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of Pathology


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Foundation

IMPACT OF NEXT-GENERATION DNA SEQUENCING AND WHOLE-GENOME ANALYSIS ON PATHOLOGY PRACTICE

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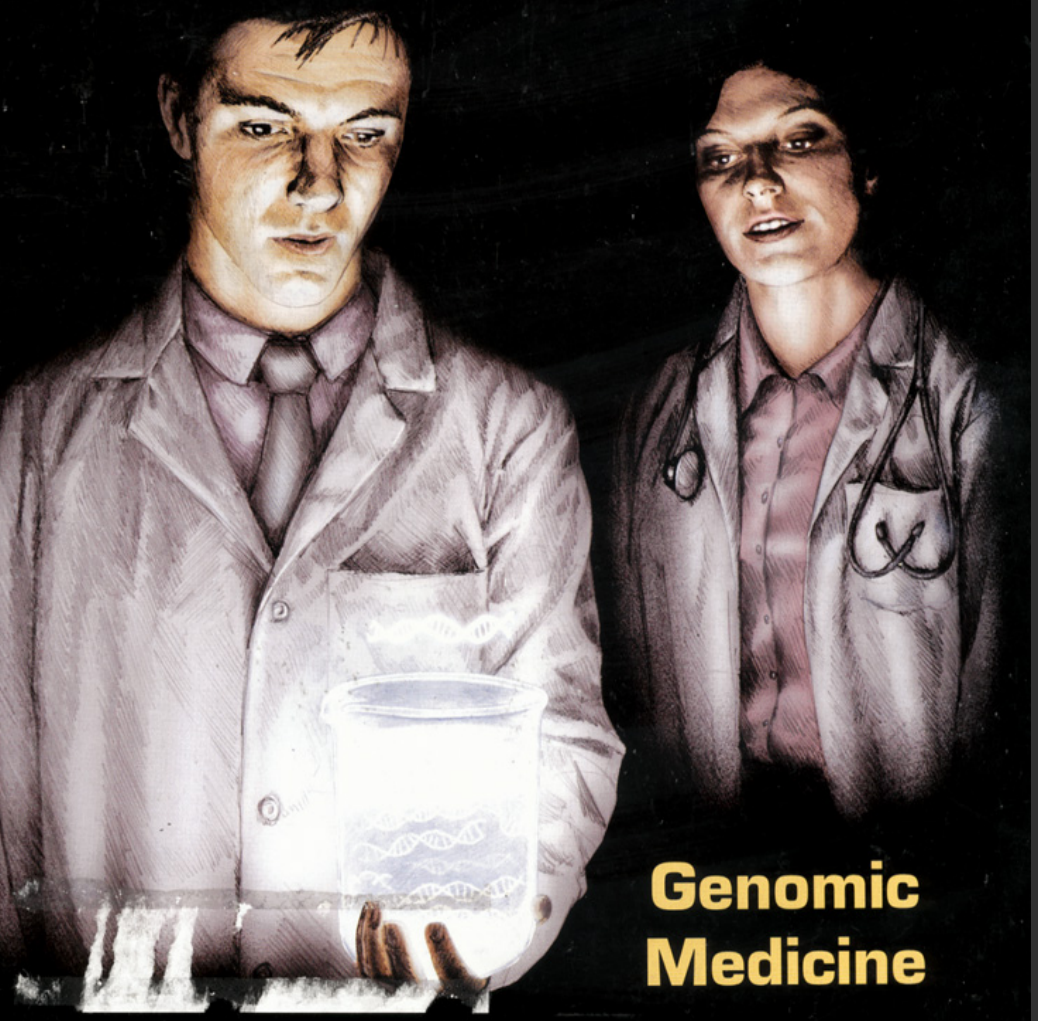


Genomic Medicine

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

24 October 2003 Science Vol. 302 No. 5645 Pages 517–728 Genomic Medicine

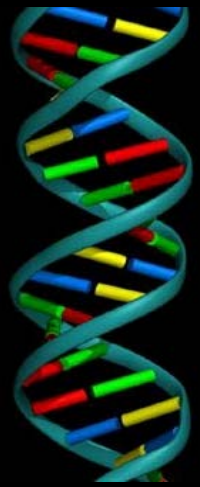
Genomic (Molecular) Medicine

- Gene-level diagnostics
- Gene-level therapeutics

What is “Personalized Medicine”?

Personalized Medicine

= Molecular Medicine



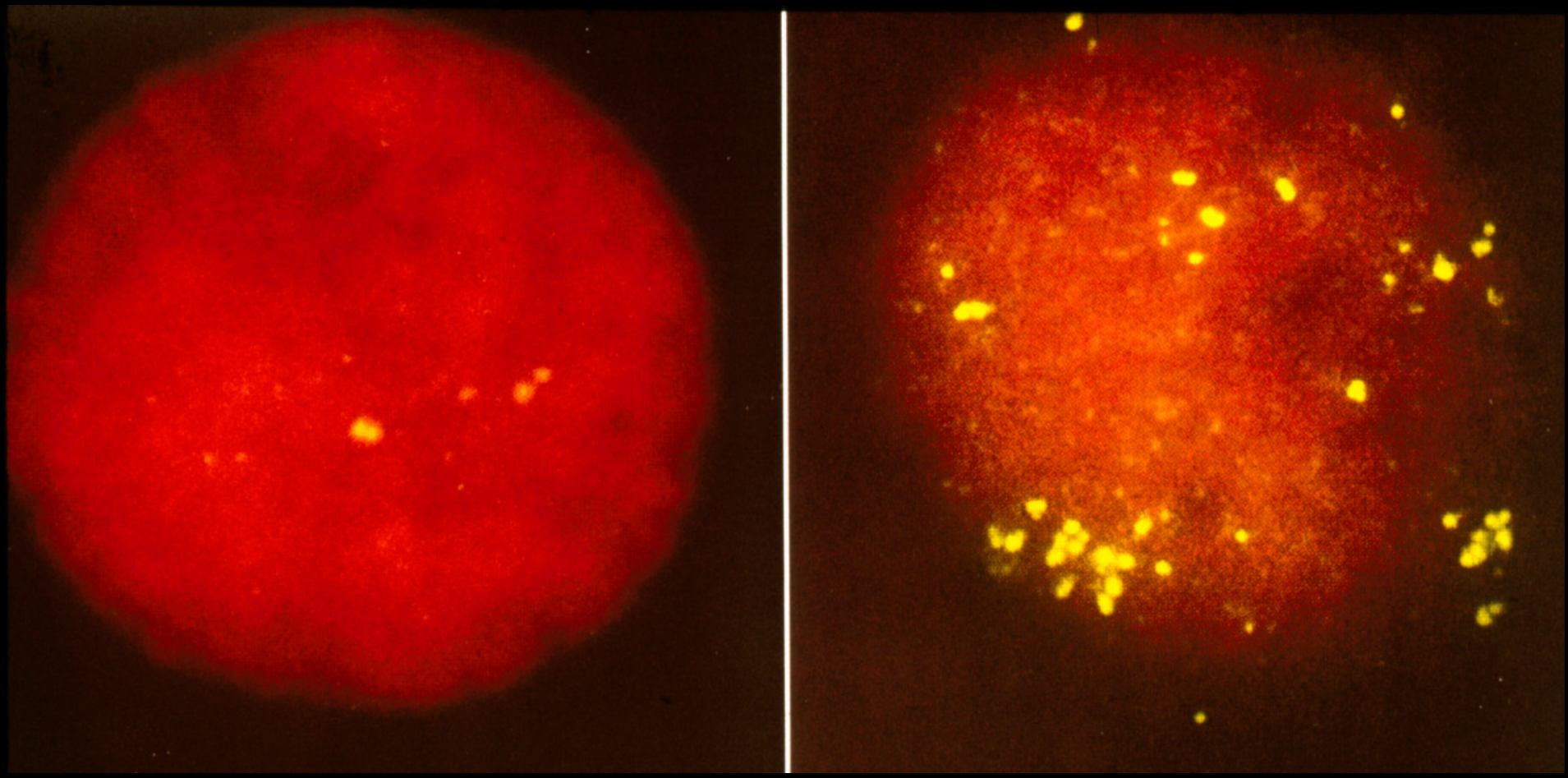
“Personalized Medicine”

