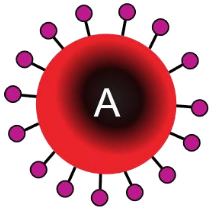
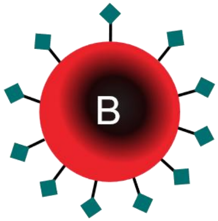
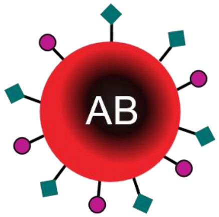
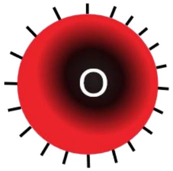
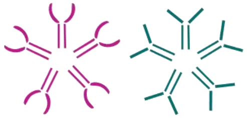
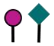
GENETICS AND POPULATION GENETICS

Genetic polymorphisms

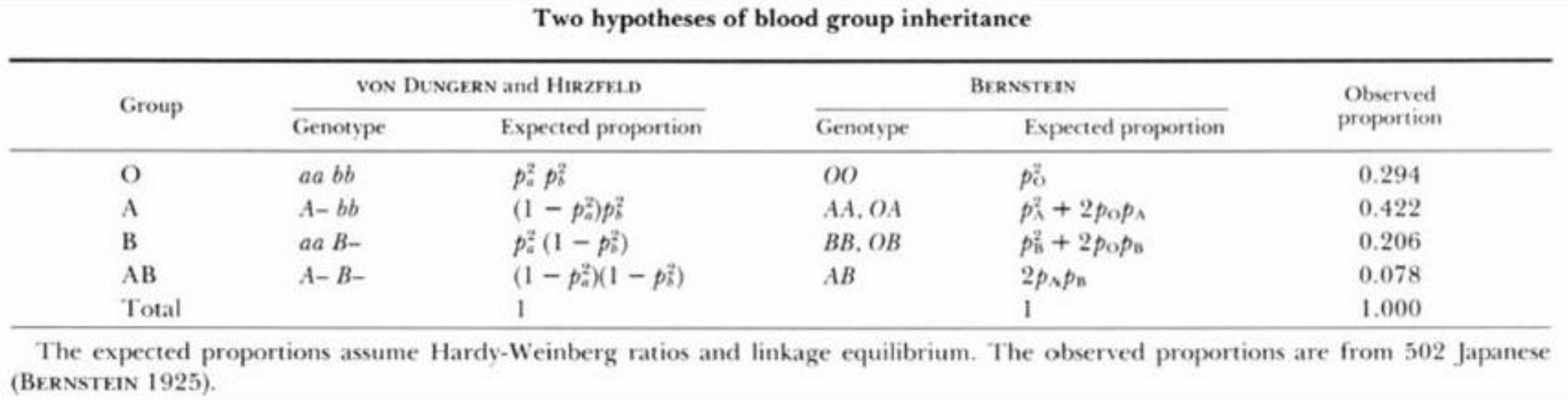
ELTE Faculty of Sciences Department of Genetics

First genetic marker: ABO blood group system

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	 A antigen	 B antigen	 A and B antigens	None

Landsteiner, 1900; Jan Jansky, W. Moss 1907;

FELIX BERNSTEIN (1933)



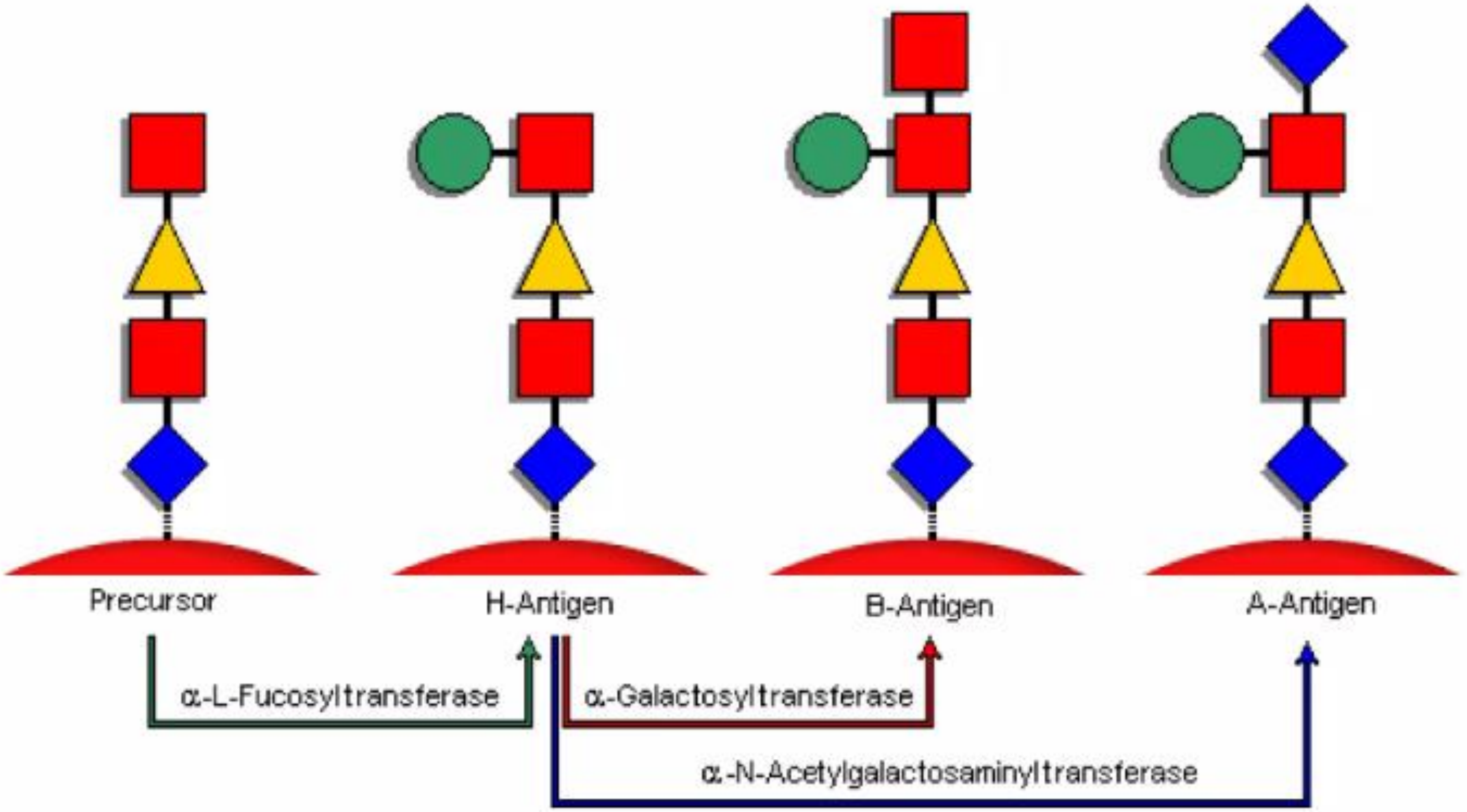
Two hypotheses of blood group inheritance

Group	VON DUNGERN and HIRZFELD		BERNSTEIN		Observed proportion
	Genotype	Expected proportion	Genotype	Expected proportion	
O	*aa bb*	$p_a^2\ p_b^2$	*OO*	p_O^2	0.294
A	*A– bb*	$(1 - p_a^2)p_b^2$	*AA, OA*	$p_A^2 + 2p_Op_A$	0.422
B	*aa B–*	$p_a^2\ (1 - p_b^2)$	*BB, OB*	$p_B^2 + 2p_Op_B$	0.206
AB	*A– B–*	$(1 - p_a^2)(1 - p_b^2)$	*AB*	$2p_Ap_B$	0.078
Total		1		1	1.000

The expected proportions assume Hardy-Weinberg ratios and linkage equilibrium. The observed proportions are from 502 Japanese (BERNSTEIN 1925).

Biochemistry of AB0-Antigens

● L-Fucose
■ D-Galactose
◆ N-Acetylgalactosamine
△ N-Acetylglucosamine



Precursor

H-Antigen

B-Antigen

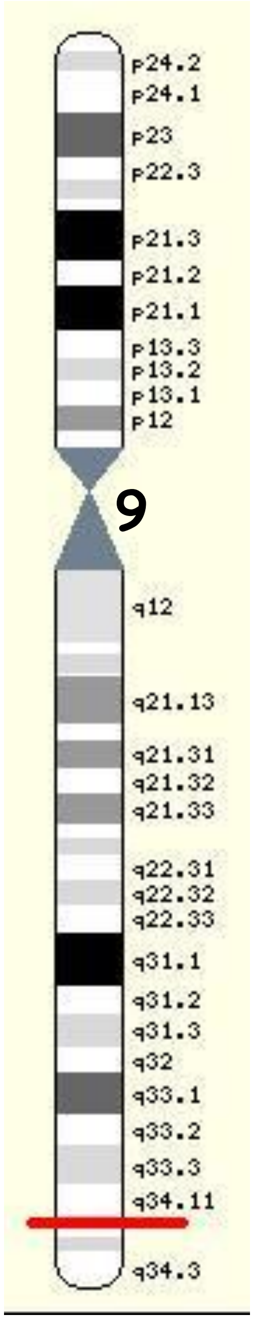
A-Antigen

α-L-Fucosyltransferase

α-Galactosyltransferase

α-N-Acetylgalactosaminyltransferase

http://www.usmlemcq.com/



Various Alleles at the ABO Locus

Exon Number	6		7														
Nucleotide Position	261	297	467	526	646	657	681	703	771	796	802	803	829	871	930	1054	1060
A alleles																	
A101	G	A	C	C	T	C	G	G	C	C	G	G	G	G	G	C	C
A102	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*
A201	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	Δ
A301	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*
Ax01	*	*	*	*	A	*	*	*	*	*	*	*	*	*	*	*	*
cis-AB01	*	*	T	*	*	*	*	*	*	*	*	C	*	*	*	*	*
B alleles																	
B101	*	G	*	G	*	T	*	A	*	A	*	C	*	*	A	*	*
B301	*	G	*	G	*	T	*	A	*	A	*	C	*	*	A	T	*
B(A)01	*	G	*	G	*	*	*	*	*	A	*	C	*	*	A	*	*
O alleles																	
O01	Δ	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
O02	Δ	G	*	*	A	*	A	*	T	*	*	*	A	*	*	*	*
O03	*	G	*	G	*	*	*	*	*	*	A	*	*	*	*	*	*
Possible Amino Acid Change	Frameshift	No change	P156L	R176G	F216I	No change	No change	G235S	No change	L266M	G268R	G268A	V277M	D291N	No change	R352W	Frameshift

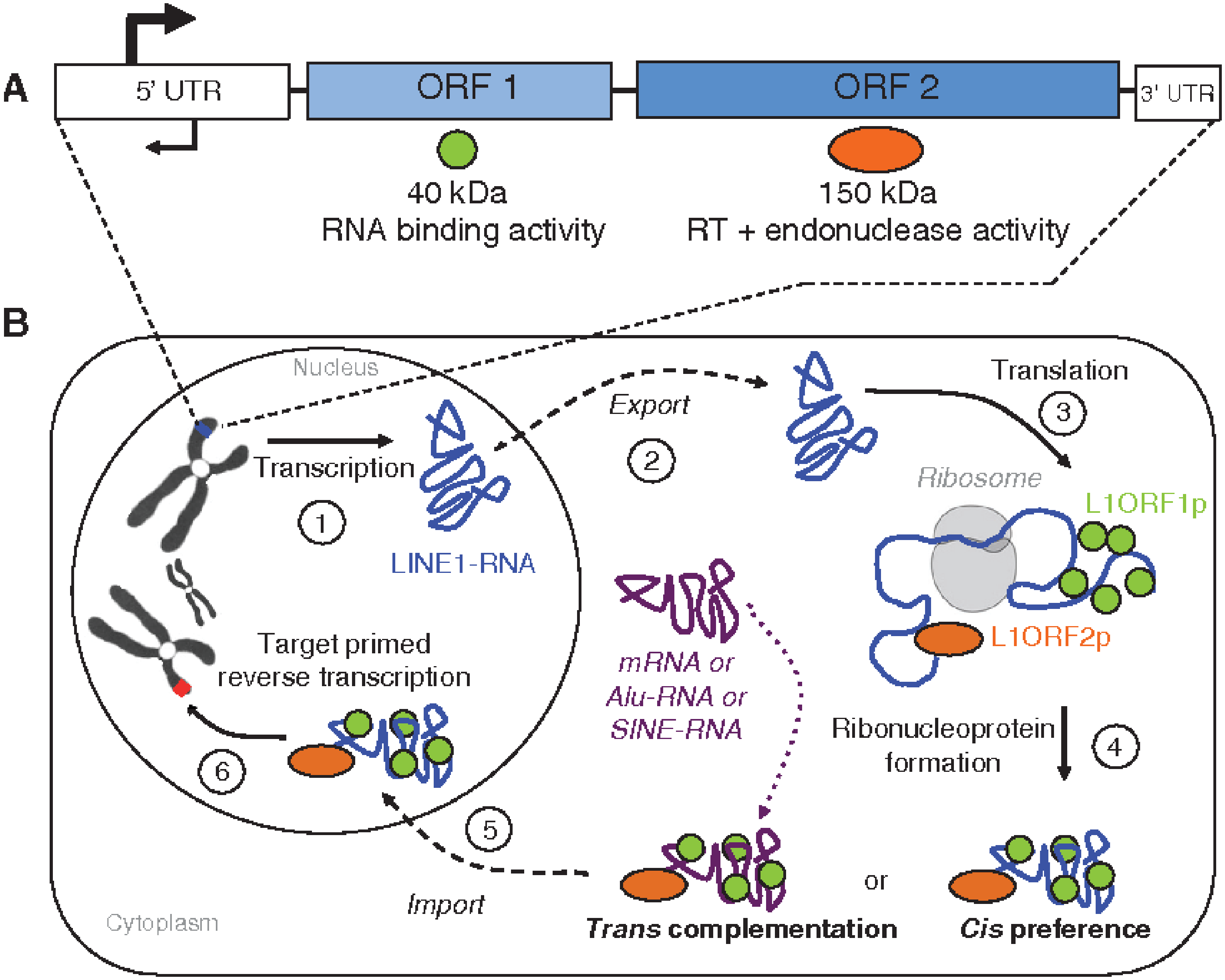
Alternative allele variation on the same gene coding a glycosyltransferase enzyme:

- Galactosyltransferase: **group B**
- N-Acetylgalactosaminyltransferase: **group A**
- Null allele (frameshift mutation): **group O**

ABO blood group belongs to pseudogenes

- Failed gene duplication events - noncoding DNA.
  - Nonprocessed pseudogene
- Processed pseudogenes: through mRNA transcript medation.
  - RT cDNA reintegration >> missing sequences >> dead-on-arrival.
  - in germ line: genes of standard metabolic function.
- Unitary pseudogenes:
  - Only one copy in the genome: usually inactivated genes.
  - Vitamin C producing gene: *L-glucono-φ-lactone oxidase*
- Polymorphic pseudogenes:
  - Active / inactive alleles segregating in the population.
  - *N-acetylgalactosaminyltransferase gene:* ABO blood group

An example to process pseudogene



Regulation of LINE-1 in mammals
DOI: https://doi.org/10.1515/bmc-2014-0018

Genetic variations

- Genes / Alleles (Mutation) >> Polymorphism
- Discontinuous variation
  - Mutation (Drosophila vestigal wings)
  - Polymorphism (ABO blood group)
- Continuous variation
  - Phenotypic gradiation (unbroken range of phenotype)
- Genotype / Phenotype: dominant / codominance
- Polymorphisms in gene DNA sequences:
- - base substitution / indel: SNP
- - tandemly repeated sequences: satellite DNA

DNA sequence based polymorphic sites

Sequence polymorphisms (SNP)

--------AGACTAGACATT--------

--------AGATTAGGCATT--------

Fragment length polymorphisms (VNTR, STR)

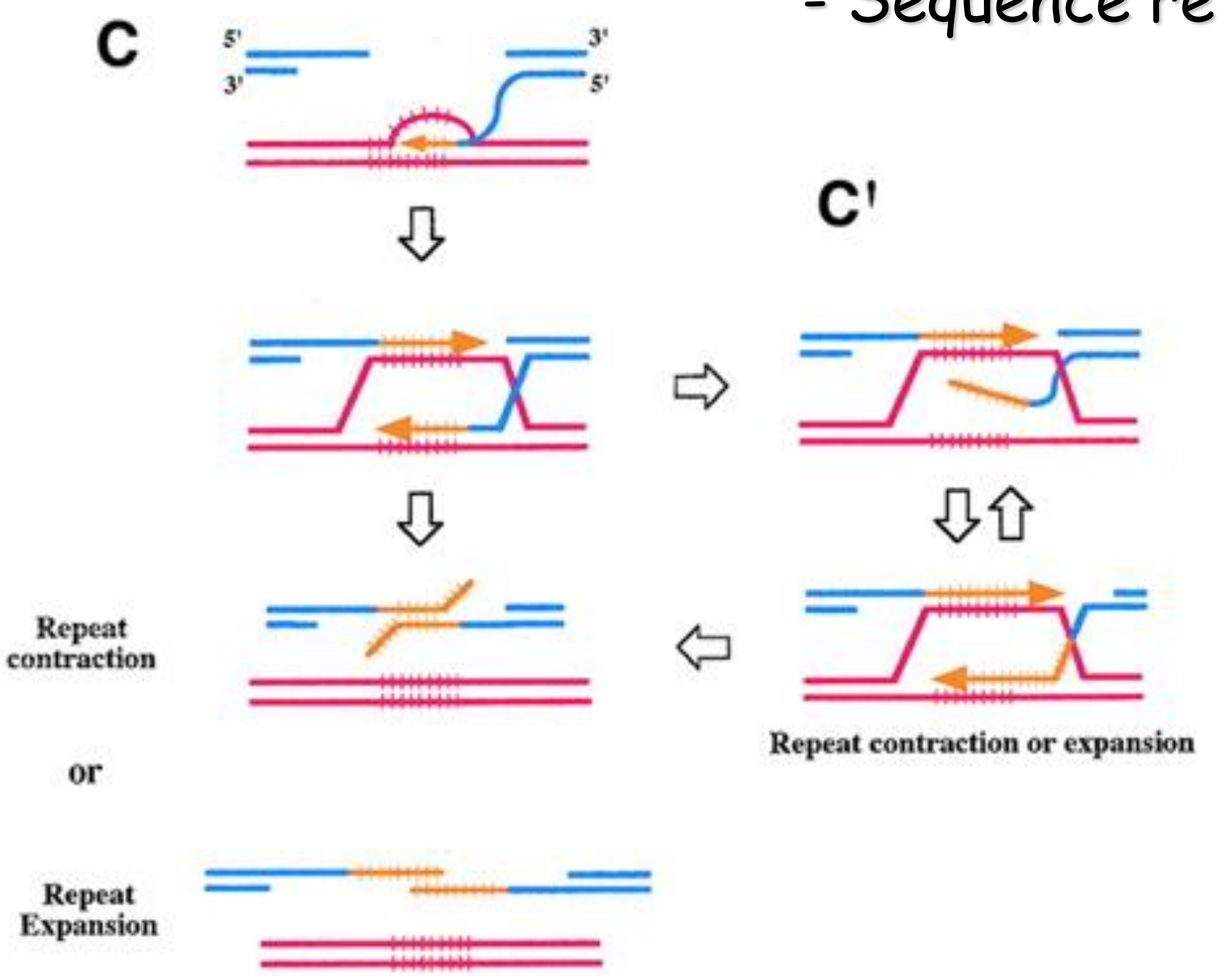
-----(AATG)(AATG)(AATG)-----

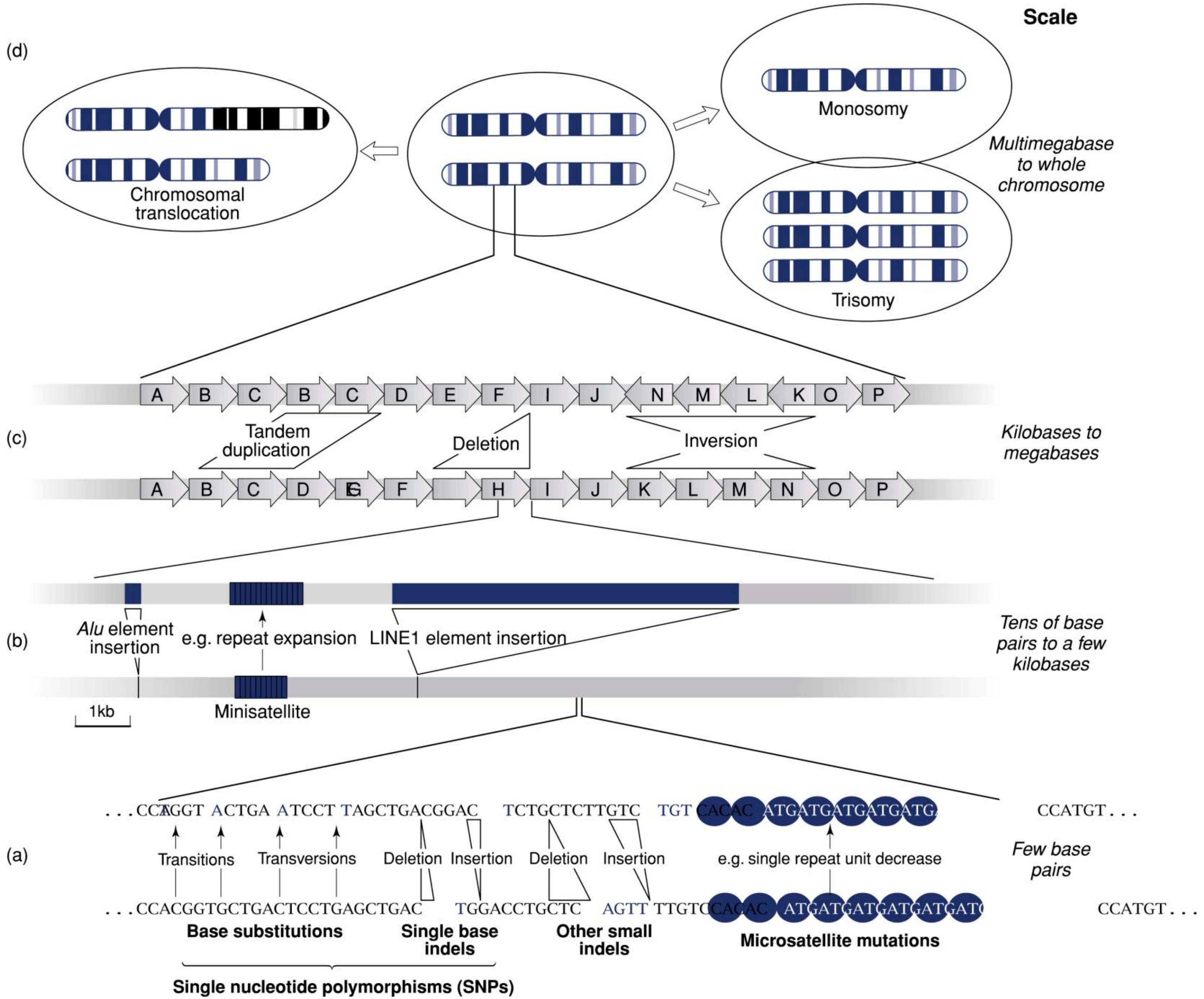
-----(AATG)(AATG)---------------

RECOMBINATION

Drive of polymorphisms:

- Single nucleotide mutation
- Sequence re-arrangement

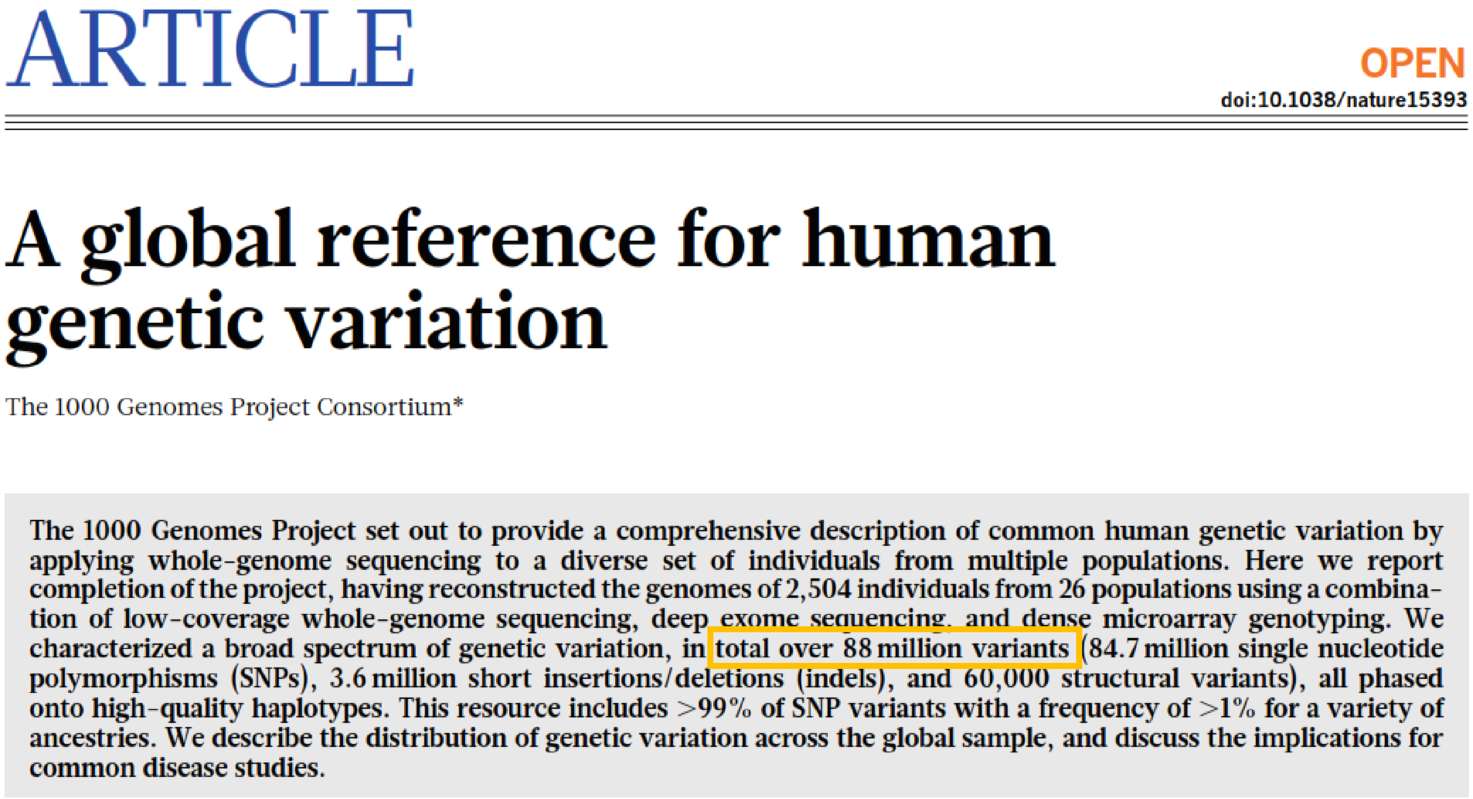



Scale
(d)
Chromosomal translocation
Monosomy
Trisomy
Multimegabase to whole chromosome
A B C B C D E F I J N M L K O P
(c)
Tandem duplication
Deletion
Inversion
A B C D E G F H I J K L M N O P
Kilobases to megabases
Alu element insertion
e.g. repeat expansion
LINE1 element insertion
(b)
1kb
Minisatellite
Tens of base pairs to a few kilobases
. . . CCATGGT ACTGA ATCCT TAGCTGACGGAC TCTGCTCTTGTC TGT CACAC ATGATGATGATGATG CCATGT . . .
(a)
Transitions
Transversions
Deletion
Insertion
Deletion
Insertion
e.g. single repeat unit decrease
Few base pairs
. . . CCACGGTGCTGACTCCTGAGCTGAC TGGACCTGCTC AGTT TTGTCCACAC ATGATGATGATGATGATG CCATGT . . .
Base substitutions
Single base indels
Other small indels
Microsatellite mutations
Single nucleotide polymorphisms (SNPs)

