Chromosome Analysis

- The best whole genome analysis technique currently available is ……..

GTG-banding of Metaphase chromosomes

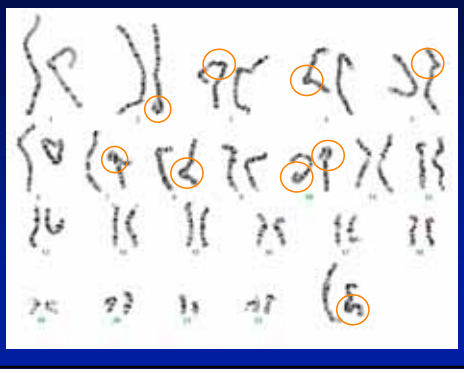
Chromosome Analysis

- G-banding provides a visual examination of the entire genome
- It therefore provides the best coverage but not the best resolution
- Banding resolution differs from preparation to preparation

Diagnostic Limits of Conventional Cytogenetic Analysis

- "Obvious" Aneuploidies & Rearrangements should be easily diagnosed
- The smaller the region of gain/loss, the harder it is to detect
- At Absolute best, imbalances in the realm of 2-5Mb may be detected
  - Most banding resolutions will allow detection of gains/deletions of >5Mb
- Absolute best is dependent on banding resolution

How Does Banding Resolution Impact Diagnostic Ability ?



How Does Banding Resolution Impact Diagnostic Ability ?

- Small & Subtle aberrations may be missed
  - Depends on Banding Resolution & Specimen Preparation
- Most clinical labs strive for 550 band level resolution in postnatal studies and greater than 400 bands in prenatal studies. The resolution in bone marrows and products of conception are often at or below the 400 band level
- Make do with what you have and accept the limitations

Molecular Cytogenetics

Expanding the resolution of conventional cytogenetic analysis

- Application of the techniques of Molecular Biology to cytogenetic preparations

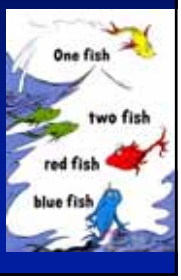
Clinical Applications of Molecular Cytogenetics

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

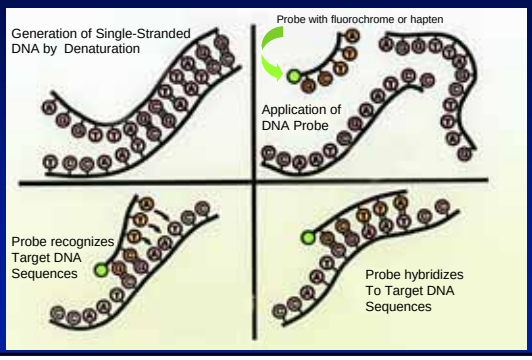
FLUORESCENCE *IN SITU HYBRIDIZATION*

FISH

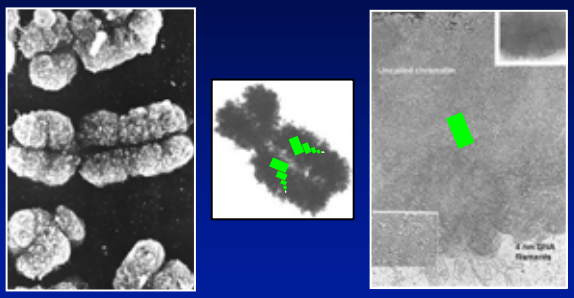
- FISH is a physical DNA mapping technique in which a DNA probe labeled with a marker molecule is hybridized to chromosomes on a slide, and visualized using a fluorescence microscope
- The marker molecule is either fluorescent itself, or is detetcted with a fluorescently labeled antibody.