- Pharmacogenetics
- “Companion” Diagnostics
- Patient-specific therapies

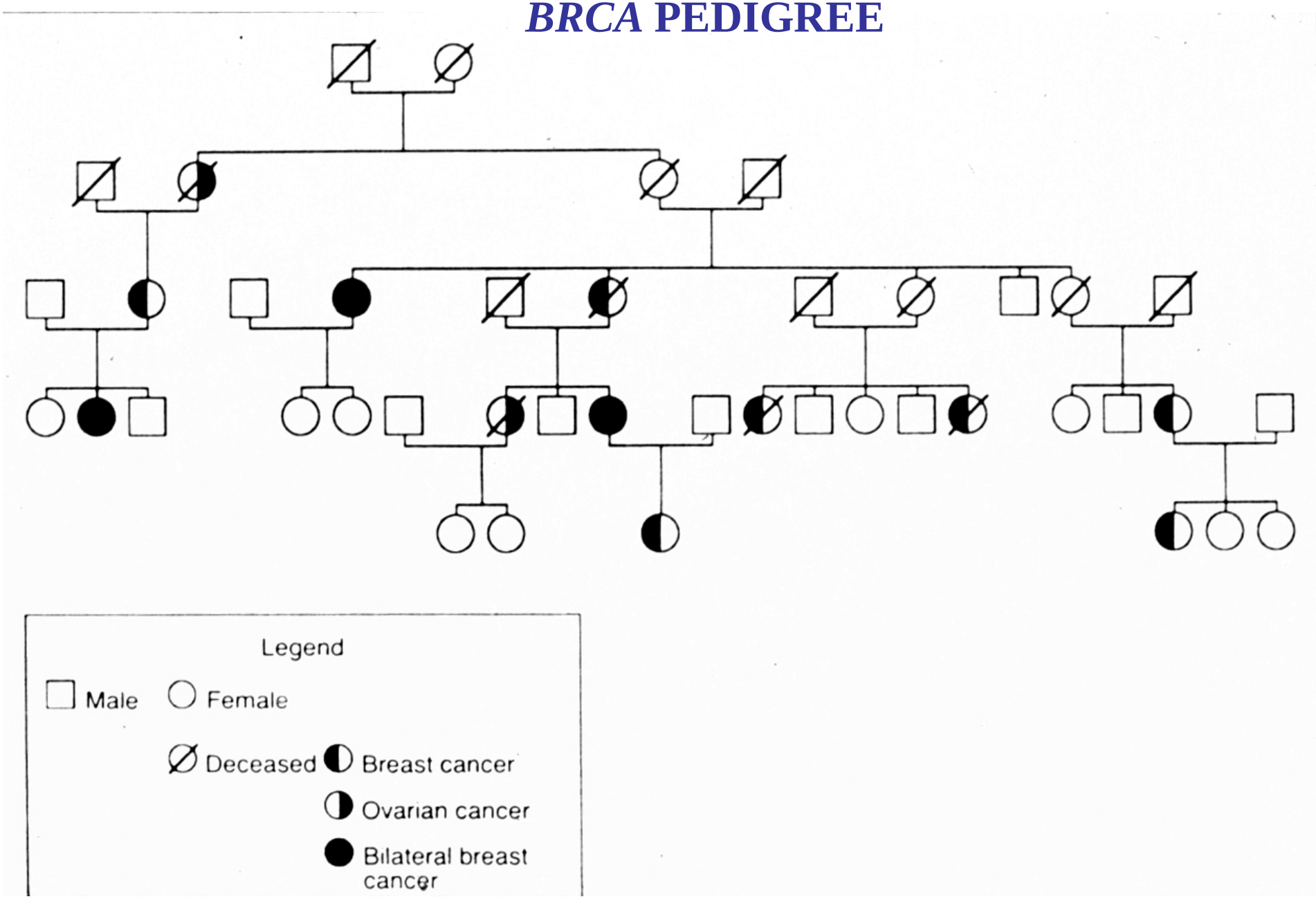
Is Whole-Genome Sequencing the Ultimate "Personalized Medicine"?

Somatic vs. Germline Mutation Testing

Detection of HER2/*neu* Amplication in Breast Cancer by FISH



BRCA PEDIGREE



Sequence Analysis of *BRCA1* and *BRCA2* Can Find the Needle in the Haystack

- ***BRCA1*: 22 coding exons, > 5,500 bp**

GGCTTTA**A**GTATCCAT

- ***BRCA2*: 26 coding exons, > 11,000 bp**

How is Routine Molecular Diagnostics Conducted Now?

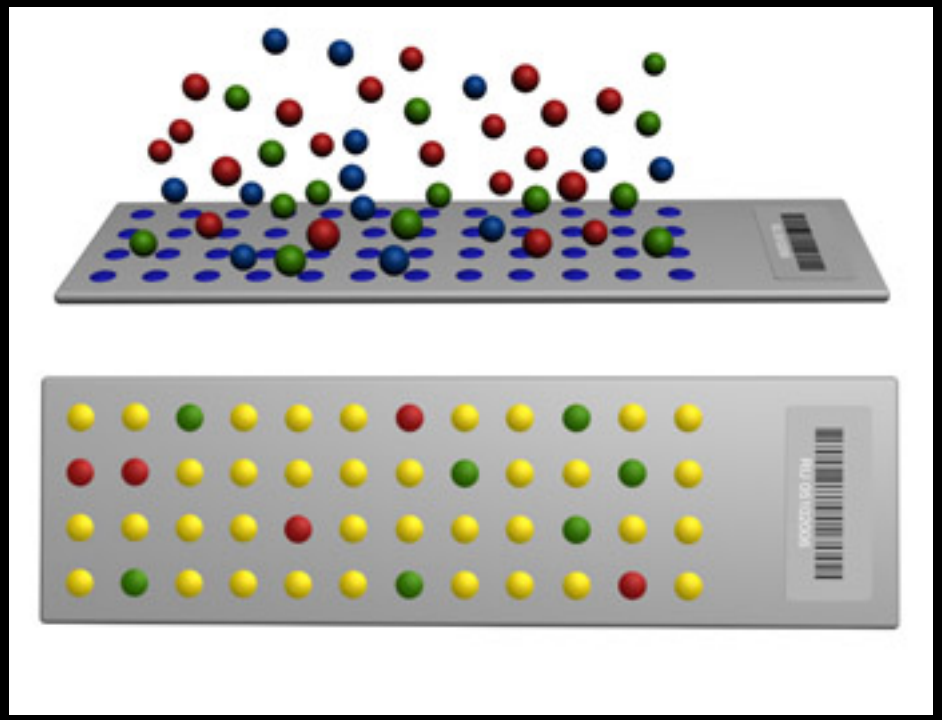
Current Techniques Applied to Molecular Pathology (one gene – one disease)

- Southern blot
- Dot blot/Reverse dot blot
- Polymerase chain reaction
- SSCP/DGGE
- RT-PCR
- DNA sequencing
- TaqMan, real-time PCR
- Invader assay
- *In situ* hybridization

