Genomic Architecture and Inheritance of Human Ribosomal RNA Gene Arrays

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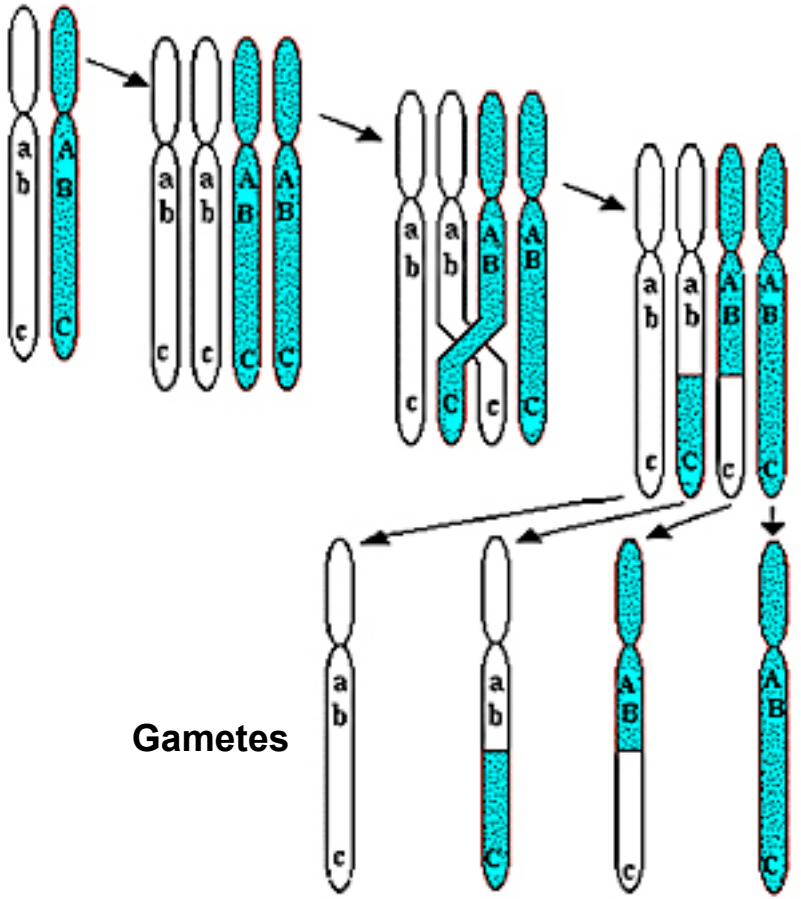
Meiosis Requires Genomic Restructuring

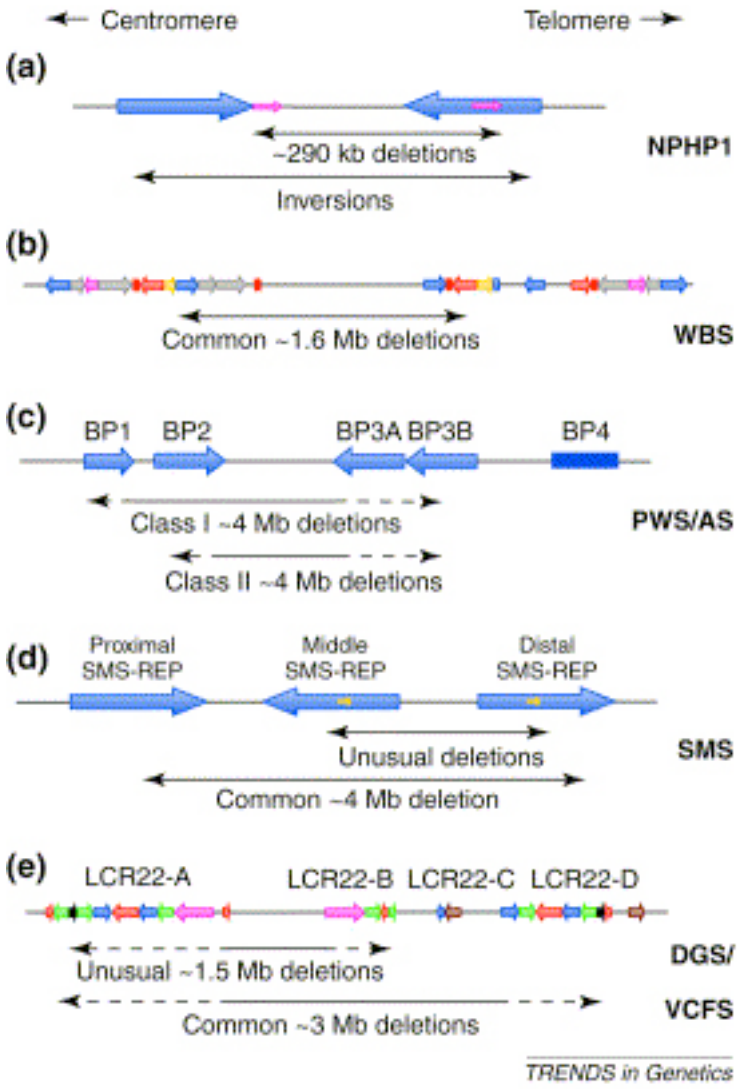


Crossing-over and Recombination In Meiosis

Adapted from: Morgan T.H., Sturtevant A.H., Muller H.J., and Bridges C.B., "The Mechanism of Mendelian Heredity", 1915
By http://www.accessexcellence.org/RC/VL/GG/comeiosis.html

