Program of Medical Genetics for Medicine students (SUN 2013-2014)

Prof. Sandro Banfi – Vincenzo Nigro

1. • The human genome: genes and organization
2. • Next generation sequencing (NGS), the exome
3. • Penetrance and expressivity, anticipation
4. • Homozygosity and compound heterozygosity
5. • Haploinsufficiency, congenital and syndromic disorders
6. • Mechanism of splicing and its alterations
7. • Classes of point mutations, transition and transversion, conservative, missense, nonsense, nonstop
8. • Insertions, deletions and frame-shift and non-duplication, gene conversion
9. • Pathological significance of the various classes of DNA variations
10. • International Nomenclature of genetic variation and significance of reporting
11. • Genetic counseling: reproductive risk
12. • Prenatal diagnosis and presymptomatic testing
13. • Karyotype analysis, the FISH
14. • Molecular karyotyping by arrayCGH

Program of Medical Genetics for Medicine students
part 2

15. Aneuploidy in abortions and risk of recurrence
16. Triploidy, tetraploidy
17. The X chromosome inactivation and PAR
18. Autosomal trisomies
19. Sex chromosome trisomies
20. Monosomies, Turner syndrome
21. Chromosomal deletions, paracentric and pericentric inversions
22. Balanced and unbalanced translocations, Robertsonian, chromosomal markers
23. Submicroscopic deletions and duplications (Williams s., diGeorge, Cri du Chat, Smith-Magenis)
24. Monoallelic Mendelian disorders with de novo mutations (craniosynostosis, achondroplasia)
25. Autosomal dominant Mendelian disorders (neurofibromatosis, Marfan, polycystic kidney disease)
26. X-Linked disorders (Duchenne and Becker muscular dystrophy, hemophilia, X-linked mental retardation, Rett syndrome)

Program of Medical Genetics for Medicine students
part 3

27. Autosomal recessive disorder (cystic fibrosis, alpha and beta thalassemia, spinal muscular atrophy, hemochromatosis, glycogen storage disorders, lysosomal storage disorders)
28. Mendelian disorders with genetic heterogeneity (limb-girdle muscular dystrophies, retinitis pigmentosa)
29. Dynamic mutations in non-coding regions (fragile X, myotonic dystrophy) and coding regions (Huntington's disease, spinocerebellar ataxias)
30. Mutations in chromosomal regions with imprinting (Prader-Willi syndrome, Angelman syndrome, Beckwith-Wiedemann syndrome, Silver-Russell), uniparental disomy
31. Mutations of mitochondrial DNA (MERFF, MELAS, LHON, KS, Leigh syndrome)
32. Genetic predisposition
33. Multifactorial traits
34. GWAS studies
35. microRNA function and role in genetic diseases
36. General principles of advanced therapies for genetic diseases

REFERENCE TEXTBOOKS:

-Tom Strachan, Andrew Read. Human Molecular Genetics, 4th edition. Garland Science

-Thompson and Thompson Genetics in Medicine. Saunders 7th edition

ORAL EXAM

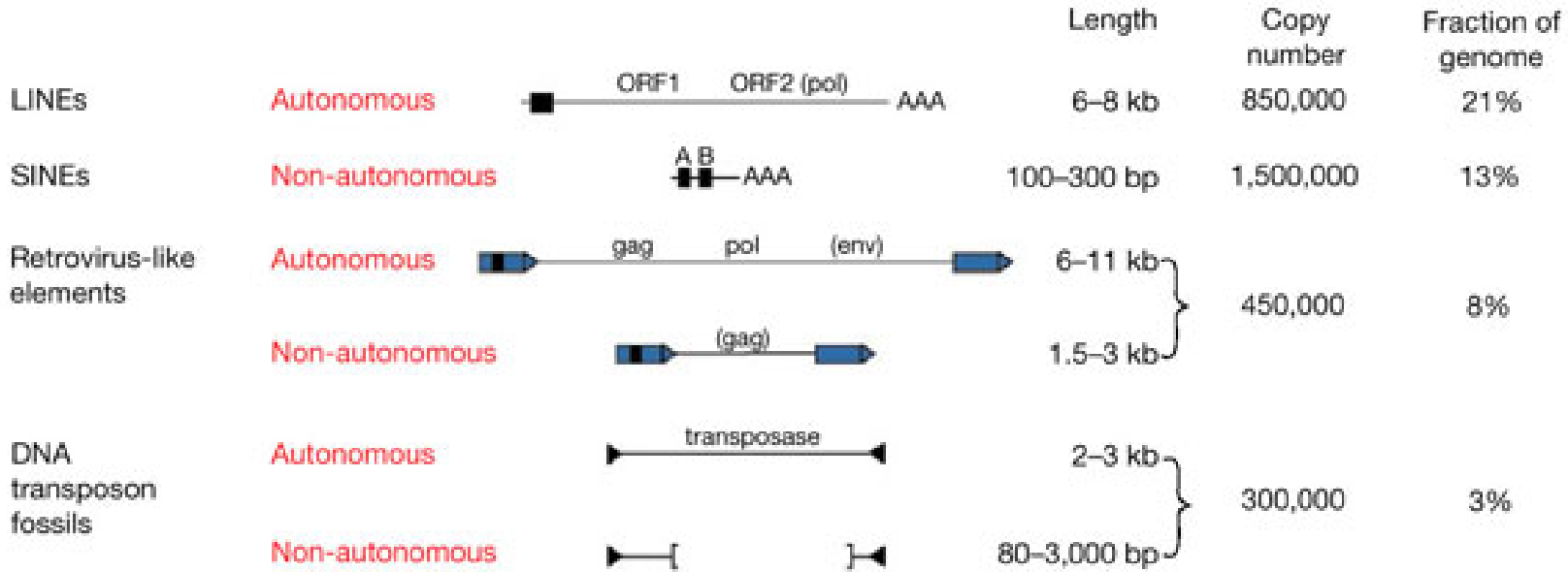
Genome

1953

- The first Polymerase chain reaction is assembled in 1985
- The Human Genome Project starts in 1985
- the entire sequencing of the human genome was completed in 2001 by two competing groups

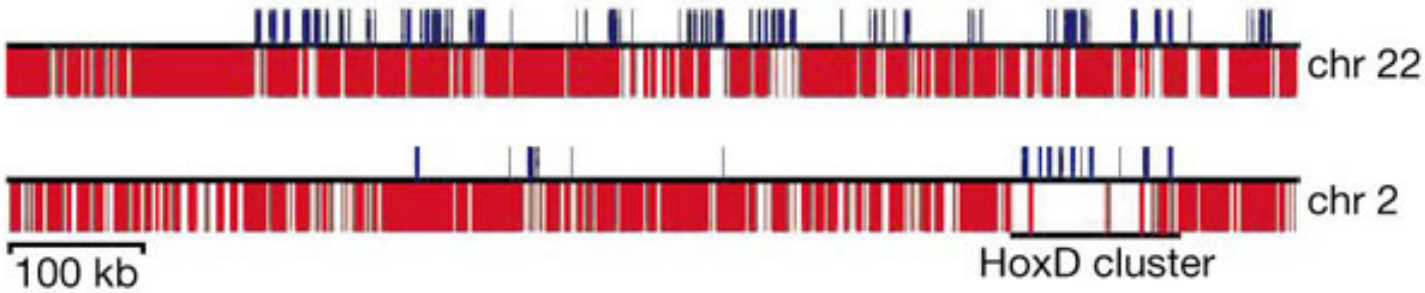
2001

Classes of interspersed repeat in the human genome

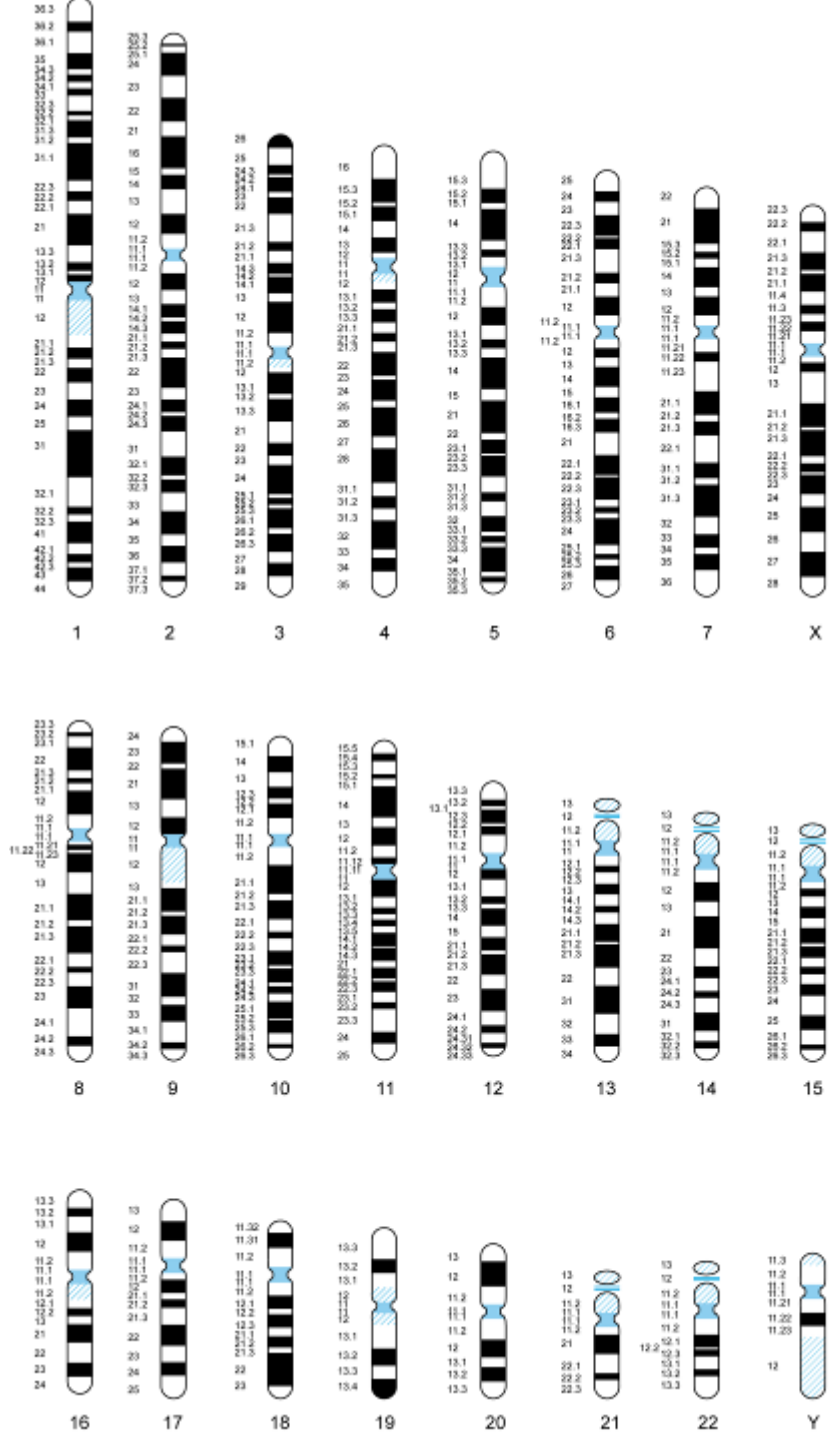


Almost all transposable elements fall into one of four types:

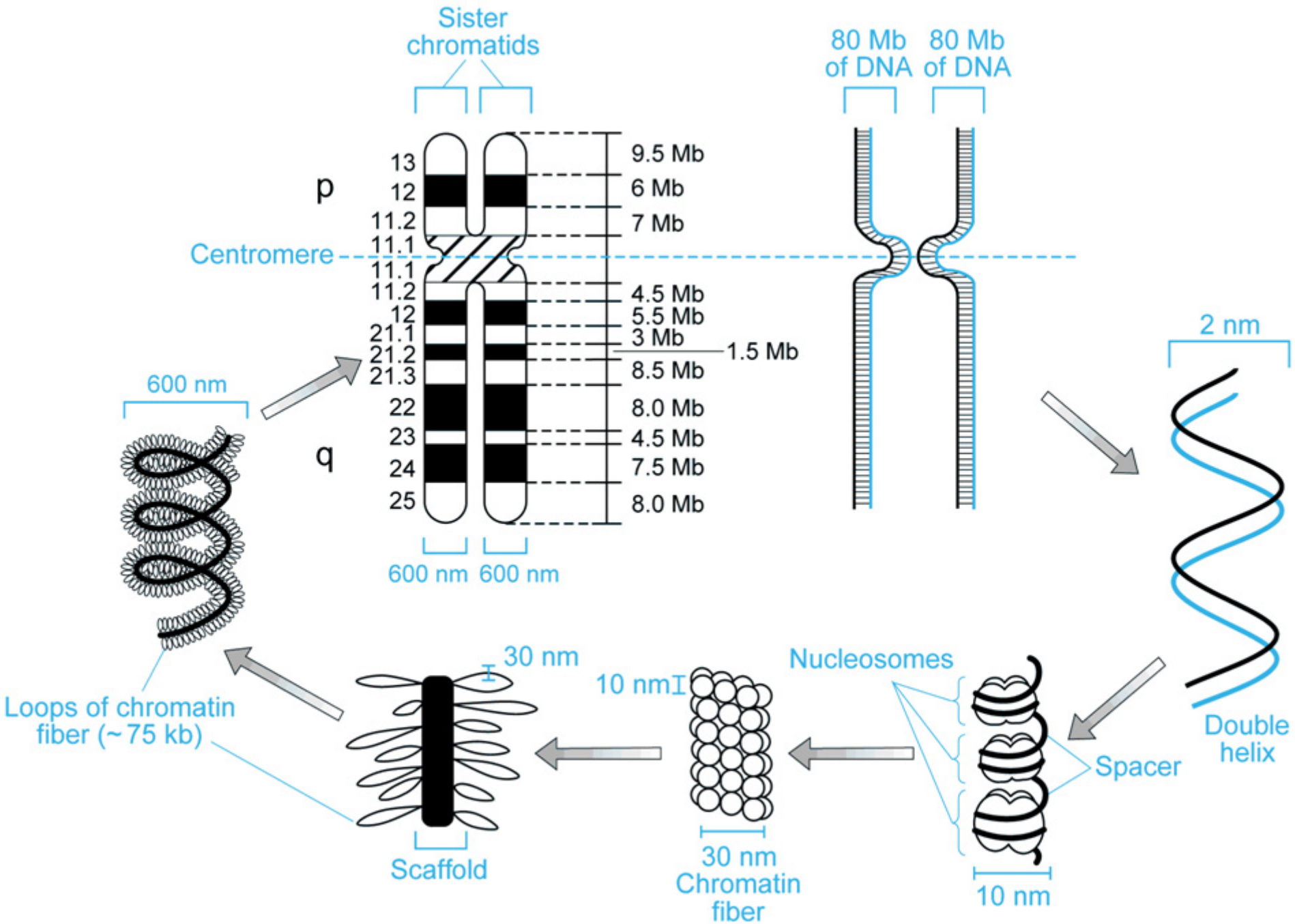
- Long interspersed elements (LINEs)
- Short interspersed elements (SINEs)
- LTR retrotrasposons
- DNA trasposons



Red bar = interspersed repeats, blue = exons

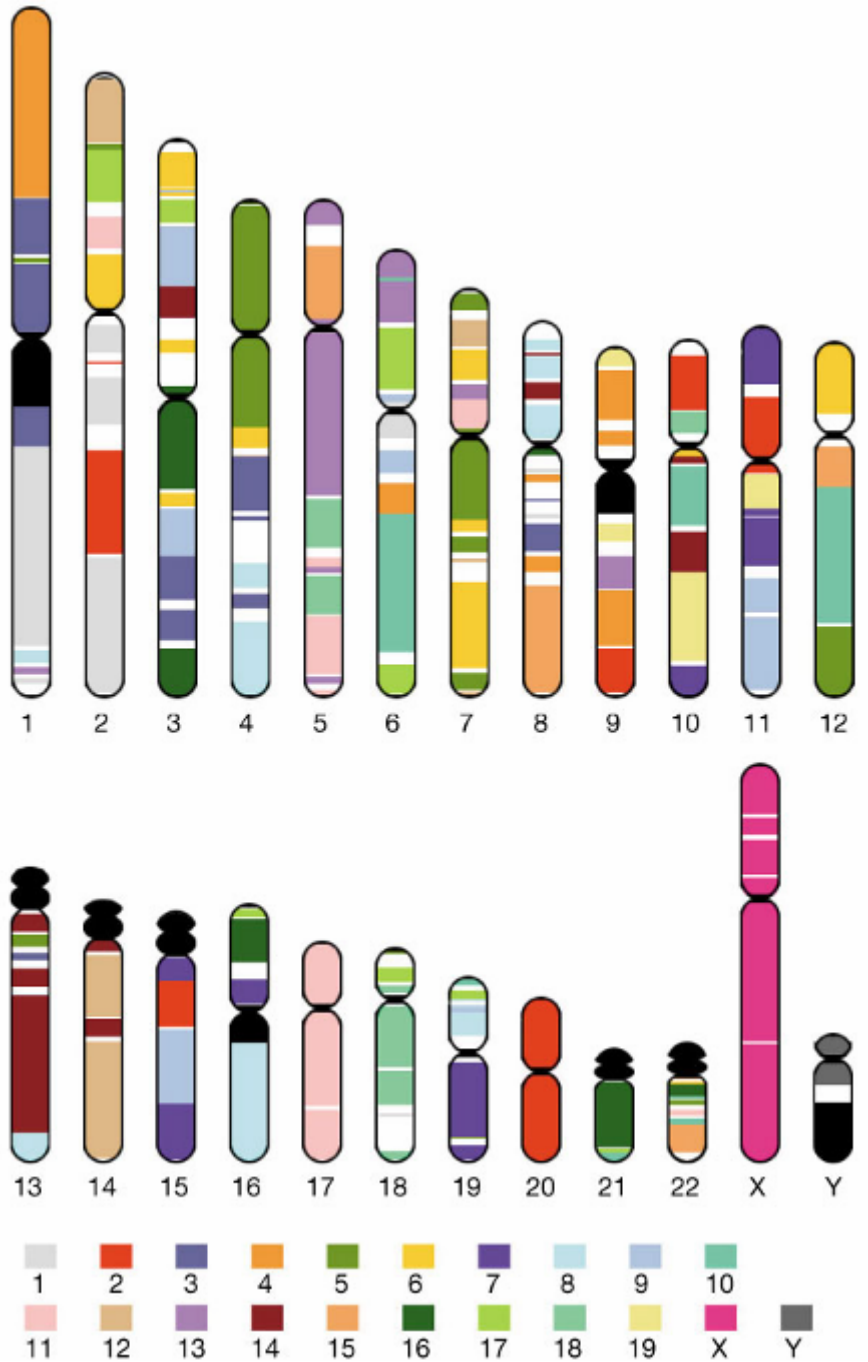


1	245,203,898	218,712,898
2	243,315,028	237,043,673
3	199,411,731	193,607,218
4	191,610,523	186,580,523
5	180,967,295	177,524,972
6	170,740,541	166,880,540
7	158,431,299	154,546,299
8	145,908,738	141,694,337
9	134,505,819	115,187,714
10	135,480,874	130,710,865
11	134,978,784	130,709,420
12	133,464,434	129,328,332
13	114,151,656	95,511,656
14	105,311,216	87,191,216
15	100,114,055	81,117,055
16	89,995,999	79,890,791
17	81,691,216	77,480,855
18	77,753,510	74,534,531
19	63,790,860	55,780,860
20	63,644,868	59,424,990
21	46,976,537	33,924,742
22	49,476,972	34,352,051
X	152,634,166	147,686,664
Y	50,961,097	22,761,097

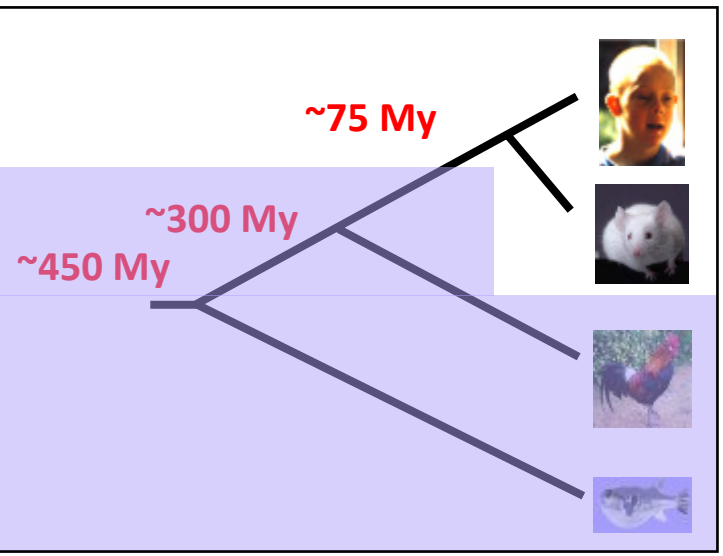

Sister chromatids
80 Mb of DNA
80 Mb of DNA
p
q
13
12
11.2
11.1
11.1
11.2
12
21.1
21.2
21.3
22
23
24
25
Centromere
9.5 Mb
6 Mb
7 Mb
4.5 Mb
5.5 Mb
3 Mb
1.5 Mb
8.5 Mb
8.0 Mb
4.5 Mb
7.5 Mb
8.0 Mb
600 nm
600 nm
600 nm
2 nm
Double helix
Spacer
Nucleosomes
10 nm
30 nm
Chromatin fiber
30 nm
Scaffold
Loops of chromatin fiber (~75 kb)

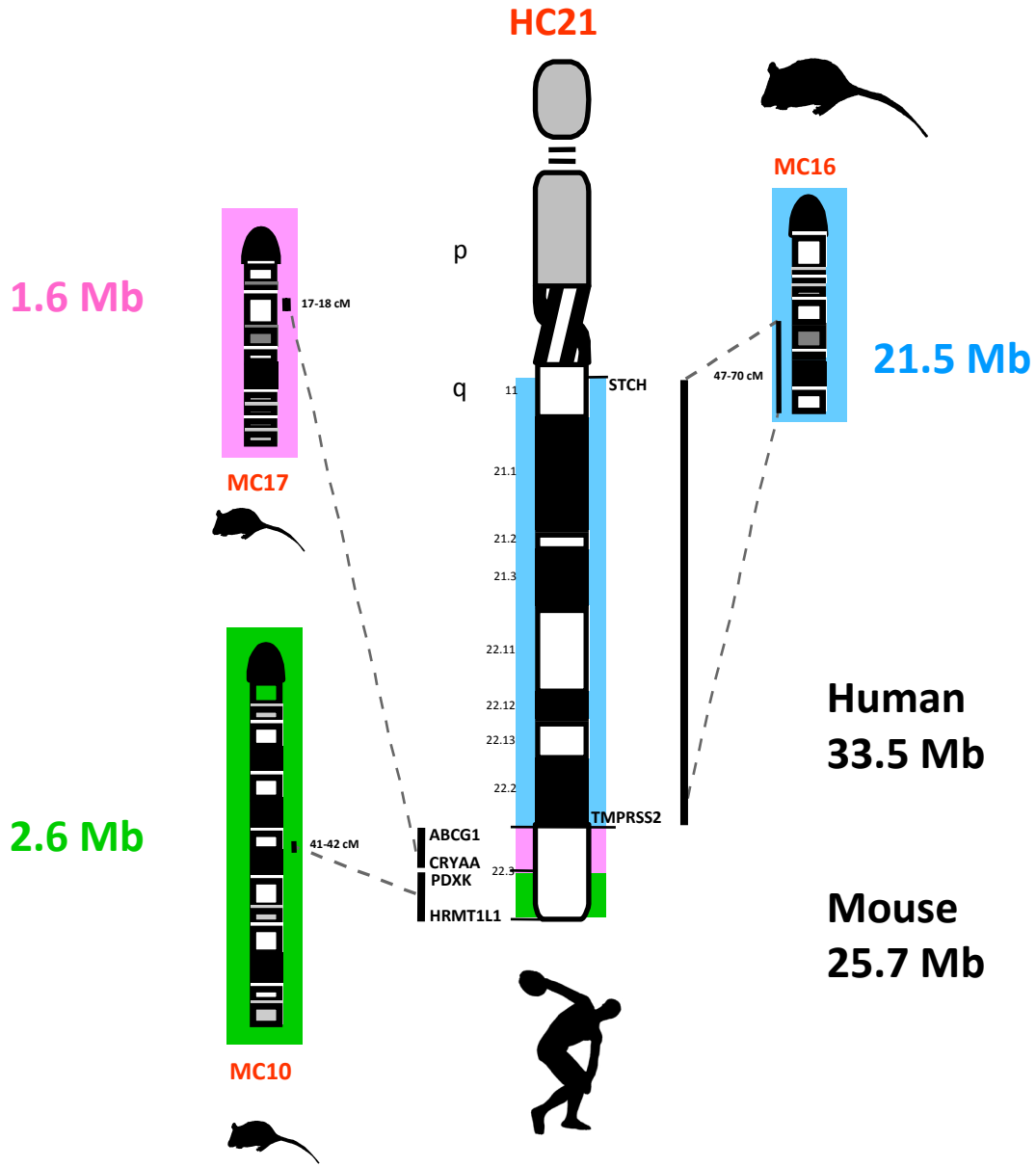
conserved segments in the human and mouse genome

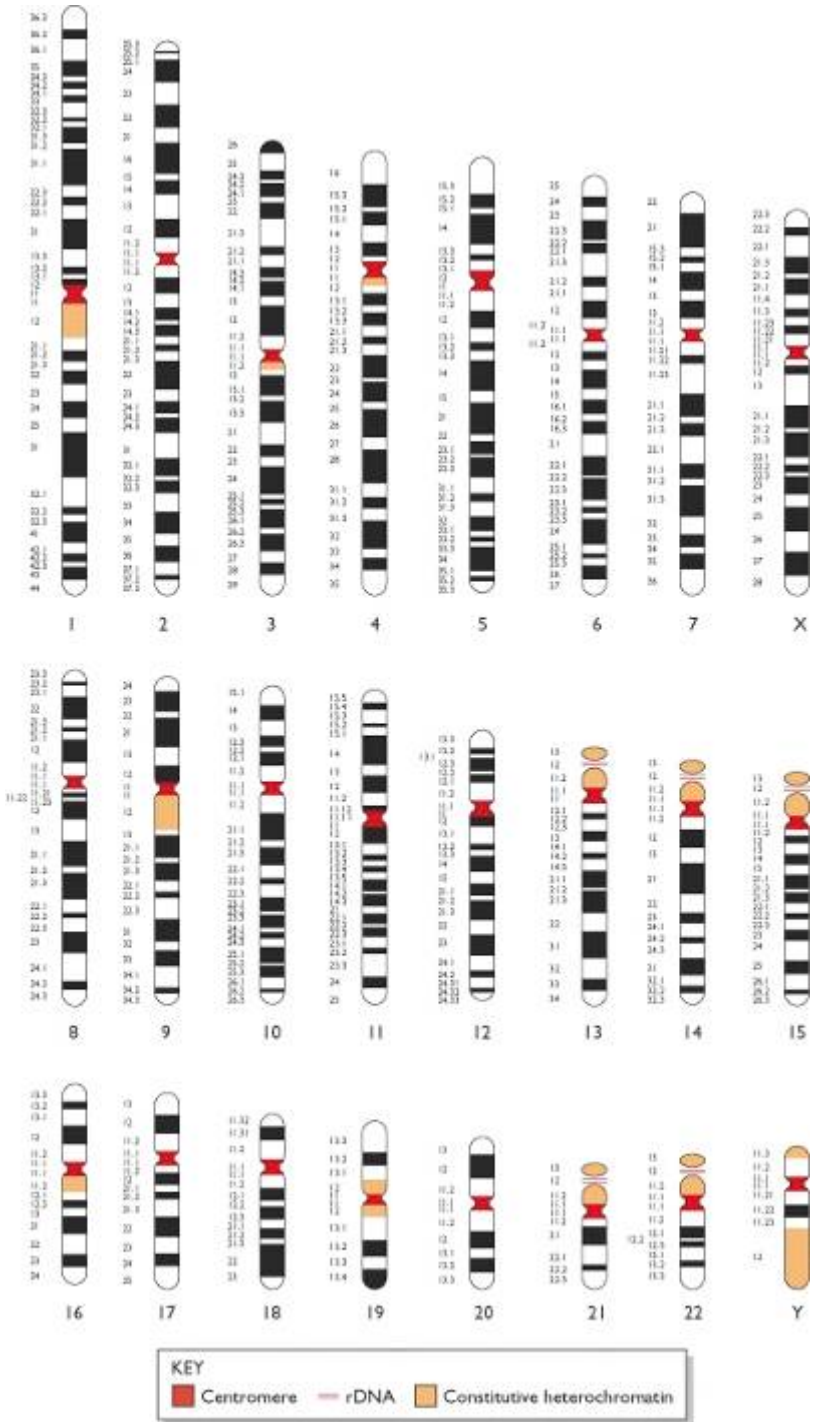
the human chromosome have the color of the corresponding regions in mouse chromosomes



