Medical Genetics and Genetic Testing: An Update

Merlin G. Butler, MD, PhD, FFACMG
Director, Division of Research and Genetics
Director, KUMC Genetics Clinic
Professor of Psychiatry & Behavioral Sciences and Pediatrics
University of Kansas Medical Center
Kansas City, Kansas

KU MEDICAL CENTER
The University of Kansas

Disclosures/Acknowledgements

- No financial disclosures although several biotechnology companies are listed by name with description with their technologies

Heritable Birth Defects

Single Gene Defects

Chromosomal Abnormalities

Multifactorial Disorders

Non-classical Disorders

Cancer Genetics

Genetic Disorders

- Single Gene (~10,000 disorders)
  - Autosomal Dominant (~5,000)
  - Autosomal Recessive (~4,500)
  - X linked (~500)
- Chromosomal (~500)
- Multifactorial (~70% of morbidity & mortality)

GENETIC TESTING

What is it?

How is it done?

Types of Genetic Tests

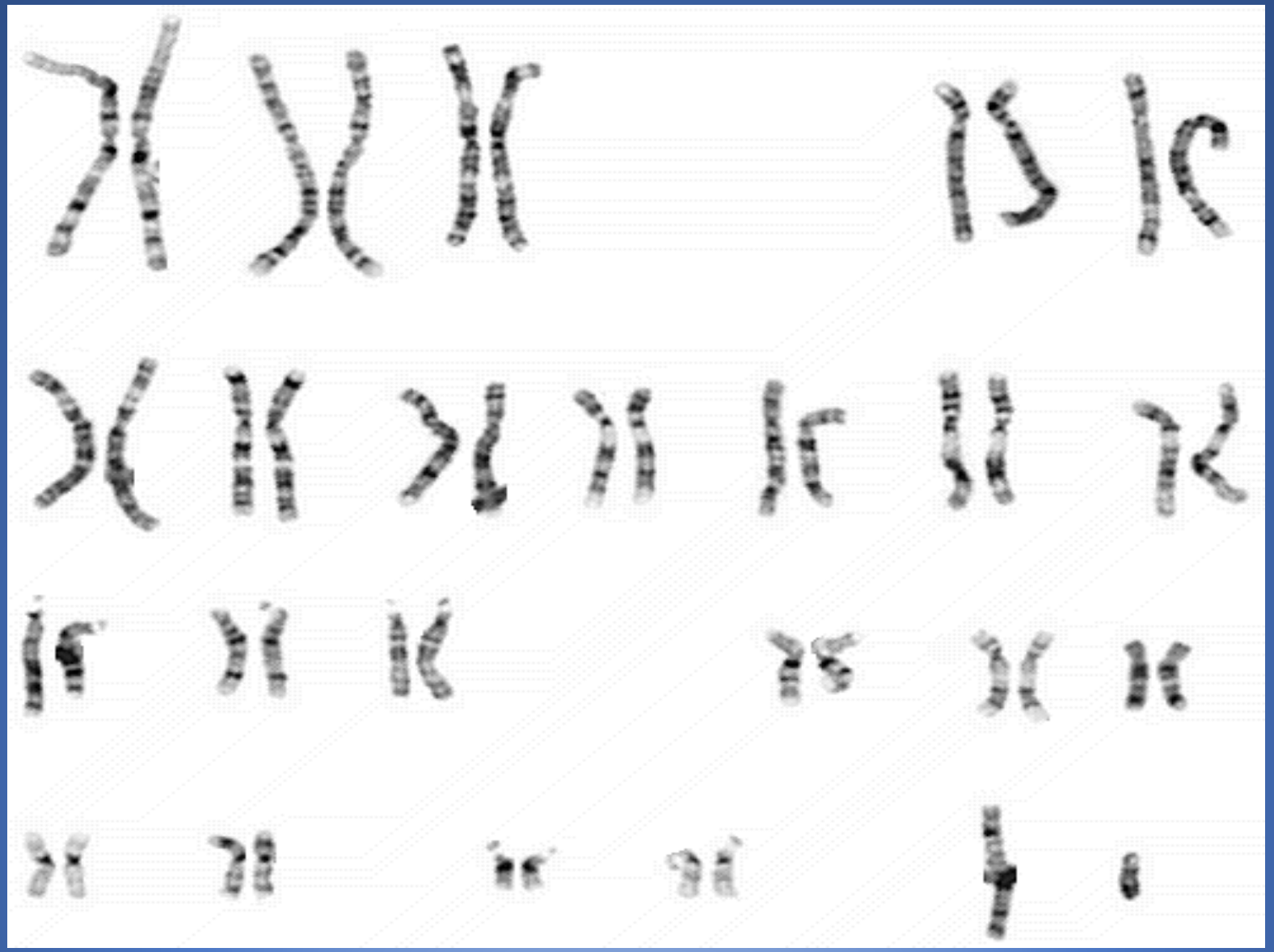
- Biochemical
- Cytogenetic
- Molecular

Biochemical Testing/ Inborn Errors of Metabolism

Testing is performed by looking for

- Accumulation of substrate behind the block
- Abnormal quantity of the enzyme which catalyzes the process
- Deficiency of the chemical reaction product
- Presence of other abnormal chemicals

Cytogenetics Testing



46, XY male karyotype

Chromosomal Abnormalities

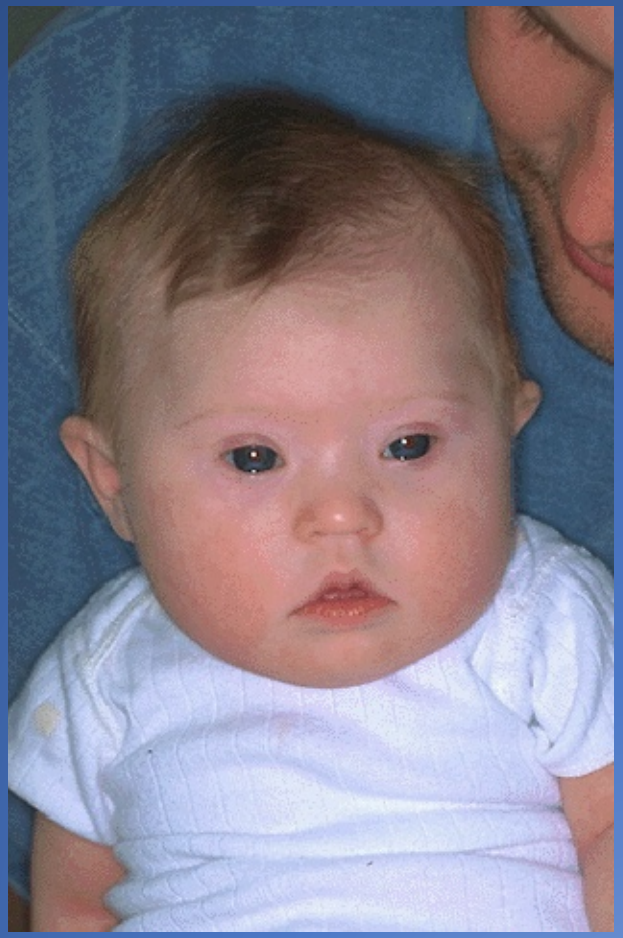
- Numerical abnormalities:

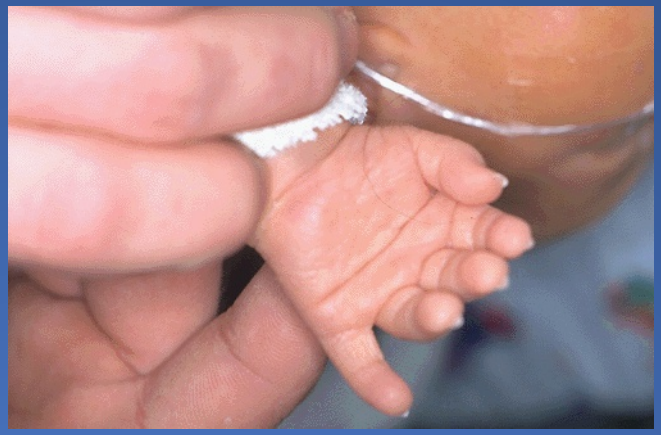
  Change in normal number of chromosomes (e.g., Down syndrome)

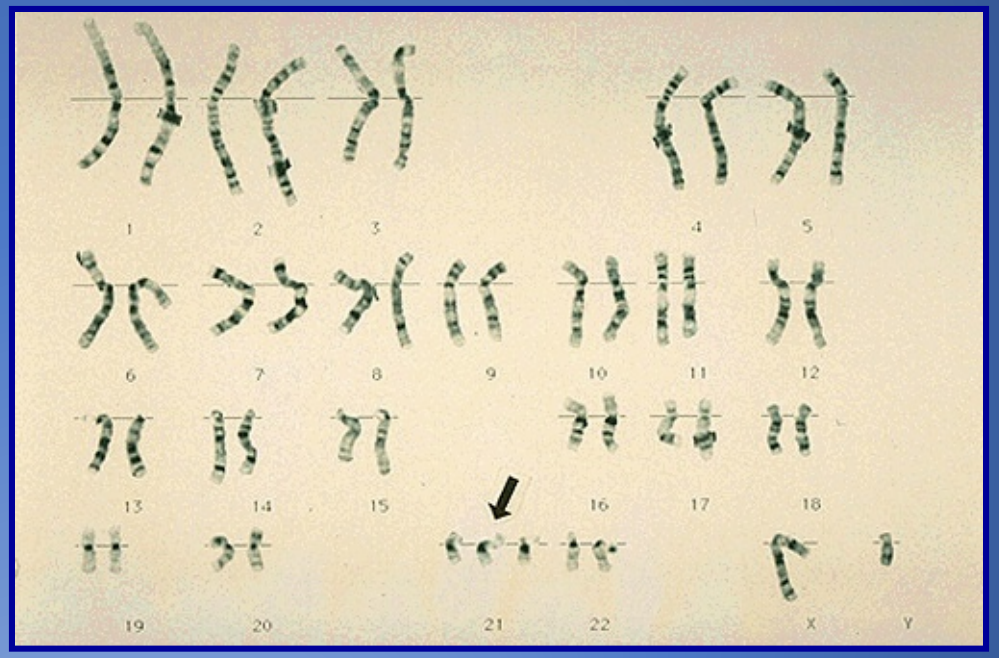
- Structural abnormalities:

  Involves chromosome breakage

  (i.e., deletion-duplication-inversion-translocation)