HYBRIDIZATION STEPS

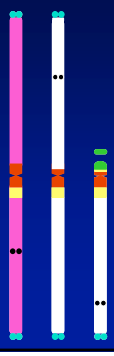


Chromatin Compaction



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**

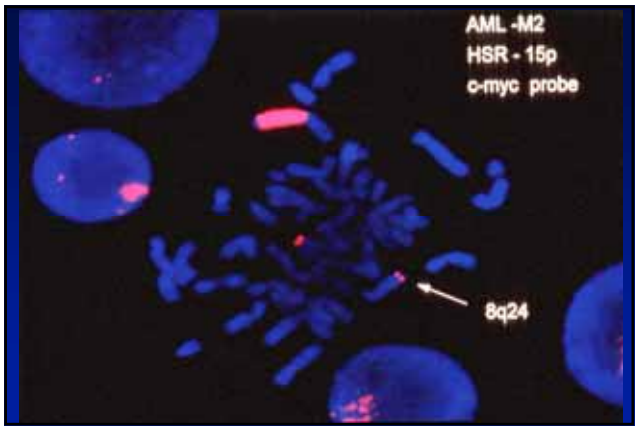
FISH APPLICATIONS

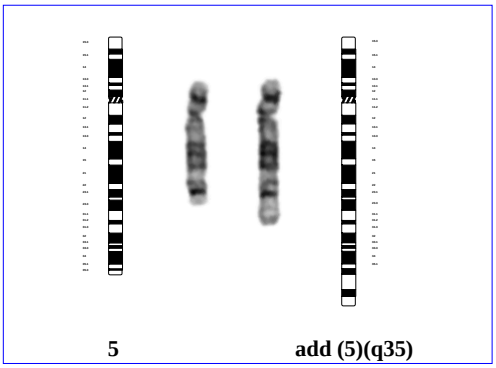
- **Gene Mapping**
- **Chromosome Identification**
- **Aneuploidy Detection**
- **Sexing for X-Linked diseases**
- **Marker chromosome Identification**
- **Total chromosome Analysis**
- **Translocation Analysis**
- **Unique Sequence DNA Detection**
- **Microdeletion Syndrome Analysis**
- **Gene Amplification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