http://biology-pages.info
Photomicrograph by Prof. Bernard John

Some Genomic Disorders Mediated by Repetitive Sequences

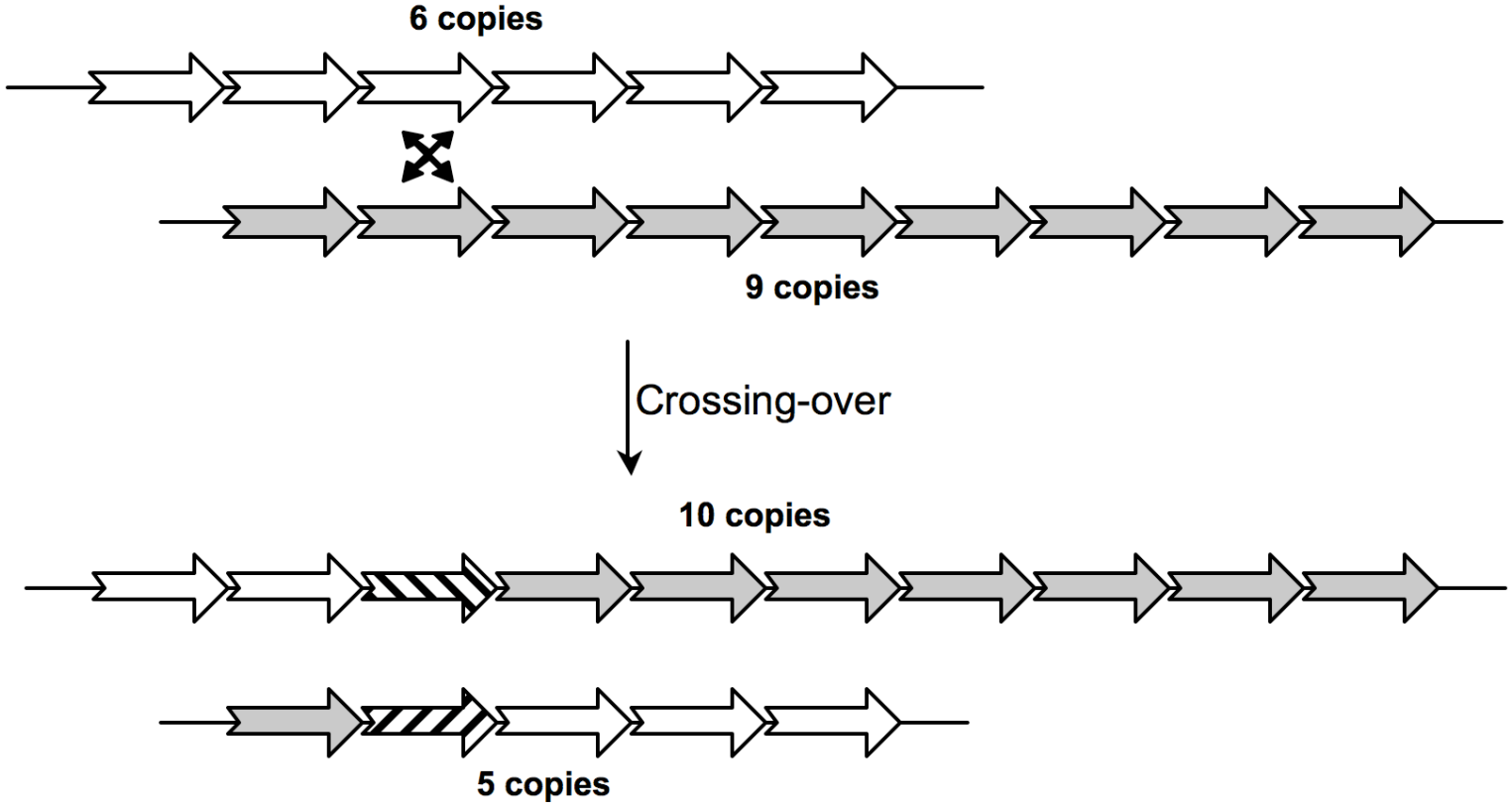


Complex structure of selected low-copy repeats (LCRs). Horizontal lines represent specific genomic regions with the centromere toward the left and telomere to the right. At the right are listed abbreviations for the disease manifested through common deletions of the regions. The colored regions refer to LCRs with the orientation given by the arrowhead. Note complex structure of LCRs consisting of both direct and inverted repeats. (a) LCRs in chromosome 2q13 responsible for rearrangements associated with familial juvenile nephronophthisis 1 (NPHP1). (b) LCRs7 flanking the Williams–Beuren syndrome (WBS) chromosome region 7q11.23. (c) LCRs15 within the Prader–Willi syndrome/Angelman syndrome (PWS/AS) chromosome region 15q11.2. (d) Smith–Magenis syndrome (SMS) repeats within 17p11.2. (e) LCRs22 within the DiGeorge syndrome (DGS) chromosome 22q11.2.

Stankiewicz P, Lupski JR. **Genome architecture, rearrangements and genomic disorders.** Trends Genet. 2002 Feb;18(2):74-82.

Instability Mechanism: Homologous Recombination

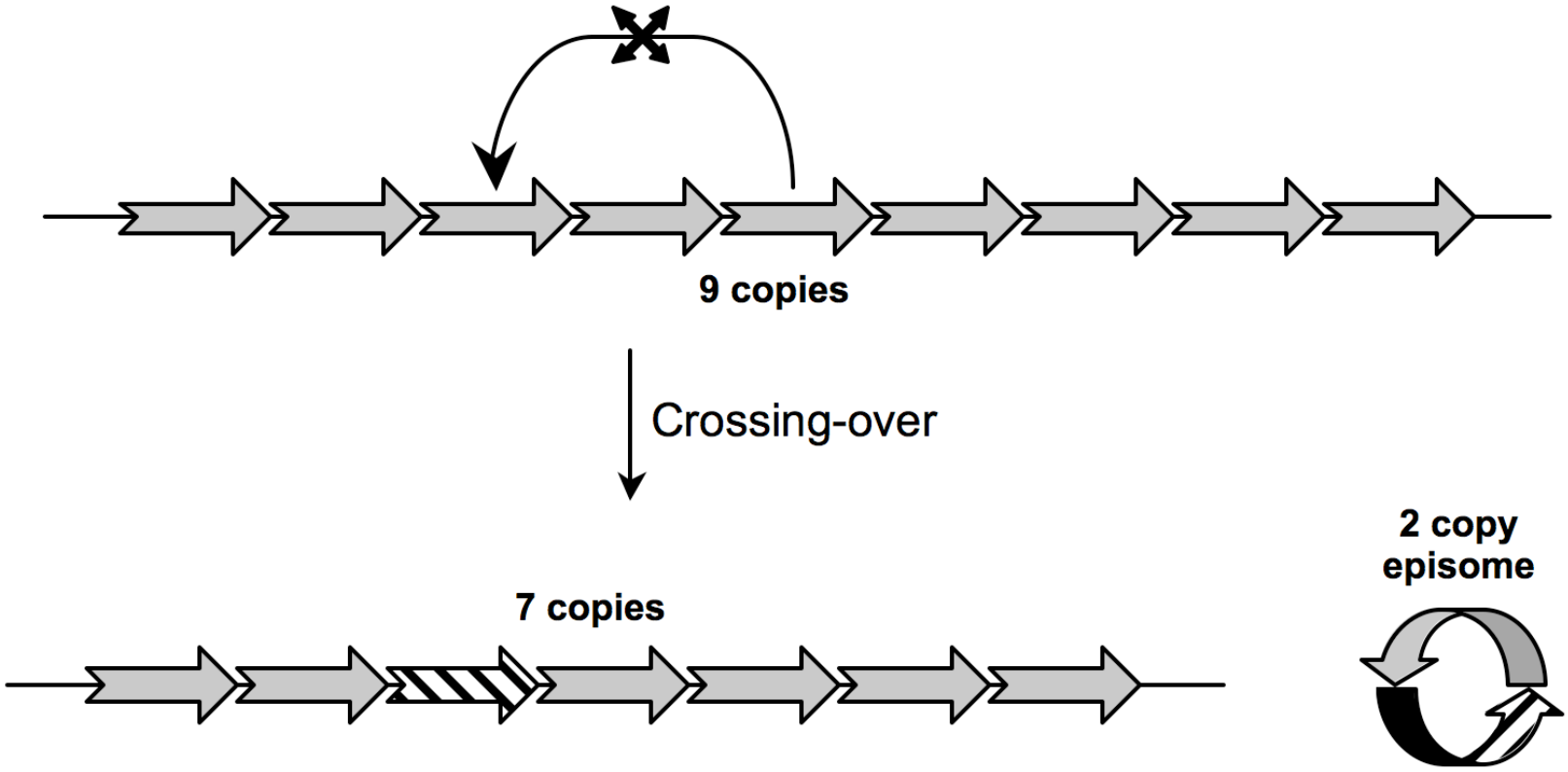
Figure 1. Intermolecular Crossing-over



white arrows and gray arrows: unit repeated elements
(arrow direction indicates relative orientation)
crossed arrows: site of crossing-over
hatched arrows: repeats in which crossover exchange has occurred

