Understanding Genetic Tests and How They Are Used

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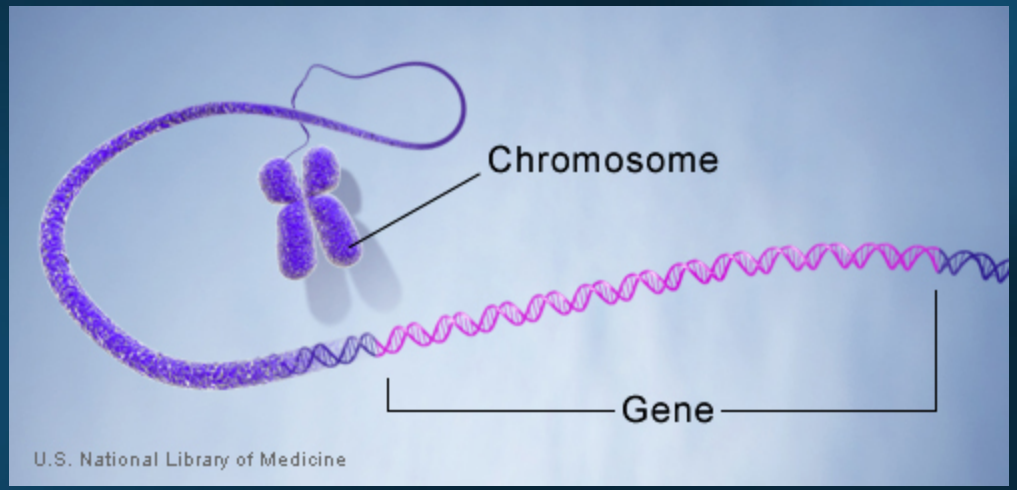
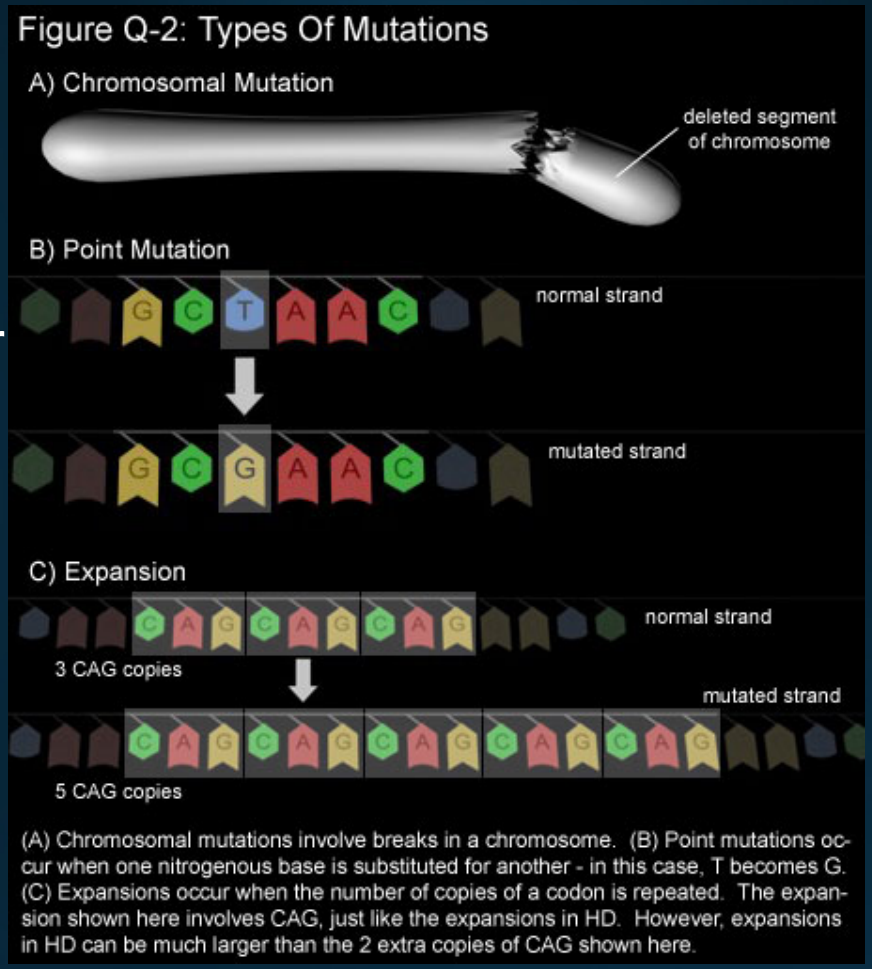
American College of Medical Genetics and Genomics

Starting Points

- Genes are made of DNA and are carried on chromosomes



- Genetic disorders are the result of alteration of genetic material
- These changes may or may not be inherited

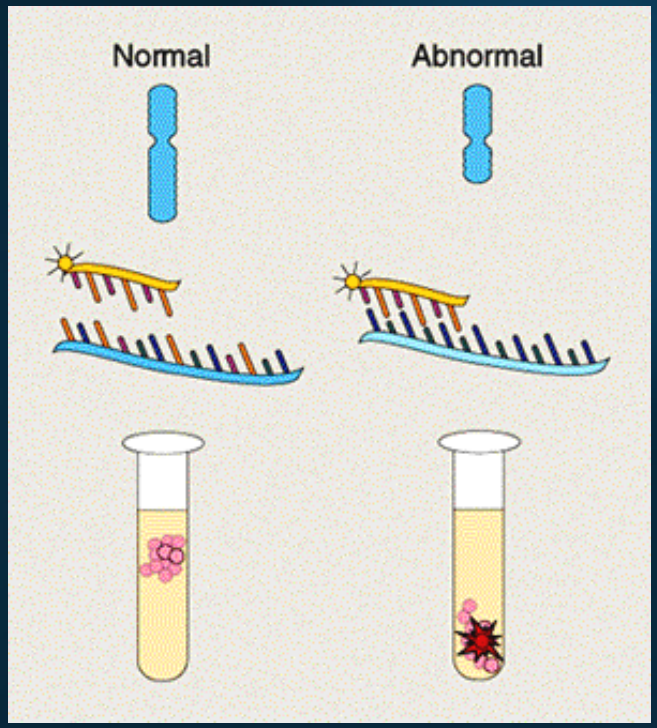


Objectives

- To explain what variety of genetic tests are now available
- What these tests entail
- What the different tests can detect
- How to decide which test(s) is appropriate for a given clinical situation

Types of Genetic Tests

- Cytogenetic
  - (Chromosomes)
- DNA
- Metabolic
  - (Biochemical)