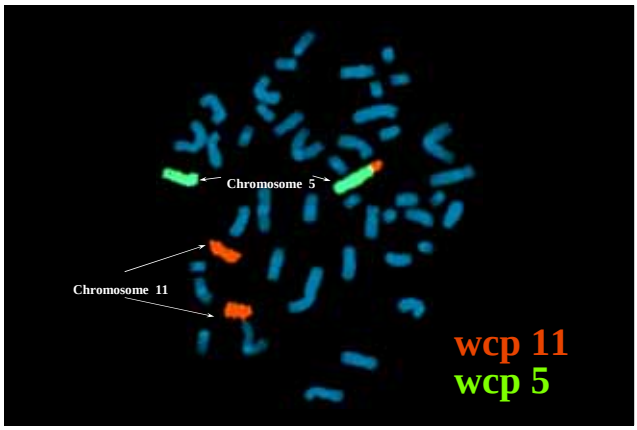
FISH TECHNIQUES

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

GENE MAPPING





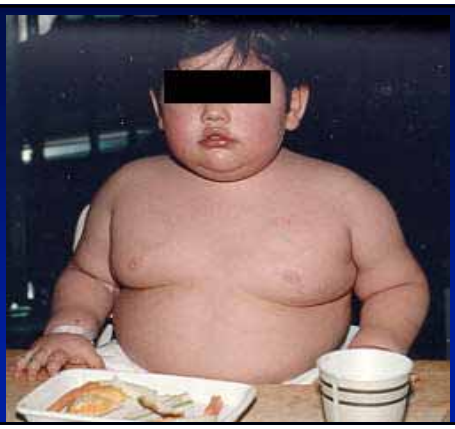


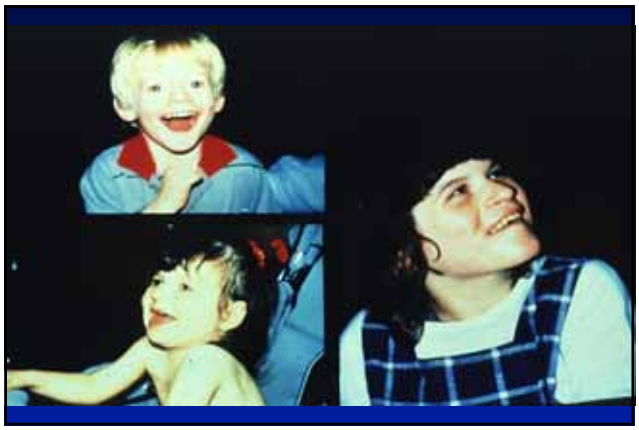
Microdeletion Syndromes

- 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

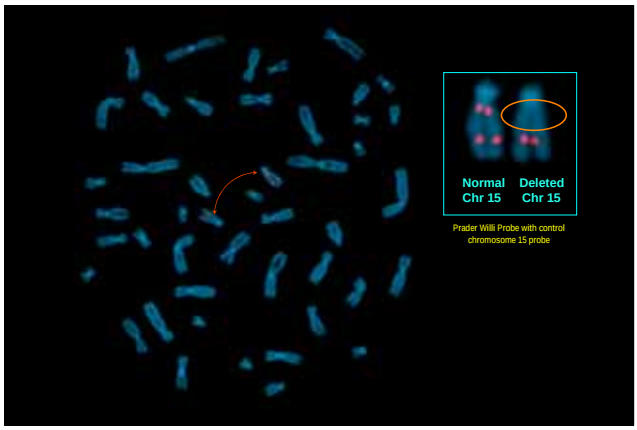


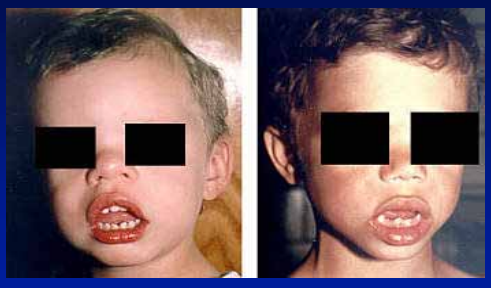


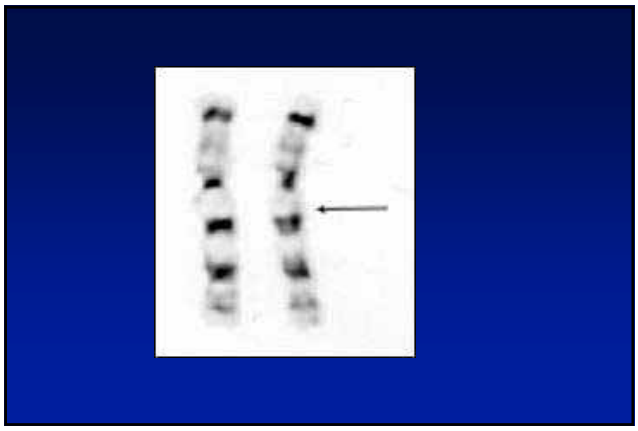
Prader - Willi Syndrome

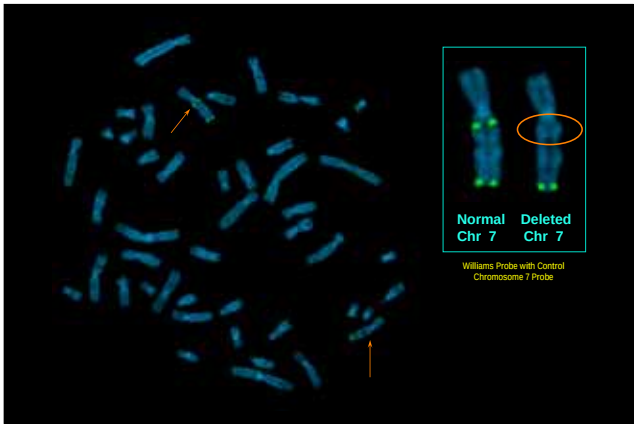
Angelman Syndrome

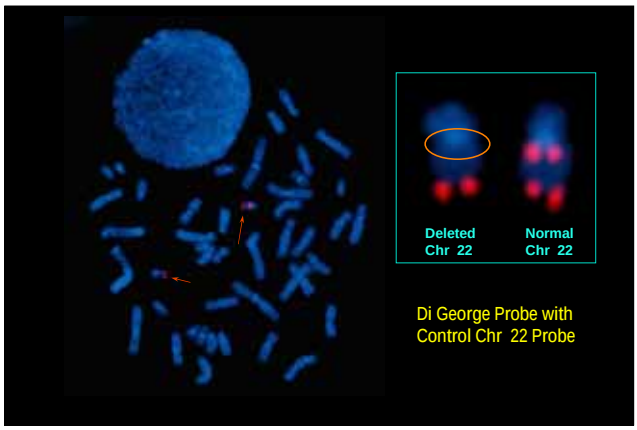
From *Medical Genetics* (Jorde, Carey, Bamshad, White; 2nd Ed.)