Instability Mechanism: Homologous Recombination

Figure 2. Intramolecular Crossing-over



gray arrows: unit repeated elements
(arrow direction indicates relative orientation)
crossed arrows: site of crossing-over
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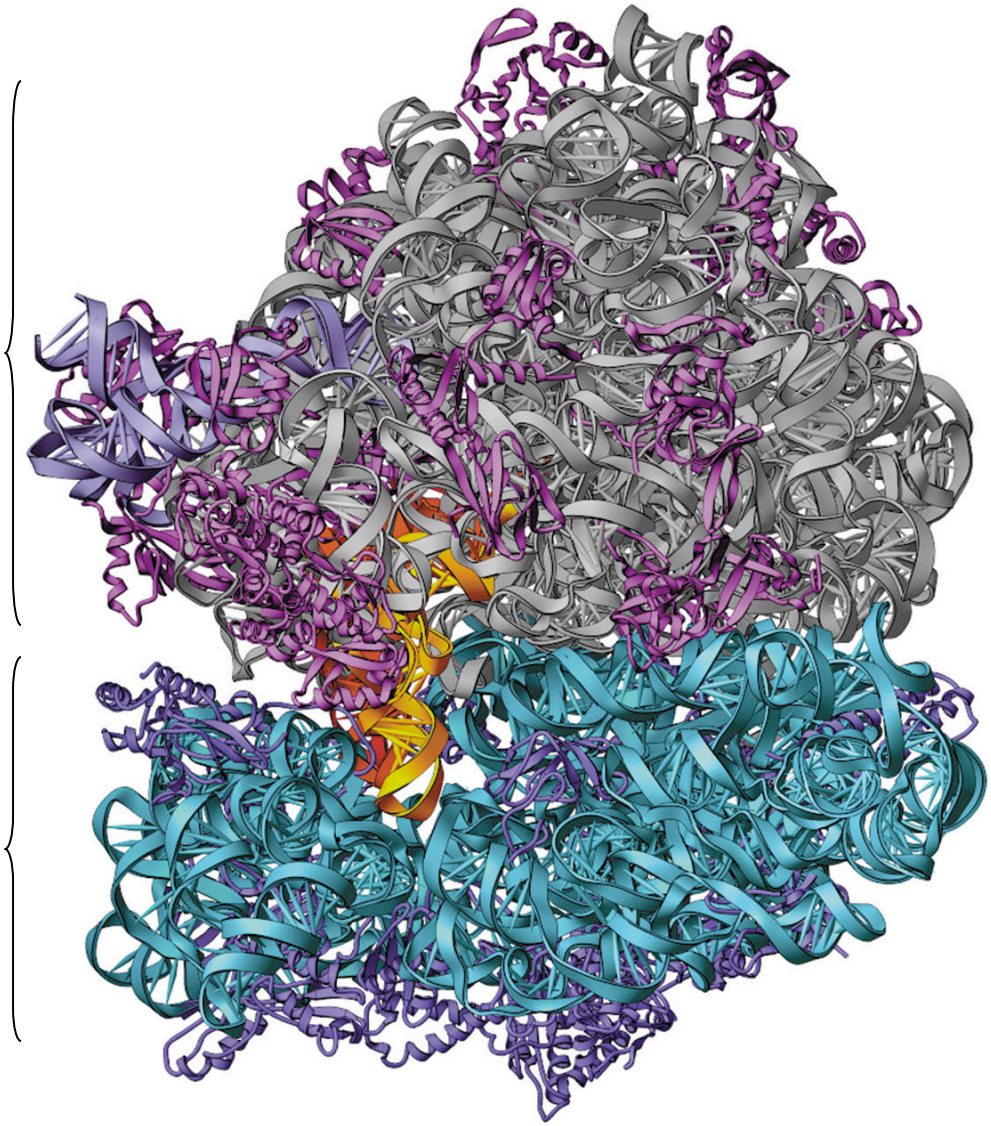
Ribosomal Composition



60S

40S

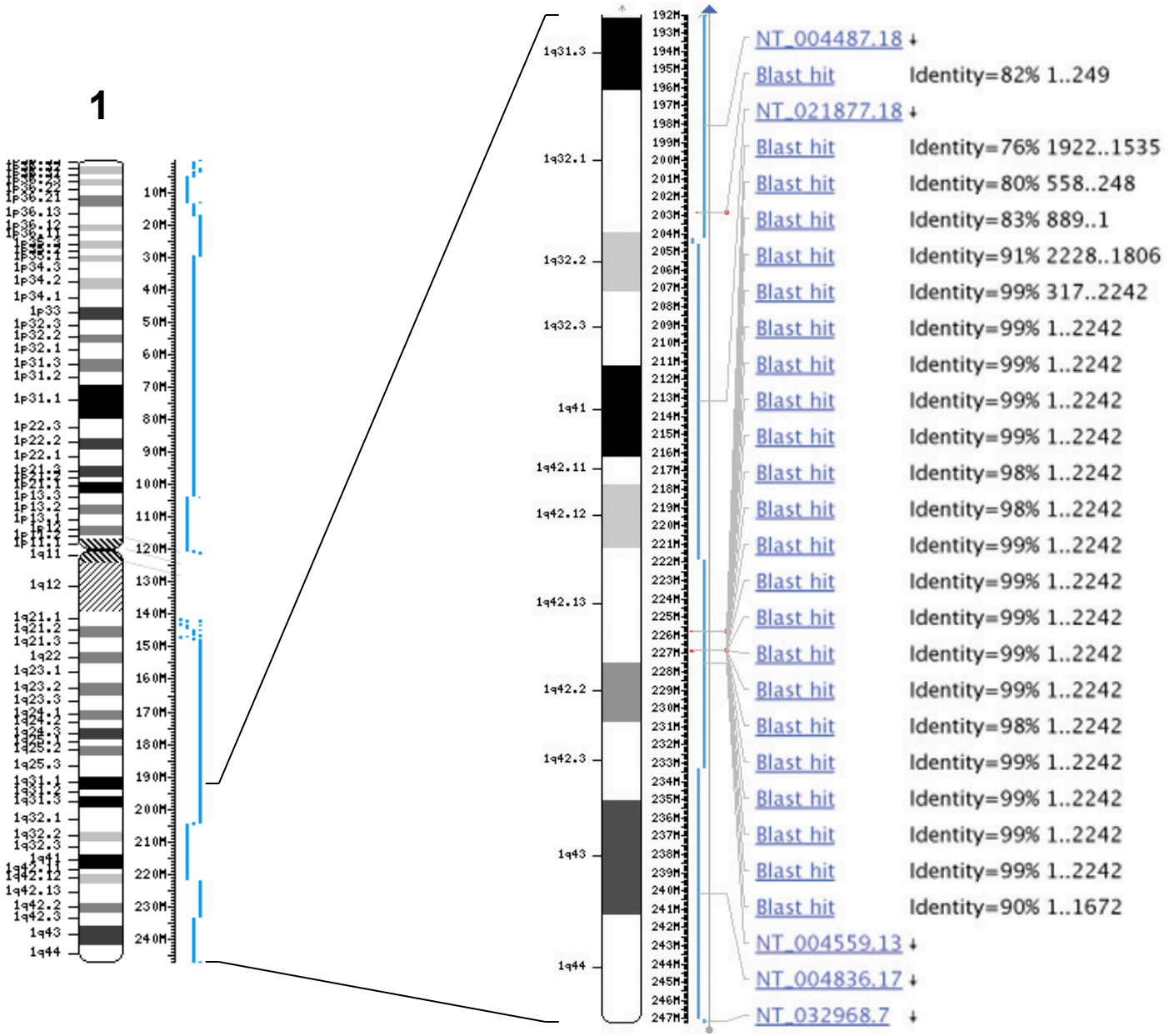
4 RNA molecules

5S, 5.8S, 28S

18S

… and 80+ proteins too

5S rDNA Arrays and the Human Genome Project



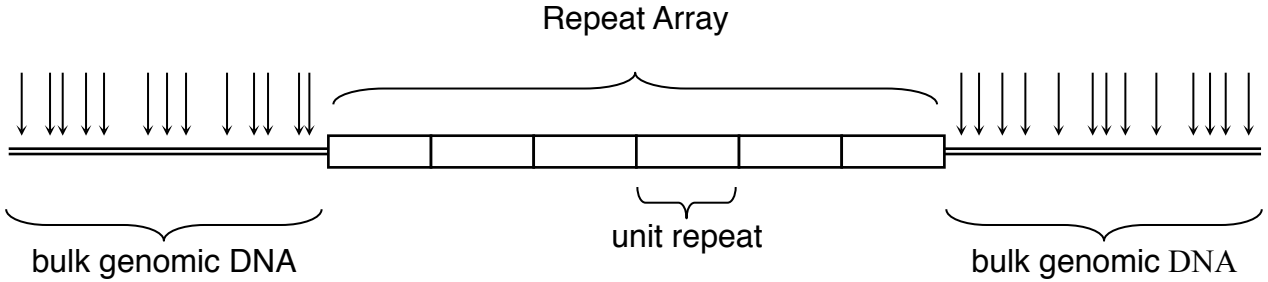
Experimental Strategy

1. **Isolate very high molecular weight genomic DNA from human peripheral blood**

