Cytogenetics in the Diagnostic Laboratory

Arslangiin Bayarmaa

Human Genetics, Ninewells Hospital, Dundee

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Cytogenetics in the Diagnostic Laboratory

- Scottish pregnancy screening
- Conventional techniques
- Clinical Applications
- Array CGH (Comparative Genomic Hybridisation)

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Scottish pregnancy screening

- Screening involving blood test
- Screening involving serum test
- Screening involving ultrasound test

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Scottish pregnancy screening

- Screening involving blood test

Routine blood test: as early as possible (8-10 weeks)

Haemoglobin, group, rhesus and antibodies, Syphillis, Hepatitis B, HIV and Rubella .

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Scottish pregnancy screening

- Screening involving serum test: 1st trimester combined test for Down's syndrome (11-13 weeks)

  - Biochemistry screeninig - PAPP-A (Pregnancy Associated Plasma Protein A), hCG (human Chorionic Gonadotropin)
  - Ultrasound screening - Nuchal Translucency (NT)

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Scottish pregnancy screening

- Screening involving ultrasound test:
  - Dating scan – 8-14 weeks
  - Fetal anomaly scan – 18-21 weeks

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Cytogenetics in the Diagnostic Laboratory

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- **Conventional techniques**
- Clinical Applications
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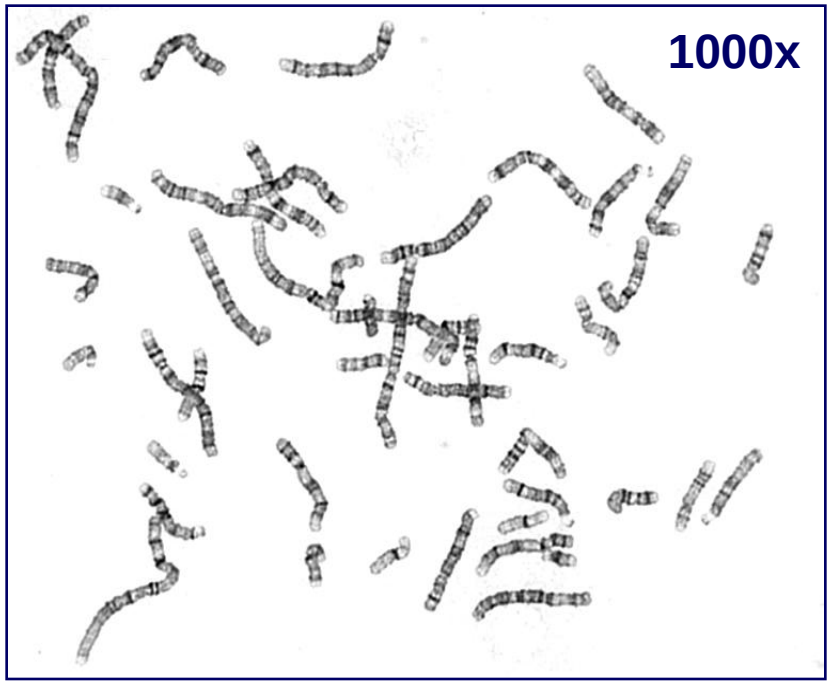
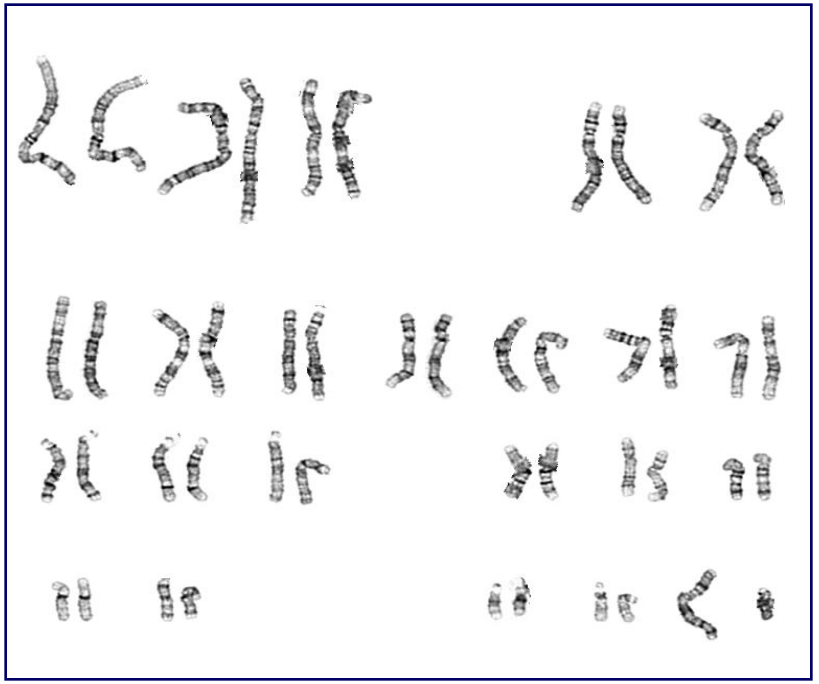
G-banded karyotype

Conventional cytogenetic analysis involves examination of a G-banded **karyotype** to detect changes in the 46 human chromosomes by light microscopy



Metaphase



Karyotype

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Cytogenetics in the Diagnostic Laboratory

- Scottish pregnancy screening
- Conventional techniques
- **Clinical Applications**
- CGH

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Why do chromosome studies?

- **Diagnosis**
- **Phenotype prediction**
- **Prognosis**
- **Recurrence risk**
- **Reproductive future**
- **Genetic counseling**
- **Prenatal diagnosis**

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Most common cytogenetic referrals

- **Constitutional postnatal**
  - Blood
  - Skin / other solid tissue
- **Constitutional prenatal**
  - Amniotic fluid
  - Chorionic villus sampling
- **Molecular Cytogenetics**
  - All samples above
  - DNA

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Postnatal: blood referrals

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Postnatal: blood referrals

- Congenital abnormalities (eg: heart defect) in neonates
- Failure to thrive/developmental delay
- Idiopathic mental retardation
- Sexual ambiguity & delayed puberty
- Recurrent miscarriage/infertility couples

- **Lithium-heparin sample**

- **Culture time ~ 3 days**
- **Results up to 28 days**
- **Urgent results within 10 days**