Sequence Comparisons Human Chromosome 21 and its Mouse Homologues

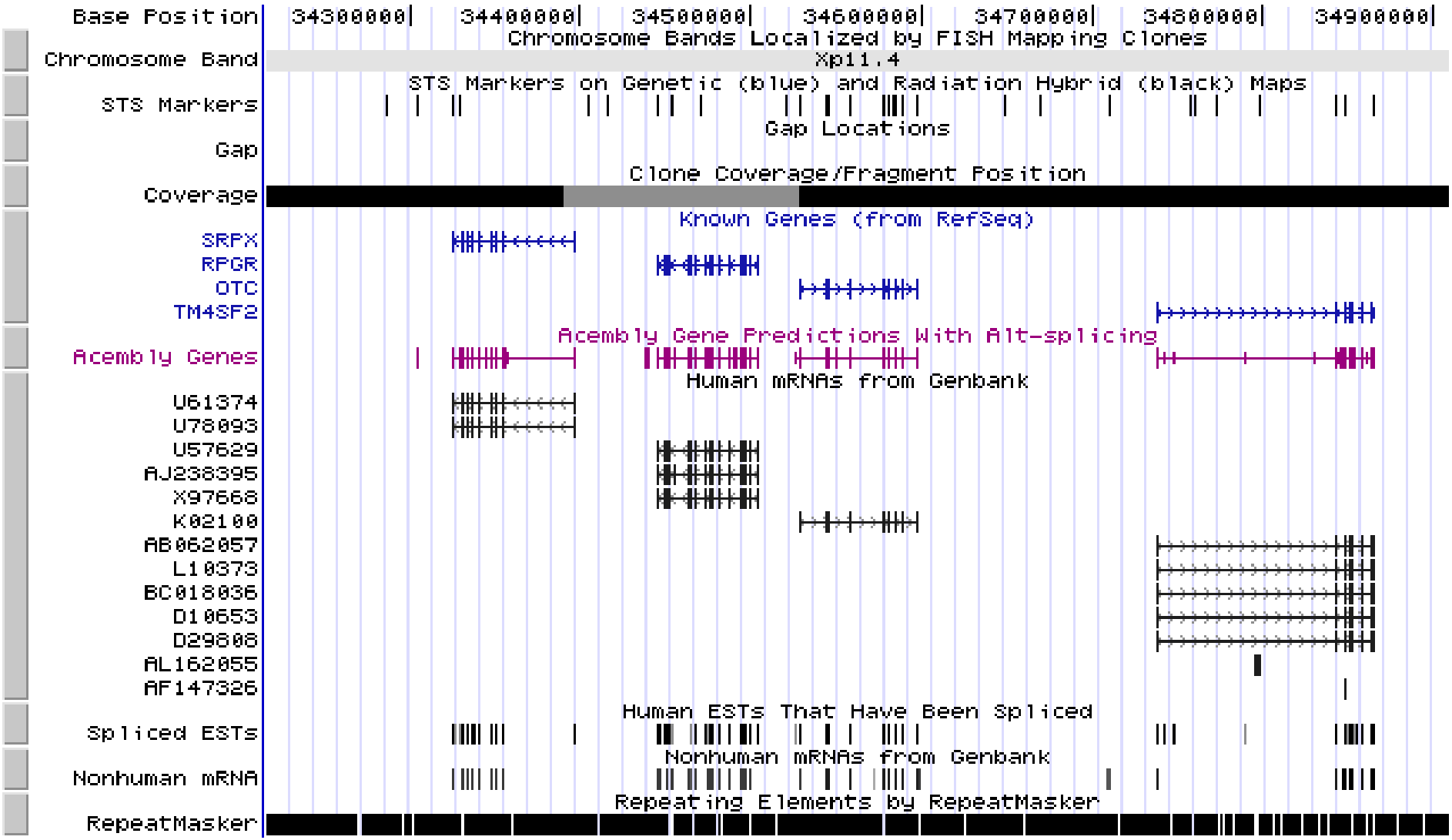






CCDS IDs per chromosome	
Chromosome	**Count**
1	2,513
2	1,548
3	1,299
4	898
5	1,028
6	1,236
7	1,094
8	807
9	921
10	971
11	1,509
12	1,240
13	385
14	749
15	711
16	967
17	1,370
18	350
19	1,616
20	672
21	282
22	530
X	967
Y	53
XY	23

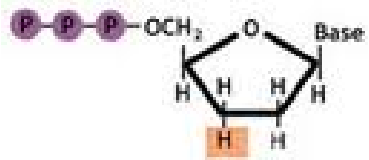
UCSC Genome Browser



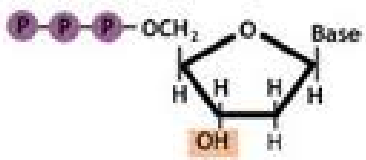
Screenshot from University of California at Santa Cruz http://genome.ucsc.edu

History and present: Sanger sequencing

Frederick Sanger
Nobel price 1958 and 1980
born August 13 1918, died November 19 2013



dideoxynucleotide (ddNTP)

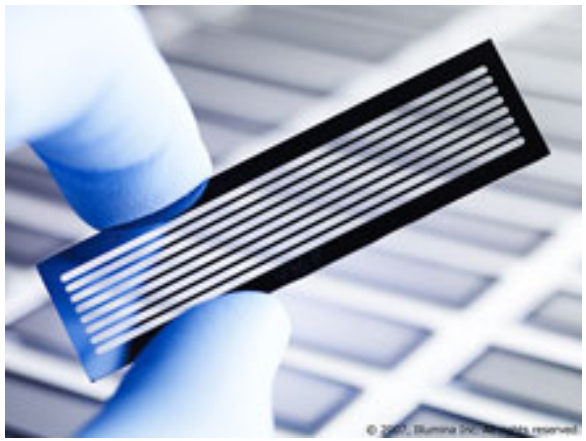


deoxynucleotide (dNTP)

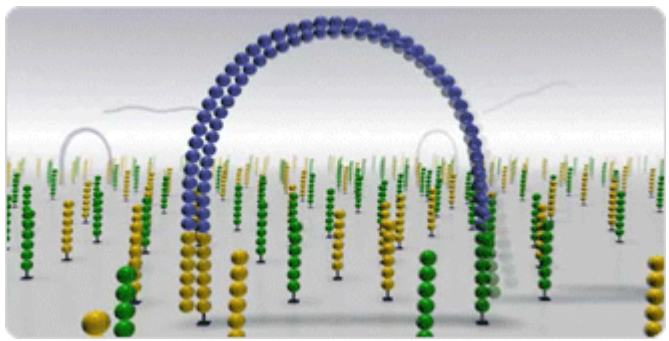
ddNTPs (ddATP, ddTTP, ddGTP, ddCTP) are terminators : they block the polymerization of DNA when inserted

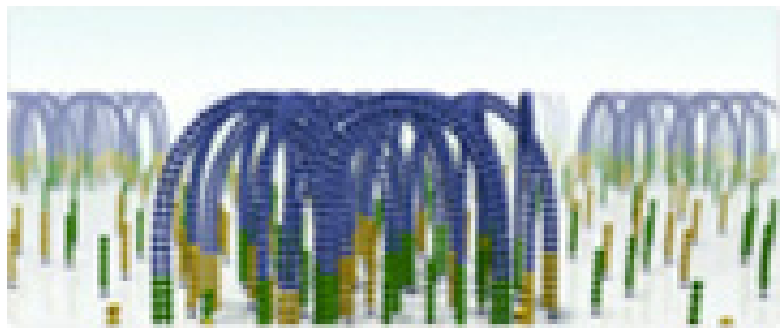
flow cell

Solid-phase amplification generates up to 2,000 M of clusters (Illumina HiSeq)



flow cell





Bridge PCR

illumina®

Whole Genome Seq

The NEW ENGLAND JOURNAL of MEDICINE

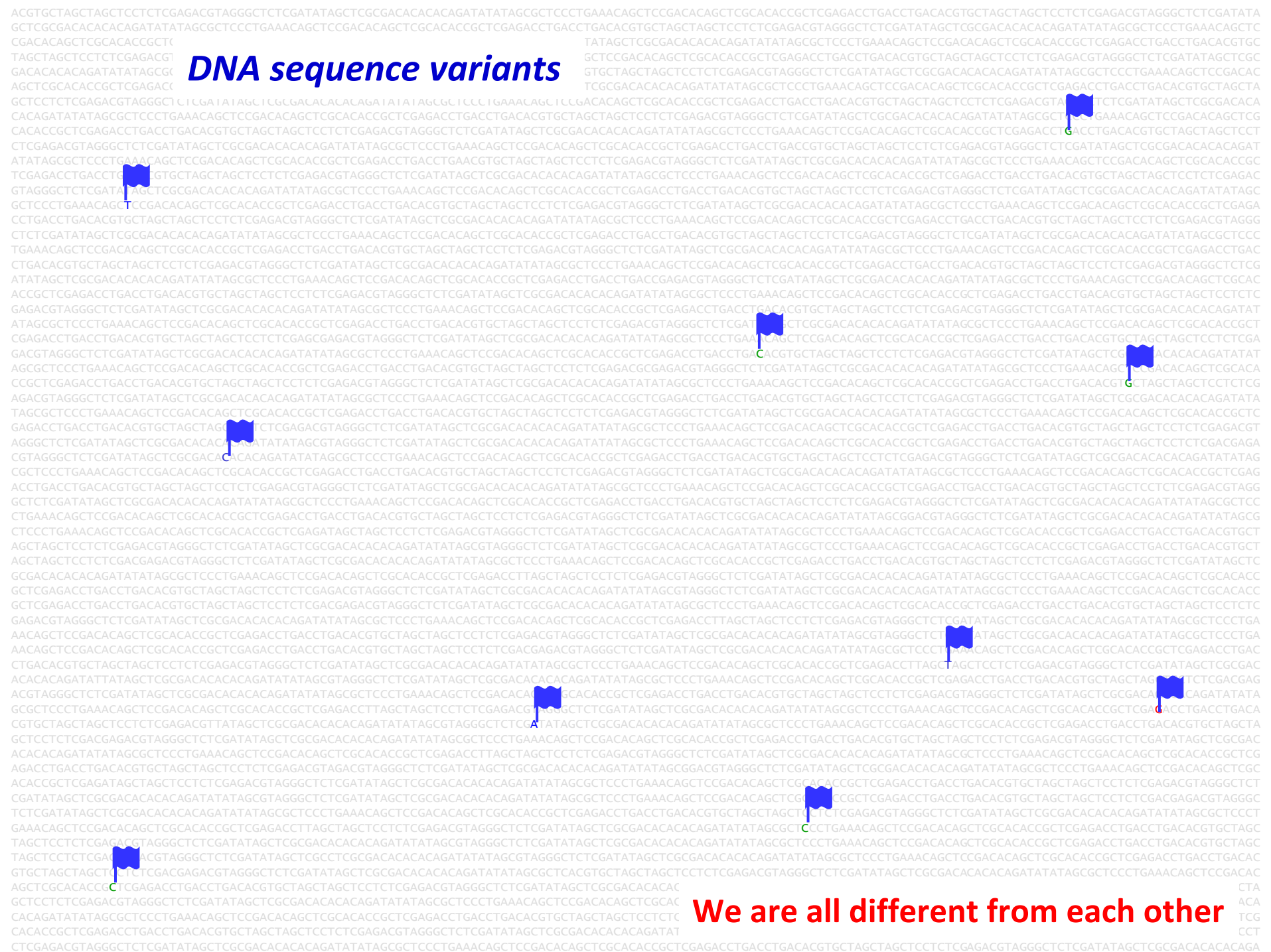
ORIGINAL ARTICLE

Whole-Genome Sequencing in a Patient with Charcot–Marie–Tooth Neuropathy

James R. Lupski, M.D., Ph.D., Jeffrey G. Reid, Ph.D., Claudia Gonzaga-Jauregui, B.S., David Rio Deiros, B.S., David C.Y. Chen, M.Sc., Lynne Nazareth, Ph.D., Matthew Bainbridge, M.Sc., Huyen Dinh, B.S., Chyn Jing, M.Sc., David A. Wheeler, Ph.D., Amy L. McGuire, J.D., Ph.D., Feng Zhang, Ph.D., Pawel Stankiewicz, M.D., Ph.D., John J. Halperin, M.D., Chengyong Yang, Ph.D., Curtis Gehman, Ph.D., Danwei Guo, M.Sc., Rola K. Irikat, B.S., Warren Tom, B.S., Nick J. Fantin, B.S., Donna M. Muzny, M.Sc., and Richard A. Gibbs, Ph.D.

ABSTRACT

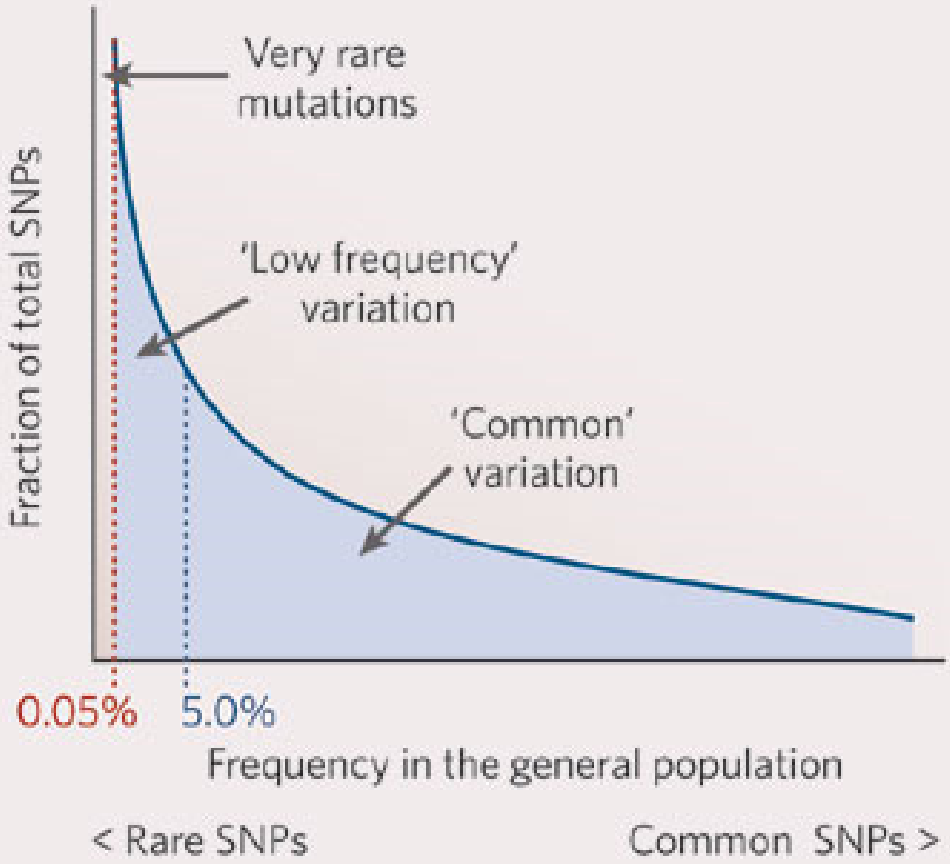
DNA sequence variants



We are all different from each other

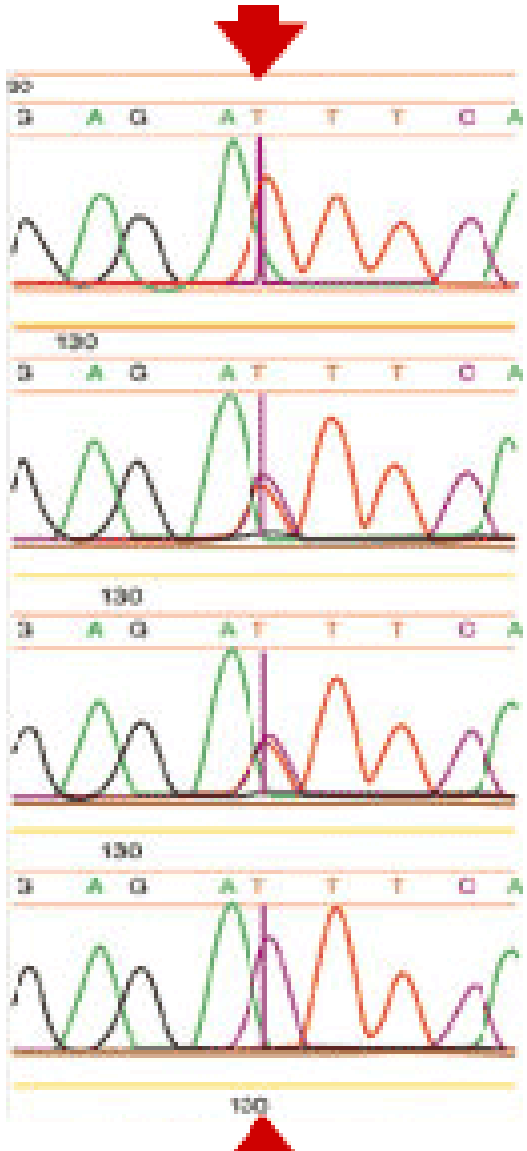
GENETIC VARIATION IN HUMANS

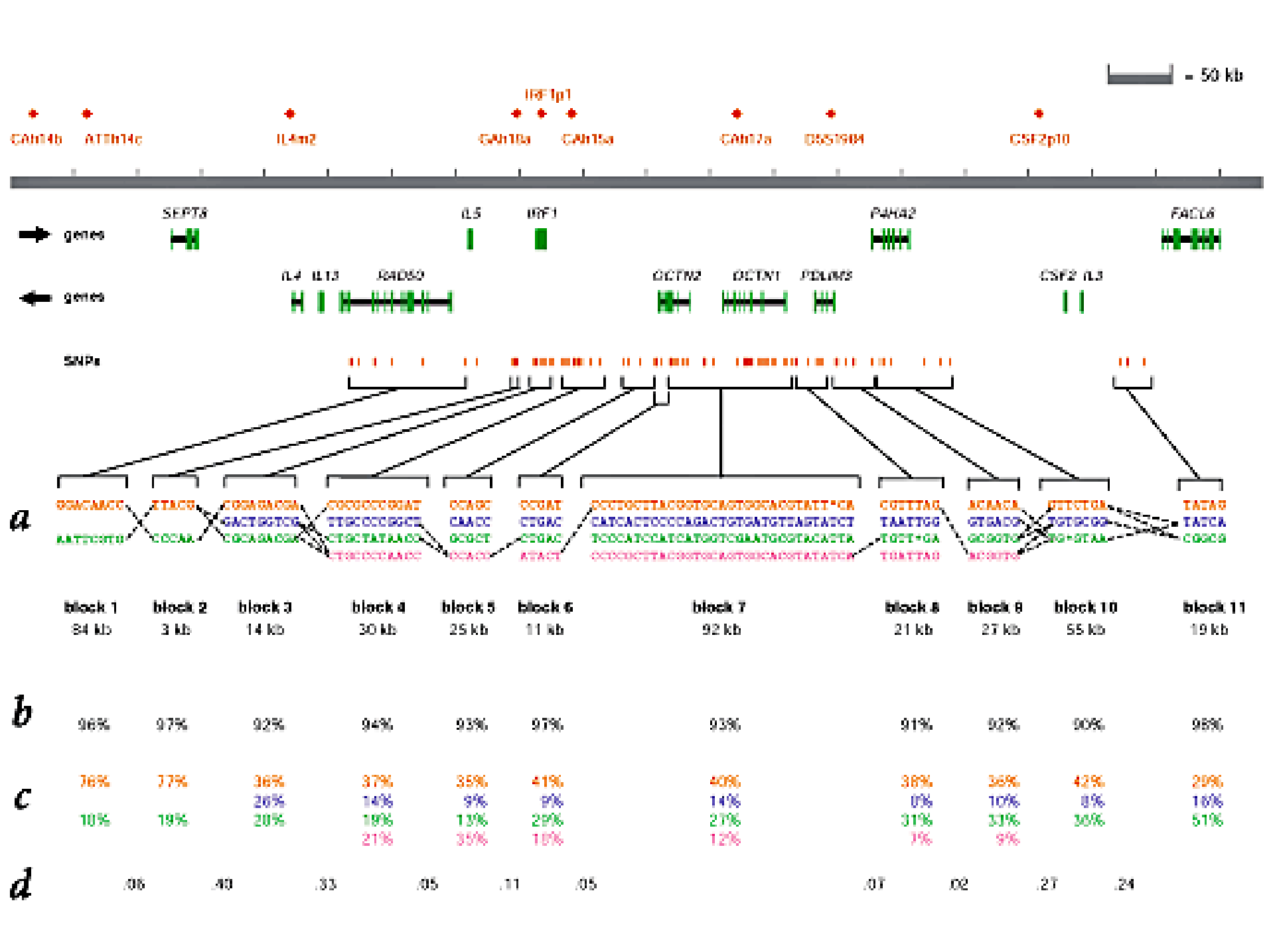
Variation is measured by
single nucleotide polymorphisms (SNPs).



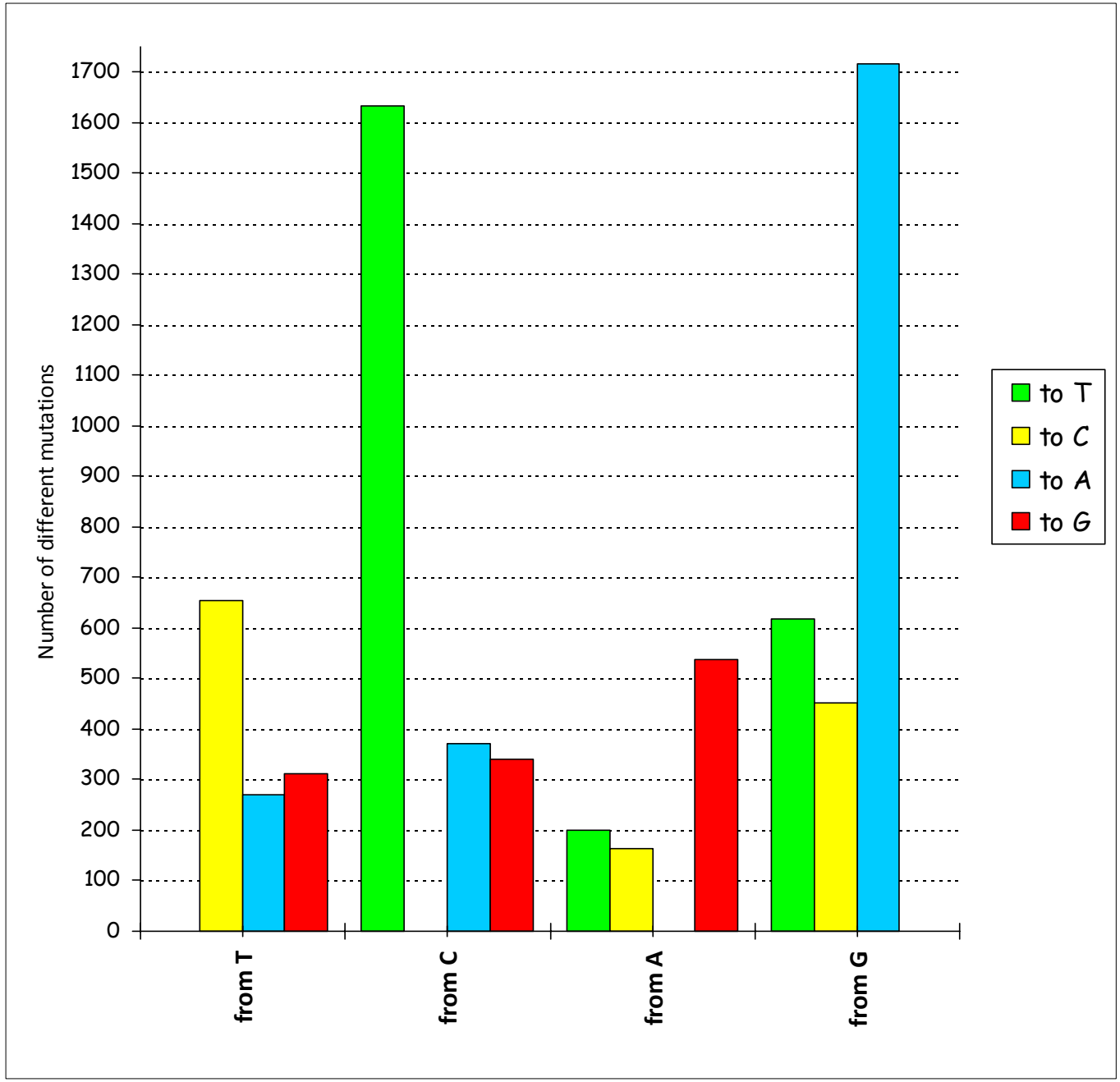
SNPs
single nucleotide polymorphisms

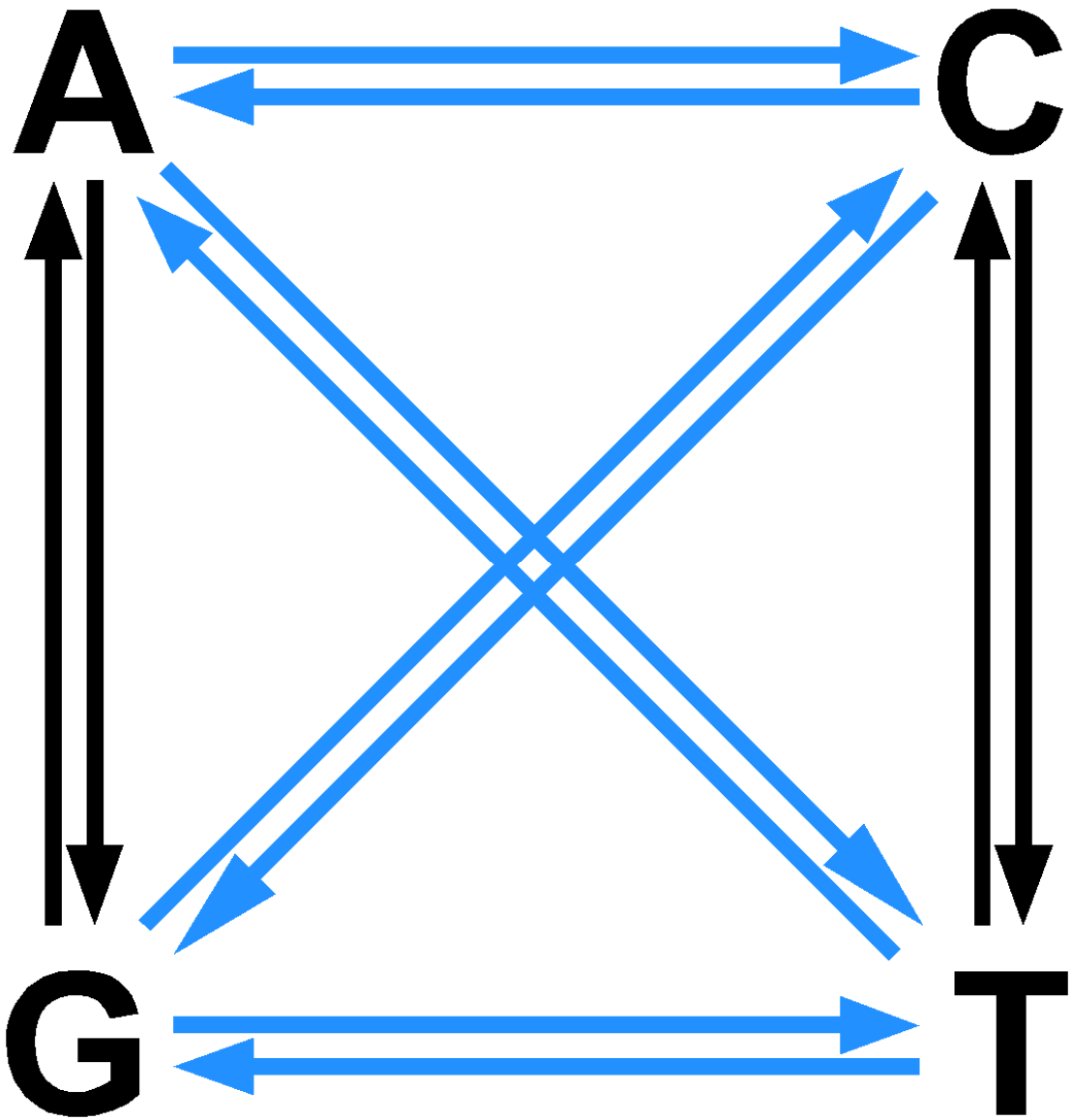
- Natural sequence variation occurs between any two copies of the human genome.
- Most variations are SNPs involving single base substitutions — the rest are insertions or deletions
- A SNP is detected by sequencing a particular region from different individuals, who may have identical (homozygous; T/T or C/C) or different (heterozygous; T/C) bases at the polymorphic site