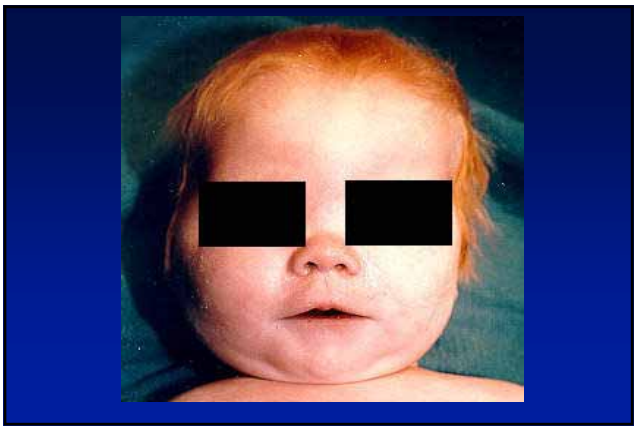












FISH on Interphase Nuclei is Useful in Specific Clinical Situations

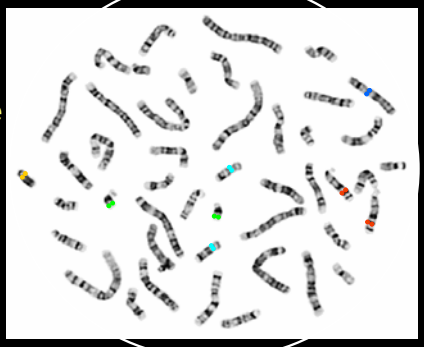
- Prenatal diagnosis aneuploidy screening by FISH looks at interphase nuclei derived from chorionic villi or amniocytes
- Preimplantation Genetic Diagnosis aneuploidy screening by FISH looks at interphase blastomere nuclei