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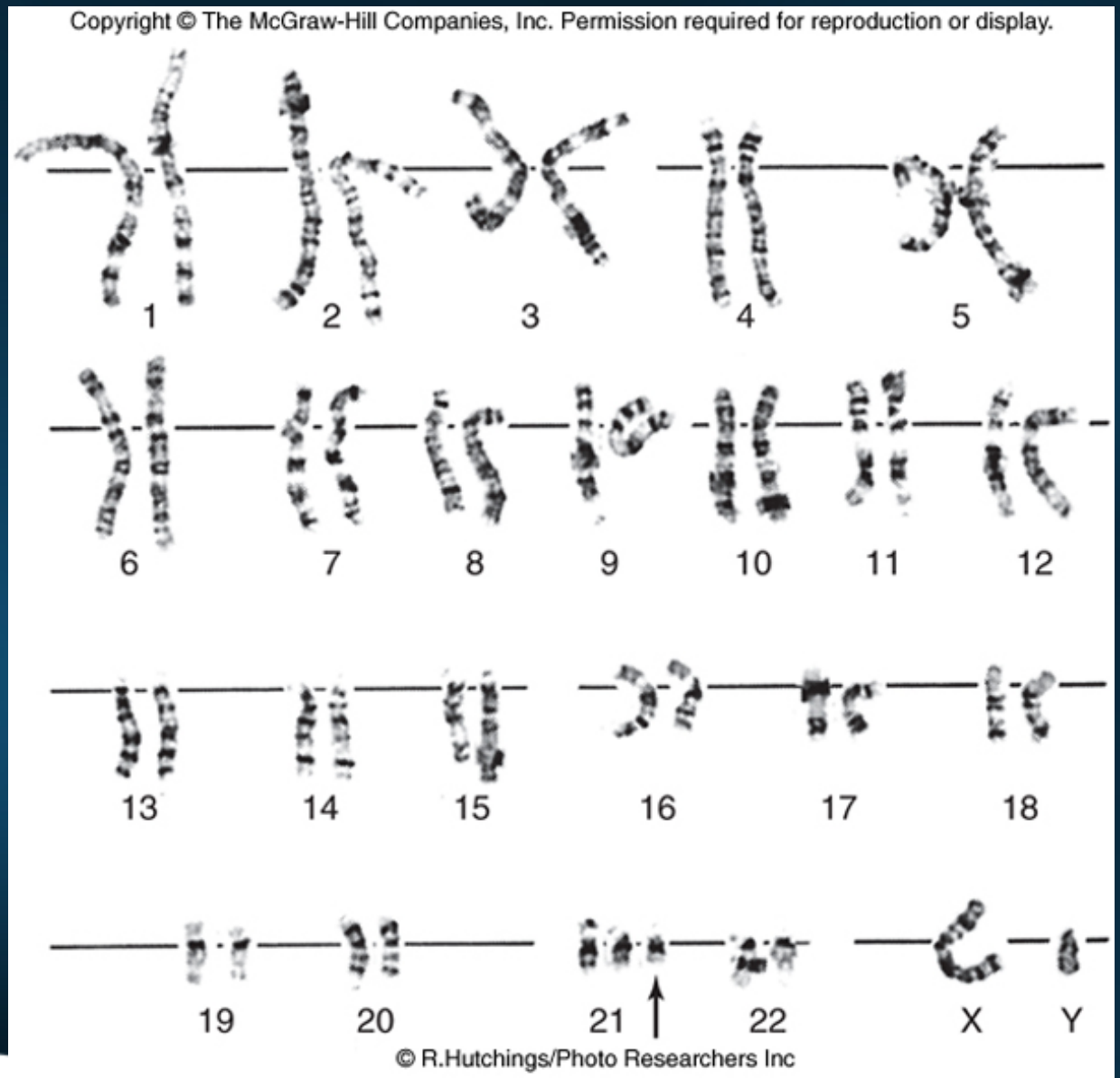
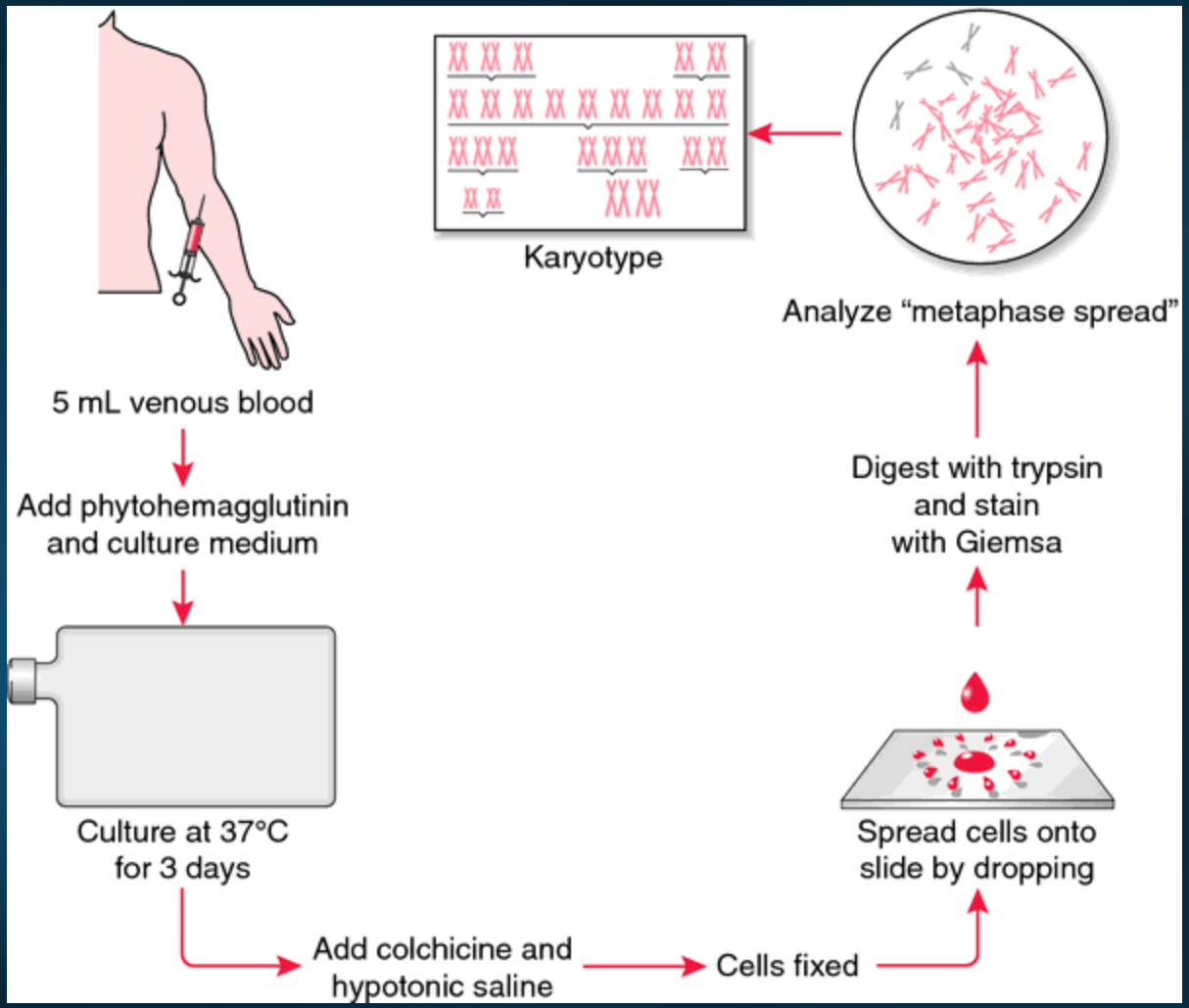
Chromosome Test (Karyotype)

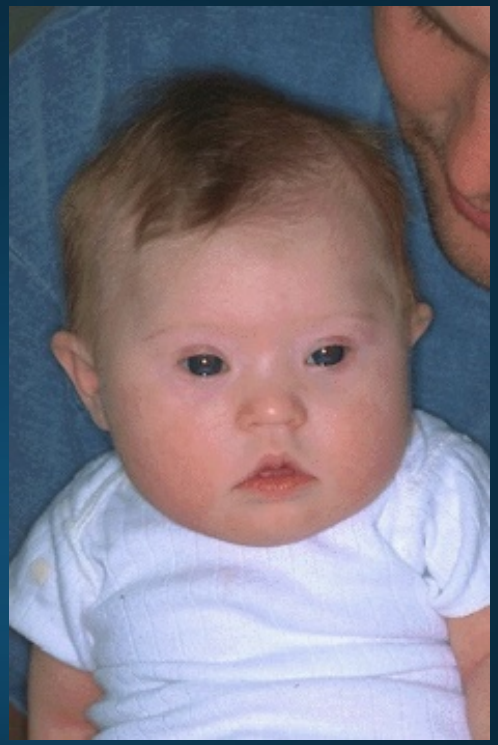


How a Chromosome test is Performed

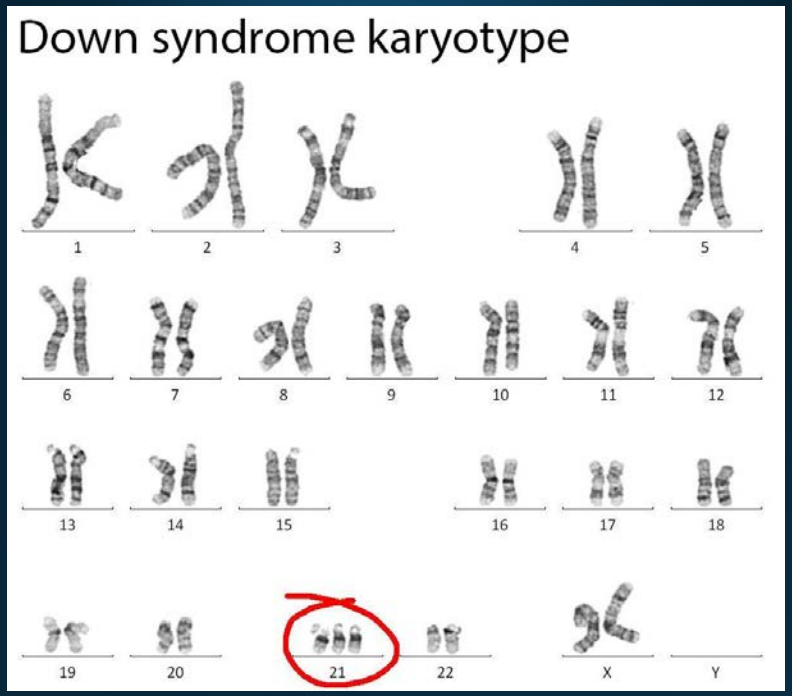


Medicaldictionary.com

Use of Karyotype



http://medgen.genetics.utah.edu/photographs/diseases/high/peri001.jpg



Karyotype Detects Various Chromosome Abnormalities

- Aneuploidy- to many or to few chromosomes
  - Trisomy, Monosomy, etc.
- Deletions – missing part of a chromosome
  - Partial monosomy
- Duplications – extra parts of chromosomes
  - Partial trisomy
- Translocations
  - Balanced or unbalanced

Karyotyping has its Limits

- Many deletions or duplications that are clinically significant are not visible on high-resolution karyotyping
- These are called “microdeletions” or “microduplications”

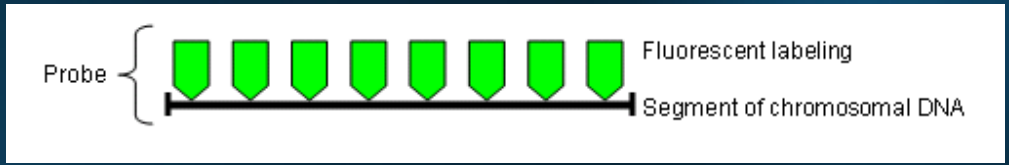
Microdeletions or microduplications are detected by FISH test

