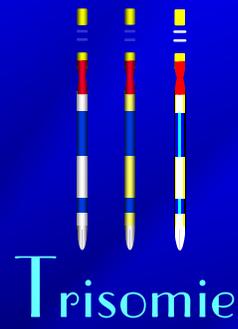
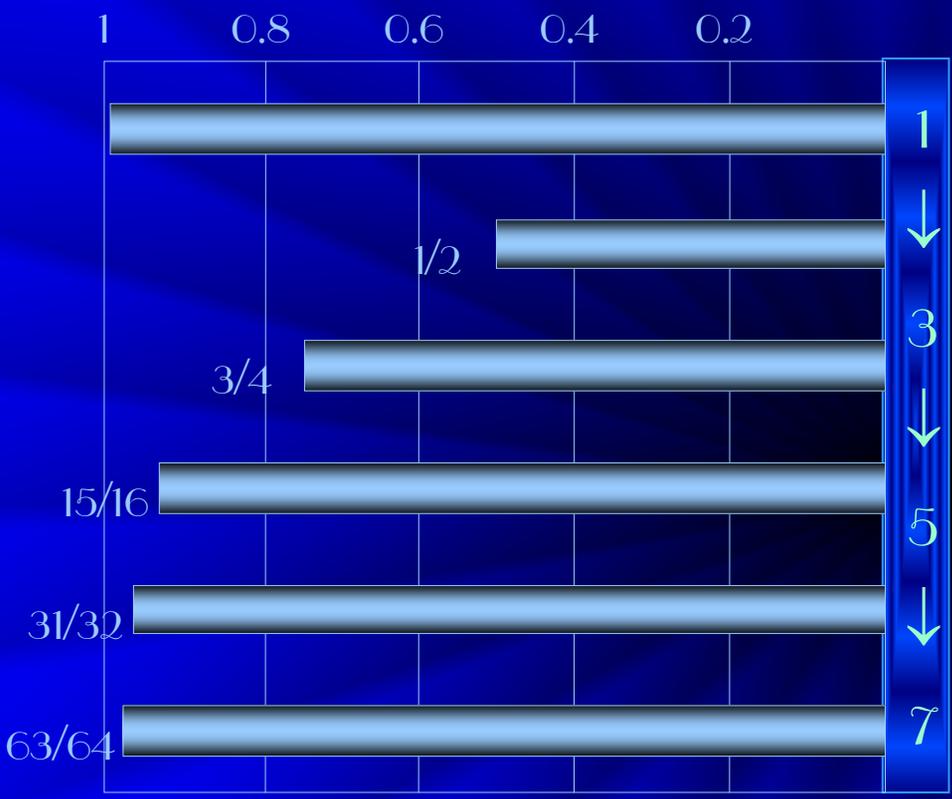
MII Non-disjonction



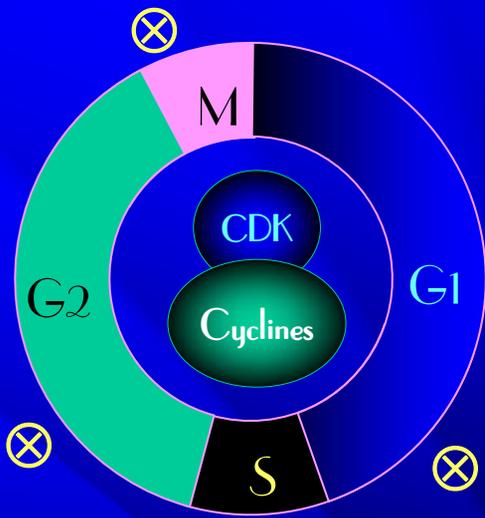
Mosaïcisme (CPM) et UPD

3%

% de cellules trisomiques



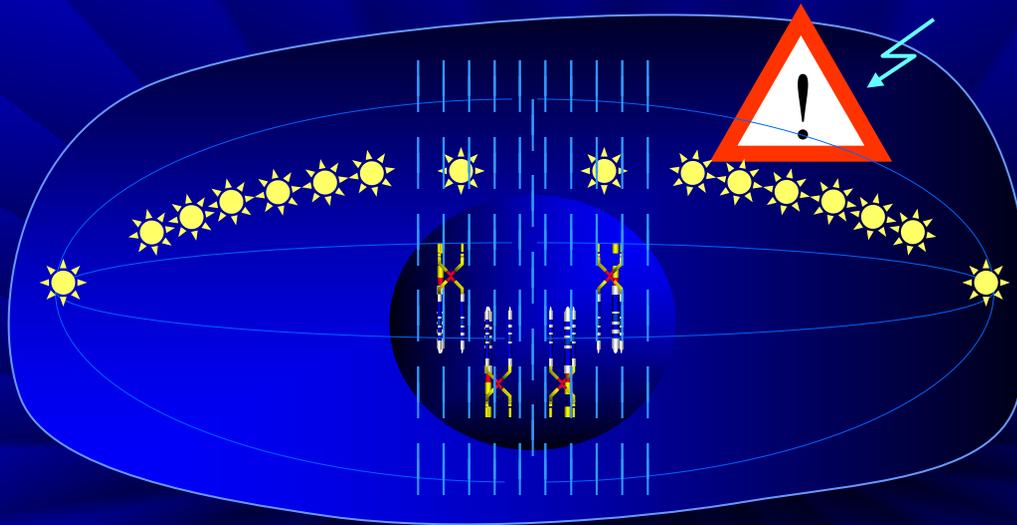
Cycle cellulaire



⊗ Check point 1

Ok dimensions cellule

Ok intégrité ADN



⊗ Check point 2

Ok rélication ADN

Ok dimensions cellule

Correction erreurs

⊗ Check point 3

Centromères-Fuseau



5 dicembre 2002

⊗ Check point 2

Correction erreurs
de réplication

Réplication
anomalies structure

Erreurs



Malformations
Tumeurs

Répartition
anomalies numériques

⊗ Check point 3

Fuseau & Centromères



5 décembre 2002

Fréquence des anomalies chromosomiques



Ovocytes 25%

Spermatozoides 2%

Personnes handicapées

> 25%

Couples infertiles

5-10%

Zygotes >30%

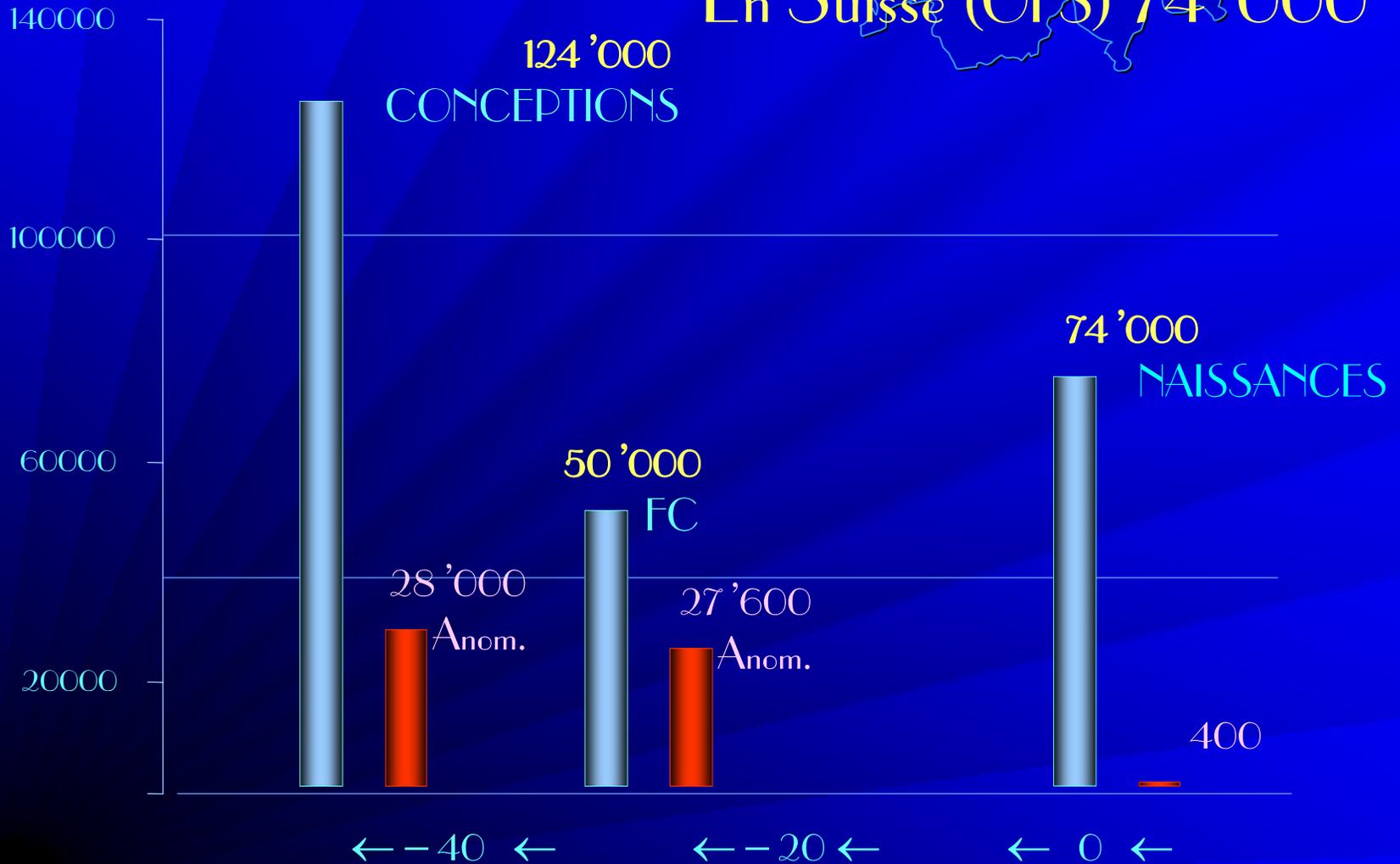
Avortements spontanés >50%

Morts-nés 5%

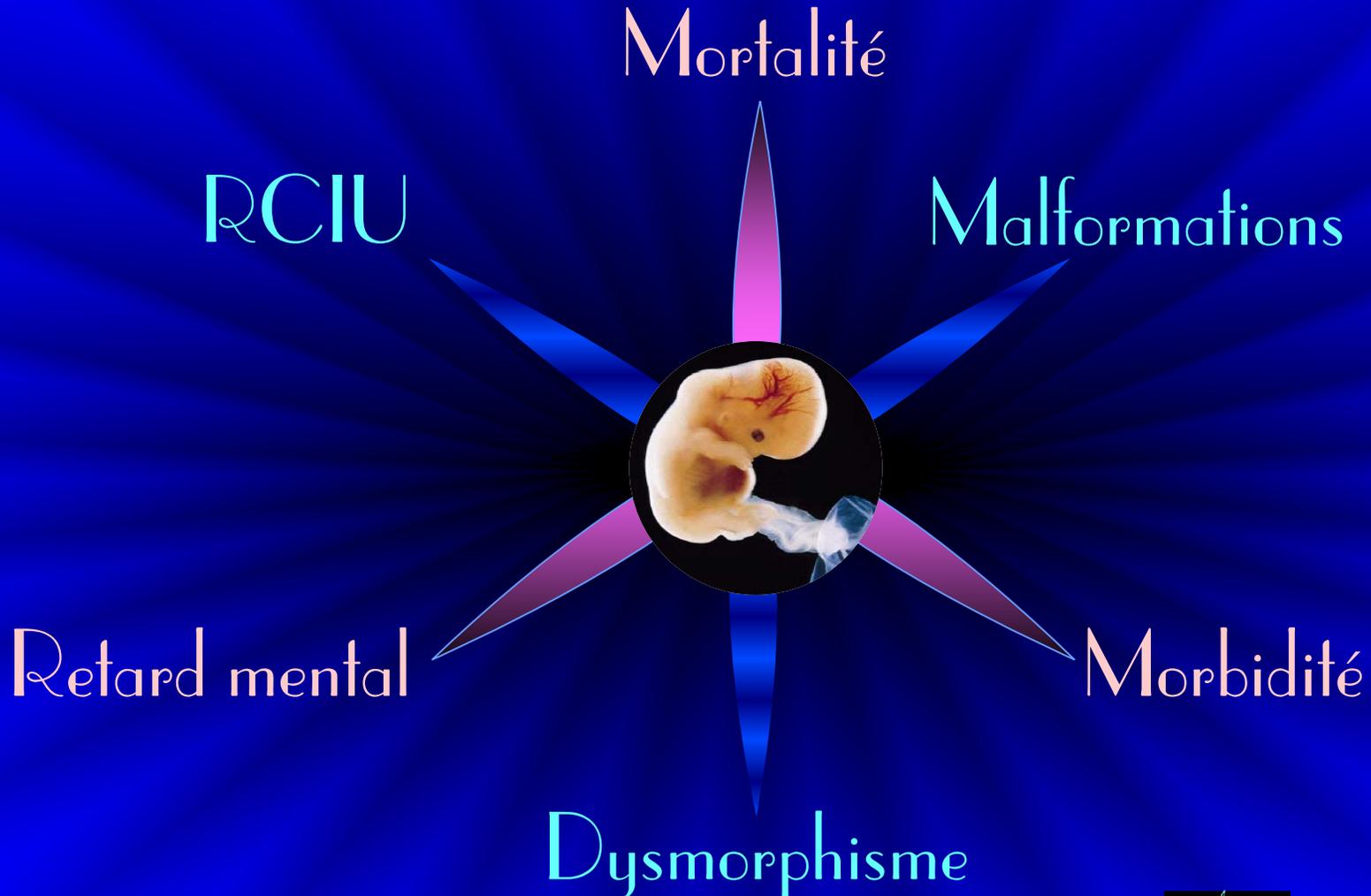
Nouveau-nés <0.3%



2001 L'Odysseé de l'espèce En Suisse (OFS) 74'000



Conséquences des déséquilibres constitutionnels



Malformations congénitales



Deaths in full-term neonates 48%

Pediatric intensive care admissions 39%

Pediatric hospital admissions 21%

Am J Hum Gent, 63, 1153, 1998



5 dicembre 2002

Handicap mental : 2-3% dont
~ 15% sur anomalie chromosomique >3-4 Mb
et ~ 10% <3-4 Mb