2. **Liberate rDNA arrays by digesting with restriction enzymes that cut external to the array**

3. **Separate arrays by size on pulsed-field gels**

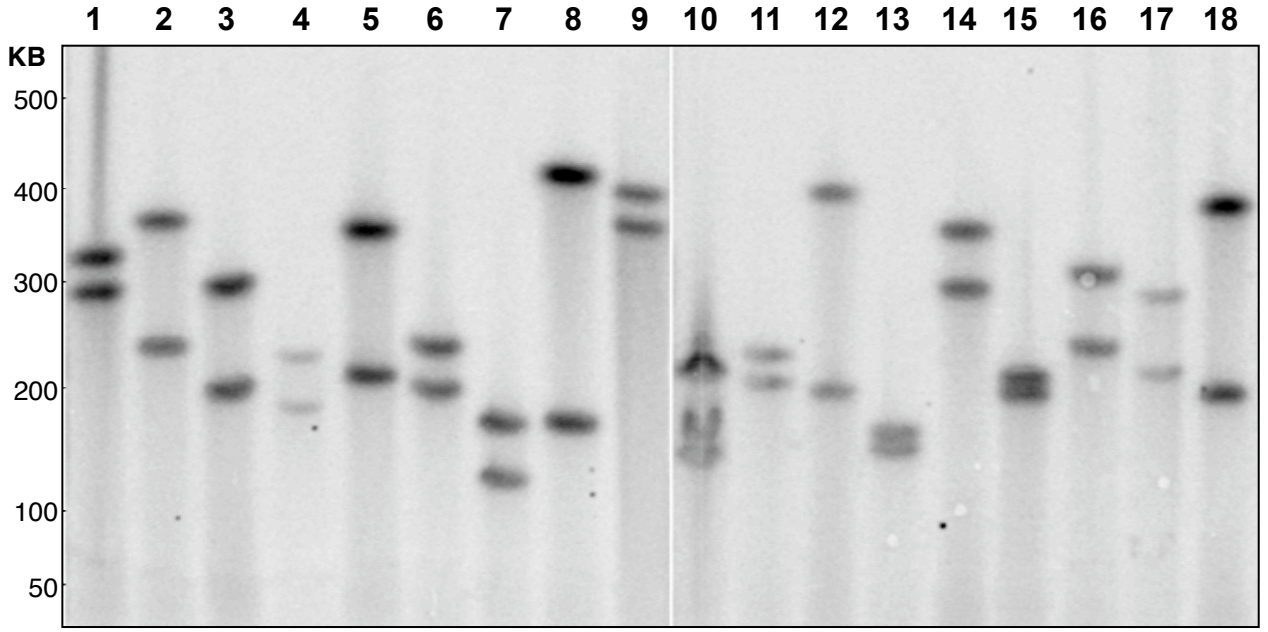
4. **Detect rDNA specific bands by Southern blotting**



Band intensity is proportional to:

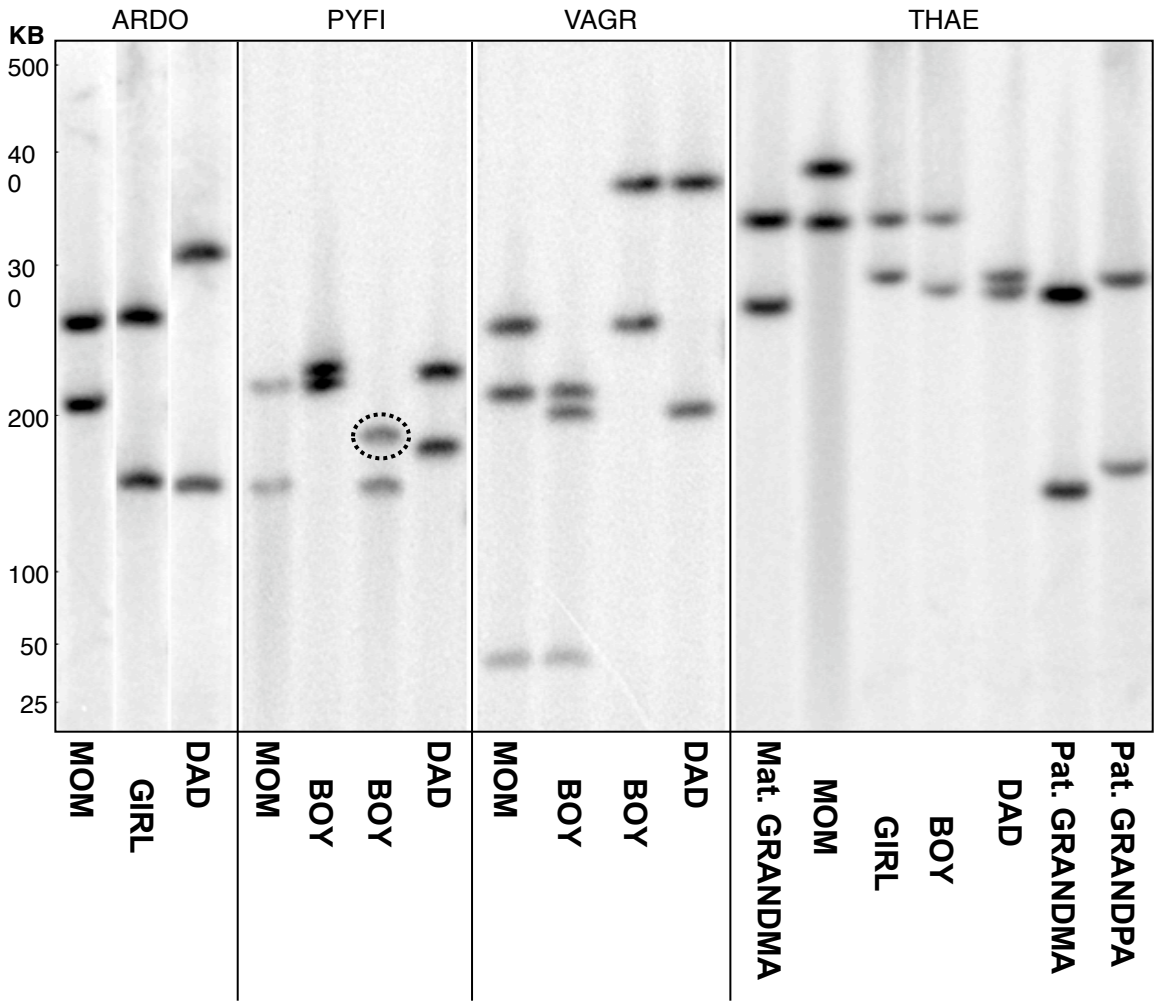
i. **Length of the array**
ii. **Fraction of cells in the population containing any given sized array**

5S rDNA Arrays from Anonymous Human Donors



- Everyone has two different sized arrays
- No two people have the same array lengths