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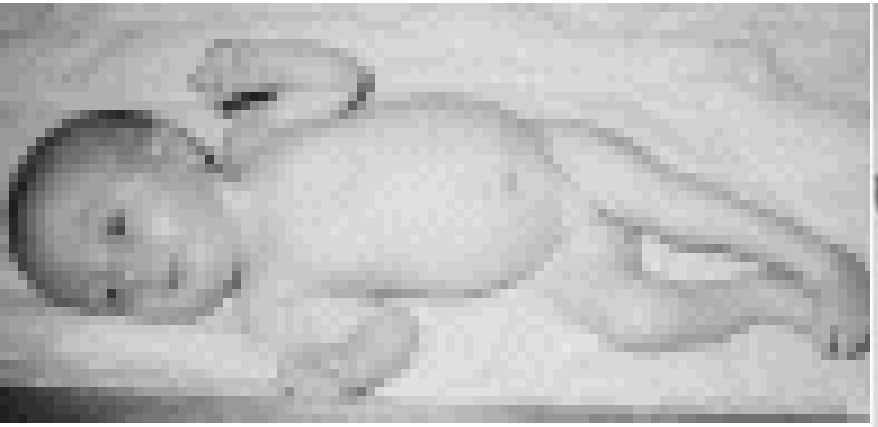
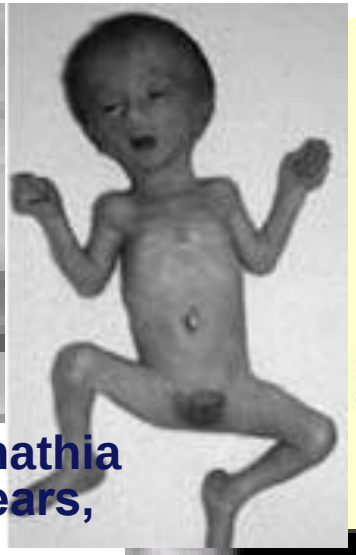
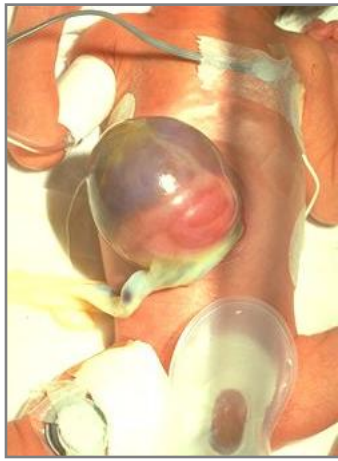
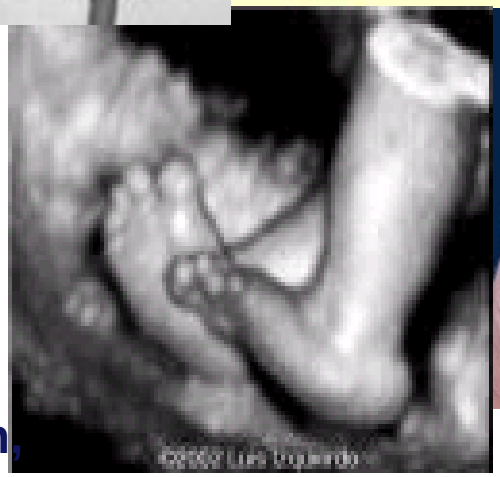
Neonate with congenital abnormalities









Rounded prominent occiput, micrognathia small posteriorly rotated "faun like" ears, small mouth, high arched palate,
Short sternum, small thorax narrow pelvis
Severe IUGR
Profound MR ~ no response
CHD ~90%
Omphalocoele 60%
Rocker bottom feet, abnormal hand clenching
V poor prognosis, most die within 1 month, unless mosaic (10%)

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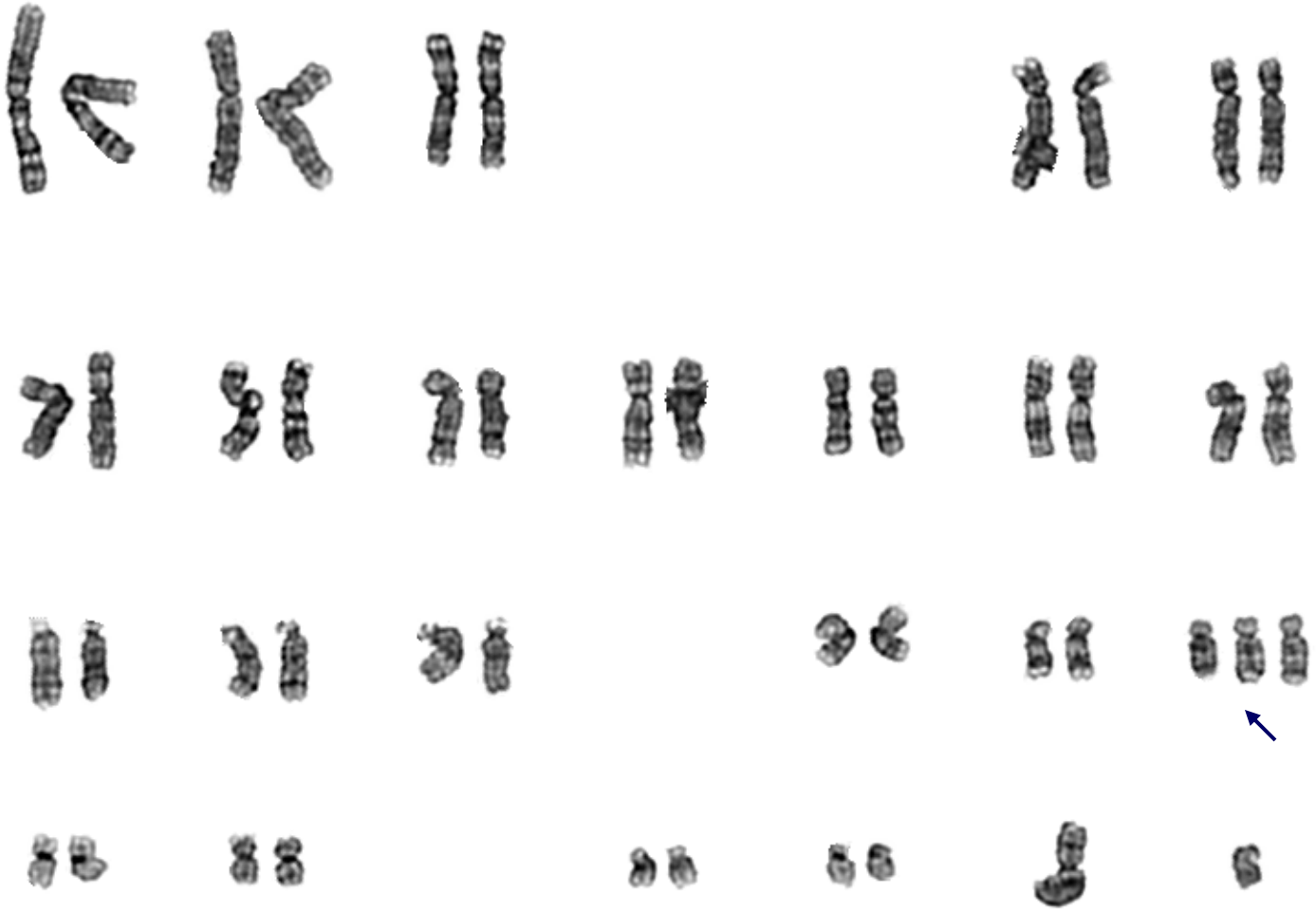
Edward syndrome (+18) ~1:5,000 livebirths