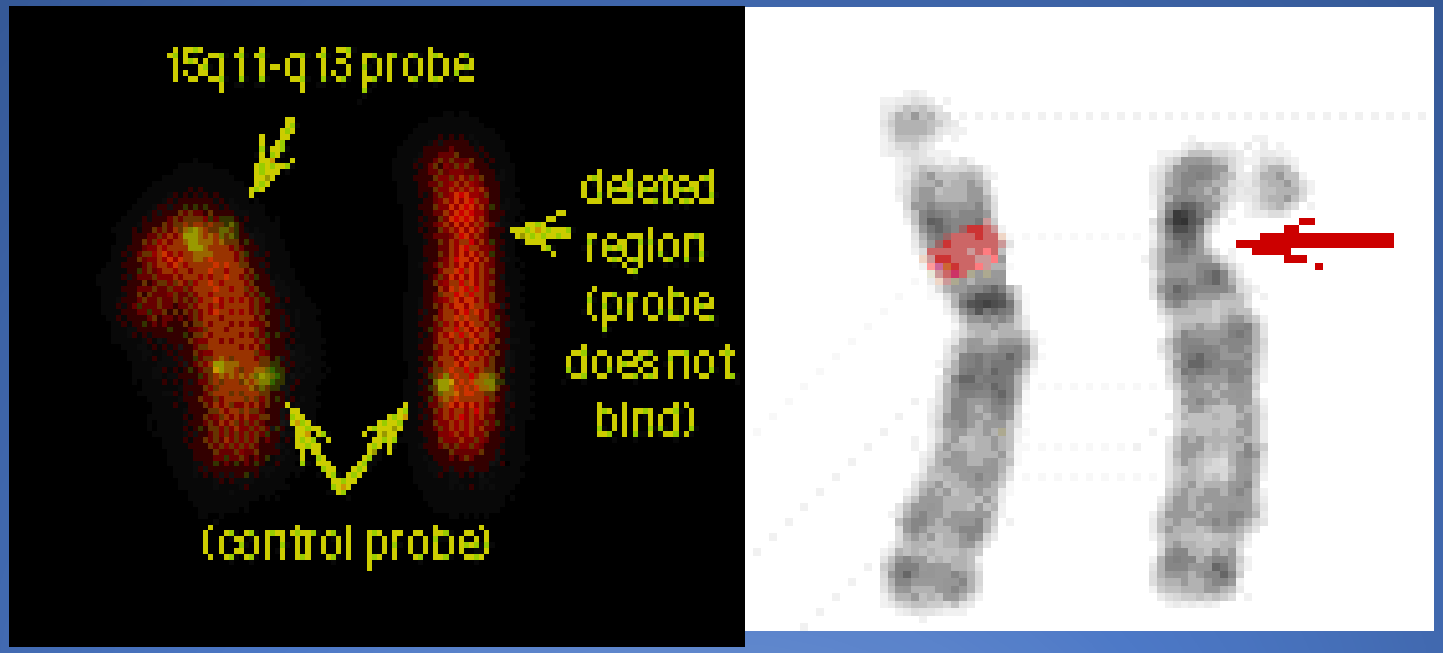




http://medgen.genetics.utah.edu/index.html

Fluorescent In Situ Hybridization (FISH)

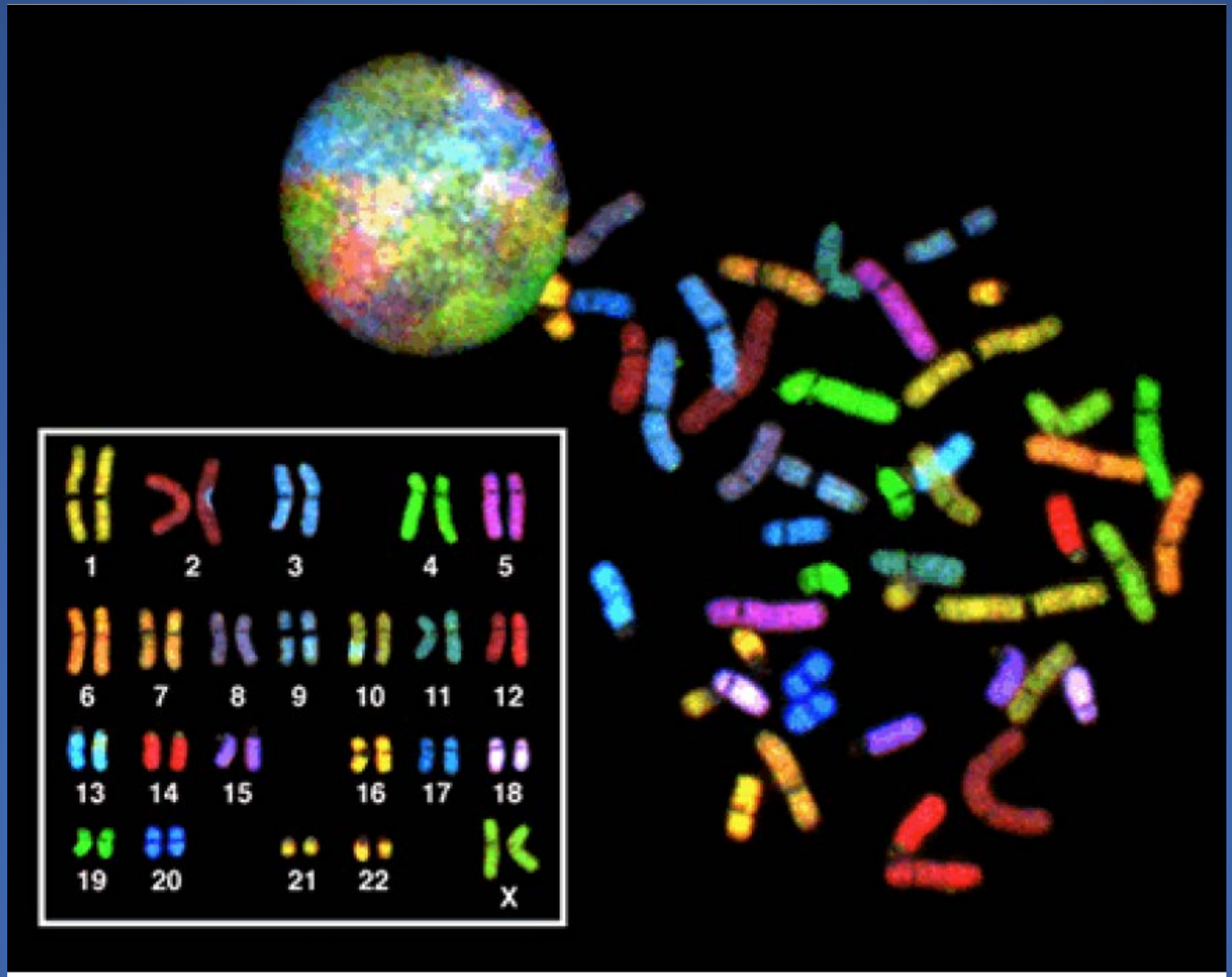
- Involves hybridizing a fluorescently labeled probe to DNA
- Uses known genetic sequences as probes
- Rapidly identifies chromosomal abnormalities
- Identifies unknown chromosomal marker material
- Developed in the 1990s and still used today in cancer cytogenetic testing


15q11-q13 probe
deleted region (probe does not bind)
(control probe)

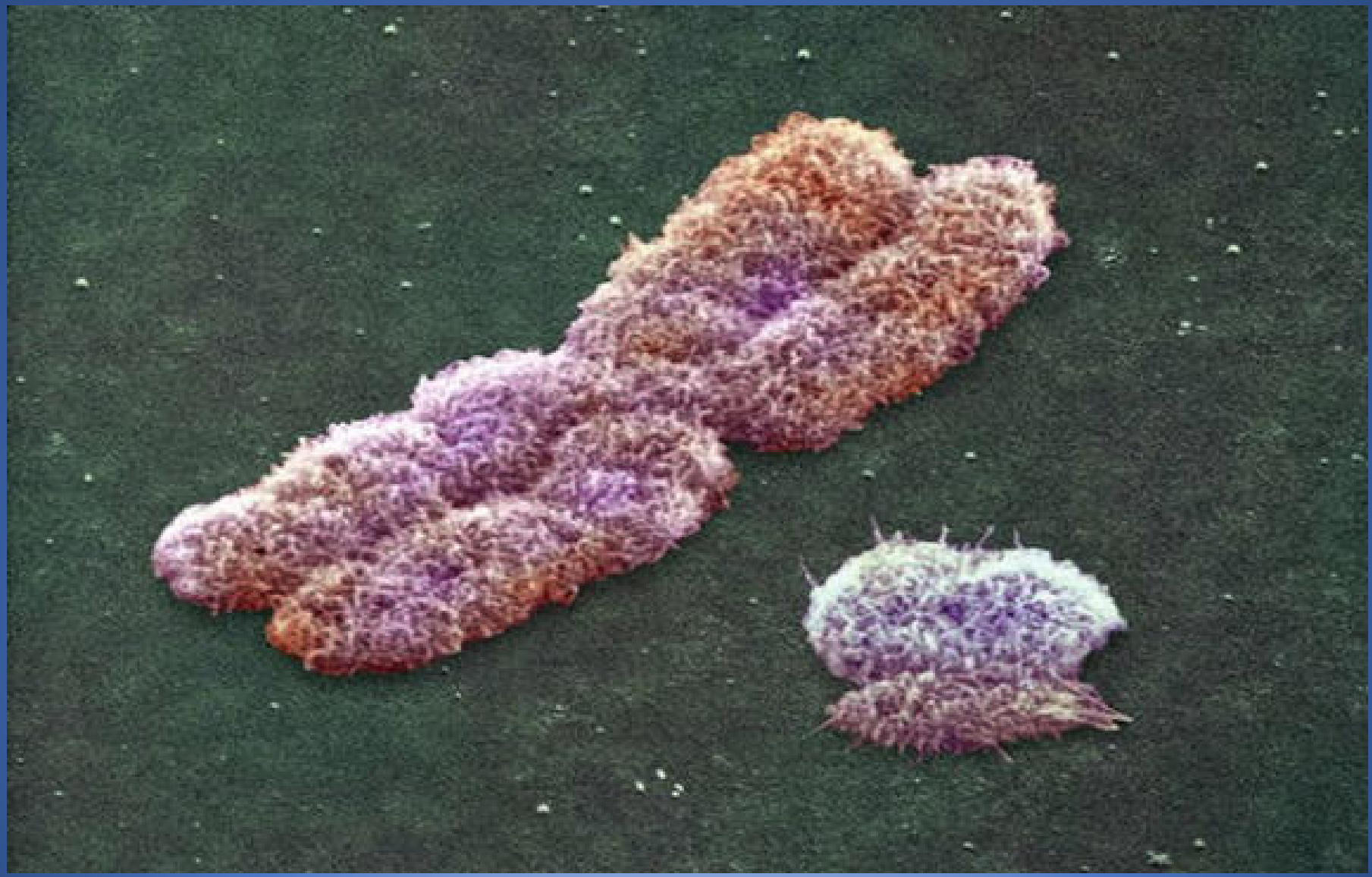
Disorders Diagnosable with FISH

Williams Syndrome	del(7)(q11.23)
Prader-Willi/Angelman	del(15)(q12)
Miller-Dieker Syndrome	del(17)(p13.3)
CMT Type 1A	dup(17)(p12)
Smith-Magenis Sx.	del(17)(p11.2)
DiGeorge Syndrome	del(22)(q11.2)