Human Evolutionary Genetics, Jobling, 2004

First results of Human Genome Project

- First draft in 2001 (Science, Nature)
- The most large whole genome determined
- Structure and organisation similare to each eukaryotes (model organizms)
- Unexpectedly low amount of protein coding genes (~20000)
- Emerging number of RNA genes (snRNA, lcnRNA, miRNA)
- Low amount of protein coding sequences (exons): < 1 %
- **Excess amount of repetitive sequences: Mobile elements?**



ARTICLE

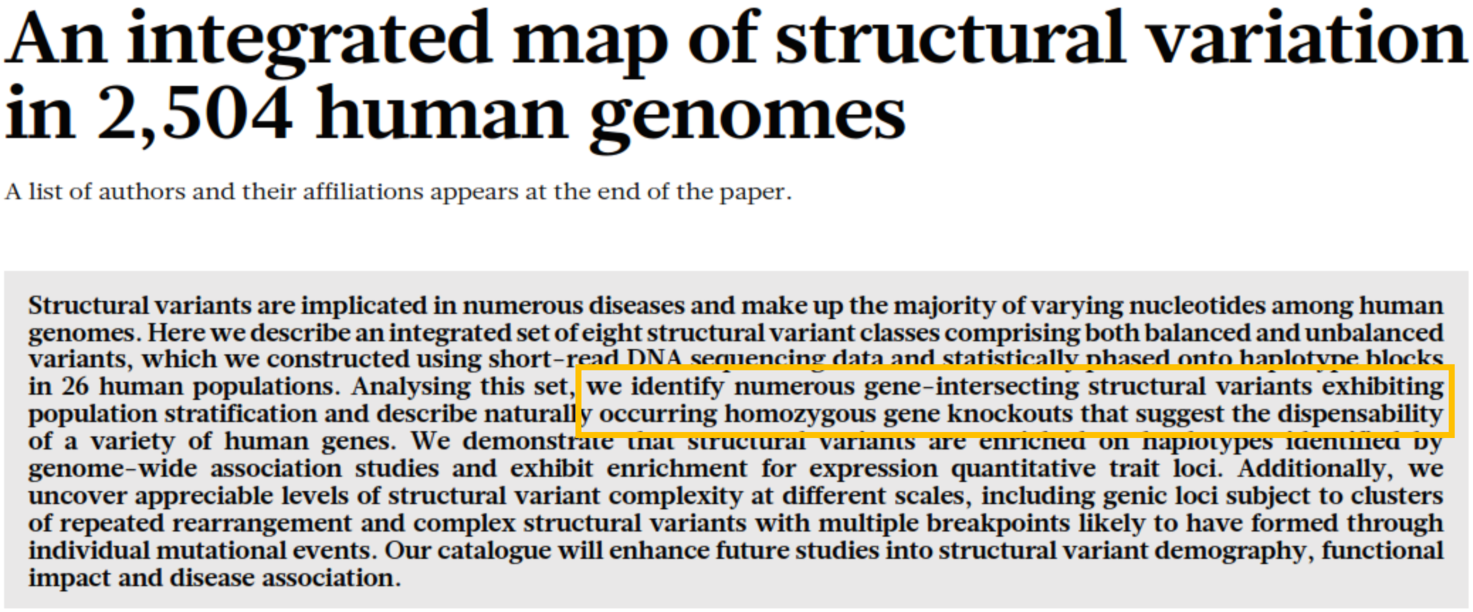
OPEN

doi:10.1038/nature15393

A global reference for human genetic variation

The 1000 Genomes Project Consortium*

The 1000 Genomes Project set out to provide a comprehensive description of common human genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations. Here we report completion of the project, having reconstructed the genomes of 2,504 individuals from 26 populations using a combination of low-coverage whole-genome sequencing, deep exome sequencing, and dense microarray genotyping. We characterized a broad spectrum of genetic variation, in total over 88 million variants (84.7 million single nucleotide polymorphisms (SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants), all phased onto high-quality haplotypes. This resource includes >99% of SNP variants with a frequency of >1% for a variety of ancestries. We describe the distribution of genetic variation across the global sample, and discuss the implications for common disease studies.

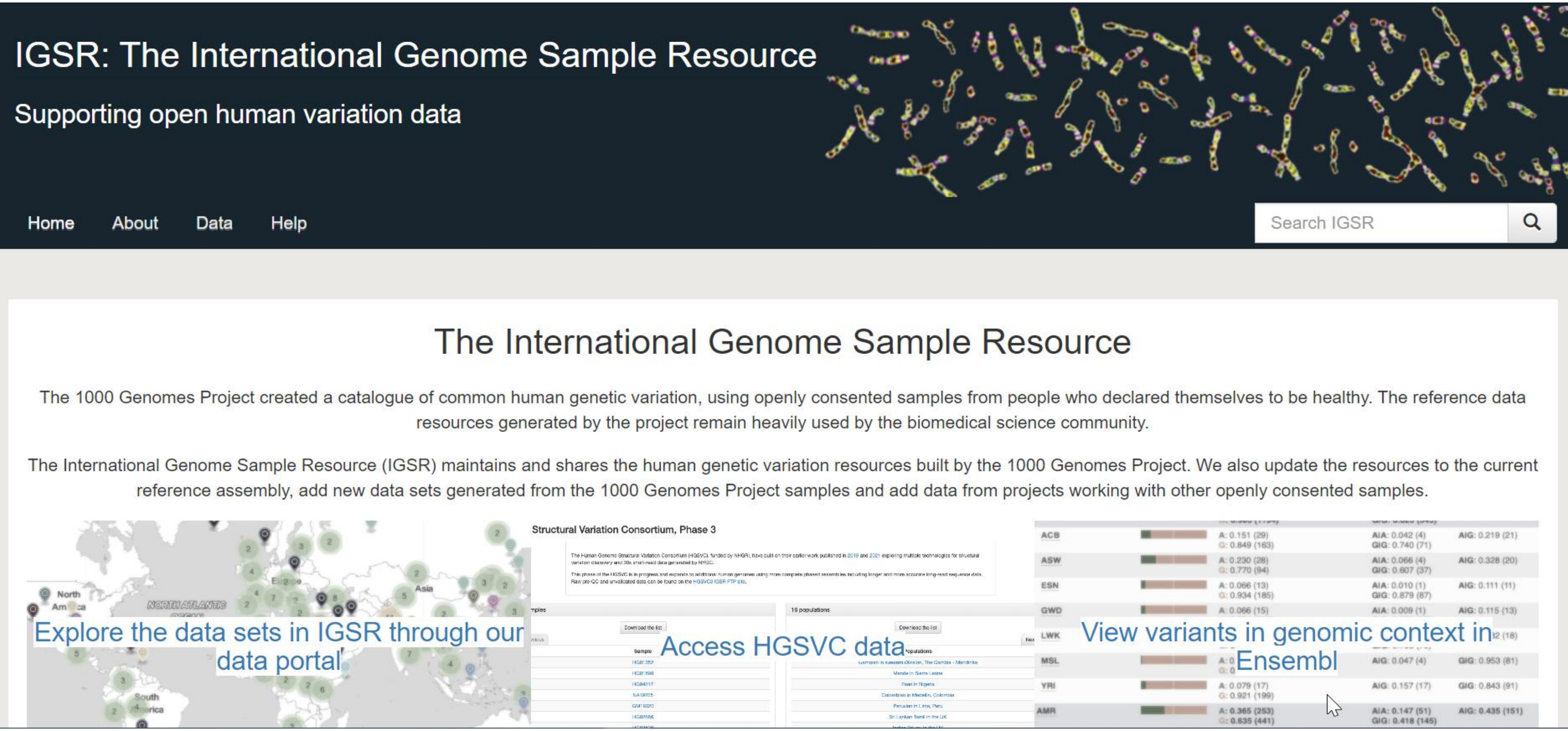


An integrated map of structural variation in 2,504 human genomes

A list of authors and their affiliations appears at the end of the paper.

Structural variants are implicated in numerous diseases and make up the majority of varying nucleotides among human genomes. Here we describe an integrated set of eight structural variant classes comprising both balanced and unbalanced variants, which we constructed using short-read DNA sequencing data and statistically phased onto haplotype blocks in 26 human populations. Analysing this set, we identify numerous gene-intersecting structural variants exhibiting population stratification and describe naturally occurring homozygous gene knockouts that suggest the dispensability of a variety of human genes. We demonstrate that structural variants are enriched on haplotypes identified by genome-wide association studies and exhibit enrichment for expression quantitative trait loci. Additionally, we uncover appreciable levels of structural variant complexity at different scales, including genic loci subject to clusters of repeated rearrangement and complex structural variants with multiple breakpoints likely to have formed through individual mutational events. Our catalogue will enhance future studies into structural variant demography, functional impact and disease association.

| NATURE | VOL 526 | 1 OCTOBER 2015



IGSR: The International Genome Sample Resource

Supporting open human variation data

The International Genome Sample Resource

The 1000 Genomes Project created a catalogue of common human genetic variation, using openly consented samples from people who declared themselves to be healthy. The reference data resources generated by the project remain heavily used by the biomedical science community.

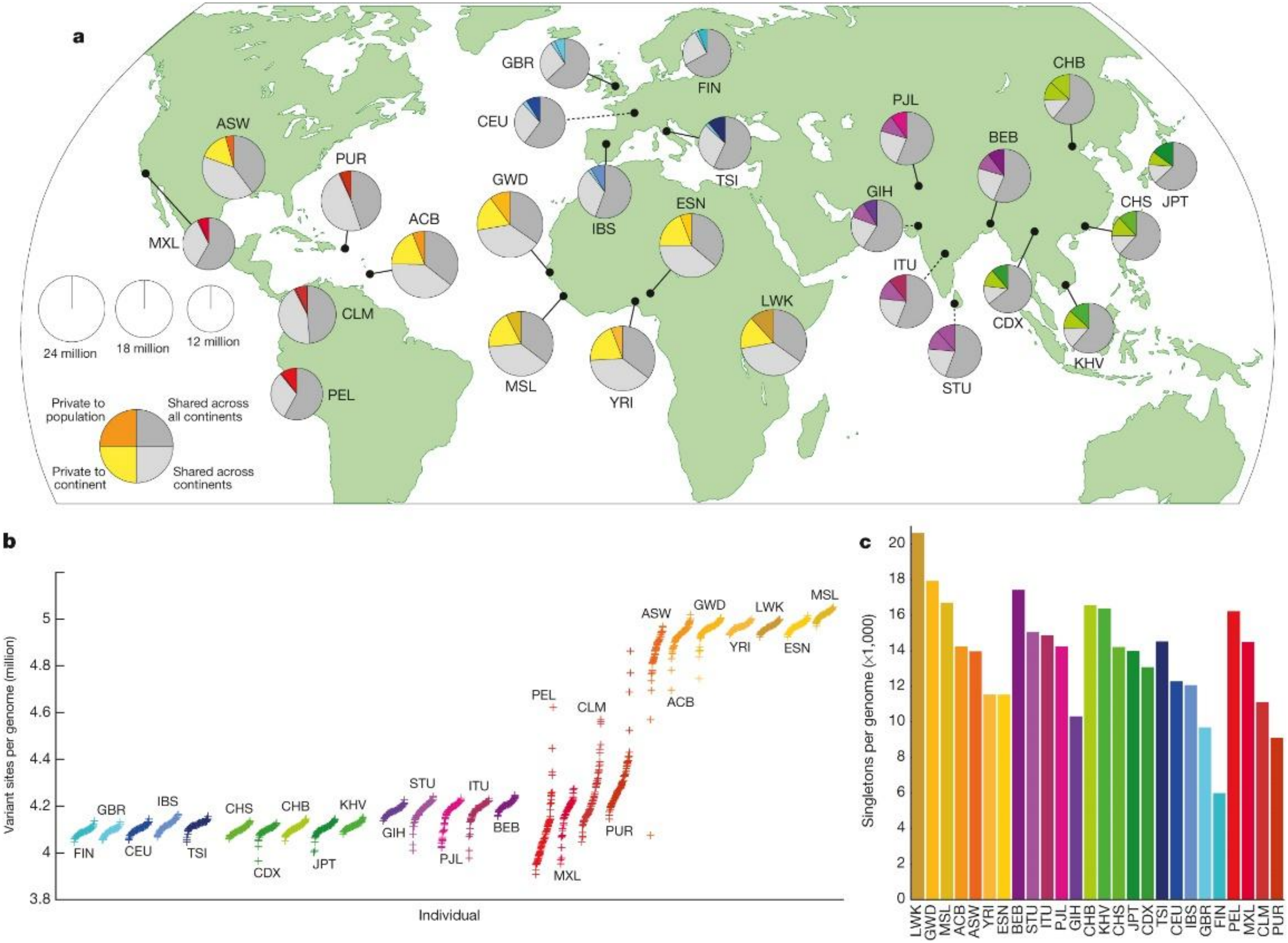
The International Genome Sample Resource (IGSR) maintains and shares the human genetic variation resources built by the 1000 Genomes Project. We also update the resources to the current reference assembly, add new data sets generated from the 1000 Genomes Project samples and add data from projects working with other openly consented samples.