= 50 kb
IRF1p1
CAh14b
ATTh14c
IL4m2
CAh15a
CAh17a
CSF2p10
genes
SEPT8
IL5
IRF1
P4HA2
FACL6
genes
IL4
IL13
RAD50
OCTN2
OCTN1
PDLIM3
CSF2
IL3
SNPs
a
block 1
84 kb
block 2
3 kb
block 3
14 kb
block 4
30 kb
block 5
25 kb
block 6
11 kb
block 7
92 kb
block 8
21 kb
block 9
27 kb
block 10
55 kb
block 11
19 kb
b
96%
97%
92%
94%
93%
97%
93%
91%
92%
90%
96%
c
36%
77%
36%
37%
35%
41%
40%
38%
36%
42%
29%
26%
14%
9%
9%
14%
0%
10%
8%
16%
10%
19%
20%
19%
13%
29%
21%
31%
33%
36%
51%
21%
35%
18%
12%
7%
9%
d
.06
.40
.33
.05
.11
.05
.07
.02
.27
.24

Single nucleotide substitutions

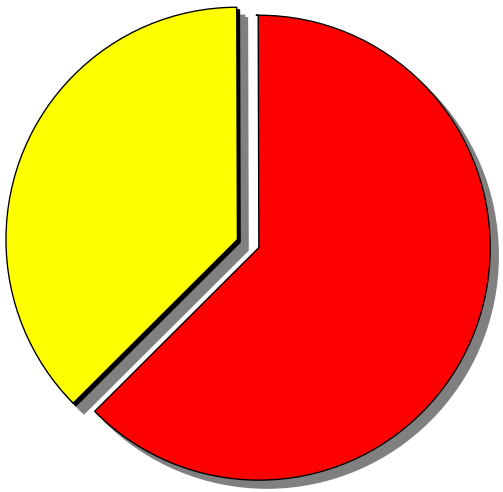



A
C
G
T

Mutations observed

T>A or G , C>G or A
G>T or C , A>T or G

transversions



transitions

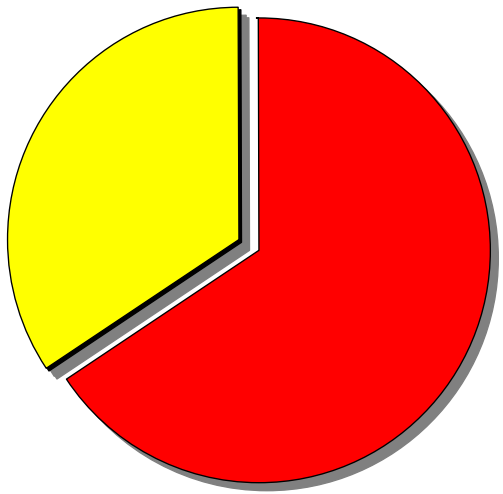
T>C, C>T, G>A, A>G

46,000

SNPs

T/A, C/G
T/G, C/A

transversions



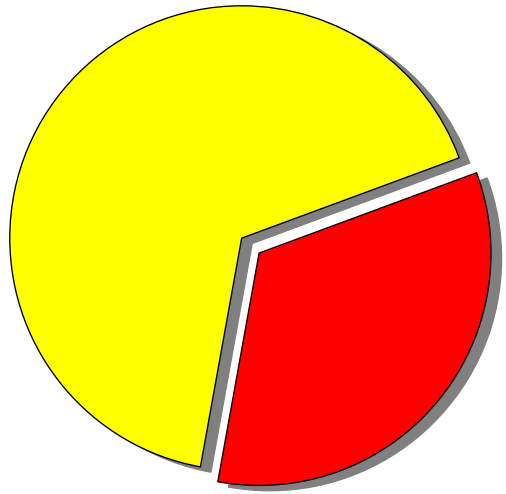
transitions

T/C, A/G

12,000,000

Expected

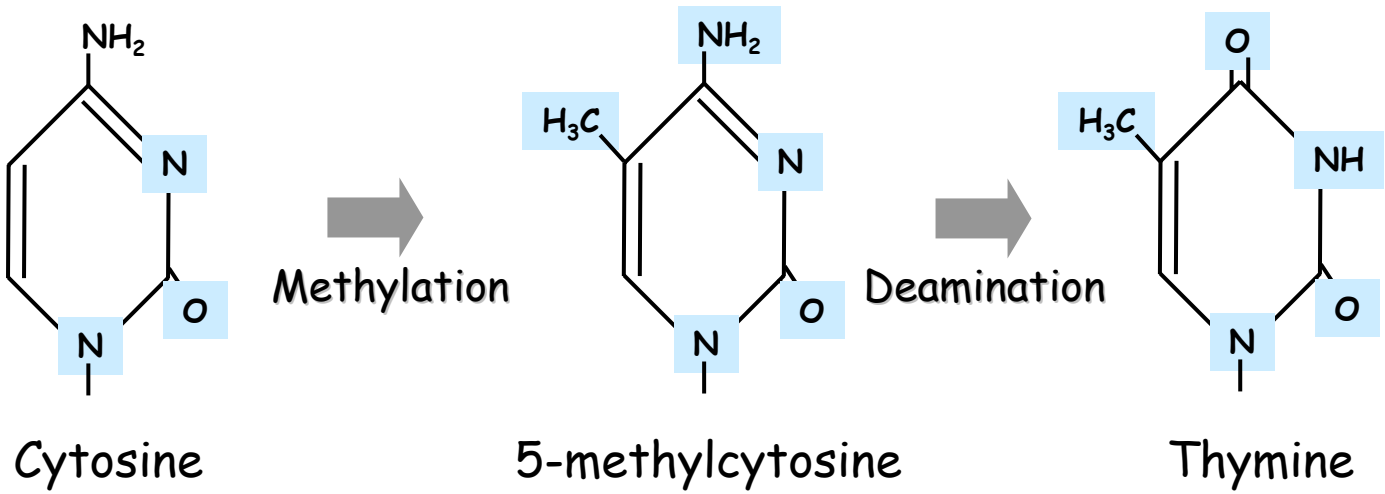
transversions



transitions

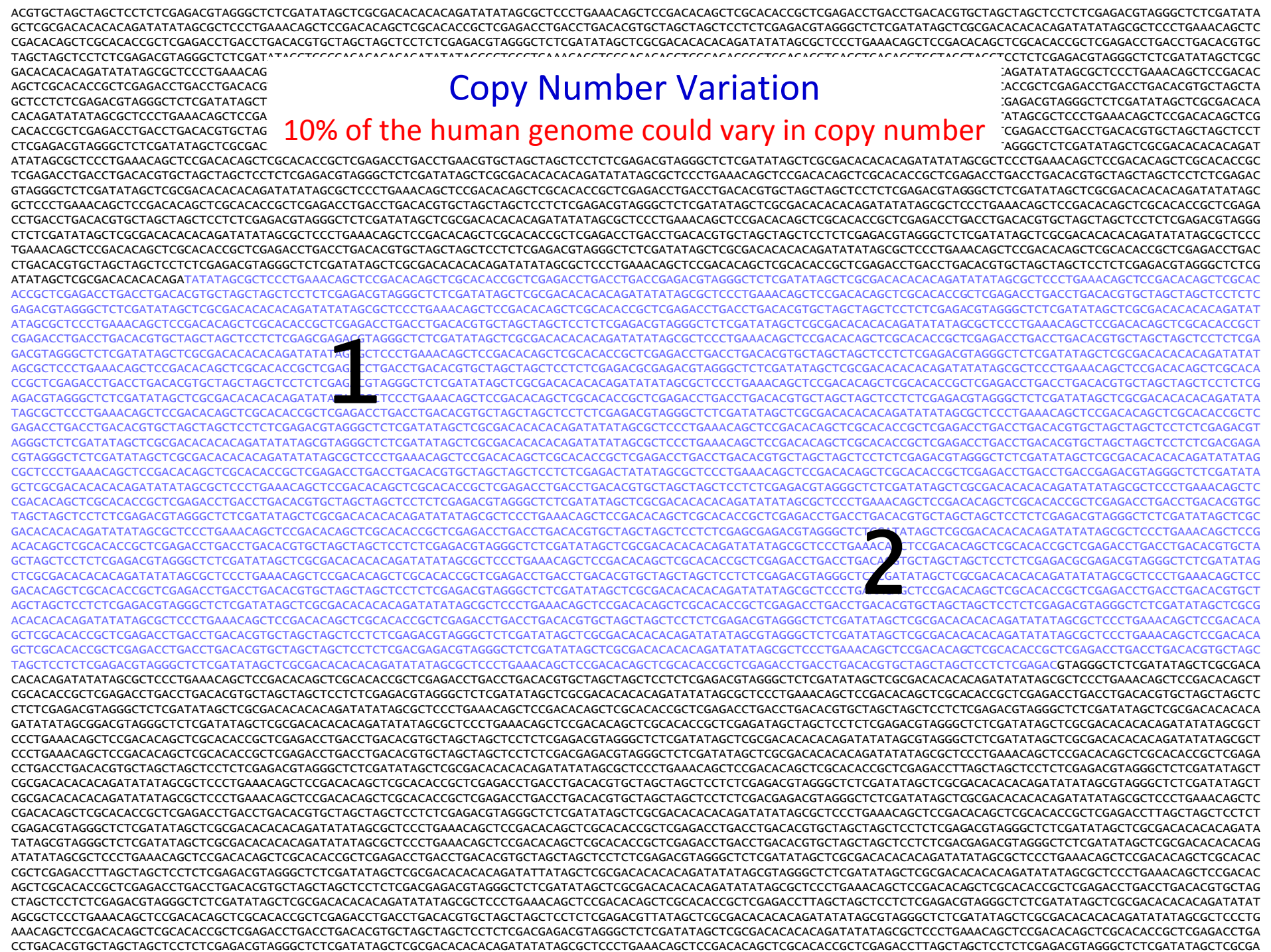
SEA 3057

the most common mutation mechanism



CG ⇒ TG

CG ⇒ CA


Copy Number Variation
10% of the human genome could vary in copy number
1
2

Genome Variation

SSR

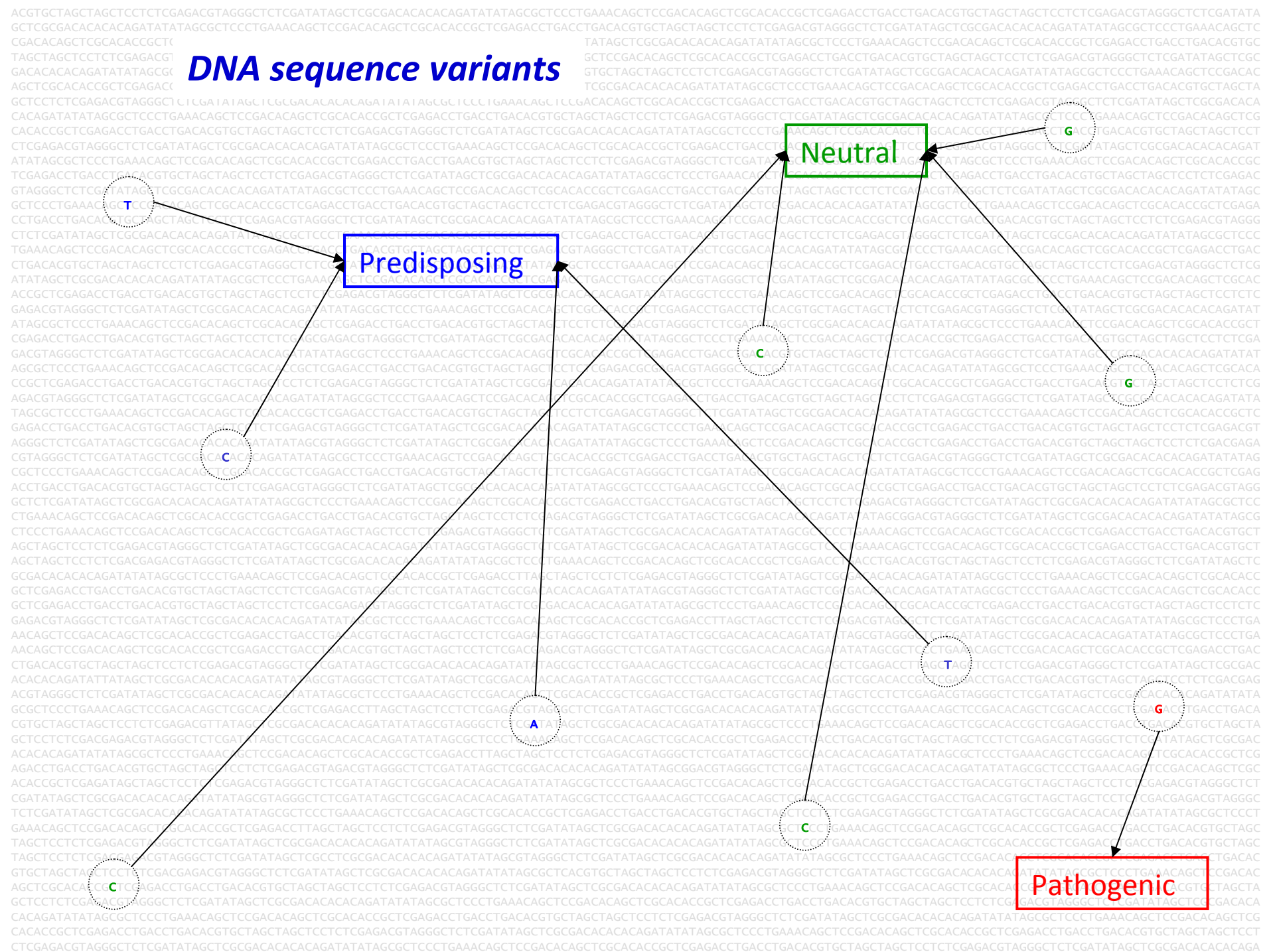
Short Sequence Repeats

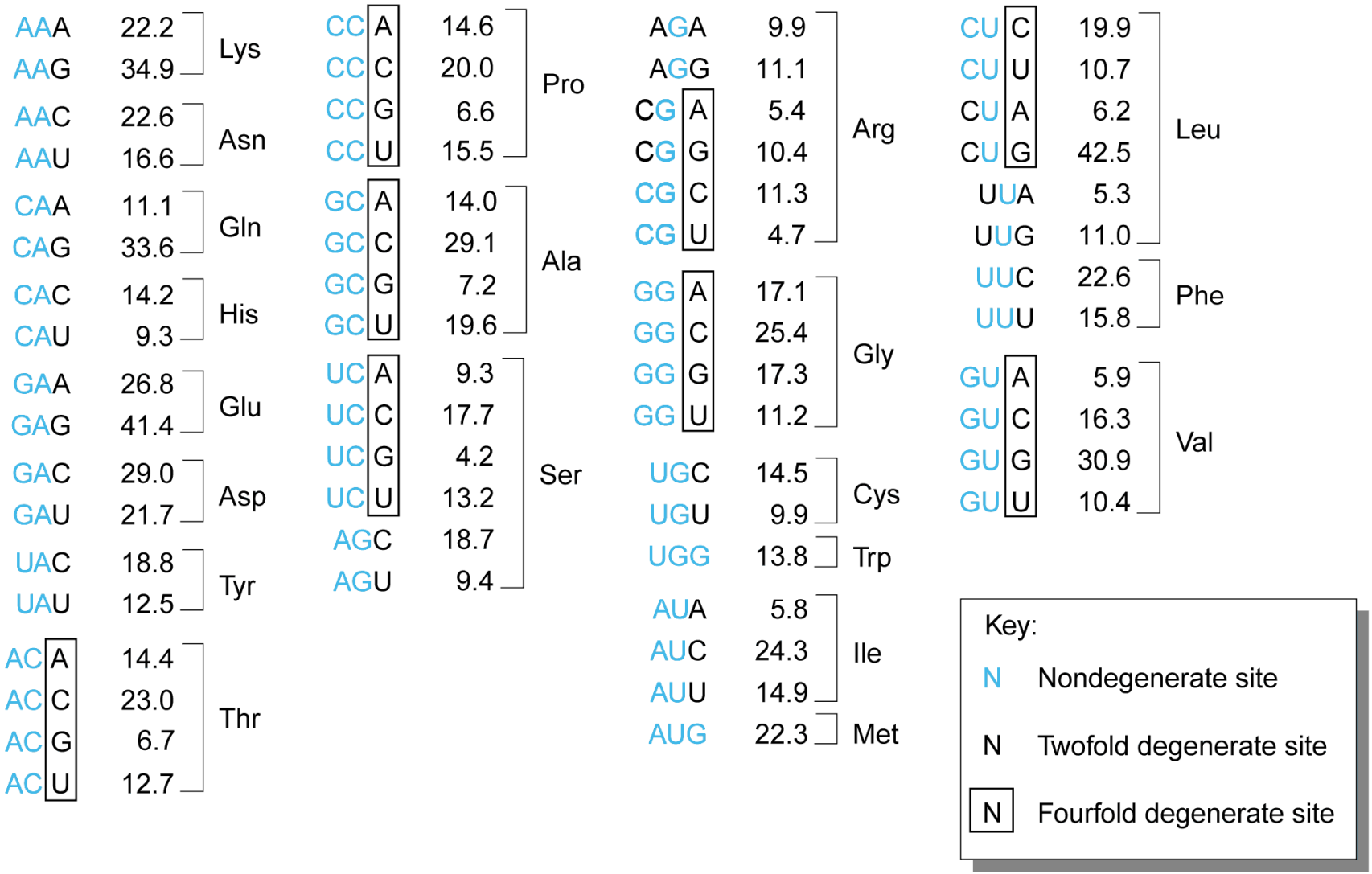
Allele 1

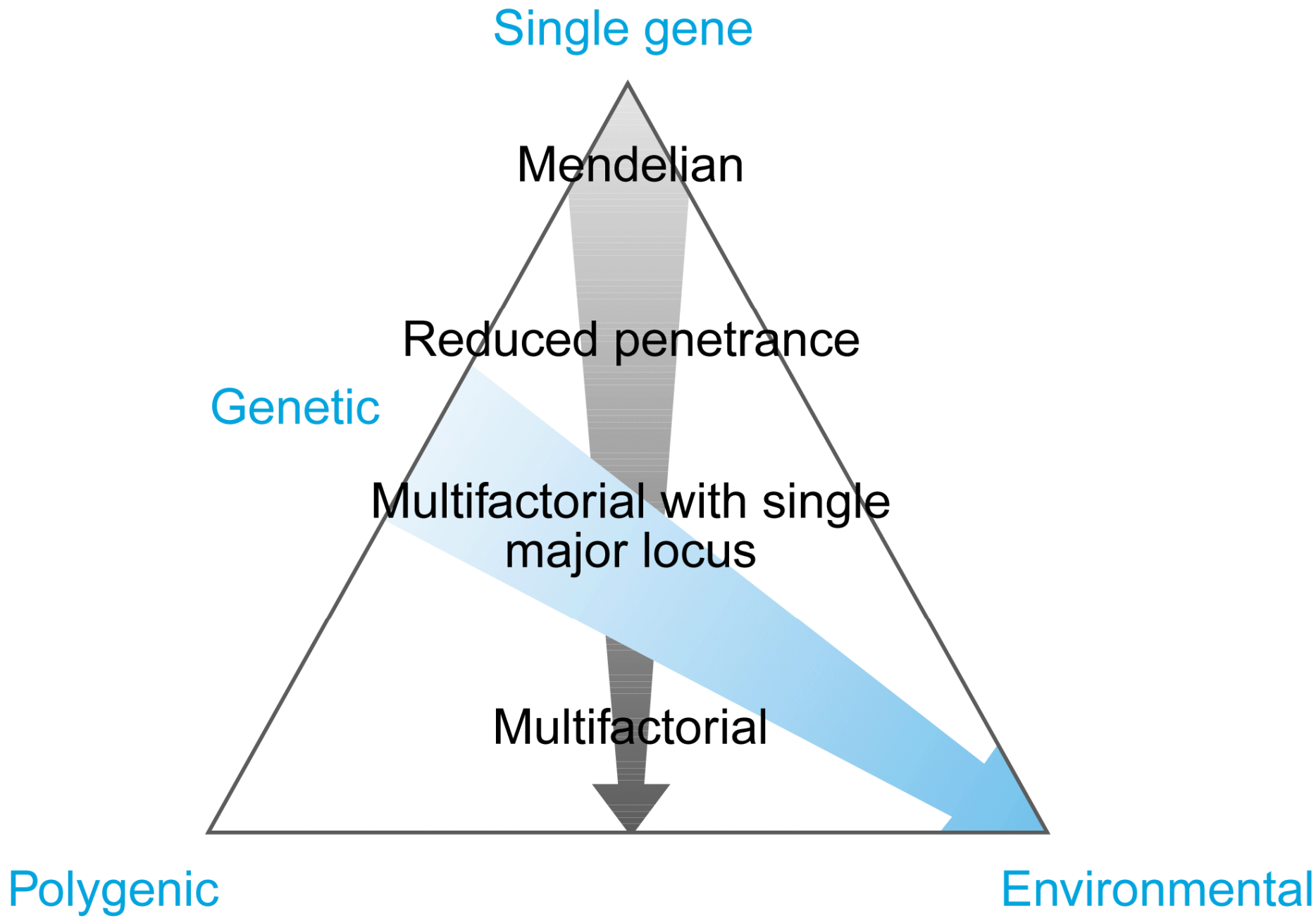
Allele 2

Allele 3

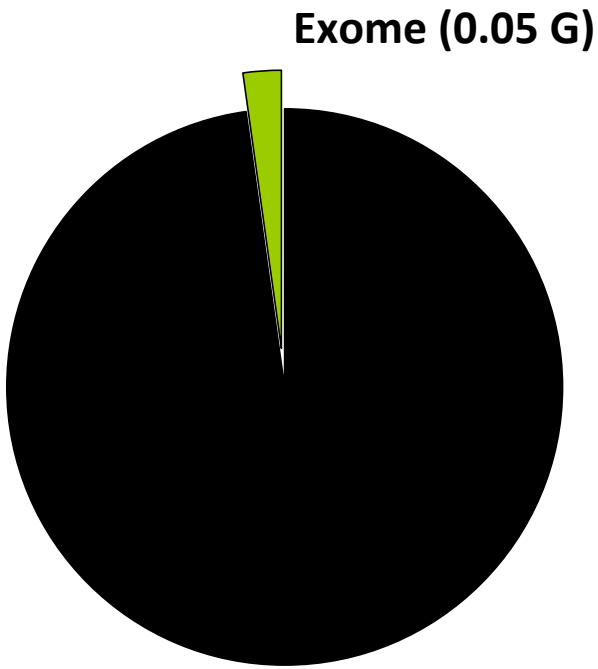
DNA sequence variants




AAA 22.2
AAG 34.9
Lys
AAC 22.6
AAU 16.6
Asn
CAA 11.1
CAG 33.6
Gln
CAC 14.2
CAU 9.3
His
GAA 26.8
GAG 41.4
Glu
GAC 29.0
GAU 21.7
Asp
UAC 18.8
UAU 12.5
Tyr
ACA 14.4
ACC 23.0
ACG 6.7
ACU 12.7
Thr
CCA 14.6
CCC 20.0
CCG 6.6
CCU 15.5
Pro
GCA 14.0
GCC 29.1
GCG 7.2
GCU 19.6
Ala
UCA 9.3
UCC 17.7
UCG 4.2
UCU 13.2
AGC 18.7
AGU 9.4
Ser
AGA 9.9
AGG 11.1
CGA 5.4
CGG 10.4
CGC 11.3
CGU 4.7
Arg
GGA 17.1
GGC 25.4
GGG 17.3
GGU 11.2
Gly
UGC 14.5
UGU 9.9
Cys
UGG 13.8
Trp
AUA 5.8
AUC 24.3
AUU 14.9
Ile
AUG 22.3
Met
CUC 19.9
CUU 10.7
CUA 6.2
CUG 42.5
UUA 5.3
UUG 11.0
Leu
UUC 22.6
UUU 15.8
Phe
GUA 5.9
GUC 16.3
GUG 30.9
GUU 10.4
Val
Key:
N Nondegenerate site
N Twofold degenerate site
N Fourfold degenerate site

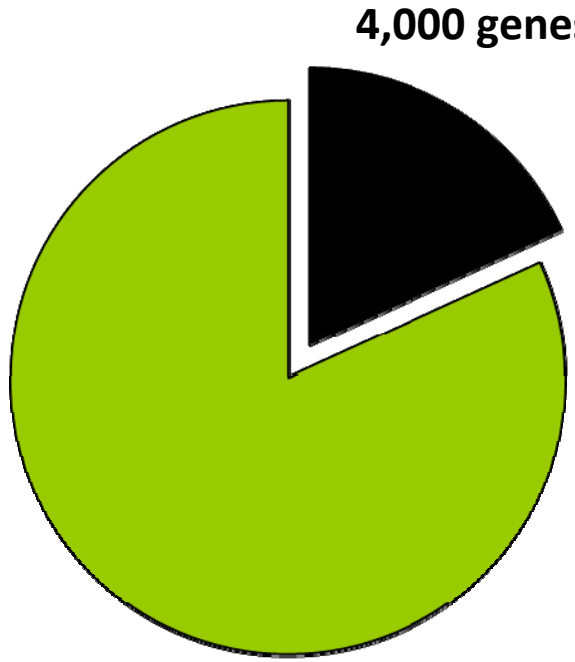

Single gene
Mendelian
Reduced penetrance
Genetic
Multifactorial with single major locus
Multifactorial
Polygenic
Environmental

Exonic part



Total ~ 3,1 Gbases

Genes with mutations causing human disorders



Total ~ 22,000 genes

HEALTH-2007-1.2-6: High throughput molecular diagnostics in individual patients for genetic diseases with heterogeneous clinical presentation

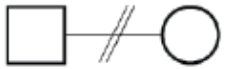
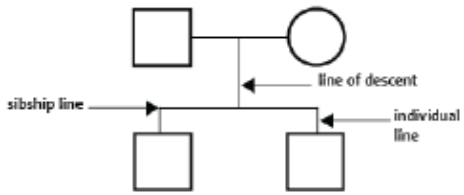
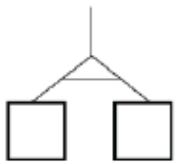
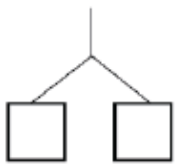
Heterogeneous genetic disorders can either be caused

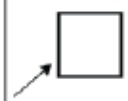
- by many different mutations in a single gene (allelic heterogeneity)
- by mutations in different genes (locus heterogeneity)

What is a mutation?