- Fluorescence In situ Hybridization

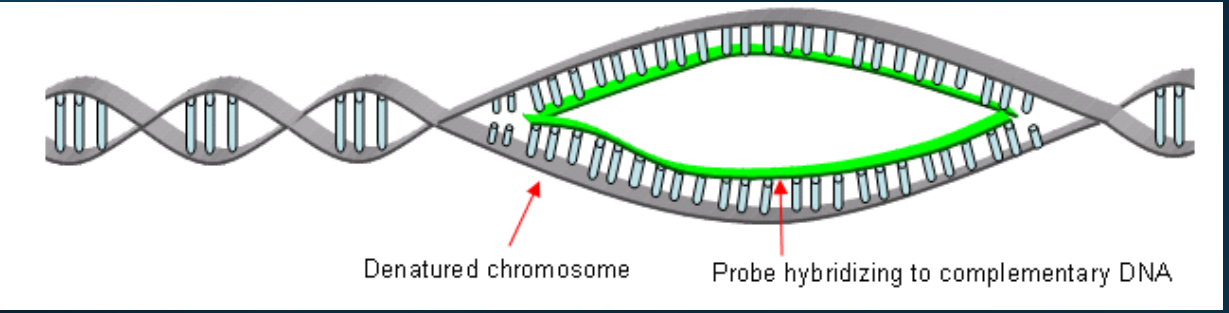
FISH

fluorescent in situ hybridization: (FISH) A technique used to identify the presence of specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently-labeled DNA probes to denatured chromosomal DNA.

Step 1. Preparation of probe. A probe is a fluorescently-labeled segment of DNA comlementary to a chromosomal region of interest.



Step 2. Hybridization. Denatured chromosomes fixed on a microscope slide are exposed to the fluorescently-labeled probe. Hybridization (attachment) occurs between the probe and complementary (i.e., matching) chromosomal DNA.



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FISH for DiGeorge/Velocardiofacial syndrome

deleted for DiGeorge chromosome region (DGCR)

FISH detects small (submicroscopic) chromosome

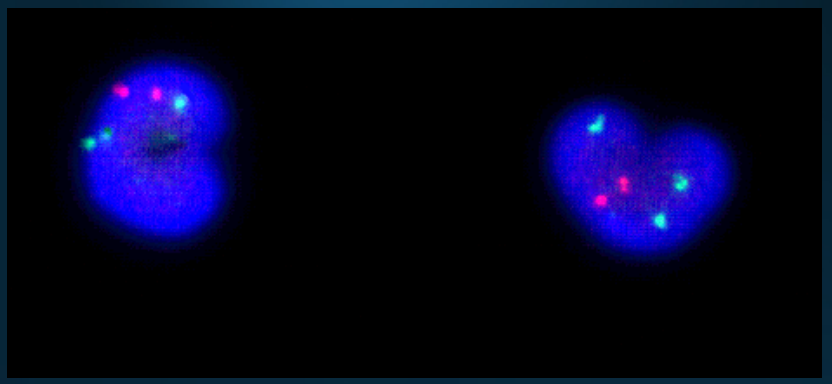
- Deletions
  - 15q11.2 deletion in Prader-Willi syndrome and Angelman syndrome
  - 22q11.2 deletion in velocardiofacial syndrome
- Duplications
  - *PMP22* – CMT1A
  - *PLP1* – Pelizeus-Merzbacher syndrome

Other Uses of FISH

- Interphase FISH for rapid diagnosis of Trisomies
- Example: Newborn with severe congenital heart disease and facial and hand anomalies.
- If it is Trisomy 18, the prognosis for survival to age 1 year is extremely poor, and cardiac surgery will be very risky
- Karyotype take 72 hours

- Interphase FISH
- Done on a blood sample
- Takes a few hours to get results

- 3 signals for chromosome 18



A Patient Who Needs Genetic Testing

- Boy who has:
  - Microcephaly
  - Hyperactivity
  - Seizures
  - Developmental delay
  - Verbal apraxia
  - Happy affect

Doctor is concerned that child may have Angelman Syndrome

- ~68% of cases have a microdeletion of a region of Chromosome 15
- So the first test to order would be FISH with a specific DNA probe that detects this region

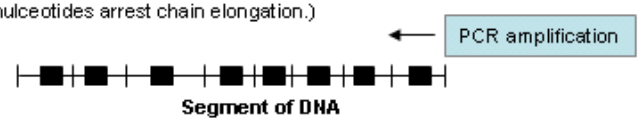
Result of the FISH test

- “NO DELETION was detected in the Angelman syndrome critical region of chromosome 15 using FISH. [ish 15q11.2(D15S10x2)]
- What next?
- ~11% of cases are caused by mutation in the UBE3A gene
- 7% have Uniparental Disomy
- 3% have an Imprinting Center defect

Sequence Analysis

Step 1: Amplification.

A. The segment of DNA to be sequenced is PCR-amplified using normal nucleotides (i.e., dATP, dTTP, dGTP, and dCTP) and fluorescently labeled, dideoxy nucleotides (i.e., ddATP, ddTTP, ddGTP, ddCTP). (Dideoxy nulceotides arrest chain elongation.)

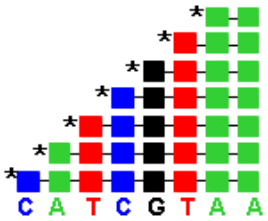


B. The DNA segment is copied when normal nucleotides are incorporated. Copying ceases when a dideoxy nucleotide is incorporated. By this process, many different-sized fluorescently labeled DNA fragments are produced.

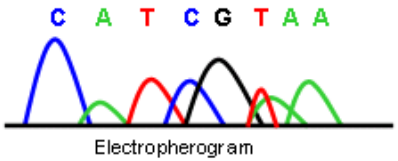
Key: Nucleotides used in PCR reaction

Normal nucleotides	Fluorescently labeled, dideoxy nucleotides
dATP	ddATP
dTTP	ddTTP
dGTP	ddGTP
dCTP	ddCTP

Step 2: Sequence determination. The fragments are sorted by length. A sequencing machine reads the fluorescent wavelengths to determine which nucleotide is at the end of each fragment.

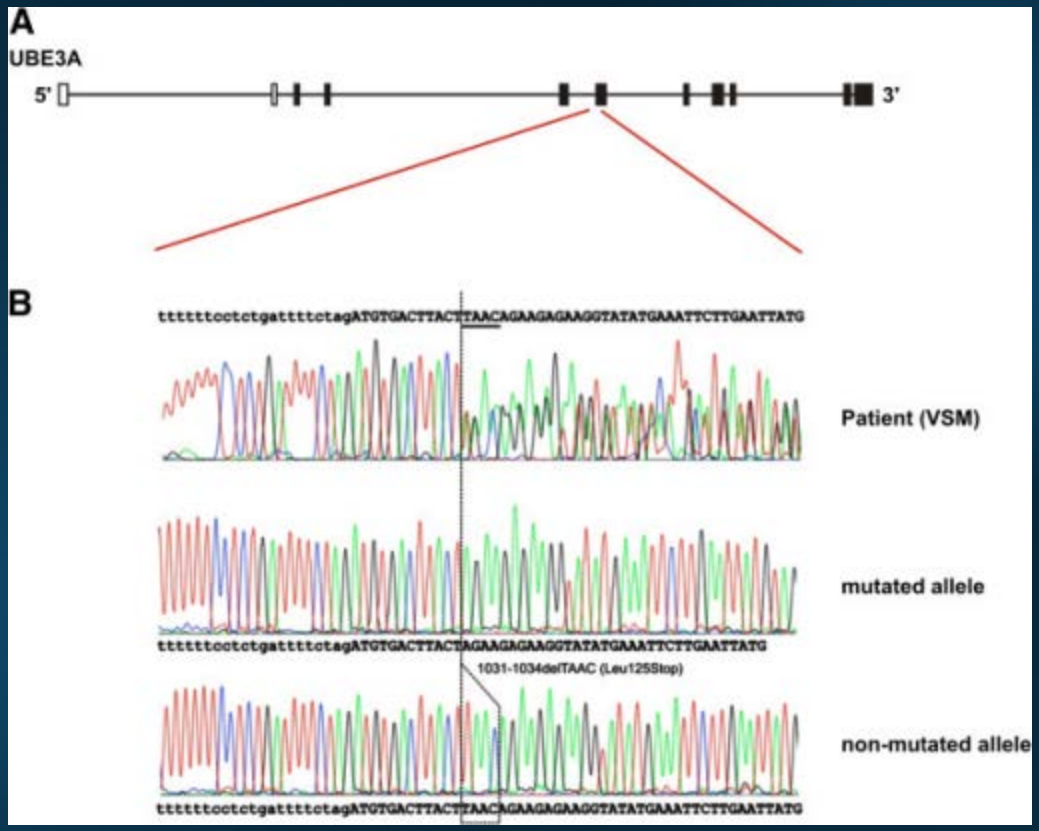


Step 3: Sequence reporting. Sequence data are typically displayed on an electropherogram as colored peaks. Each peak represents a nucleotide, corresponding to the letter above it.