Délétions
interstitielles

Délétions
télémériques

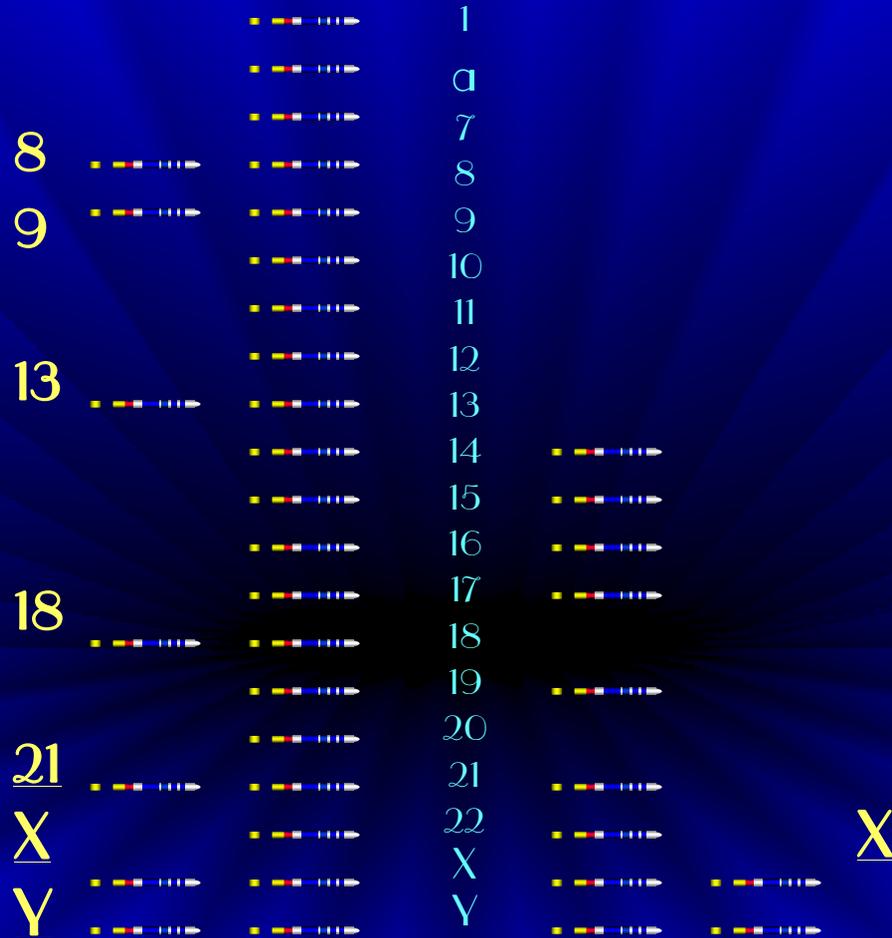
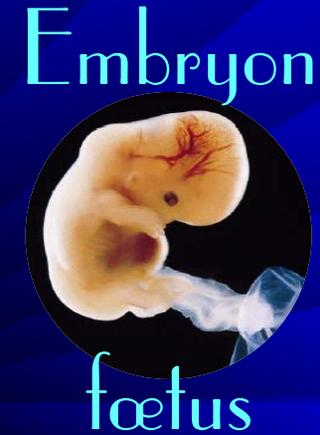


Total ~ 25%

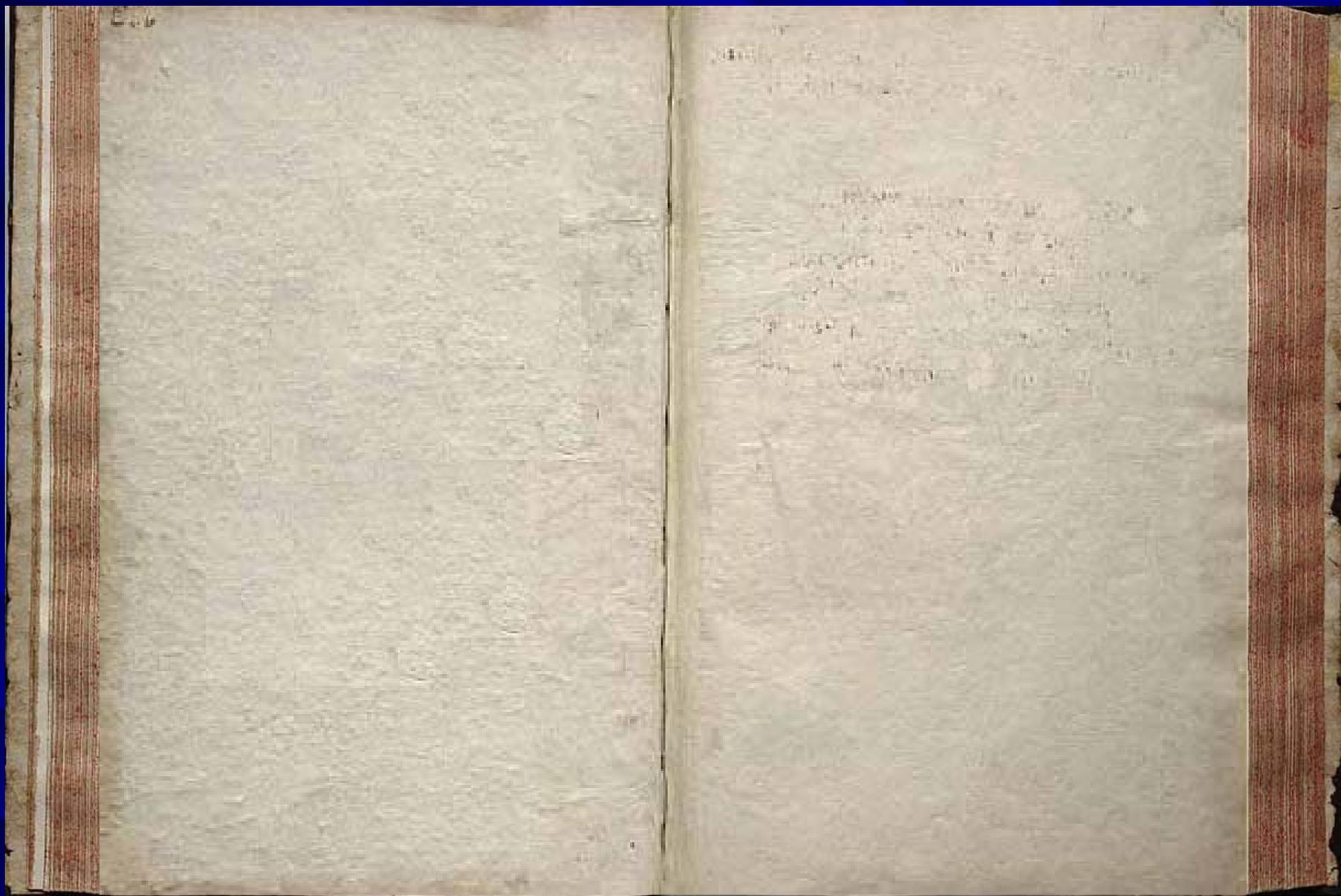
Aneuploidies

Trisomies

Monosomies







12 décembre 2002

1876



Premier portrait d'une personne
atteinte de S. de Down

FRASER & MITCHELL, 1876

Juillet 1959

« Nous proposons le terme de
TRISOMIE 21 »



Jérôme Lejeune
(1926-1994)

LE MONGOLISME PREMIER EXEMPLE
D'ABERRATION AUTOSOMIQUE HUMAINE

Jérôme LEJEUNE, R. TURPIN et Marthe GAUTIER

Annales de Génétique, Vol.1, n°2, juillet 1959, 41-49.

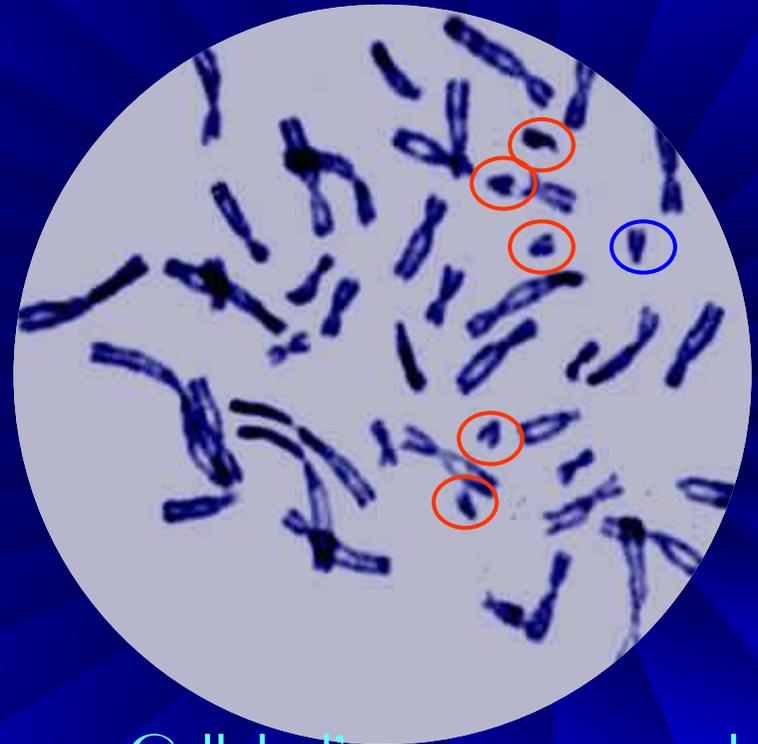


Fig. 5. Cellule d'un garçon mongolien,
après dispersion des chromosomes.