A variation of the DNA sequence

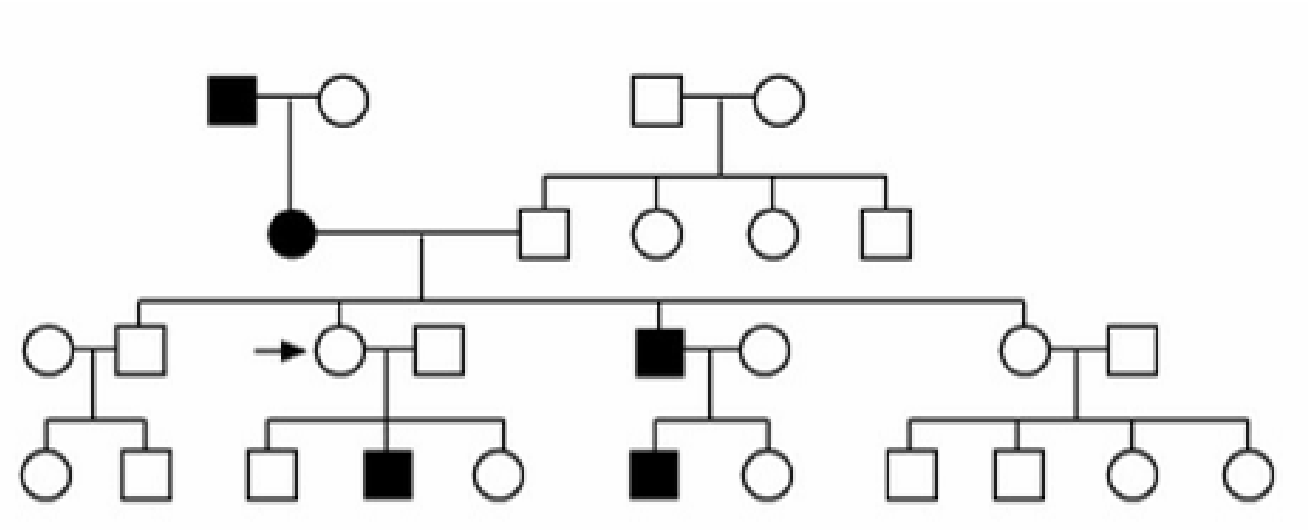
- that is only found in affected individuals
- that is never found in non affected individuals
- that accounts for the pathological process/status
- that, when corrected in time, disease is rescued

Marriage/partnership	
Divorce/separation	
Where the partners are blood relatives (consanguineous relationship)	
Children/siblings	
Identical twins (monozygotic)	
Non-identical twins (dizygotic)	

	Male	Female	Sex Unknown
Individual			
Affected individual (symbol coloured in)			
Multiple individuals			
Deceased			
Pregnancy (the unborn baby of a pregnant mother)			
Miscarriage			
Person providing pedigree information			

..that is only found in affected and that is never found in non affected

incomplete penetrance

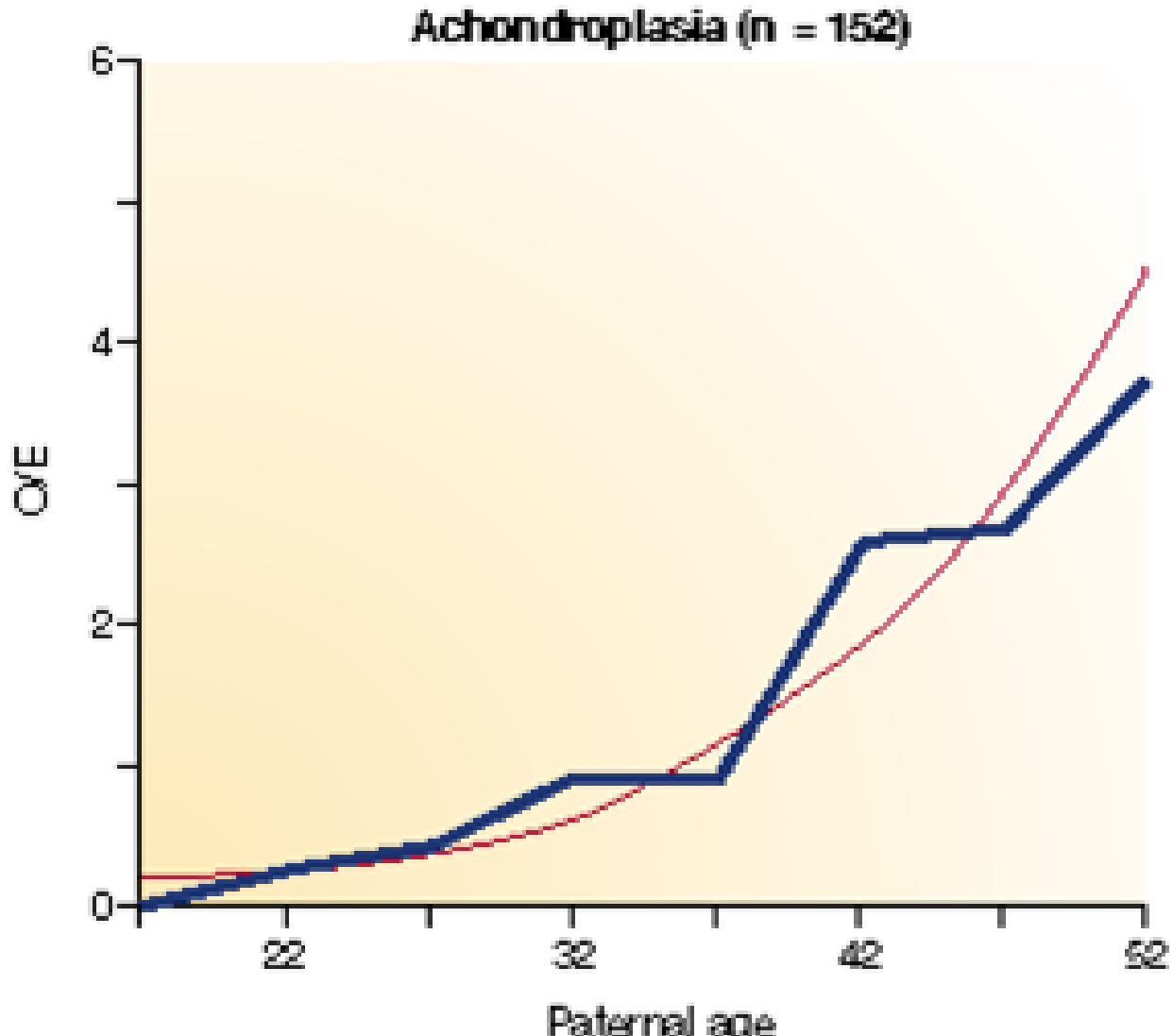


that is more often found in affected
than in non affected...

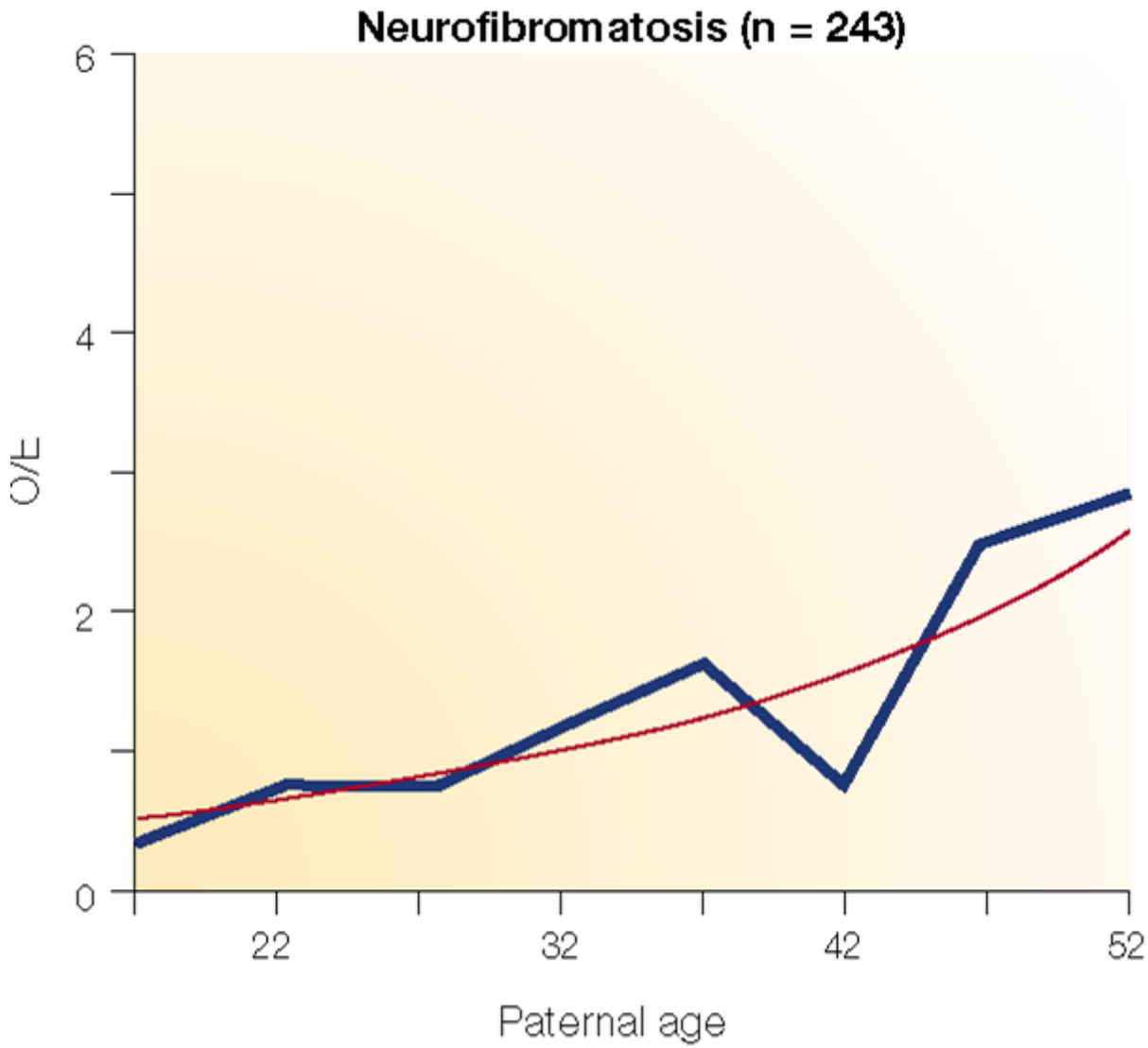
1-allele diseases

- monoallelic mutations may be responsible for dominant or X-linked disorders
- new **random** mutations are the rule with an unpredictable pattern of distribution

relative frequency of *de novo* achondroplasia for different paternal ages

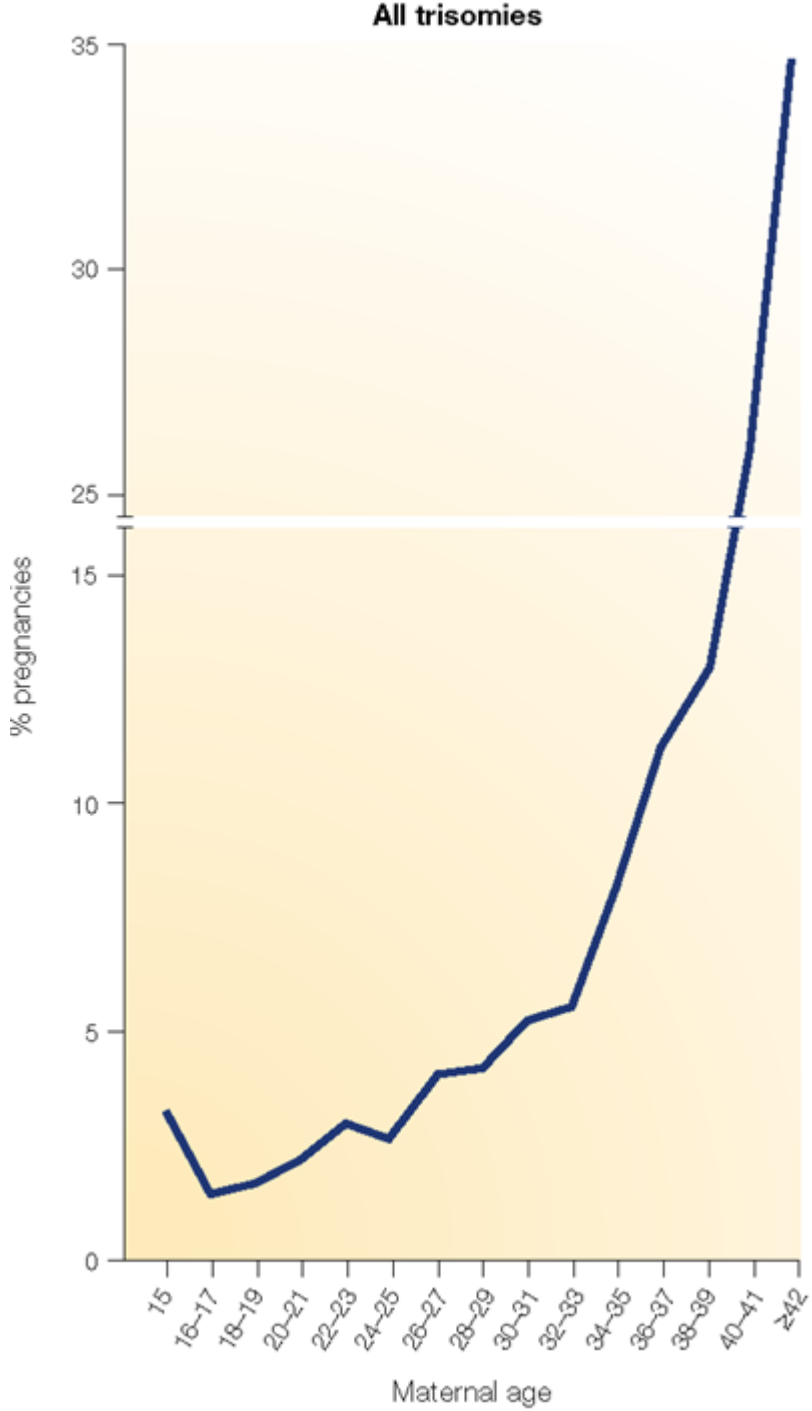


relative frequency of *de novo* neurofibromatosis for different paternal ages

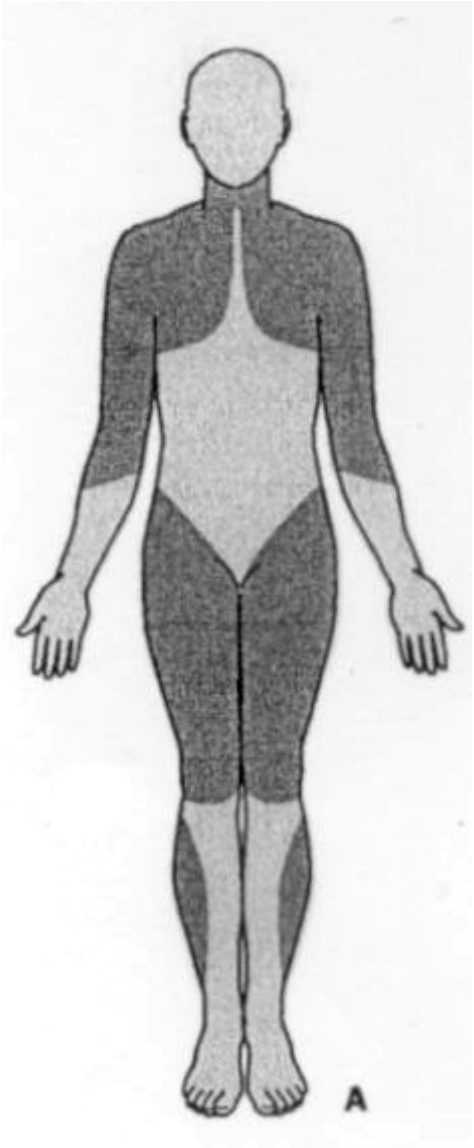


the number of male germ-cell divisions

Age	Chromosome replications
15	35
20	150
30	380
40	610
50	840


All trisomies
35
30
25
15
10
5
0
% pregnancies
15
16–17
18–19
20–21
22–23
24–25
26–27
28–29
30–31
32–33
34–35
36–37
38–39
40–41
≥42
Maternal age

X-linked disorders

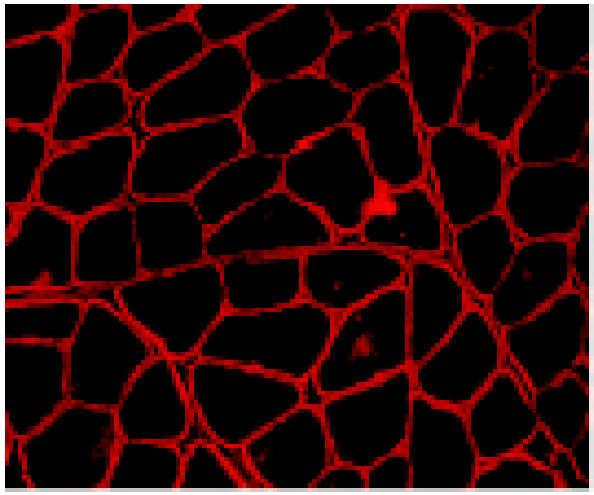


- **DMD Duchenne Muscular Dystrophy**
  - 1/3,500 boys
  Onset -- Early childhood - about 2 to 6 years
  - **Laboratory -- CK (50x to 1.000x), LDH5, ALT, AST, aldolase increase**

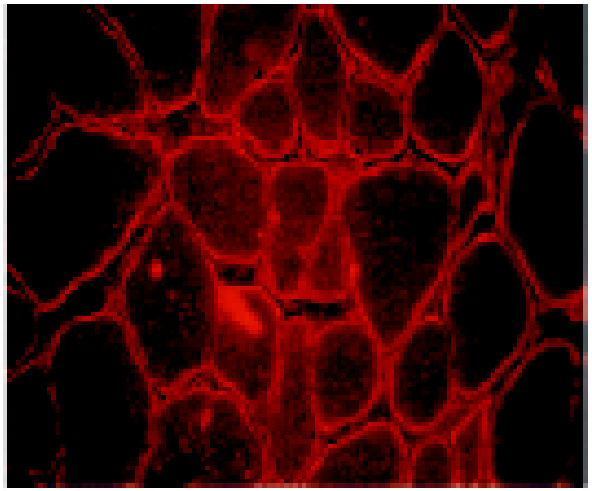
  Symptoms -- Generalized weakness and muscle wasting affecting proximal limb muscles first. Calves often enlarged. Heart involvement
  Progression -- Disease progresses slowly but will affect all voluntary muscles. Survival possible beyond late twenties

- **BMD Becker Muscular Dystrophy**
  - 1/10,000 boys
  Onset -- Adolescence or adulthood
  Symptoms -- Almost identical to Duchenne but often much less severe. Heart involvement
  Progression -- Slower and more variable than DMD with survival well into mid to late adulthood