5S rDNA Arrays from Human Families – Inheritance



Sample Source:		Mother	(Other)	Child	(Focus)	Father	(Alleged)	Exclusion Status*	Direct Index	Column Not Used
Name:		PYFI-10-MA		PYFI-02-TY		PYFI-20-BR				
Racial Database Used:		Caucasian-American		Caucasian-American		Caucasian-American				
Date Collected:		Unknown		Unknown		Unknown				
STR Locus	Allele Range	Alleles Called		Alleles Called		Alleles Called				
D3S1358	(12 - 20)	14	15	15	16	15	16	OK	2.072	---
TH01	(4 - 13.3)	6	10	6	9.3	9.3		OK	2.820	---
D21S11	(24 - 38)	32.2		29	32.2	29	32.2	OK	1.933	---
D18S51	(8 - 27)	12	16	12	13	13	16	OK	3.510	---
Penta E	(5 - 24)	7	12	5	12	5	12	OK	7.478	---
D5S818	(7 - 16)	11	12	11	12	12		OK	1.357	---
D13S317	(7 - 15)	11	12	12		10	12	OK	1.552	---
D7S820	(6 - 14)	10	13	12	13	11	12	OK	4.719	---
D16S539	(5 - 15)	11	13	9	13	9	11	OK	5.333	---
CSF1PO	(6 - 15)	10	11	10	12	11	12	OK	1.667	---
Penta D	(2.2 - 17)	9	10	9		9	13	OK	2.915	---
Amelogenin	(XX - XY)	Female (XX)		Male (XY)		Male (XY)		N/A	N/A	---
vWA	(10 - 22)	15	19	17	19	17		OK	3.800	---
D8S1179	(7 - 18)	12	15	12	14	14		OK	5.375	---
TPOX	(6 - 13)	8	11	8	11	8		OK	1.265	---
FGA	(16 - 46.2)	20	23	20	21	21	23	OK	2.730	---
NOT USED										
NOT USED										
NOT USED										
NOT USED										
NOT USED										
NOT USED										
NOT USED										
NOT USED										

Laboratory Batch Number:	63613130C1	Notable Events: Exclusions-None; Infrequent Events-None. See COMMENTS section for additonal information.

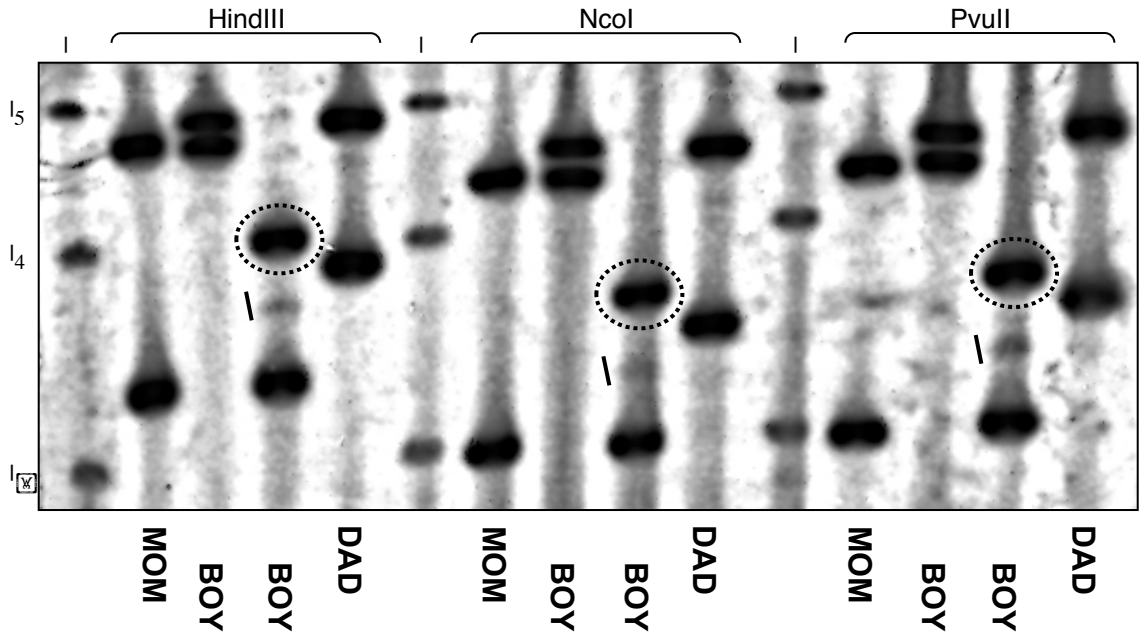
Statement of Results: Alleged relationship is not excluded.

Based on the DNA analysis, the alleged Father, PYFI-20-BR, cannot be excluded as the biological Father of the Child, PYFI-02-TY, because they share genetic markers. Of the genetic identity systems tested, 15 of 15 match. (99.999945964304% of the Caucasian-American male population is excluded from the possibility of being the biological Father). The probability of the stated relationship is indicated below, as compared with an untested, unrelated Caucasian-American male. Analyses, with the exception of sample collection, were conducted in accordance with the Standards for PCR DNA analysis set forth by the AABB.

Statistical Results:

Combined Direct Index	5,383,421	.....Probability =	99.999981424455%
Prior Probability	0.50	.....Statistical Constant	

5S rDNA Arrays – Inheritance and Somatic Mosaicism



Meiotic rearrangement: Band length not represented in either parent
(dotted circle)

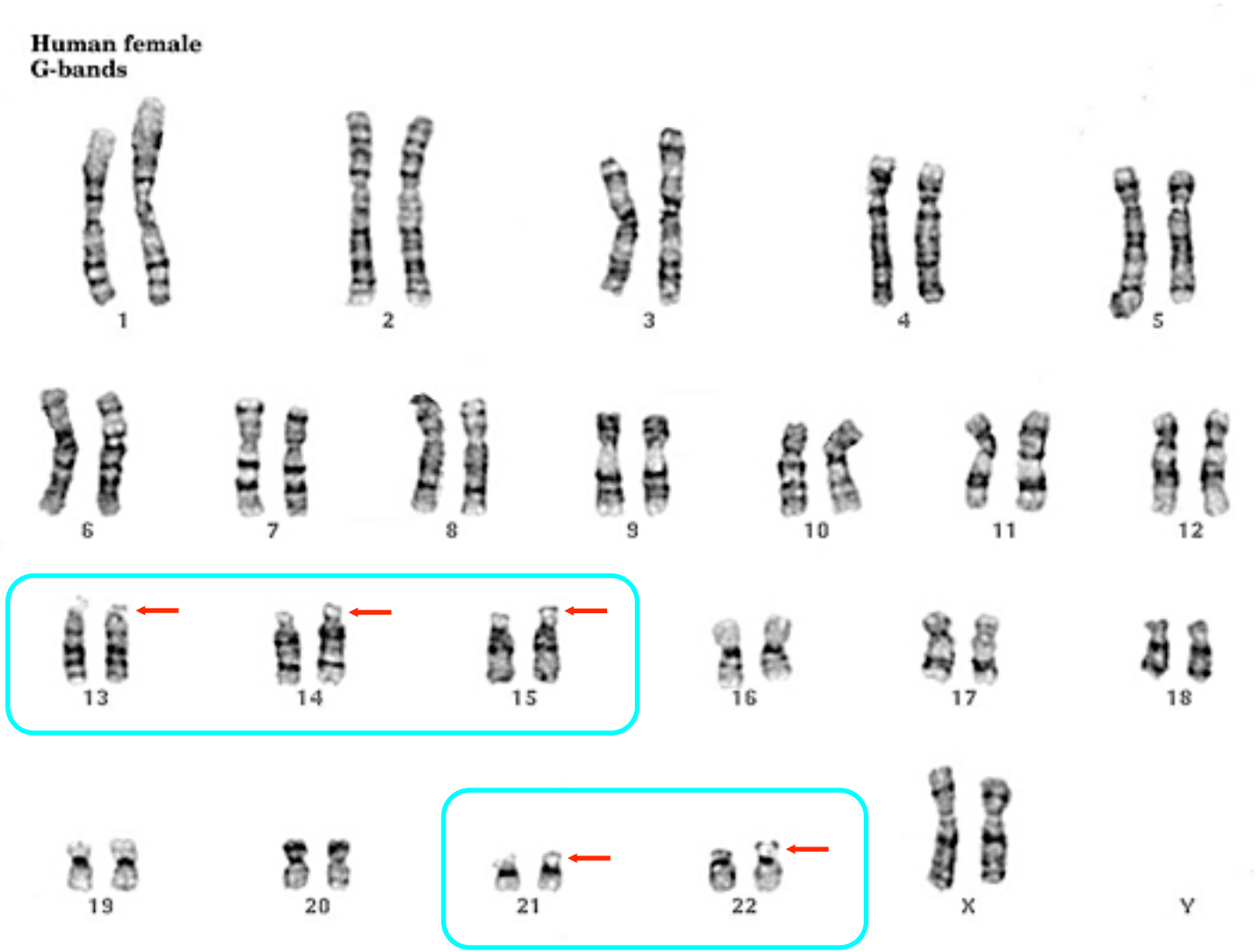
Somatic mosaicism: Band of reduced intensity relative to length
(square bracket)