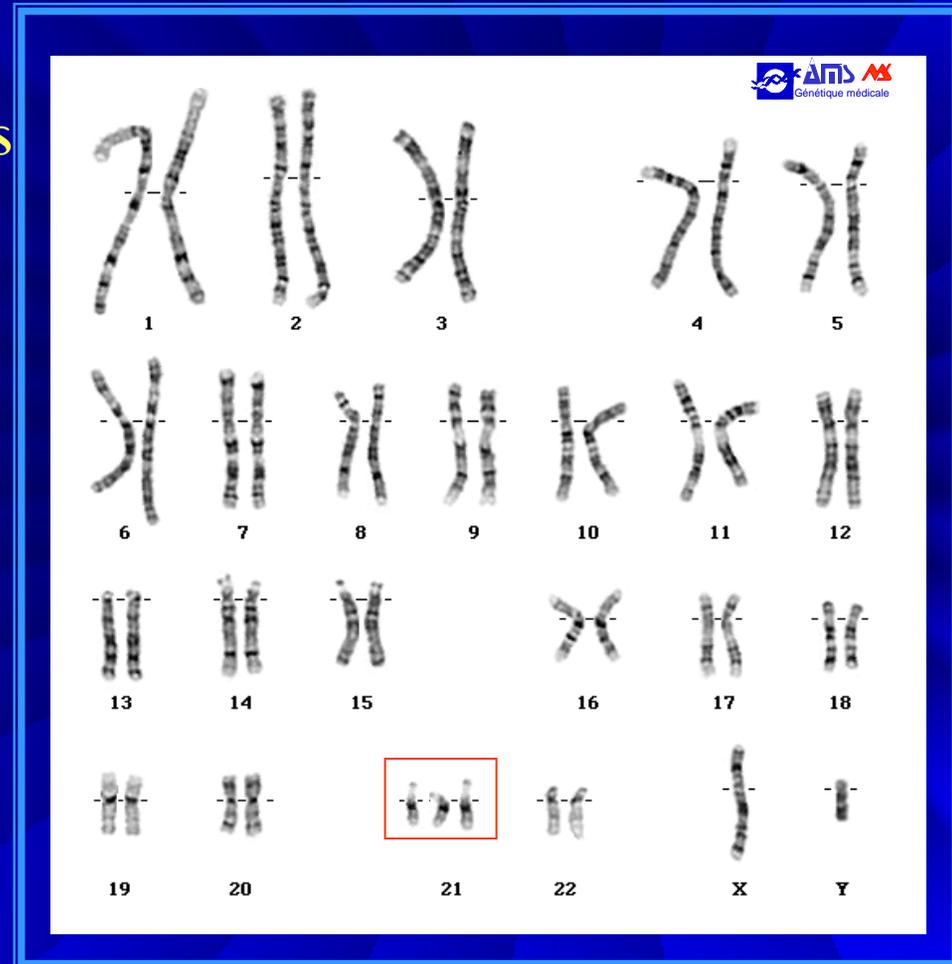
Caryotype en bandes G

(500 bandes) : 47,XY, Trisomie 21 libre

Résolution ~ 3-4 Mb

Correspondant à ~ 20 gènes

1975





Depuis 1959 plus de 300'000 diagnostics

Trisomie 21





Depuis 1959 plus de 10'000 diagnostics

Trisomie 21

 Libre

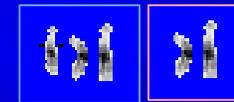
97%

Homogène



94%

Mosaïque

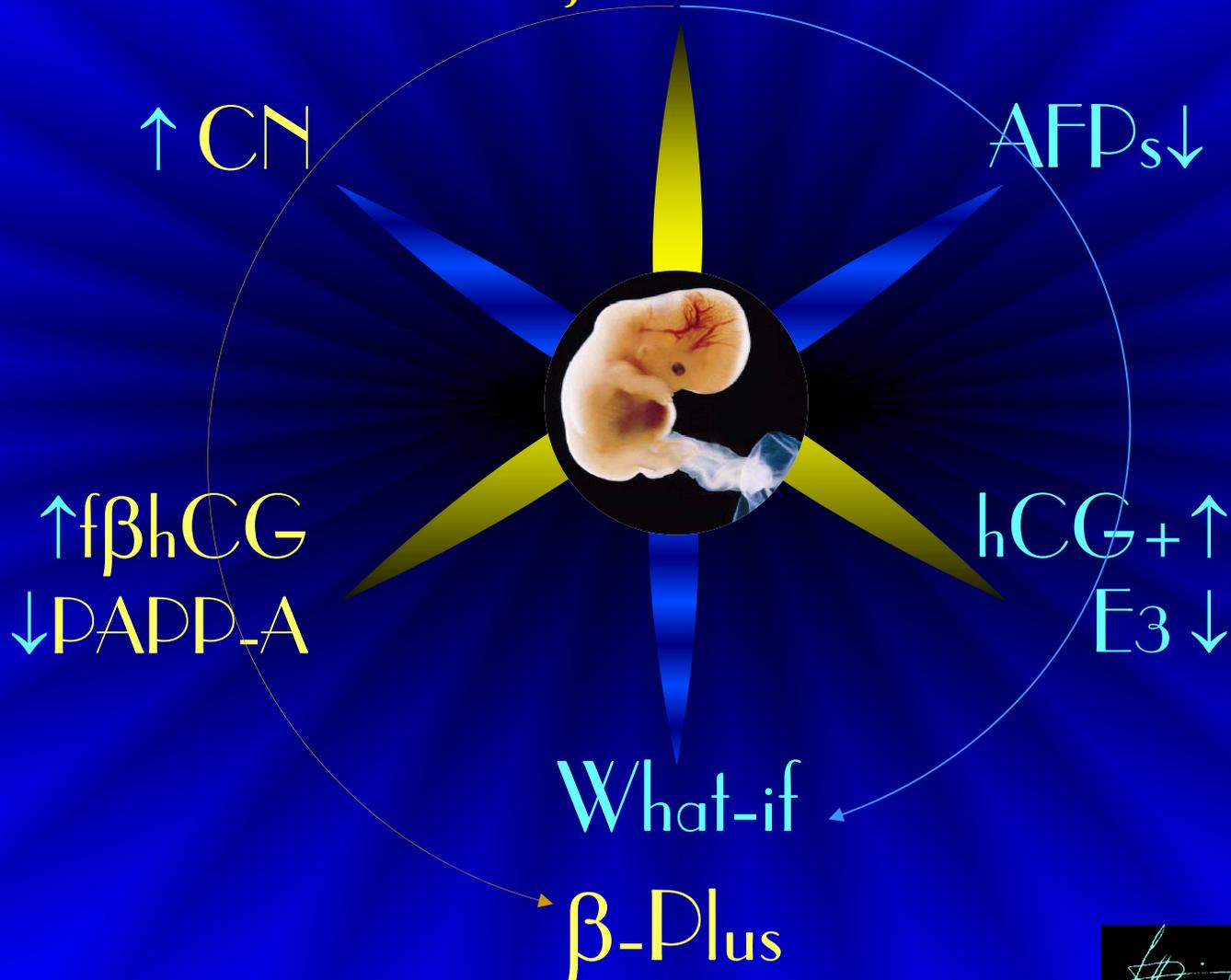


3%



Dépistages : facteurs de risque

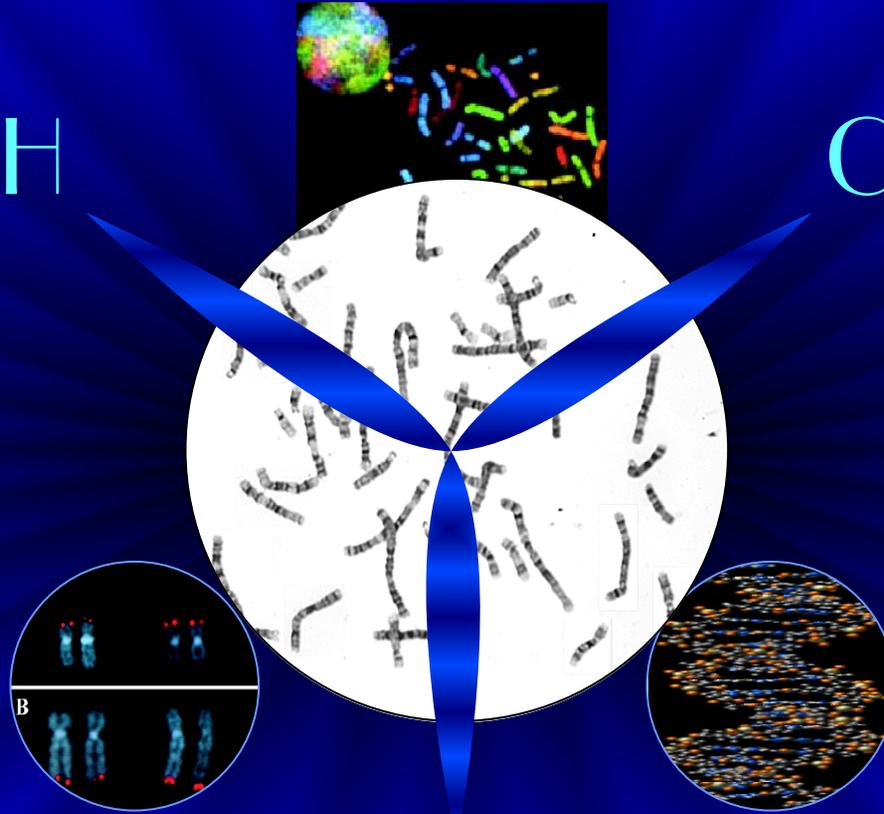
Age maternel



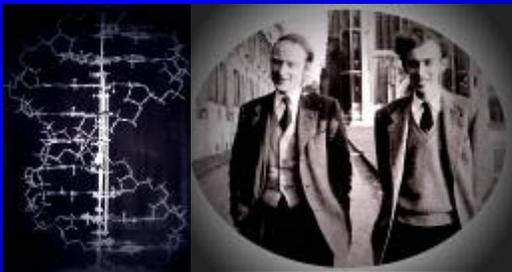
Diagnostic : les nouvelles méthodes

FISH

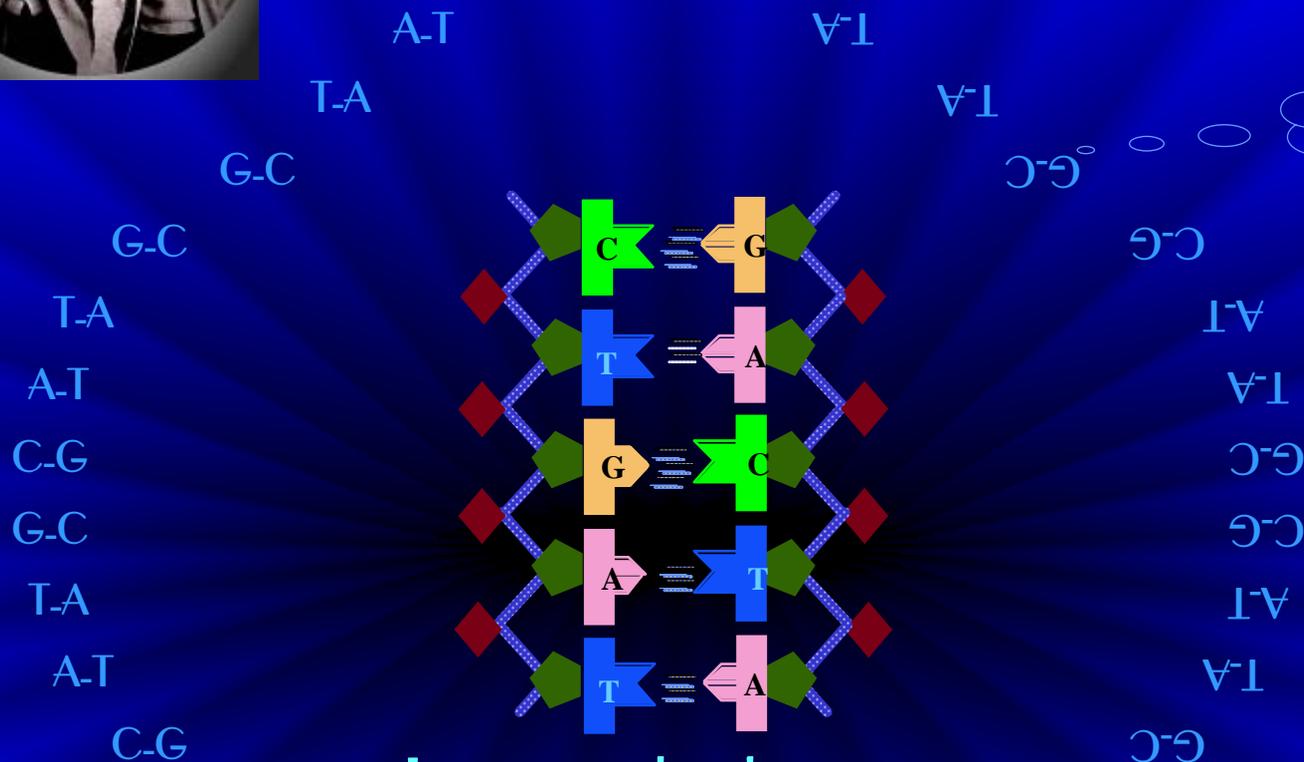
CGH



SKY



La loi de complémentarité



A-T

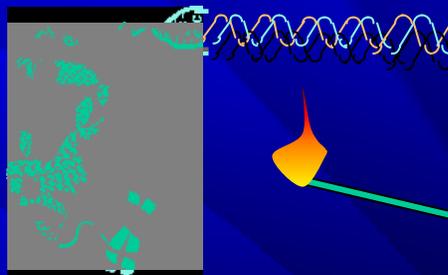
C-G



5 dicembre 2002

Principe de l'hybridation moléculaire

1. Dénaturation thermique



A T G T G A A T A C A C
A T G T G
* * * * *

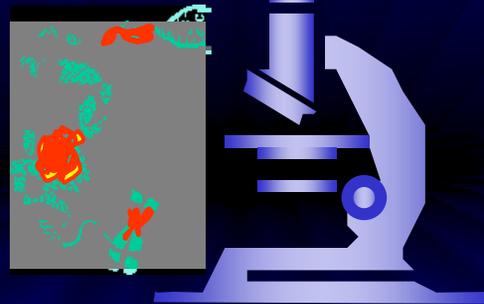