Wavelength	Nucleotide
Red	T
Green	A
Blue	C
Black	G

Result of Sequencing UBE3A Gene



DeMolfetta, et al.BMC Med Genet. 2012; 13: 124.

Sequencing Results Can be Complex

- ACMG and AMP Joint Policy Statement:”Standards and guidelines for the interpretation of sequence variants” (2015)

- Pathogenic
- Likely Pathogenic
- Benign
- Likely Benign
- Uncertain Significance

Possible explanations for a false negative test result if a sequence change is not detected

- Patient does not have a change in the tested gene but there is another gene that also produces the phenotype
- Patient has a sequence change that cannot be detected by sequence analysis (e.g., a large deletion)
- Patient has a sequence change in a region of the gene (e.g., an intron or regulatory region) not covered by this laboratory's test

Another Useful Test

- Chromosome microarray

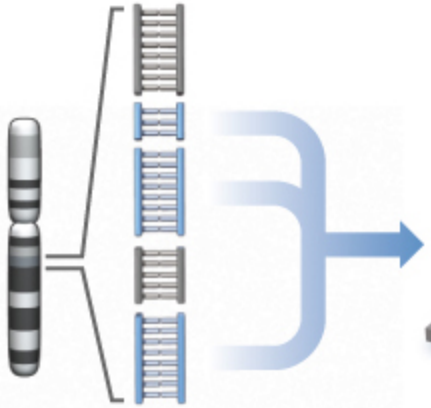
What is Chromosome Microarray?

- A gene chip which uses Comparative Genomic Hybridization to detect missing regions of chromosomes or extra segments of chromosomes
- Essentially it is performing thousands of FISH tests simultaneously

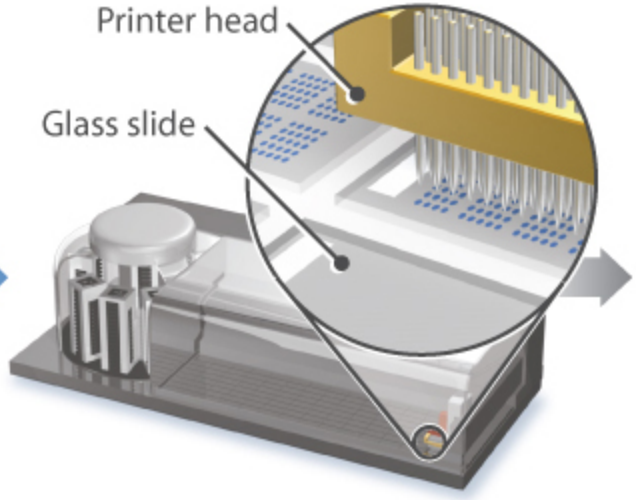
What is a Microarray?

Understanding CGH Technology

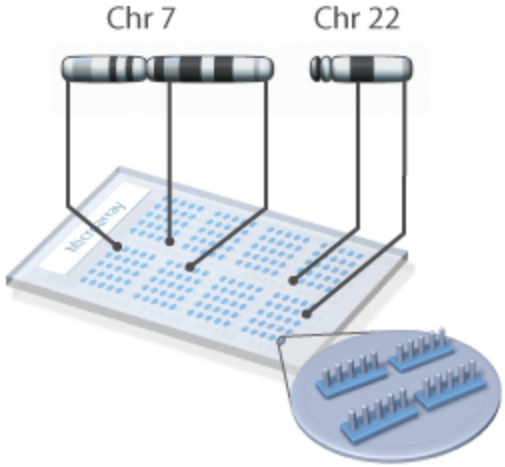
Short segments of DNA (such as bacterial artificial chromosomes, BACs) containing regions of interest are printed onto a glass slide.



Selected segments from human genome



Array printer



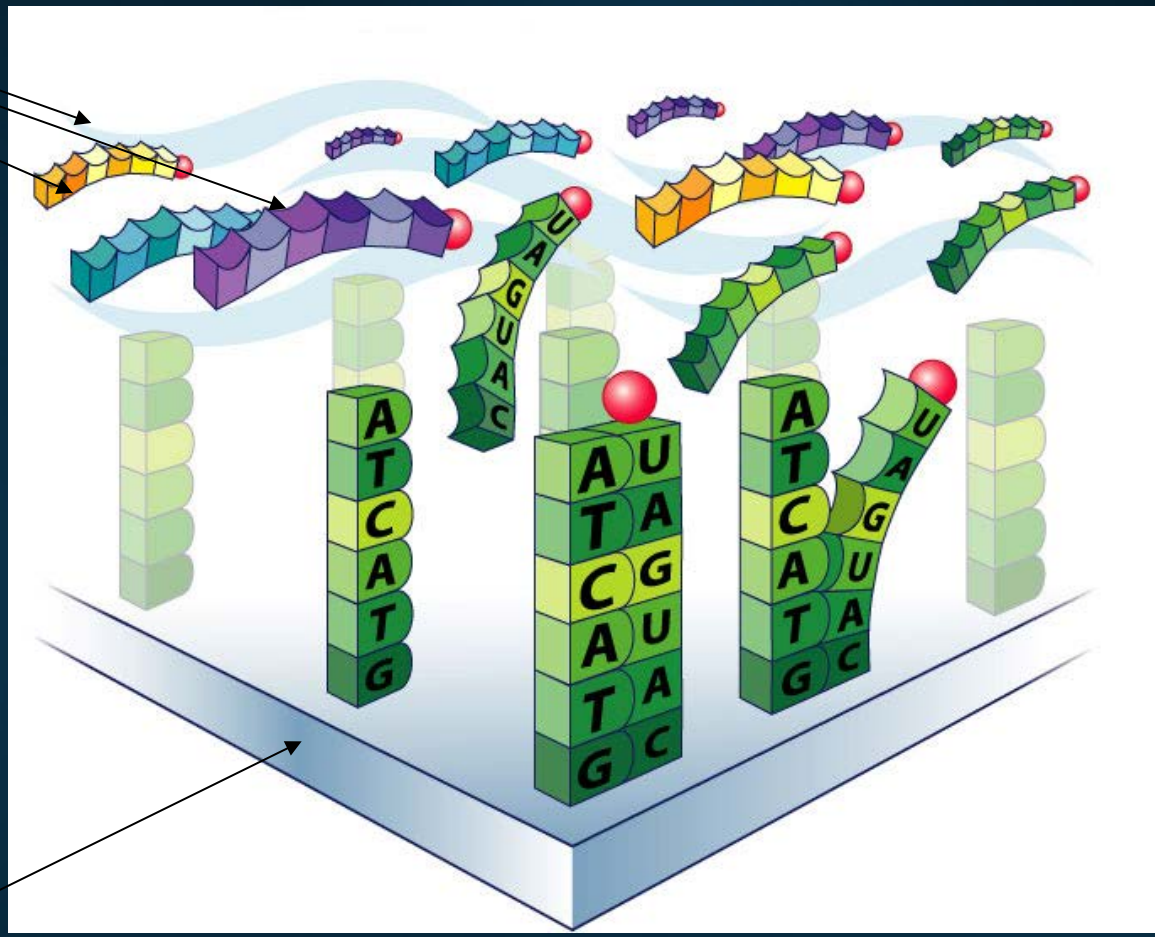
Microarray

Chromosome Microarray on a Gene Chip

- The probes attached to the gene chip are unique segments of every chromosome
- Depending on the number of probes, it could represent every genetic region of the entire genome