Spectral Color Karyotype

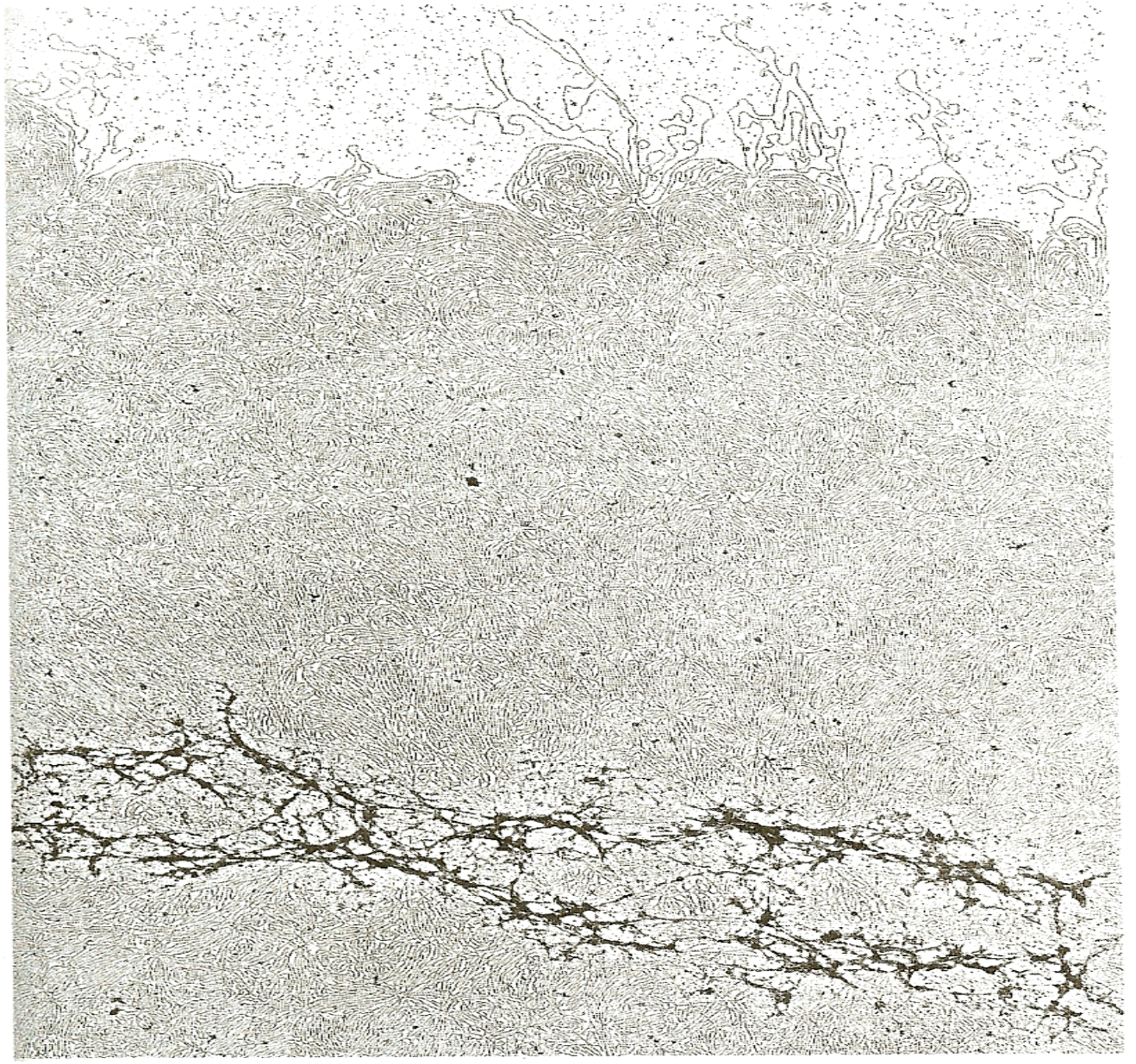


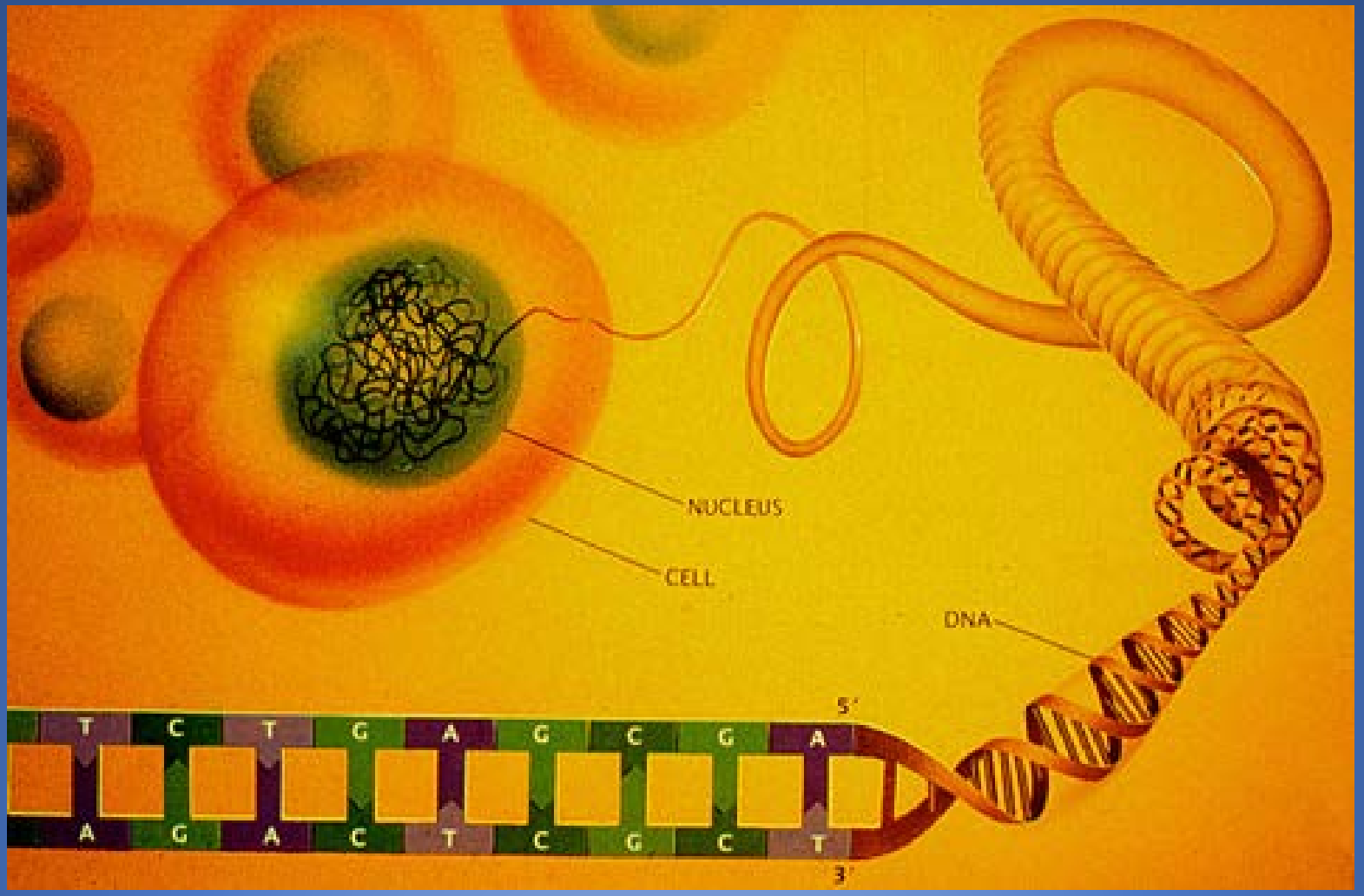
Human X and Y Chromosomes



DNA from a single chromosome.

The DNA molecule is one meter in length in each human cell.




NUCLEUS
CELL
DNA
5'
T C T G A G C G A
A G A C T C G C T
3'

Entire Human Genome
3,000,000,000 base pairs

Average Chromosome
120,000,000 base pairs

Average Gene
2,000-200,000 base pairs

Mutation That Can Cause Disease
1 base pair

DNA TESTING

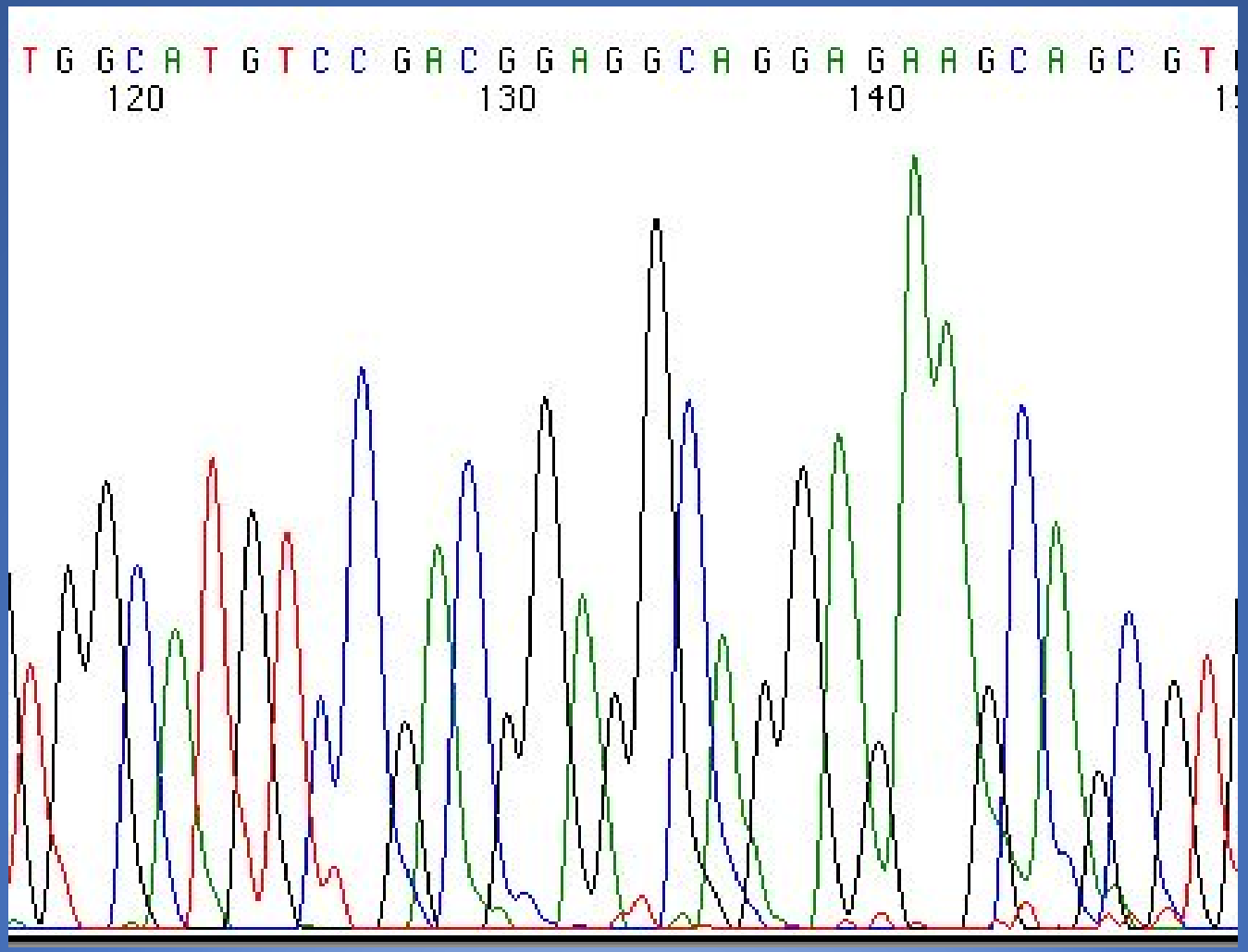
Direct Method of DNA Testing

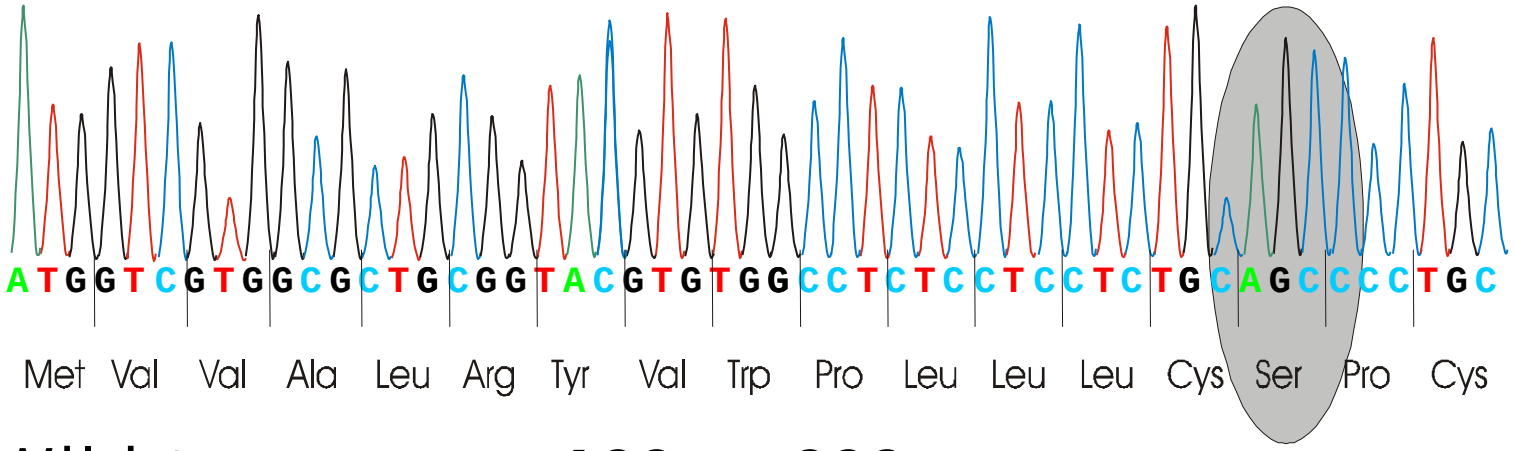
- Direct DNA testing involves identifying abnormalities within a specific gene
- Can be performed on cells from a variety of tissues
- Using PCR, it requires very little tissue