2. Hybridation

T A C A C T A T G T G A

A T G T G
* * * * *

A T G T G
* * * * *

A T G T G
* * * * *

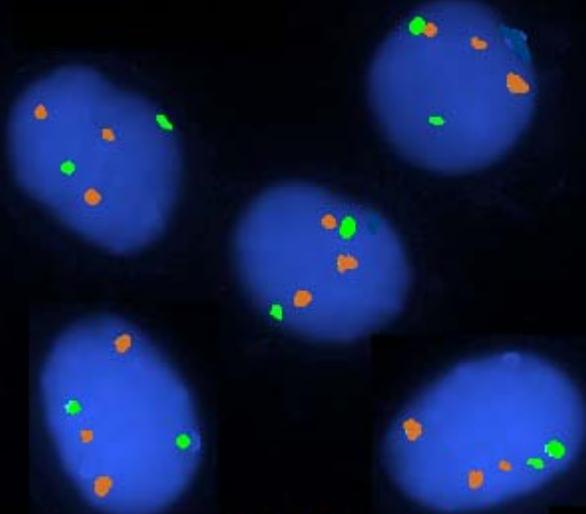


3. Observation

Sondes fluo



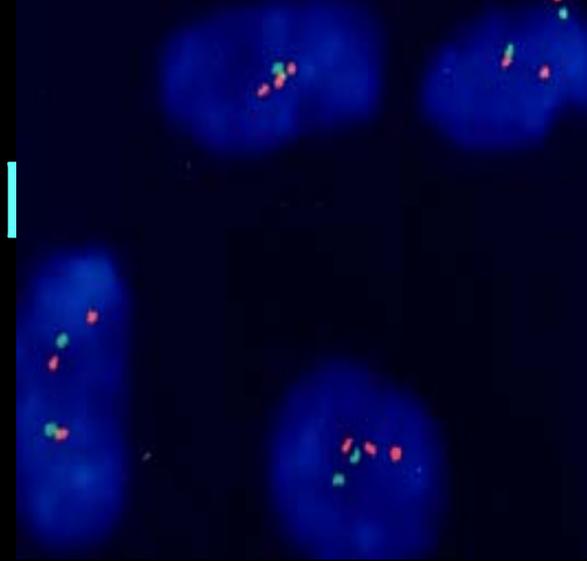
Hybridation in situ = FISH



AMS GE 00 07 17

Trisomie 21

Sperme



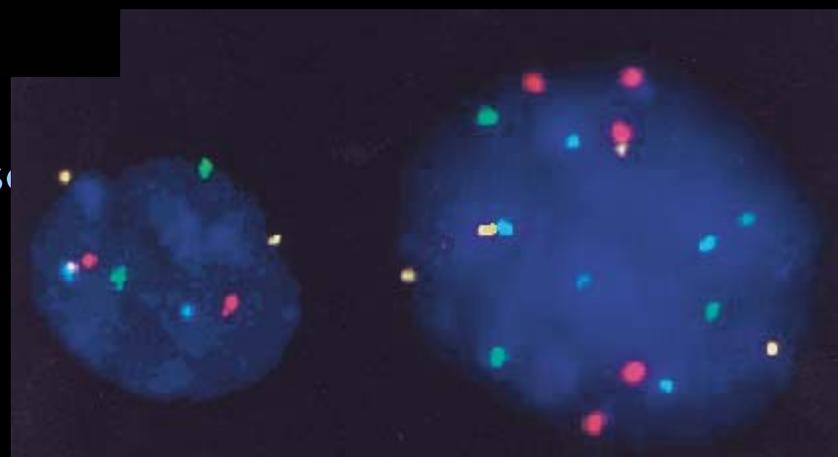
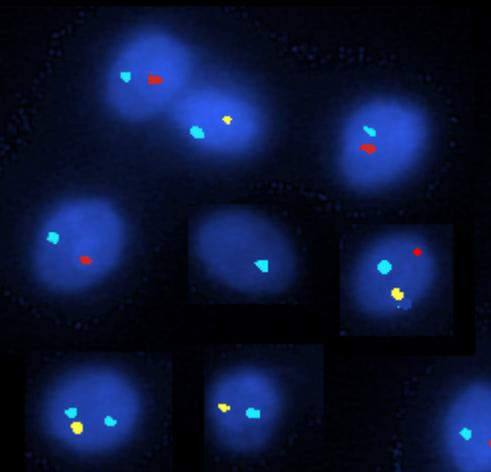
Mosaïcisme



HER-2

Cancer du sein

Cancer de la vessie



Caryotype spectral

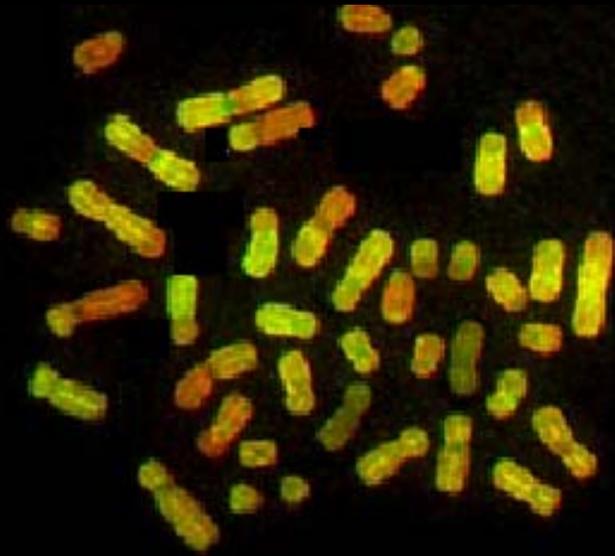


Handwritten signature

Comparative Genomic Hybridization

Hybridation avec
ADN du patient seul

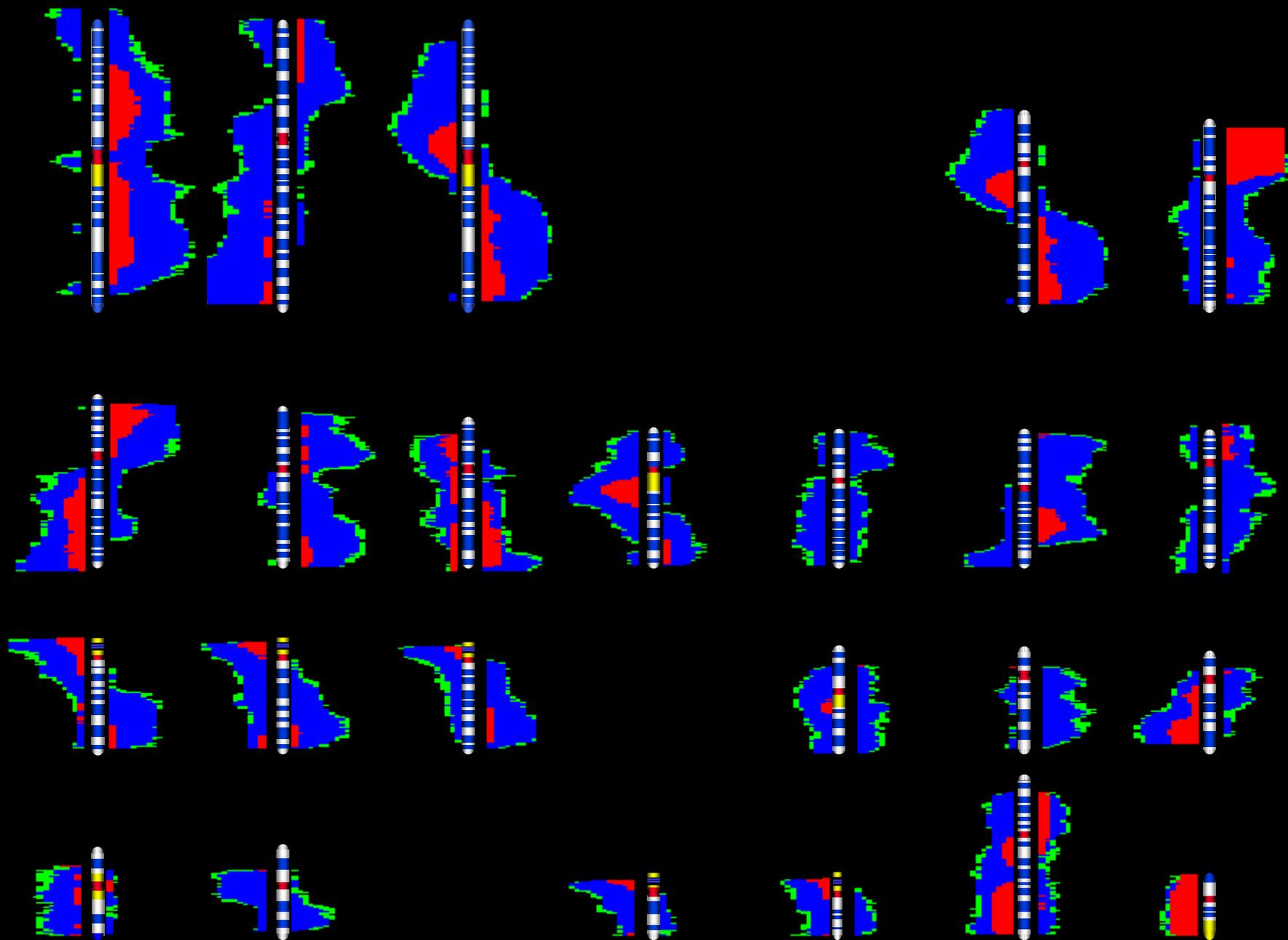
Hybridation avec
ADN normal seul



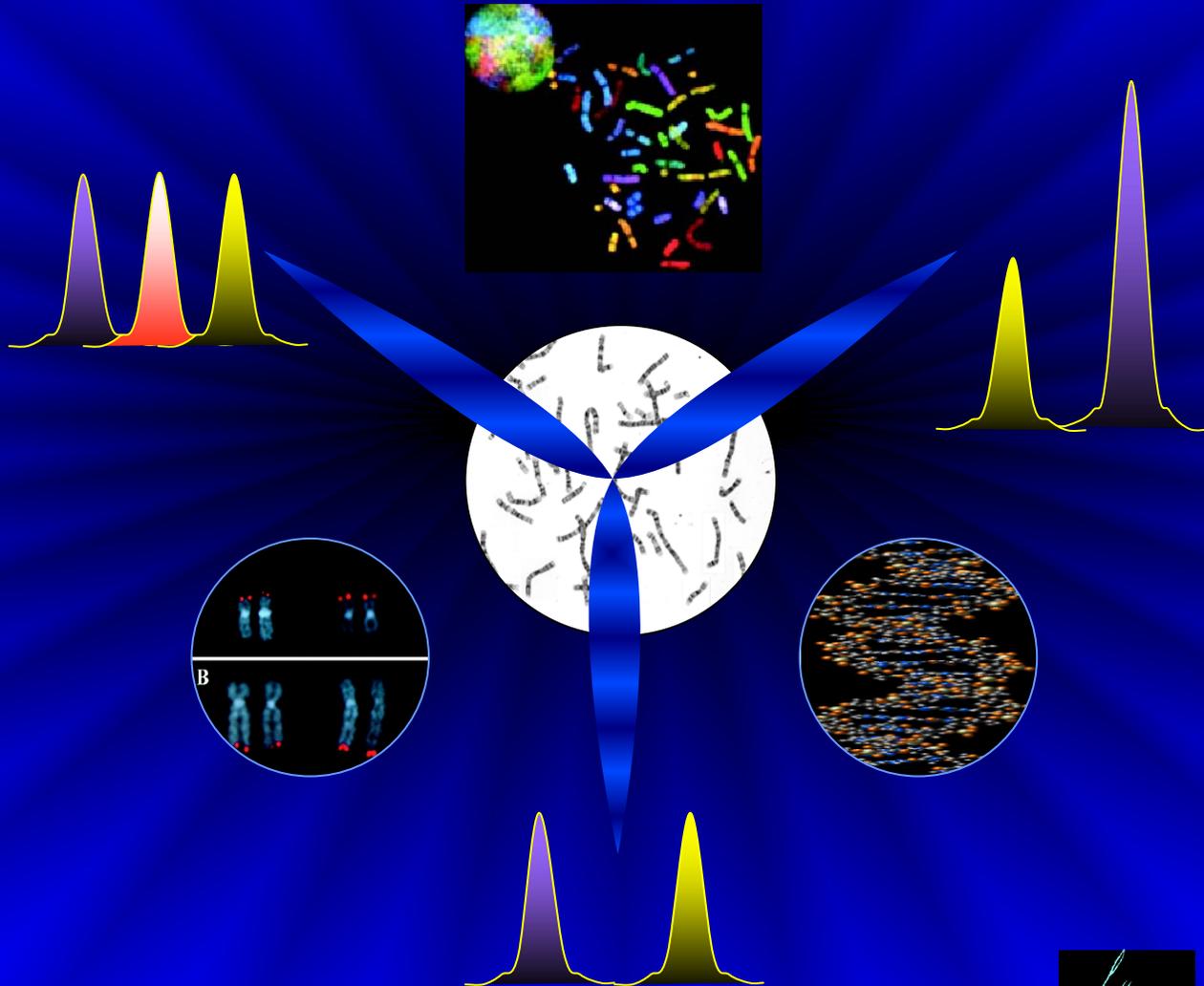
Métaphase
Normale

Mélange
ADN patient + ADN normal

A handwritten signature in white ink, located in the bottom right corner of the slide.