New Techniques Coming to Molecular Pathology *(all genes – all diseases)*

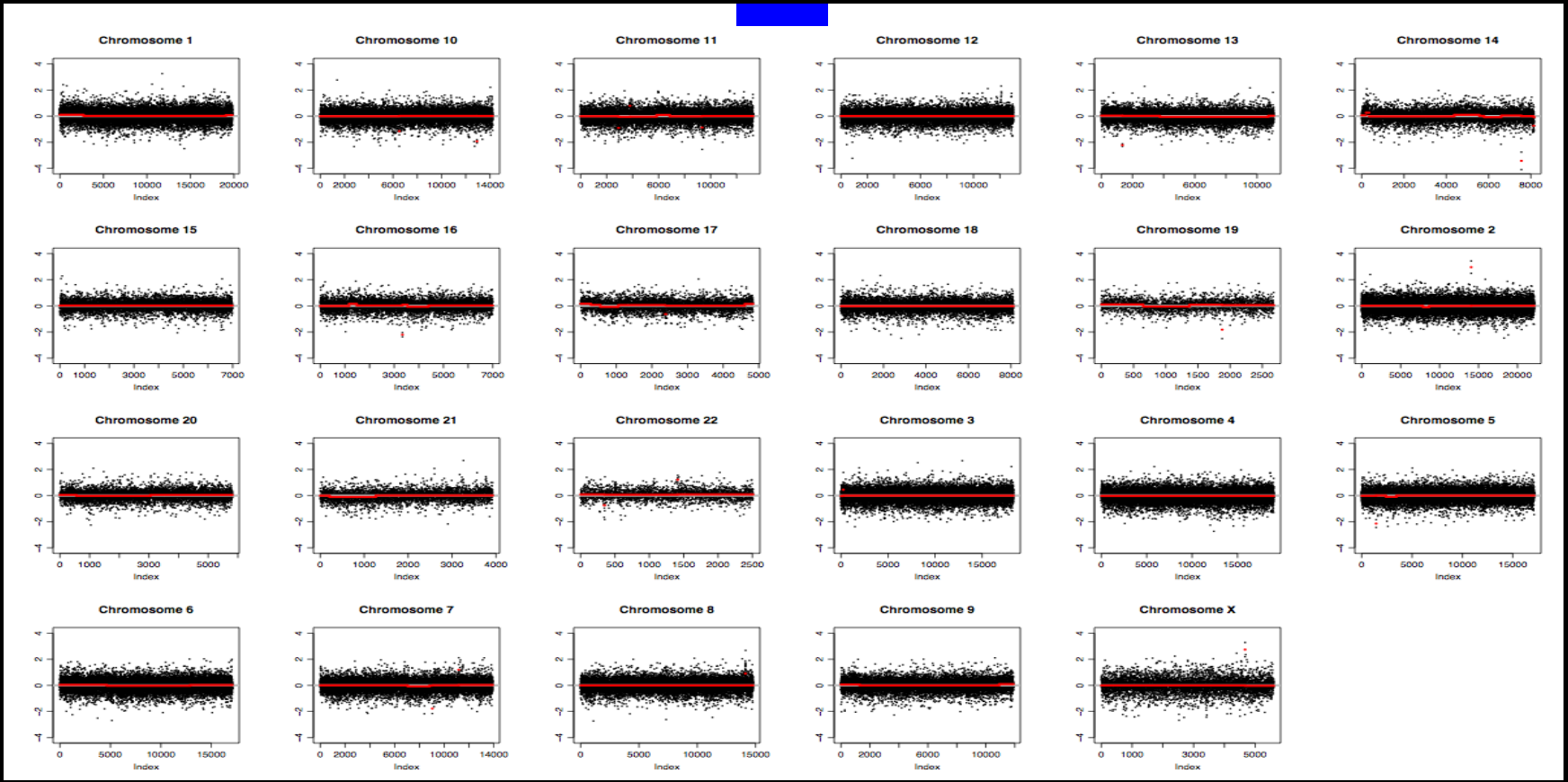
- Southern blot
- Dot blot/Reverse dot blot
- Polymerase chain reaction
- SSCP/DGGE
- RT-PCR
- DNA sequencing
- TaqMan, real-time PCR
- Invader assay
- *In situ* hybridization
- Microarray hybridization
- *High-density microarray hybridization*
- *Array comparative genomic hybridization*
- *Whole-genome sequencing*

Array Comparative Genomic Hybridization (aCGH)



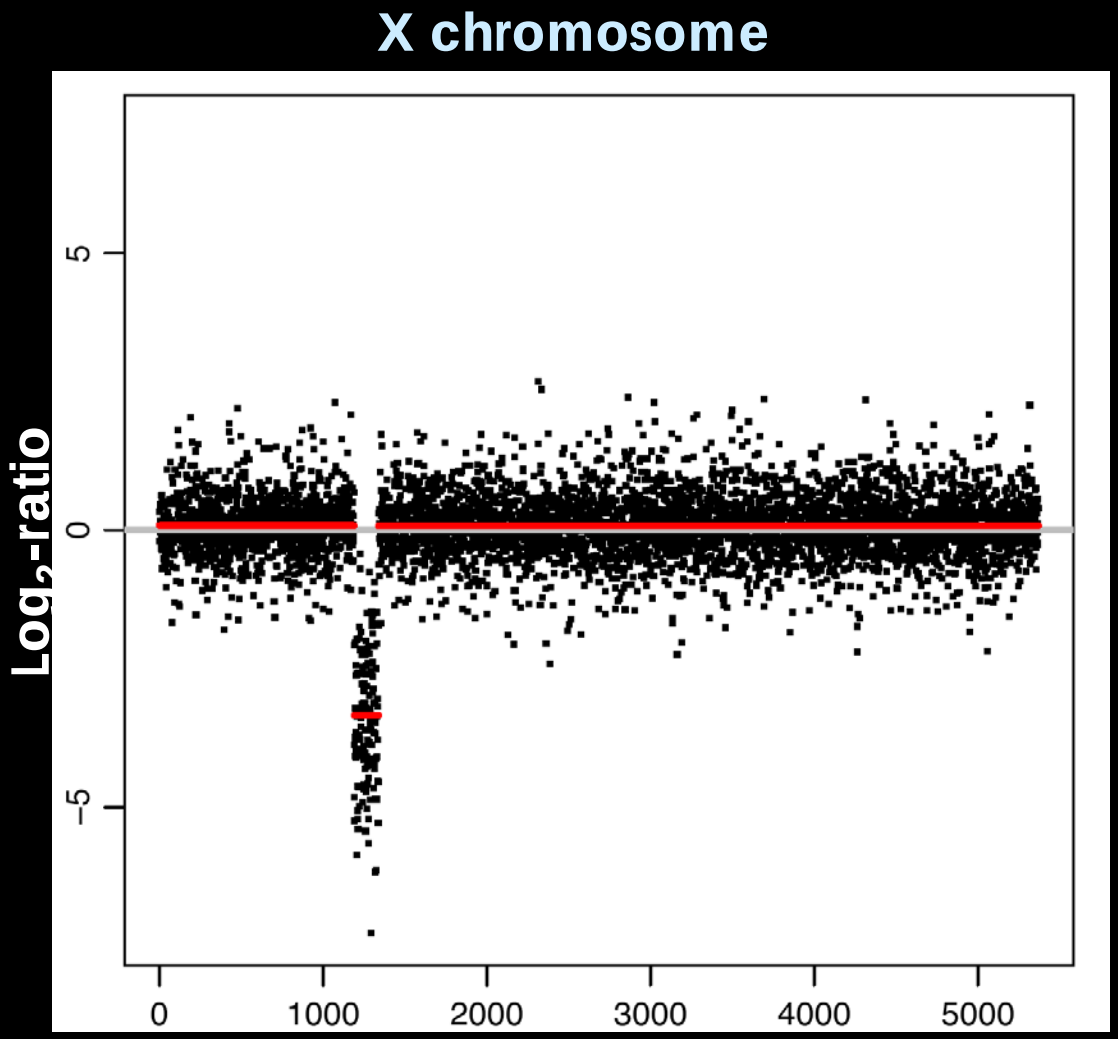
Whole Genome Data Is Acquired

- ✓ Patient below without any known genetic disease
- ✓ All chromosomes but Y represented



