Molecular Cytogenetics

Expanding the resolution of conventional cytogenetic analysis

- Application of the techniques of Molecular Biology to cytogenetic preparations

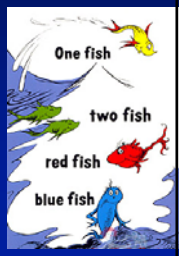
Clinical Applications of Molecular Cytogenetics

- Molecular cytogenetic techniques provide a way to detect complicated, cryptic and submicroscopic rearrangements that remain undetected or undecipherable by conventional cytogenetic analysis

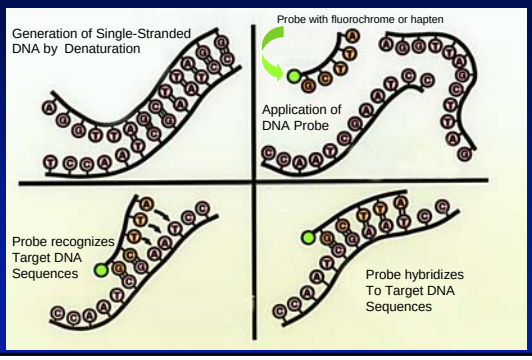
FLUORESCENCE IN SITU HYBRIDIZATION

FISH

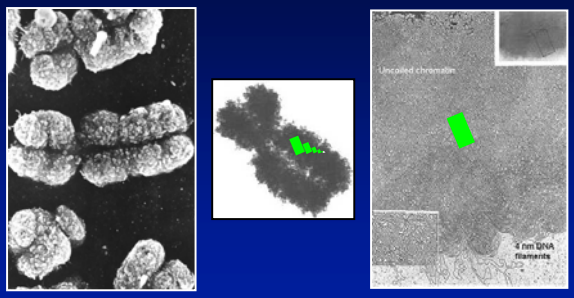
- FISH is a physical DNA mapping technique in which a DNA probe labeled with a marker molecule is hybridized to chromosomes on a slide, and visualized using a fluorescence microscope
- The marker molecule is either fluorescent itself, or is detetcted with a fluorescently labeled antibody.



HYBRIDIZATION STEPS

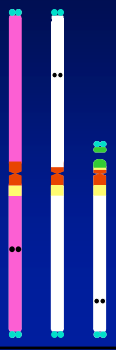


Chromatin Compaction



Metaphase chromosome is compacted into a structure that is 50,000 times shorter than its extended length

Classification of Chromosomal Sequences



- Beta satellite
- Alpha satellite
- Classical satellite
- Telomeric sequences
- Unique gene sequences
- Partial chromosome paints
- Whole chromosome paints

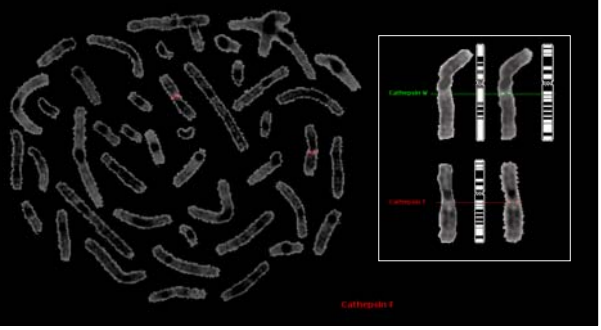
FISH APPLICATIONS

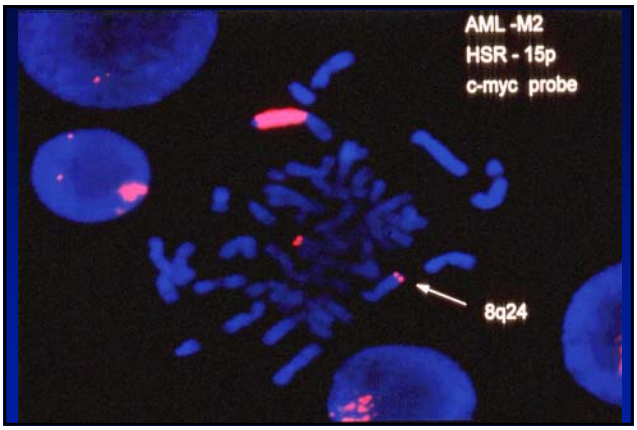
- Gene Mapping
- Chromosome Identification
- Aneuploidy Detection
- Sexing for X-Linked diseases
- Marker chromosome Identification
- Total chromosome Analysis
- Translocation Analysis
- Unique Sequence DNA Detection
- Microdeletion Syndrome Analysis
- Gene Amplification Analysis
- Mouse Chromosome Research

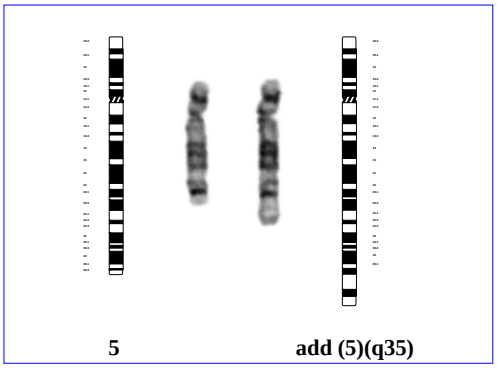
FISH TECHNIQUES

- Metaphase FISH
- Interphase FISH
- Reverse FISH
- Multi-color FISH (M-FISH; SKY)
- PRINS
- Fiber FISH
- CGH
- DNA Arrays (Chip technology)