TYPES OF GENETIC TESTING

- Carrier Identification
- Prenatal Diagnosis
- Newborn Screening
- Late-onset Disorders
- Identification

Sequencing

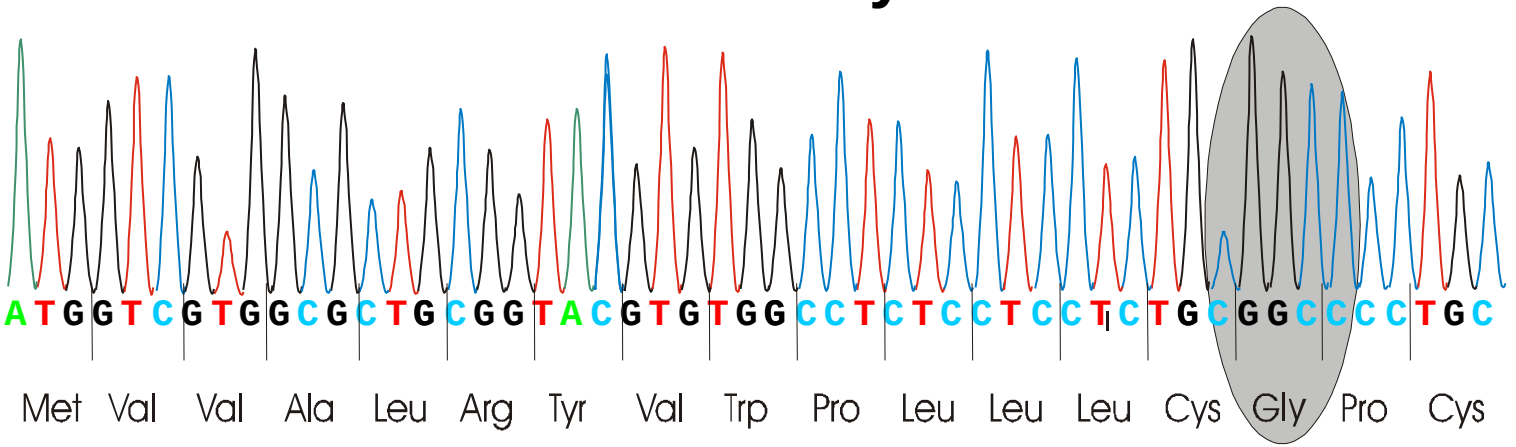




Wild type

AGC > GGC

Ser > Gly



Mutant

A > G mutation causes large size change at conserved amino acid

Effects of Mutations

- Loss of function: Reduces or eliminates the amount of functional protein. Ex: CF
- Gain of function: Increases production of a normal protein. Ex: Achondroplasia
- Novel property mutations: confers a novel property to the protein (changes function)
  - Example: sickle cell anemia. The mutation doesn't affect O_2 transport but causes sickling of red cells.
  - Example: Huntington disease
- Dominant negative: An allele that disrupts the function of a wildtype allele in the same cell: it's worse than having none! Ex: Osteogenesis Imperfecta.

The Humble Beginning

Humans have 46 chromosomes, 1956

THE CHROMOSOME NUMBER OF MAN

By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

Sanger Sequencing is born, 1977

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage ϕX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

Contributed by F. Sanger, October 3, 1977

ABSTRACT A new method for determining nucleotide sequences in DNA is described. It is similar to the "plus and minus" method [Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* 94, 441–448] but makes use of the 2′,3′-dideoxy and arabinonucleoside analogues of the normal deoxynucleoside triphosphates, which act as specific chain-terminating inhibitors of DNA polymerase. The technique has been applied to the DNA of bacteriophage ϕX174 and is more rapid and more accurate than either the plus or the minus method.

a stereoisomer of ribose in which the 3′-hydroxyl group is oriented in *trans* position with respect to the 2′-hydroxyl group. The arabinosyl (ara) nucleotides act as chain terminating inhibitors of *Escherichia coli* DNA polymerase I in a manner comparable to ddT (4), although synthesized chains ending in 3′ araC can be further extended by some mammalian DNA polymerases (5). In order to obtain a suitable pattern of bands from which an extensive sequence can be read it is necessary

Advances in Genetic Testing

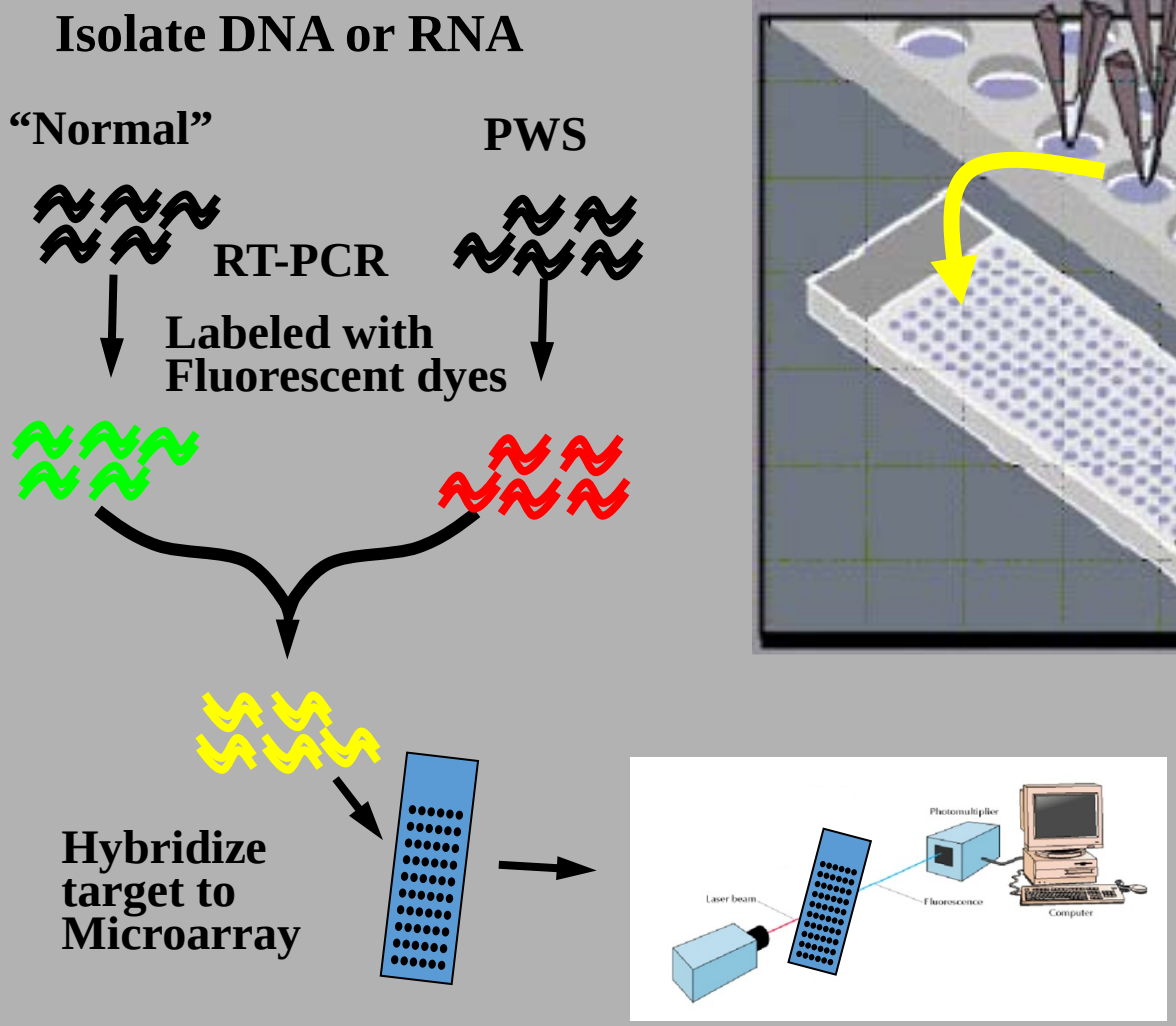
DNA microarray (aCGH) analysis

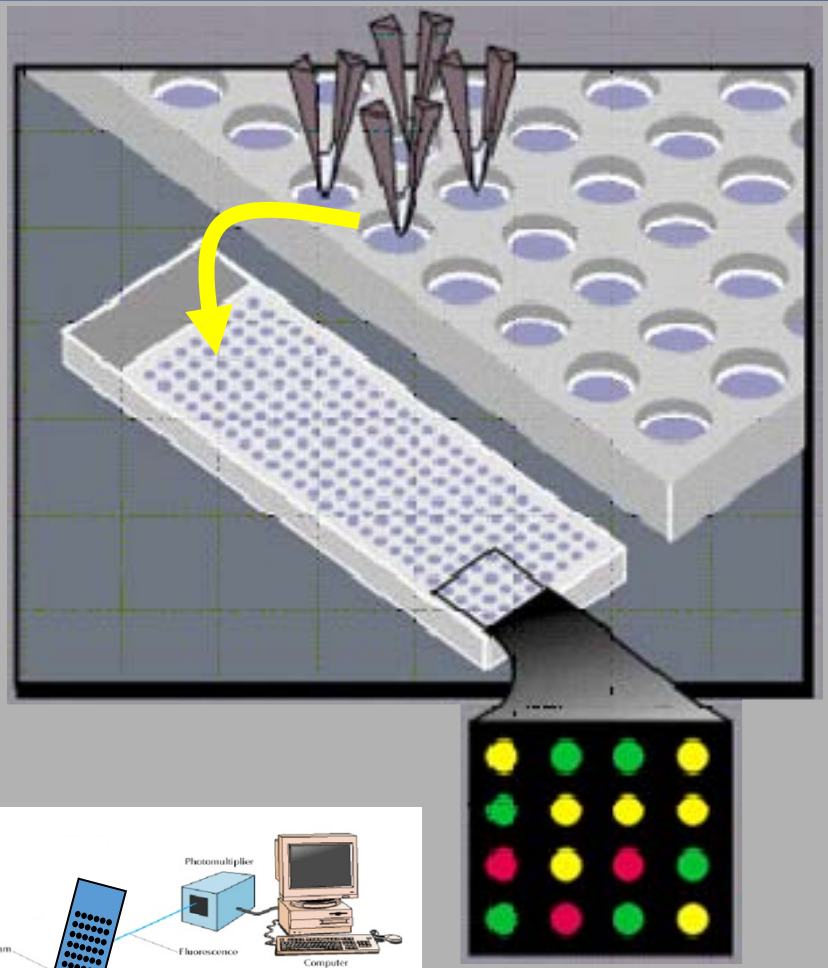
- Copy number variants (CNVs)
- Single nucleotide polymorphisms (SNPs)
- CNV/SNP