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

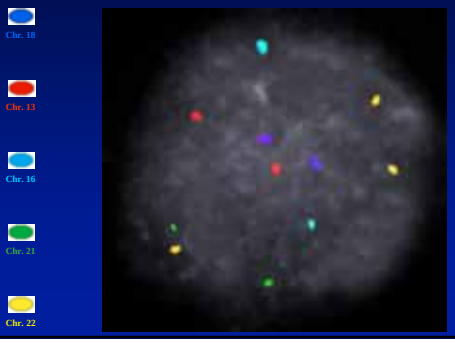
Embryo Biopsy – Acid Tyrodes

Courtesy of Kathleen Miller, RMA of NJ

Embryo Biopsy

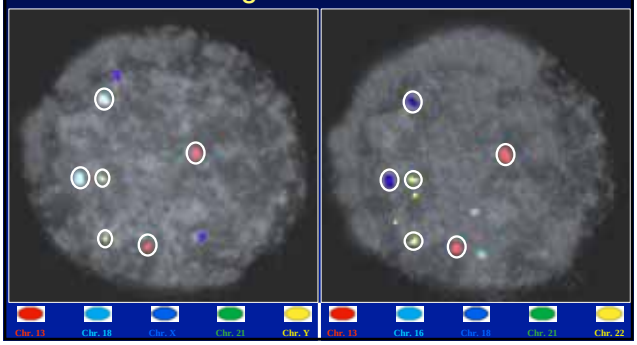
Courtesy of Kathleen Miller, RMA of NJ

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



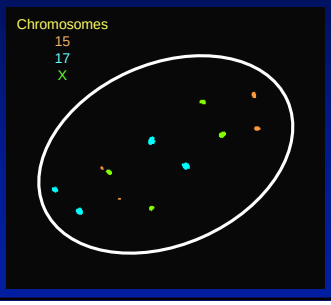
Trisomy 22

Multiple Hybridizations Add Diagnostic Power



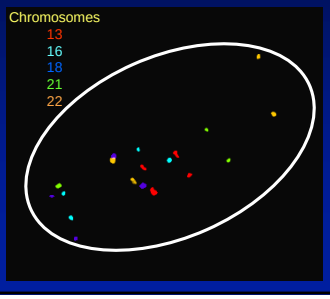
Mosaicism

Post-Zygotic Non–Disjucntion

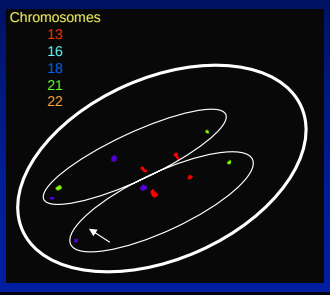


Mosaicism

Post-Zygotic Non–Disjucntion



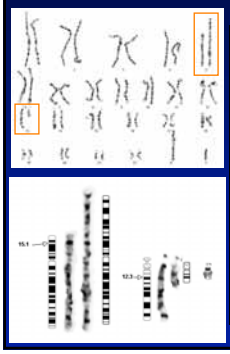
Mosaicism
Post-Zygotic Non–Disjucntion

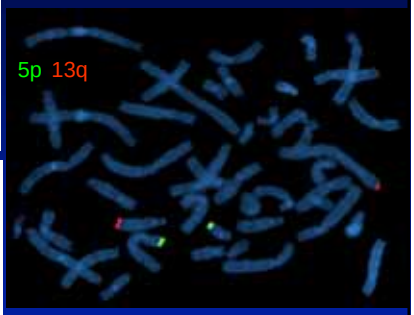


Telomeres

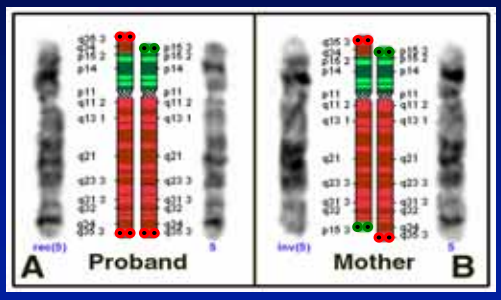
- Highest concentration of genes of any chromosomal region – therefore sub-microscopic deletions and duplications would have a significant impact
- Increased genetic recombination at telomeres
  - Male rate higher than female for most chromosomes
  - Telomeres play a critical role in chromosome pairing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

FISH using Subtelomere Probes





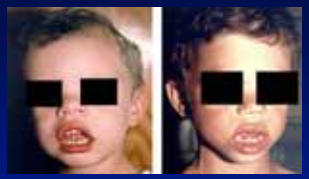
Pericentric Inversion

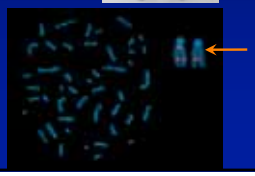


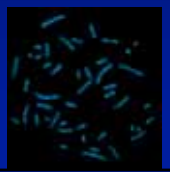
Pericentric Inversion FISH

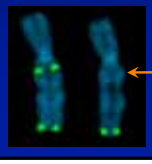
Mother 5p 5q Proband

The Clinical Phenotype Guides the Choice of FISH Test









The Clinical Phenotype Guides the Choice of FISH Test

What FISH test do we do in this case ???

How do we determine the origin of this marker chromosome?

Options:

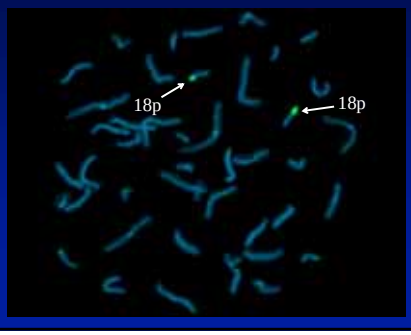
- Sequential trial & error with centromeric probes
- Sequential trial & error with whole chromosome paints

Detection of Partial Aneuploidies – An expensive FISHing Expedition

- Unbalanced rearrangements
- Marker chromosomes
- Cryptic translocations
- Cryptic deletions
- Suspected Microdeletions with non-specific clinical abnormalities

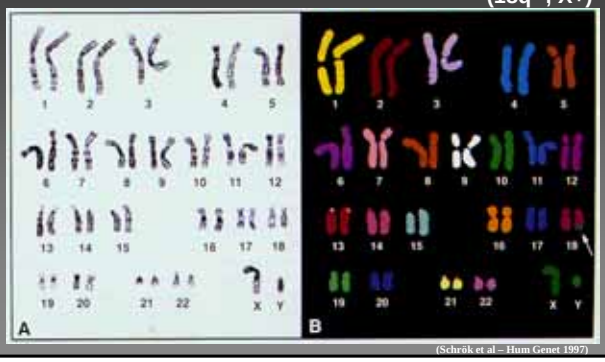
The Need for New Technologies

REVERSE FISH FOLLOWING MARKER CHROMOSOME MICRODISSECTION



Spectral KarYotyping

(18q- ; X+)