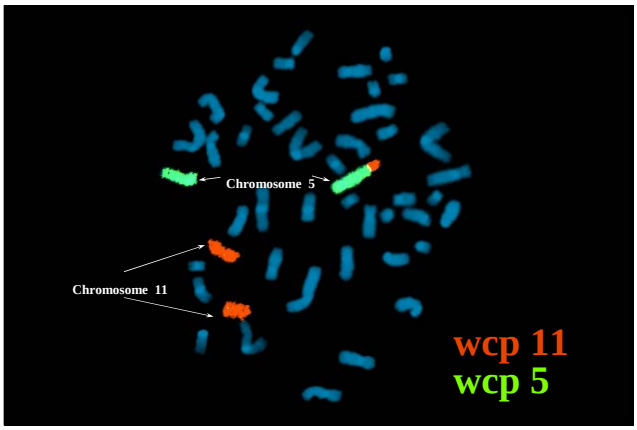
GENE MAPPING







5 add (5)(q35)

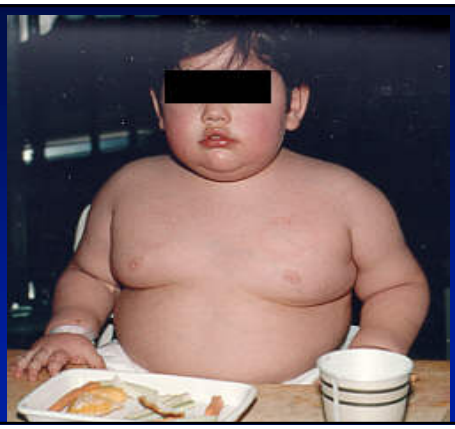


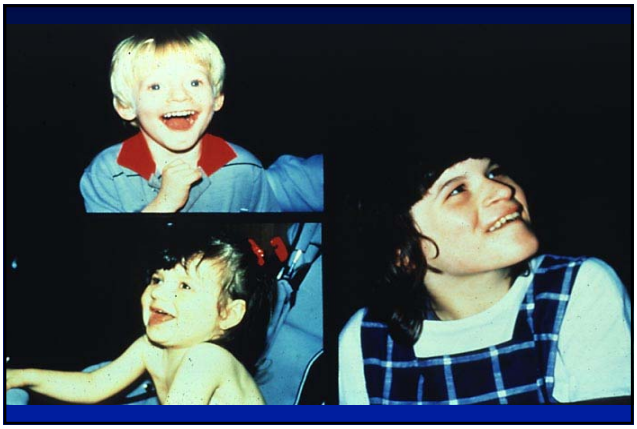
Microdeletion Syndromes

- Deletions of a megabase or so of DNA that are most often too small to be seen under the microscope
- Produce well defined contiguous gene syndromes which demonstrate superimposed features of several different mendelian diseases(X-linked or autosomal)
- Defined by high resolution banding or molecular cytogenetic techniques

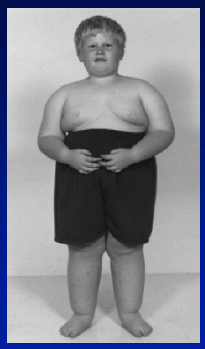
Microdeletion Studies Using *FISH*

Syndrome	Chromosome Location	Probe/Gene Locus
DiGeorge	22q11.2	D22S75
Velocardiofacial	22q11.2	D22S76
Miller-Dieker	17p13.3	D17S379
Smith-Magenis	17p11.2	D17S29
Prader-Willi	15q11.2	SNRPN
Angelman	15q11.12	D15S10
Williams	7q11.23	Elastin
Cri du chat	5p15.2	D5S23
Wolf-Hirschhorn	4p16.3	D4S96