Trisomy 18 in a neonate
47,XY,+18



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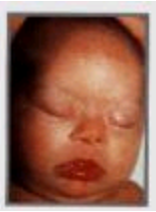
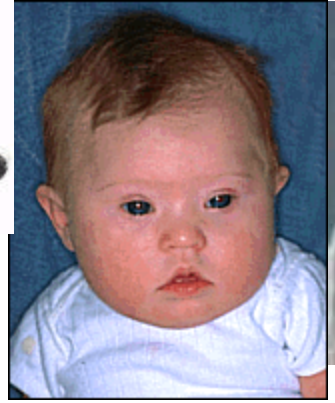
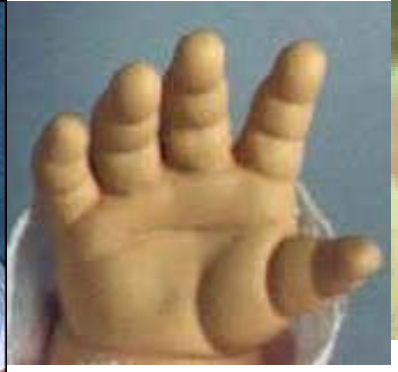
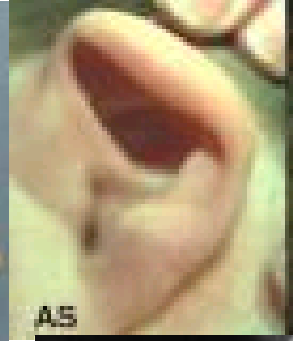
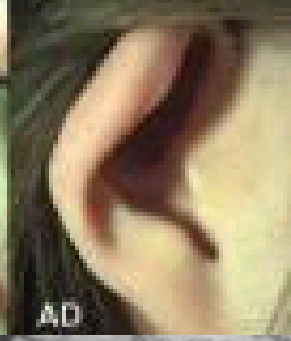
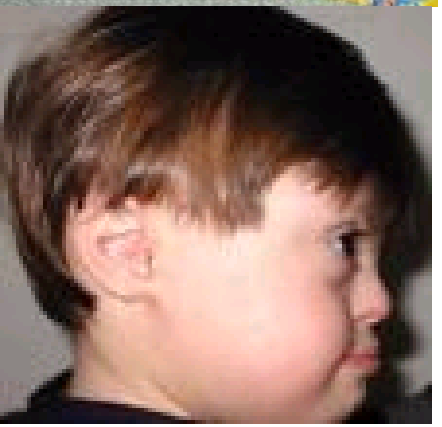


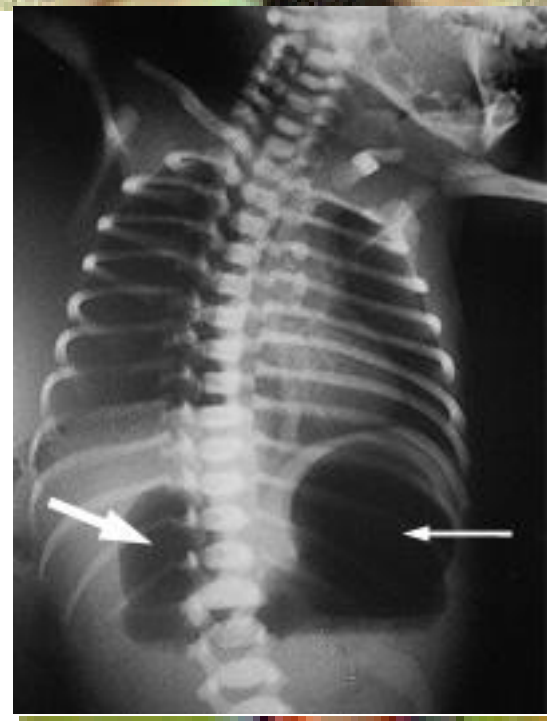


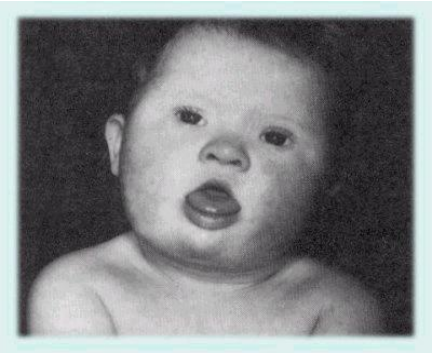






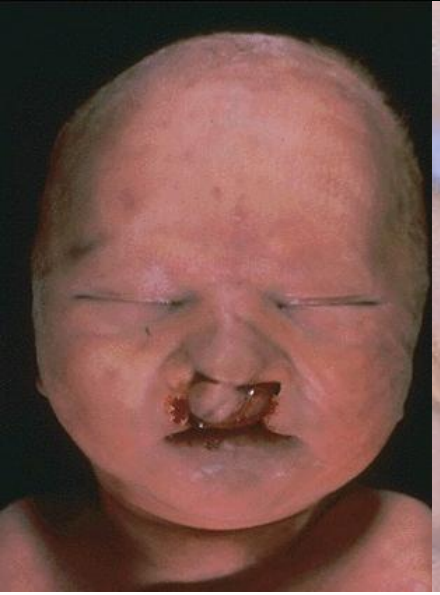
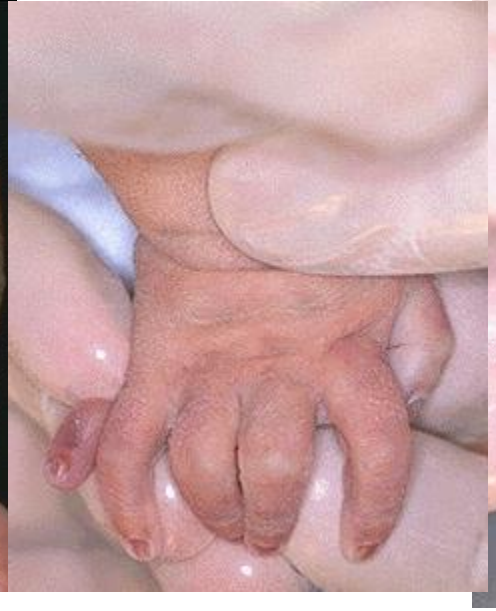
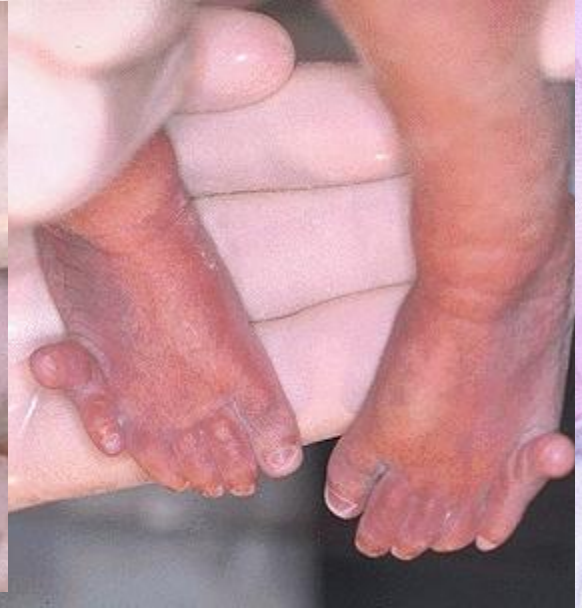






- Mid face hypoplasia
- Short upturned nose
- Upward slanting palpebral fissures
- Epicanthic folds
- Small mouth and relative macroglossia
- Brachydactyly
- Single palmar crease ~50%
- Heart defects ~50%
- Duodenal/oesophageal, anal atresia~3%
- Premature ageing
- Most common cause of MR
- Mean IQ 50