QF-PCR



Chaque parent contribue par
une moitié à la
constitution génétique de chaque enfant





A O



B O



AB
RR



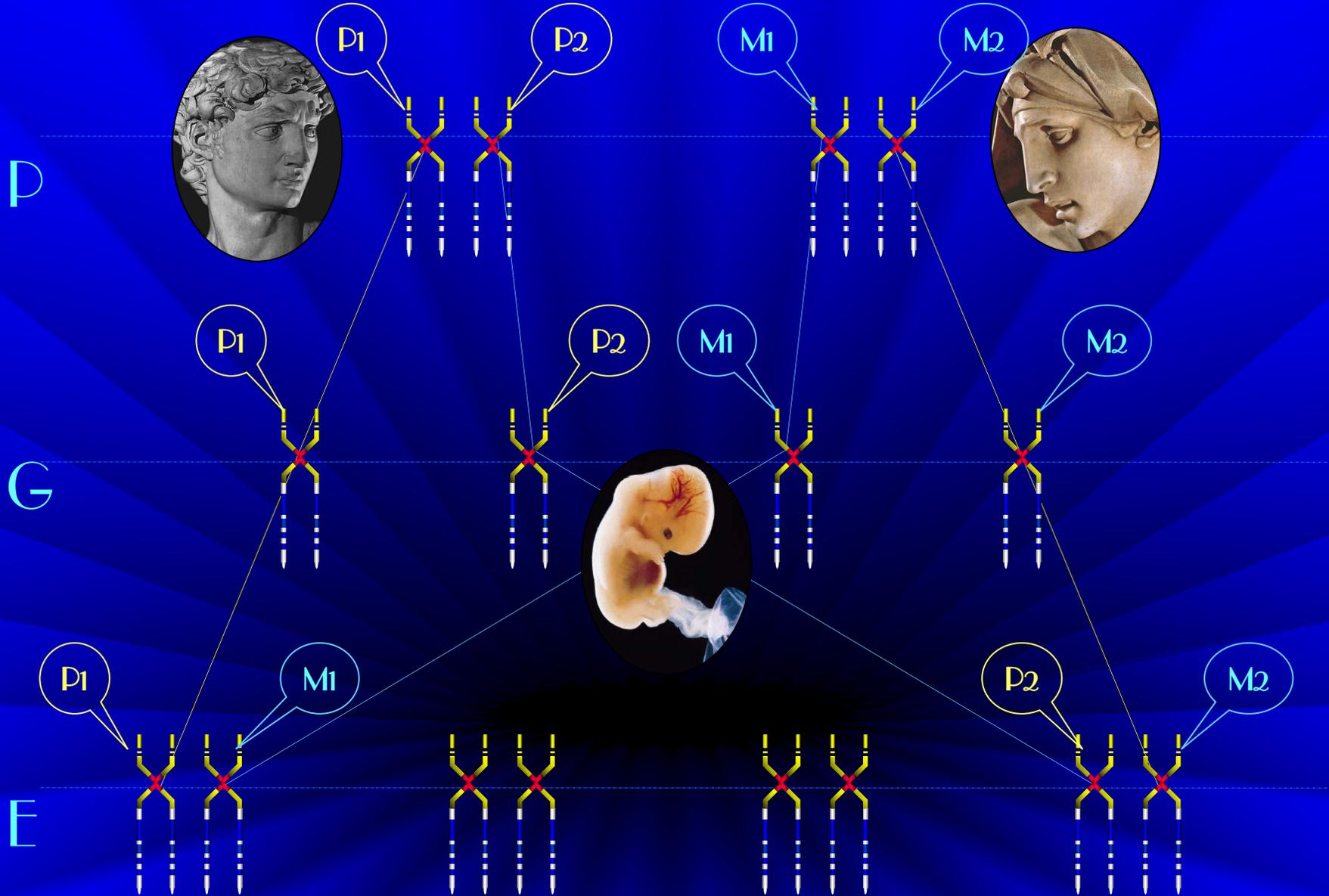
AO
RV



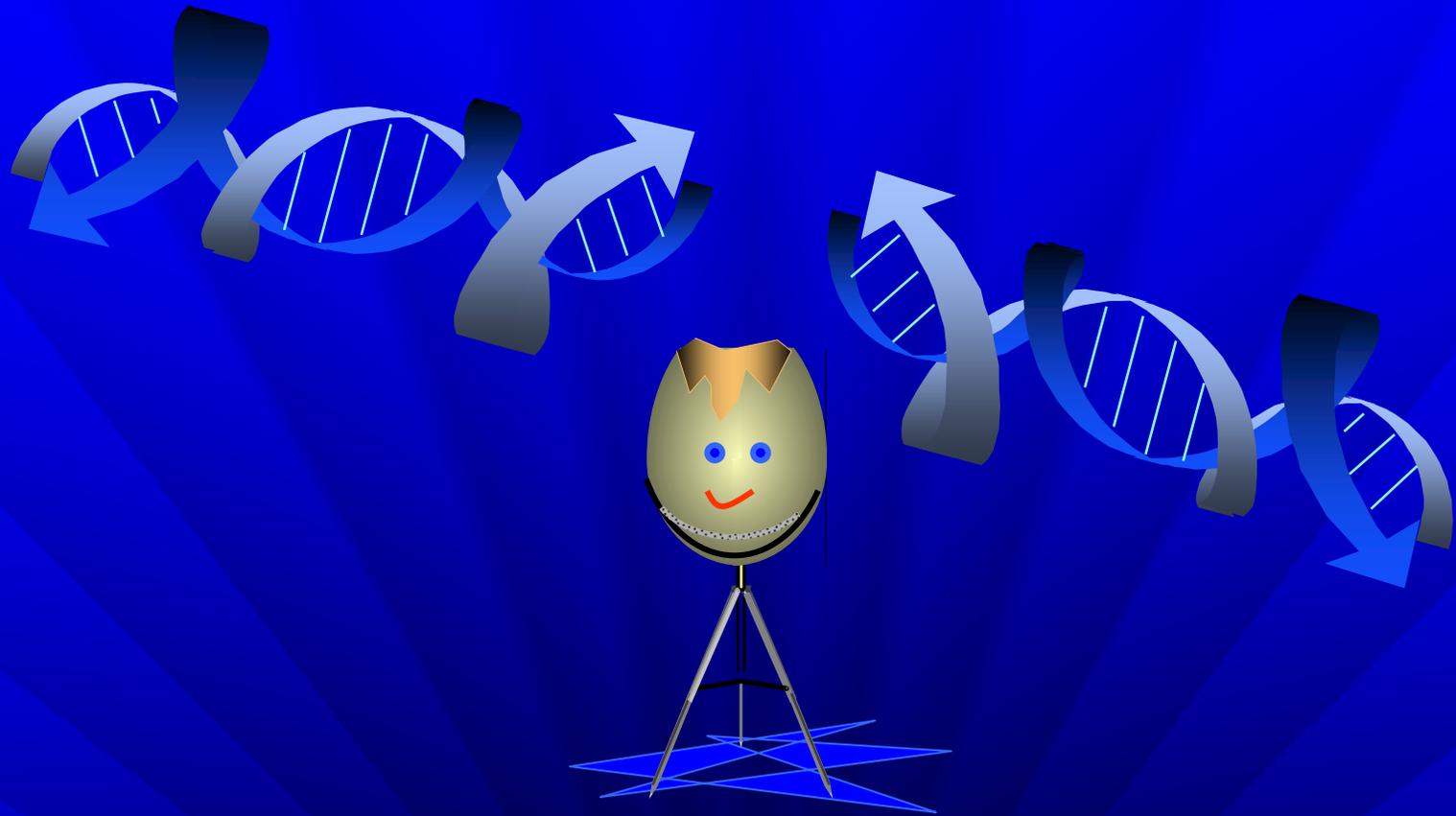
OB
VR



OO
VV



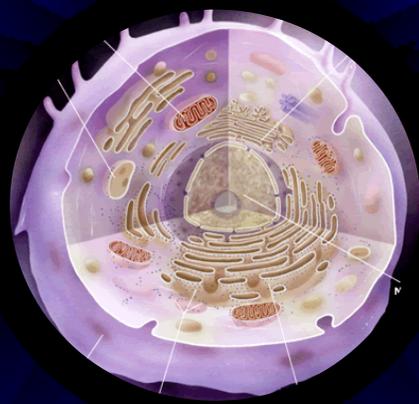
Croissez et multipliez (Dieu)



« *Qui fait un œuf, fait du neuf* »

10^{90} sortes de gamètes





6 pg d 'ADN \Rightarrow 170 cm

24 molécules

3 Milliards de pb

46 Mille gènes

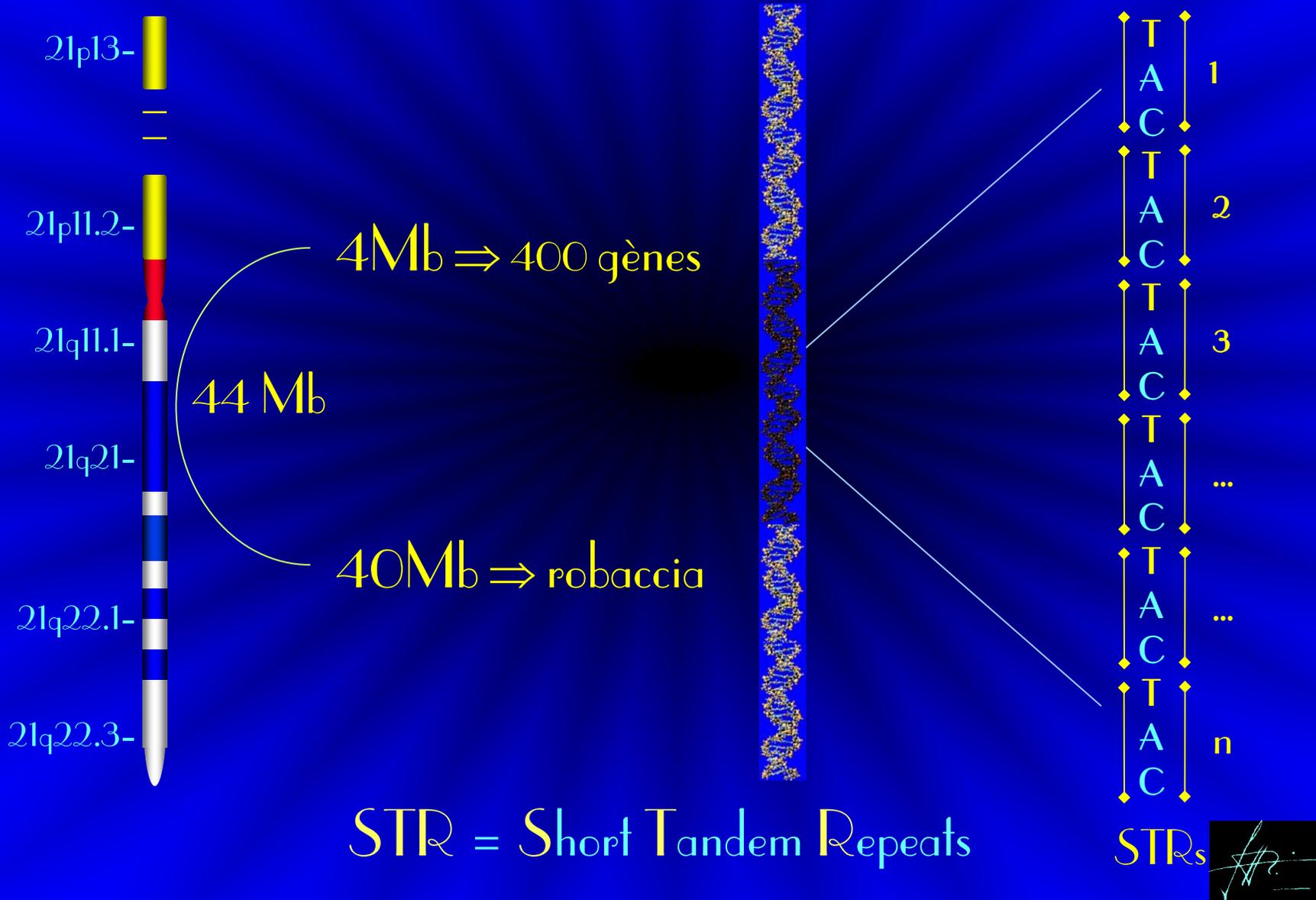
3%

97%

Junk DNA (robaccia)