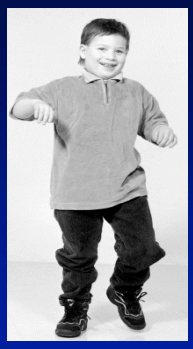




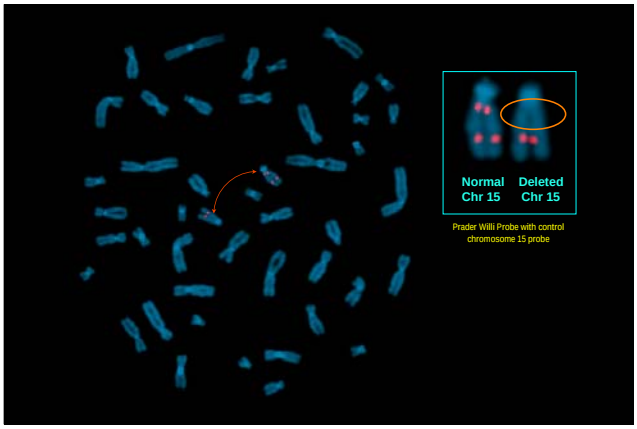
Prader-Willi Syndrome

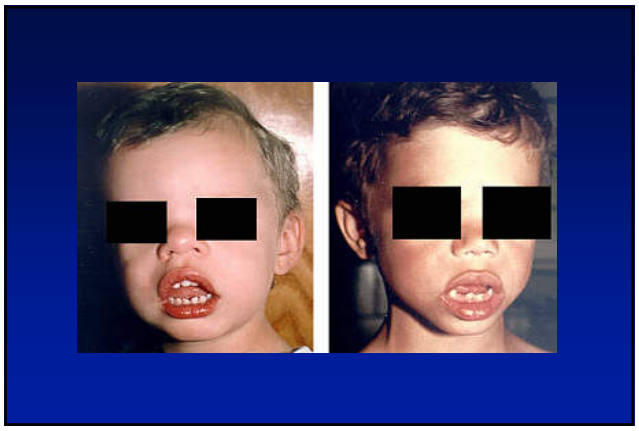


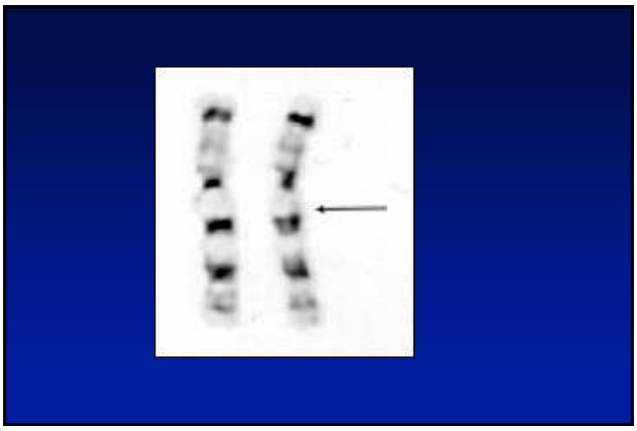
Angelman Syndrome

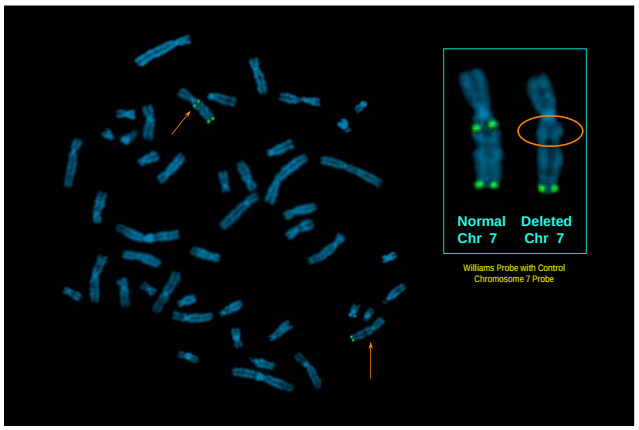


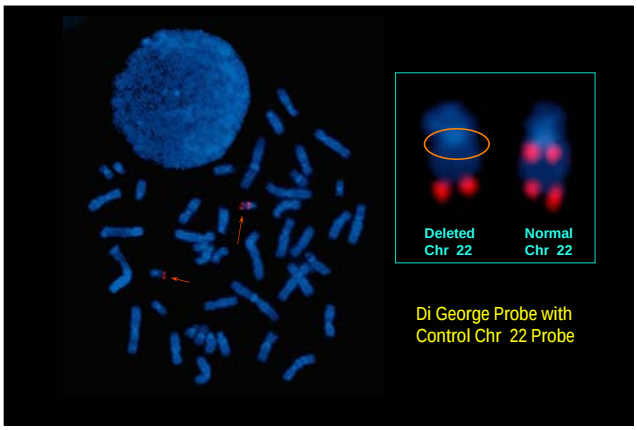
From *Medical Genetics* (Jorde, Carey, Bamshad, White; 2nd Ed.)