Explore the data sets in IGSR through our data portal
Structural Variation Consortium, Phase 3
Access HGSVC data
View variants in genomic context in Ensembl

https://www.internationalgenome.org/

nature

Population sampling



nature

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doi:10.1038/nature15393

A global reference for human genetic variation

The 1000 Genomes Project Consortium*

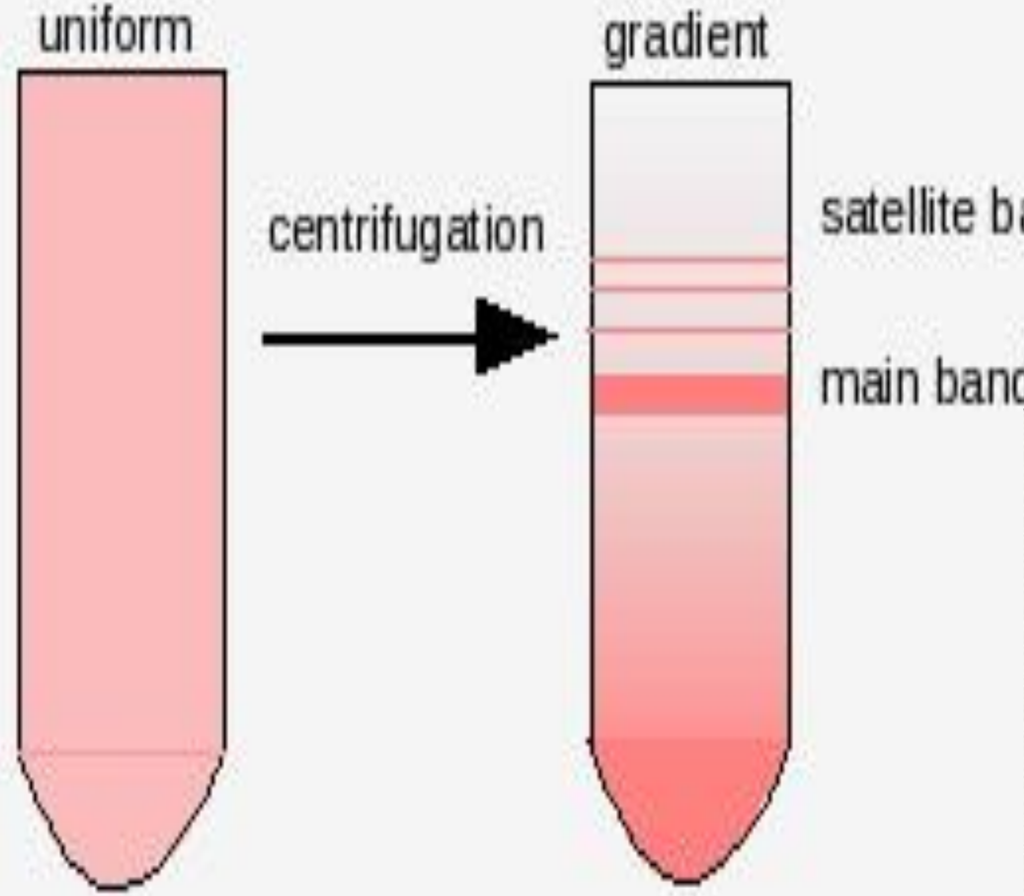
Table 1 | Median autosomal variant sites per genome

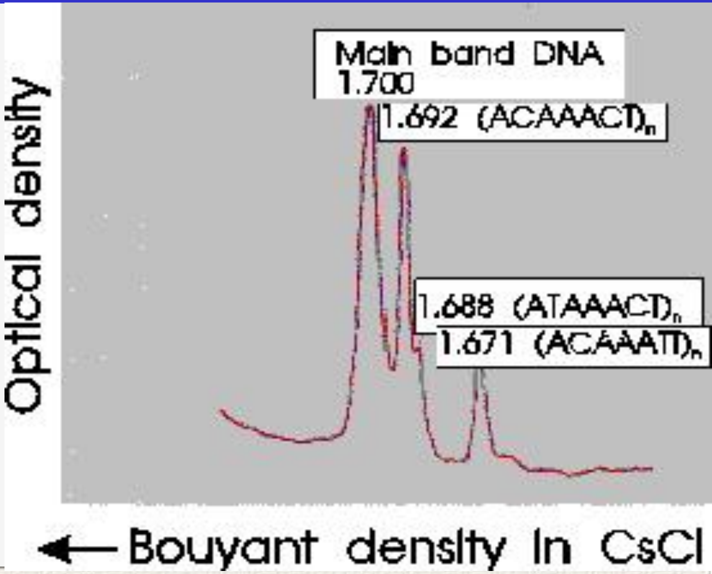
	AFR		AMR		EAS		EUR		SAS	
Samples	661		347		504		503		489	
Mean coverage	8.2		7.6		7.7		7.4		8.0	
	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons
SNPs	4.31M	14.5k	3.64M	12.0k	3.55M	14.8k	3.53M	11.4k	3.60M	14.4k
Indels	625k	-	557k	-	546k	-	546k	-	556k	-
Large deletions	1.1k	5	949	5	940	7	939	5	947	5
CNVs	170	1	153	1	158	1	157	1	165	1
MEI (Alu)	1.03k	0	845	0	899	1	919	0	889	0
MEI (L1)	138	0	118	0	130	0	123	0	123	0
MEI (SVA)	52	0	44	0	56	0	53	0	44	0
MEI (MT)	5	0	5	0	4	0	4	0	4	0
Inversions	12	0	9	0	10	0	9	0	11	0
Nonsynon	12.2k	139	10.4k	121	10.2k	144	10.2k	116	10.3k	144
Synon	13.8k	78	11.4k	67	11.2k	79	11.2k	59	11.4k	78
Intron	2.06M	7.33k	1.72M	6.12k	1.68M	7.39k	1.68M	5.68k	1.72M	7.20k
UTR	37.2k	168	30.8k	136	30.0k	169	30.0k	129	30.7k	168
Promoter	102k	430	84.3k	332	81.6k	425	82.2k	336	84.0k	430
Insulator	70.9k	248	59.0k	199	57.7k	252	57.7k	189	59.1k	243
Enhancer	354k	1.32k	295k	1.05k	289k	1.34k	288k	1.02k	295k	1.31k
TFBSs	927	4	759	3	748	4	749	3	765	3
Filtered LoF	182	4	152	3	153	4	149	3	151	3
HGMD-DM	20	0	18	0	16	1	18	2	16	0
GWAS	2.00k	0	2.07k	0	1.99k	0	2.08k	0	2.06k	0
ClinVar	28	0	30	1	24	0	29	1	27	1

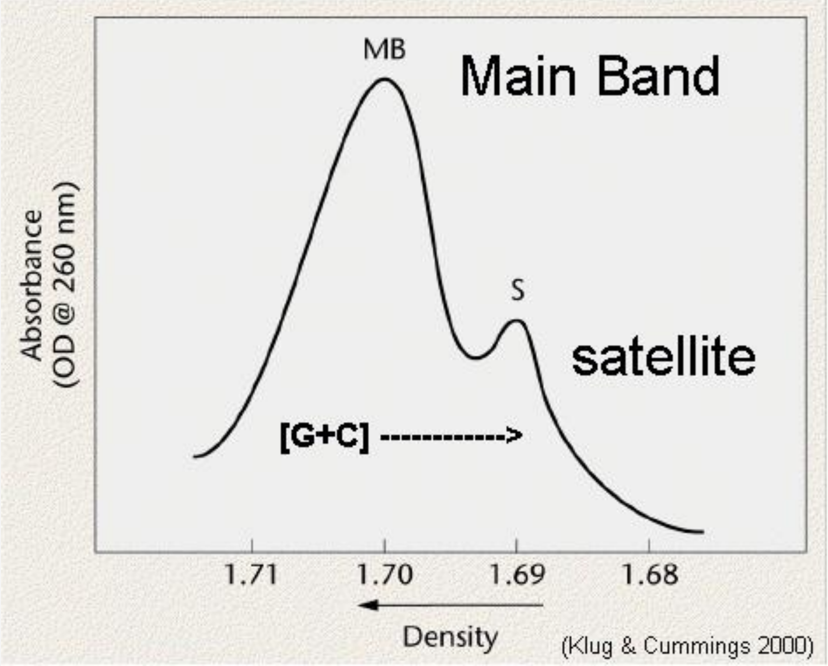
See Supplementary Table 1 for continental population groupings. CNVs, copy-number variants; HGMD-DM, Human Gene Mutation Database disease mutations; k, thousand; LoF, loss-of-function; M, million; MEI, mobile element insertions.

- a typical genome differs from the reference human genome at 4.1 million to 5.0 million sites.
- >99.9% of variants consist of SNPs and short indels.
- structural variants affect more bases:
- typical genome contains an estimated 2,100 to 2,500 structural variants (1,000 large deletions, 160 copy-number variants, 915 Alu insertions, 128 L1 insertions, 51 SVA insertions, 4 NUMTs and 10 inversions) affecting 20 million bases of sequence.

Satellite DNA







(Klug & Cummings 2000)

Restriction Fragment Length Polymorphism (RFLP) - „DNA fingerprinting"