15% répétitif

Chromosome 21 : 2,5 cm d'ADN



STR = Short Tandem Repeats

Chromosome paternel



TAC TAC TAC TAC TAC 5

ATAC ATAC ATAC 3

AGATA AGATA AGATA

Chromosome maternel



TAC TAC TAC TAC 4

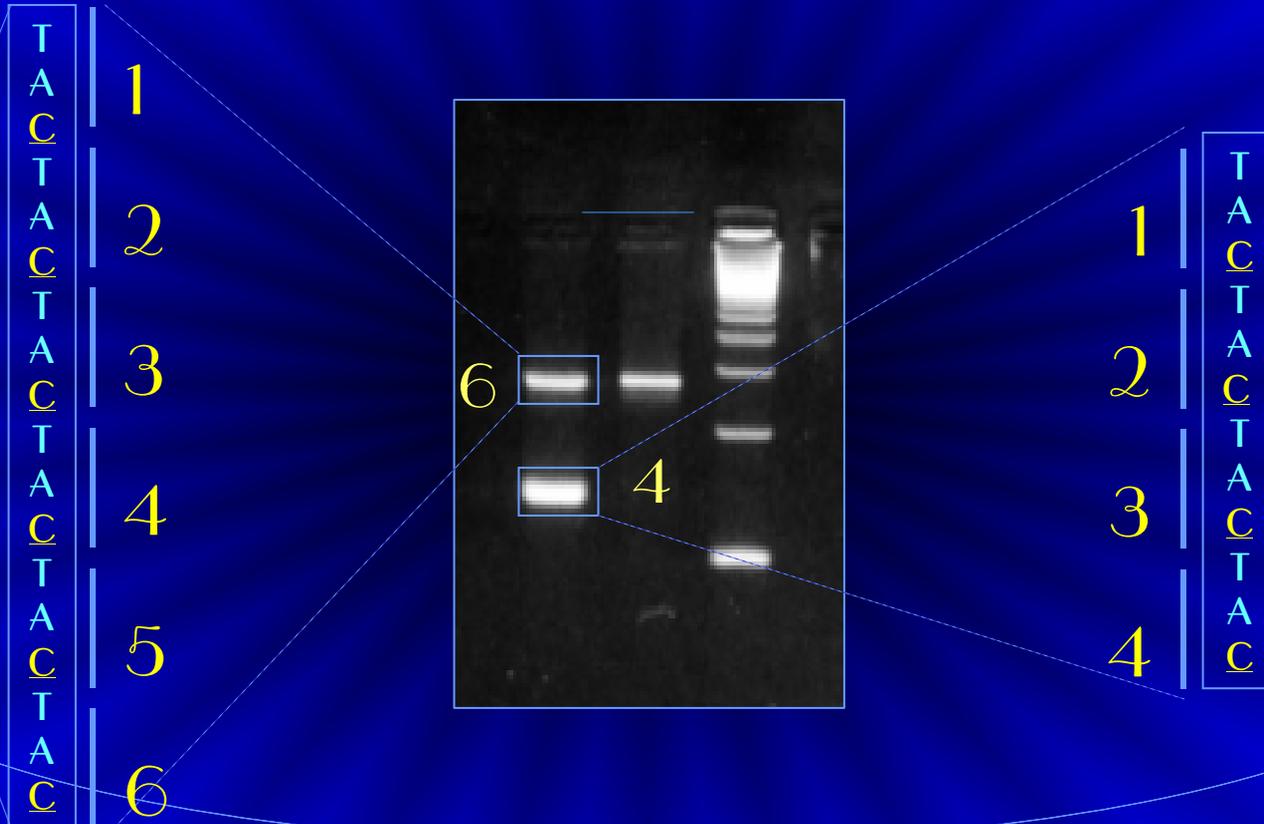
ATAC ATAC 2

AGATA AGATA

STR = Short Tandem Repeats



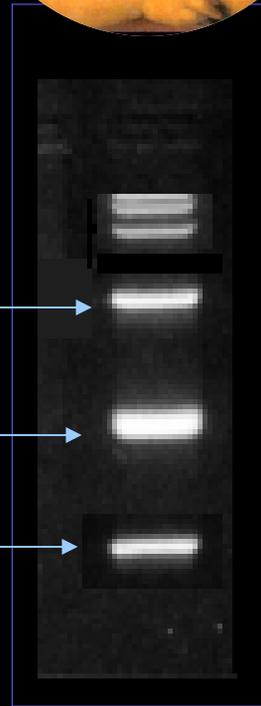
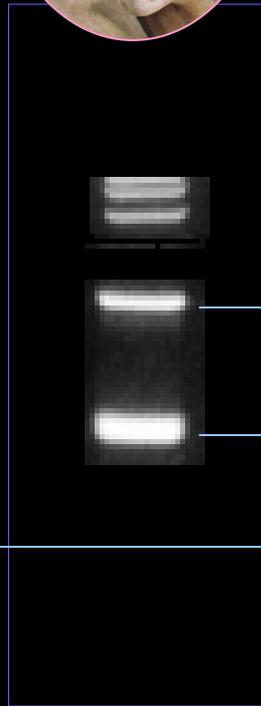
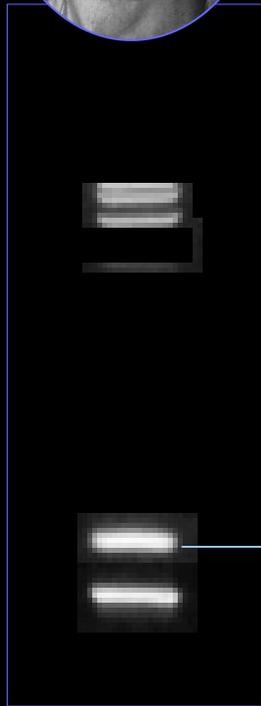
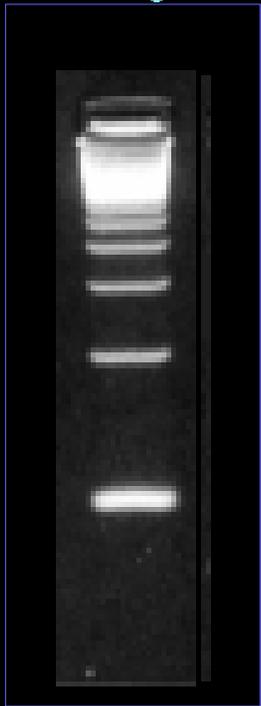
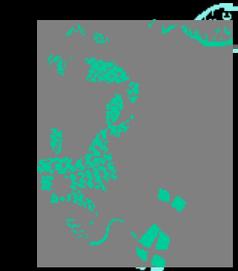
STR = Short Tandem Repeats