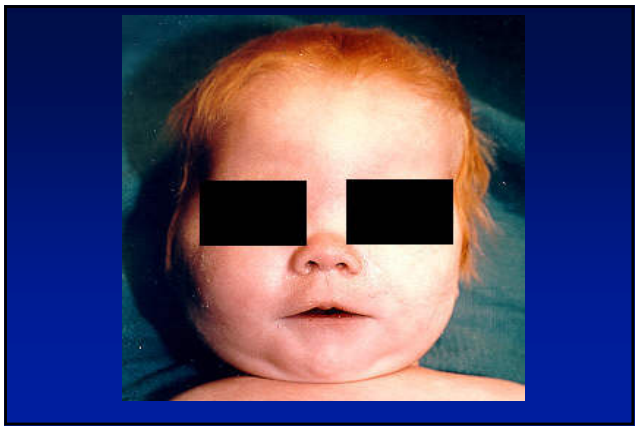


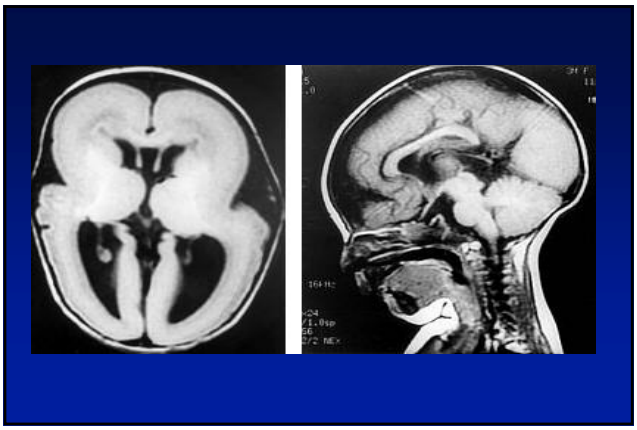





Normal
Chr 7
Deleted
Chr 7
Williams Probe with Control
Chromosome 7 Probe


Deleted
Chr 22
Normal
Chr 22
Di George Probe with
Control Chr 22 Probe





FISH on Interphase Nuclei is Useful in Specific Clinical Situations

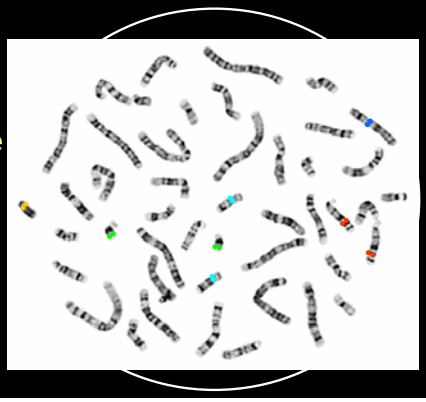
- Prenatal diagnosis aneuploidy screening by FISH looks at interphase nuclei derived from chorionic villi or amniocytes
- Preimplantation Genetic Diagnosis aneuploidy screening by FISH looks at interphase blastomere nuclei

Interphase FISH vs Metaphase FISH

- Prenatal diagnosis aneuploidy screening by FISH looks at interphase nuclei derived from chorionic villi or amniocytes
- Preimplantation Genetic Diagnosis aneuploidy screening by FISH looks at interphase blastomere nuclei

From Metaphase

To Interphase



Chromosomes Enumeration by Rapid Prenatal Interphase FISH

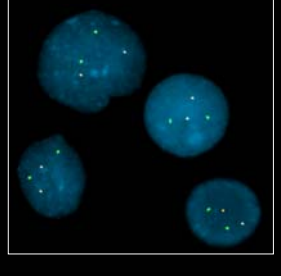
- Trisomies 13, 18 & 21 and Monosomy X are the most common aneuploidies related to maternal age or fetal abnormality
- Routine chromosome analysis take 7-10 days
- Prenatal Interphase FISH provides a rapid way to screen for the common aneuploidies in uncultured amniotic fluid cells in about 1-2 days

Chromosomes Enumeration in Chorionic Villi and Amniocytes by Rapid Prenatal Interphase FISH

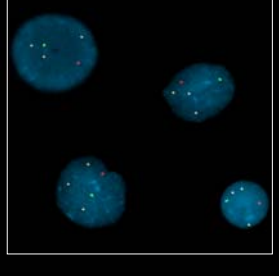
Interphase Amniocyte

X Y 21

Disomy 21 Female



Trisomy 21 Male



Benefits of Prenatal Interphase FISH

- Trisomies 13, 18 & 21 and Monosomy X are the most common aneuploidies related to maternal age or fetal abnormality
- Routine chromosome analysis take 7-10 days
- Prenatal Interphase FISH provides a rapid way to screen for the common aneuploidies in uncultured amniotic fluid cells in about 1-2 days
  - Reduces emotional burden on the patient and/or physician in the face of an increased risk for chromosome abnormalities following an abnormal screening result
    - Opportunity to reduce anxiety through earlier decision making

Sensitivity of Prenatal Interphase FISH

Abnormality	Sensitivity	Specificity	PPV	NPV
Trisomy 13	98.6%	100%	100%	99.98%
Aneuploidy 18	99.5%	100%	100%	99.8%
Trisomy 21	100%	100%	100%	100%
45,X	100%	99.98%	98.5%	100%
Other Sex Chr.	100%	100%	100%	100%
All detectable	99.6%	99.98%	99.8%	99.96%

From Tepperberg et al. Prenatal Diagnosis 2001; 21:293-301

- Study looked at 5197 pregnancies
- Review of Lit includes ~ 30,000 pregnancies

Preimplantation Genetic Diagnosis

- PGD is a very early form of prenatal diagnosis
- Oocytes or embryos obtained *in vitro* through assisted reproductive techniques are biopsied
  - Polar bodies for the oocytes
  - Blastomeres for the embryos
- Only Embryos shown to be free of the disease under consideration are subsequently used for transfer

Preimplantation Genetic Diagnosis

- Method
  - Generate Oocytes/embryos in vitro by ART
  - Biopsy Polar Body/1-2 cells from embryos
  - FISH or PCR for genetic diagnosis
- Patients
  - Repeated terminations
  - Moral or religious objections to termination
  - Repeated miscarriages due to chromosome abnormality
  - Infertile couples

Biopsy

- Polar Body
- Cleavage Stage
- Blastocyst

Polar Body Biopsy

From Veeck – Atlas of Embryology

- Used by few groups in USA
- Need the first and second polar body
- Labour intensive
- Only maternal chromosomes examined

Day 3 - Cleavage Stage

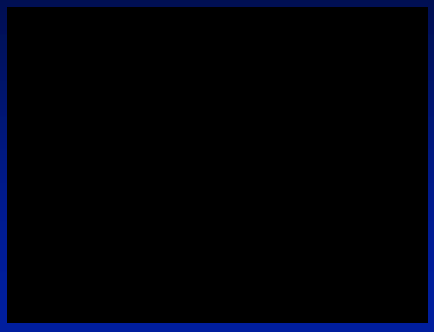
From Veeck – Atlas of Embryology

- Used by majority of groups
- Biopsy at 6-10 cell stage
- Blastomeres totipotent
- 1-2 cells for analysis