Double-stranded DNA

Restriction enzymes

Gel electrophoresis

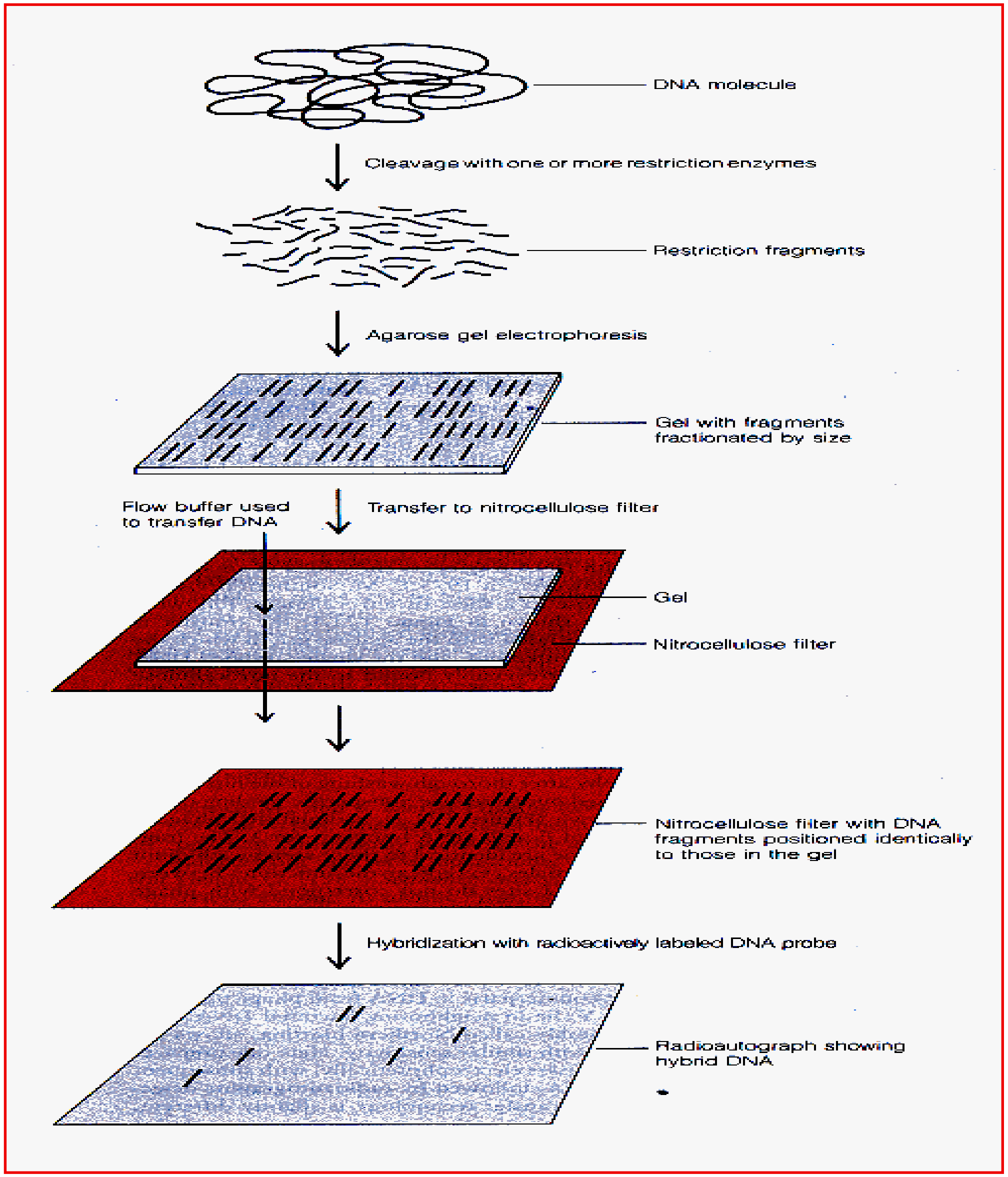
Southern-blot

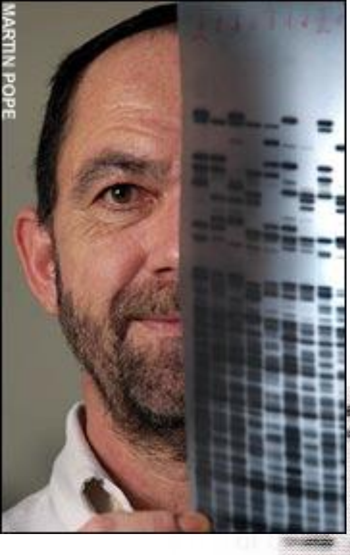
Probe hibridization

Autoradiogram

- MLP-RFLP

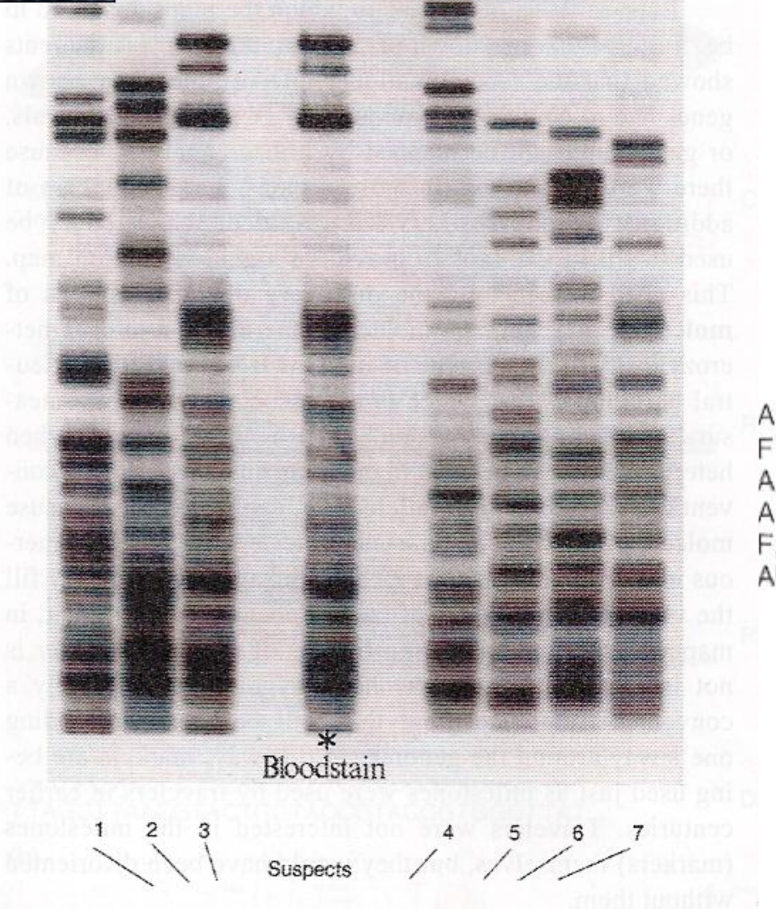
- SLP-RFLP

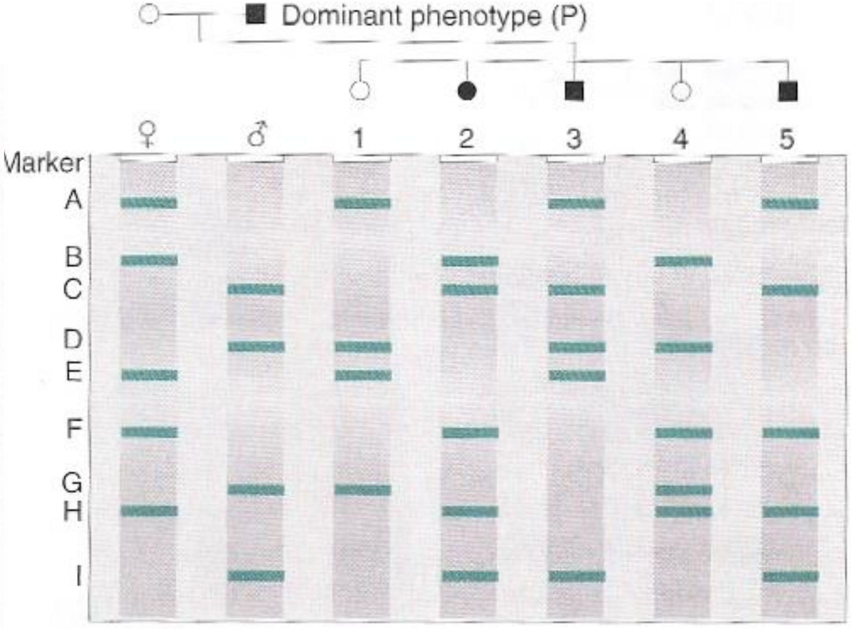

DNA molecule
Cleavage with one or more restriction enzymes
Restriction fragments
Agarose gel electrophoresis
Gel with fragments fractionated by size
Flow buffer used to transfer DNA
Transfer to nitrocellulose filter
Gel
Nitrocellulose filter
Nitrocellulose filter with DNA fragments positioned identically to those in the gel
Hybridization with radioactively labeled DNA probe
Radioautograph showing hybrid DNA



VNTR assay markers: RFLP analytics

1985 – Sir Alec Jeffreys





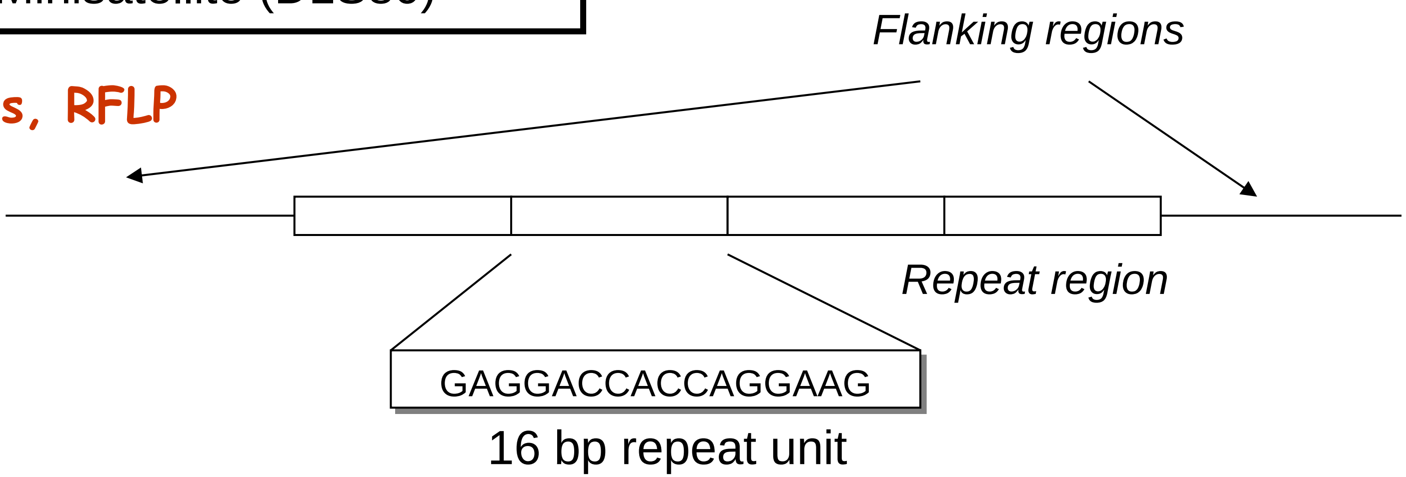
ANALYSIS EXAMPLES

F and H	Always inherited together — linked?
A and B	In progeny, always *either* A *or* B — "allelic"?
A and D	Four combinations; A and D, A, D, or neither — unlinked?
F, H, and E	Always *either* F and H *or* E — closely linked in trans?
Allele *P*	Possibly linked to I and C.

Genetic mapping

Minisatellite (D1S80)

VNTRs, RFLP



Flanking regions

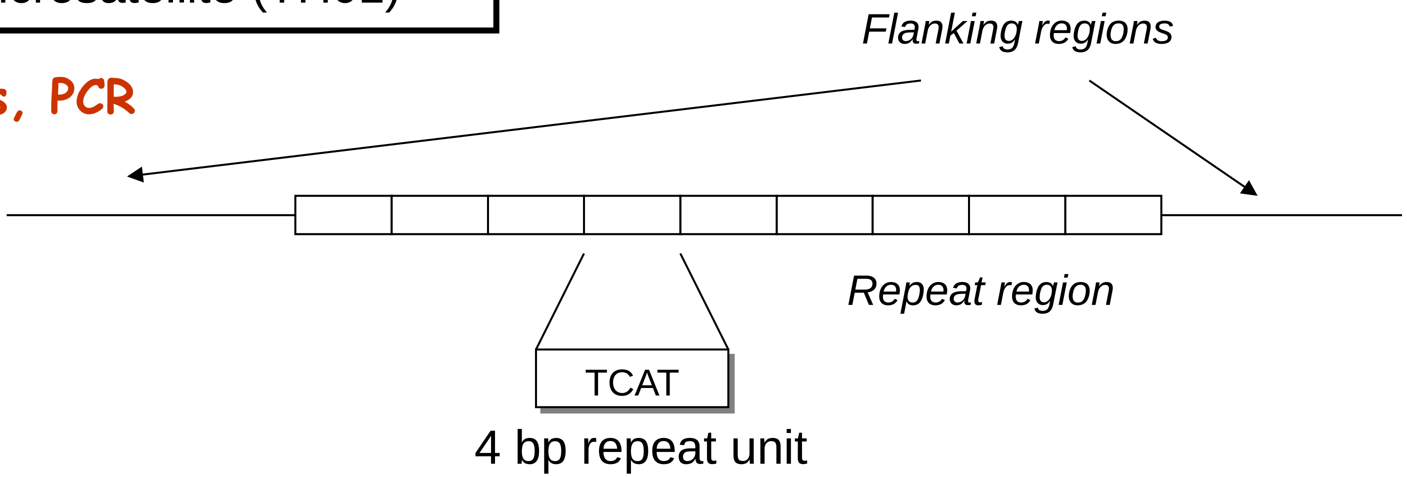
Repeat region

GAGGACCACCAGGAAG

16 bp repeat unit

Microsatellite (TH01)