5S rDNA Arrays – Human Statistics

(N = 27)

	Each Haploid Array	Diploid Totals
Average number of repeats	**98 ± 35**	**195 ± 51**
Fewest repeats observed	**35**	**51**
Most repeats observed	**175**	**299**

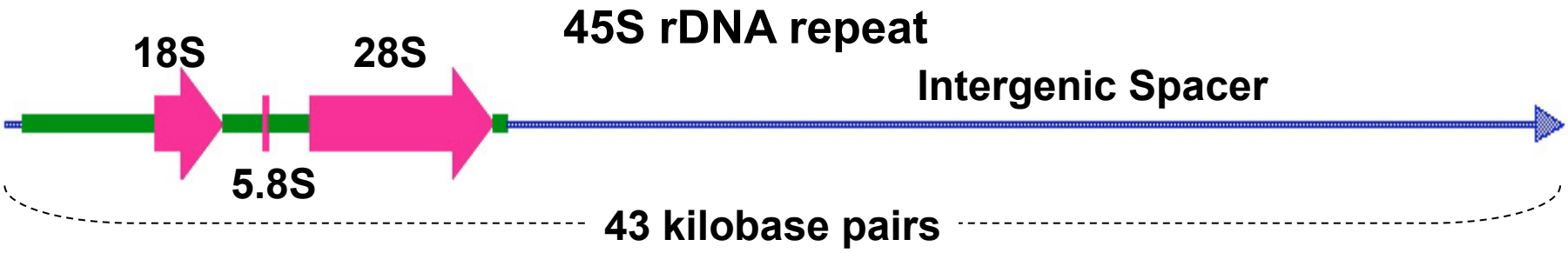
45S Ribosomal DNA in Human Karyotypes

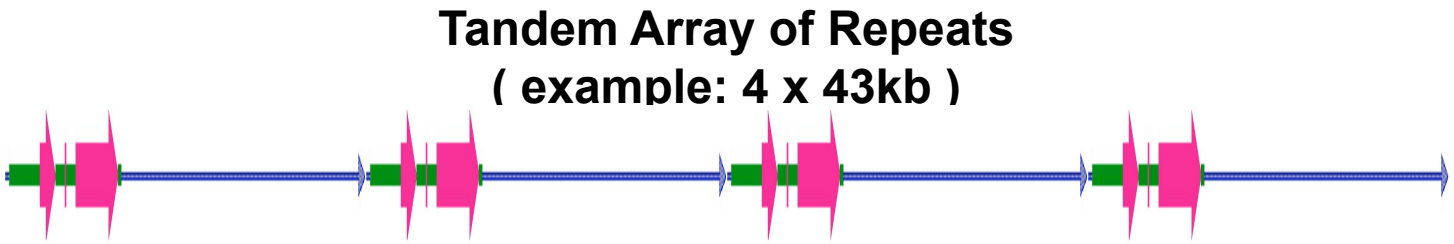


http://www.pathology.washington.edu/galleries/Cytogallery/main.php

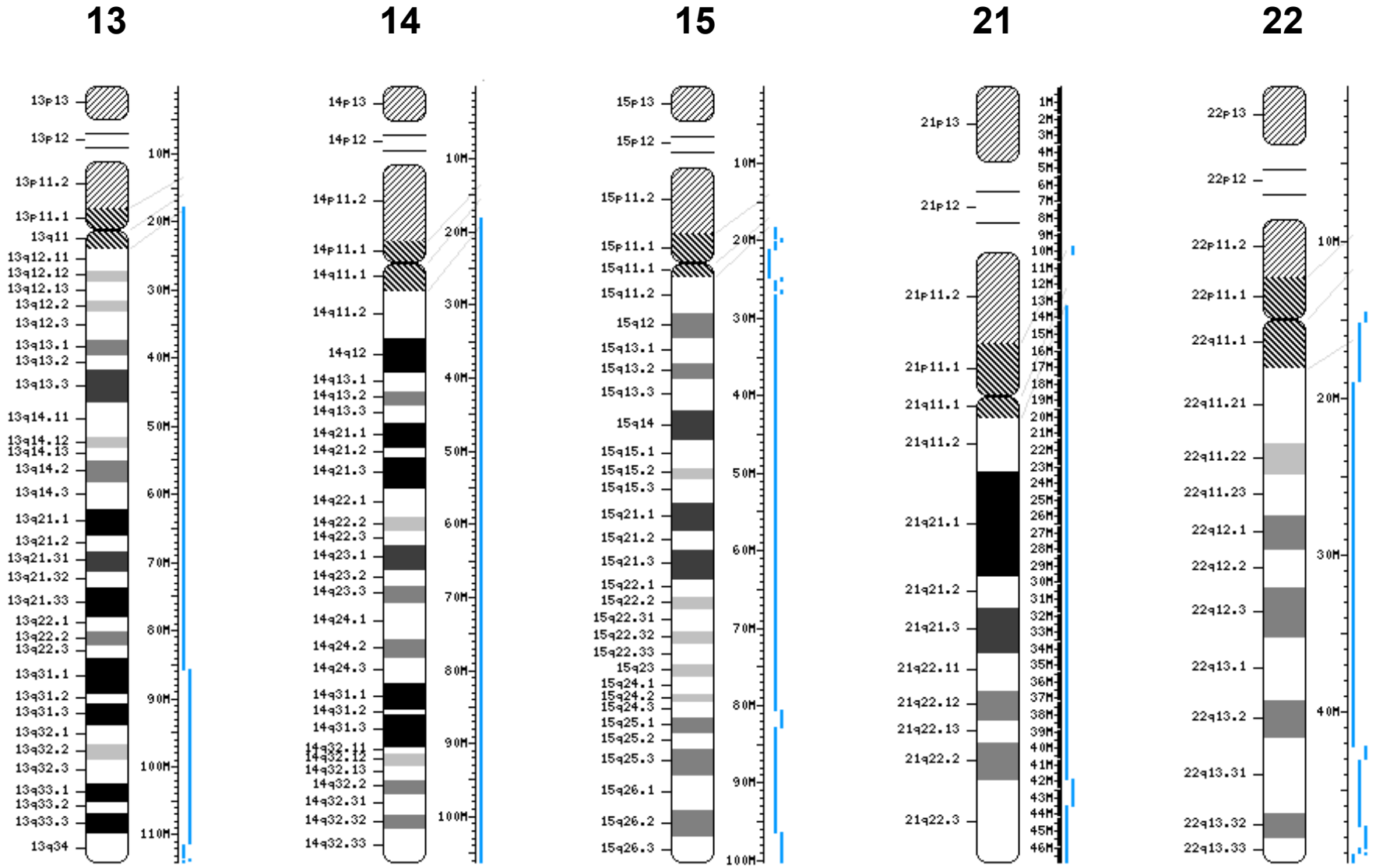
Ribosomal DNA Repeats

- generally structured in tandem head-to-tail arrays
- unit repeats completely sequenced