Xp21 Complex Glycerol Kinase Deficiency

- ✓ 6.66 Mb deletion
- ✓
- ✓

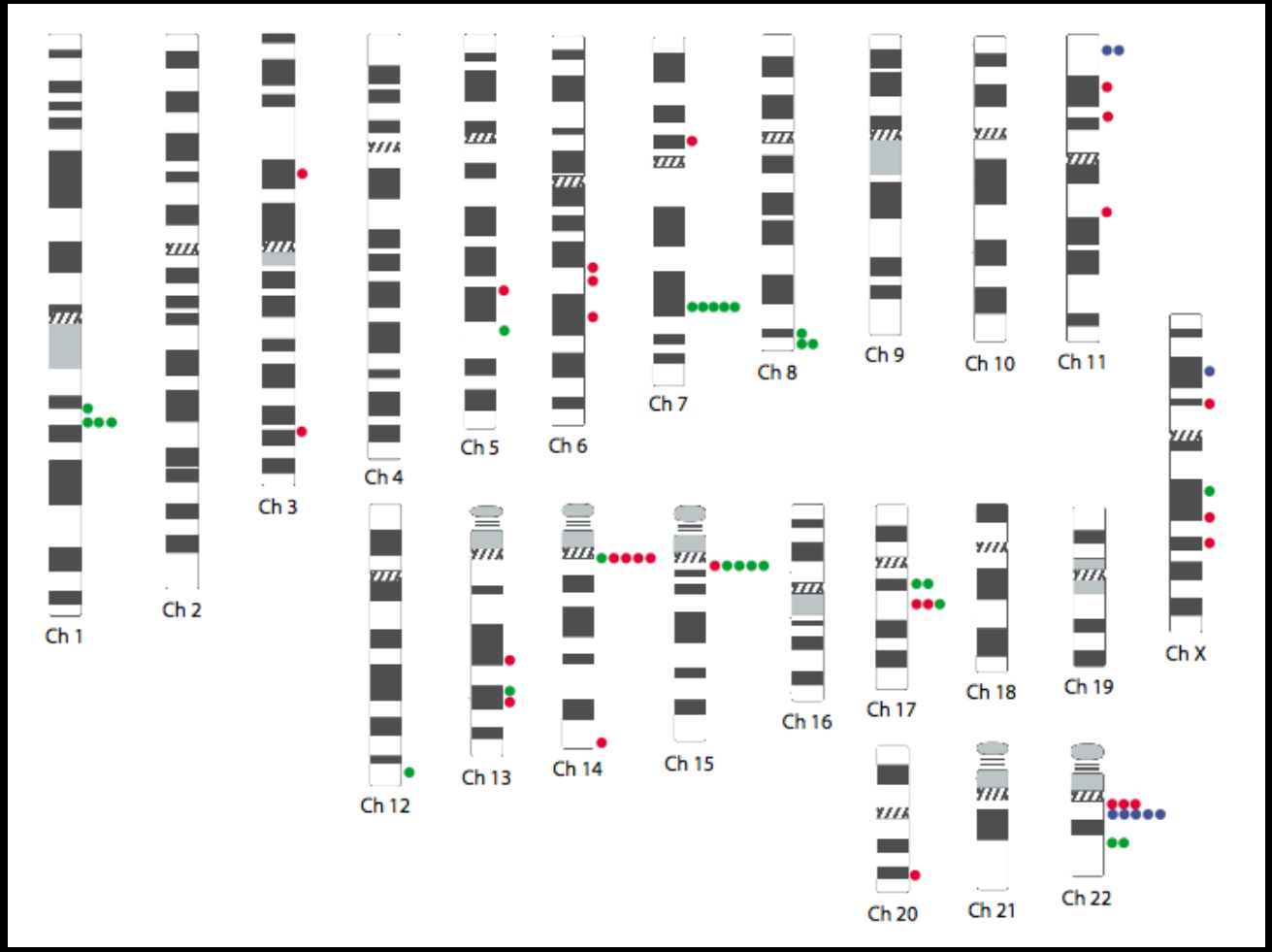


September 28, 2010

ACMG Recommends Replacing Karyotyping with Chromosomal Microarrays as 'First-Line' Postnatal Test

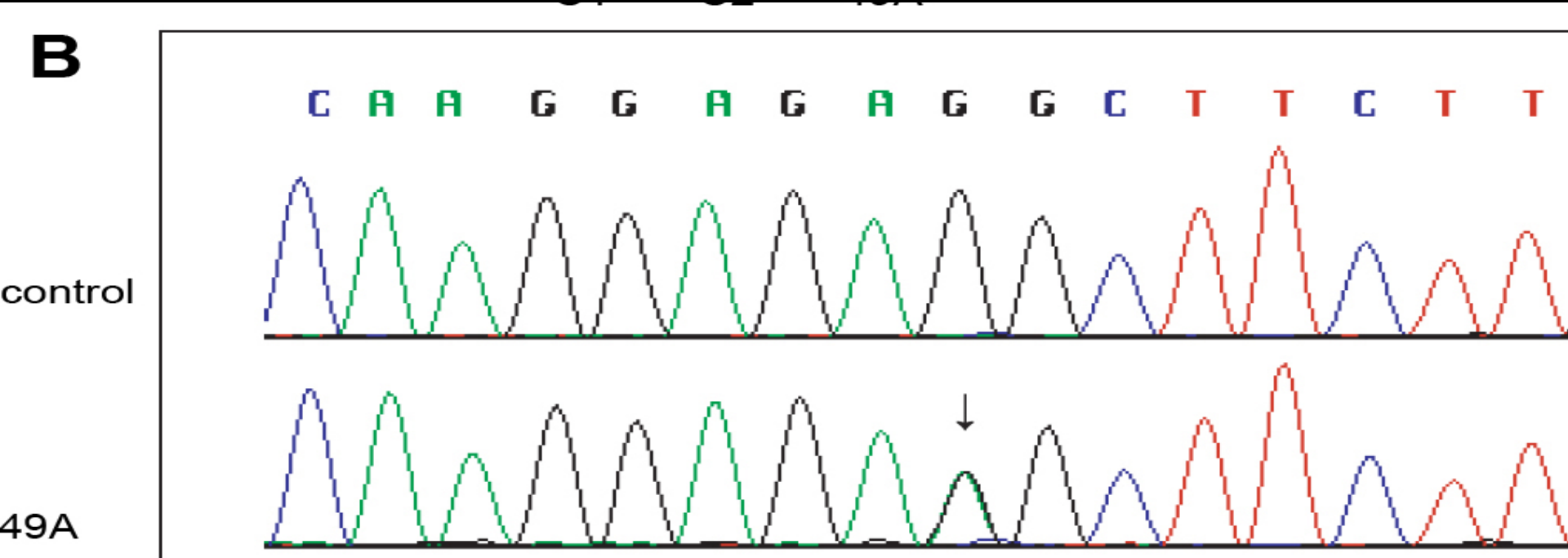
Microarrays should be used instead of G-banded karyotyping as the first test to detect genetic abnormalities in postnatal evaluations, according to the American College of Medical Genetics.

CNVs are common in all genomes surveyed …



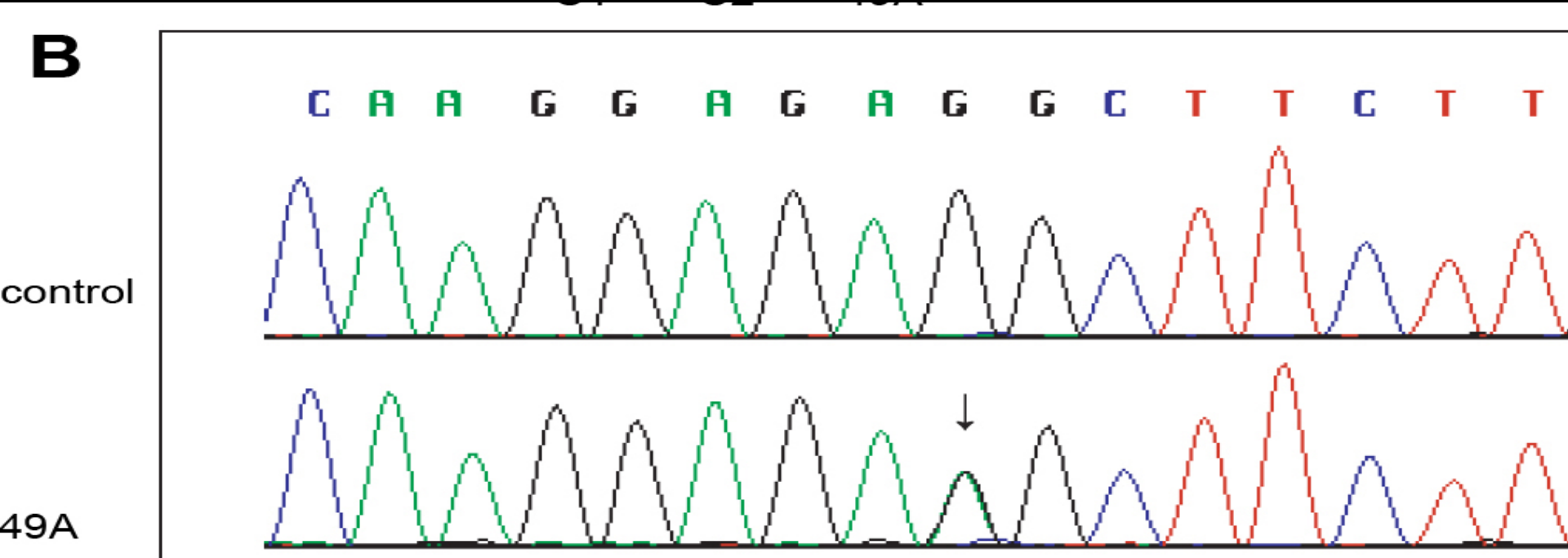
- **Blue** = pathogenic
- **Red** = deletion
- **Green** = duplication

And sequence variants are even more common...



And sequence variants are even more common...