STRs, PCR



Flanking regions

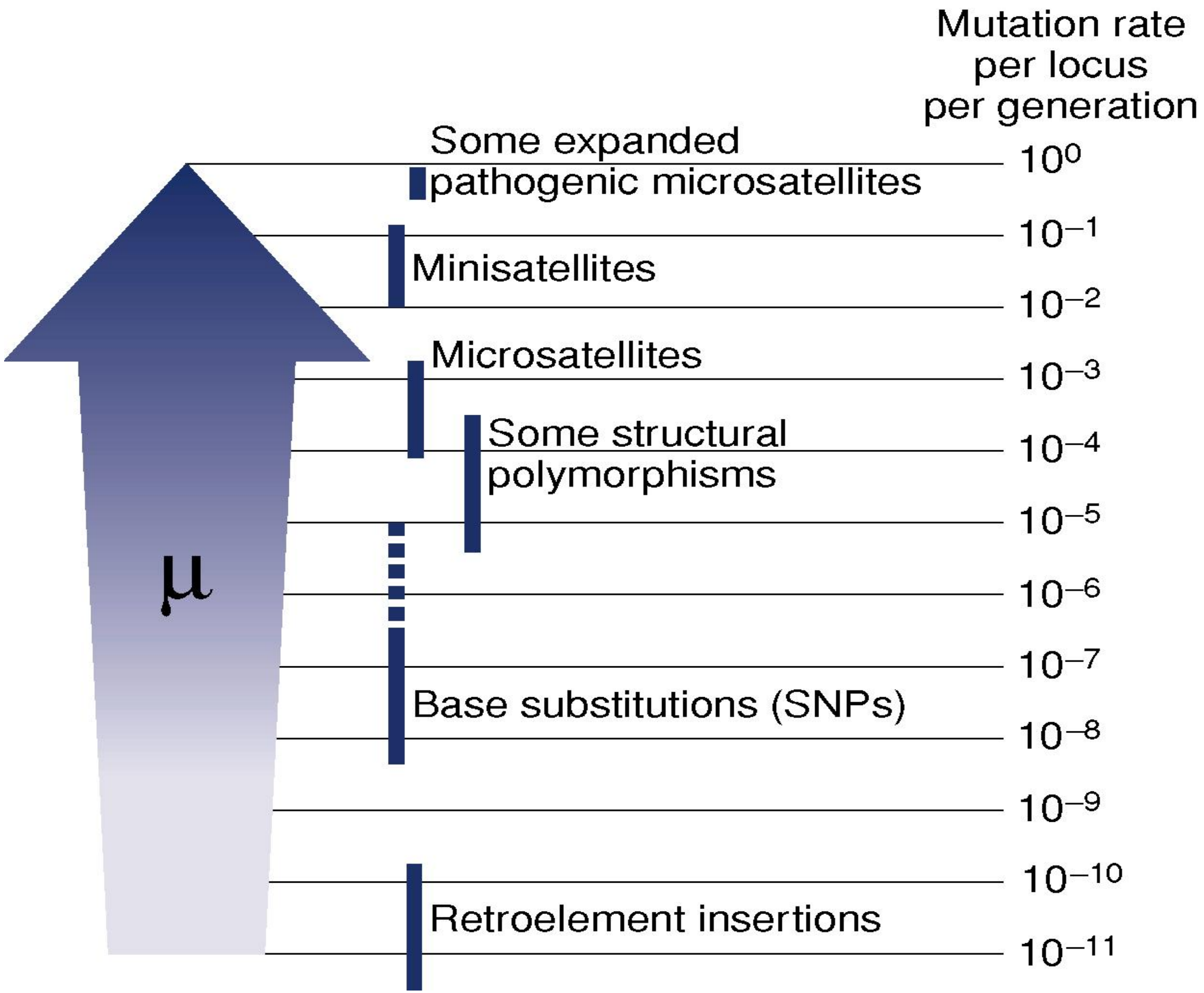
Repeat region

TCAT

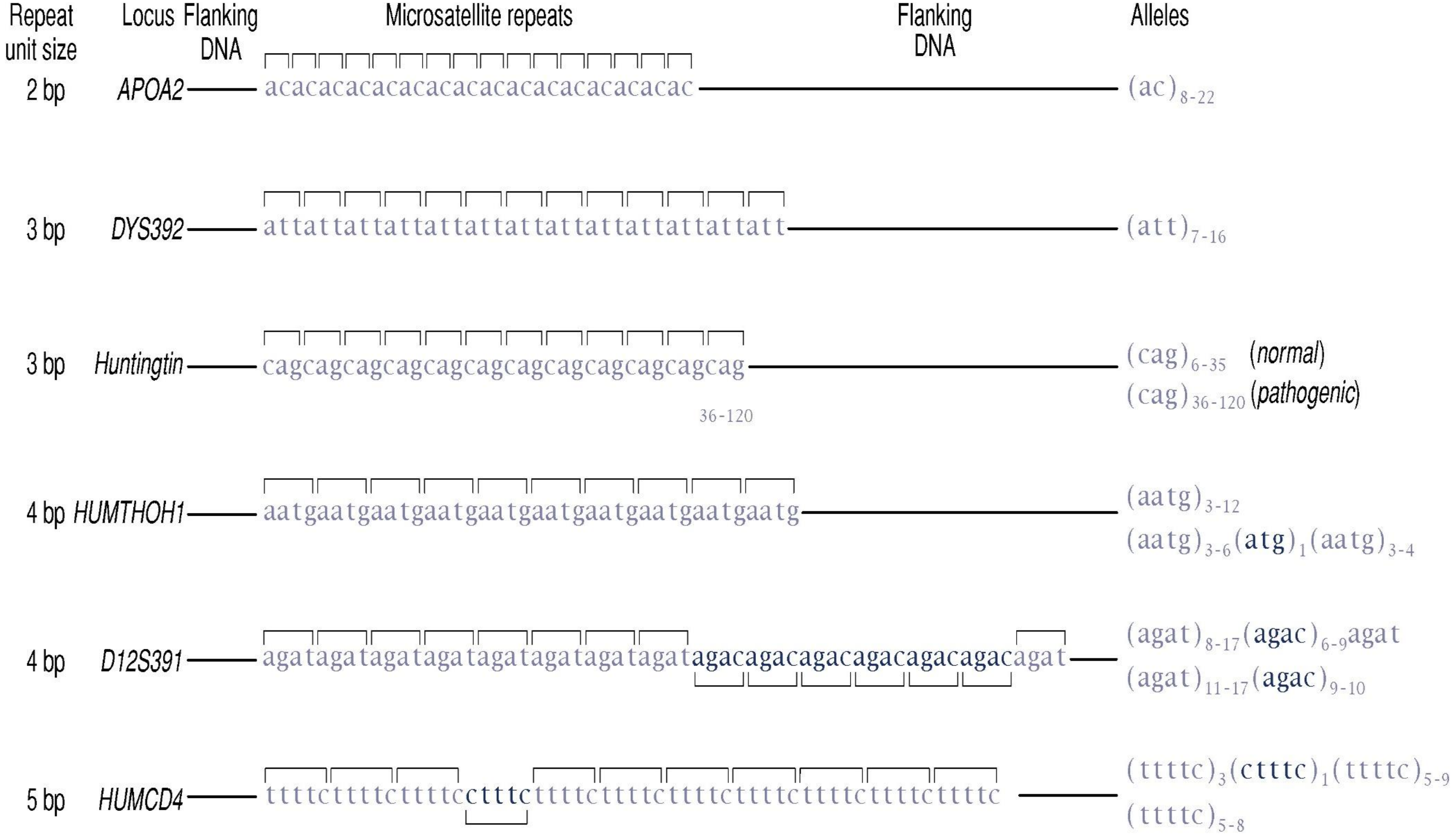
4 bp repeat unit

Forensic DNA Typing, 2nd Edition, J. Butler, 2005

Mutation rate of polymorphic sequences (µ)



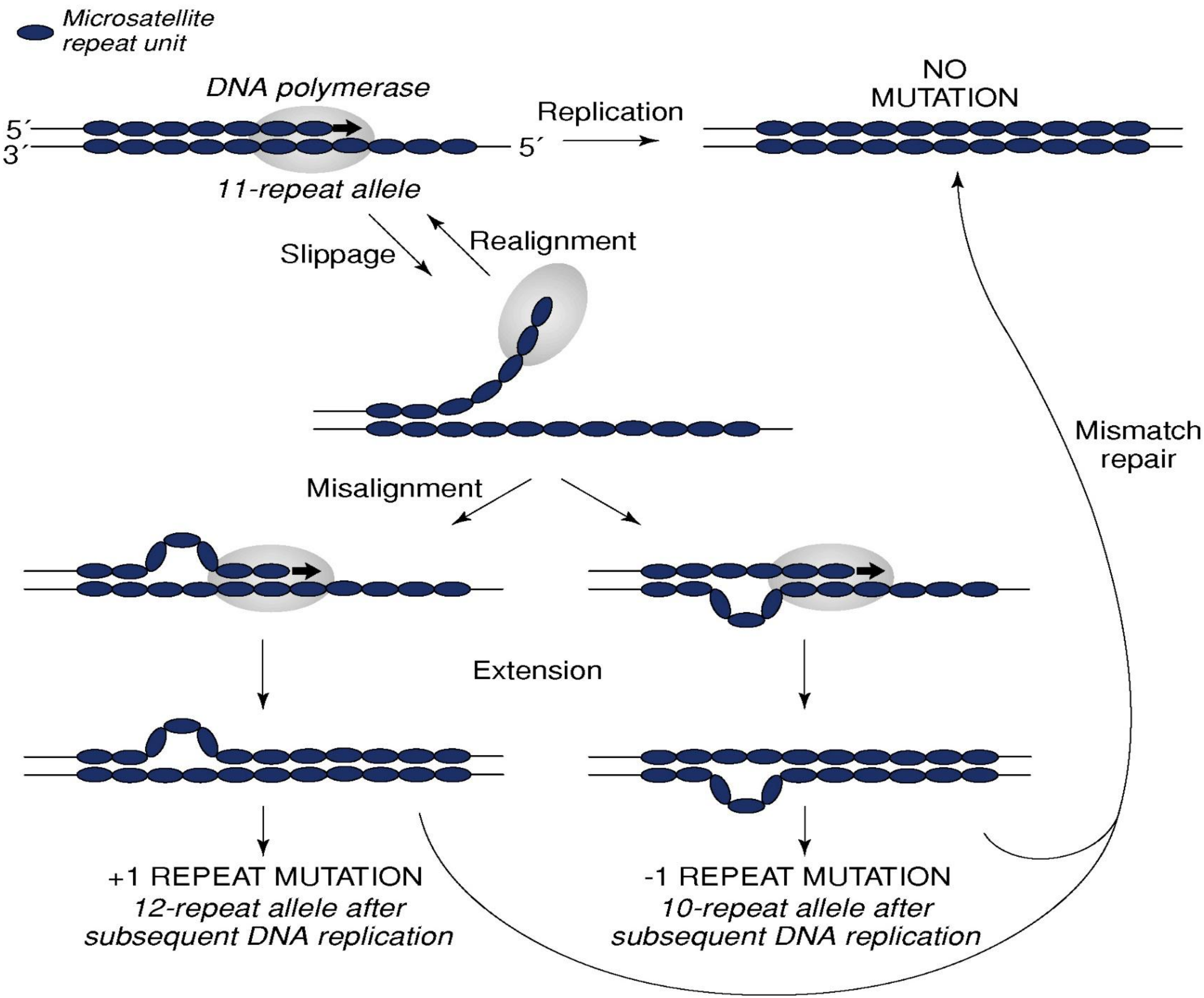
Microsatellite structure



Repeat unit size	Locus	Flanking DNA	Microsatellite repeats	Flanking DNA	Alleles
2 bp	*APOA2*		acacacacacacacacacacacacacacacac		$(ac)_{8-22}$
3 bp	*DYS392*		attattattattattattattattattattattattatt		$(att)_{7-16}$
3 bp	*Huntingtin*		cagcagcagcagcagcagcagcagcagcagcagcag 36-120		$(cag)_{6-35}$ *(normal)* $(cag)_{36-120}$ *(pathogenic)*
4 bp	*HUMTHOH1*		aatgaatgaatgaatgaatgaatgaatgaatgaatgaatg		$(aatg)_{3-12}$ $(aatg)_{3-6}(atg)_{1}(aatg)_{3-4}$
4 bp	*D12S391*		agatagatagatagatagatagatagatagatagacagacagacagacagacagacagat		$(agat)_{8-17}(agac)_{6-9}$agat $(agat)_{11-17}(agac)_{9-10}$
5 bp	*HUMCD4*		ttttcttttcttttcctttcttttcttttcttttcttttcttttcttttcttttc		$(ttttc)_{3}(ctttc)_{1}(ttttc)_{5-9}$ $(ttttc)_{5-8}$

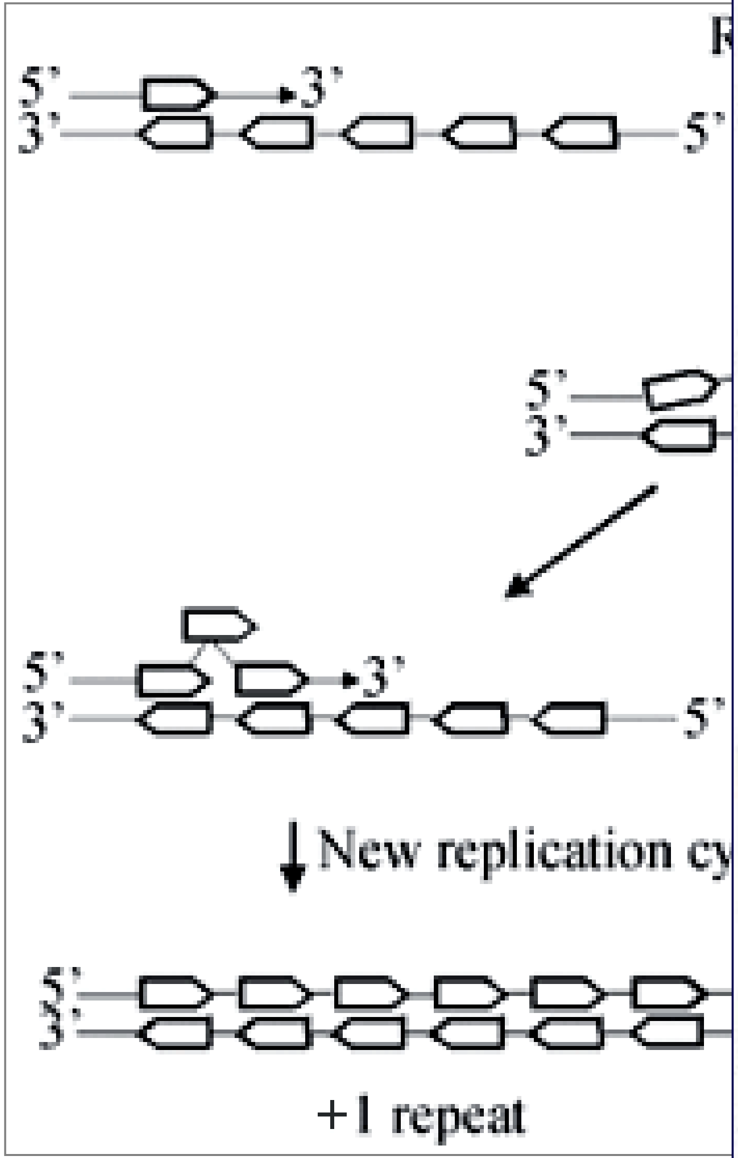
Human Evolutionary Genetics, Jobling, 2004