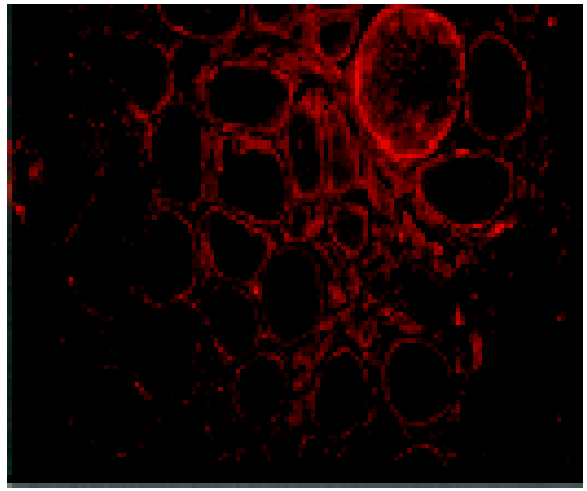
Normal



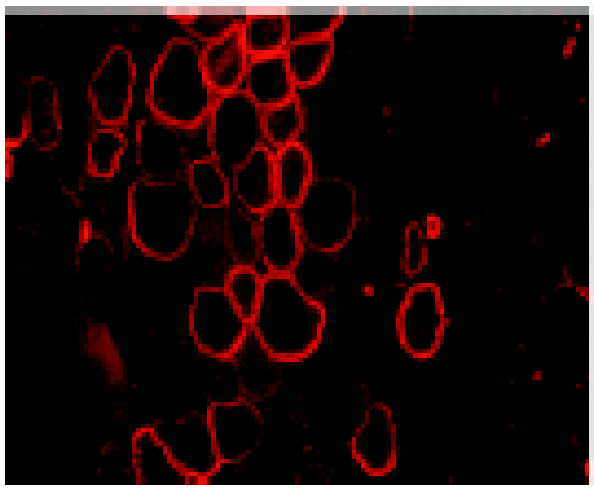
Mild BMD



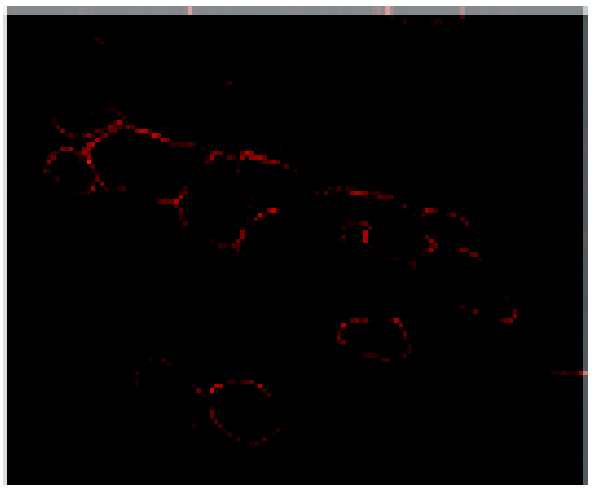
Severe BMD



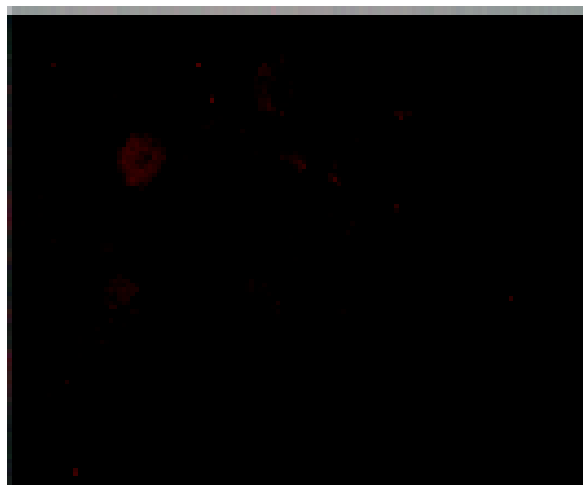
Manifesting carrier of DMD

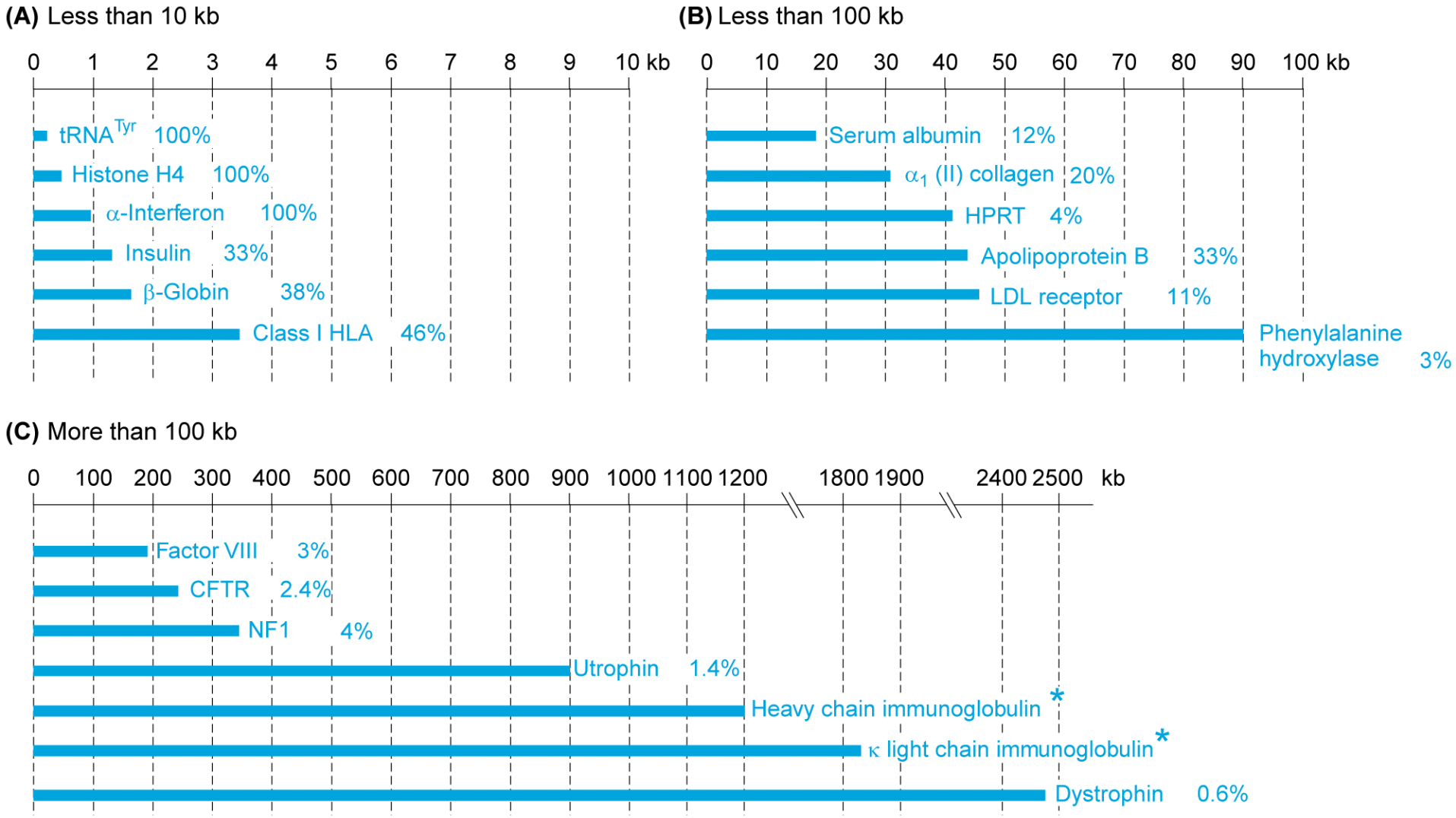


Revertant fibres in a patient with IMD

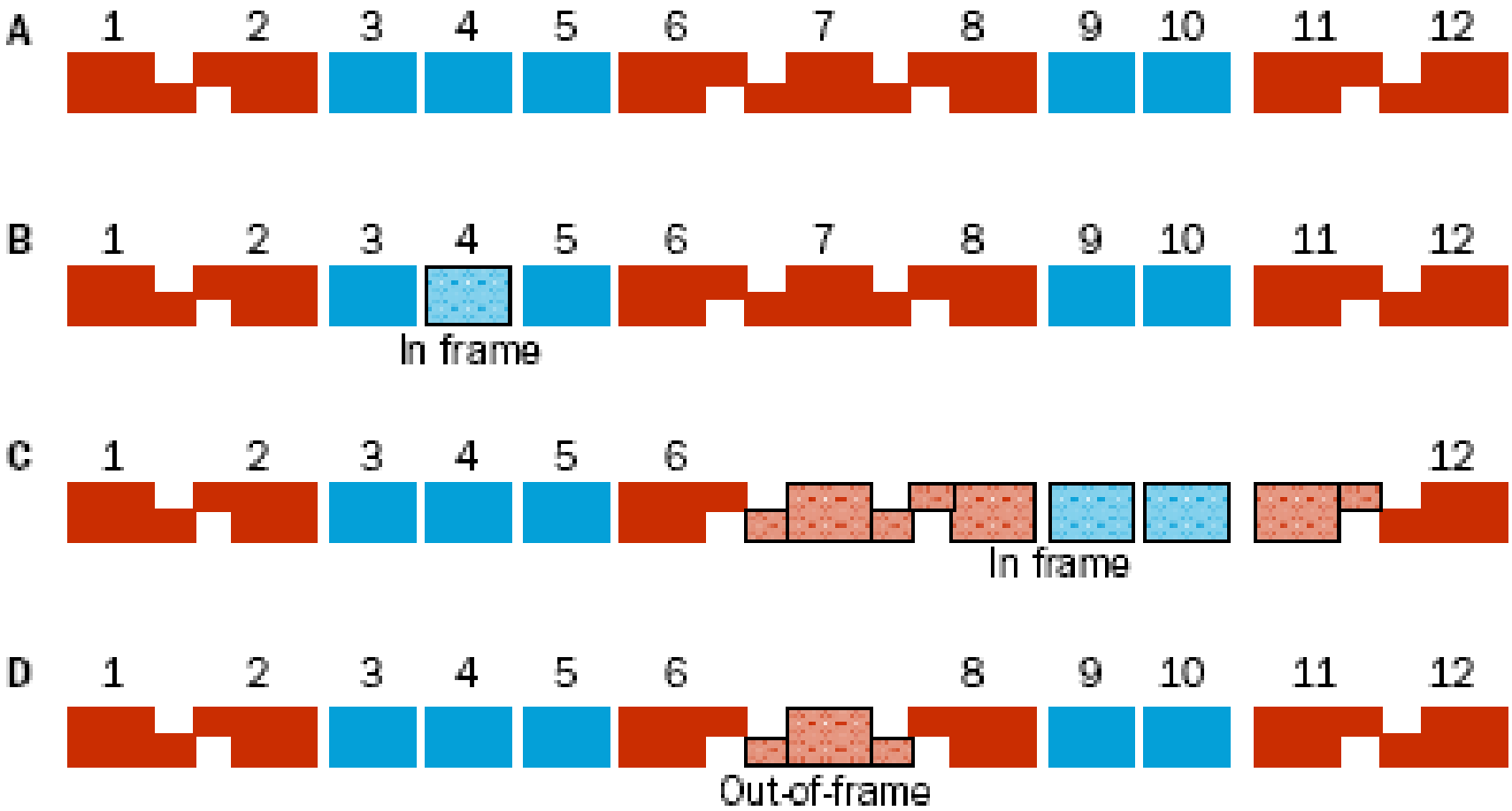


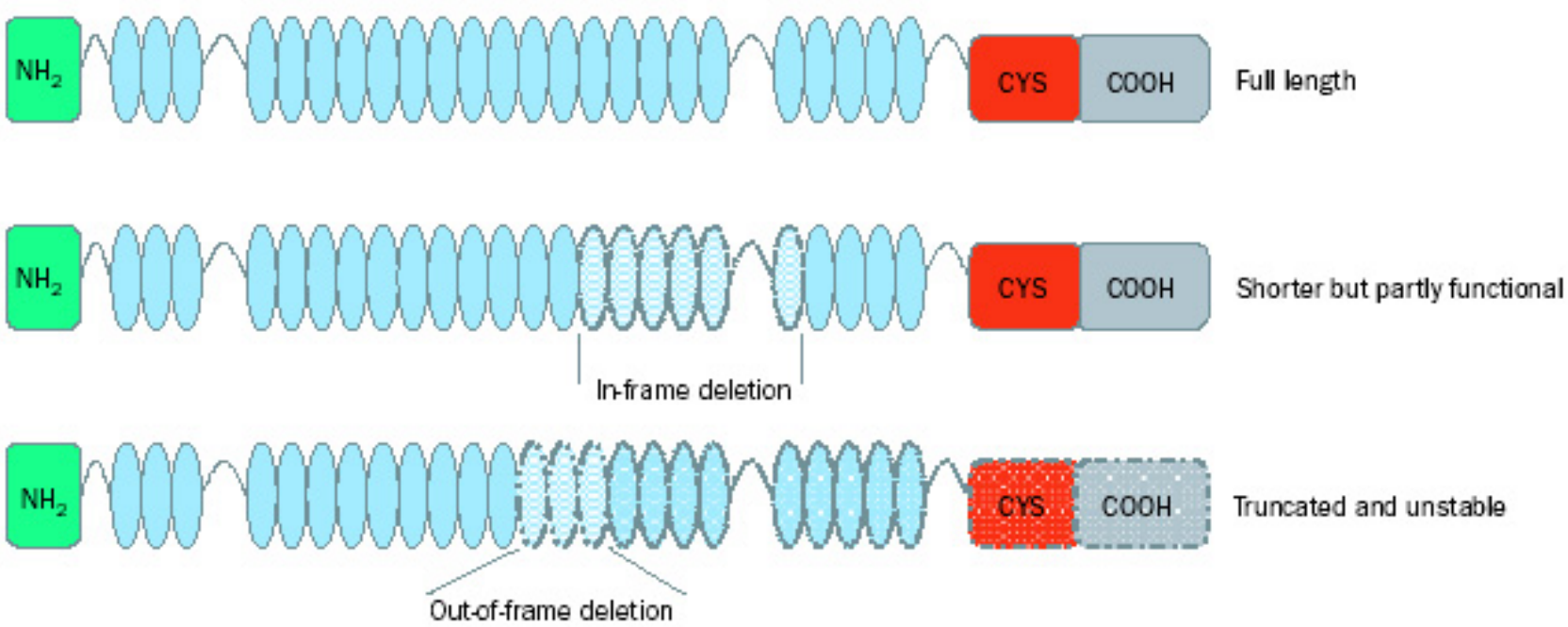
DMD





DMD gene is the largest of the human genome encompassing 2.22 Mb on the short arm of the X-chromosome

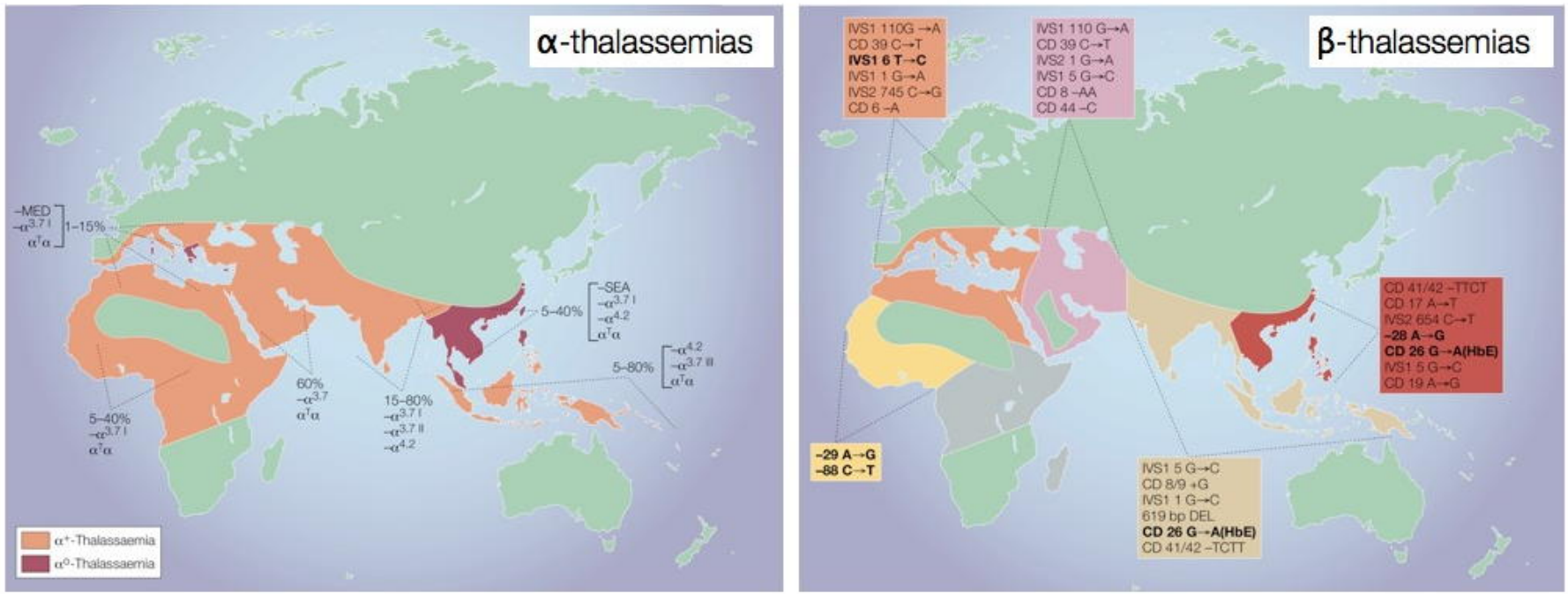

A
1
2
3
4
5
6
7
8
9
10
11
12
B
1
2
3
4
5
6
7
8
9
10
11
12
In frame
C
1
2
3
4
5
6
12
In frame
D
1
2
3
4
5
6
8
9
10
11
12
Out-of-frame


NH2
CYS
COOH
Full length
NH2
CYS
COOH
Shorter but partly functional
In-frame deletion
NH2
CYS
COOH
Truncated and unstable
Out-of-frame deletion

2-allele diseases

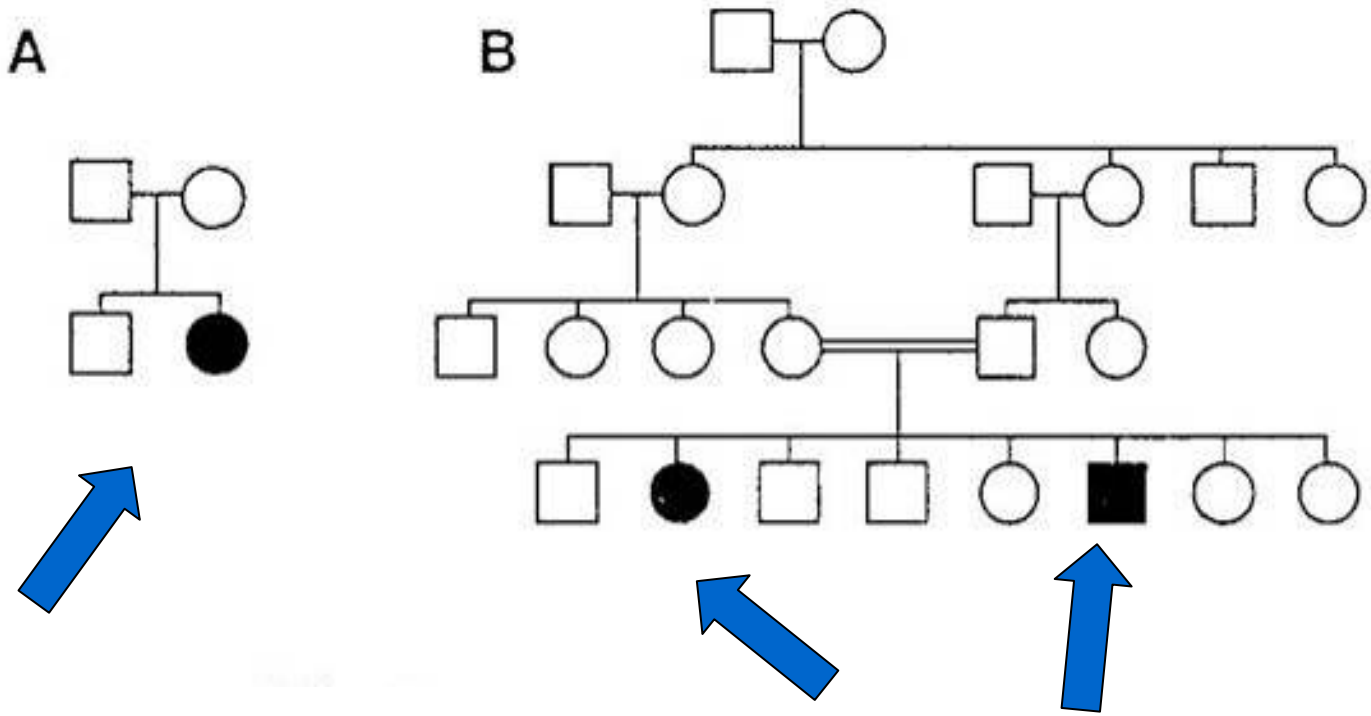
- novel mutations are rare, usually mutations have a long history (100-1,000 generations)
- mutations have an **ethnical signature** with a predictable pattern of distribution and frequency in specific geographic areas
- biallelic mutations may be responsible for **autosomal recessive** disorders
- polymorphisms and private variants may be more easily discriminated vs true mutations

Worldwide distribution of α- and β-thalassemias



Weatherall DJ *Nat Rev Genetics* 2:245, 2001

2-allele diseases



high carrier frequency

is a risk factor for

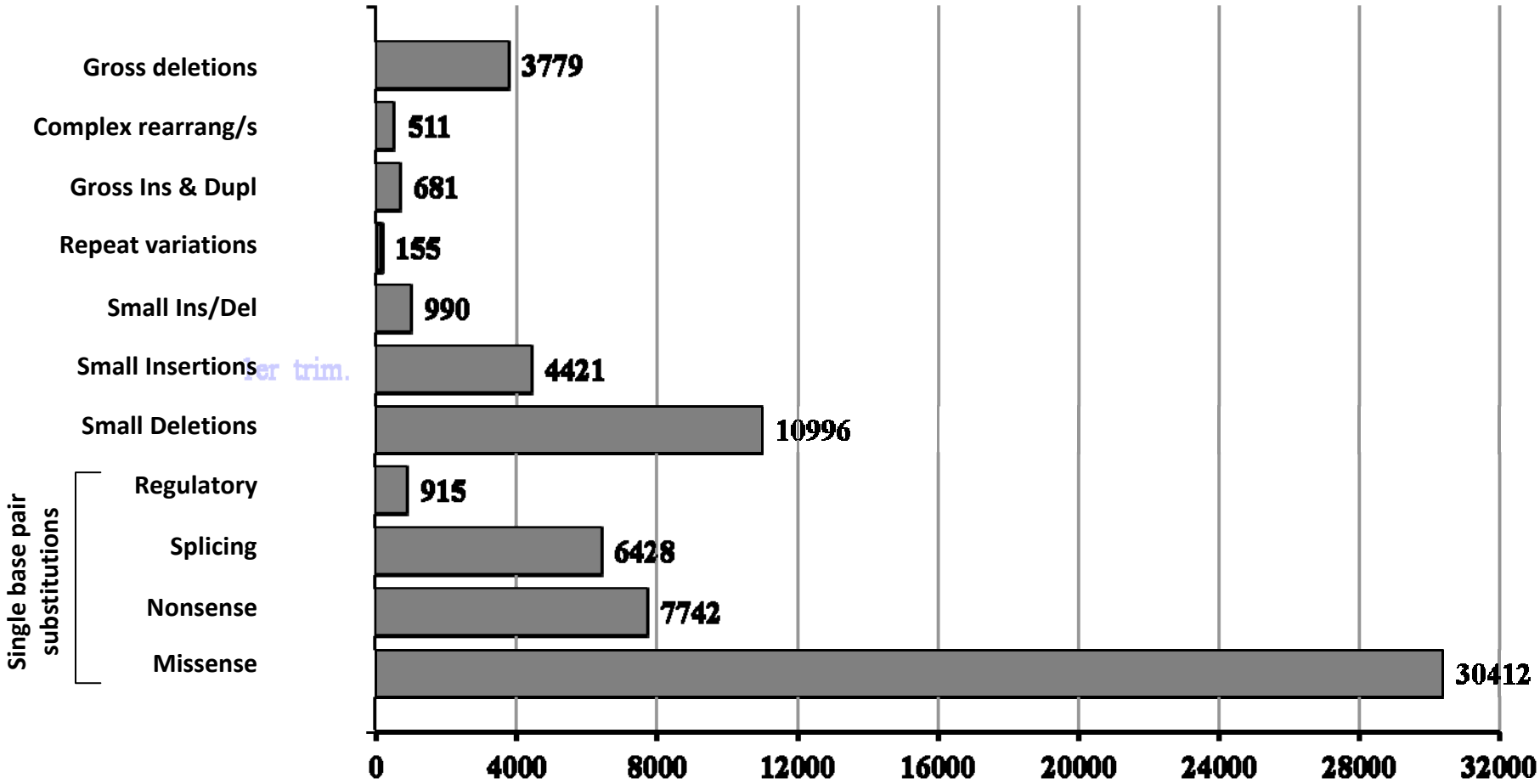
compound heterozygosity

consanguineity is a risk factor for

homozygosity, even if disease alleles

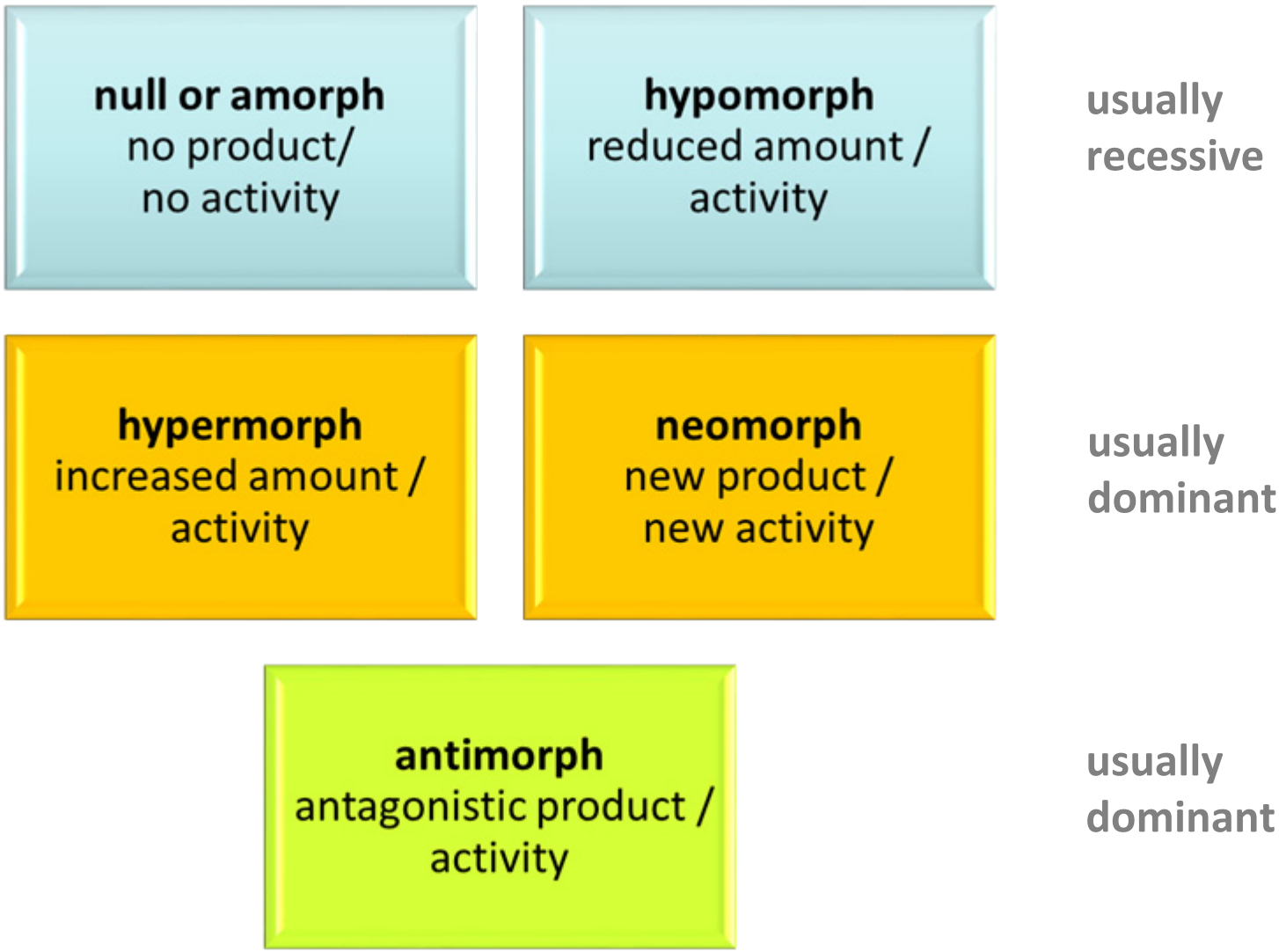
are very rare

The Human Gene Mutation Database (HGMD)

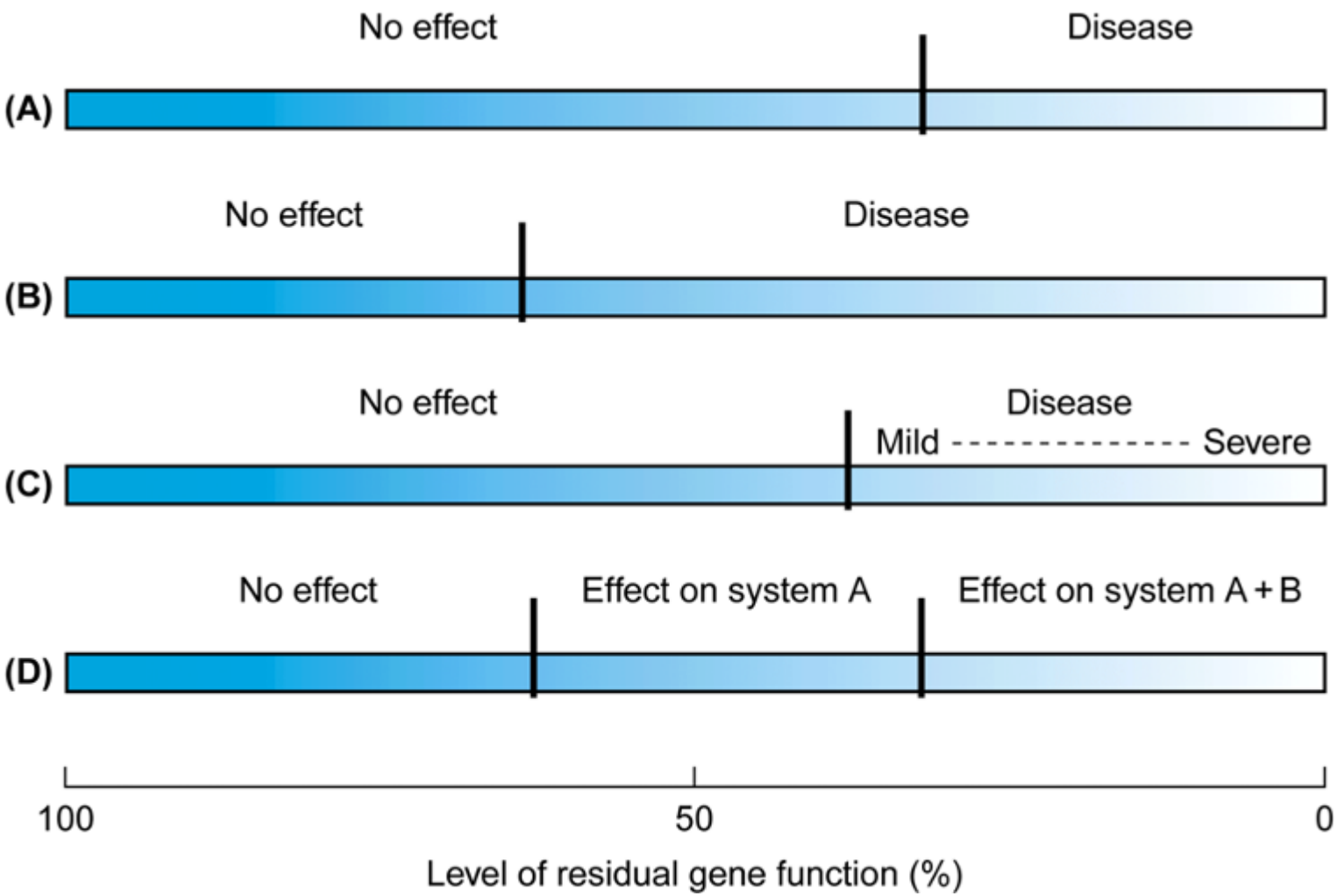


67030 mutations in 2478 genes

five effects of a mutated allele

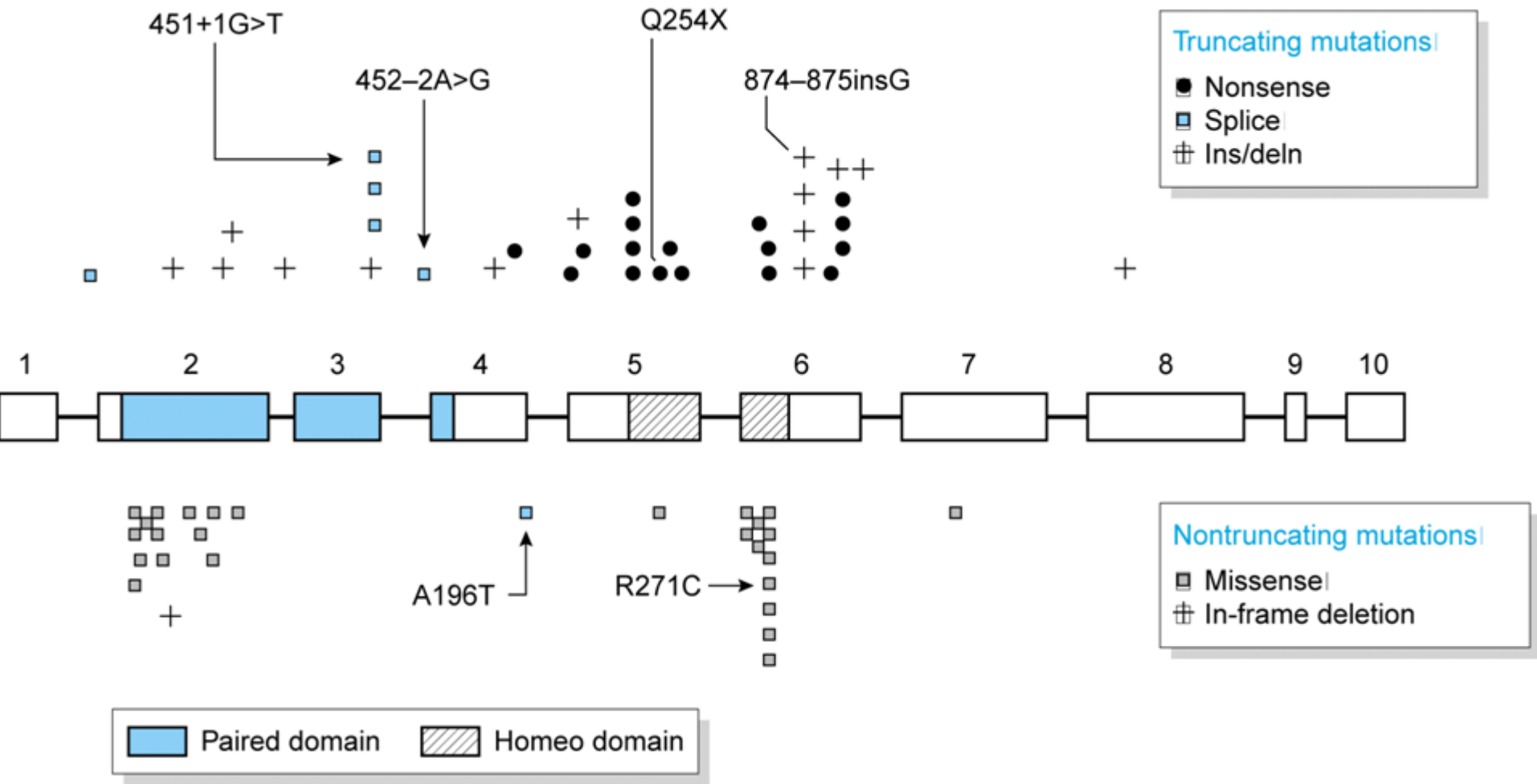


With an amorph or hypomorph heterozygous allele, why the phenotype may be also dominant?