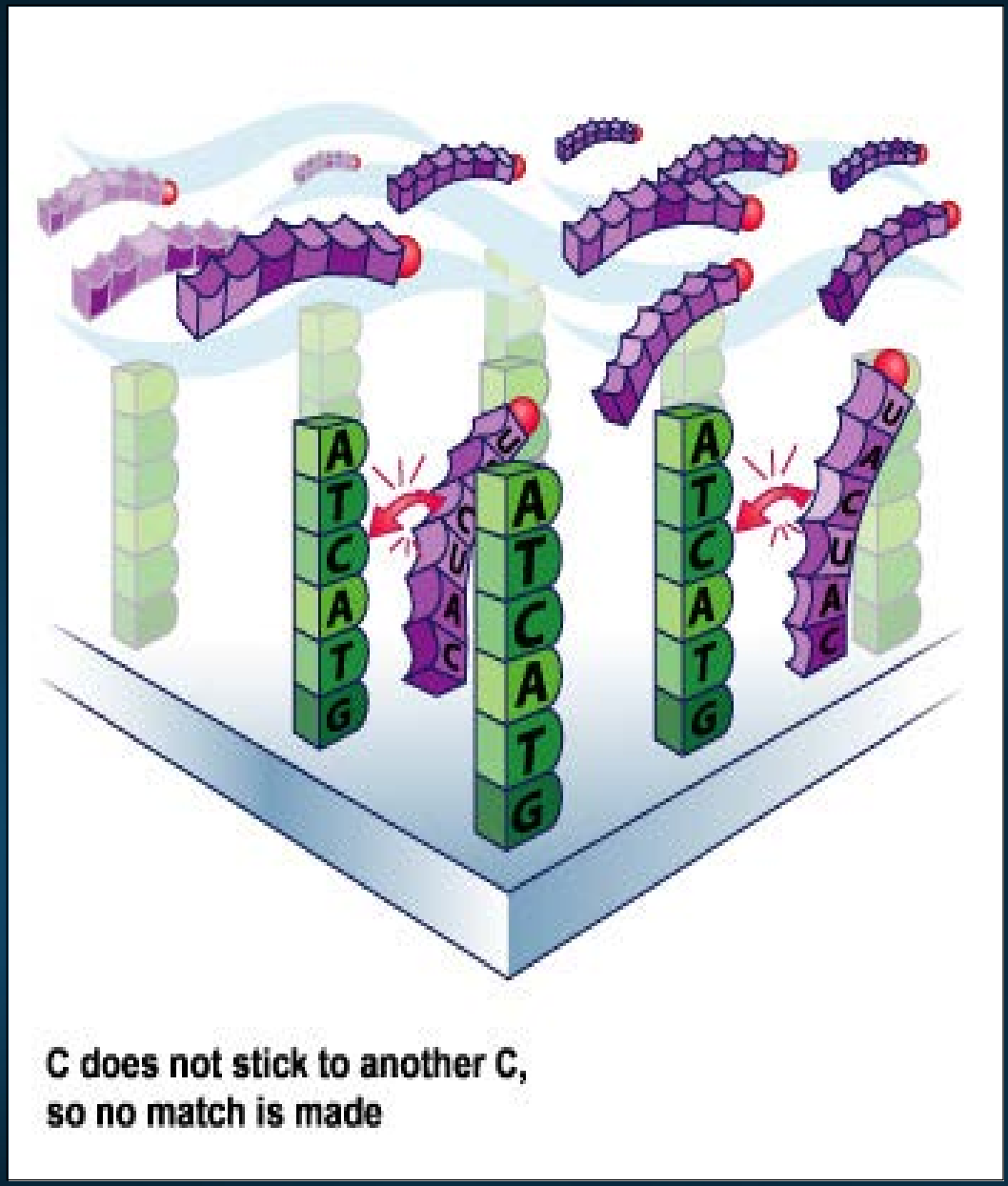
Microarray: Hybridization

Labelled cDNA A



Labelled mRNA hybridise to corresponding probe

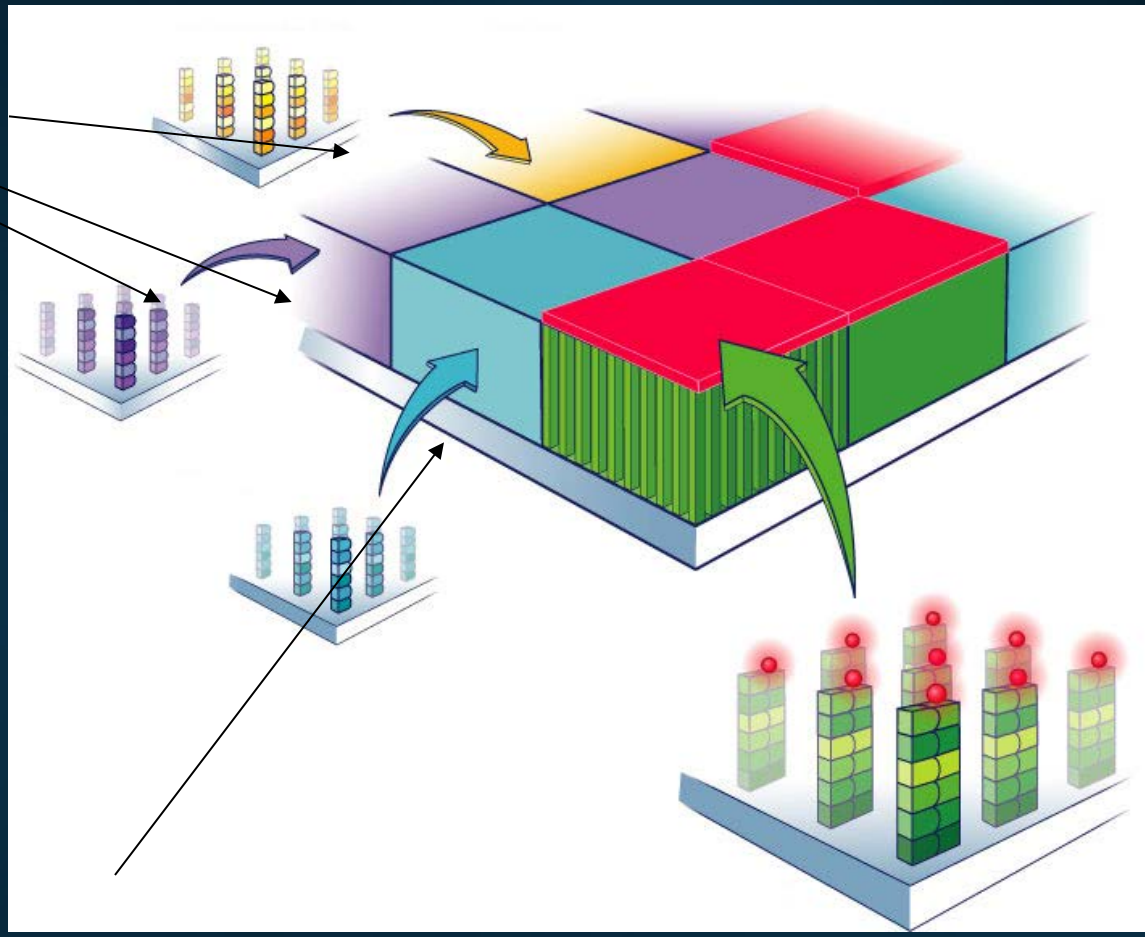
Signature Genomics



C does not stick to another C,
so no match is made

Microarray: Measurement

Genes not expressed in the source tissue



A gene expressed in the source tissue

Comparative Genomic Hybridization

- DNA from subject tissue and from normal control tissue (reference) is labeled with different colors.
- After mixing subject and reference DNA the mix is hybridized to a slide containing hundreds or thousands of defined DNA probes.
- The The fluorescence color ratio at each probe location on the array is used to evaluate regions of DNA gain or loss in the subject sample.

Microarray can detect

- Duplicated genomic material
- Deleted genomic material
- Multiple deletions and or duplications of genomic regions