Down Syndrome: 47,XY,+21


Rare and most lost before term

- **IUGR & profound MR**
- **Moderate microcephaly**
- **All degrees of holprosencephaly (failure of forebrain cleavage)**
- **Lip and palate clefting**
- **Post-axial polydacytly of hand and feet**
- **Ears low set & flattened**
- **Scalp vertex anomalies**
- **Heart abnormality**
- **Kidney anomalies**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Patau Syndrome: 47,XY,+13

Mental retardation & developmental delay

- **Usually young children**
- **Often associated with dysmorphism**
- **Unbalanced karyotype causes phenotype**
  - **Unbalanced translocations**
  - **deletions**
  - **duplications**
  - **Additional “markers”**
  - **sometimes only detectable by FISH (Fluorescence in situ hybridisation)**

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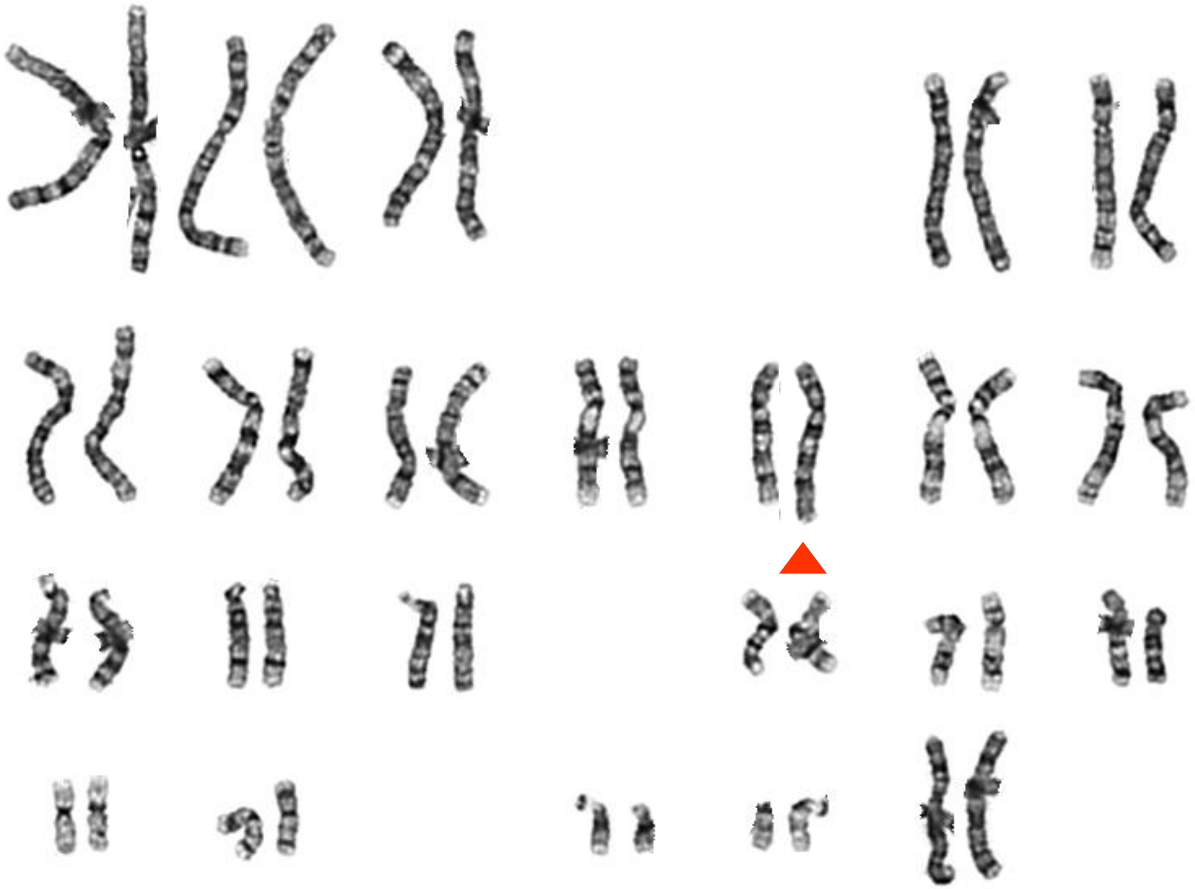
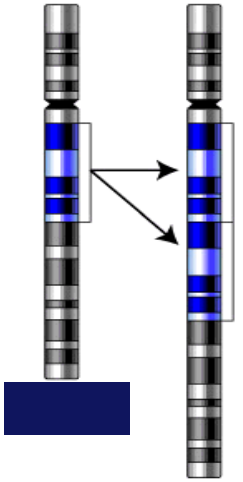
Duplication





- 8 year old girl
- Global developmental delay
- Dysmorphic features
- Facial asymmetry
- Marked language delay

46,XX,dup(10)(q26.1q26.3)

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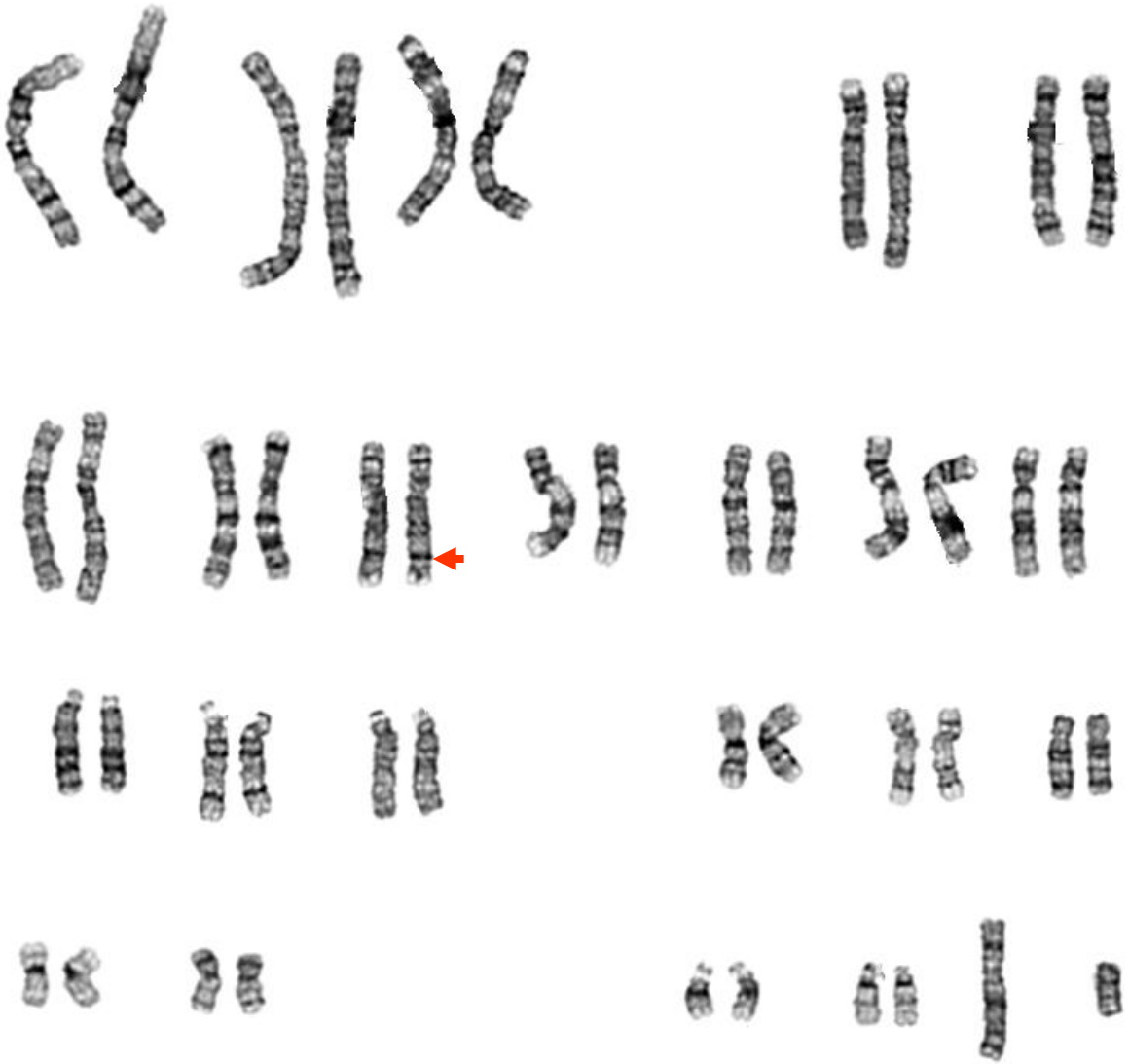
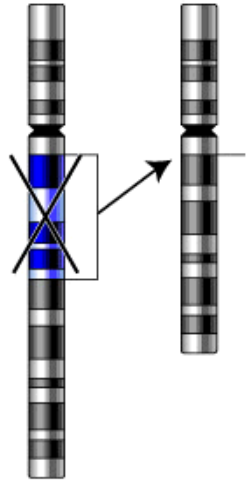
Deletion