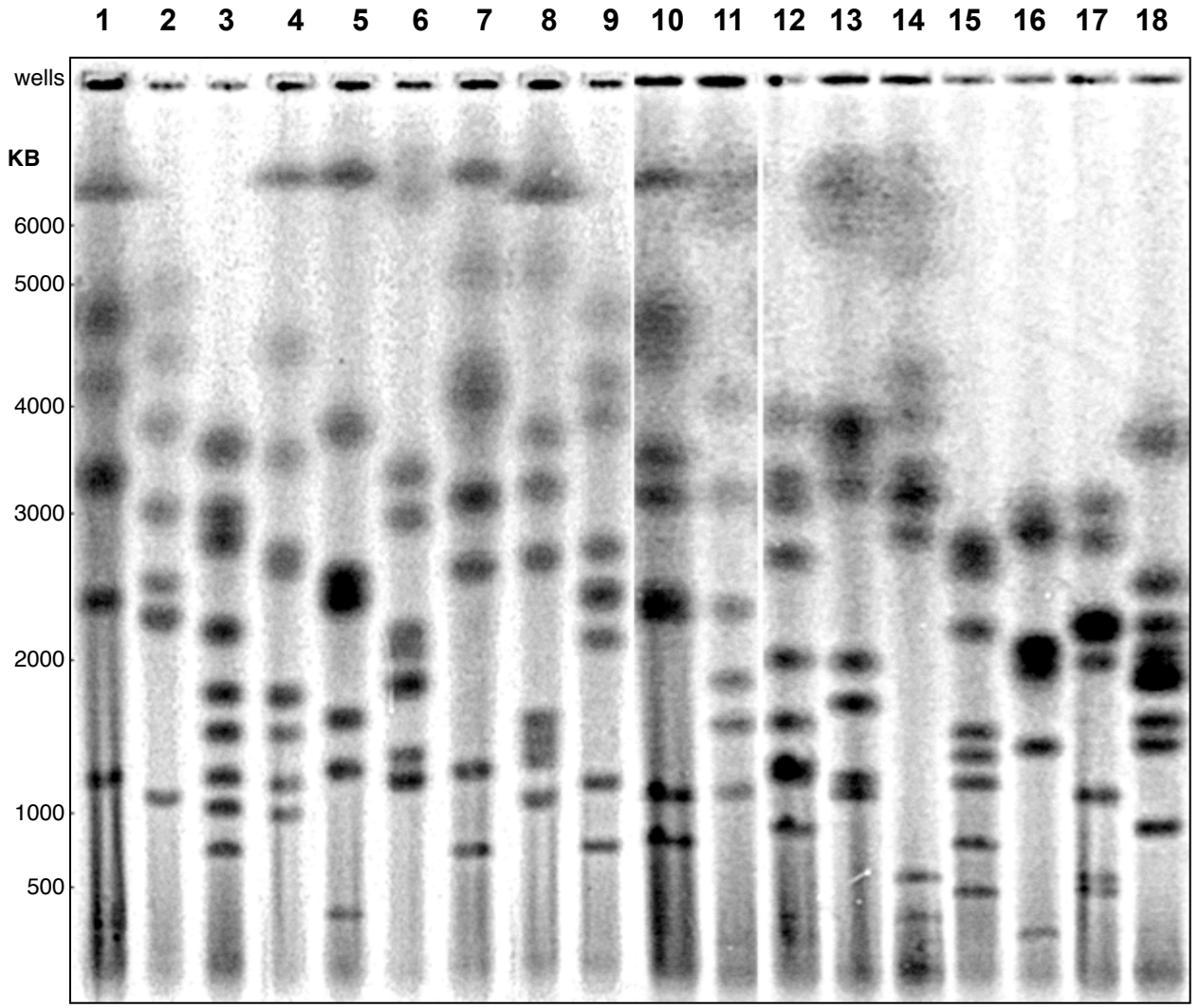




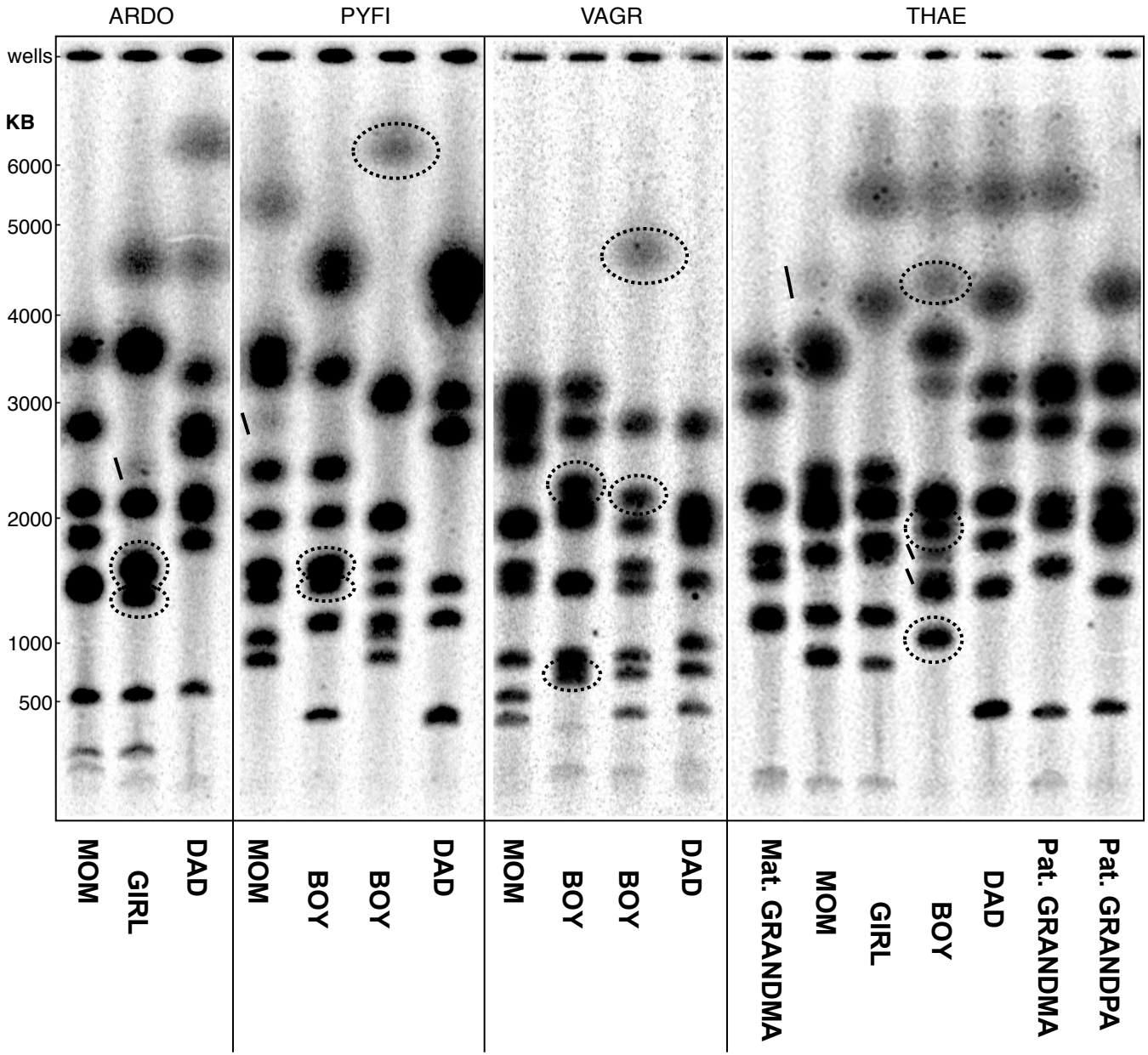
45S rDNA Arrays and the Human Genome Project