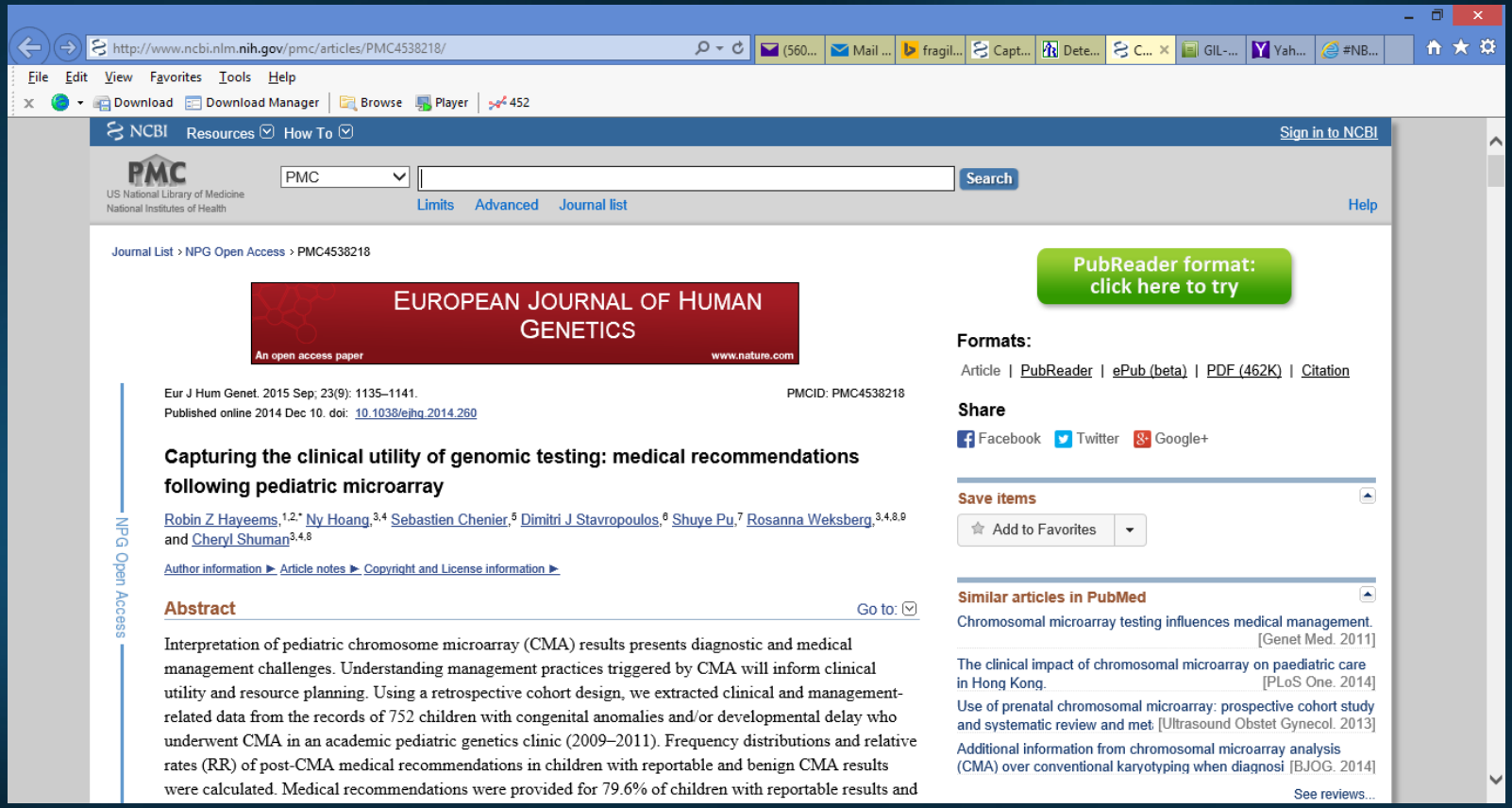
Microarray cannot

- Determine if deletion or duplication is due to a Chromosome Translocation

Microarray Results

- Microarray makes 10-15% more diagnoses than karyotyping in the evaluation of patients with Idiopathic Learning disability
- Some studies report as high as 28% diagnosis rate with microarray
- ACMG Practice Guideline 2010 affirming use of Chromosome Microarray as a first-tier genetic test in evaluating patients with intellectual disability and/or multiple congenital anomalies

Clinical Utility of Chromosome Microarray



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Capturing the clinical utility of genomic testing: medical recommendations following pediatric microarray

Robin Z Hayeems,[1,2,*] Ny Hoang,[3,4] Sebastien Chenier,[5] Dimitri J Stavropoulos,[6] Shuye Pu,[7] Rosanna Weksberg,[3,4,8,9] and Cheryl Shuman[3,4,8]

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Abstract

Go to:

Interpretation of pediatric chromosome microarray (CMA) results presents diagnostic and medical management challenges. Understanding management practices triggered by CMA will inform clinical utility and resource planning. Using a retrospective cohort design, we extracted clinical and management-related data from the records of 752 children with congenital anomalies and/or developmental delay who underwent CMA in an academic pediatric genetics clinic (2009–2011). Frequency distributions and relative rates (RR) of post-CMA medical recommendations in children with reportable and benign CMA results were calculated. Medical recommendations were provided for 79.6% of children with reportable results and

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Chromosomal microarray testing influences medical management. [Genet Med. 2011]

The clinical impact of chromosomal microarray on paediatric care in Hong Kong. [PLoS One. 2014]

Use of prenatal chromosomal microarray: prospective cohort study and systematic review and met. [Ultrasound Obstet Gynecol. 2013]

Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosi [BJOG. 2014]

See reviews...

Possible Results from a Chromosome Microarray Test

- A **normal result** means that no duplications or deletions of genetic material were found.
- A **likely pathogenic result** means that a duplication or deletion of genetic material was found, and this is likely to cause health or learning problems. Your doctor might be able to make predictions on how this genetic change will affect a person.
- A **likely benign result** means that a genetic change was found, but it **is not** likely to cause health or learning problems. Every person has slight differences in his or her genes. These differences make each person individual and unique. Benign changes (duplications or deletions that do not cause learning or health problems) are part of normal human variation.

Possible Results from a Chromosome Microarray Test(continued)

- A **variant of unknown significance** is a genetic change that has not been reported before in other individuals. It is unclear whether the genetic change might cause learning or health problems, or if it is **benign**.

- When a **variant of unknown significance** is found on microarray, the lab recommends testing the parents to see if either of them has the same genetic change.
  - If either parent **is** found to have the same genetic change and has no learning or health concerns, then the variant is more likely to be benign.
  - If the parents are **not** found to have the same genetic change, it is still difficult to tell whether the change is normal human variation or if it could cause any health or learning problems. In these cases, we watch to see if any other individuals are reported with a similar genetic difference. Over time we may learn more about what this change means.

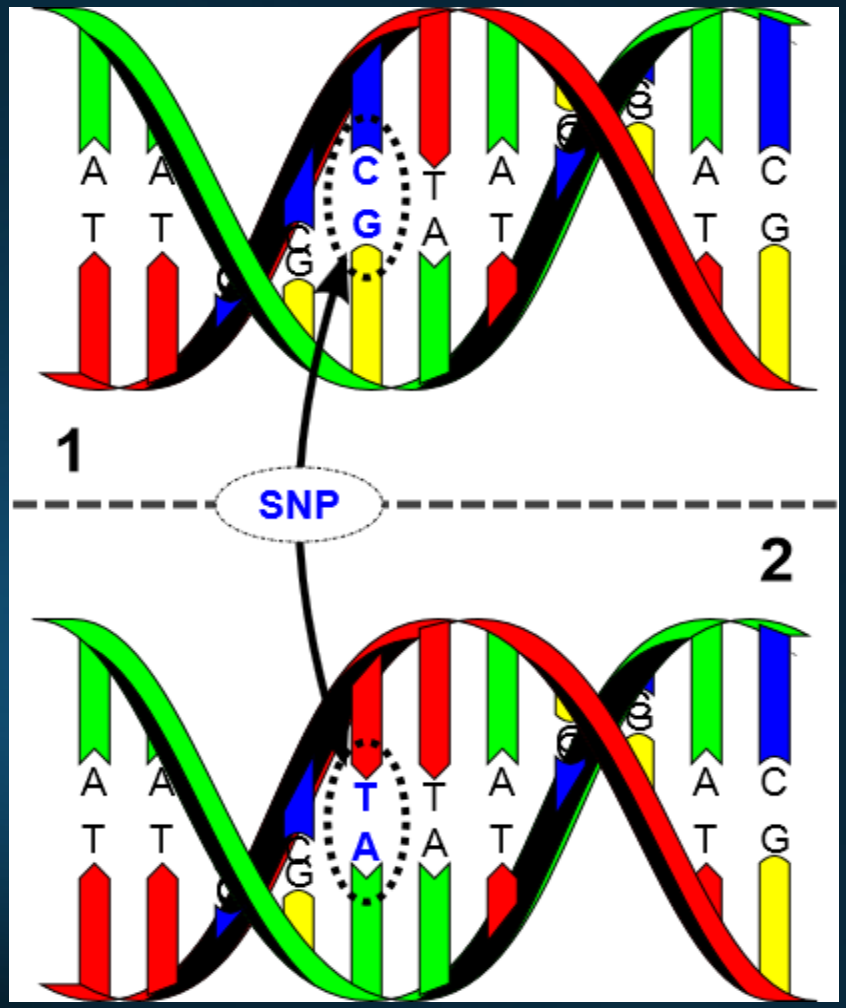
What other types of microarrays?

- “SNiP” array

Single Nucleotide Polymorphisms (“SNiPs”)

- A **Single Nucleotide Polymorphism** (SNP) is the variation of a single base pair in the DNA sequence between either the members of a species or between the paired chromosomes of an individual.

SNP



- Here there is a single nucleotide difference in the sequence of part of a gene between these 2 individuals:
  - Individual 1 has TTCCCTACCAC
  - Individual 2 has TTCCTTACCAC
- This change doesn't necessarily change the function of the gene