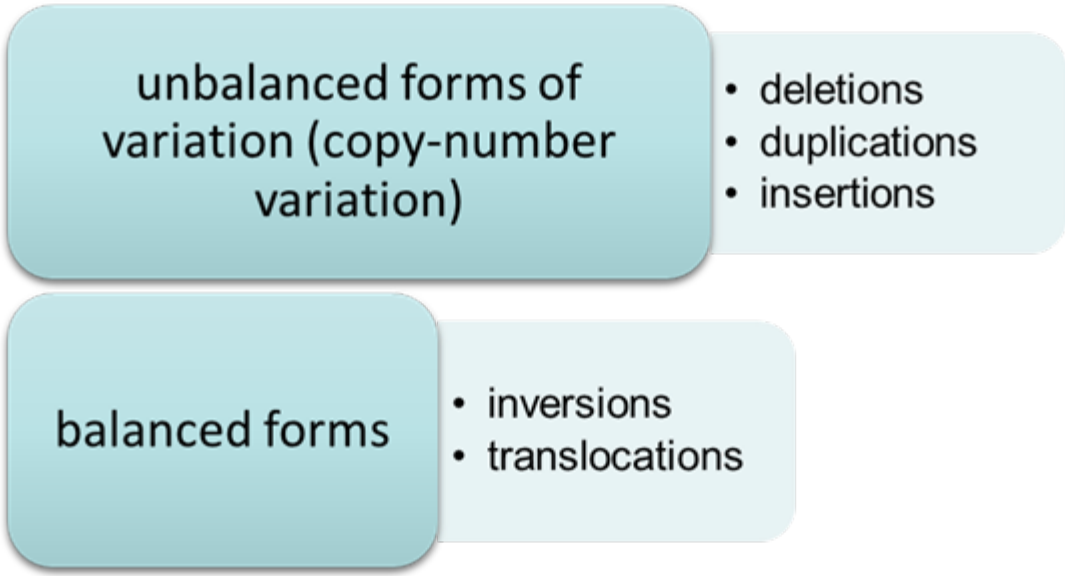


dominant loss of function mutations in the PAX3 gene (Waardenburg syndrome)

haploinsufficiency



Structural genomic variants



they are the most difficult to interpret with respect to their functional consequences

Structural genomic variants

at least 50 base pairs (bp) in size

- usually heterozygous
- high locus-specific formation rates
- 0.5–1% sequence differences between individuals

new technologies

- microarray based comparative genome hybridization (array CGH)
- high-throughput paired-end DNA next-generation sequencing

in the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (**DECIPHER**) 20% of disorders are caused by duplications and 80% by deletions

Phenotype/gene dosage correlation

duplications

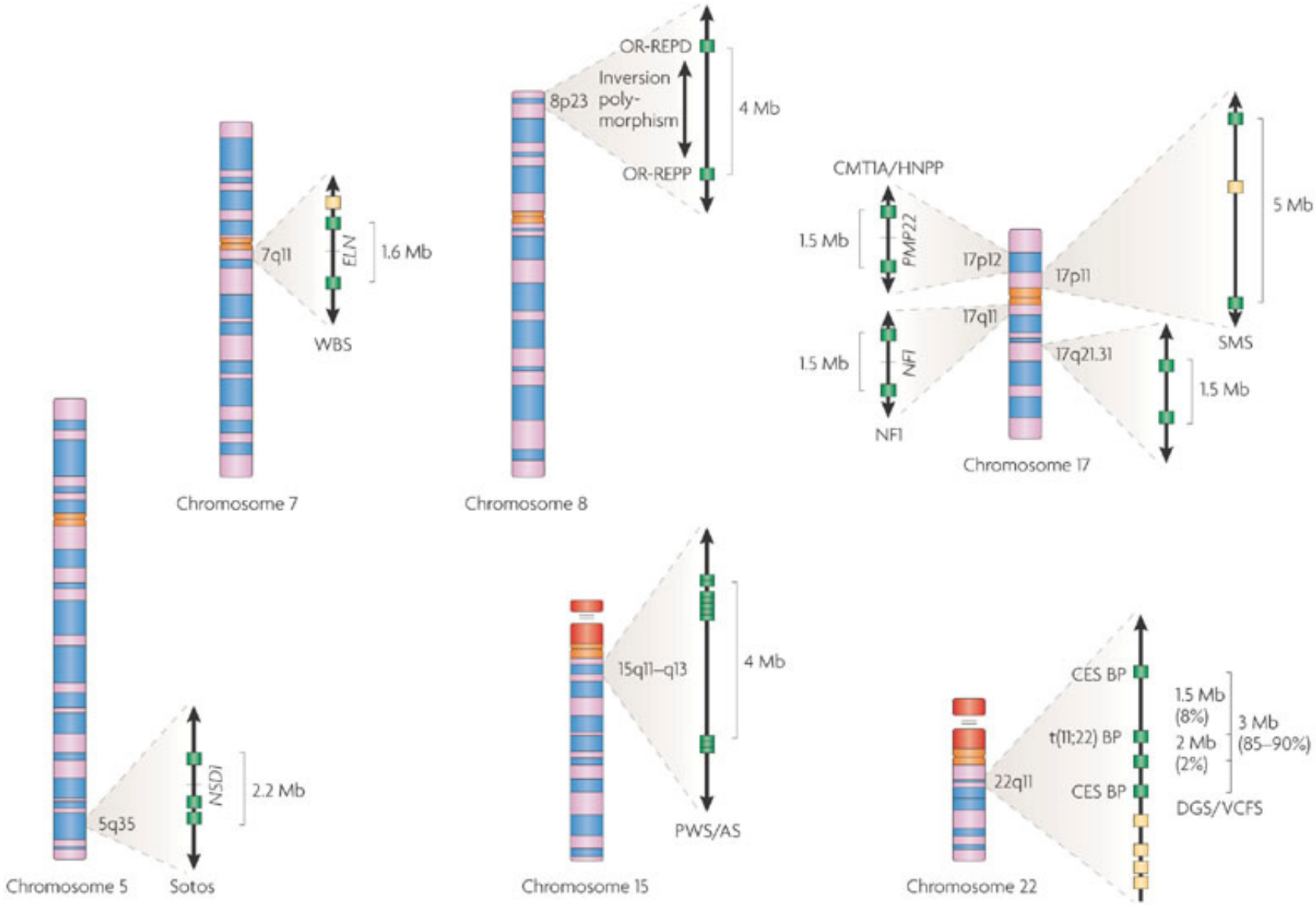
- result in a 3:2 variant-to-normal gene dosage ratio
- duplications are usually associated with a less-severe phenotype grading than deletions

deletions

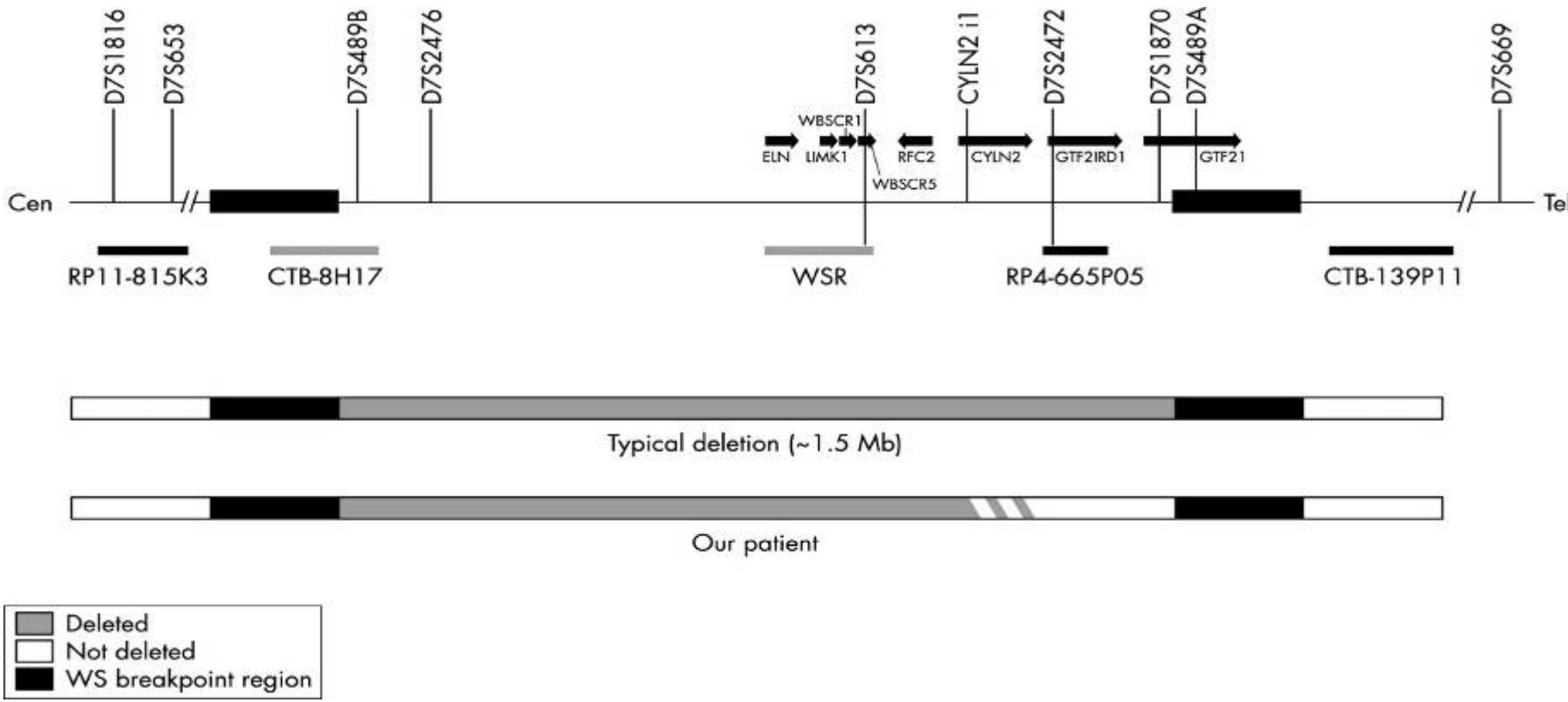
- result in a 1:2 variant-to-normal gene dosage ratio
- unmask recessive alleles on the other chomosome
- expose inactive imprinted genes
- result in **haploinsufficiency**

Humans have two copies of most genes, one from the mother and one from the father. This provides a back-up copy, should one copy be lost through mutation

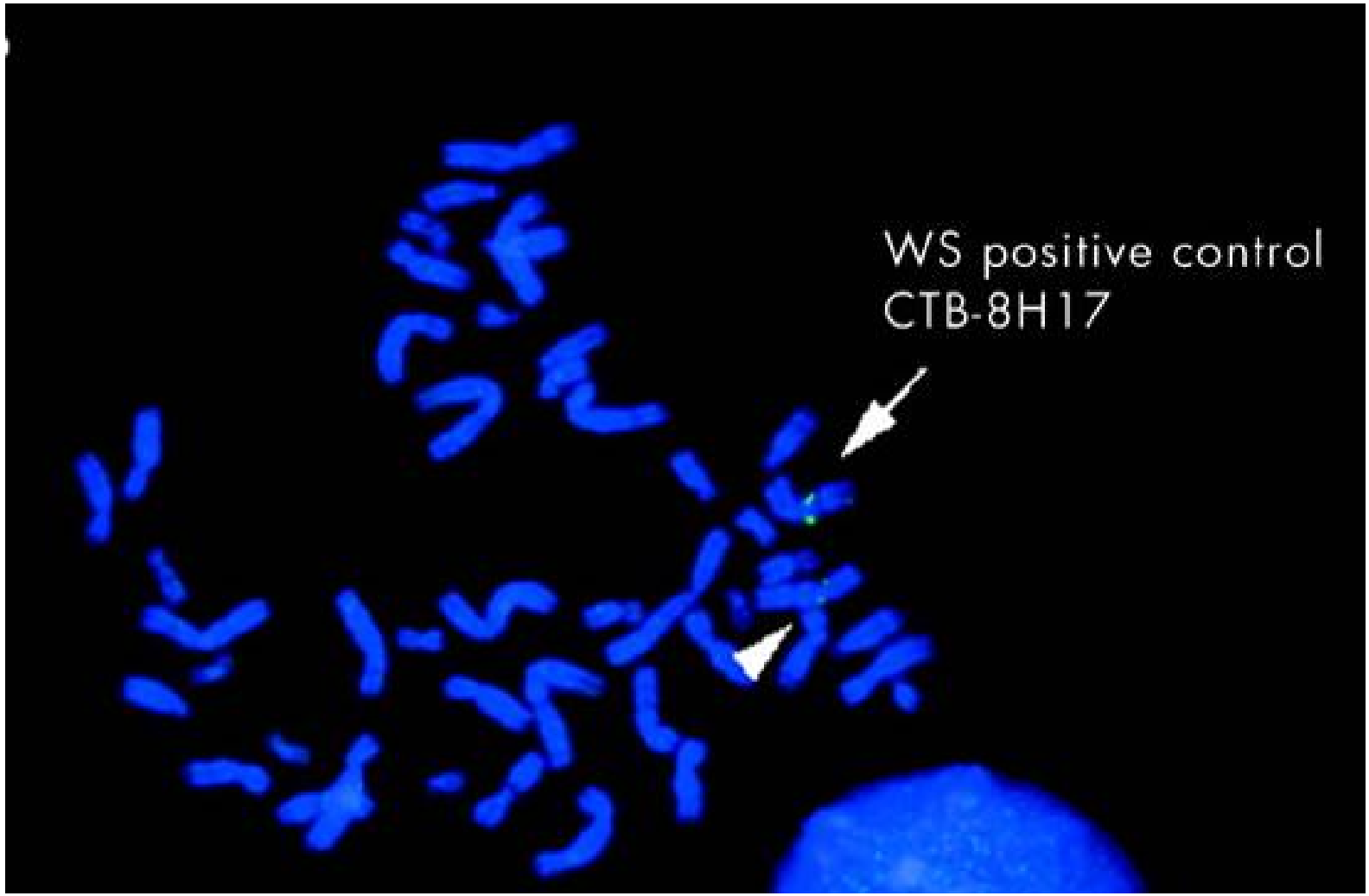
For at least 300 genes, one functional copy is not enough to sustain normal human function, and mutations causing the loss of function of one of the copies of such genes are a major cause of childhood developmental diseases


7q11
ELN
1.6 Mb
WBS
Chromosome 7
8p23
Inversion
poly-
morphism
OR-REPD
OR-REPP
4 Mb
Chromosome 8
CMT1A/HNPP
1.5 Mb
PMP22
17p12
17p11
17q11
5 Mb
SMS
1.5 Mb
NF1
17q21.31
1.5 Mb
NF1
Chromosome 17
5q35
NSD1
2.2 Mb
Sotos
Chromosome 5
15q11–q13
4 Mb
PWS/AS
Chromosome 15
CES BP
1.5 Mb
(8%)
t(11;22) BP
2 Mb
(2%)
3 Mb
(85–90%)
22q11
CES BP
DGS/VCFS
Chromosome 22

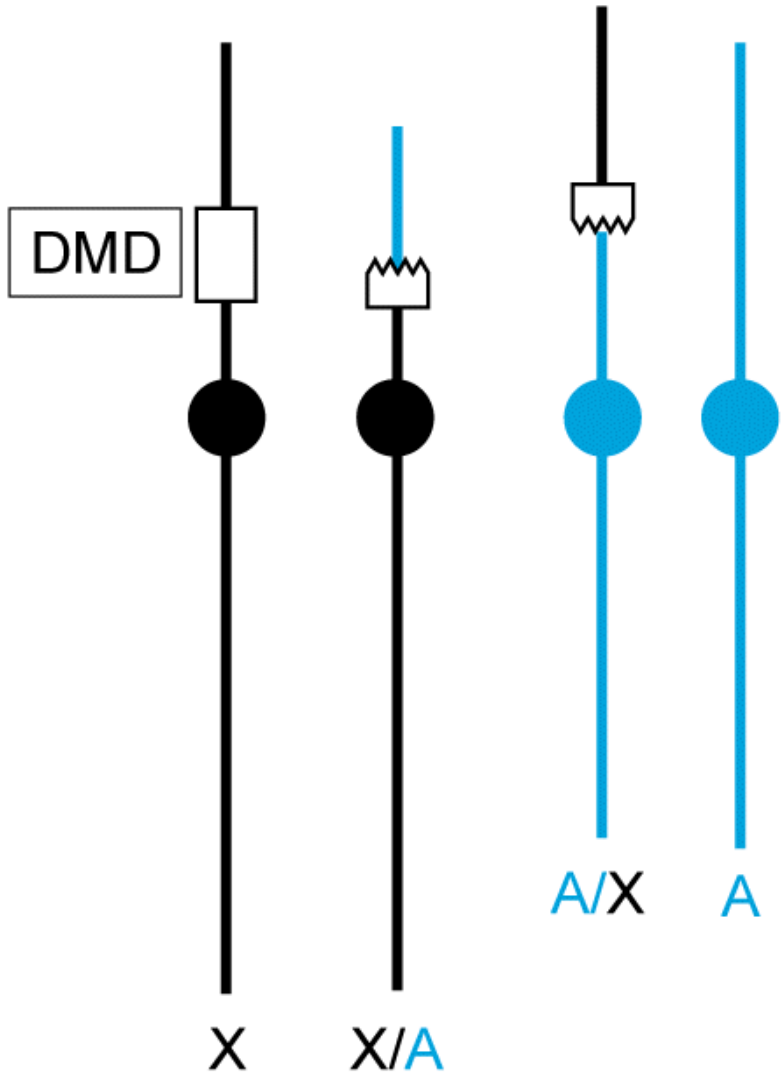
Williams Syndrome



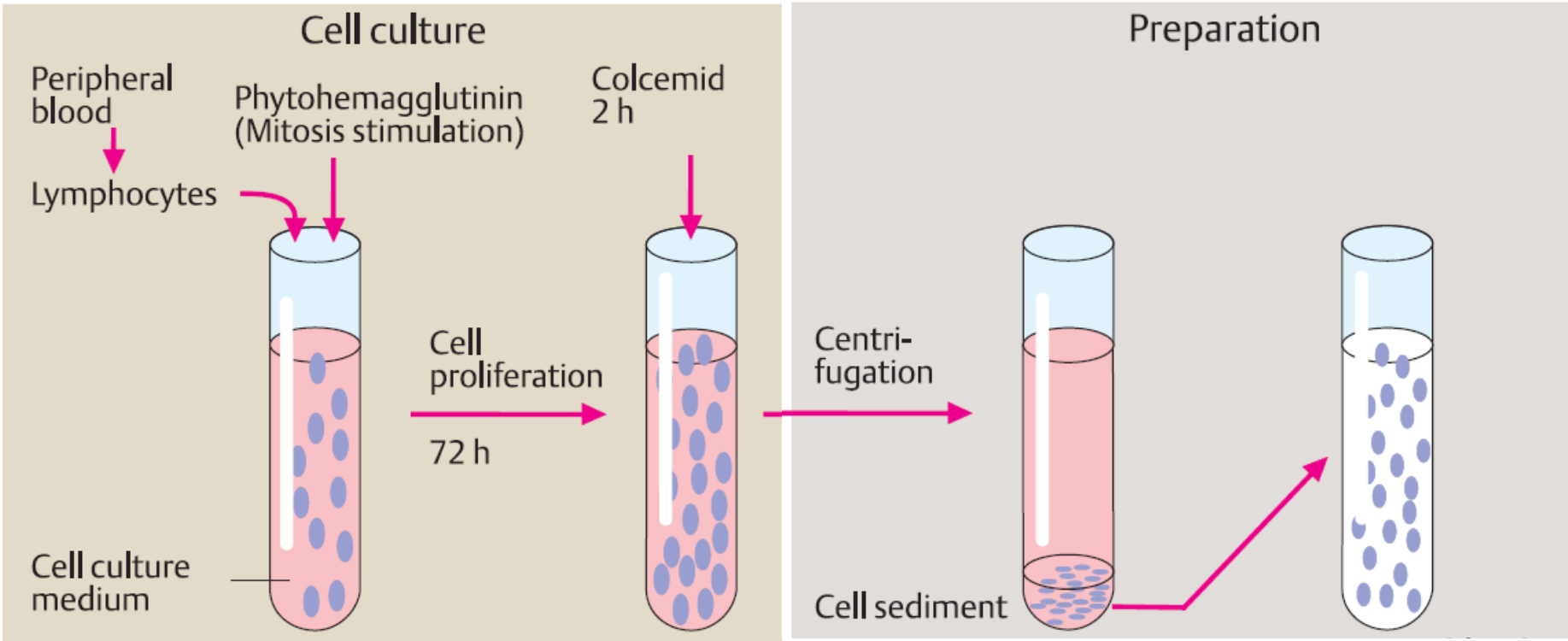
Williams s.
FISH : heterozygous deletion 7q11.23

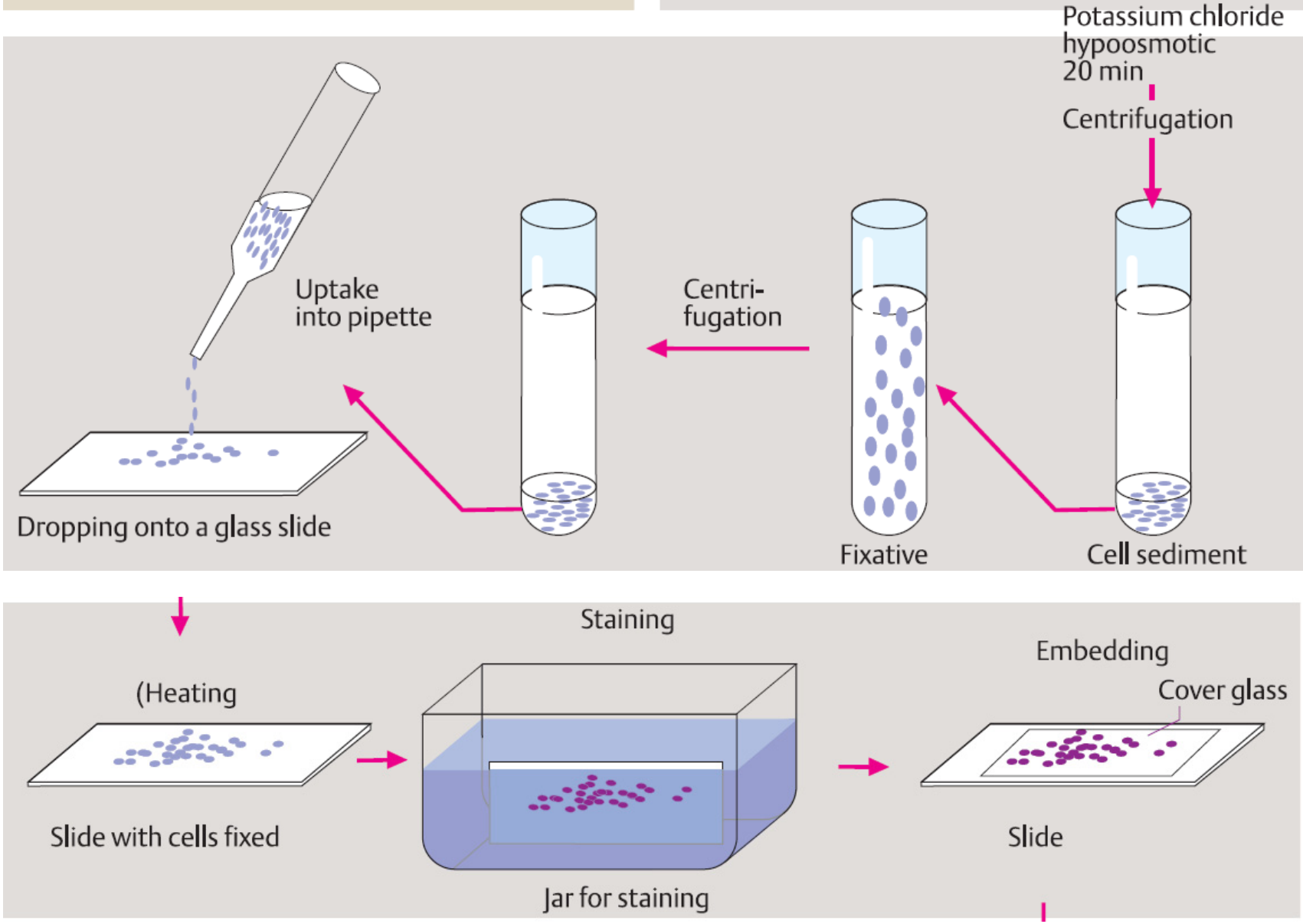


Carrier of a balanced reciprocal X-autosome translocation



cytogenetics




Potassium chloride
hypoosmotic
20 min
Centrifugation
Cell sediment
Fixative
Centri-
fugation
Uptake
into pipette
Dropping onto a glass slide
(Heating
Slide with cells fixed
Staining
Jar for staining
Embedding
Cover glass
Slide