- 32 year old man
- Short stature
- Multiple exostoses
- Brachydactyly
- MR
- ?Trichorhino

  -Phalangeal syndrome

Deletion at 8q24

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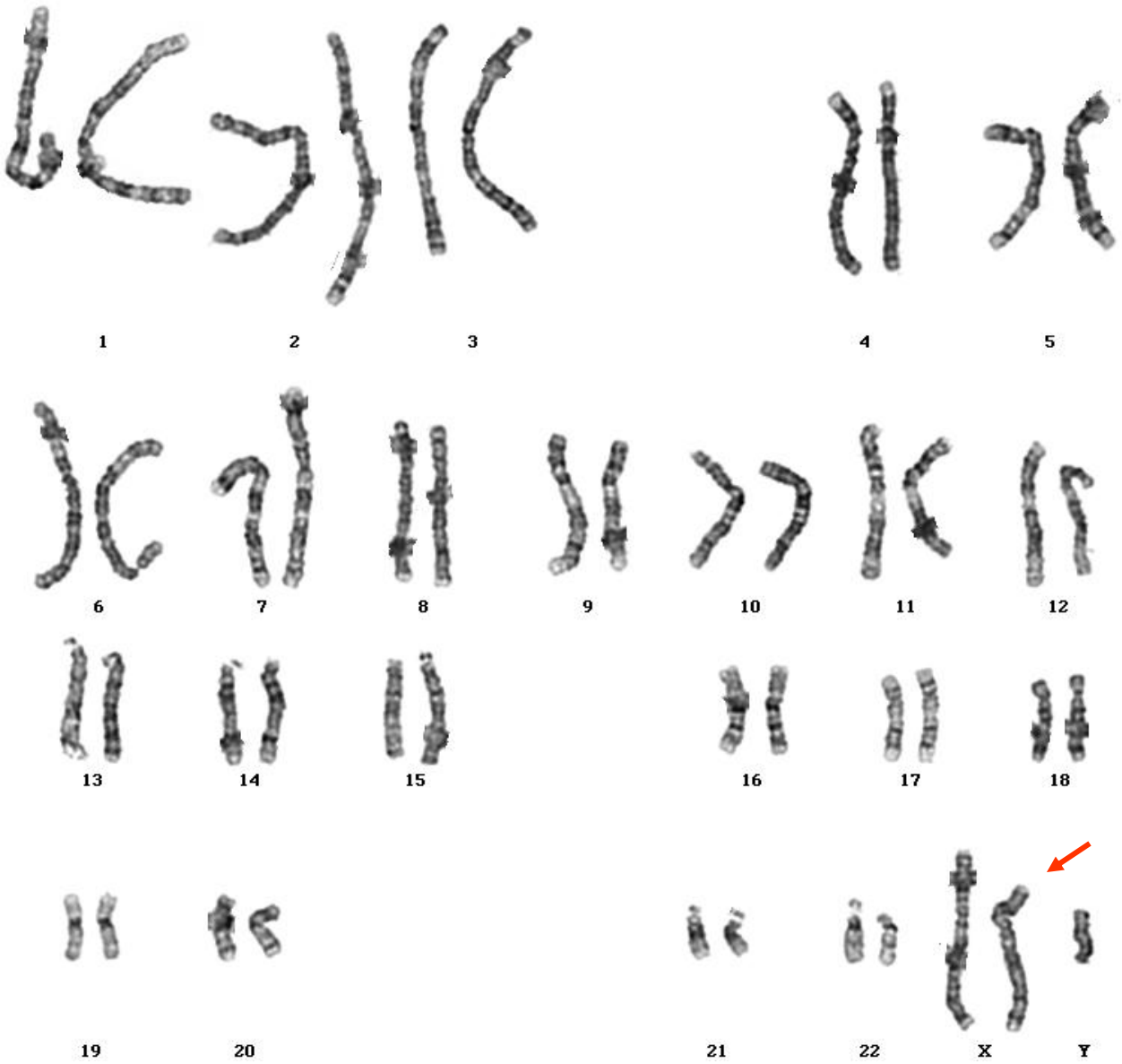
Problems of sexual development



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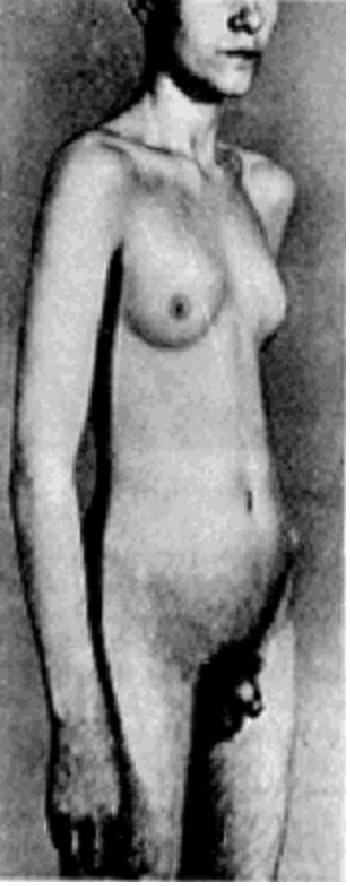
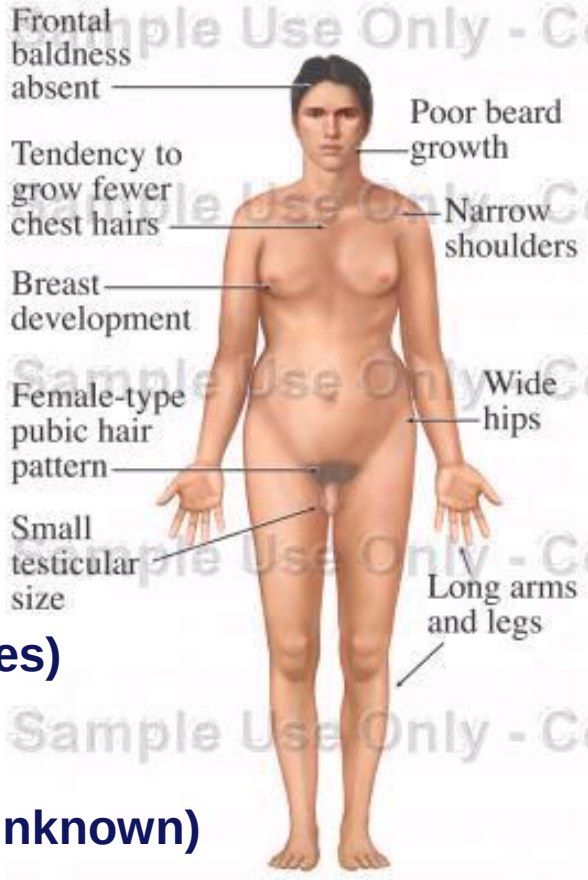