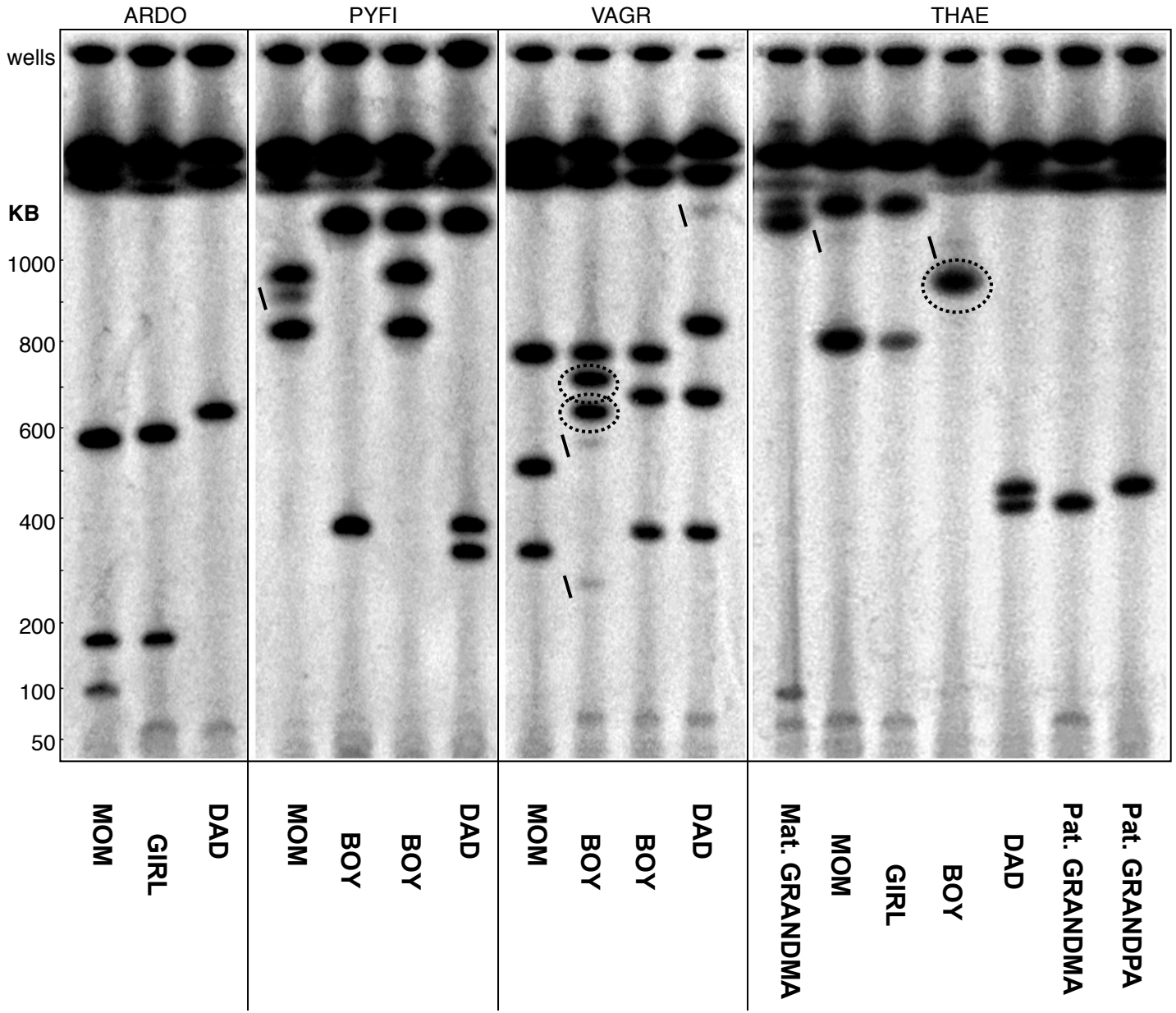
45S rDNA Arrays from Anonymous Human Donors



45S rDNA Arrays from Human Families – Inheritance



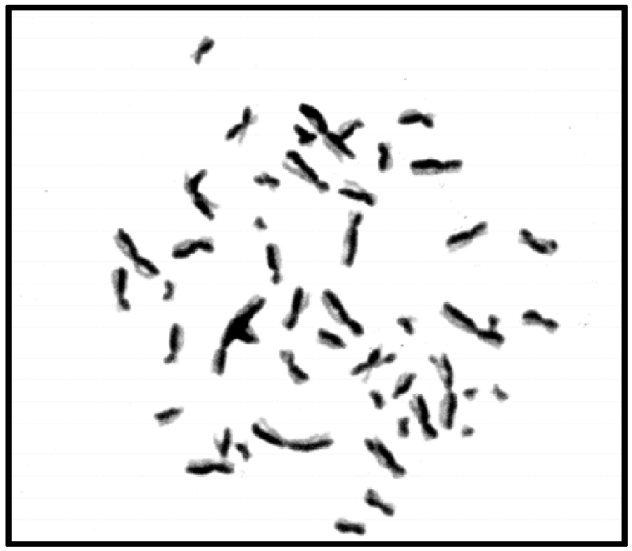
High Resolution 45S Arrays – Inheritance and Mosaicism



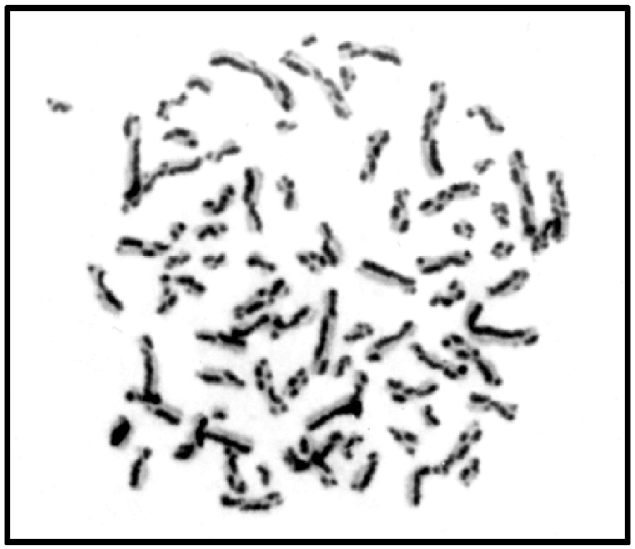
Conclusions

- Array lengths vary from person to person
- Array lengths are not coordinated between chromosome homologs
- Individual arrays are usually inherited in Mendelian manner but undergo meiotic recombination with a frequency of ~10% per array
- Mitotic recombination is capable of altering rDNA architecture in different cells in the same person

Bloom's Syndrome



Normal cell metaphase



Bloom's syndrome cell metaphase

Amor-Gueret M. **Bloom's syndrome**. Orphanet Encyclopedia. February 2004

- proportionate pre- and postnatal growth deficiency
- sun-sensitive
- telangiectatic
- hypo- and hyperpigmented skin
- **chromosomal instability, predisposition to malignancy**

BLM protein in RecQ-helicase family with Werner Syndrome (WRN), Rothmund-Thomson Syndrome (RecQ4), and two other non-disease-associated members RecQL, RecQ5b

Acknowledgements

Dawn Stults

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