Electrophorèse



Les profils d'ADN

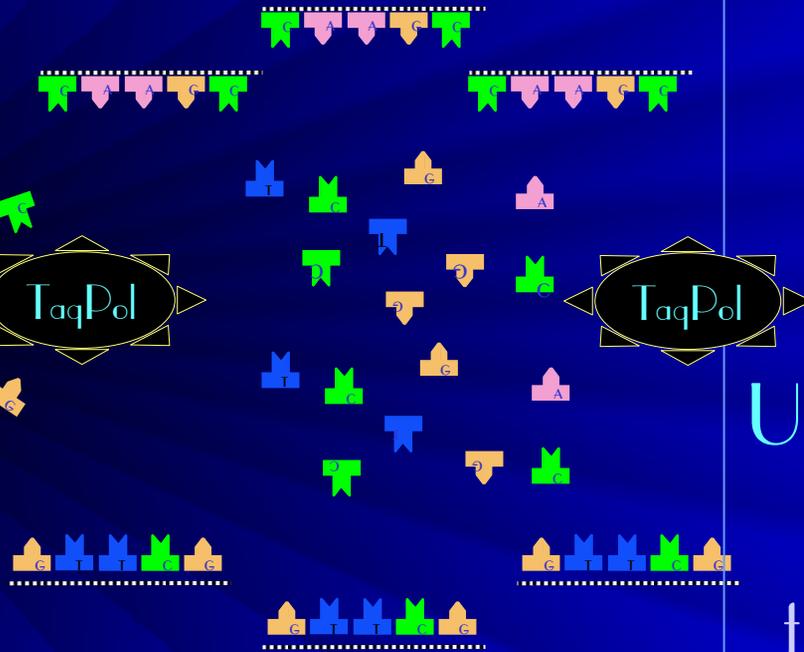




Kary Mullis, 1944



L'ADN cible



PCR: La recette du chef

Quelques pg d'ADN

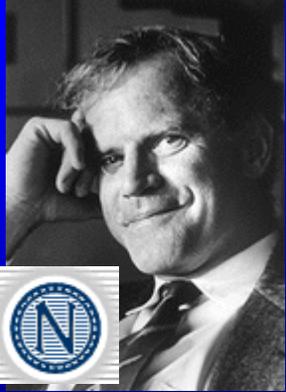
Un bouquet d'amorces

Une bonne pincée
de nucléotides

Un soupçon de polymérase

Mettre dans le
thermocycleur, couvrir et
laisser cuire 2 heures





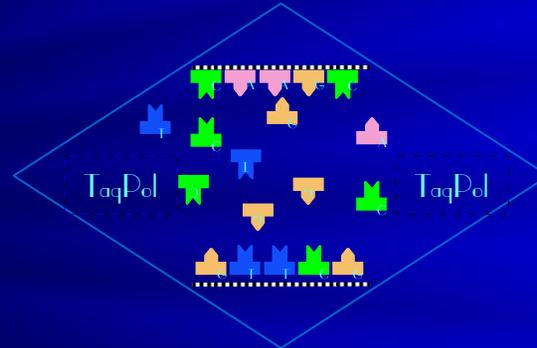
Kary Mullis, 1944

1^{er} Cycle

Dénaturation

Extension

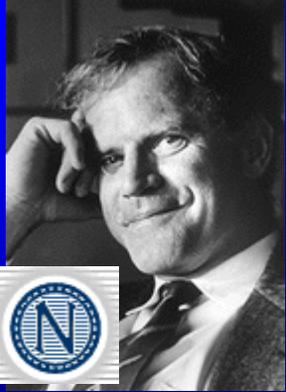
Hybridation



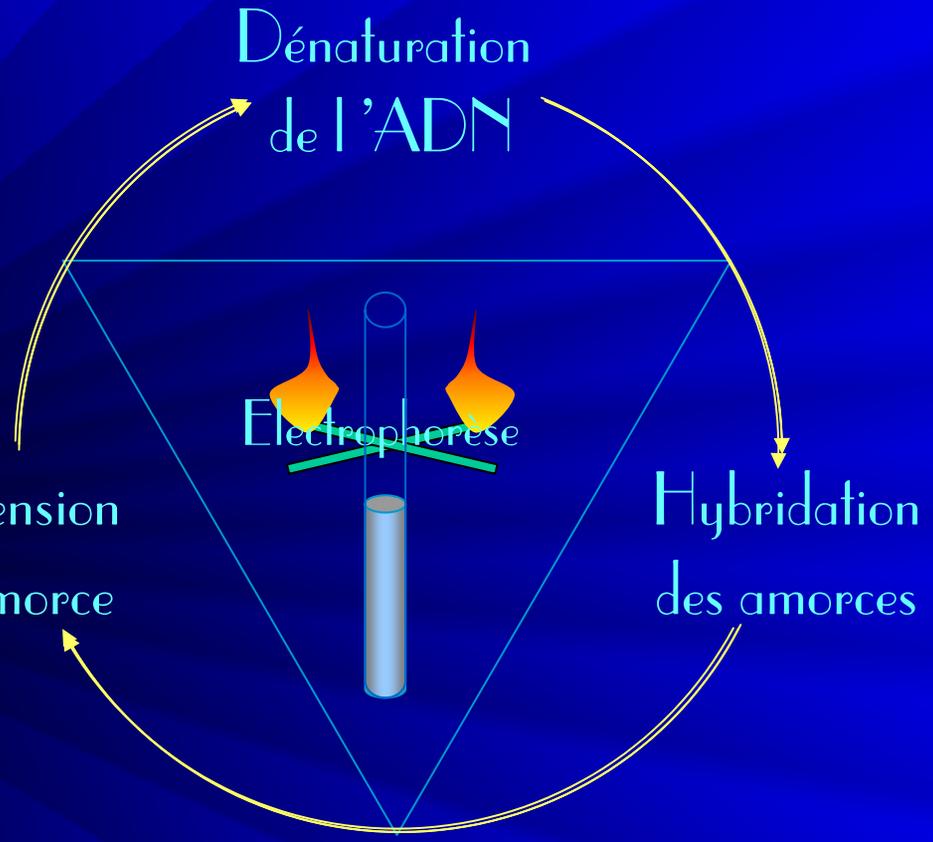
Hybridation

Extension



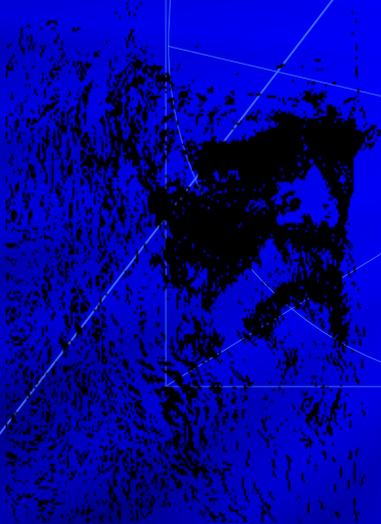


Kary Mullis, 1944

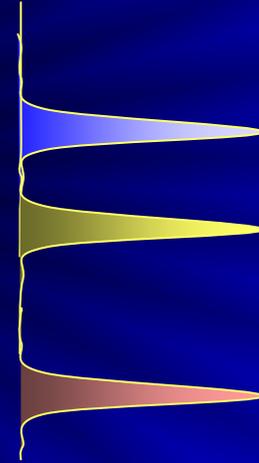
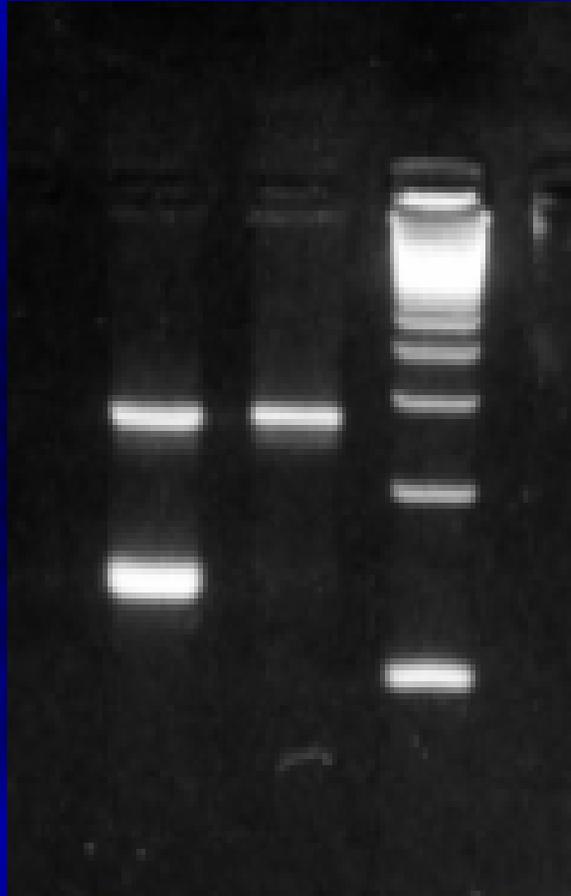
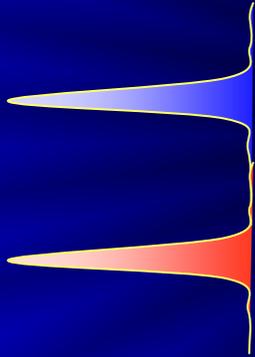


Amplimère
 $2^n \Rightarrow 2^{20} = 1 \text{ million}$

Elémentaire
mon cher Pesca



Polymorphisme



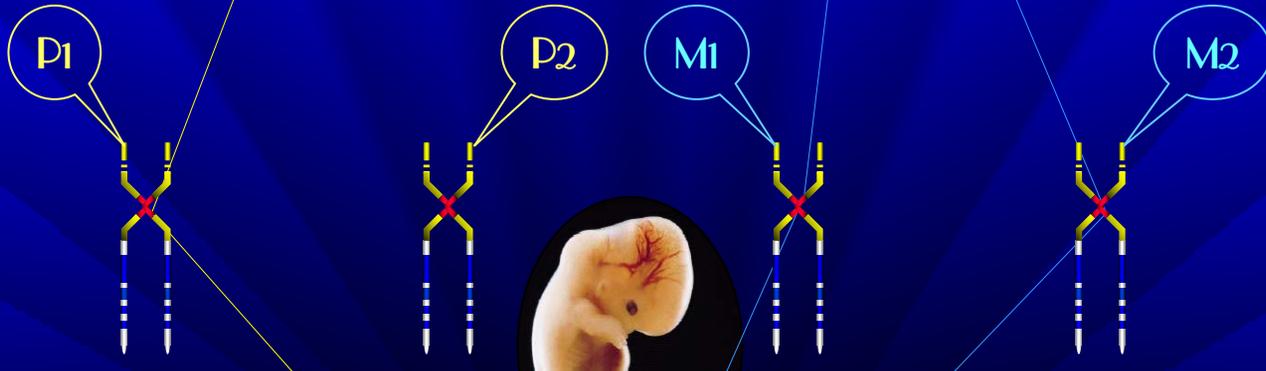
PCR et électrophorèse



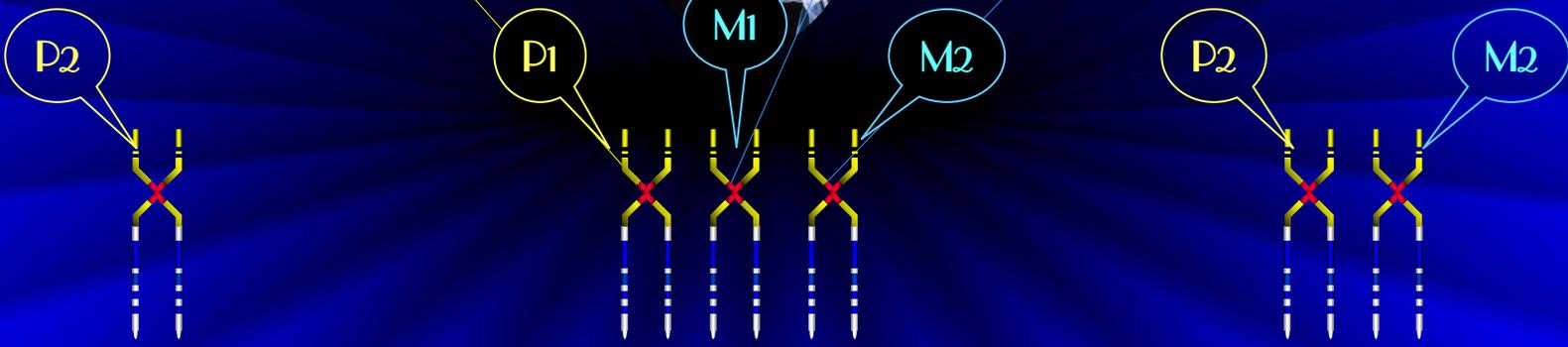
P



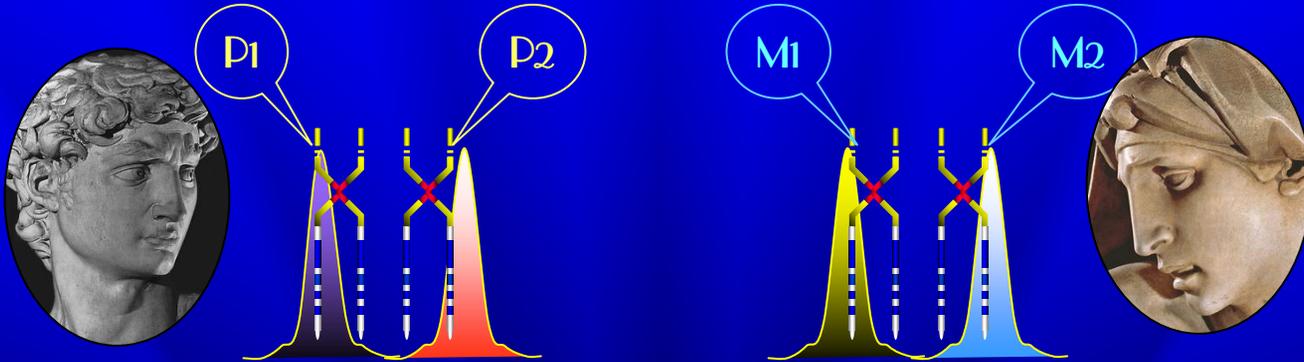
G



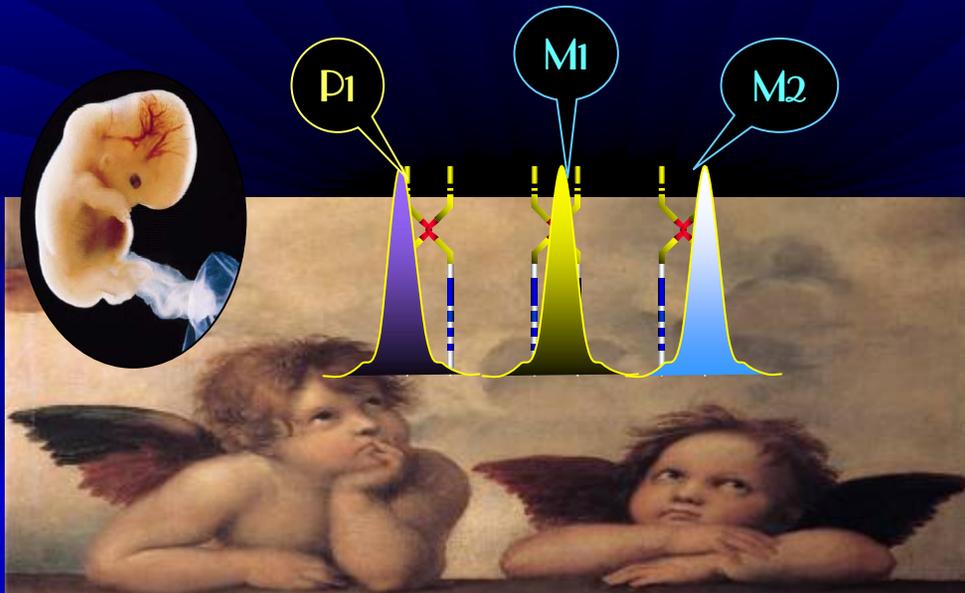
E



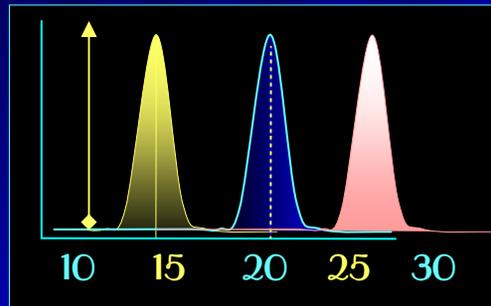
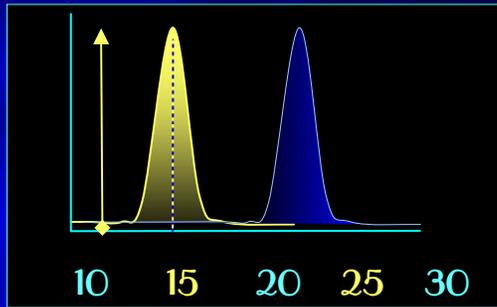
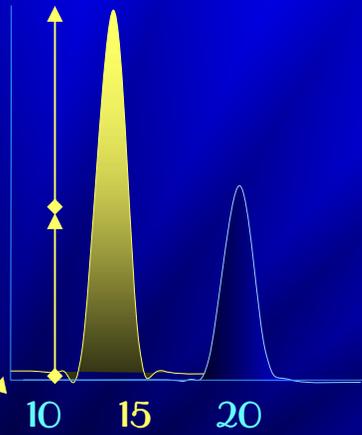
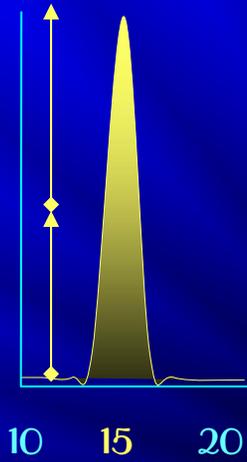
Trisomie