(Schrök et al – Hum Genet 1997)

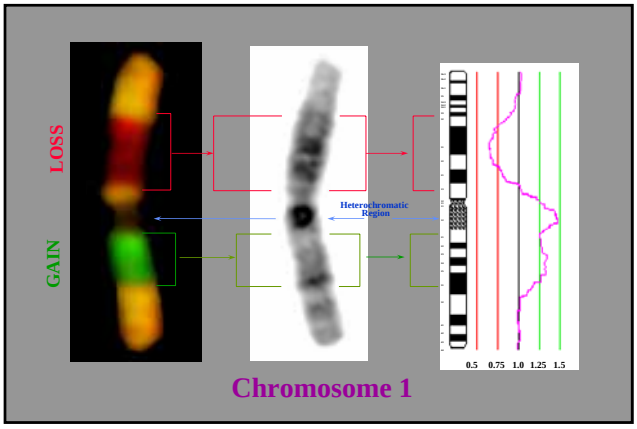
COMPARATIVE GENOMIC HYBRIDIZATION

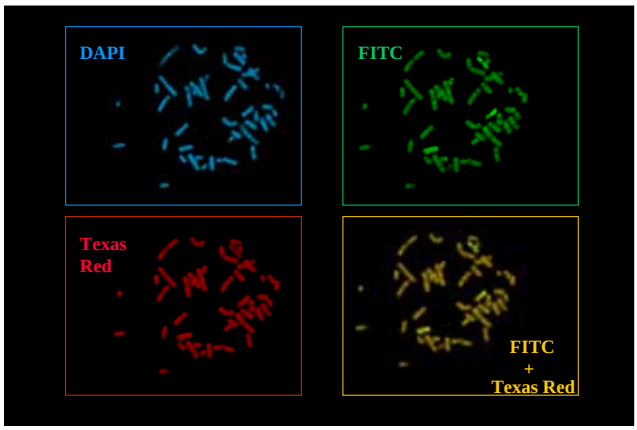
C G H

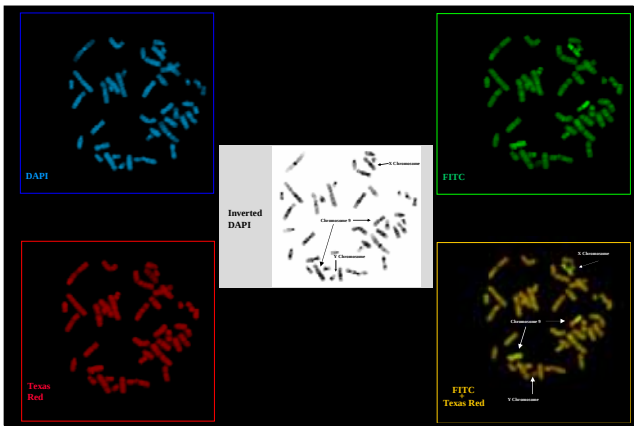
- Identifies chromosomal gains and losses in a **single** hybridization procedure
- Effectively reveals any DNA sequence copy number changes (i.e., gains, amplifications, losses and deletions) in a particular specimen and maps these changes on normal chromosomes

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.