Embryo Biopsy – Acid Tyrodes

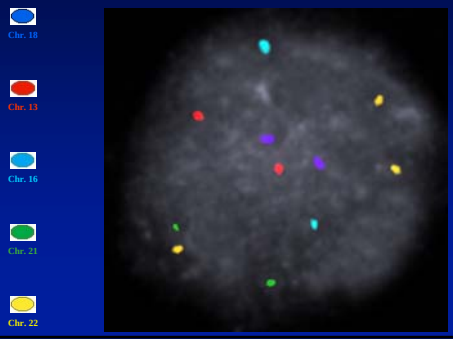
Courtesy of Kathleen Miller, RMA of NJ

Embryo Biopsy



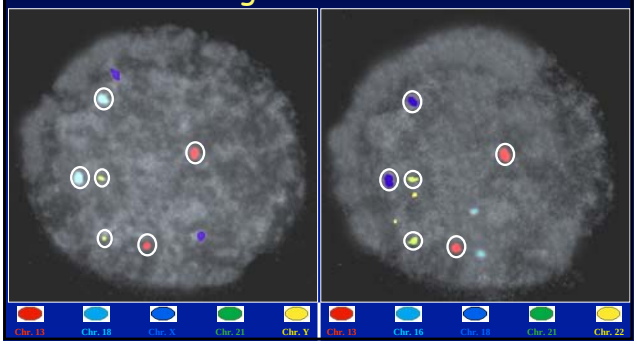
Courtesy of Kathleen Miller, RMA of NJ

Aneuploidy Analysis of Embryos for Chromosomes 13, 16, 18, 21 & 22



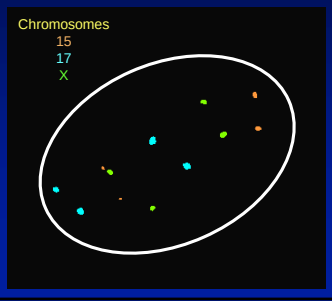
Trisomy 22

Multiple Hybridizations Add Diagnostic Power

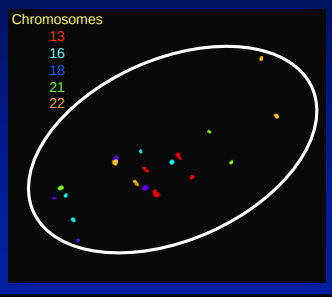


Mosaicism

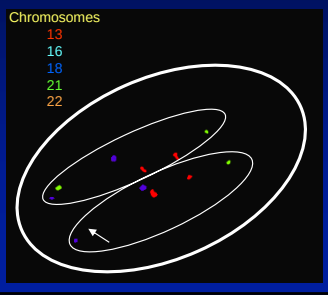
Post-Zygotic Non–Disjucntion



Mosaicism
Post-Zygotic Non–Disjucntion



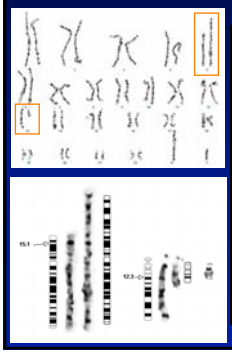
Mosaicism
Post-Zygotic Non–Disjucntion

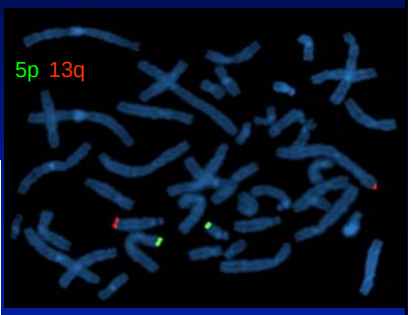


Telomeres

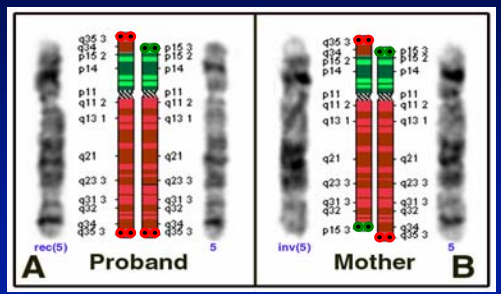
- Highest concentration of genes of any chromosomal region – therefore sub-microscopic deletions and duplications would have a significant impact
- Increased genetic recombination at telomeres
  - Male rate higher than female for most chromosomes
  - Telomeres play a critical role in chromosome pairing at meiosis