Genereviews.org

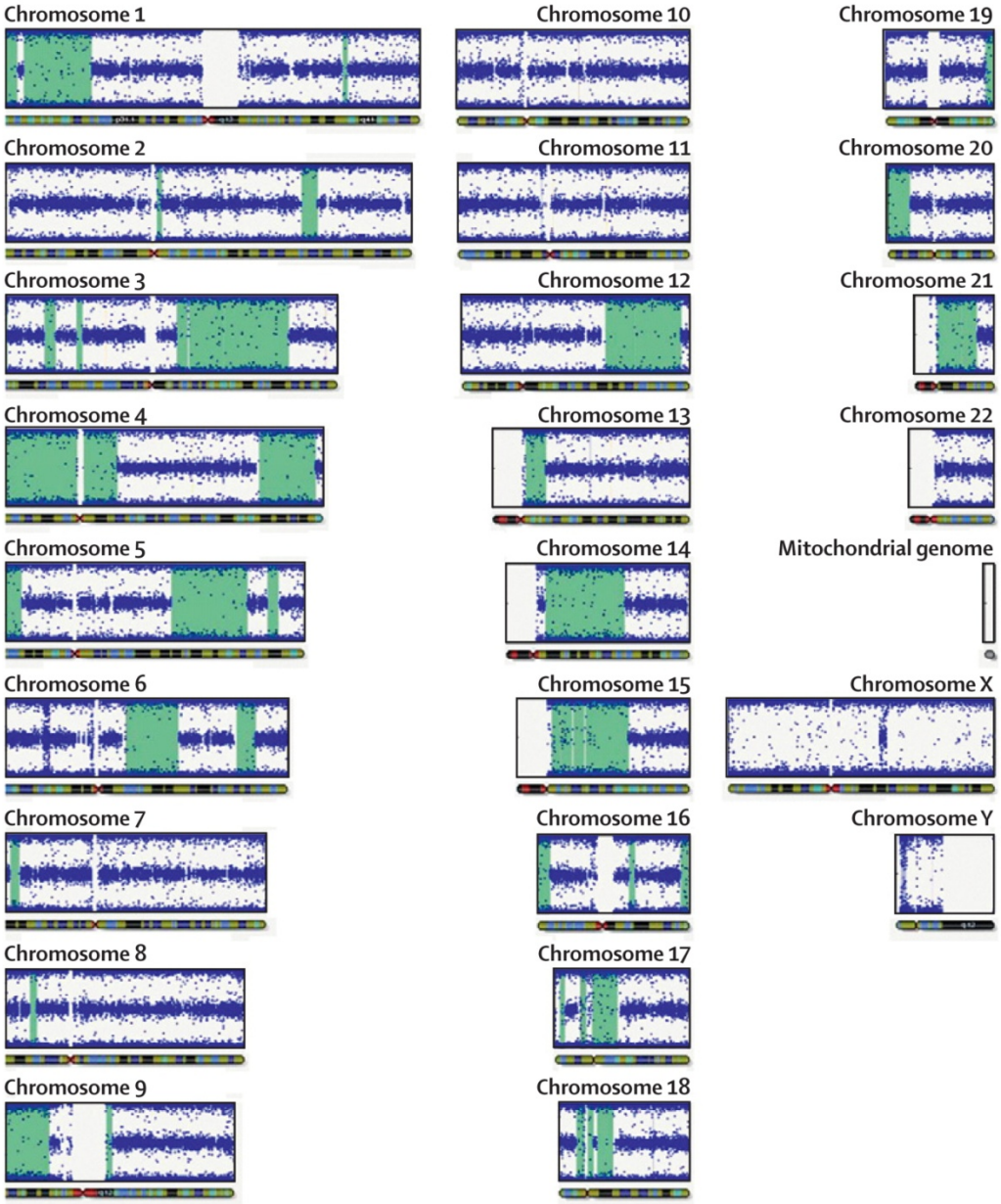
SNiP Arrays

- Currently have 1.8 million probes for SNiPs
- If the tested DNA matches the sequence of a specific SNiP in a specific gene = positive result
- Like chromosome microarrays, can also detect small deletions or duplications
- But,
- Can also yield surprising information beyond deletion or duplication of genomic regions
  - “Loss of Heterozygosity”

Identification of incestuous parental relationships by SNP-based DNA microarrays

CP Schaaf, DA Scott, J Wiszniewska and AL Beaudet

The Lancet
Volume 377, Issue 9765, Pages 555-556 (February 2011)
DOI: 10.1016/S0140-6736(11)60201-8



Source: The Lancet 2011; 377:555-556 (DOI:10.1016/S0140-6736(11)60201-8)

Case I saw Back in Georgia

- Internationally adopted girl with MR, non-specific facies, no family or pre-natal history available
- Karyotype, Chromosome microarray, DNA tests for MECP2, Angelman syndrome, all negative
- SNiP array showed high degree of loss of heterozygosity
  - Corresponding to biologic parents being closely related, and thus the girl must have some sort of autosomal recessive disorder

Another Case

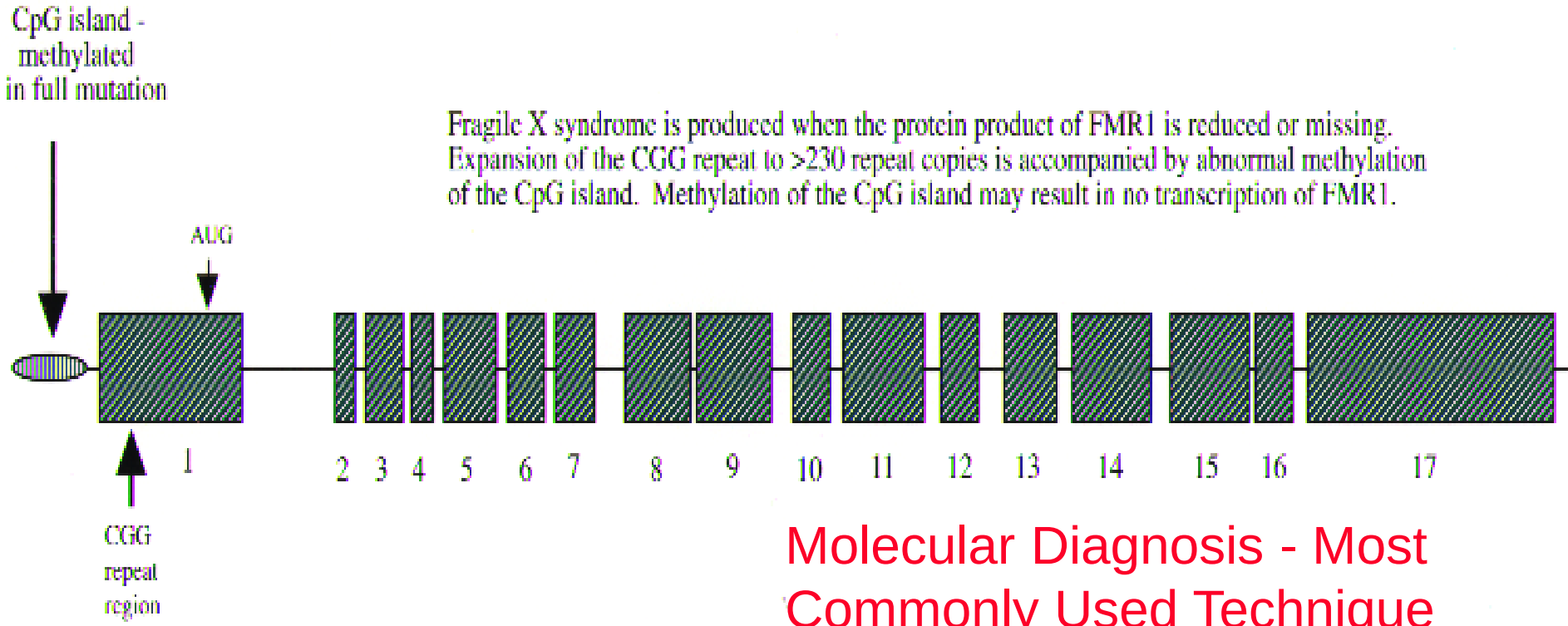
- Girl with complex phenotype, with MR, non-specific dysmorphism, multiple congenital anomalies, endocrine dysfunction
- Extensive work-up has been negative
- SNiP array showed areas of loss-of-heterozygosity
  - one of the areas has gene for Bardet-Biedl syndrome type 7
    - Patient has a few features compatible with BBS, but lacks many of the most characteristic features (pigmentary retinal dystrophy, polydactyly,renal malformation) but we are proceeding with sequencing the BBS7 gene

Let’s Turn to Another Patient Situation

- 3 year old boy who is not walking and has only a few word vocabulary
- Growth is normal
- He has a long facial profile
- Family history is not contributory
- First test to evaluate the underlying cause?

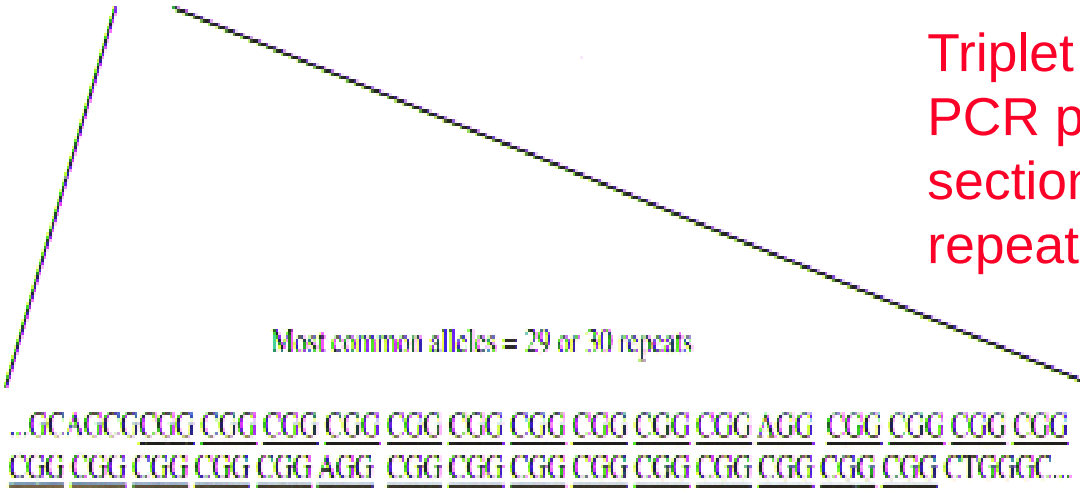
Chromosome Microarray

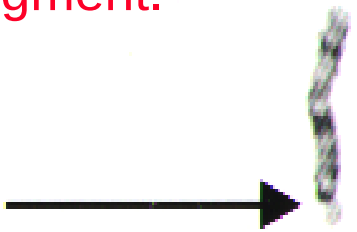
- Normal
- What next?
- The most common cause of intellectual disability in males is Fragile-X syndrome,
- So Dr. sends blood for Fragile-X testing