Trisomie



Normal



Trisomie



INFORMATIVITE = 99.96%

1/2 '550

	<u>Marqueurs</u>	<u>Hétérozygotie</u>	<u>Nombre d'allèles</u>	<u>Position</u>
21p13-				
21p11.2-				
21q11.1-	-D21S11	.90	≥ 15	17'218'418
21q21-22.1-	-D21S1270	.86	≥ 20	27'918'928
21q22.11-	-D21S226	.59	≥ 6	28'284'058
21q22.3-	-D21S1411	.93	≥ 13	40'704'046



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Collectif et résultats cytogénétiques

46,XX	95	
46,XY	83	
<u>Total normaux</u>		<u>178</u>
Trisomie 13	1	
Trisomie 18	3	
Trisomie 21	12	
Triploidie	3	
Dysgonosomies	3	
<u>Total anormaux</u>		<u>22</u>
<u>Total</u>		<u>200</u>



Résultats

Tous identifiés correctement

QF-PCR

CARYOTYPES	XX	XY	T13	T18	T21	TX	KI	X	69
XX	95	95							
XY	83	83							
T13	1		1						
T18	3			3					
T21	12				12				
47,XXX	1					1			
47,XXY	1						1		
45,X	1							1	
69,XXX	3								3



$$\text{Spécificité} = d : (b+d) = 100$$

	M_+	M_\emptyset	
T_{est+}	a	b	$a : (a+b) = \text{VPP} \sim 100$
$T_{est\ \emptyset}$	c	d	$d : (c+d) = \text{VPN} \sim 100$

$$\text{Sensibilité} = a : (a+c) = 99.96$$



Algorithm

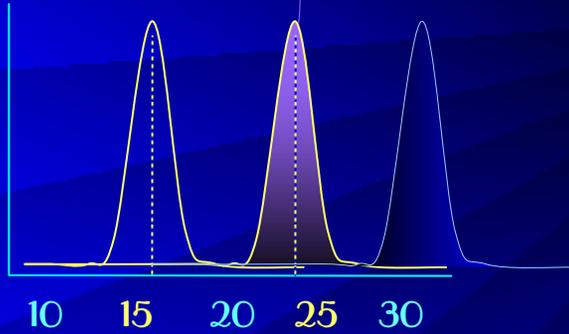


Valeur ajoutée

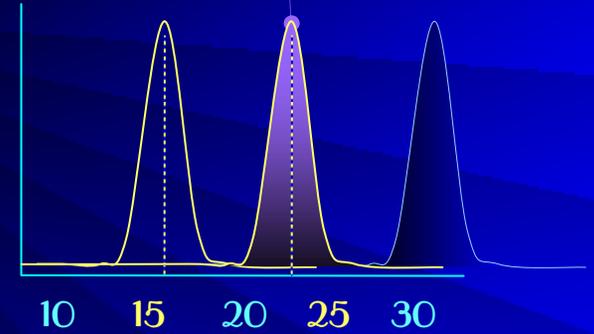
- * Faible cellularité du LA
- * Contamination maternelle
- * Mosaïques
- * Méthode extensible
- * Automatisable



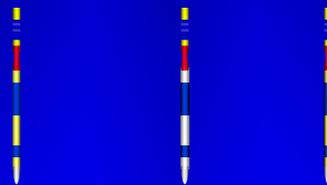
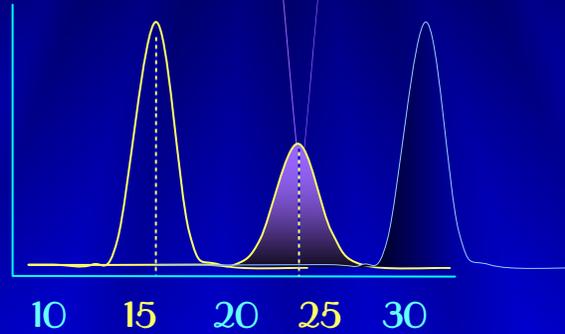
Trisomie



Clone Trisomie



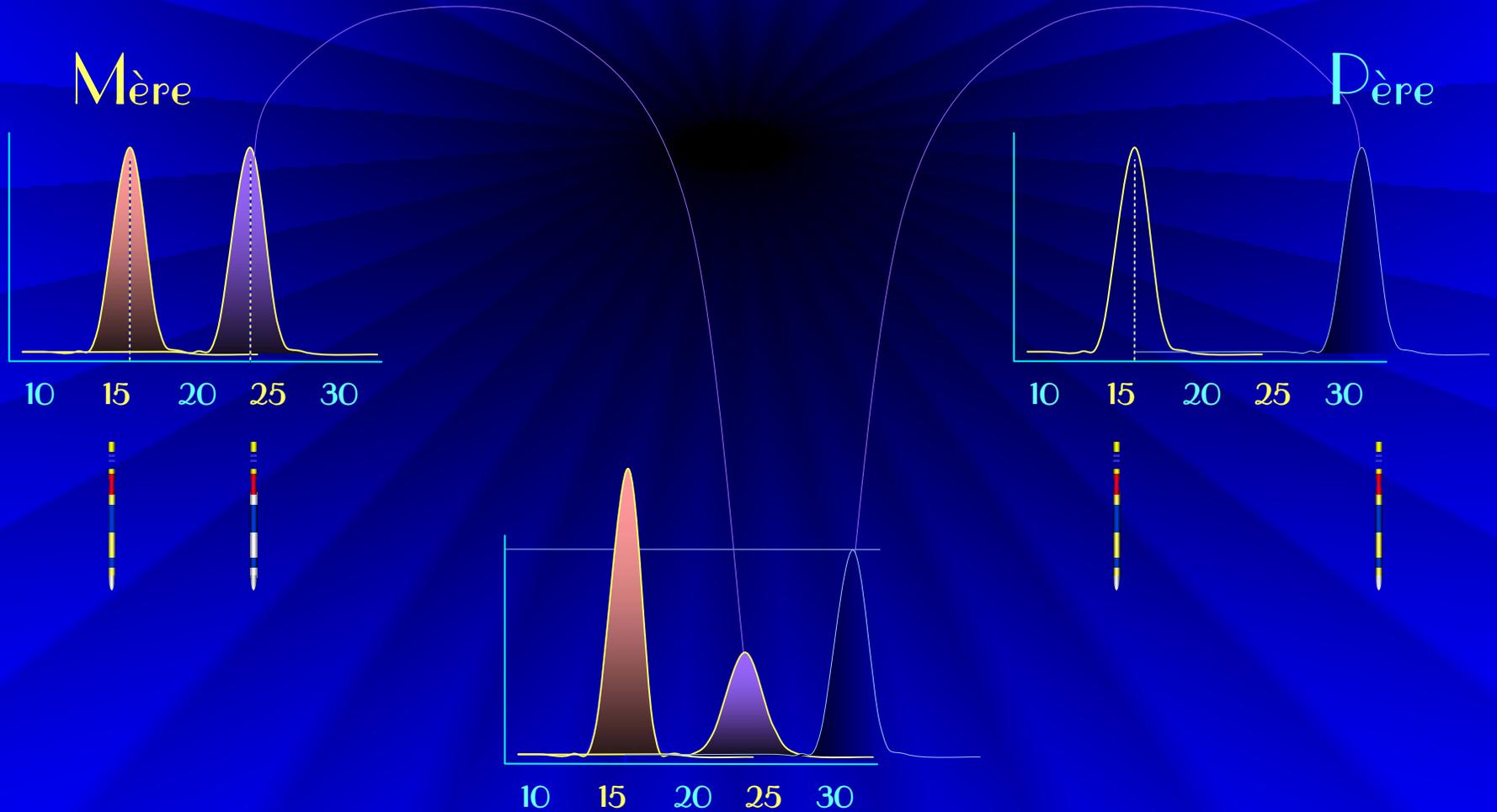
Mosaïcisme



Marqueurs d'un seul chromosome



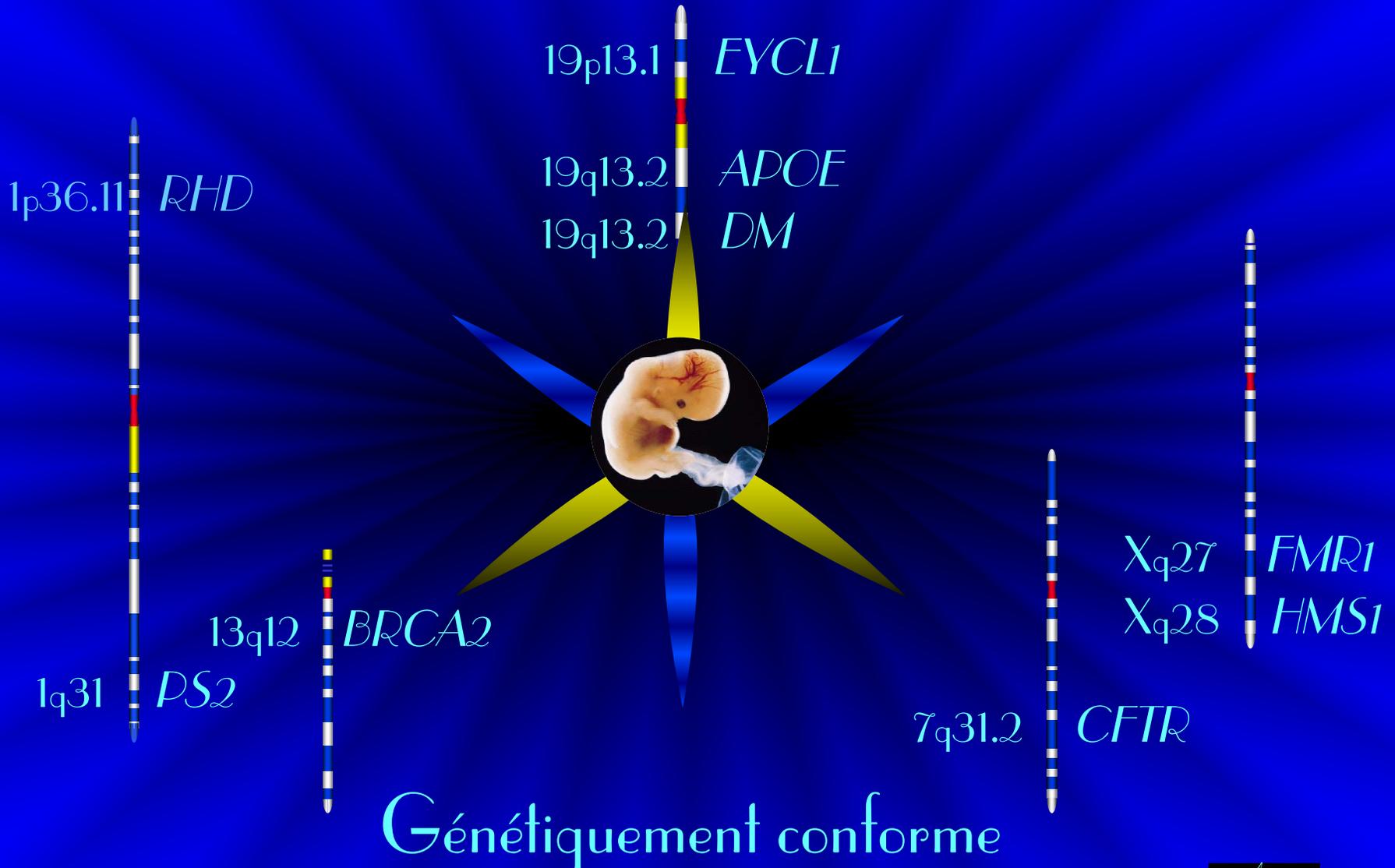
Contamination



Marqueurs de tous les chromosomes



Développements ou escalade ?



Génétiquement conforme



Dépistage ou diagnostic ?



Nouveautés : résumé

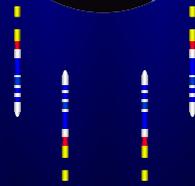
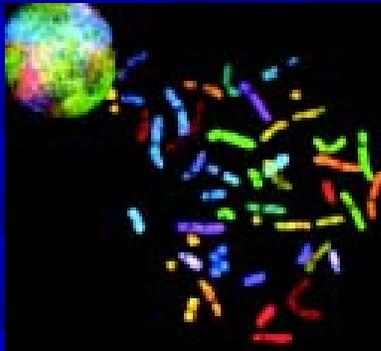
ELSI

Techniques

Cliniques

Hybridations ADN

Dépistages



Art. 24 octies
Art. 24 novies
Const. Nouveau

FISH, SKY, CGH
QF-PCR

Age, AFP, What-if
PAPP-A, CN