Next generation sequencing (coding and noncoding)

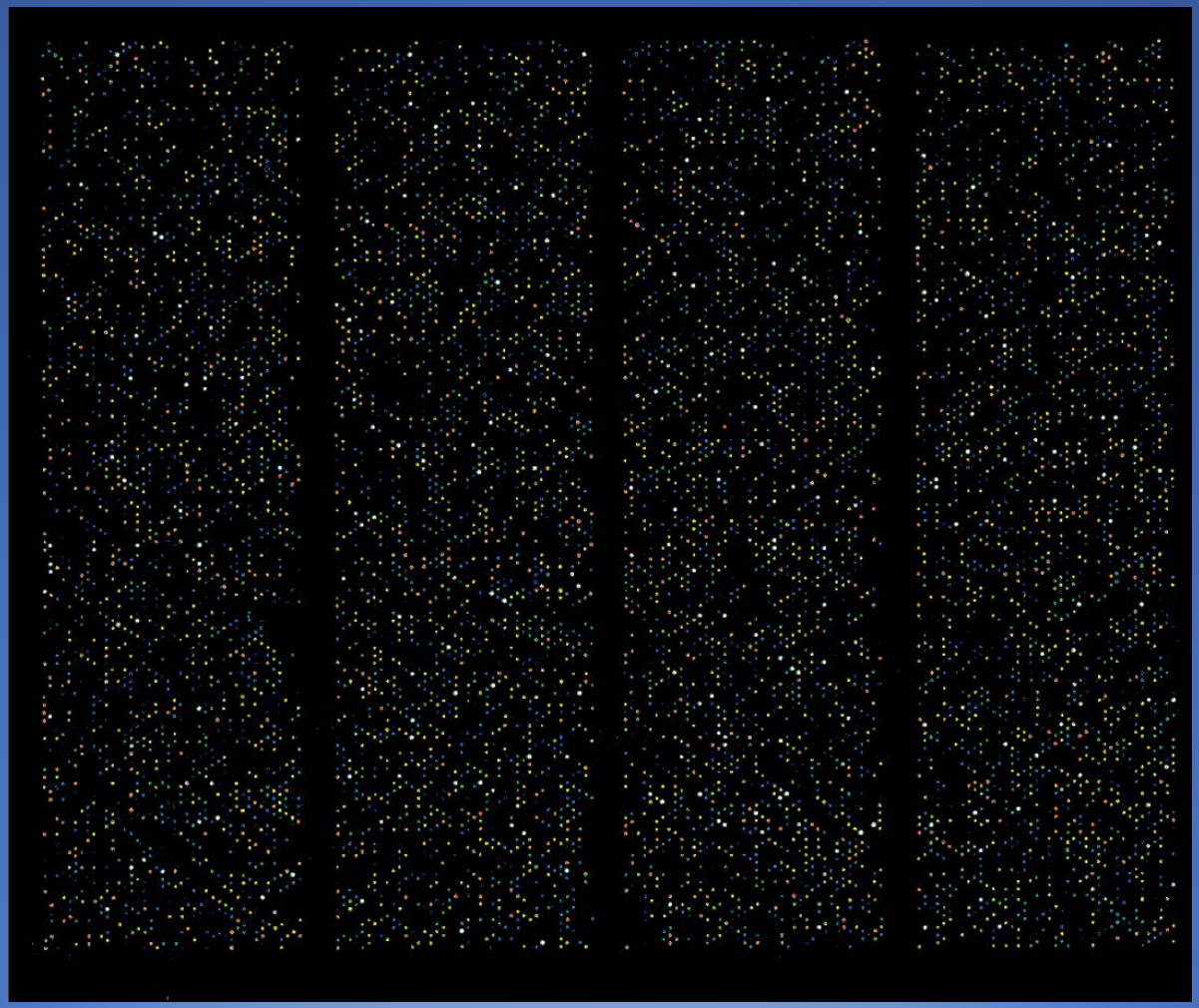
- Whole genome
- Exome (exon) - protein coding genes
- MicroRNA - noncoding regulatory function

Microarray Procedure





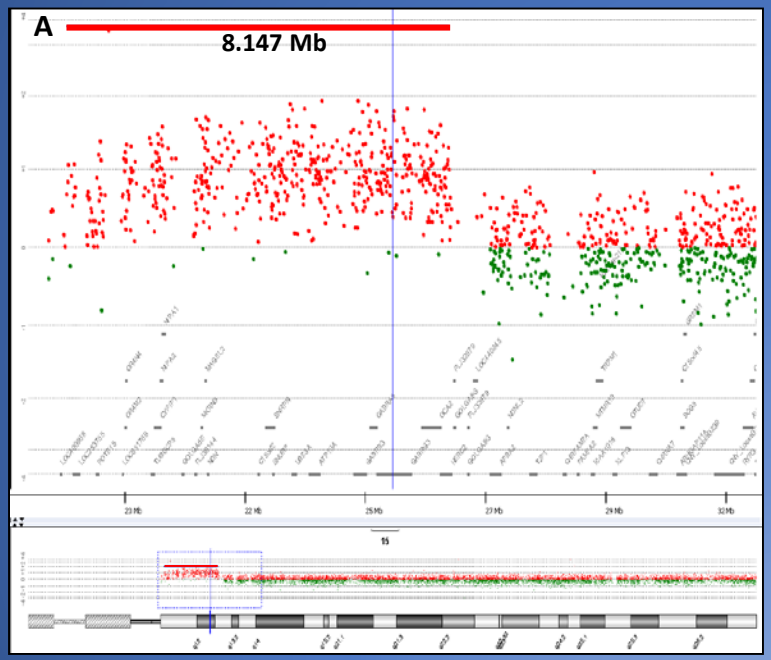
Microarray Slide



DNA Microarray Study in Prader-Willi Syndrome

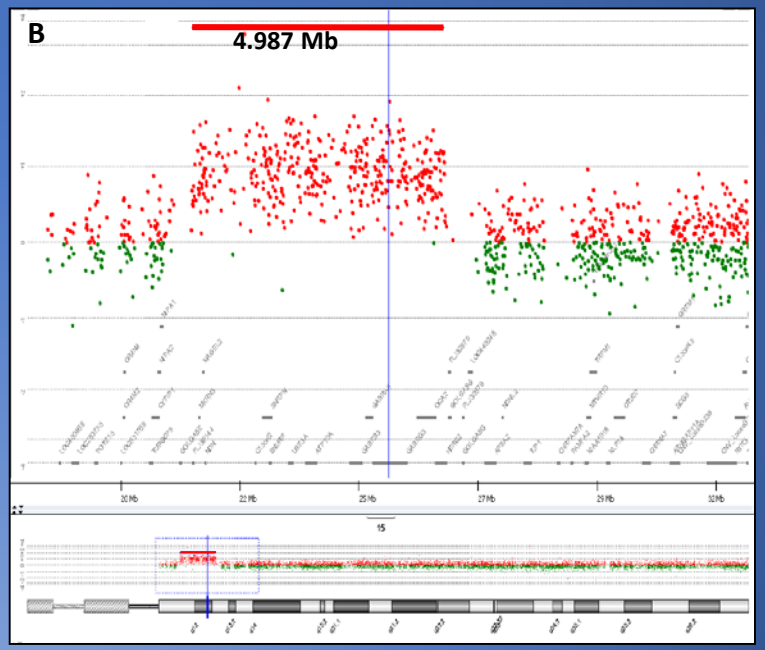
Representative examples of chromosome 15 using array comparative genomic hybridization (aCGH) of individuals with Prader-Willi syndrome.