Klinefelter syndrome 47,XXY



- ~1:500-1,000 males
- Prepubertally normal
  12-14 yrs testosterone plateaus
- Testes remain small and firm
- Tall stature (? Due to extra stature genes)
  but eunochoid body habitus
- Fat distribution female
- Gynaecomastia in ~30% (mechanism unknown)
- Most common genetic cause of
  male infertility
- This phenotype is also variable –
  many men never know they have
  KS until routine investigations
  for infertility

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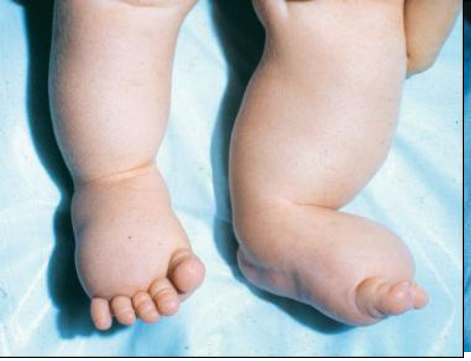
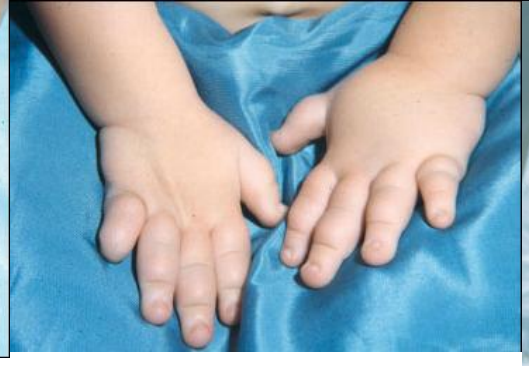
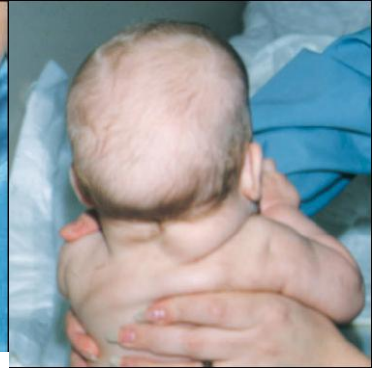
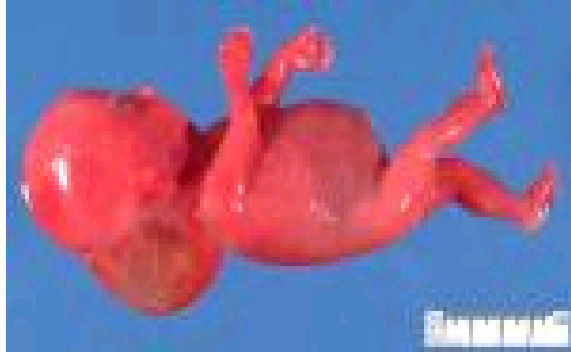
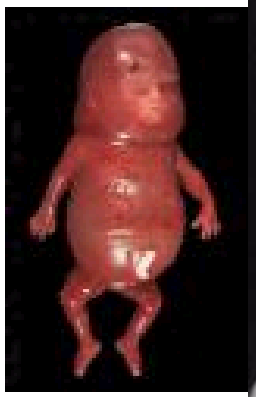
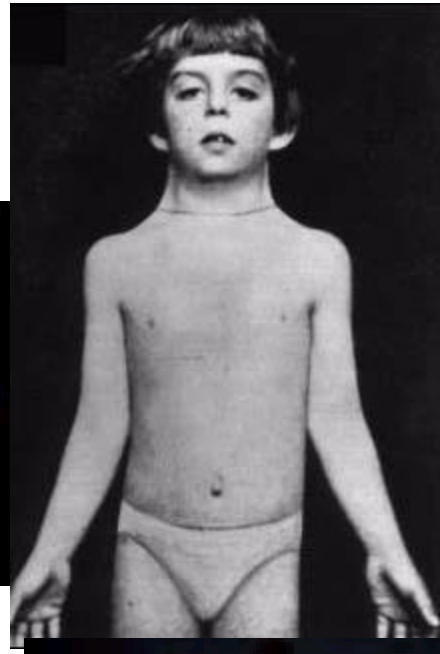





Turner Syndrome: 45,X & variants

- Prenatal: Cystic hygroma, heart defects
- Newborn:
  - redundant neck webbing,
  - peripheral lymphoedema
- Later childhood:
  - short stature,
  - broad chest
  - low hairline to nape
  - neck webbing
  - cubitus valgus
  - 20% co-arctation of aorta or ASD
- Adults: primary or secondary amenorrhoea
  - (gonadal dysgenesis or failure)
  - Many mosaic (may ameliorate phenotype)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Recurrent miscarriages

- 29 year old woman
- Mother had several pregnancy losses
- Carries balanced translocation
- Patient has had losses & abnormal neonatal deaths due to unbalanced meitotic segregants
- Prenatal diagnosis by CVS or AF