FISH using Subtelomere Probes

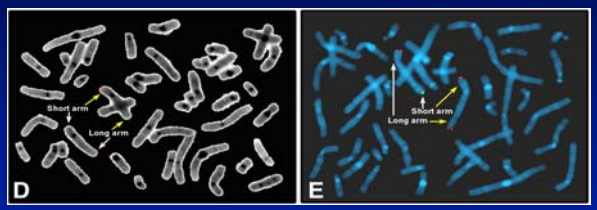




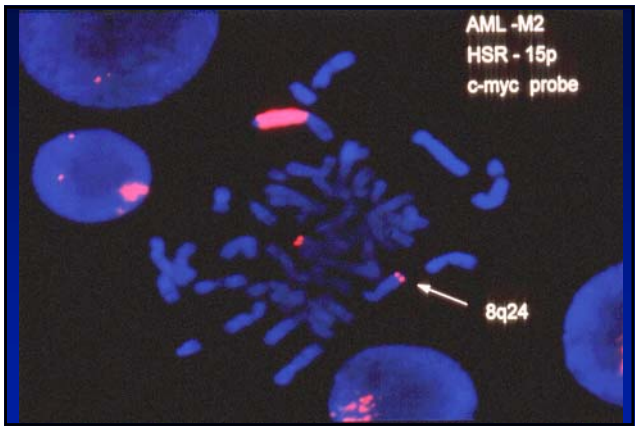
Pericentric Inversion

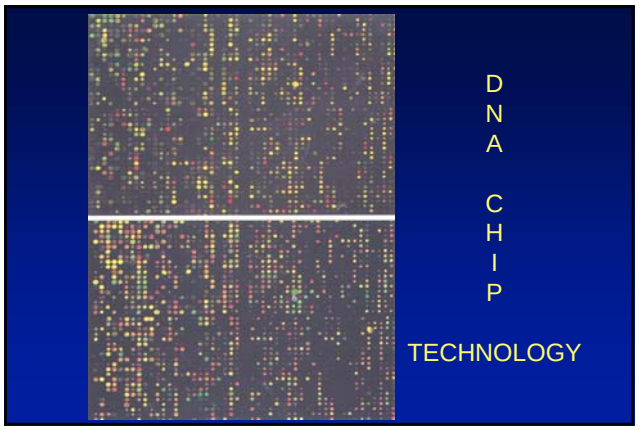


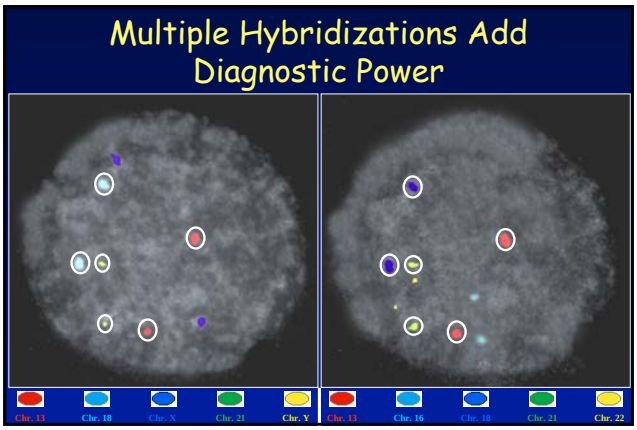
Pericentric Inversion FISH



Mother 5p 5q Proband



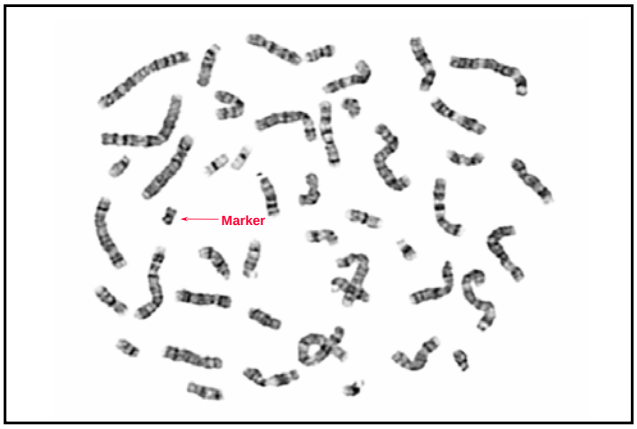




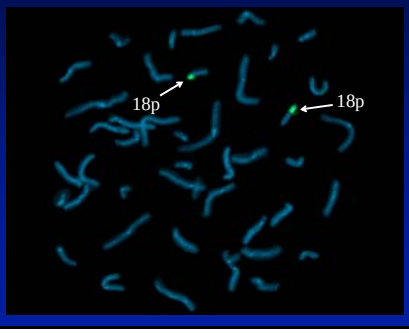
Detection of Partial Aneuploidies – An expensive FISHing Expedition

- Unbalanced rearrangements
- Marker chromosomes
- Cryptic translocations
- Cryptic deletions
- Microdeletions

The Need for New Technologies

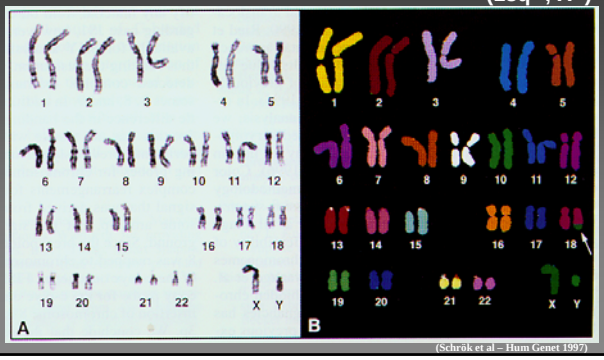


REVERSE FISH FOLLOWING MARKER CHROMOSOME MICRODISSECTION



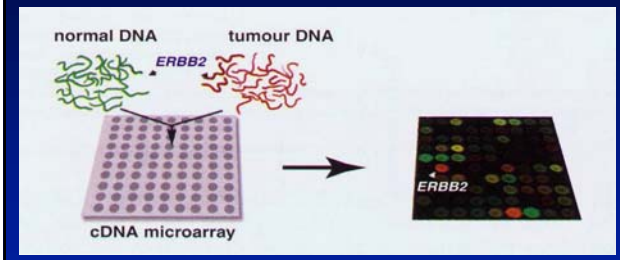
Spectral KarYotyping

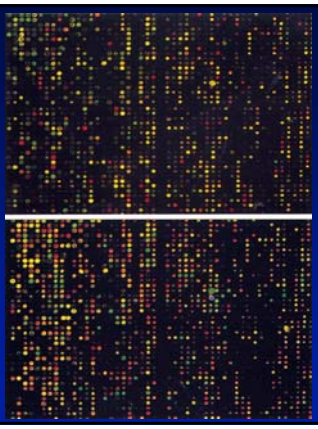
(18q- ; X+)