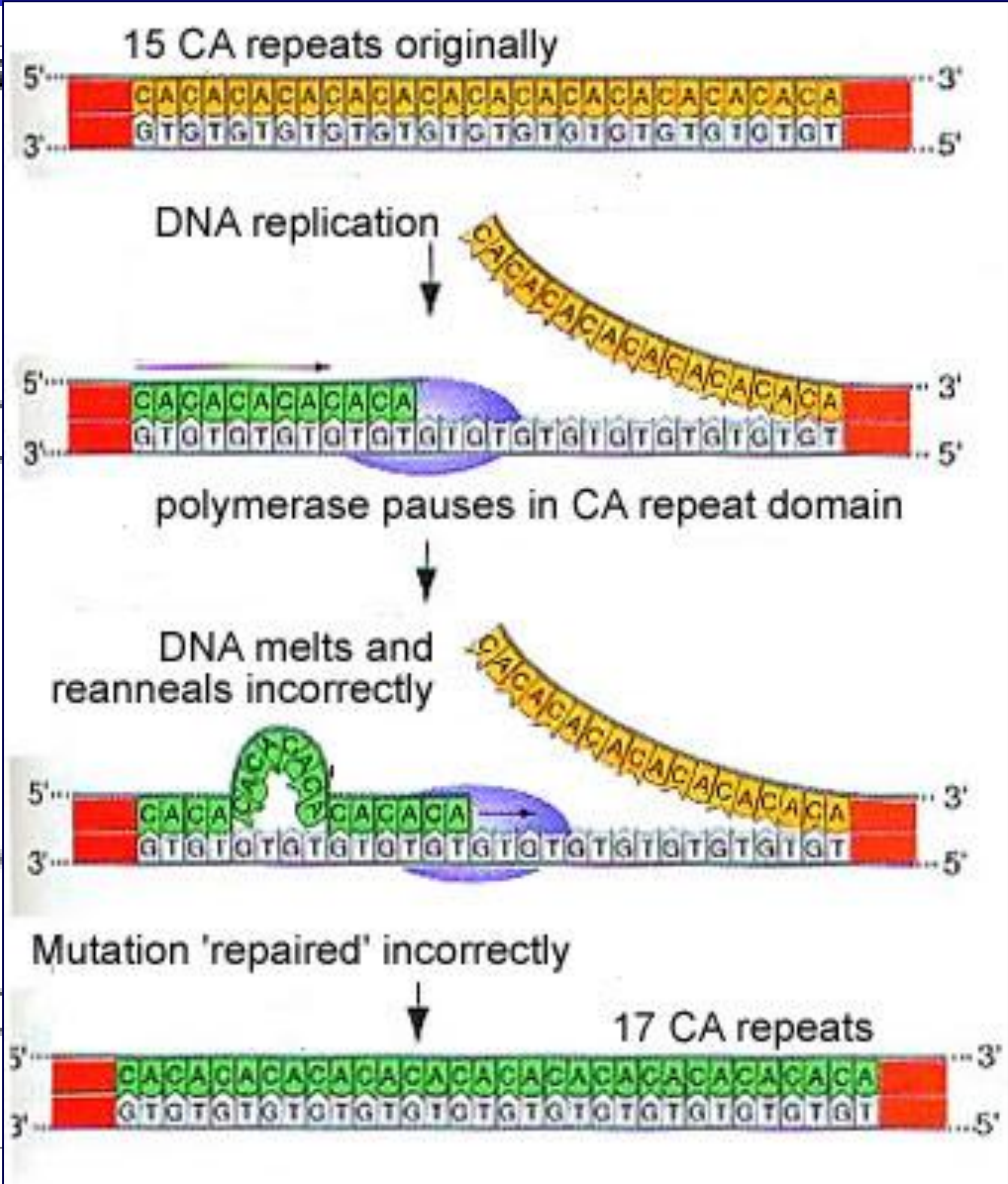
„Replication slippage" – Microsatellite mutation



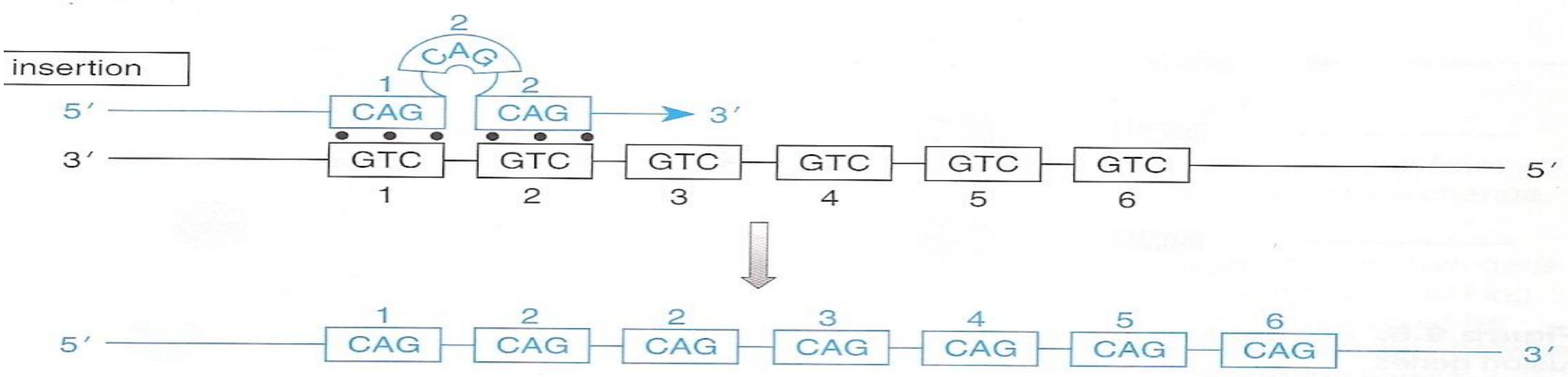
Human Evolutionary Genetics, Jobling, 2004

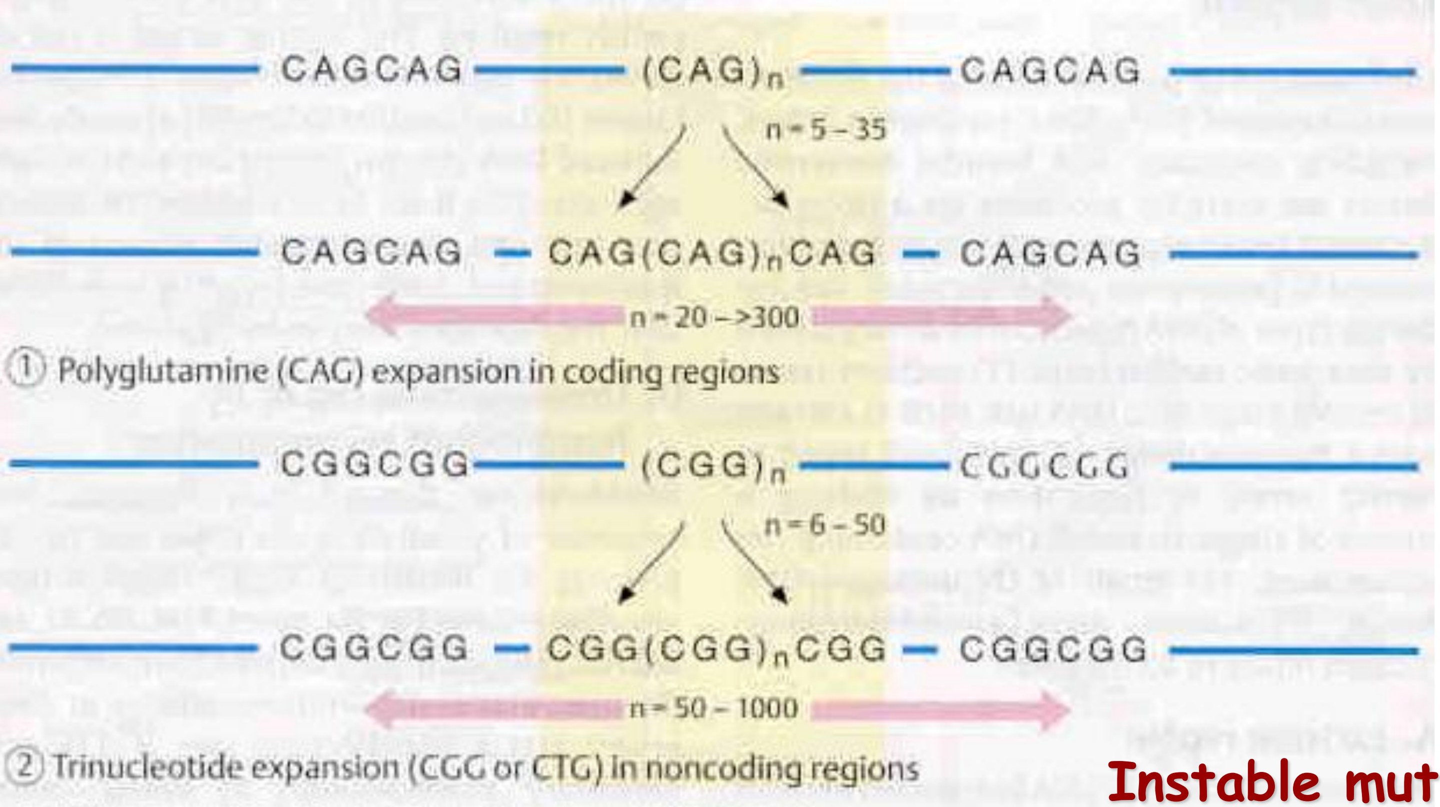
Microsatellite evolution





Trinucleotide repeat expansion





① Polyglutamine (CAG) expansion in coding regions

② Trinucleotide expansion (CGG or CTG) in noncoding regions

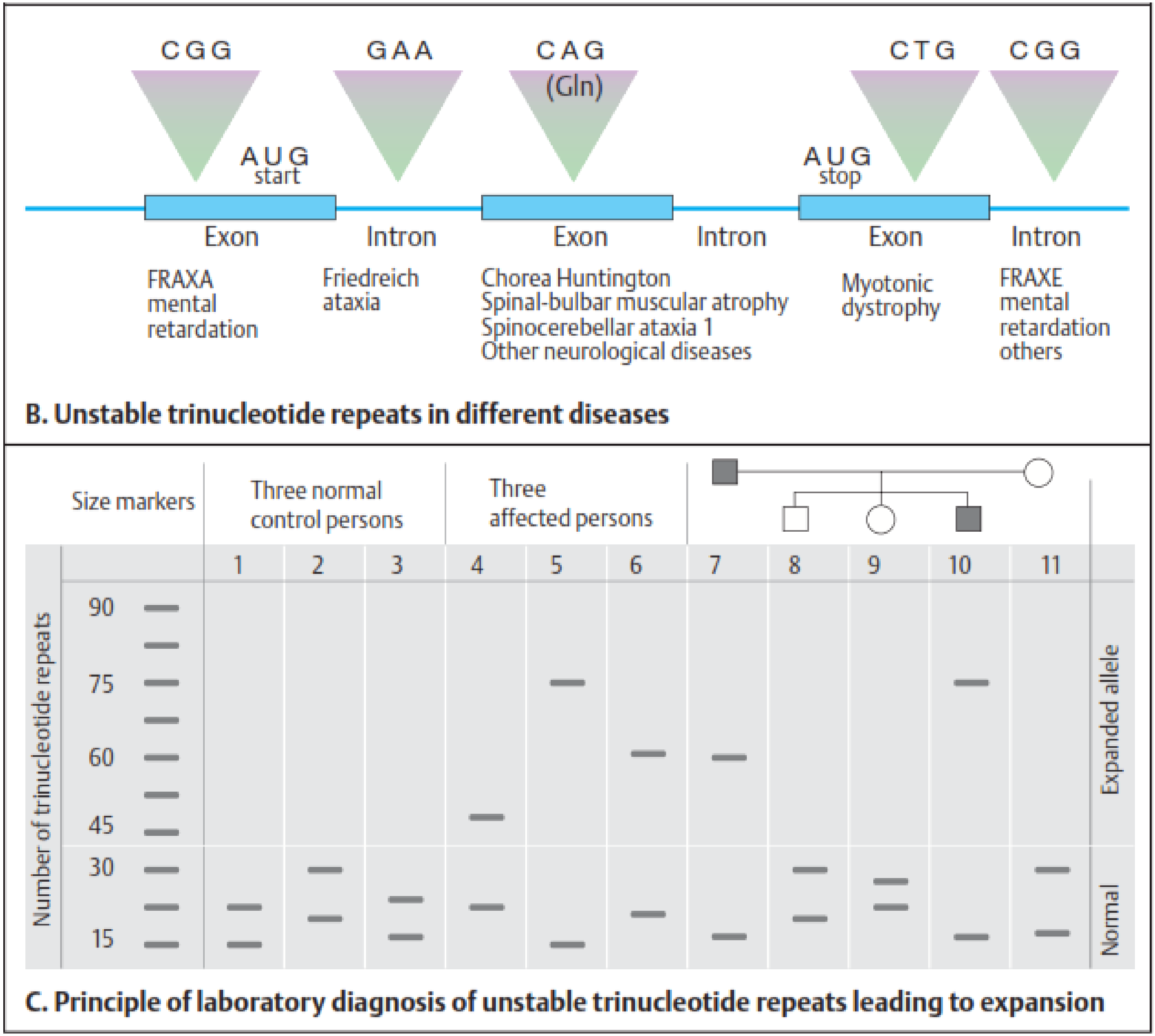
A. Different types of trinucleotide repeat expression