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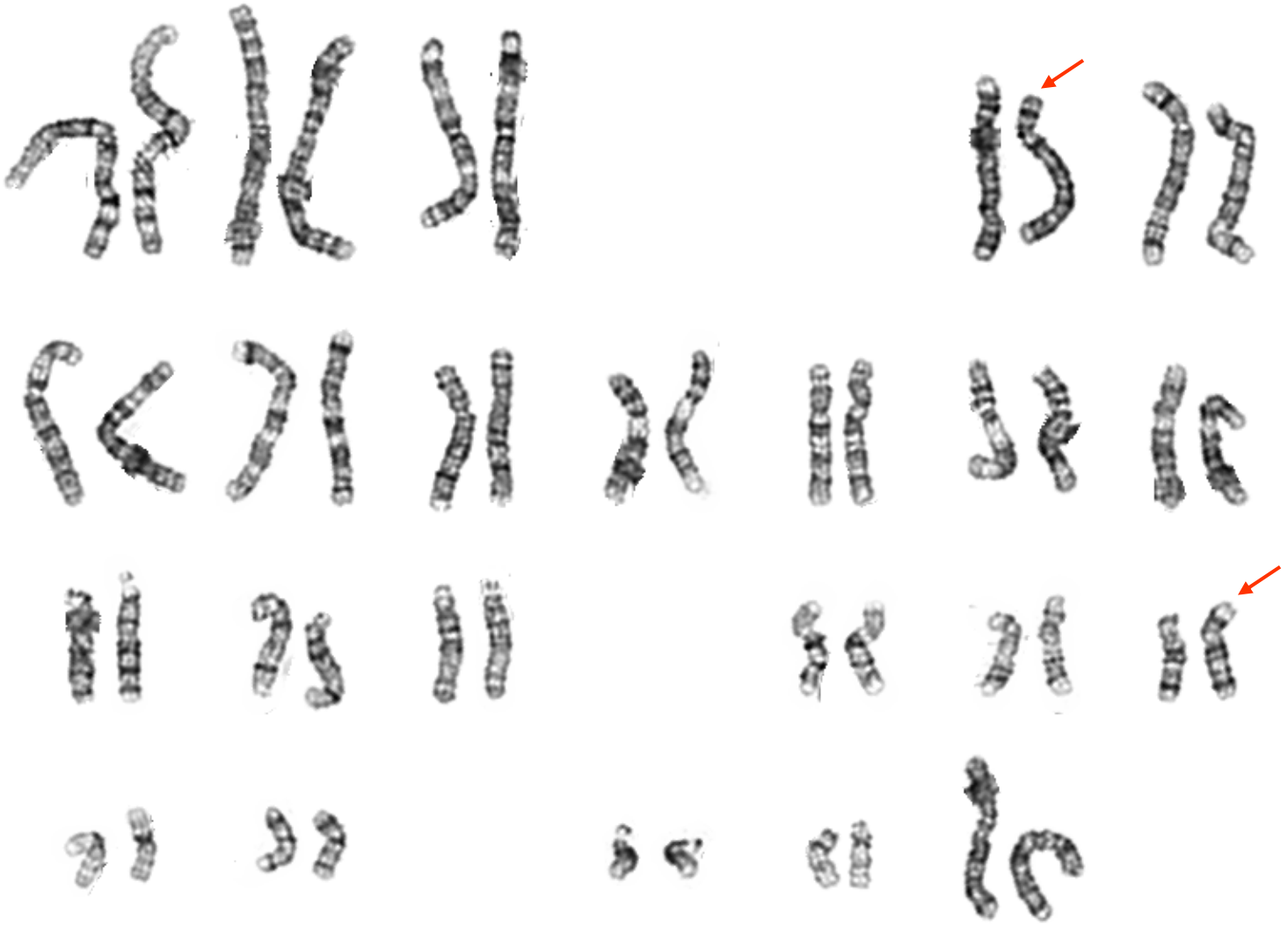
Maternal blood: balanced t(4;18) translocation



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Prenatal: CVS and amniotic fluid samples

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Prenatal: CVS and amniotic fluid samples

Reasons for referral include :

- Abnormal ultrasound scan
- Carrier of a structural rearrangement
- Elevated risk of a chromosome abnormality indicated by biochemical and/or ultrasound maternal screening
- Previous chromosome anomaly
- Maternal age >35
- FH of chromosome abnormality

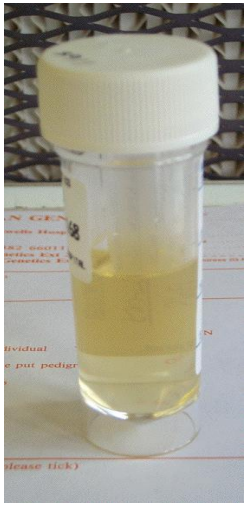
Prenatal diagnosis is normally carried out using one or more of the following sample types:

- Amniotic fluid
- Chorionic villi
- Fetal blood

Prenatal: CVS and amniotic fluid samples

- **Amniotic fluid:**

~15ml at 16/40 gestation



Culture time 2-3 weeks

Results in 2-3 weeks

RTG – 14 days

Rapid aneuploidy Screening by QF-PCR (1-2 days)

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Prenatal: CVS and amniotic fluid samples

- **CVS:**

~10-25mg at 10-13/40 weeks gestation

***Long term* culture time:**
2-3 weeks
Results: 2-3 weeks
RTG – 14 days

***Direct preparations*:**
1-2 days

Rapid aneuploidy Screening by QF-PCR (1-2 days)

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Amniotic fluid with unbalanced translocation

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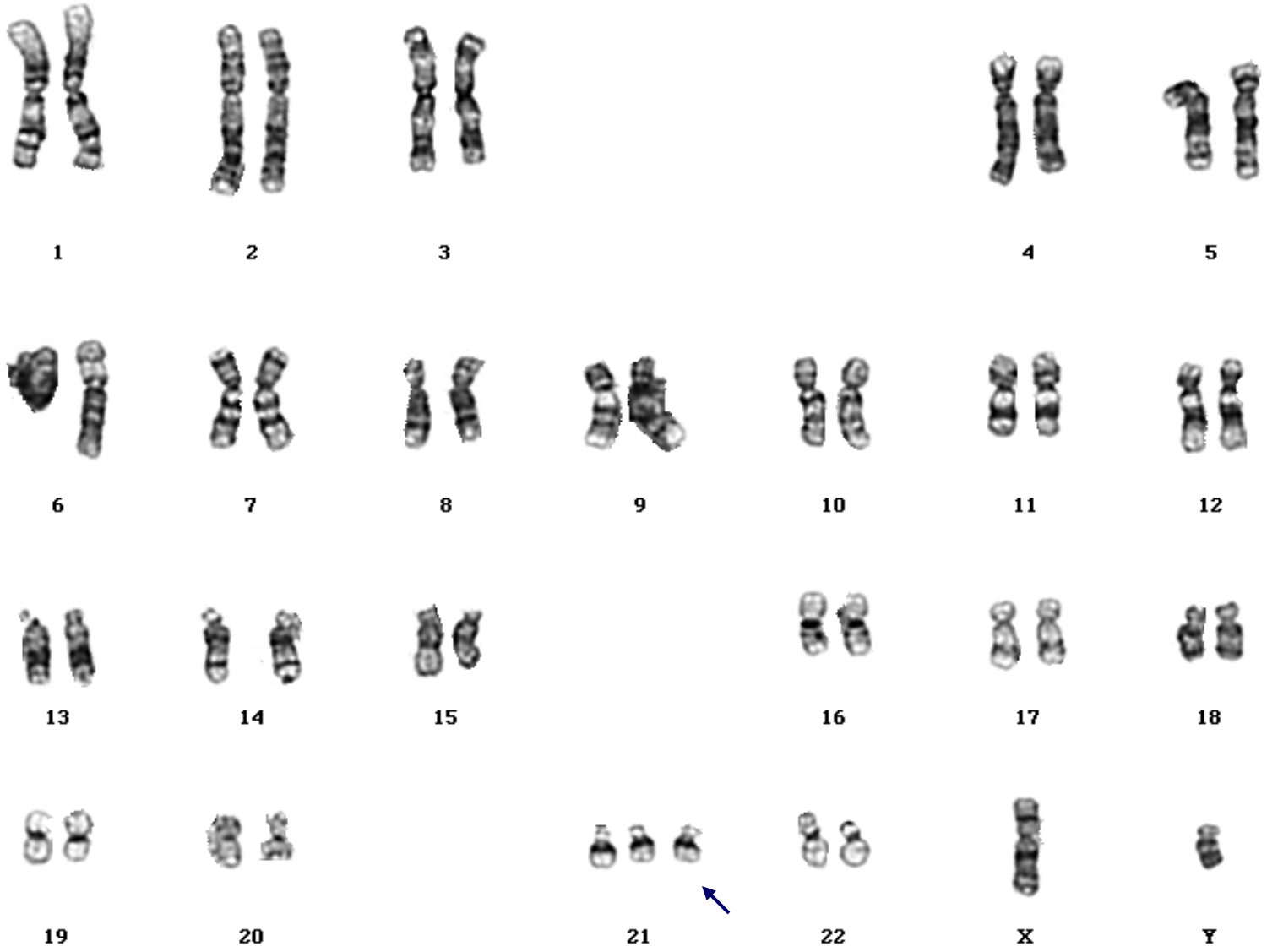
Trisomy 21 in amniotic fluid