Type I Deletion

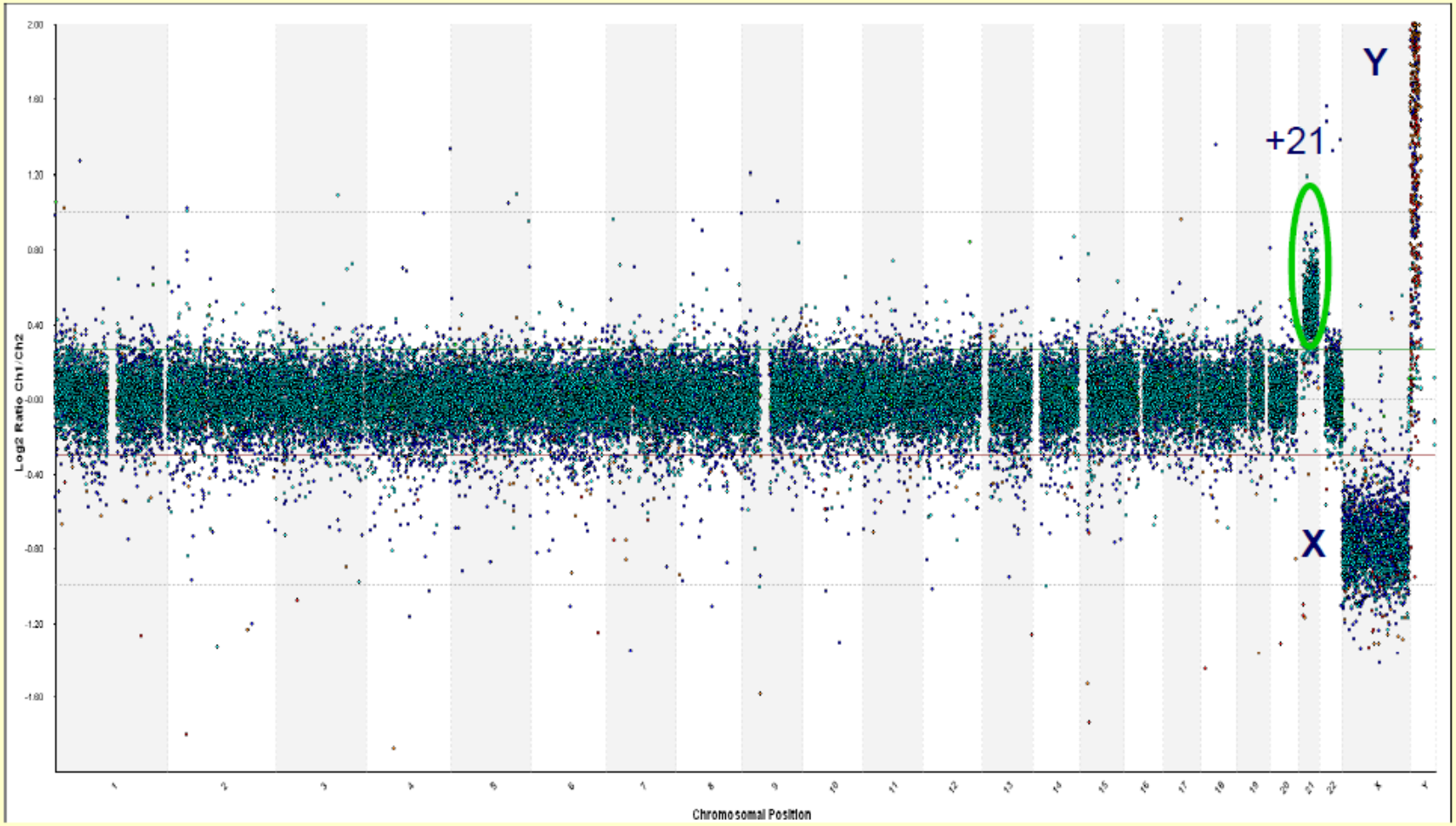


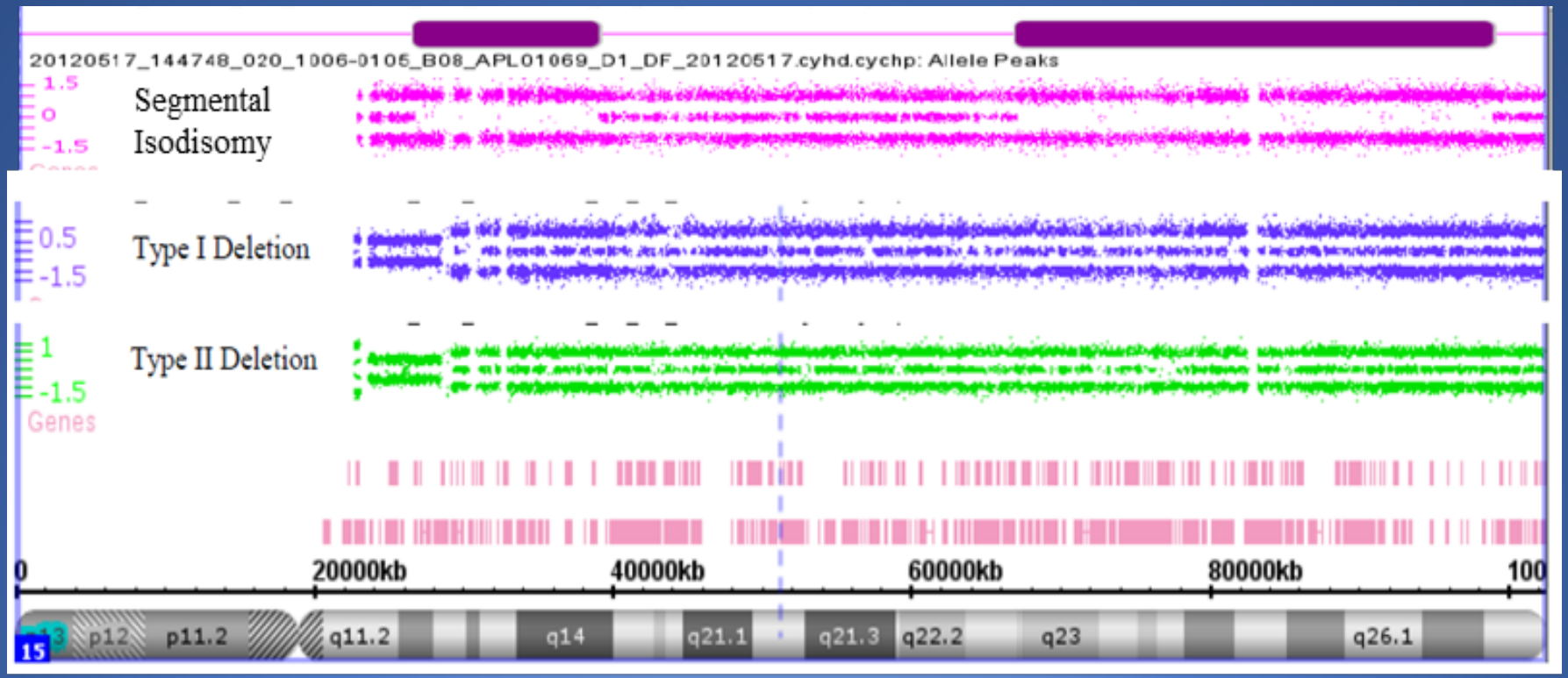
deleted

Type II Deletion



Male, Trisomy 21





Chromosome microarray using CNV and SNP probes to identify maternal disomy 15 (segmental isodisomy) subtype and the 15q11-q13 Type I or Type II deletions in PWS.

Next Generation Sequencing

- Automated sequencers have been available since the mid-1980's using Sanger technology
- In the mid-1990's, new technology such as pyrosequencing became available
- In 2004, 454 Life Sciences marketed a version for clinical use
- Since 2008, many vendors now sell this technology

Next Generation Sequencing

- A number of technologies can be used for high-throughput parallel sequencing
  - Reversible dye terminator technology
  - Parallel pyrosequencing
  - Ligation-based sequencing
  - Ion semiconductor sequencing
  - Several others

NGS Applications are Diverse

- Hotspots, gene panels, exomes, or whole genomes
- Germline (Constitutional) DNA
- Somatic DNA
  - Solid and hematological tumors
  - Liquid biopsy (circulating tumor DNA or cells)
  - Immune repertoire and neoantigen sequencing
  - Germline mosaicism
- Non-invasive prenatal screening
- RNA sequencing and gene expression
- Epigenetics…

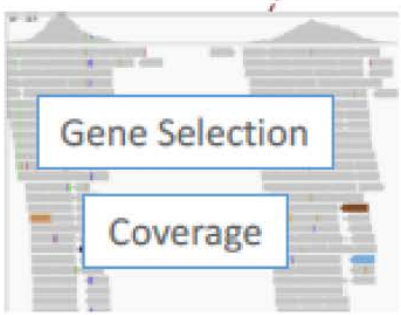
NGS Workflow

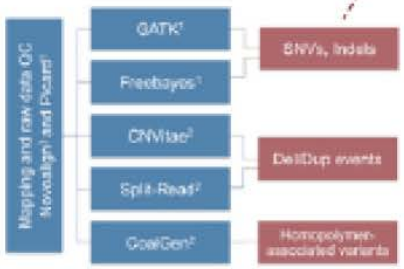
Generally standardized processes

Processes that can vary considerably between labs

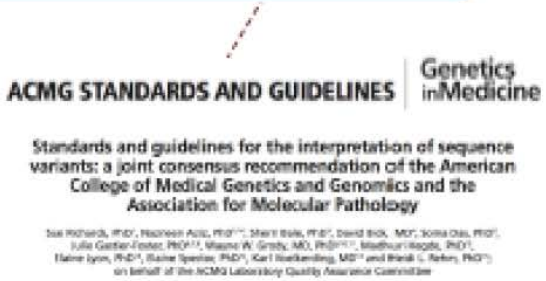
DNA extraction and quality control → Target Prep → Next-generation sequencing → Bioinformatics → Variant classification