(Schrök et al – Hum Genet 1997)

DNA Microarrays





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TECHNOLOGY

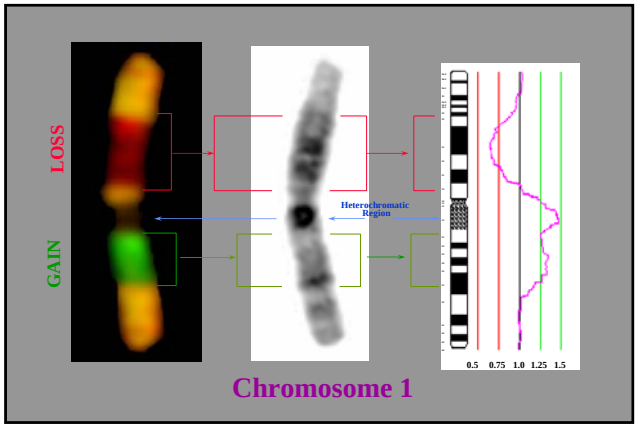
COMPARATIVE GENOMIC HYBRIDIZATION

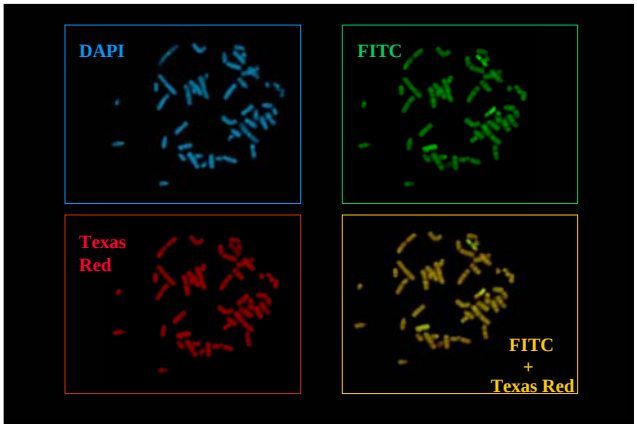
C G H

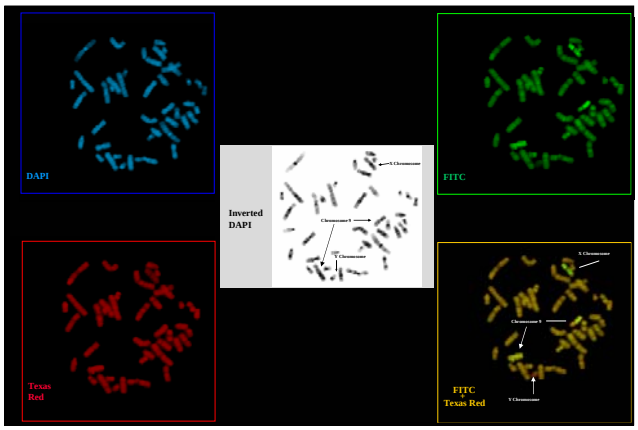
- Identifies chromosomal gains and losses in a **single** hybridization procedure
- Effectively reveals any DNA sequence copy number changes (i.e., gains, amplifications, losses and deletions) in a particular specimen and maps these changes on normal chromosomes

CGH

- *In situ* hybridization of differentially labelled specimen DNA & normal reference DNA to normal human metaphase chromosome spreads.
- Specimen & reference DNA can be distinguished by their different fluorescent colors.







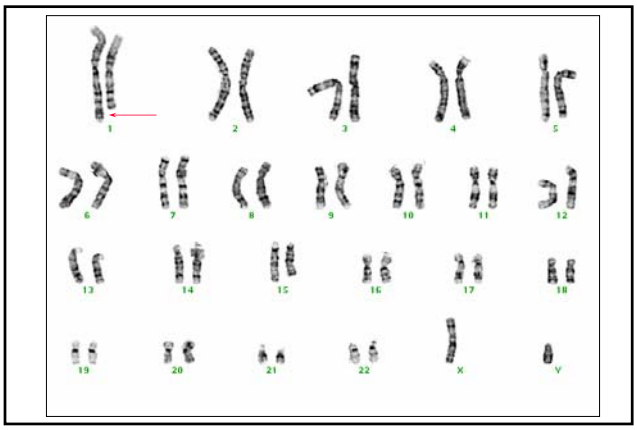
OVERVIEW OF CGH

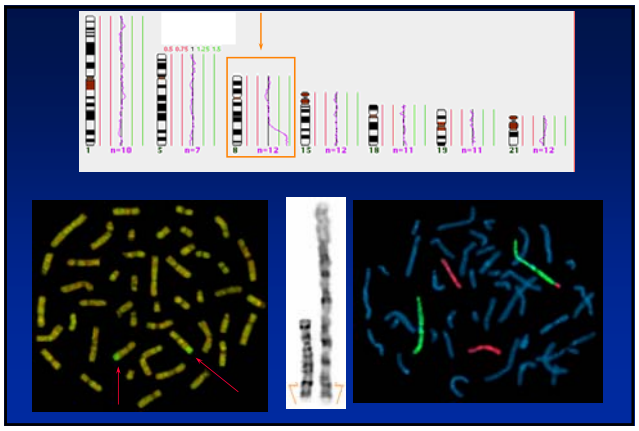
The major steps in CGH involve:

- Preparation of normal metaphase spreads
- Isolation of high molecular weight DNA from specimen (test) and reference (normal) samples.
- Labelling of specimen & reference DNA with different color fluorochromes

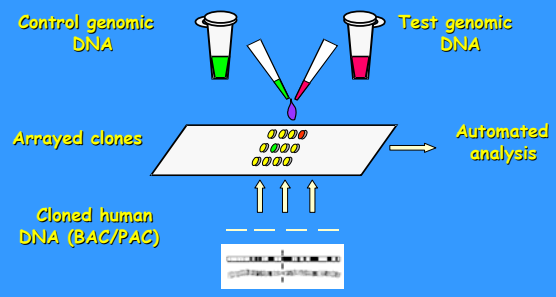
OVERVIEW OF CGH (cont)

- *In situ* hybridization of the labelled specimen & reference DNAs to normal metaphase spreads
- Washing off unbound DNA
- Counterstaining metaphase spreads with DAPI
- Fluorescent microscopy to visualize & capture color ratio differences along the chromosomes

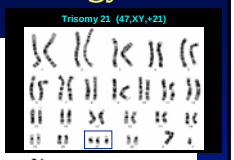




CGH Microarray Methodology



CGH Microarray Methodology



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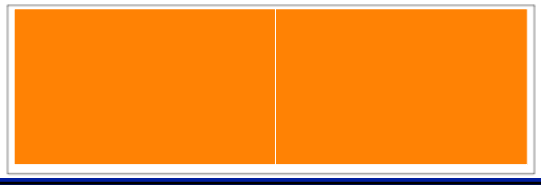
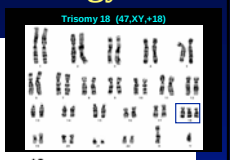
SignatureChip® Profile for Trisomy 21



CGH Microarray Methodology



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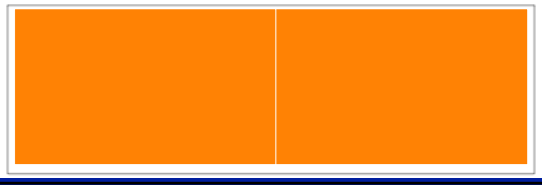
SignatureChip® Profile for Trisomy 18



CGH Microarray Methodology

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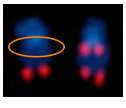
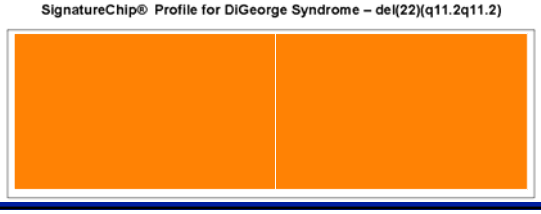


SignatureChip® Profile for DiGeorge Syndrome – del(22)(q11.2q11.2)



Interpreting a Normal CGH Result in a Karyotypically Normal Individual with Clinical Abnormalities

- A normal CGH result has to be interpreted within the boundaries of the test's limitations
- A normal CGH result does NOT rule out balanced cryptic rearrangements
- A normal CGH result does NOT rule out sub-microscopic imbalances such as microdeletions

CGH IN CLINICAL CYTOGENETICS

- Precise identification of extra or missing material
  - Important for diagnostic and prognostic value
  - Important for identifying those genes causative of the clinical phenotype
- Single step global genome scan prevents FISHing expedition
- DNA based analysis
  - Quality of metaphase spreads is not a consideration
  - Non-viable tissues are amenable to analysis

Benefits of CGH Analysis in Clinical Genetics

The ability of CGH to define more precisely the chromosomal material comprising marker chromosomes and unbalanced translocations may help to further define critical chromosomal regions which are associated with normal and adverse phenotypic outcomes and thus provide prognostic information for genetic counseling.

Benefits of CGH Analysis in Clinical Genetics

It is therefore prudent for investigators who are utilizing the newer molecular cytogenetic techniques, such as reverse FISH and CGH, to report their findings in conjunction with the clinical presentation so that a comprehensive database can be constructed.

Benefits of CGH Analysis in Clinical Genetics

Information derived from such a database would directly benefit prenatally ascertained cases of chromosomal imbalance, providing couples with a means to make rational and informed decisions concerning the pregnancy. In pediatric cases, such information may provide the parents with a realistic prognosis and be important for the clinical management of the infant.

Peripheral Blood Cultures

- Complete Media - Microcultures
  - Whole blood NOT buffy coat
  - Only need 2 - 3 cc of blood
- Collection tube must contain Sodium Heparin (green top tube). **BE SURE TO INVERT TUBE TO PREVENT CLOTTING.**
- Short term culture takes 72hrs to complete. **SPECIALIZED ADDITIONAL STUDIES MUST BE NOTED AT THE ONSET TO ENSURE THAT THE CULTURE IS SET UP APPROPRIATELY.**

FISH Studies

- All FISH requests require routine chromosome analysis
  - Turn around time = 72hrs (routine culture) + hybridization/FISH analysis time
  - Final report issued when **all studies** completed
- Probe selection must be focused !!
  - Dependent on clinical indication
  - Use of multiple probes = $$$$$$$ & Time

FISH Studies (Cont)

- Probe availablity should be cleared with the laboratory before specimen is collected
  - Limited stock kept in lab (*Shelf life*)
  - Vendor QA
    - Availability may cause time delay
  - Research probes have limited availability
    - Publication does not = immediate clinical application