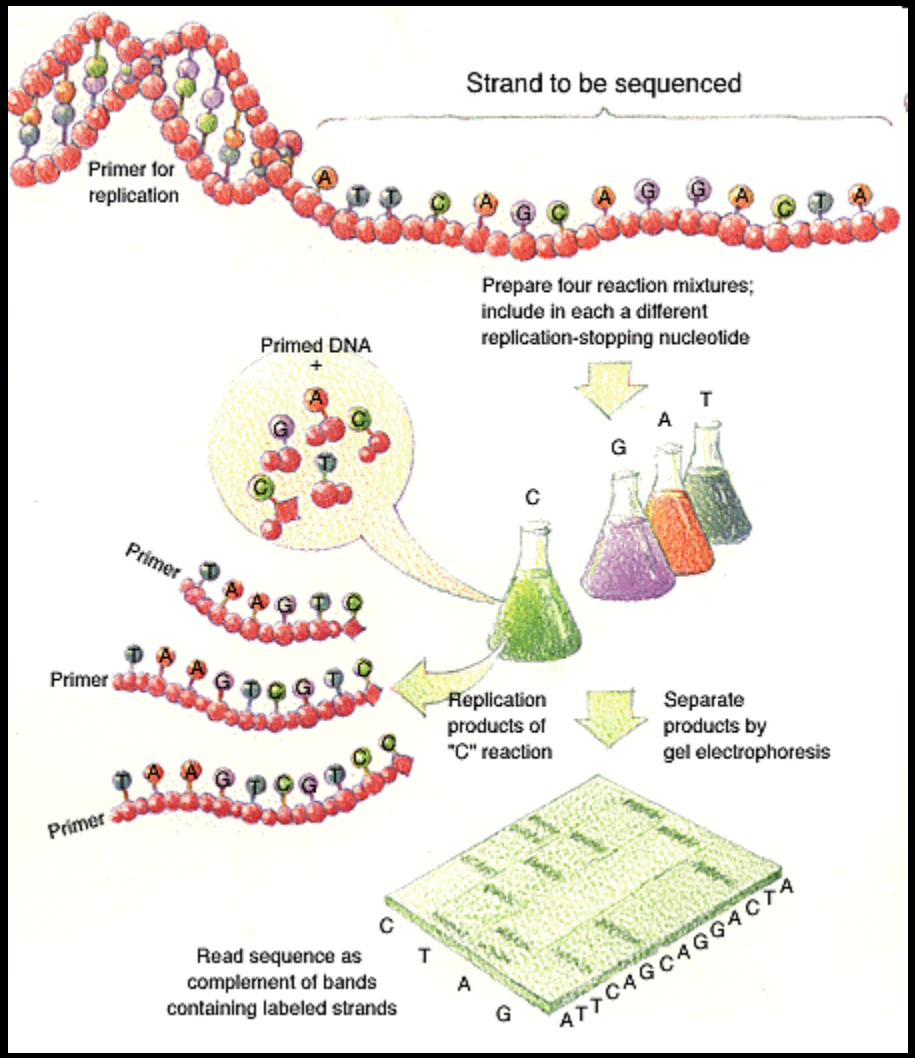
Incidentalome



THE HUMAN GENOME PROJECT
Timeline

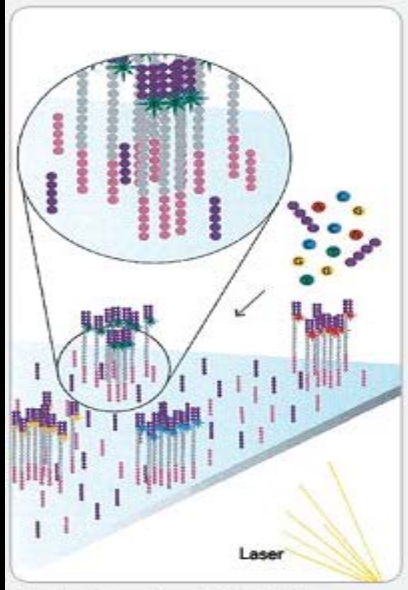
1985	Exploratory conferences held at UC-Santa Cruz and Santa Fe
1986	Human Genome Initiative announced by DOE
1987	NIH funding commences; 15-year plan formulated
1988	HUGO founded
1989	ELSI established
1990	15-year NIH-DOE project formally begins; $3 billion in funding pledged
1991	Genome Database established
1992	Low-resolution linkage map of entire human genome published
1993	First 5-year plan revised
1994	First 5-year goal achieved one year ahead of schedule
1995	High-resolution physical maps of chromosomes 16 and 19 completed
1996	Yeast genome sequence completed
	Human genome physical map with 30,000 STS's achieved
1997	NCHGR becomes NHGRI
	Task Force on Genetic Testing releases report
	E. coli genome sequence completed
	High-resolution physical maps of chromosomes X and 7 completed
1998	New 5-year plan announced for project completion by 2003
	C. elegans genome sequence completed
1999	First human chromosome (#22) completely sequenced
	Target date for draft sequence of entire human genome revised from 2001 to 2000

Sanger Sequencing



Next-Generation DNA Sequencing

7. DETERMINE FIRST BASE

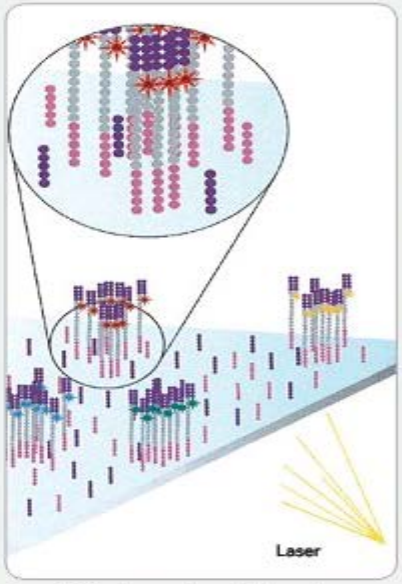


First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE

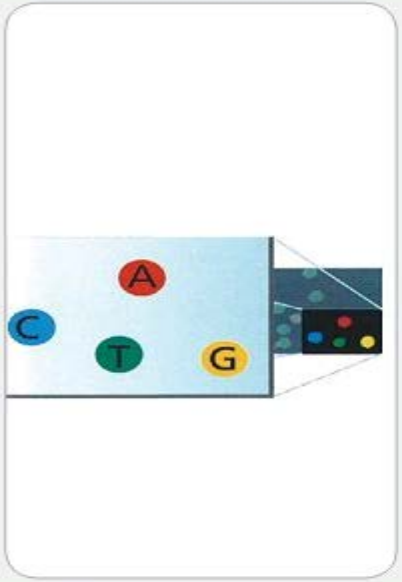
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

9. DETERMINE SECOND BASE



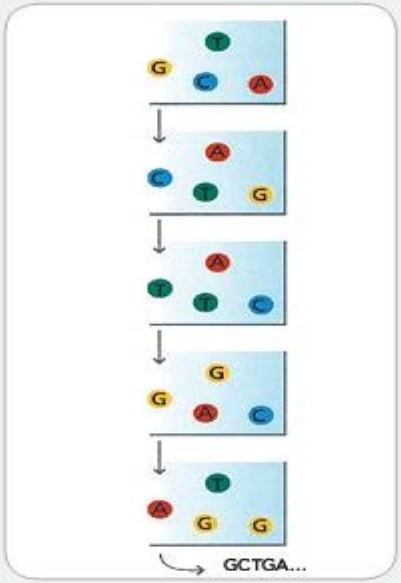
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE



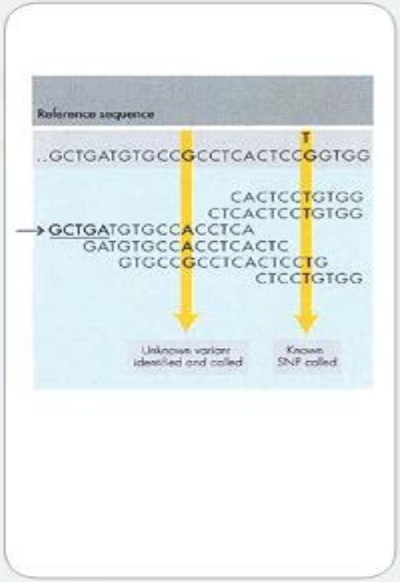
After laser excitation, collect the image data

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine

12. ALIGN DATA



Align data, compare to a reference, and

“Next-Generation” Sequencers

Sequencing System[a]	Estimated system cost	Consumable cost per single-end run (paired-end run)	Read Length per single-end run (paired-end)	Gigabases sequenced per single-end run (paired-end)	Run time per single-end run (paired-end)	Raw accuracy
454 Genome Sequencer FLX	$500,000[b]	n/a[c]	250-300 bp (2 X 110 bp)[d]	0.1 Gb[e] (0.1 Gb)	7.5 hours (7.5 hours)	99.5%
Illumina Genome Analyzer	~$400,000	$3000 (n/a)[f]	36 bp[g] (2 X 36 bp)	1.5 Gb (3.0 Gb)	2.5 days (5 days)	>98.5%
ABI SOLiD™ System	$525,000	$3390[h] ($4390)	35 bp (2 X 25 bp)[i]	3 Gb[j] (4 Gb)	5-7 days[k] (10 days)	99.94%
Helicos Heliscope	n/a	n/a	25-35 bp[l]	7.5-10 Gb	3-7 days	>99%

TenBosch & Grody, *J. Molec. Diagn*. (2008)

“Next-Next-” or “Third”-Generation Sequencing Technologies

- Pacific Biosciences
- Oxford Nanopore
- Ion Torrent
- Others…

Journal of Molecular Diagnostics, Vol. 10, No. 6, November 2008

DOI: 10.2353/jmoldx.2008.080027

Review

Keeping Up With the Next Generation

Massively Parallel Sequencing in Clinical Diagnostics

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and Wayne W. Grody, M.D., Ph.D.*†‡