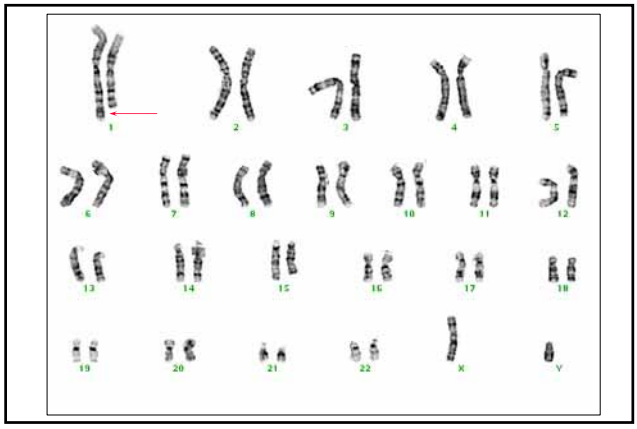
OVERVIEW OF CGH

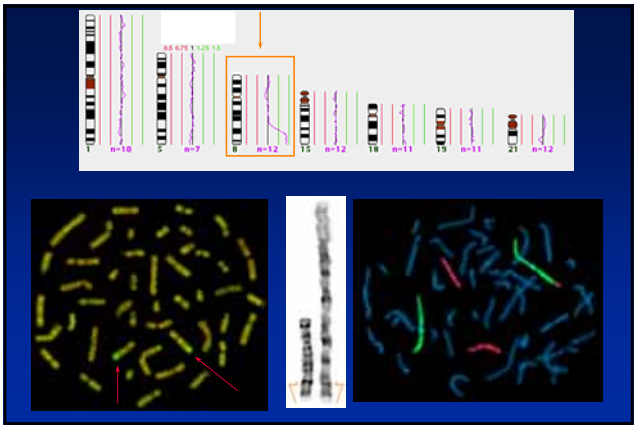
The major steps in CGH involve:

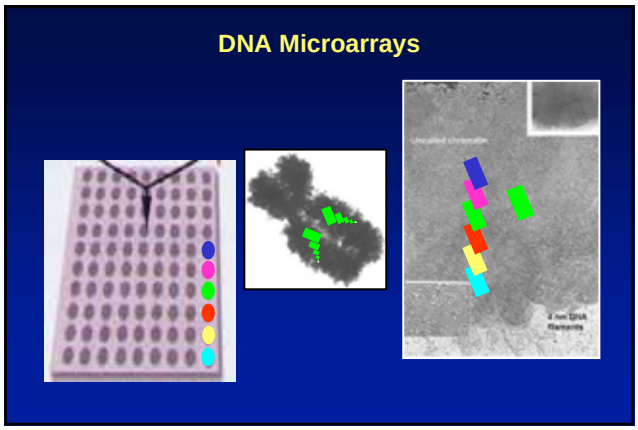
- **Preparation of normal metaphase spreads**
- **Isolation of high molecular weight DNA from specimen (test) and reference (normal) samples.**
- **Labelling of specimen & reference DNA with different color fluorochromes**

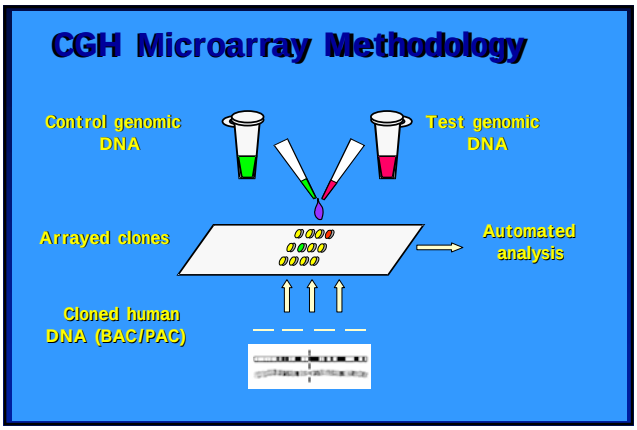
OVERVIEW OF CGH (cont)

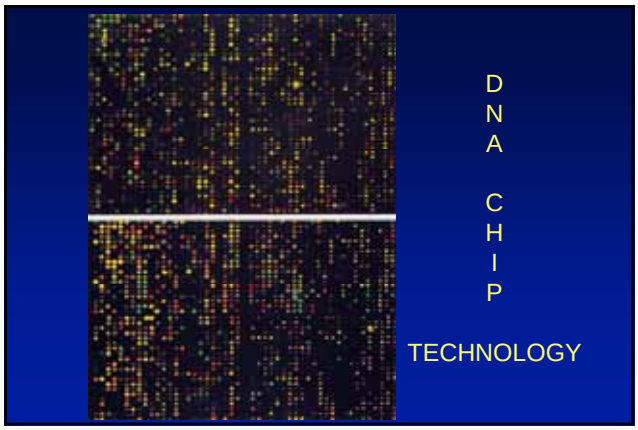
- ***In situ* hybridization of the labelled specimen & reference DNAs to normal metaphase spreads**
- **Washing off unbound DNA**
- **Counterstaining metaphase spreads with DAPI**
- **Fluorescent microscopy to visualize & capture color ratio differences along the chromosomes**