Molecular Diagnosis - Most Commonly Used Technique

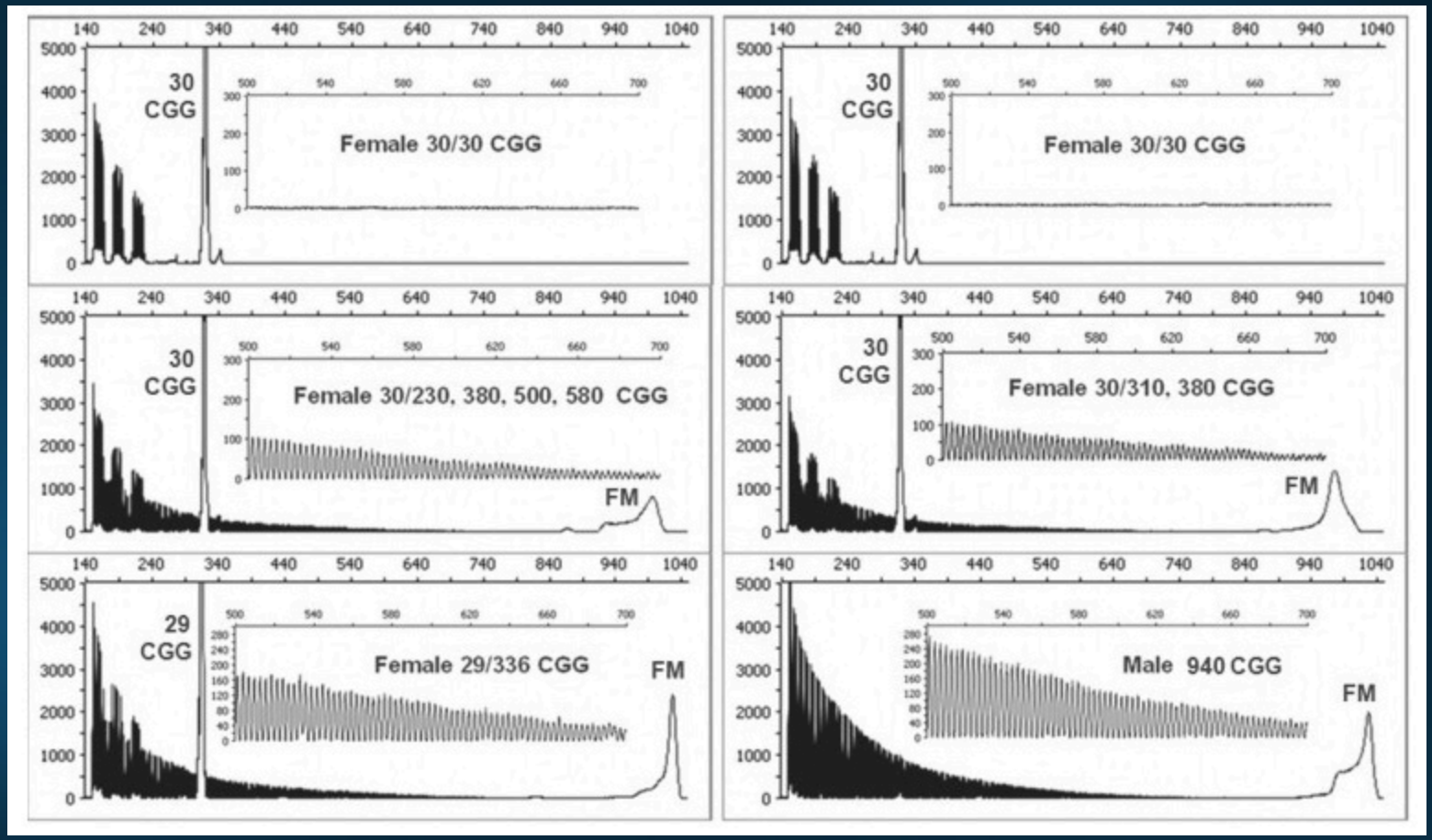
Triplet Repeat Primed PCR: uses 3 PCR primers, two that flank the repeat section and a third located within the repeat segment.





Courtesy of J Tarleton,PhD

Triplet Repeat Primed Polymerase Chain Reaction

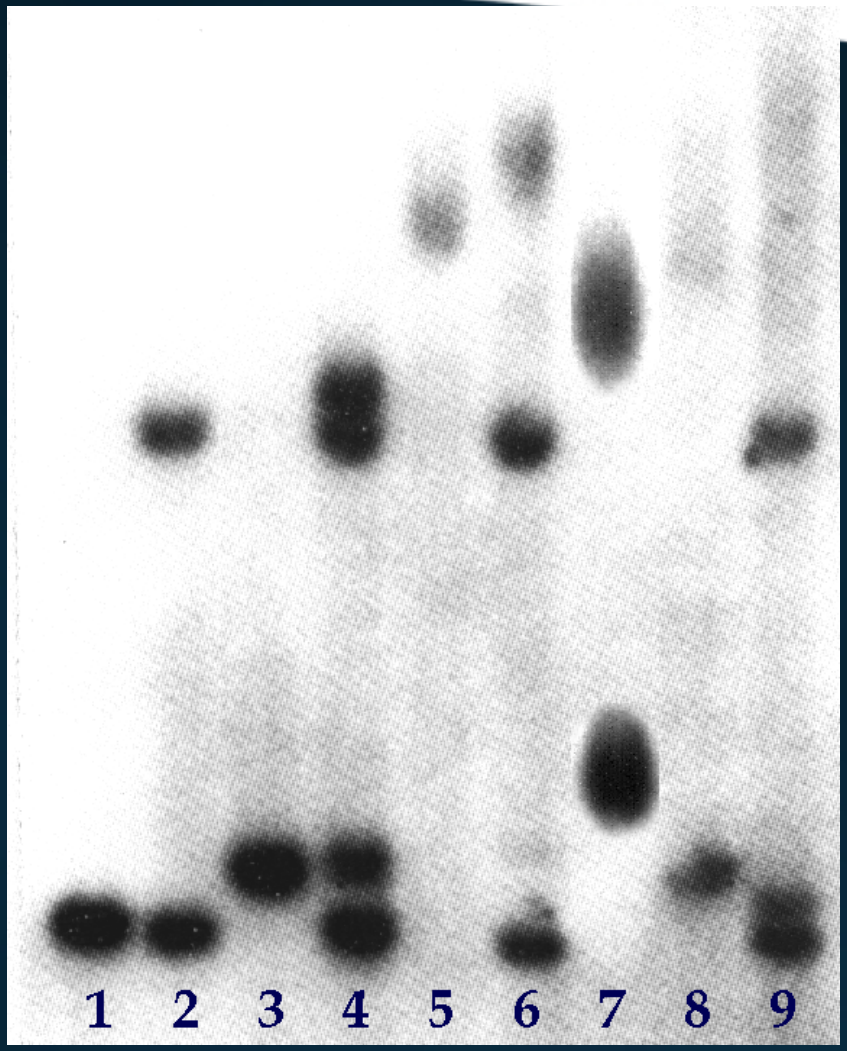


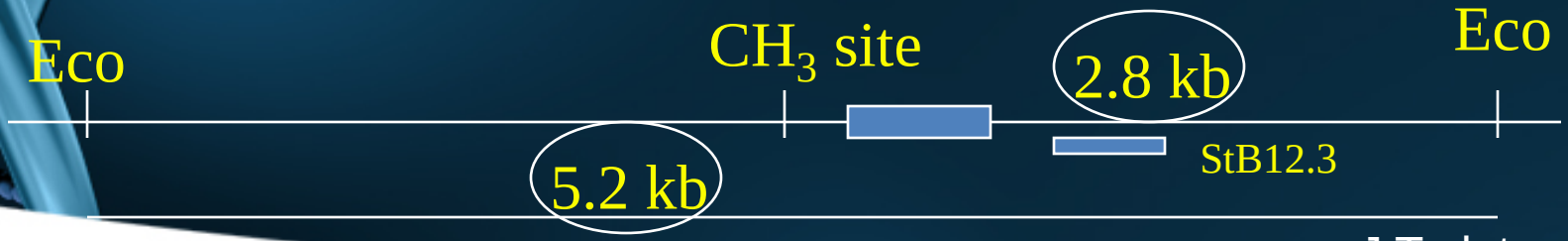
Chen, et al 2010 J Mol Diag 12: 589

J Tarleton, PhD

Southern blot analysis

Older technology but still occasionally useful to resolve the myriad of molecular rearrangements that occur in FMR1





J Tarleton, PhD

Patient results

- 330 CGG repeats
- Mom needs to be tested because the risk of having another affected male increases depending on how many repeats she has
- AND
- Her father should be tested because he may have a pre-mutation expansion, which places him at risk for developing **Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) as he ages**

Examples of Other Trinucleotide Repeat Disorders

- Huntington Disease
- Spinocerebellar Ataxias
- Myotonic Dystrophy

THANK YOU