From the Departments of Human Genetics, Pathology & Laboratory Medicine,† and Pediatrics,‡ University of California at Los Angeles School of Medicine, Los Angeles, California*

since the initial derivation of the technique by Maxam and Gilbert[1] and Sanger et al.[2] Cumbersome chemical methods gave way to enzymatic procedures, and manual techniques were replaced by even-faster automated instruments using capillary electrophoresis or high-density microarrays.[3] More recently, the advent of massively par-

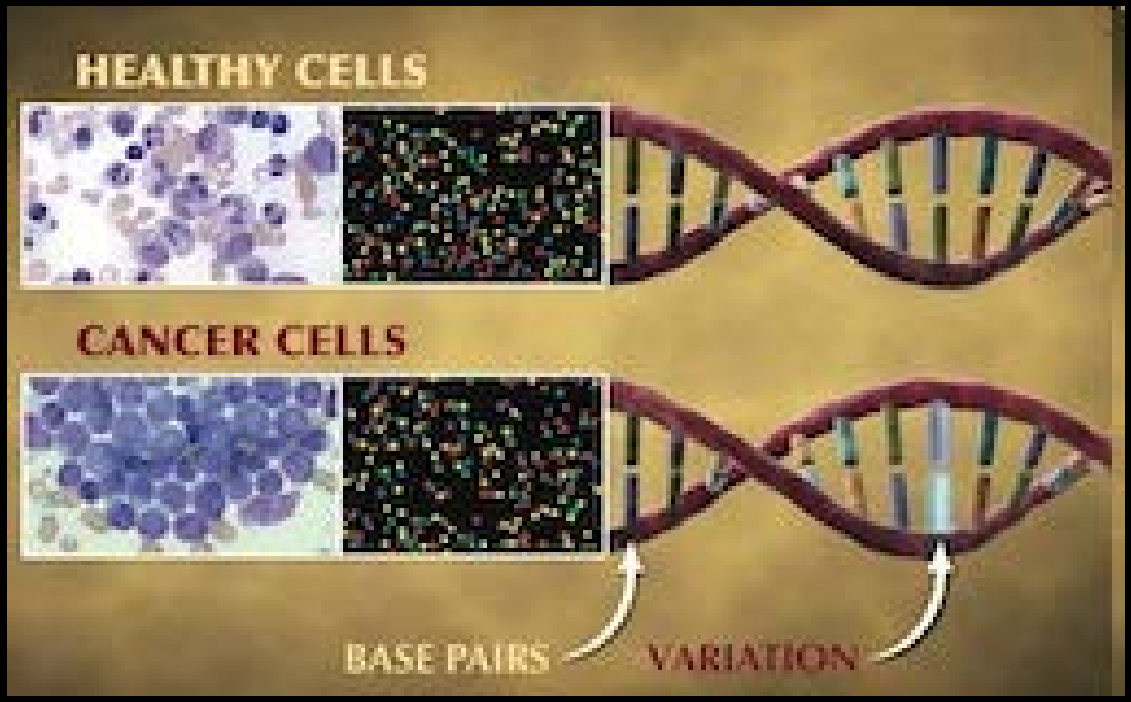
J. Molec. Diagn. 2008; 10:484-492

Potential Disease Gene Panels for Next-Generation Sequencing

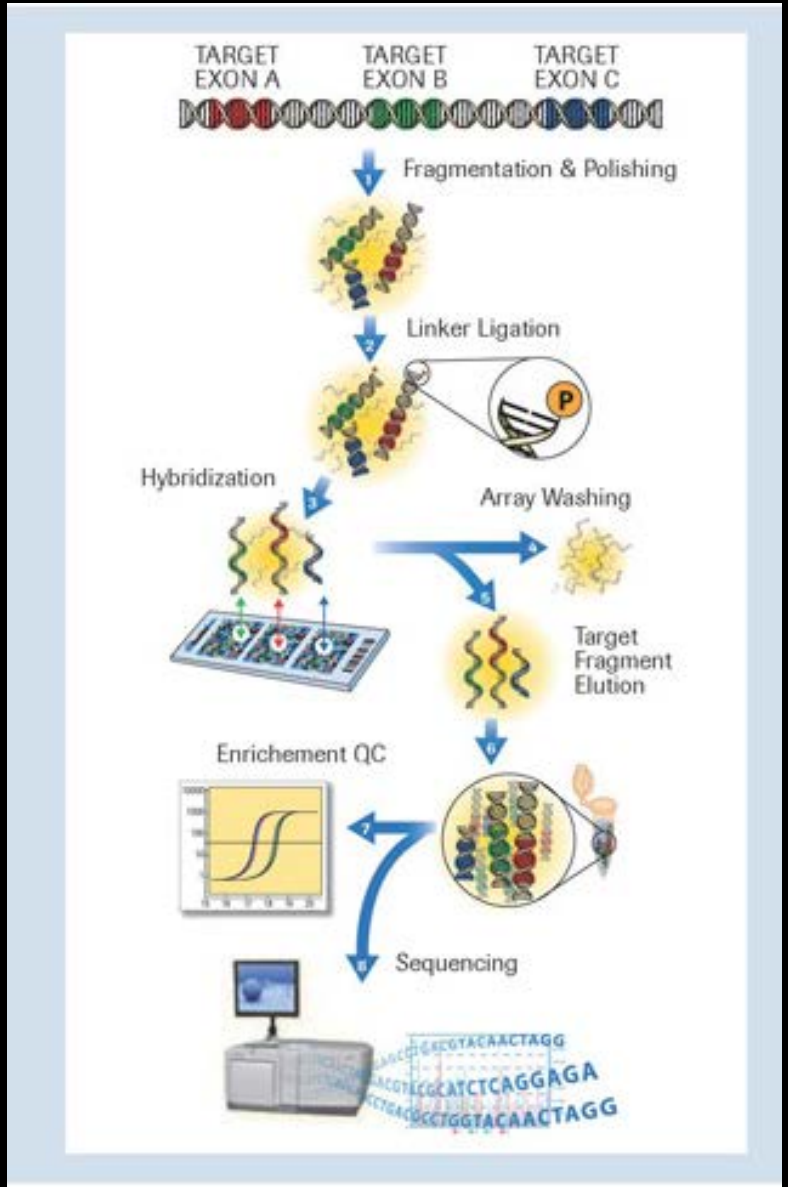
- Hypertrophic cardiomyopathy
- Dilated cardiomyopathy
- Hereditary arrhythmias (channelopathies)
- Retinitis pigmentosa
- Albinism
- Mental retardation
- Hearing loss

Development of Personalized Tumor Biomarkers Using Massively Parallel Sequencing

Whole-Genome Sequencing of Tumors



Whole-Exome Sequencing of Germline DNA



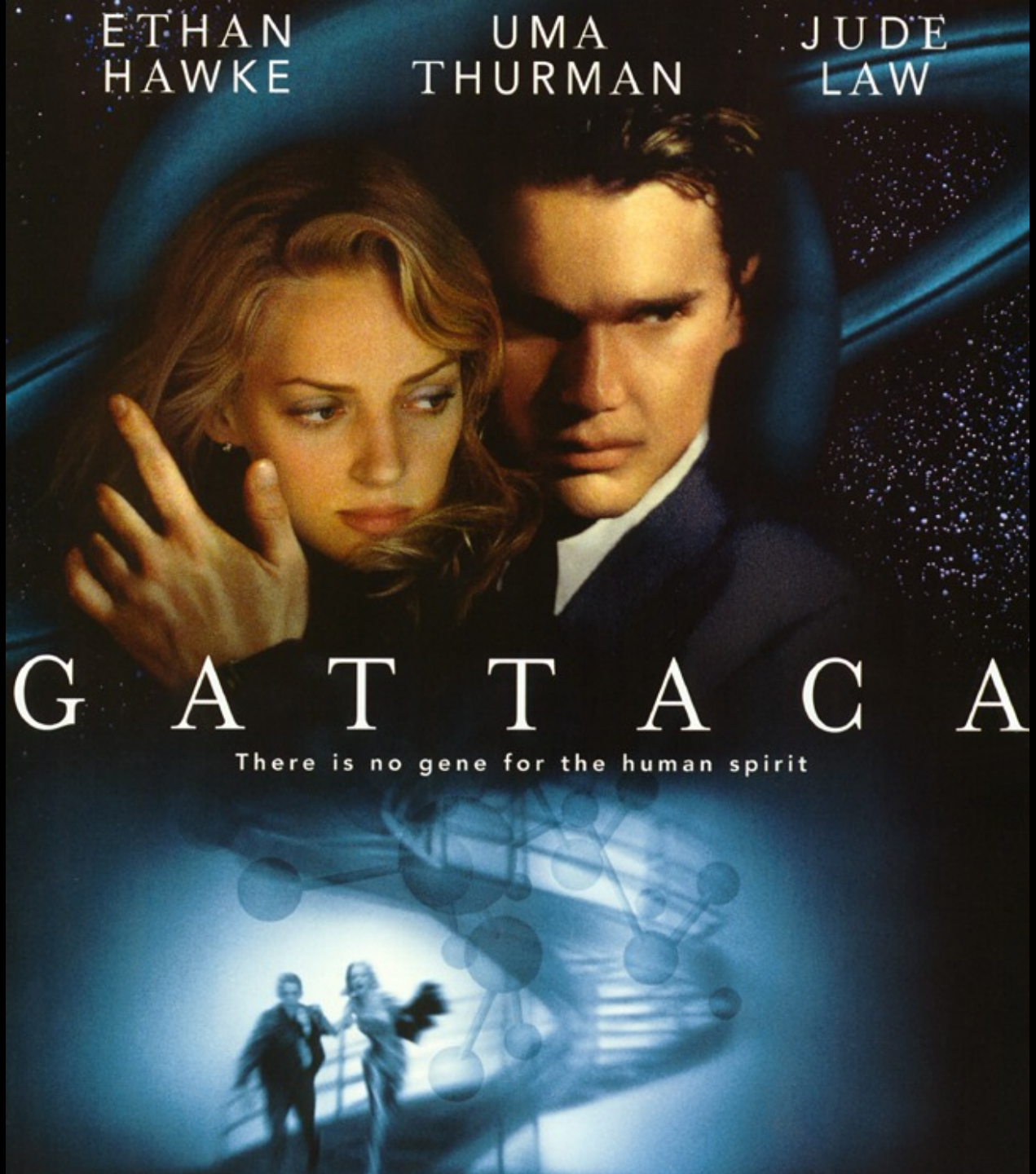
Should whole-genome/exome sequencing be applied to:

Newborn screening?

Prenatal diagnosis?

Couple screening?

Population screening?


ETHAN HAWKE
UMA THURMAN
JUDE LAW
GATTACA
There is no gene for the human spirit

NIH Task Force on Genetic Testing

Testing of Children

Genetic testing of children for adult onset diseases should not be undertaken unless direct medical benefit will accrue to the child and this benefit would be lost by waiting until the child has reached adulthood. The Task Force agrees with the American Society of Human Genetics and the American College of Medical Genetics that "Timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents."[48] Although

GINA


What's in your genes
is YOUR BUSINESS
SAY NO
TO
GENETIC
DISCRIMINATION
Contact your Congressman. Get Senate Bill 1053
OFF THE DESK and ON THE FLOOR!

Classes of Novel/Unexpected Sequence Variants Identified by Whole Genome Sequencing

- Missense variants of uncertain significance in known gene
- Variants and deleterious mutations in unknown gene(s)
- Deleterious mutations in unintended target (e.g., *BRCA* mutations in a baby)

WGS Represents a Sea-Change in Clinical Laboratory Testing:

For the first time, patients will need to choose beforehand what portions of the test results they wish to receive or not receive.

Informed Consent for Whole Genome Sequencing: Patient Choices

- Receive *all* information (CD, DVD?)
- Receive relevant/targeted information
- Receive medically actionable information for patient's age
- Receive medically actionable information for future
- Receive medically actionable information for relatives

Ethical Dilemmas of Whole Genome Sequencing

- Revelation of “off-target” mutations
- Many revealed disorders will have no prevention or treatment
- Revelation of nonpaternity, consanguinity, incest
- Costs of genetic counseling and follow-up
- Possible forensic uses of data
- Data storage and privacy
- Huge number of novel missense variants

College of American Pathologists

Commission on Laboratory Accreditation

Inspection Checklist

MOLECULAR PATHOLOGY

Section: 12

CAP Laboratory Accreditation No: ☐☐☐☐☐☐-☐☐

Laboratory:

Section Name:

Address:

City: | State: | ZIP:

Laboratory Director:

Inspector: | Inspection Date:

A Little Taste of the Challenge Ahead:
Sequencing Experience With *BRCA1&2*

- Complete sequencing of both genes in >150,000 people at Myriad Genetics alone
- >10,000 mutations and benign or uncertain variants identified

[B. Ward, *personal communication*]

A Little Taste of the Challenge Ahead:
Sequencing Experience With *BRCA1&2*

- Complete sequencing of both genes in >150,000 people at Myriad Genetics alone
- >10,000 mutations and benign or uncertain variants identified
- *Yet every week, detect 10-20 new missense variants never seen before*

[B. Ward, *personal communication*]

The Storefront Genome

Within a decade, anyone will be able to read his or her genetic makeup for a few hundred dollars. We will have to deal with this information, both as individuals and as a society.

Predictive, Preventive, and Personalized Medicine – A look ahead
Leroy Hood

Health Records, Genealogy, and Genetics – Lessons from Iceland About the Tension Between Public and Private Good
Kari Stefansson

Genomics & the Law: Forensics, Privacy, Responsibility
Mark Rothstein

Genomics and Human Identity: Who are Your People?
Eric Juengst

Knowledge and its Consequences: Decision Making and Patient Care in the Genomic Era
Nancy Wexler

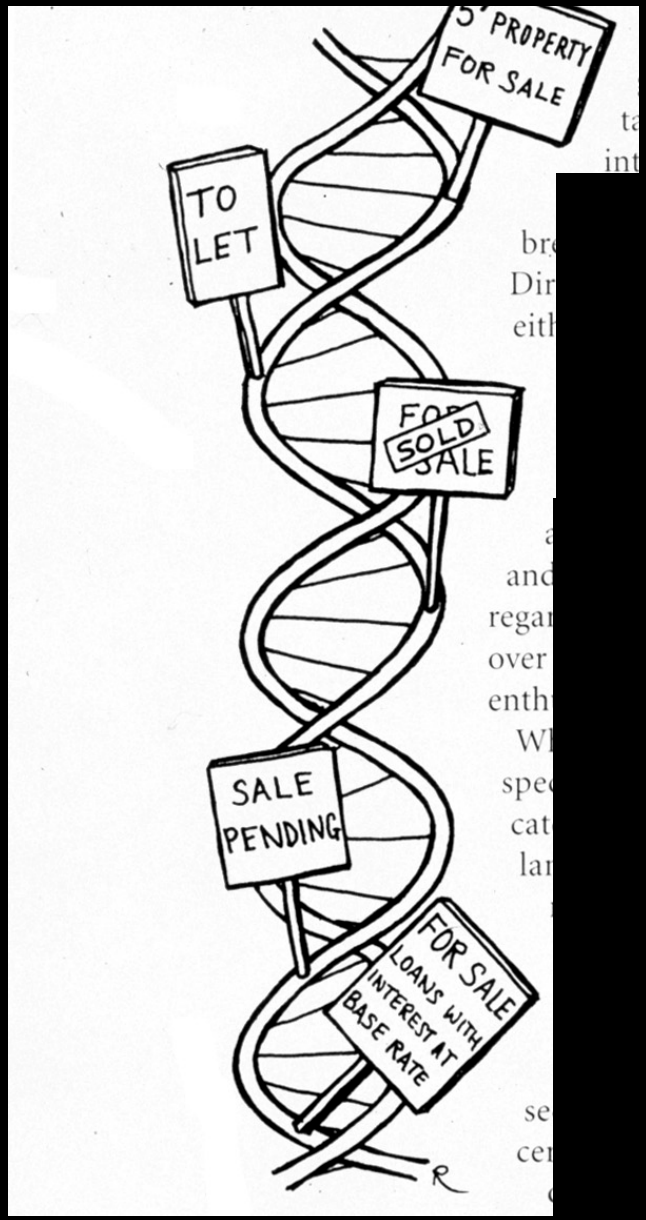
Genomics and the Future of Insurance
J. Alexander Lowden

Choosing our Children's Genes: Promises and Perils
Bonnie Steinbock

Moderator
Director Program on Medicine, Technology and Society, UCLA
Gregory Stock

Sunday, January 26, 2003
9:00 am - 4:00 pm
Sunset Village
Conference Center, UCLA
Open to the Public - Free

presented by
UCLA
CENTER FOR SOCIETY, THE INDIVIDUAL AND GENETICS
http://www.arc2.ucla.edu/csig/