Type I

Type II

Instable mutation

Trinucleotide repeat expansion



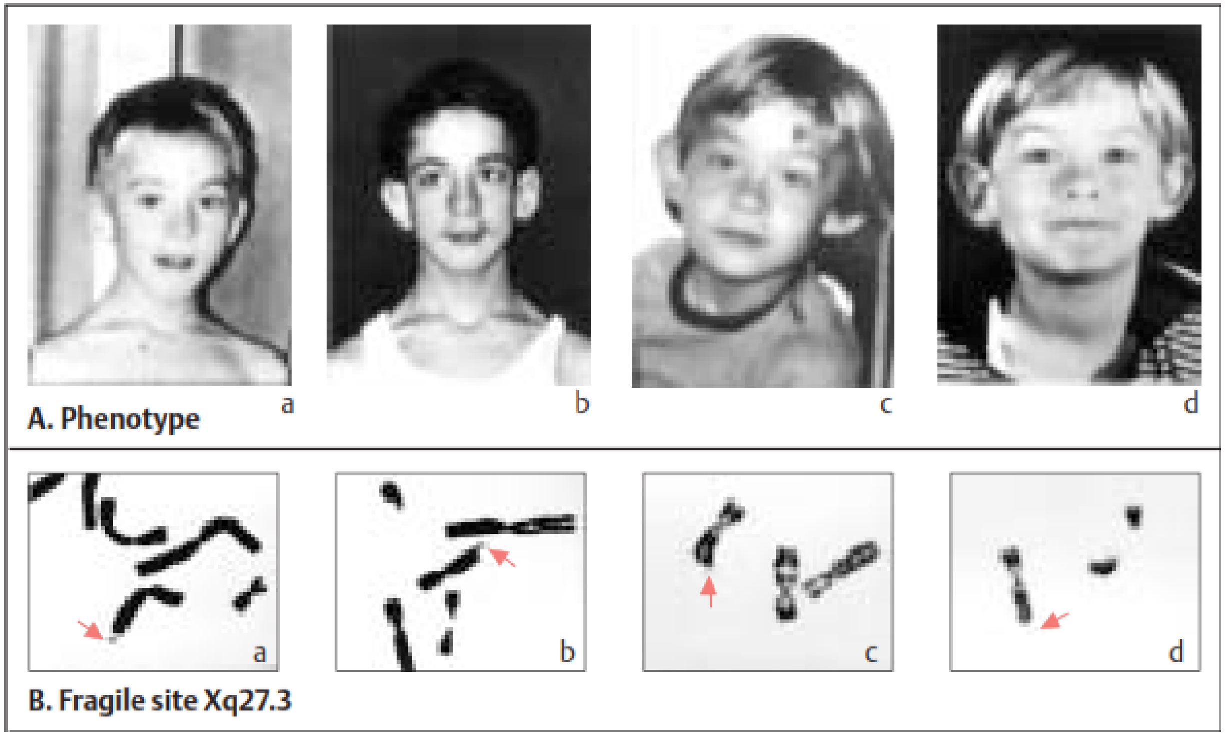
B. Unstable trinucleotide repeats in different diseases

C. Principle of laboratory diagnosis of unstable trinucleotide repeats leading to expansion

Passarge, 2001

Genetic diseases due to repeat expansion

Disease (Examples)	Gene	Frequency	Tri-nucleotide	Normal Number	Mutant Allele	Chromosome
Huntington disease	*HD*	1:10 000	$(CAG)_n$	0–26	36–121	4p16.3
Fragile X syndrome	*FMR1*	1:5 000	$(CGG)_n$	6–50	52–500	Xq27.3
Myotonic dystrophy	*DMPK*	1:8 000	$(CTG)_n$	5–37	50–500	19q13.2
Spinal-bulbar muscular atrophy (Kennedy)	*SBMA*	<1:50 000	$(CAG)_n$	11–31	36–65	Xq11-12


A. Phenotype

B. Fragile site Xq27.3

Fragile X

Huntington disease

Myotonic dystrophy

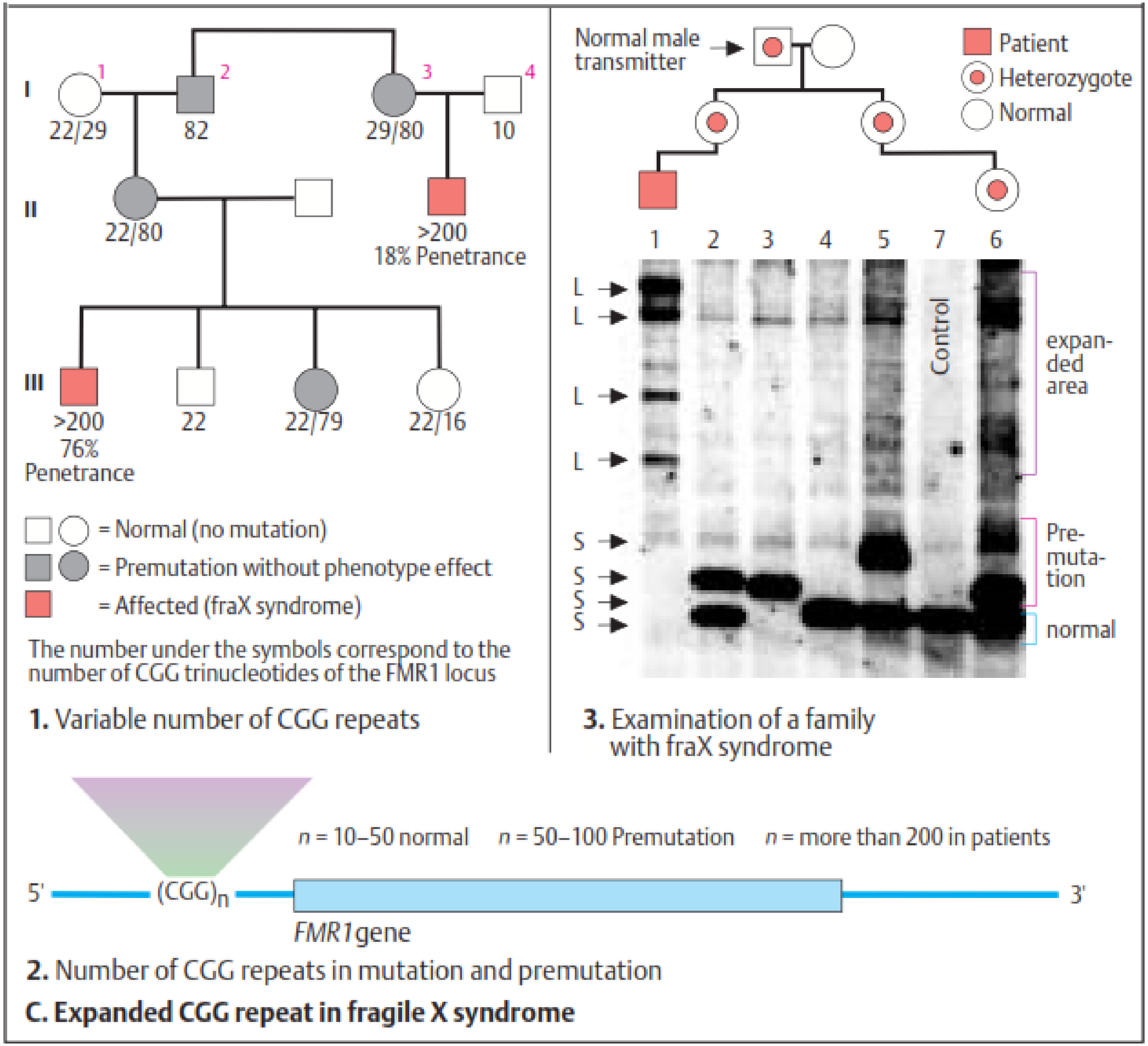
Friedrich ataxia

SMA

etc.

Passarge, 2001

Diagnostics of expanded CGG repeats in Fragile X



□ ○ = Normal (no mutation)

■ ● = Premutation without phenotype effect

🟥 = Affected (fraX syndrome)

The number under the symbols correspond to the number of CGG trinucleotides of the FMR1 locus

1. Variable number of CGG repeats

3. Examination of a family with fraX syndrome

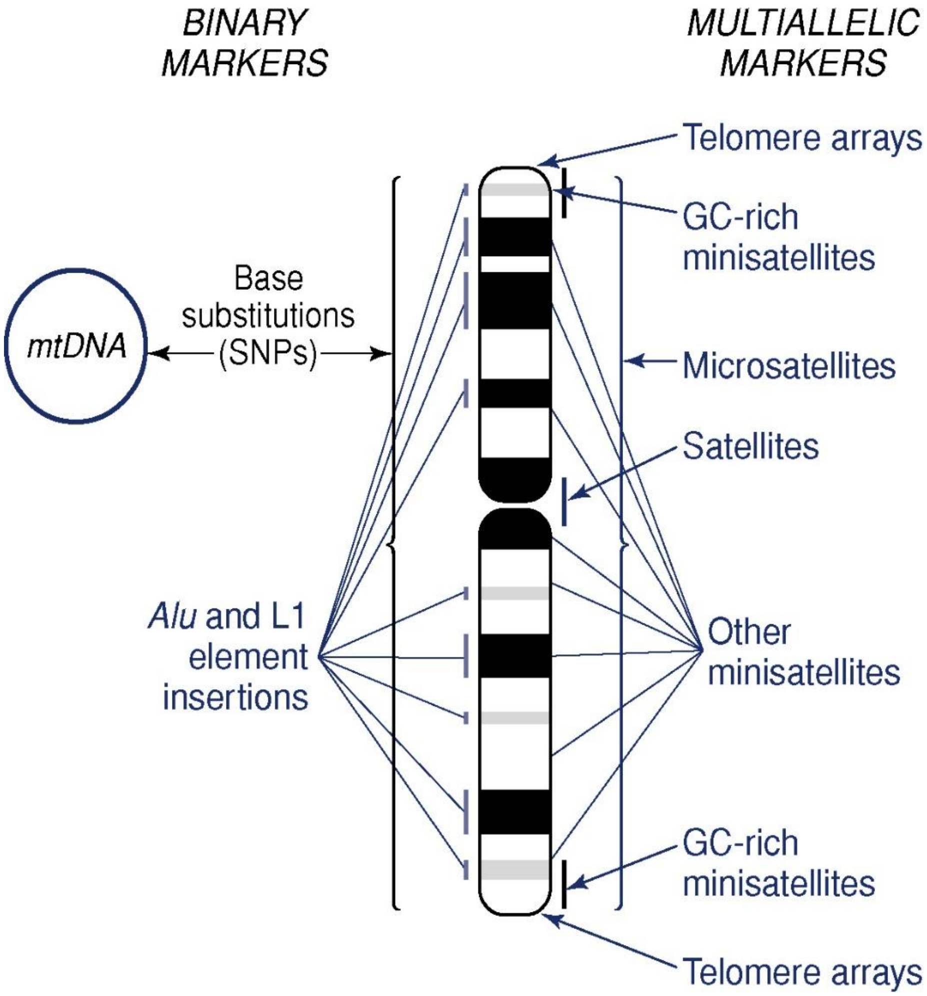
n = 10–50 normal *n* = 50–100 Premutation *n* = more than 200 in patients

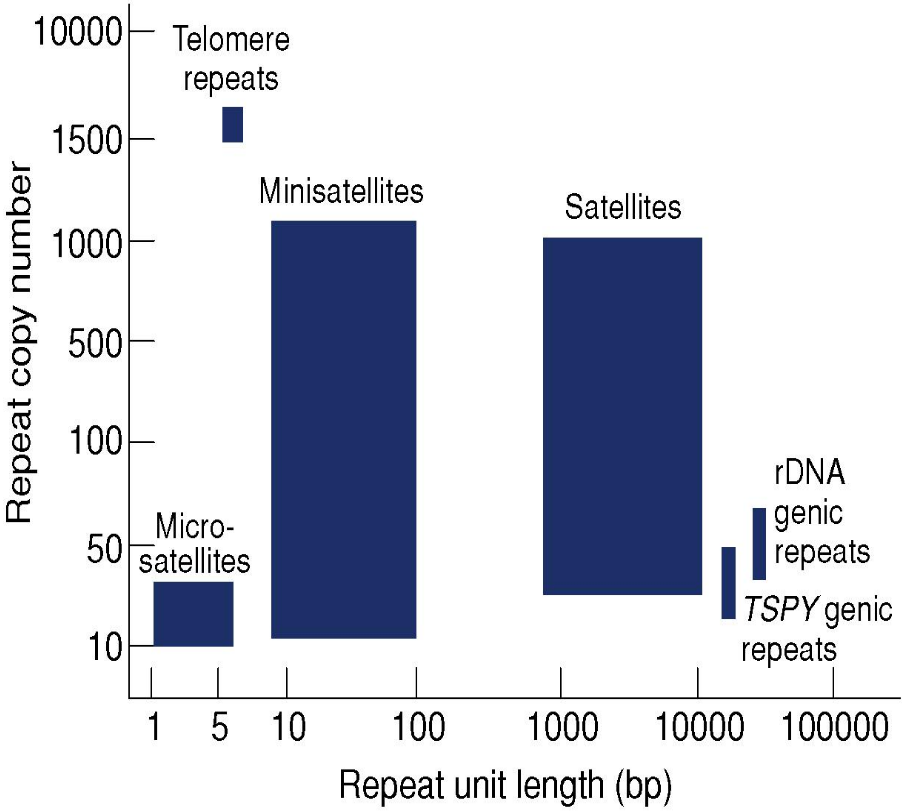
2. Number of CGG repeats in mutation and premutation

C. Expanded CGG repeat in fragile X syndrome

Passarge, 2001

Distribution of polimorphic markers in the genome





Human Evolutionary Genetics, Jobling, 2004