Validation



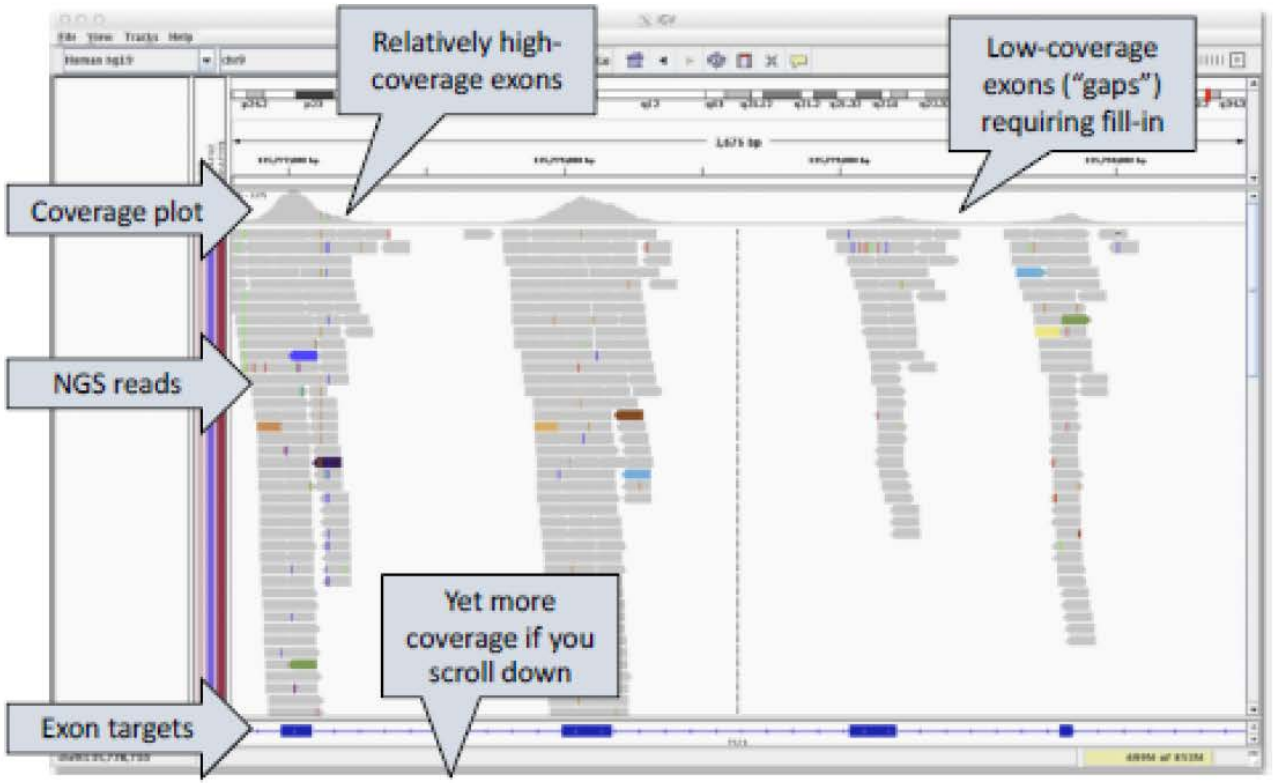


e.g. Lincoln *et al., J Mol Diag* 2015



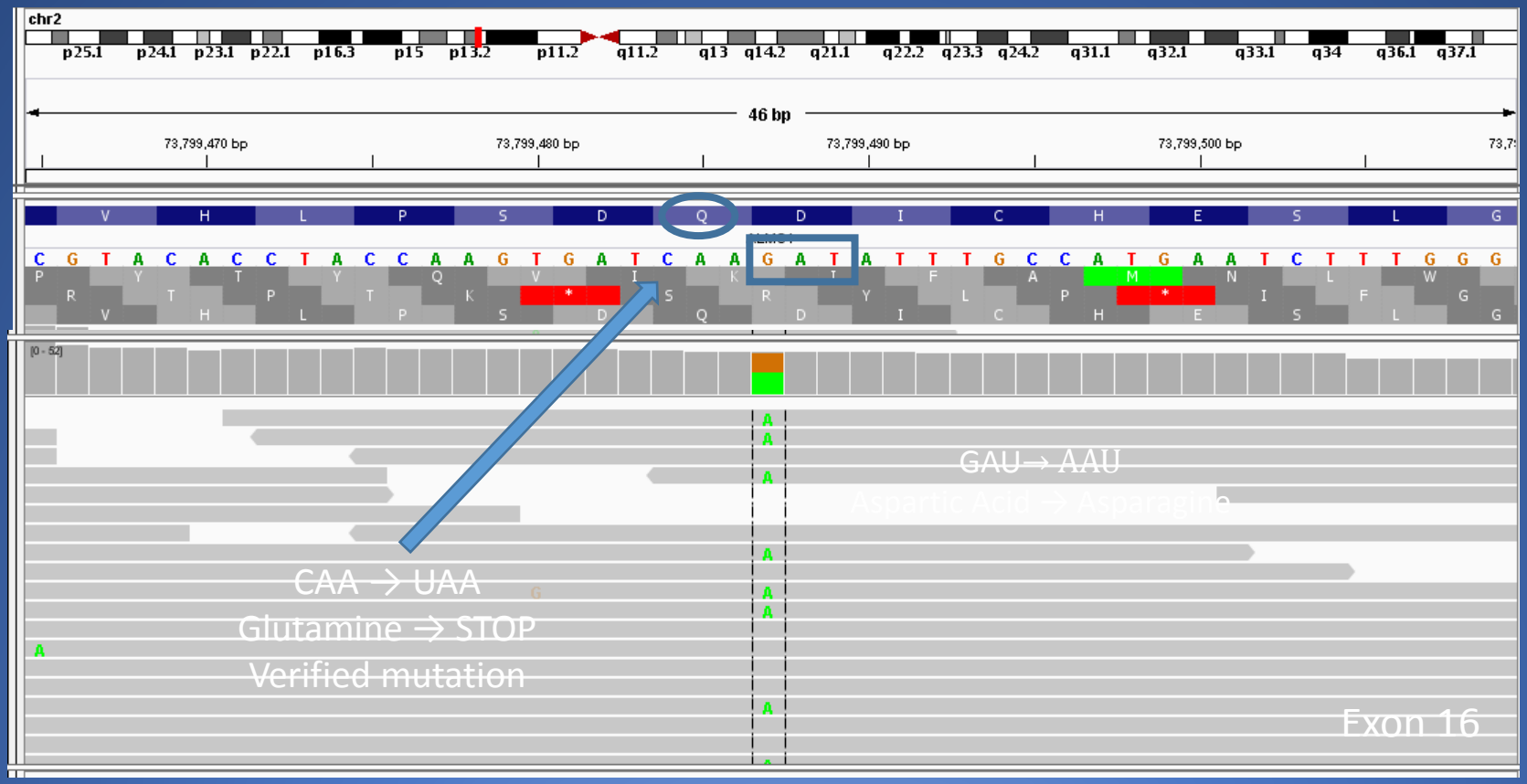
Richards *et al., Genet Med* 2015

NGS Coverage (A.K.A. Read-Depth)



- Depth varies by site both due to sequencing and (even more) due to targeting chemistry biases (panels/exomes)
- Average depth and minimum depth can be very different numbers

RNA Sequencing of ALMS1 Gene Mutation



Known mutation: c.10483C>T; p.Gln3495*

- C to T substitution
- STOP codon generated
- Terminates translation processes

Exome Sequencing

- Sequencing of all coding regions from all genes (accounts for about 3% of the total DNA sequences)
- Estimated that 85% of all mutations will be in the exome
- Cost of exome sequencing:
  - June 2010: $2,500 through a few centers
  - June 2012: $999 through dozens of centers
  - January 2013: $698 with an internet connection and an active credit card

Clinical Example of Exome Sequencing "Kabuki Syndrome"

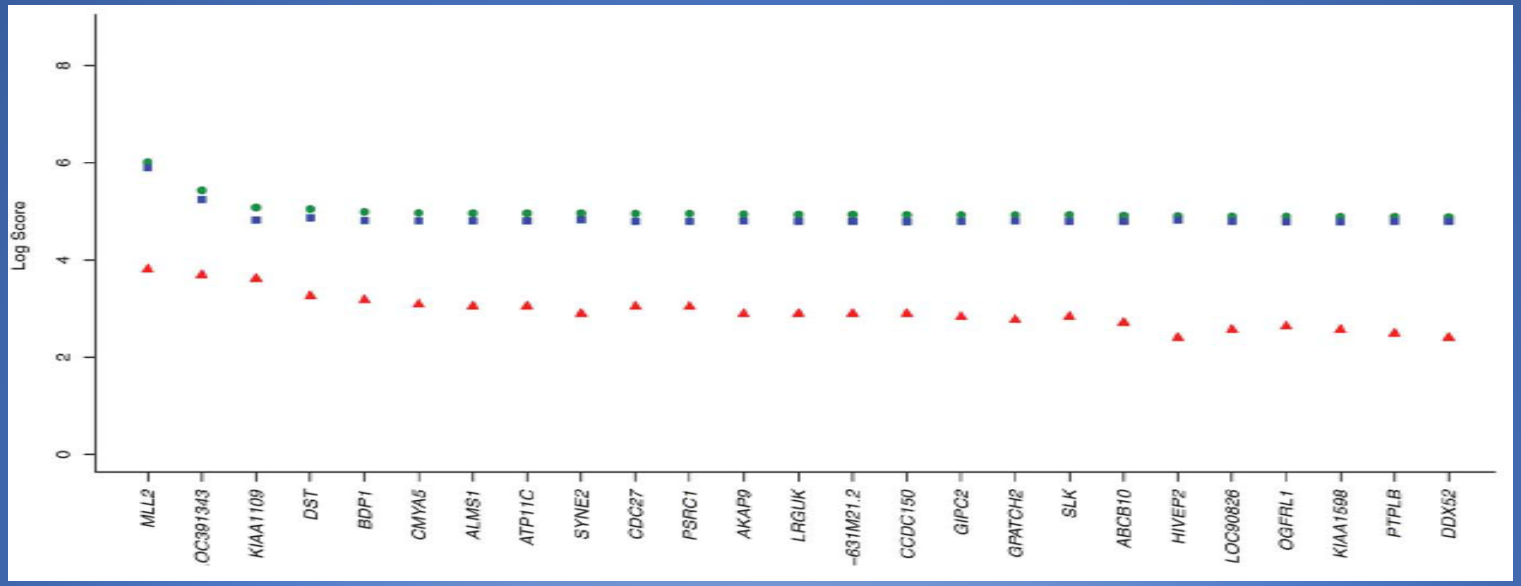
- Short stature
- Mild-moderate MR
- Craniofacial changes
- Skeletal and cardiac anomalies
- "The etiology of this disorder is unknown"
  - KL Jones, 2006

Kabuki Syndrome

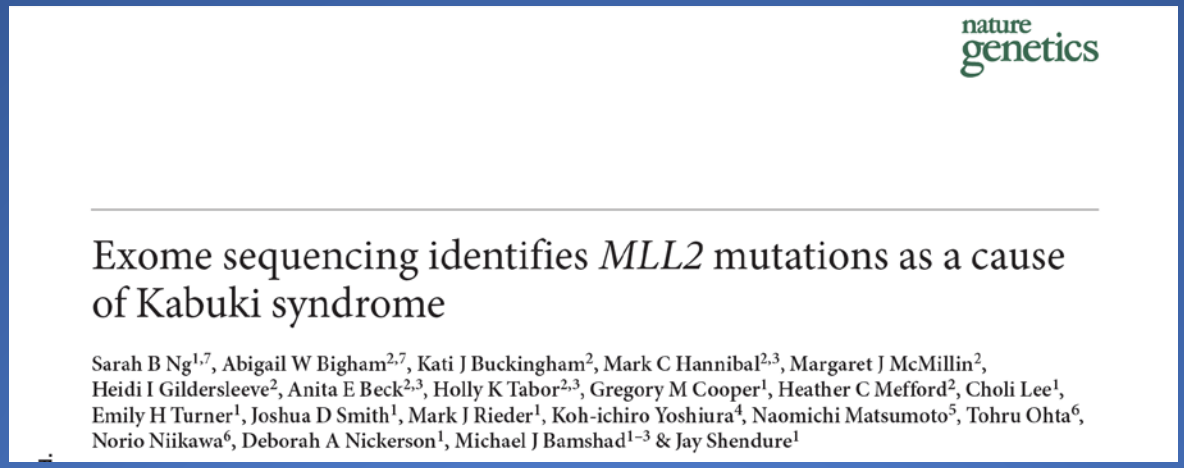
- Ten unrelated probands with clinical diagnosis of Kabuki syndrome
- Targeted exomes performed on all ten with average of 40x coverage …
- …and found 1,459 nonsynonymous, splice-site acceptor/donor, and other potentially pathogenic mutations

Kabuki Syndrome

- Comparison to dbSNP, 1000 genomes, or control exomes decreased the number to twenty-five candidate genes



Kabuki Syndrome



nature genetics

Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome

Sarah B Ng[1,7], Abigail W Bigham[2,7], Kati J Buckingham[2], Mark C Hannibal[2,3], Margaret J McMillin[2], Heidi I Gildersleeve[2], Anita E Beck[2,3], Holly K Tabor[2,3], Gregory M Cooper[1], Heather C Mefford[2], Choli Lee[1], Emily H Turner[1], Joshua D Smith[1], Mark J Rieder[1], Koh-ichiro Yoshiura[4], Naomichi Matsumoto[5], Tohru Ohta[6], Norio Niikawa[6], Deborah A Nickerson[1], Michael J Bamshad[1–3] & Jay Shendure[1]

- *MLL2* mutation present in nine of ten probands
- *MLL2* mutation present in 35 of 58 additional patients with clinical findings of Kabuki syndrome
- Around 70% of patients with *KS* have a mutation on clinical testing, which is now routine

Exome Sequencing Reveals *VCP* Mutations as a Cause of Familial ALS

Janel O. Johnson
John Q. Trojanow
Maria Martinez-L
Jeffrey Rothstein
Maria Rosaria Mo
The ITALSGEN C
Adriano Chiò,[13,2

REPORT

Exome Sequencing Identifies *CCDC8* Mutations in 3-M Syndrome, Suggesting that CCDC8 Contributes in a Pathway with CUL7 and OBSL1 to Control Human Growth

Dan Hanson,[1,2] I
Sanjeev S. Bhaska
Kate Chandler,[2,3

Exome Sequencing Identifies *SMAD3* Mutations as a Cause of Familial Thoracic Aortic Aneurysm and Dissection With Intracranial and Other Arterial Aneurysms

REPORT

Exome Sequencing Identifies *WDR35* Variants Involved in Sensenbrenner Syndrome

Christian Gilissen,[1,3] Heleen H. Arts,[1,3] Alexander Hoischen,[1,3] Liesbeth Spruijt,[1] Dorus A. Mans,[1] Peer Arts,[1] Bart van Lier,[1] Marloes Steehouwer,[1] Jeroen van Reeuwijk,[1] Sarina G. Kant,[2] Ronald Roepman,[1] Nine V.A.M. Knoers,[1] Joris A. Veltman,[1] and Han G. Brunner[1,*]

Whole Genome Sequencing

- The "Holy Grail" of Genetic Testing
- Cost of whole genome sequencing:
  - 1990-2003: $2,700,000,000 (Human Genome Project)
  - 2004-2007: $100,000,000 (J. Craig Venter)

Whole Genome Sequencing

- Cost of Whole Genome Sequencing with Next-Generation Technology:
  - January-April 2008: “less than $1.5 million” (James Watson)
  - June 2009: $48,000 (Complete Genomics)
  - June 2010: $19,500 (Complete Genomics)
  - 2012: $7,000-10,000 from several companies

Genome Basics

- 5% of Human Genome is *functionally active*
- ~1.5% “Genes” - Code for proteins
  - 20,000-25,000 genes
  - 3,000,000 kb/3,000Mb
- ~3.5% Functionally active but do not code for proteins
  - Gene regulatory elements
  - Chromosome functional elements
  - Unknown functional elements
- Other 90 - 95% of Human Genome are introns, repetitive DNA or promoter/modifier sequences

	Sanger	Next-Generation	Third-Generation
Launched	1977 Basic chemistry 1998 Modern form	2005 with significant improvements since	2010 with significant improvements since
Key Idea	Continuous reaction with terminator mixture; Size separation by electrophoresis	Homogeneous amplification of single molecules; Stepwise reactions; Short reads but high depth	Single molecule detection Continuous long reads High-depth sequencing possible
Cost			
Throughput			
Currently Available Platforms	Applied Biosystems*	Illumina Ion Torrent* Qiagen (Europe) Complete Genomics (China)**	Pacific Biosciences Oxford Nanopore
Clinical Uses	Many (but dwindling)	Many (and growing)	Niche uses (today)

*Part of Thermo Fisher **Part of BGI

NGS Benefits	NGS Limitations
Highly flexible core platform with many clinical and research applications	Requires significant expertise to implement at top performance
Produces lots of data at low cost	Can produce more data than we know how to interpret or clinically utilize, today
Rapidly evolving Multiple competing platforms Active community of vendors and third-party developers	Hard to keep up
Can be highly accurate	…but isn't always. Has limitations.
Emerging "standard" for clinical use	Not very standardized: Every lab can be different even when using the same platform. Third-generation platforms may change everything, yet again.