Nomenclatura

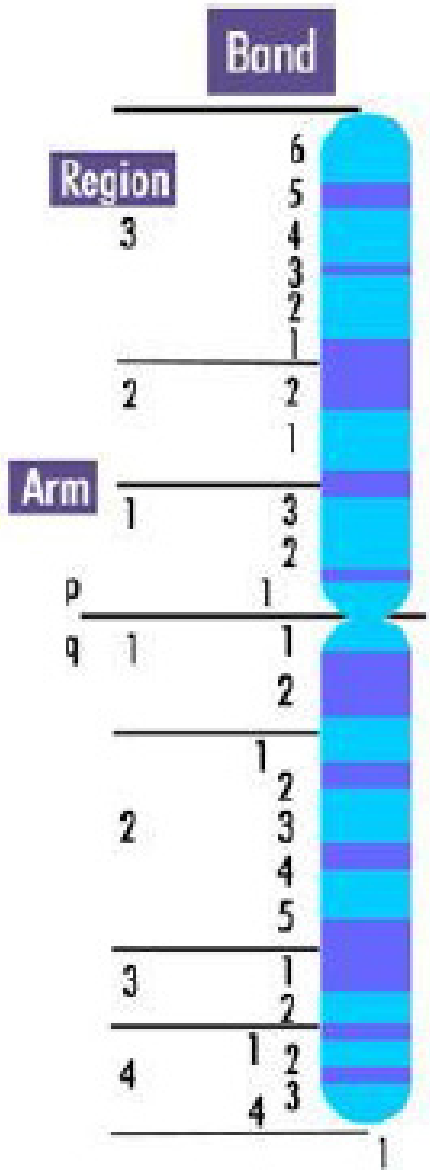
ISCN

International

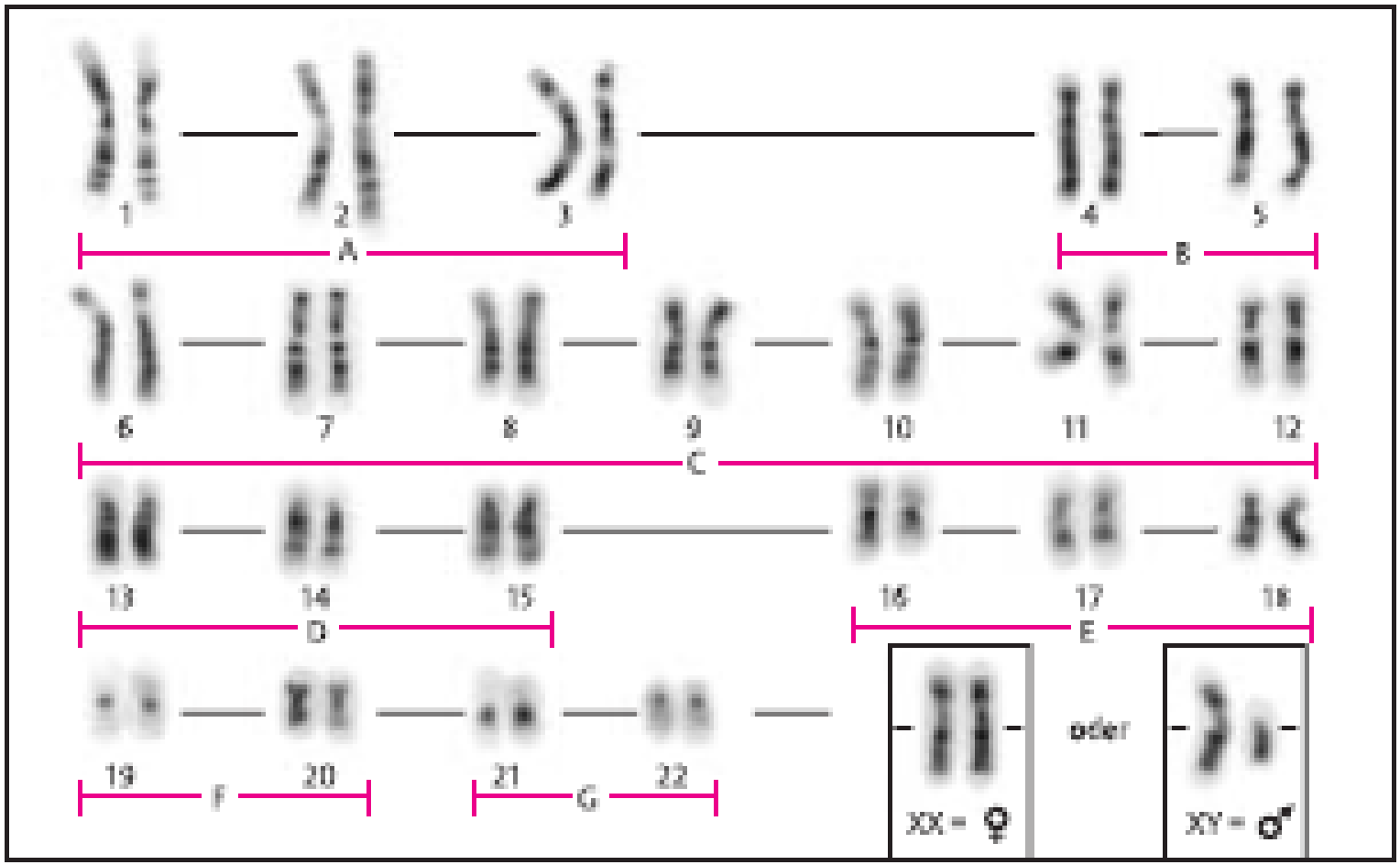
System for human

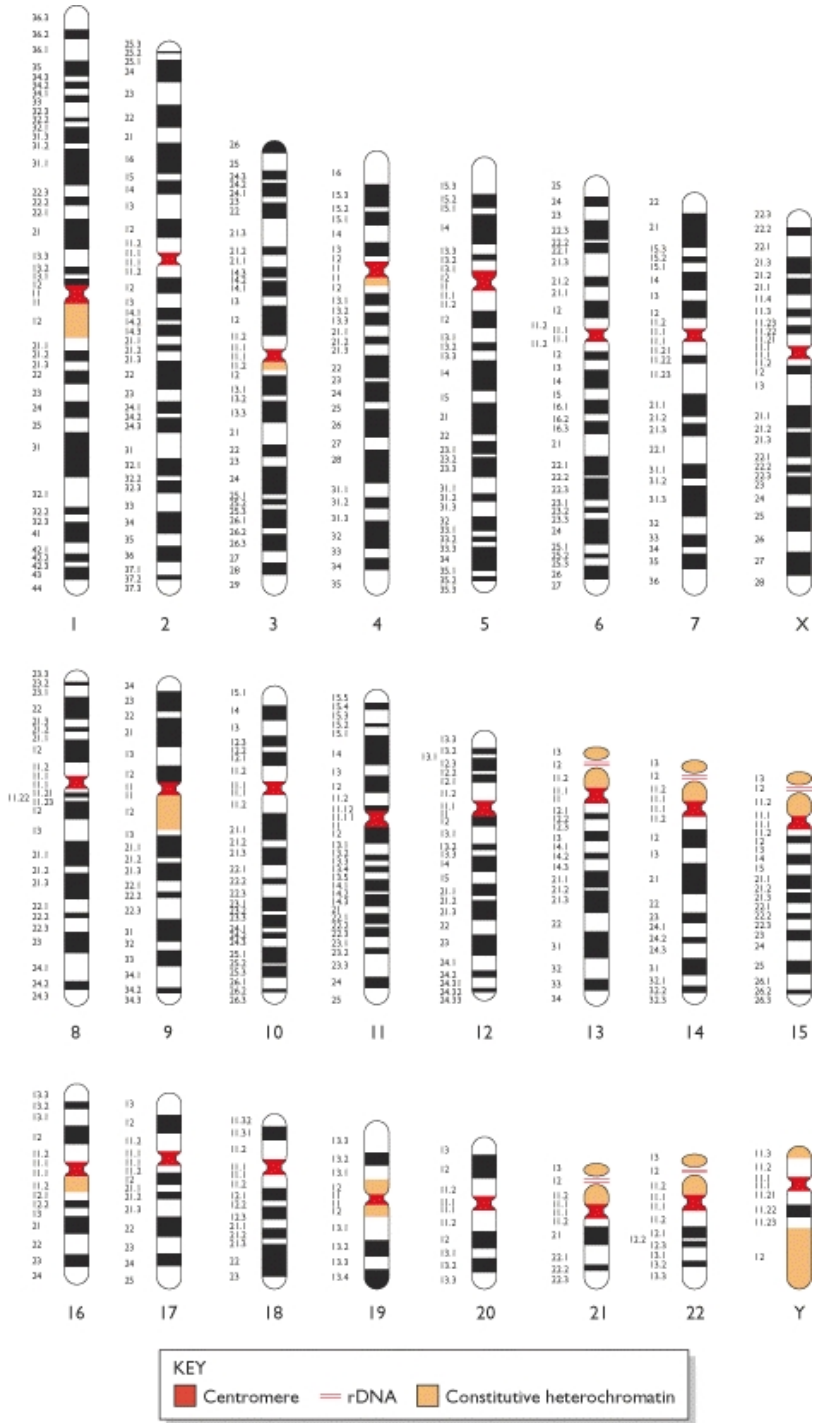
Cytogenetics

Nomenclature

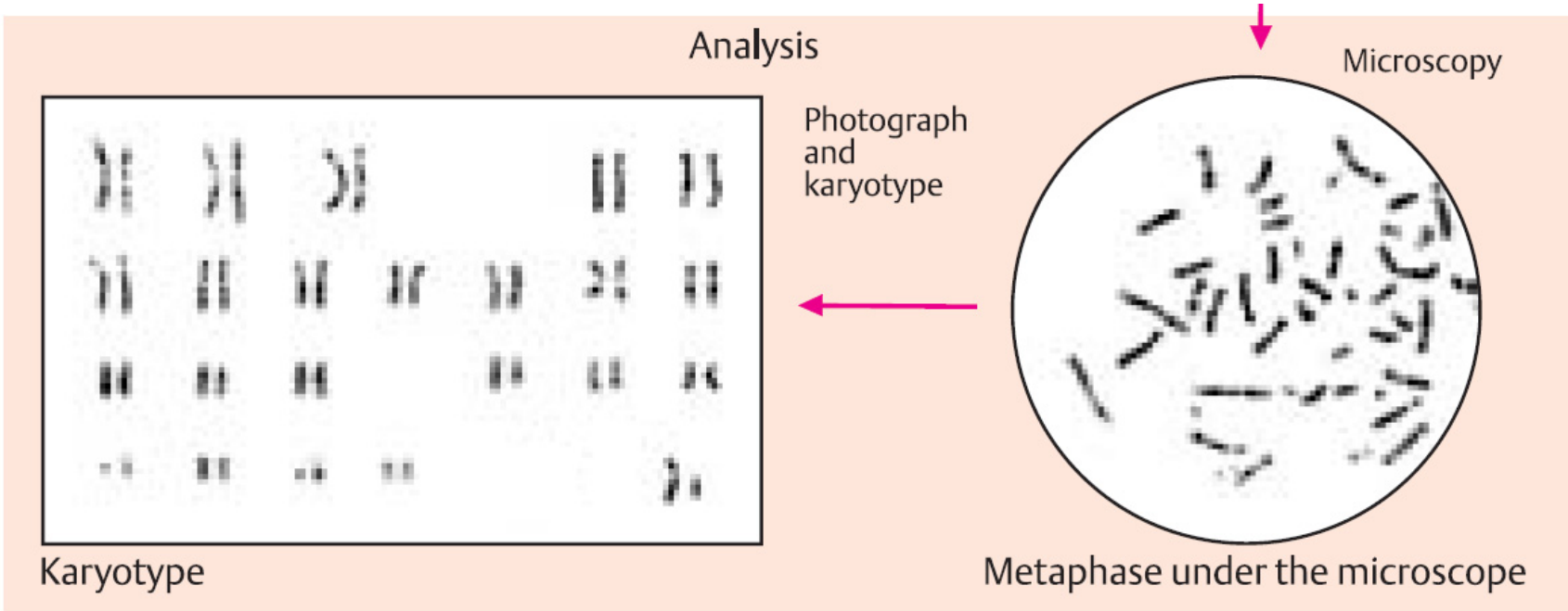


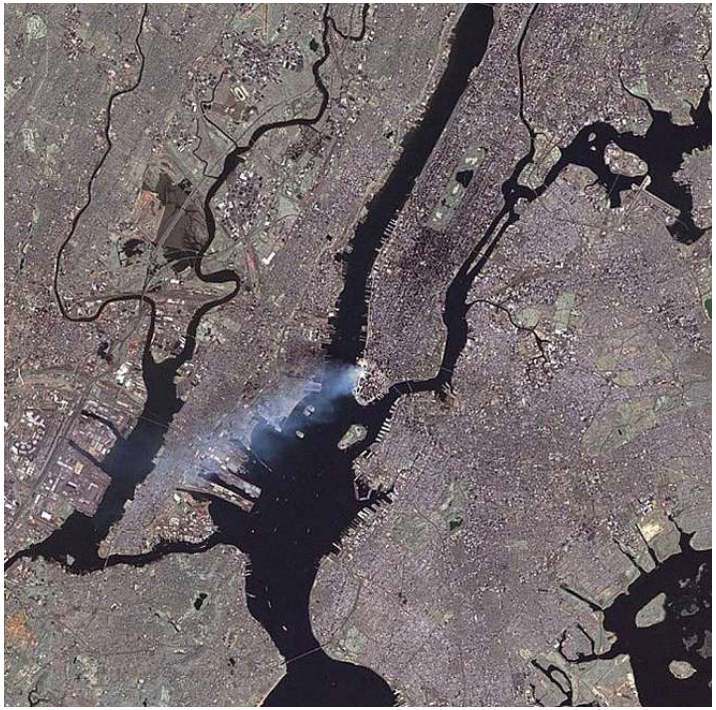
	Procedure	Banding pattern
G banding	the chromosomes are subjected to controlled digestion with trypsin before staining with Giemsa, a DNA-binding chemical dye	dark bands are known as G bands, pale bands are G negative


1
2
3
4
5
A
B
6
7
8
9
10
11
12
C
13
14
15
16
17
18
D
E
19
F
20
21
G
22
oder
XX = ♀
XY = ♂



CCDS IDs per chromosome	
Chromosome	**Count**
1	2,513
2	1,548
3	1,299
4	898
5	1,028
6	1,236
7	1,094
8	807
9	921
10	971
11	1,509
12	1,240
13	385
14	749
15	711
16	967
17	1,370
18	350
19	1,616
20	672
21	282
22	530
X	967
Y	53
XY	23

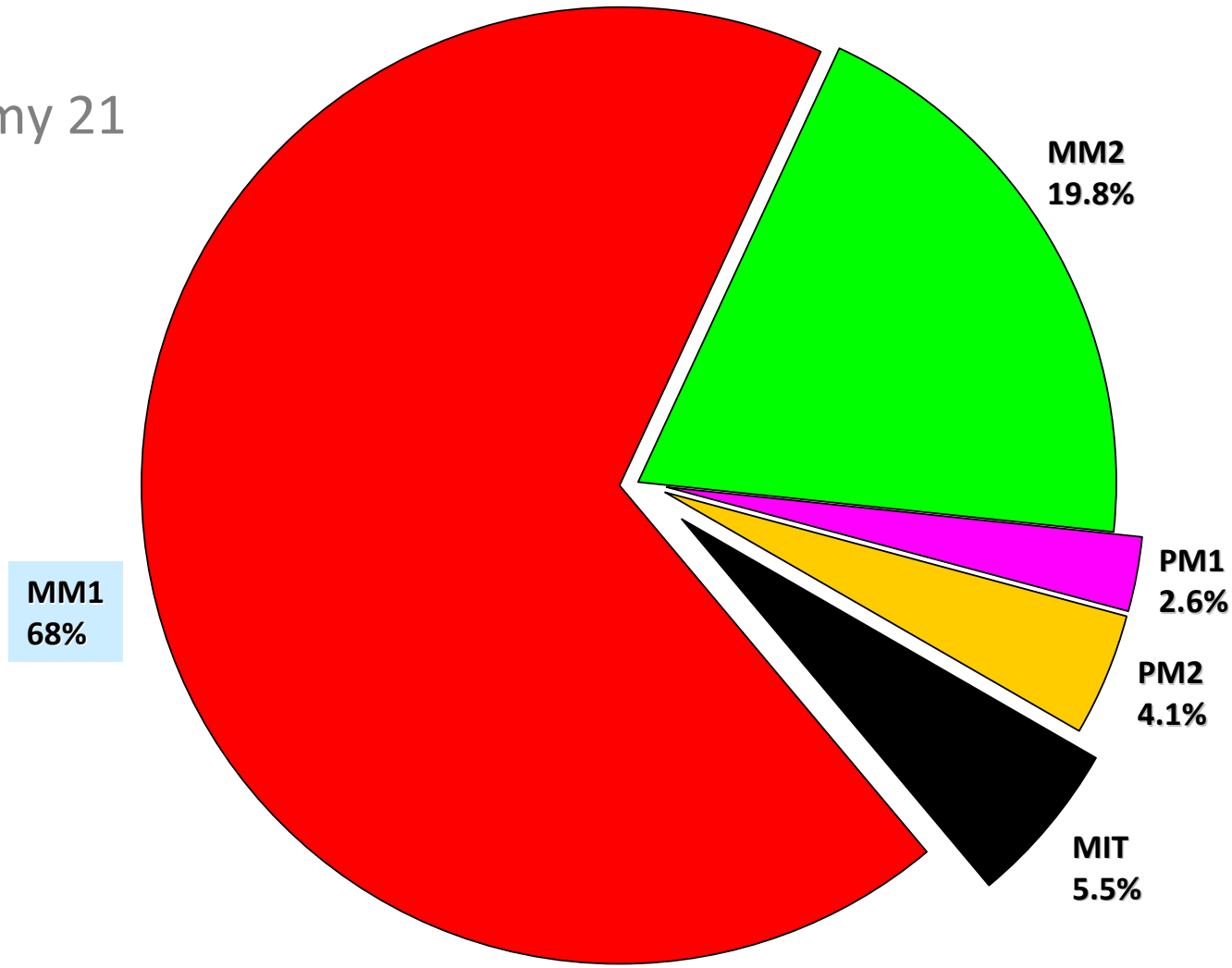

Analysis
Microscopy
Photograph
and
karyotype
Karyotype
Metaphase under the microscope



9/11 cytogenetics

9/11 NGS

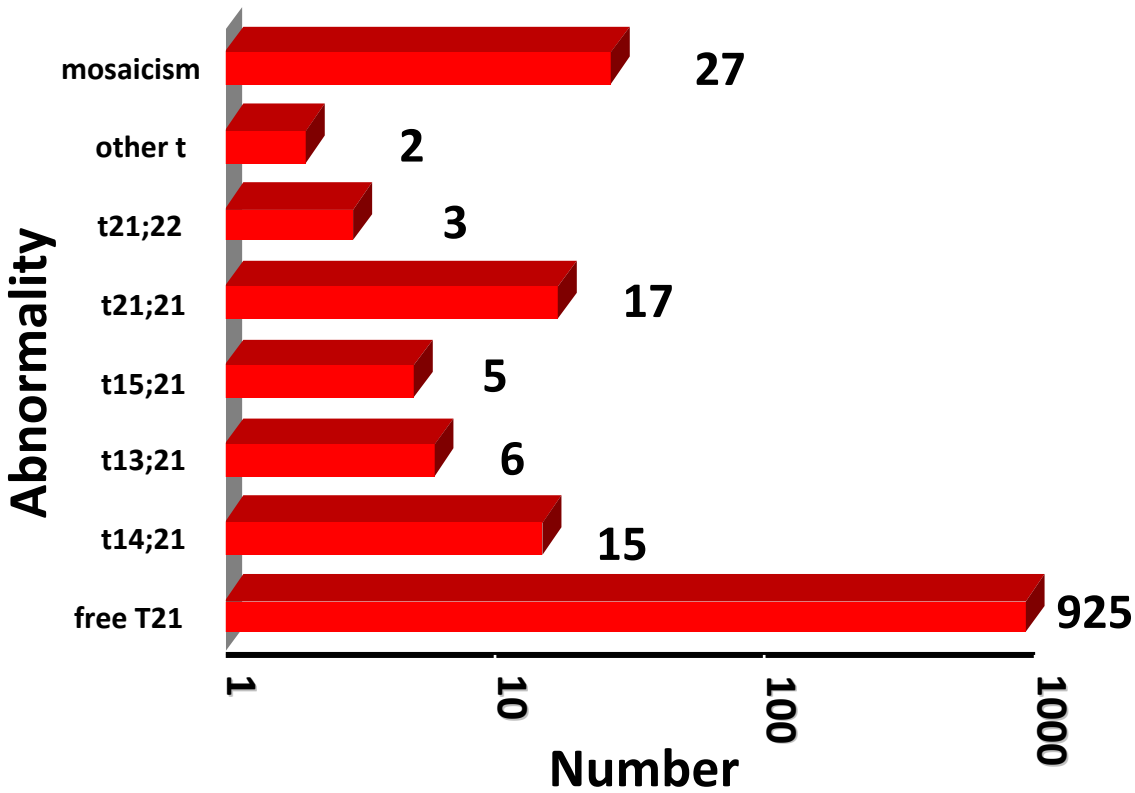
Origin of the extra chromosome 21 in 510 families with free trisomy 21

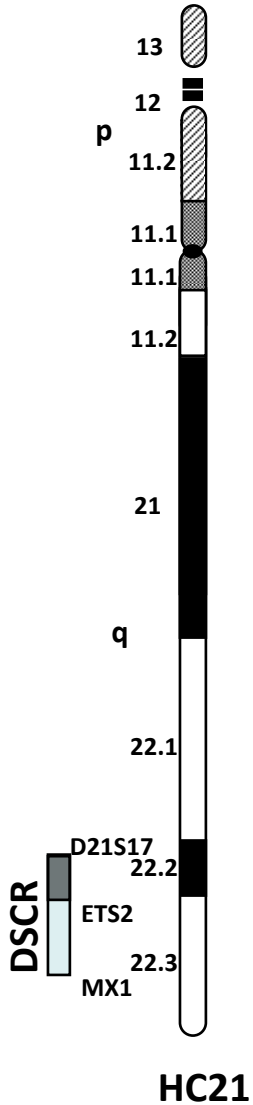


Data from the Antonarakis and Hassold laboratories

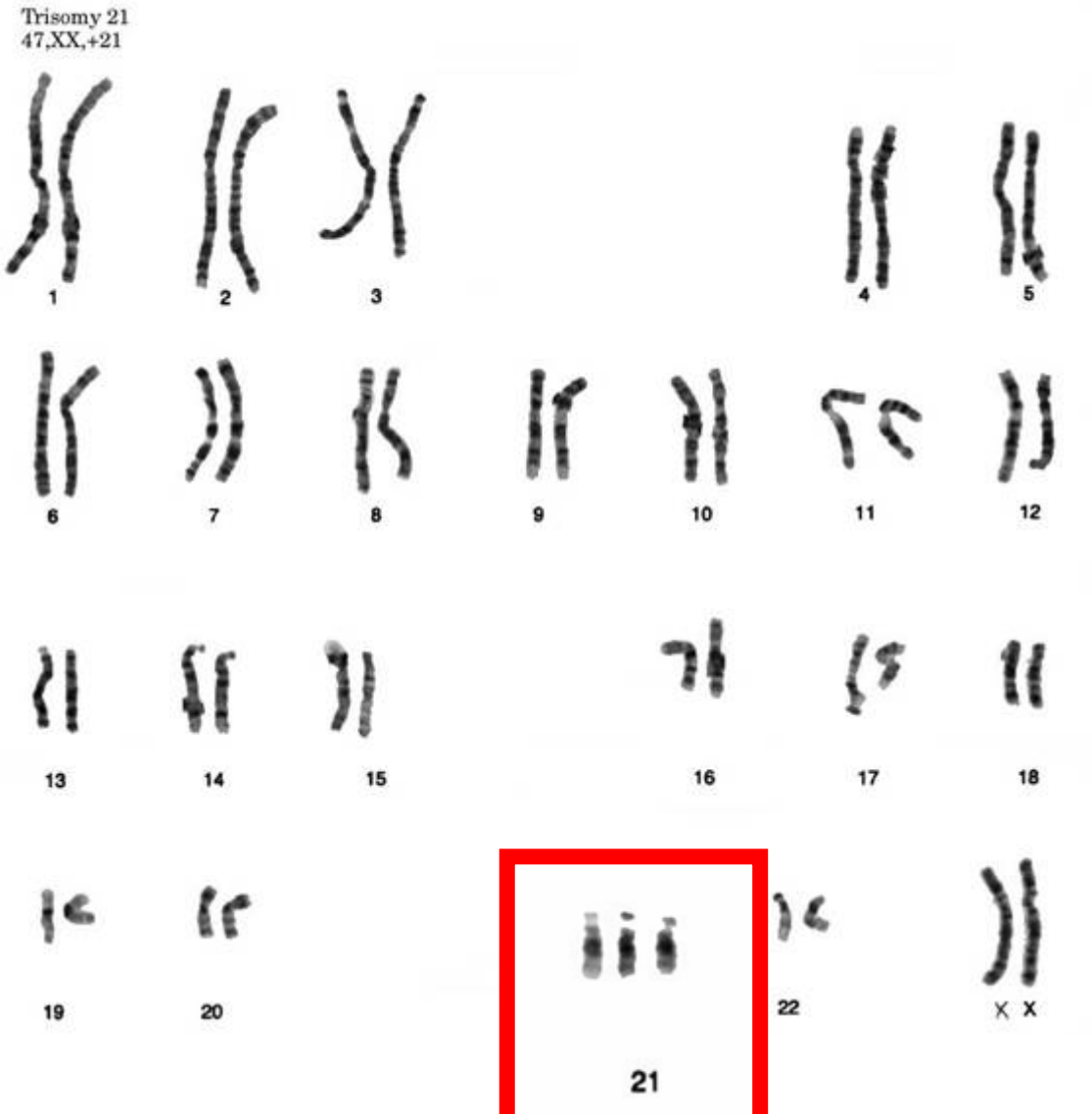
Chromosomal Abnormalities in Down Syndrome

- Most common chromosomal abnormality
- 1 in 700 live births





trisomy 21 Down s

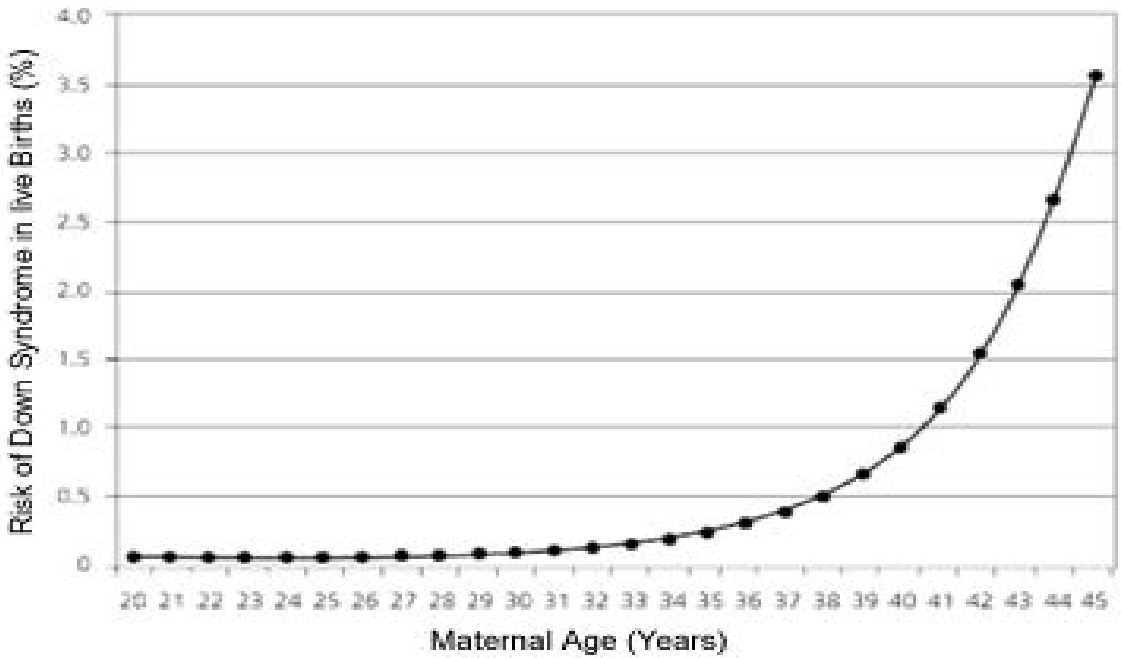


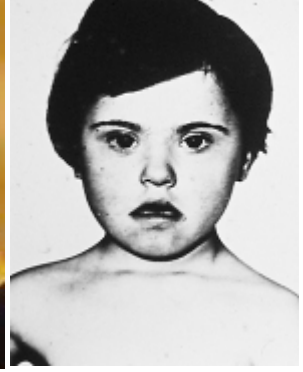
70% pregnancy loss

	Months											
Years	0	1	2	3	4	5	6	7	8	9	10	11
25	1376	1372	1367	1363	1358	1353	1348	1343	1338	1333	1328	1322
26	1317	1311	1306	1300	1294	1289	1283	1277	1271	1264	1258	1252
27	1245	1239	1232	1225	1219	1212	1205	1198	1191	1183	1176	1169
28	1161	1154	1146	1138	1130	1123	1115	1107	1099	1090	1082	1074
29	1065	1057	1048	1040	1031	1022	1014	1005	996	987	978	969
30	960	951	942	932	923	914	905	895	886	877	867	858
31	848	839	829	820	810	801	791	782	772	763	753	744
32	734	725	716	706	697	687	678	669	660	650	641	632
33	623	614	605	596	587	578	570	561	552	544	535	527
34	518	510	502	494	486	478	470	462	454	446	439	431
35	424	416	409	402	395	387	381	374	367	360	354	347
36	341	334	328	322	316	310	304	298	292	287	281	275
37	270	265	259	254	249	244	239	235	230	225	221	216
38	212	207	203	199	195	191	187	183	179	175	171	168
39	164	161	157	154	151	147	144	141	138	135	132	129
40	126	124	121	118	116	113	111	108	106	103	101	99
41	97	94	92	90	88	86	84	82	81	79	77	75
42	73	72	70	69	67	65	64	63	61	60	58	57
43	56	54	53	52	51	49	48	47	46	45	44	43
44	42	41	40	39	38	37	36	35	35	34	33	32
45	31	31	30	29	29	28	27	27	26	25	25	24

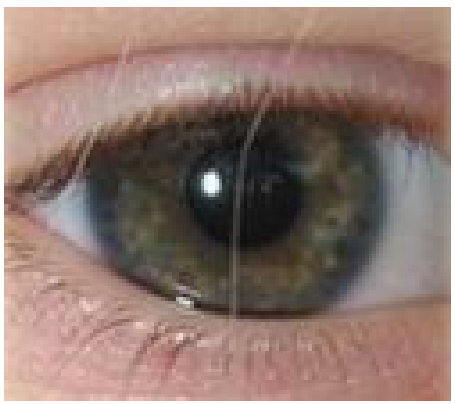
Risk of 1/239 having a child with Down s.

age	Frequency (live births)
< 35	< 0.3 %
37	0.5 %
40	1 %
50	10 %

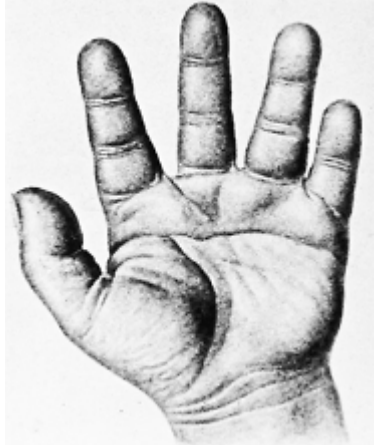




40,000 in Italy



Phenotype	%
Neurological :	
Mental retardation	100
Alzheimer disease over 35	100
Muscle :	
Hypotonia	100
Growth :	
Short stature	70
Head :	
Brachycephaly	75
Eyes :	
Epicanthic folds	60
Iris Brushfield spots	55
Mouth :	
Protruding tongue	45
Ears :	
Folded/dysplastic ear	50