DNA Microarrays


CGH Microarray Methodology
Control genomic DNA
Test genomic DNA
Arrayed clones
Automated analysis
Cloned human DNA (BAC/PAC)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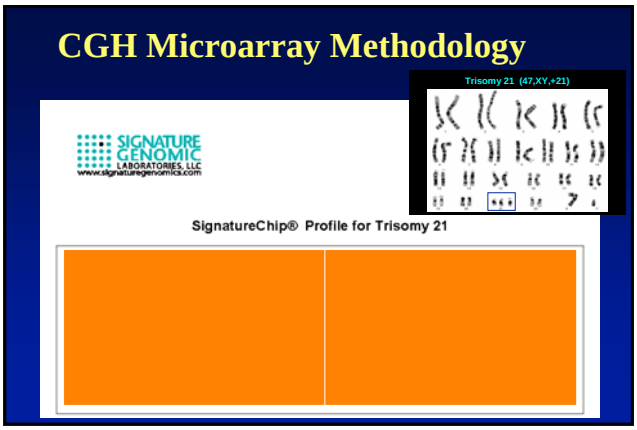
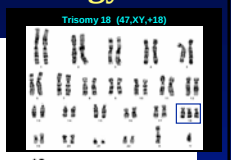
TECHNOLOGY

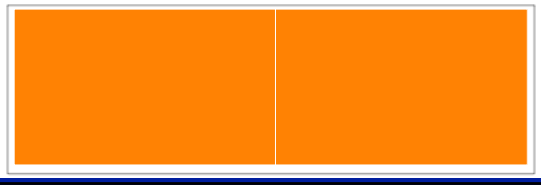

CGH Microarray Methodology
Trisomy 21 (47,XY,+21)
SIGNATURE GENOMIC LABORATORIES, LLC
www.signaturegenomics.com
SignatureChip® Profile for Trisomy 21

CGH Microarray Methodology



SIGNATURE GENOMIC LABORATORIES, LLC
www.signaturegenomics.com

SignatureChip® Profile for Trisomy 18



CGH Microarray Methodology

SIGNATURE GENOMIC LABORATORIES, LLC
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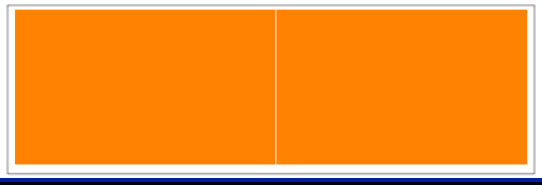
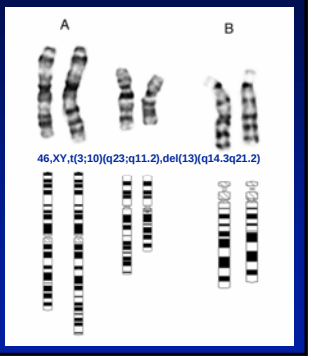
SignatureChip® Profile for DiGeorge Syndrome – del(22)(q11.2q11.2)



Patient Clinical Info

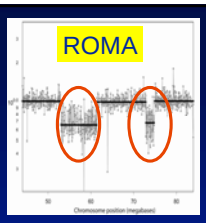
Delayed major motor milestones, including rolling over at 4 months, sitting without support at 8 months, and walking at 22 months. He had been receiving regular physical therapy for delay in gross motor skills. He spoke in short sentences and understood complex commands. There were no facial dysmorphisms besides slightly cupped ears and no medical problems except for severe eczema.

Normal development status at 3 yrs, except for mild delay in gross motor skills and coordination.

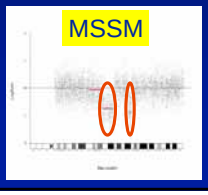


Data Analysis

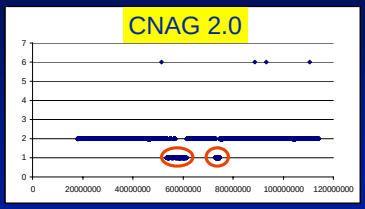
ROMA



MSSM



CNAG 2.0



MICROARRAYS 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/Microaray Analysis in Clinical Genetics

The ability 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 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.