What Would You Do Next?

A. Single gene test
B. Gene panel
C. Exome/genome sequencing
D. Nothing

A list of connective tissue disorders where NGS panel would be helpful

- Osteogenesis Imperfecta
- Marfan Syndrome
- Ehlers-Danlos Syndrome
- Thoracic Aneurysm and Aortic Dissection Syndrome
- Stickler Syndrome
- Loeys-Dietz Syndrome
- Beals Syndrome

List of connective tissue disorder genes

COL1A1
COL1A2
COL2A1
COL3A1
COL5A1
COL5A2
COL9A1
COL9A2
COL11A1
COL11A2
SLC2A10

SMAD3
ACTA2
MYH11
MYLK
TGFBR1/2
FBN1
FBN2
CRTAP
FKBP10
LEPRE1
PPIB

SERPINF1
SERPINH1
SP7

Clinical Applications of Genetic Testing

- Diagnostic
  - Identification of recognized or new syndromes
  - Anticipate co-morbidities
  - Guide treatment & improve outcomes

- Pharmacogenetics
  - Target hepatic cytochrome P450 enzyme function
  - Improve efficacy & reduce drug interactions due to metabolic side effects

- Pharmacogenomics
  - Influences on gene activity/function
  - Tailor therapy to optimize outcomes
  - Outside the scope of metabolism

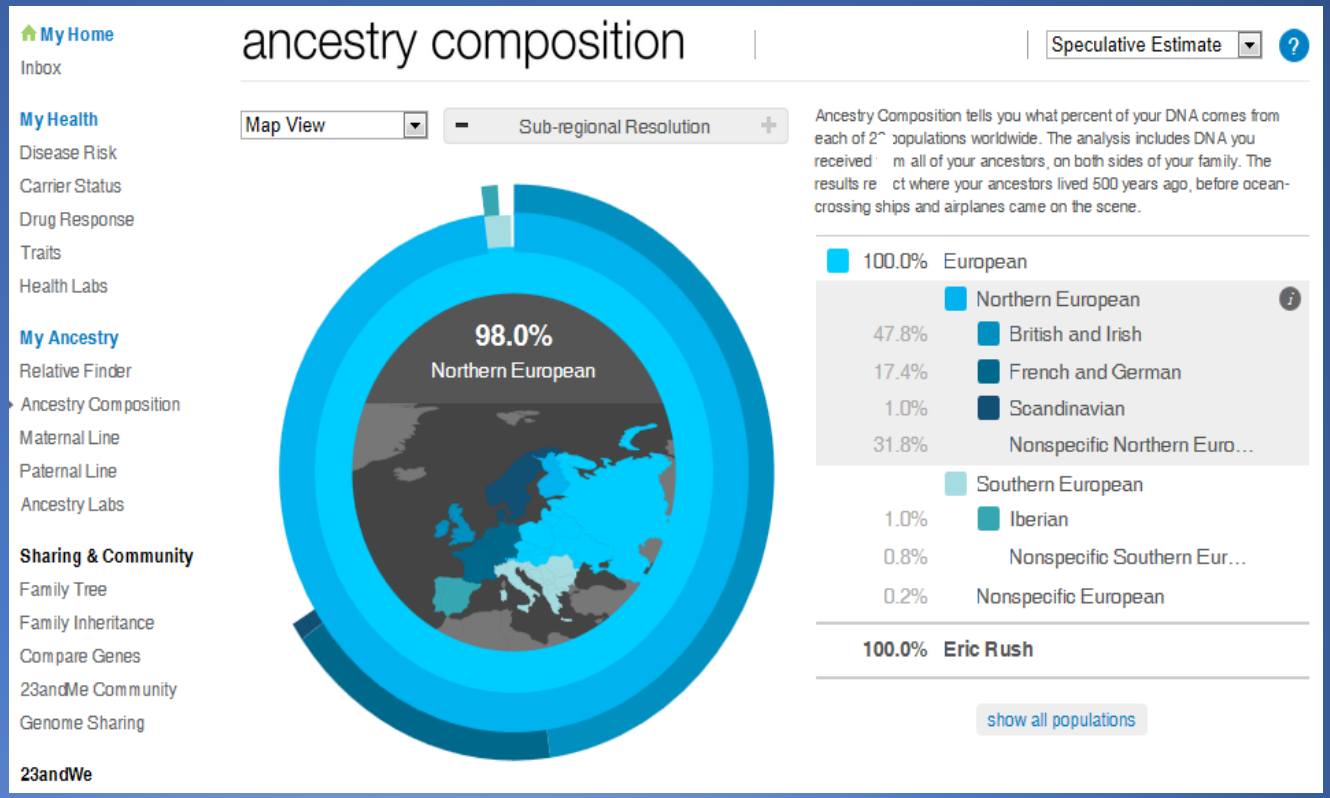
Direct-To-Consumer (DTC) Genetic Technology

What is it?

- Genetic Testing sold to a consumer without a health care provider serving as an intermediary
- Companies frequently have a large internet footprint
- Offerings vary greatly
- Use genetics and evolutionary biology to trace human migration

Pedigree versus DTC Ancestry

- 85.1% British and Irish
- 13.2% German
- 1.7% Spanish



Direct-to-consumer for Health

- Four different types of information typically tested:
  - Carrier status for recessive disease
  - Response to various drugs, both common and uncommon
  - Risk profiling for common disease
  - Traits

Four reasons why consumers seek DTC testing

- Gain information
  - Carrier testing – CF, PKU
  - Traits – Eye color, Alopecia androgenetica
  - Fun – Caffeine metabolism, Asparagus metabolism
- Seek Prevention
  - Disease Risk
  - Family planning
- Seek specific intervention
  - Response to Alzheimer or BRCA
- Help others

Potential Benefits

- Patients may feel reassured about nothing major “lurking” in their DNA
- A few high profile cases where information was very impactful in care
- Carrier testing may prove beneficial for family planning, etc.
- Testing frequently less costly than traditional genetic testing

Potential Risks

- Walking without a Guide
  - Only one company requires clients to speak to a certified genetic counselor
  - "I'm a ZZ, I'm not sure what that means…"
- Informed Consent
  - Risks and benefits should be explained
  - Must delineate between increased risk versus guarantee of developing disease

Pharmacogenetics

- CYP450 enzymes - a large enzyme superfamily (>50 members)
- 90% of all drugs metabolized by 6 different CYP450 enzymes: CYP1A2, CYP3A5, CYP2C19, CYP2D6, CYP3A4, CYP3A5
- 20% of the 121 FDA recognized pharmacogenetic markers are for psychiatric medications

CYP450 Genetics

- Every person has two copies of an Allele.
- An individual inherits two *alleles* for each gene, one from each parent.
- Alleles are referred to as “wild type” or “variant,”

CYP450 Characteristics

- Extensive metabolizer or Normal metabolizer
  - individual carrying two alleles
- Ultrarapid metabolizer
  - individual carrying more than two copies of functional allele
- Intermediate metabolizer
  - individual carrying one reduced and one nonfunctional allele
- Poor metabolizer
  - individual carrying no functional alleles

CYP2D6 Characteristics

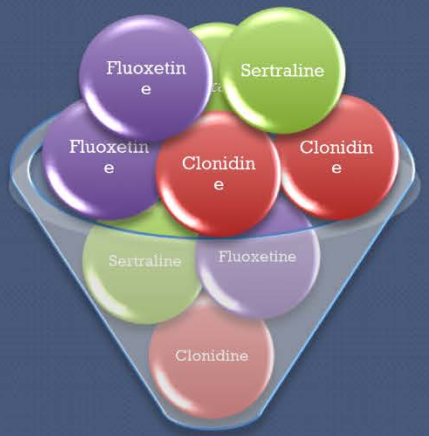
- Inhibitor: Bupropion. Fluoxetine, Paroxetine, Duloxetin, Venlafaxin, Quinidine
- Inducer: Dexamethasone, rifampin

CYP1A2 Characteristics

- Inhibitor
  - Fluvoxamine
  - Ciprofloxacin
  - Cimetidine
- Inducer
  - Broccoli, brussels sprouts
  - Char-grilled meats
  - Inhaled smoke

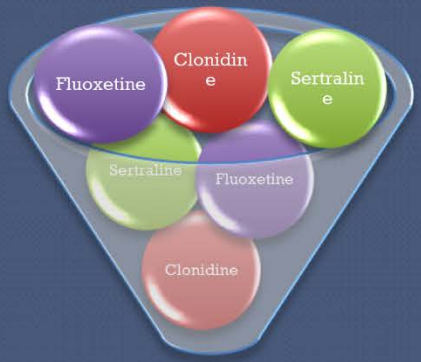
Pharmacogenetics Medicine

Poor Metabolizer



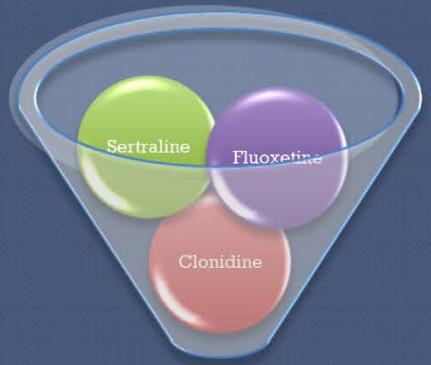
CYP2D6*4/*5
(Case Report)

Extensive Metabolizer



CYP2D6

Ultrarapid Metabolizer



CYP2D6L
(Duplication)

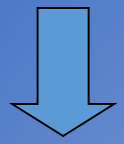
Objective

- How much do we know about OTC medication interaction with psychiatry medications?
- Pharmacogenetic testing application in psychiatry and general medical use

Concerns

- Drug-drug interaction
- Food-drug interaction
- Concern about Metabolizers (UM, PM)

Should we follow the patients for years to come to the conclusion



OR

Genotype so that we can better guide patient care

Genomics and Public Health (Current)

- Pharmacogenomic approaches to treat cardiovascular disease, infectious diseases, depression and pain control
  - ivacaftor targets the *G551D* mutation of the *CFTR* gene in 4% of patients with cystic fibrosis
  - *CYP2C19* gene variants conferring risk for drug metabolism of clopidogrel
  - other hepatic metabolic enzymes (e.g., CYP3A4, CYP2D6) impact psychotropic substrates by inhibiting (e.g., fluoxetine, haloperidol) or inducing (e.g., carbamazepine, modafinil) drug effects and interactions for clinical relevance and treatment

Thank you