RIGHT LEFT

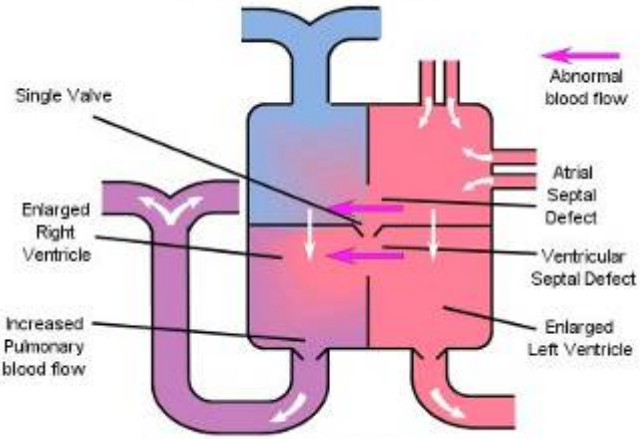
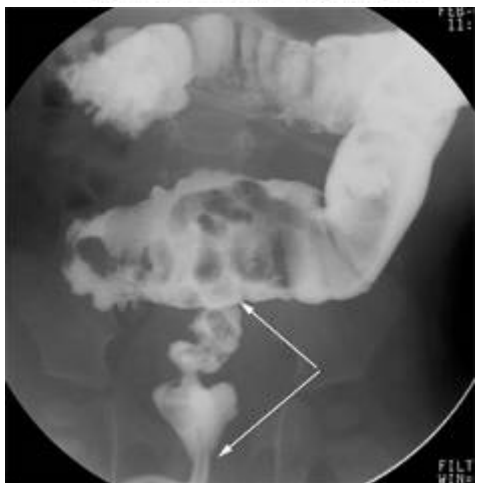
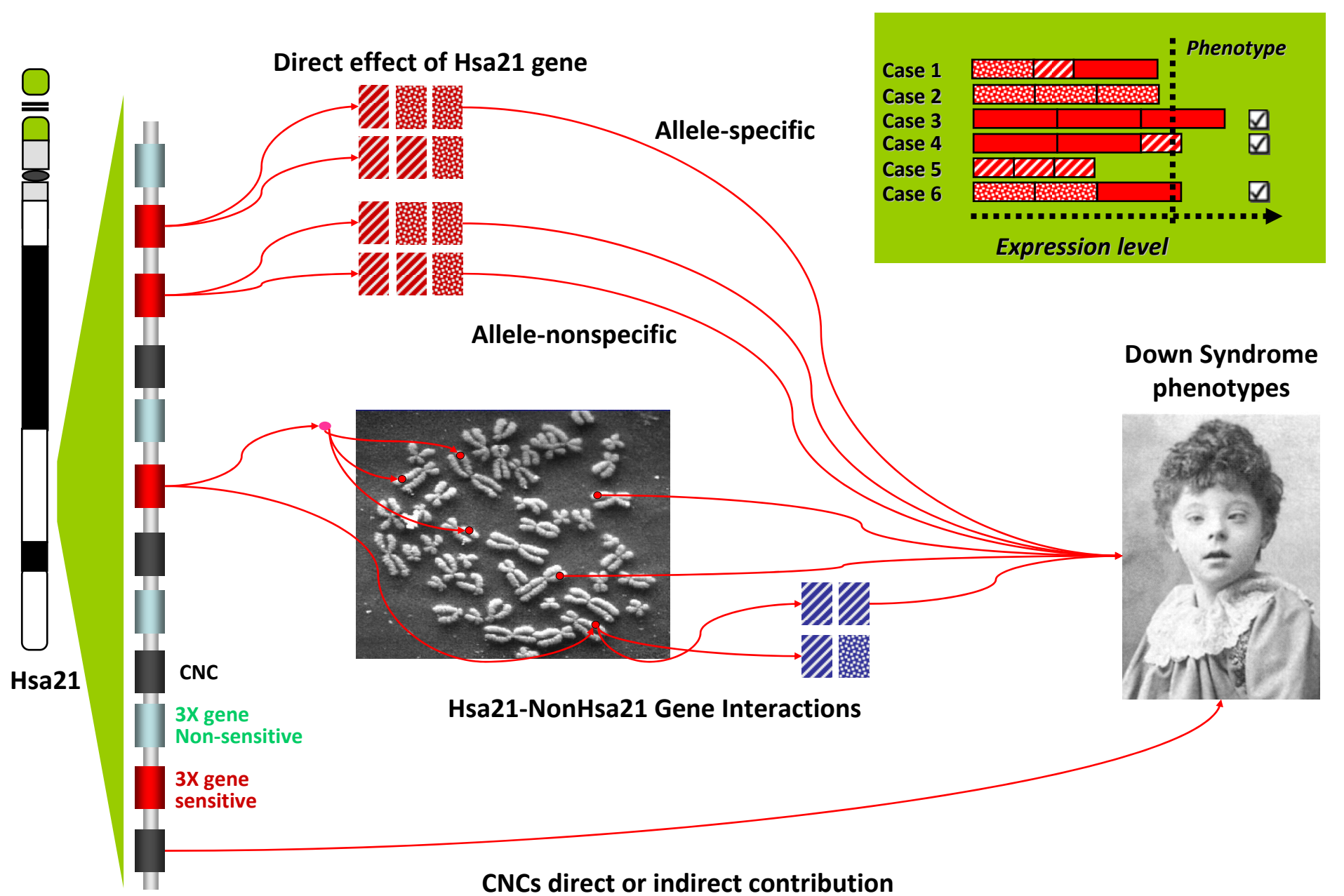


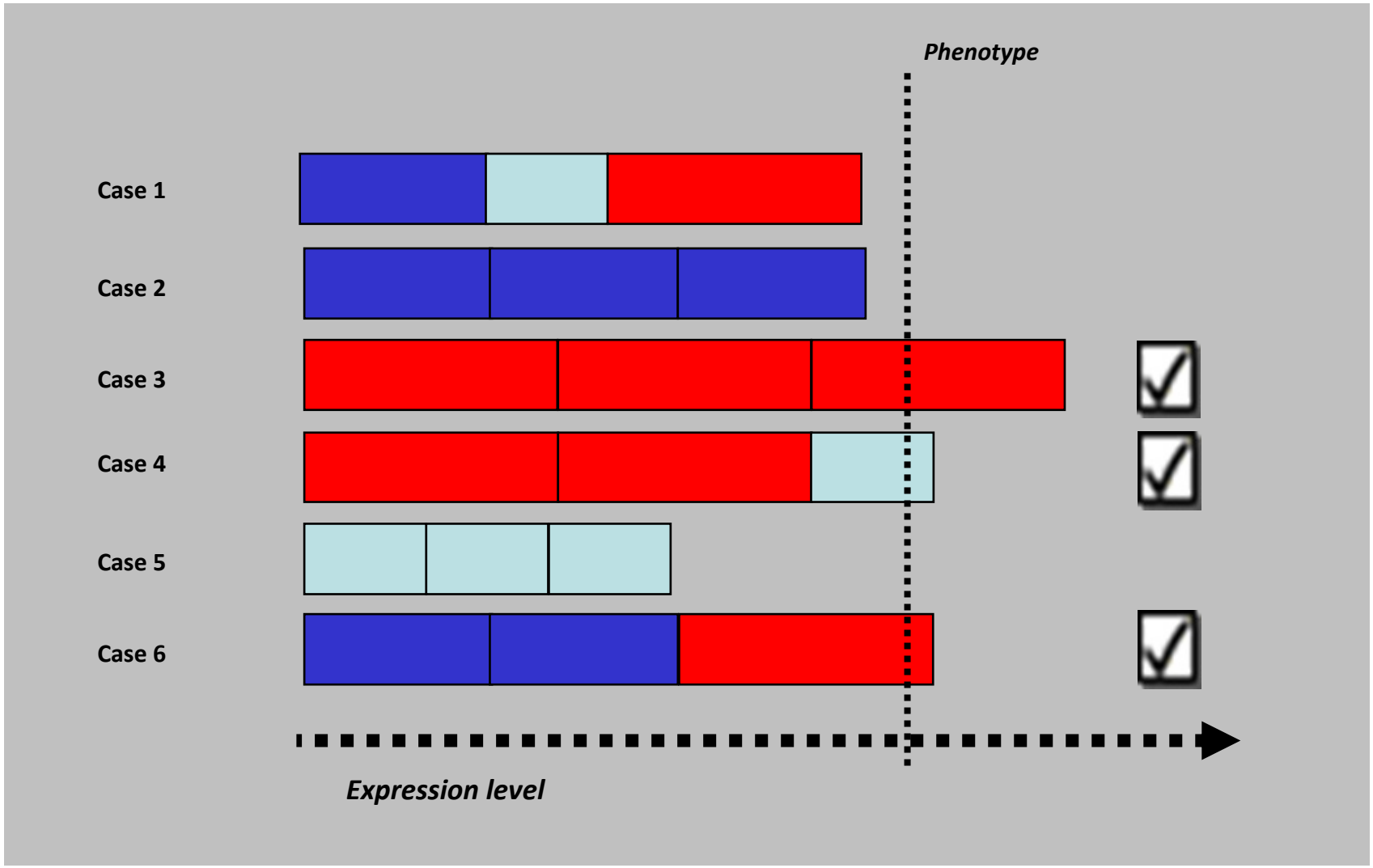
Diagram shows the heart looking at the patient



Phenotype	%
Limbs :	
Short, broad hands	65
Short 5th finger	60
Skin :	
Characteristic dermatoglyphics	85
Cardiac :	
Congenital heart defect	40
Atrioventricular canal	16
Gastrointestinal abnormalities :	
Duodenal stenosis/atresia	250x
Imperforate anus	50x
Hirschsprung disease	300x
Blood :	
Acute megakaryocytic leukemia	200-400x
Leukemia (both ALL and AML)	10-20x

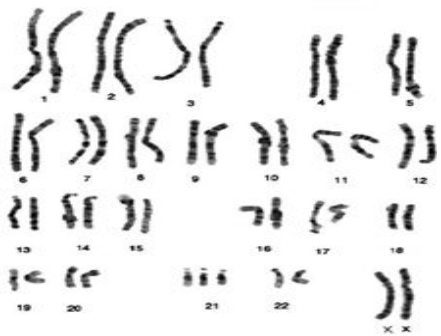


Models for the Pathogenesis of Down Syndrome

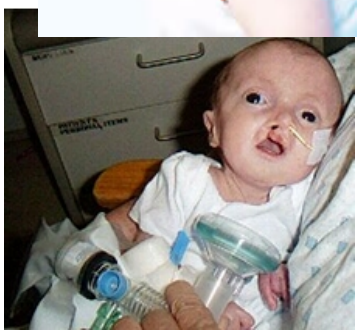


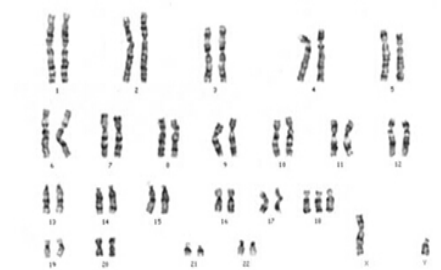
Gene Expression threshold hypothesis for the Pathogenesis of Down Syndrome

Trisomy 21, 18, 13 screening

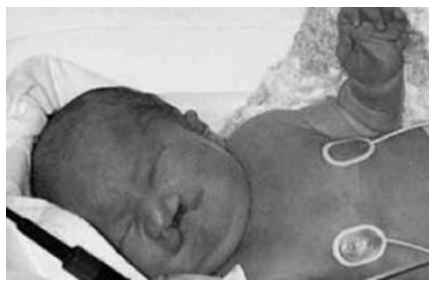


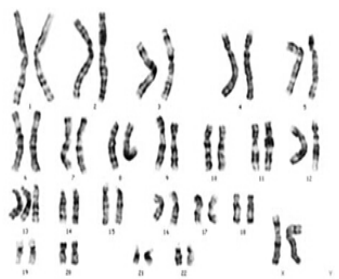
Trisomy 21 (Down syndrome)





Trisomy 18 (Edwards syndrome)





Trisomy 13 (Patau syndrome)

Classical Down syndrome screening

NT (mm)		PAPP-A (MoM)		B-HCG (MoM)	
Normal :	2.0	Normal :	1.0	Normal :	1.0
T21 :	3.4	T21 :	0.5	T21 :	2.0
T18 :	5.5	T18 :	0.2	T18 :	0.2
T13 :	4.0	T13 :	0.3	T13 :	0.5

Nicolaides et al 2008

NIPT

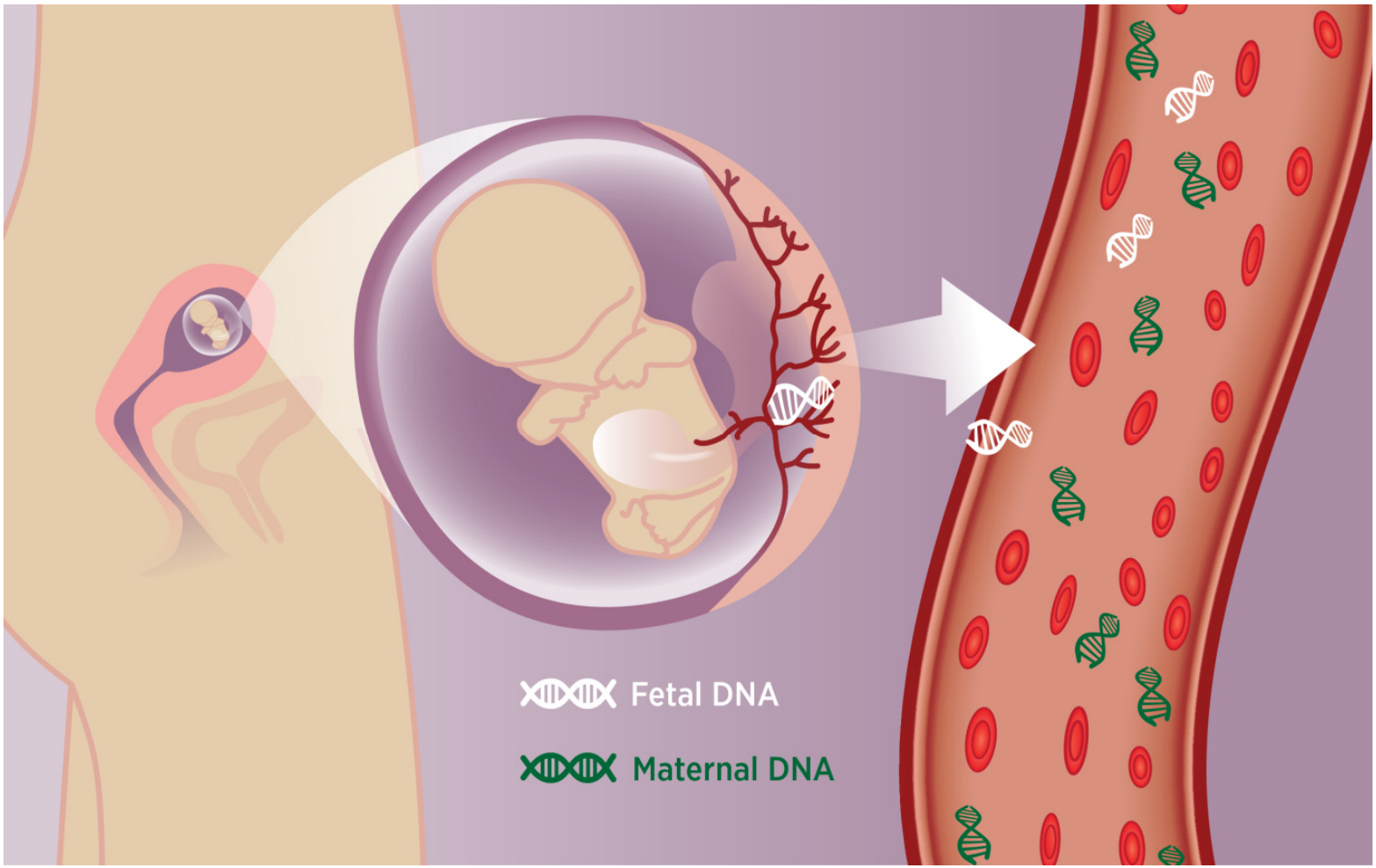
NON – **I**NVASIVE **P**RENATAL **T**ESTING

Testing of cff DNA (cell free fetal DNA)

From maternal blood during pregnancy starting from 10 weeks

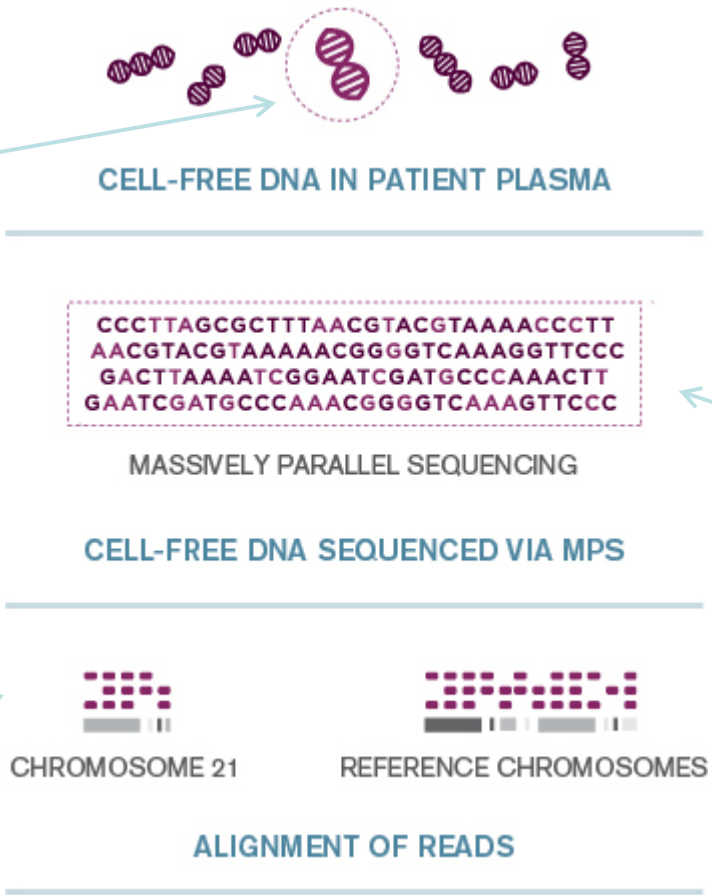
Useful for trisomies 13, 18 ,21

Cell Free Fetal DNA (cff DNA) in Maternal Blood



DNA SEQUENCING USING CELL FREE DNA

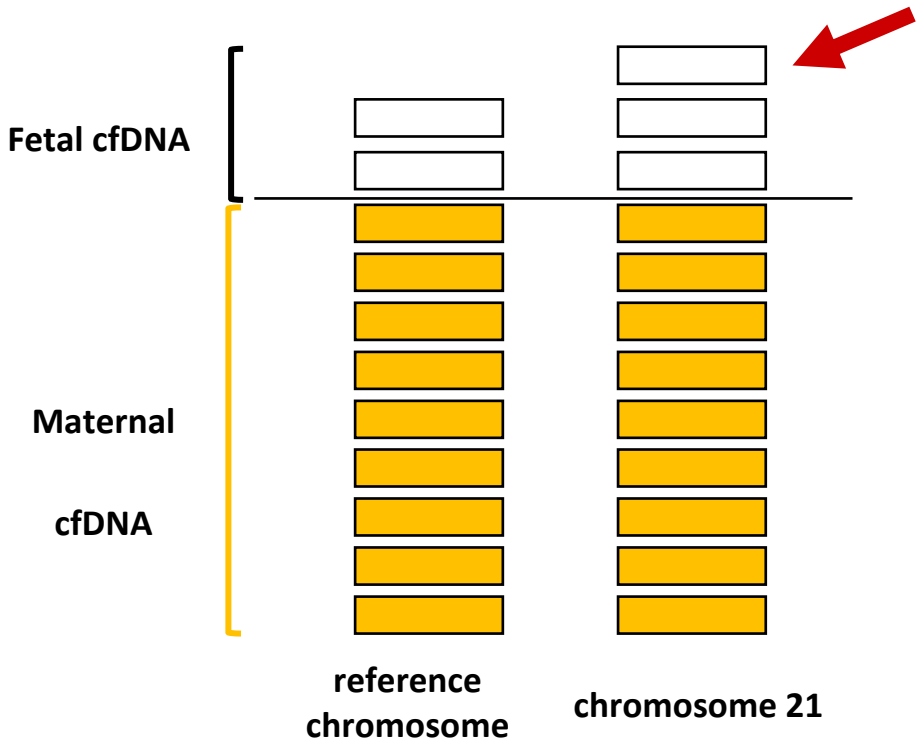
Fetal DNA fragments in maternal blood.



Cell free DNA fragments are then sequenced.

Compare the individual sequenced chromosomes against a reference for analysis.

importance of fetal fraction



Fetal Fraction	Expected ratio for Trisomy
4%	1.02
10%	1.05
20%	1.10
40%	1.20

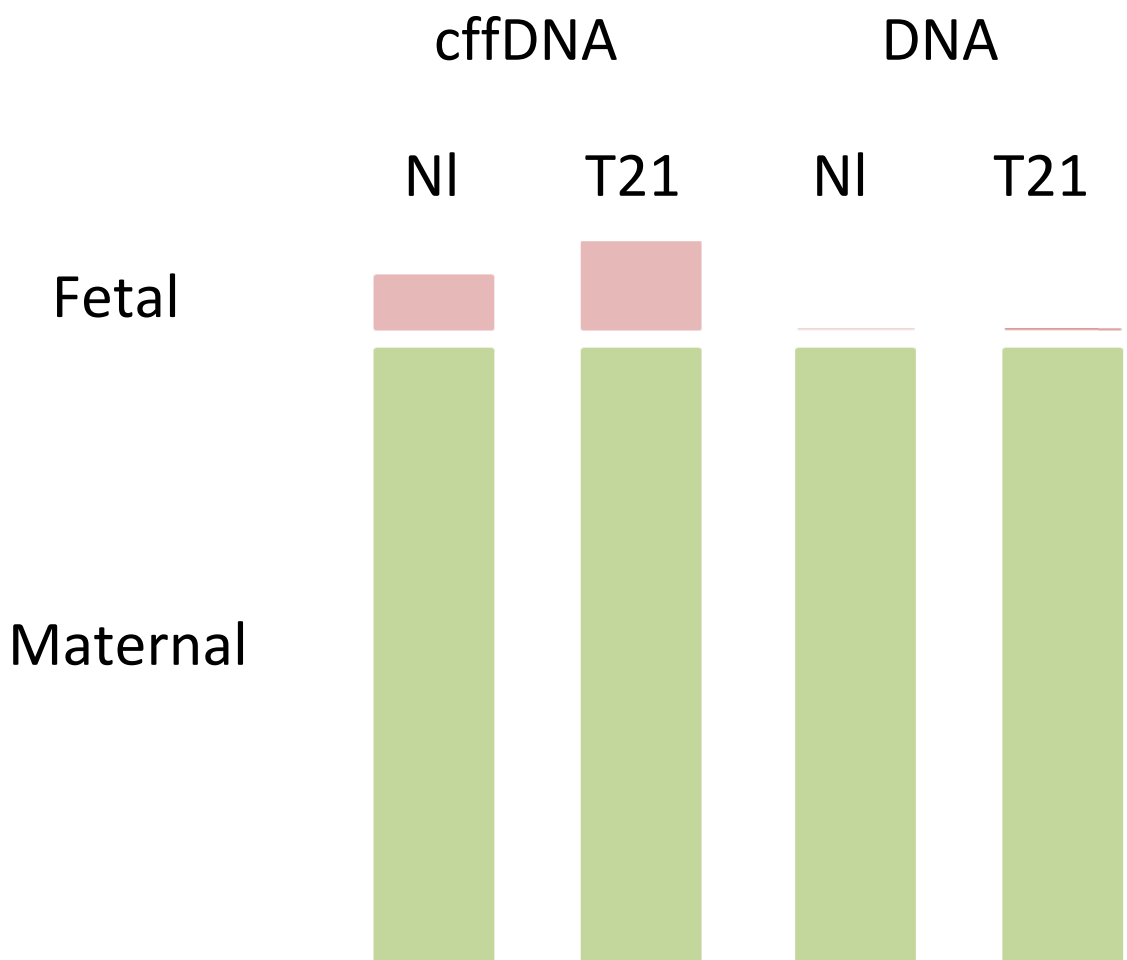
NIPT cff DNA

< 1 % of total DNA in maternal circulation is fetal

5-30 % of cell-free DNA in maternal circulation is fetal

NIPT measures the ratio of chromosome 21 sequence versus control chromosome sequence to exclude trisomy 21

NIPT cffDNA



Sensitivity NIPT for T21, T18, T13

Sensitivity

T21 : 99.5 %

T18 : 98 %

T13 : 90 %

False-negatives

If NIPT is normal, the residual risk for trisomy 21, 18, 13 : < 1 / 10.000

Specificity NIPT for T21, T18, T13

Specificity

T21 > 99.9 %

T18 > 99.9 %

T13 > 99.9 %

False-positives

If NIPT is abnormal, the risk that the fetus has no trisomy 21, 18, 13 :

small (high risk population)

? (low risk population)

NO NIPT for sex aneuploidies

- Phenotype for sex aneuploidies is highly variable
- Mosaicism in the fetus is a problem
- Mosaicism in the mother is a problem
- NIPT for sex aneuploidies is less accurate

NIPT Indications

NIPT is the test of choice when there is :

- Increased maternal age
- Increased risk on Combination or triple test
- Anxiety for invasive procedure (AC / CVS)

NIPT Contra indications

NIPT is NOT the test of choice when there is :

- Fetal anomalies on ultrasound
- A triplet pregnancy
- Vanished twin
- Known genetic anomalies that cannot be diagnosed by NIPT

NIPT Advantages versus combi test with AC / CVS

- High sensitivity (few false-negatives)
- High specificity (few false-positives)
- More than T21
- Non-invasive : no fetal risk
    - CVS : Risk of miscarriage : 1-2 %
    - AC : Risk of miscarriage : 0.5 %

NIPT Disadvantages

- Expensive (690 Euro)
  Combi test : 150 Euro
  Combitest + AC + karyotype : 1000 Euro ?
- Only testing 3 chromosomes, and gender
- Failure rate (after 1 or 2 tests): < 1 %
- Specific kits
- Not available everywhere

Companies offering NIPT

- ARIOSA (US)
- VERINATA (US)
- NATERA (US)
- SEQUENOM (US)
- BGI (China)
- LIFE-CODEXX (Germany)

NIPT results

1. **Normal result :** no specific follow up necessary,
   unless ultrasound examination of the fetus reveals anomalies

2. **Test failure :** in 3 % pregnancies not enough fetal DNA :
   NIPT repeated at no extra cost.

3. **Abnormal NIPT result :** amniocentesis or chorion biopsy

NIPT failures

If less than 4 % of cf DNA is fetal

1. **High amounts of maternal cf DNA :**

   Maternal obesitas

2. **Low amounts of fetal cf DNA :**

   - **Trisomy 18**
   - **Triploidy ??**

NIPT versus classical Down syndrome screening

	Classical	NIPT
False negatives	30 - 40 %	0.3 %
False positives	5 % (> 95 % of positives)	< 0.1 % (?)
Result	> Week 13	> Week 12
Price	150 euro	590 Euro

NIPT versus classical screening in a country with 10 million inhabitants

	Classical	NIPT
Number screenings	100.000	100.000
Expected T21	200 (1/500)	200 (1/500)
Detection rate	73 %	< 99 %
T21	**146**	**199**
False-negatives	**54 (27 %)**	**< 1 (0.3 %)**
False-positives	**4990 (4.8 %)**	**< 100 (0.03 %)**
Iatrogenic Miscarriage	**50**	**1**

NIPT : the future

1. Array CGH
   - All chromosomes
   - Small deletions - duplications

2. Detection common monogenic mutations
   - CF

3. Whole exome / genome sequencing