PROPERTY FOR SALE
TO LET
SOLD
SALE
SALE PENDING
FOR SALE
LOANS WITH INTEREST AT BASE RATE

A Sample of Genetic Testing Patents

- 5,753,441 BRCA1
- 5,753,438 Hereditary hemochromatosis
- 5,741,645 Spinocerebellar ataxia Type 1
- 5,693,470 Non-polyposis colorectal cancer
- 5,691,144 CMT-X
- 5,686,240 Niemann-Pick disease
- 5,681,699 Ulcer. colitis and Crohn's disease
- 5,679,635 Canavan disease
- 5,670,320 Dystonia, Leber's optic neuro.
- 5,658,729 Premature atherosclerosis
- 5,654,138 Von Hippel-Lindau (VHL)
- 5,650,282 Williams syndrome
- 5,650,281 Colorectal cancer
- 5,645,995 Breast or ovarian cancer
- 5,645,993 HNLPP
- 5,639,614 Idiopathic dilated cardiomyopathy
- 5,639,607 Lead sensitivity
- 5,565,323 Sporadic Alzheimer disease
- 5,550,021 Compulsive disorder
- 5,541,060 Early-onset diabetes mellitus
- 5,518,880 XSCID
- 5,508,167 Alzheimer disease
- 5,506,101 Ototoxic deafness
- 5,500,343 Compulsive disorder (cocaine)
- 5,498,521 Retinal degenerative diseases
- 5,494,794 Alzheimer, Parkinson
- 5,492,808 Familial colon cancer (FCC)
- 5,429,923 Hypertrophic cardiomyopathy
- 5,387,506 Familial dysautonomia
- 5,374,525 Hypertension
- 5,306,616 CMT-1A
- 5,296,349 Myoclonic epilepsy
- 5,266,459 Gaucher disease
- 5,210,016 Compulsive disorder (alcohol)
- 5,045,449 Vascular aneurysms

"Cease and desist…"

- Spinocerebellar ataxia (types 1, 2, 3 and 6)
- Charcot-Marie-Tooth syndrome
- BRCA1&2 mutations (non-Ashkenazi)
- Hereditary hemochromatosis
- Immunoglobulin & TCR gene rearrangements
- Congenital hearing loss (connexin-26 and -30)
- MTHFR variants
- BCR-ABL mutations
- FLT3 mutations

Genetic Testing Patents

- **Claim covers the observation of an individual's genetic makeup at a disease-associated locus when done for diagnostic purposes**
  - **Includes (bars) <u>all</u> methods of looking at the locus**
  - **Permits monopolization of a medical practice**
  - **Permits "ownership" of a disease**

Impact on Healthcare

- **Limited access and noncompetitive pricing**
- **Increased healthcare costs**
- **Lack of peer review and comparison**
- **Hampered quality assurance**
- **Potential undetected systematic errors**
- **Interference with medical training**
- **Restricted opportunity and incentive for test improvements and advancement of the field**
- ***Missing or masked targets on microarrays and whole-genome sequencing?***

ACLU

AMERICAN CIVIL LIBERTIES UNION

Association for Molecular Pathology *et al*.

v.

Myriad Genetics, United States Patent and Trademark Office, *et al*.

Key Plaintiffs in the ACLU Suit

- Association for Molecular Pathology
- American College of Medical Genetics
- American Society for Clinical Pathology
- College of American Pathologists
- Academic geneticists whose *BRCA* testing was shut down
- Breast Cancer Action Network
- Individual breast cancer patients

Key Arguments in the ACLU Suit

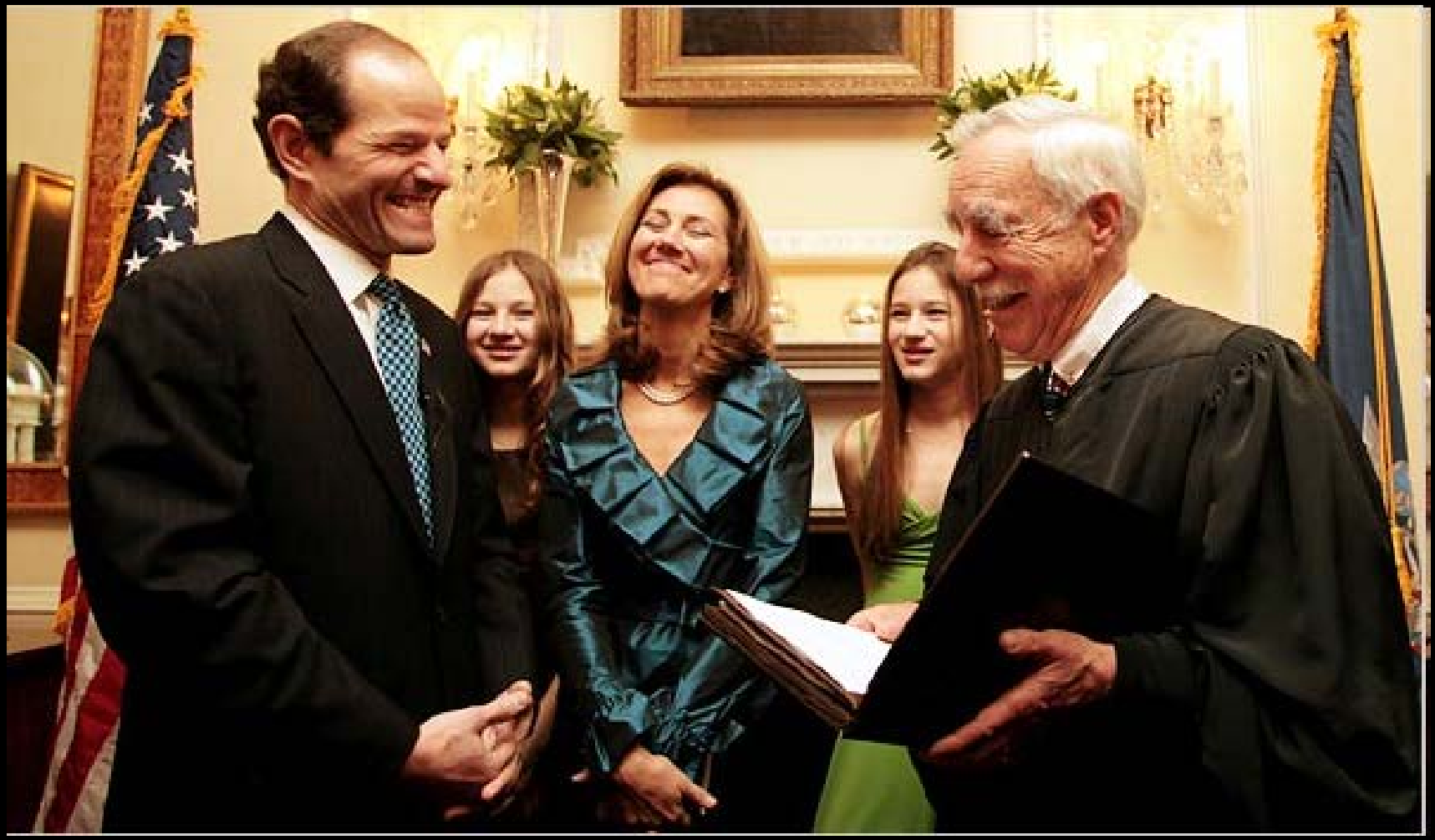
- Genes are products of nature, not inventions.
- It is unconstitutional to patent a person's individuality.
- Patients are prevented from seeking a "second opinion".
- Gene patents are overly broad.
- Legal principles bar patenting of laws of nature, products of nature, and abstract ideas.
- Gene patents violate the First Amendment by inhibiting free speech and access to information.

Key Arguments of the Defendants

- The 7 patents deal with *"isolated" BRCA* genes.
- "These isolated molecules are man-made chemical compositions, structurally and functionally distinct from any substance found in the human body – indeed, in all of nature."
- The method claims involve unique molecular tools such as DNA probes and primers.
- The inventions made familial breast/ovarian cancer testing practical.
- "Plaintiffs' case is nominally directed to Myriad, but actually imperils the entire biotechnology industry – molecular diagnostics, therapeutic drugs, agricultural applications, animal husbandry, etc."
- There is no evidence that Myriad has exerted any "adverse legal interest" or damages on the plaintiffs.

Progress of the ACLU Suit

- Filed May 2009 in New York Southern District Federal Court
- Immediate Move to Dismiss rejected
- Judge Robert Sweet issues Intention to Hear the Case, November 2009
- Judge Sweet issues Ruling, March 29, 2010
- Myriad appeals the decision to Court of Appeals for the Federal Circuit
- Depending on that outcome, case could be appealed to the U.S. Supreme Court



Key Arguments in Judge Sweet's Ruling

- "DNA represents the physical embodiment of biological information, distinct in its essential characteristics from any other chemical found in nature."
- "DNA's existence in an isolated form alters neither this fundamental quality…nor the information it encodes."
- "Therefore, the patents at issue directed to 'isolated DNA' containing sequences found in nature are unsustainable as a matter of law and are deemed unpatentable subject matter under 35 U.S.C. §101."


We Can
Do
It


They're our breast cancer genes—we identified them.
It's kind of you to let us have the disease for free
ABORATO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