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Cytogenetics in the Diagnostic Laboratory

- Department Workload
- Conventional techniques
- Clinical Applications
- **Array CGH**

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Principle of CGH

- Comparative Genomic Hybridisation
- Global karyotype assay - does not require informed probe choice
- **Hybridise to a slide with clones or oligonucleotides dotted on**
- Differentially label test DNA green & control reference DNA red
- Compare fluorescence ratios using software to give a CGH profile
- Analyse profile to demonstrate amplifications or deletions of test DNA relative to control

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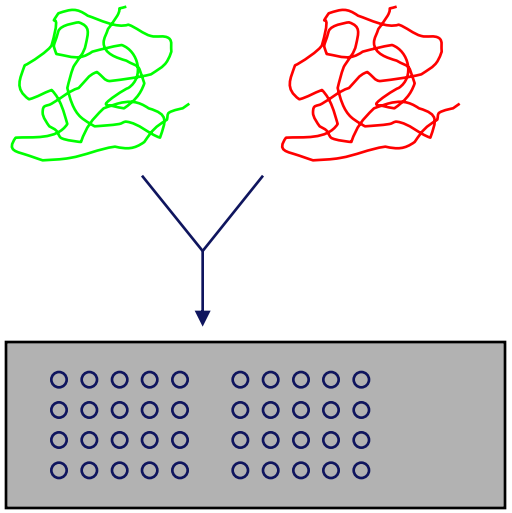
Test **Reference**

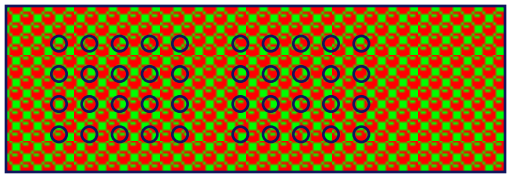
Differential labelling

Label



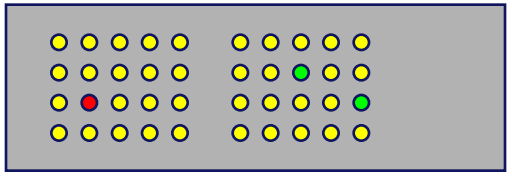
Hybridisation under competitive conditions: specific binding

Hybridise



Washing to remove unbound material

Wash



Scan

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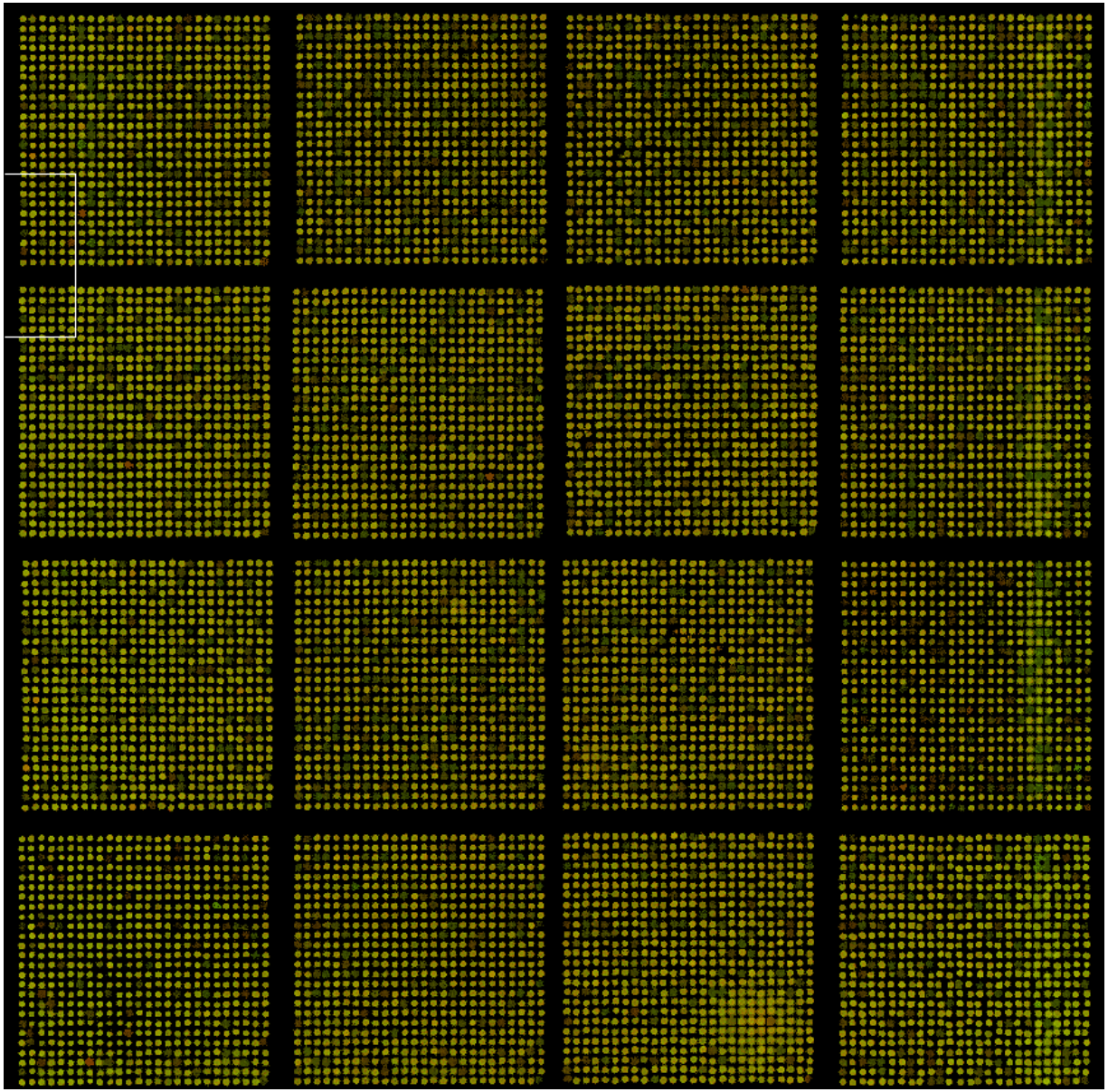
Scan Image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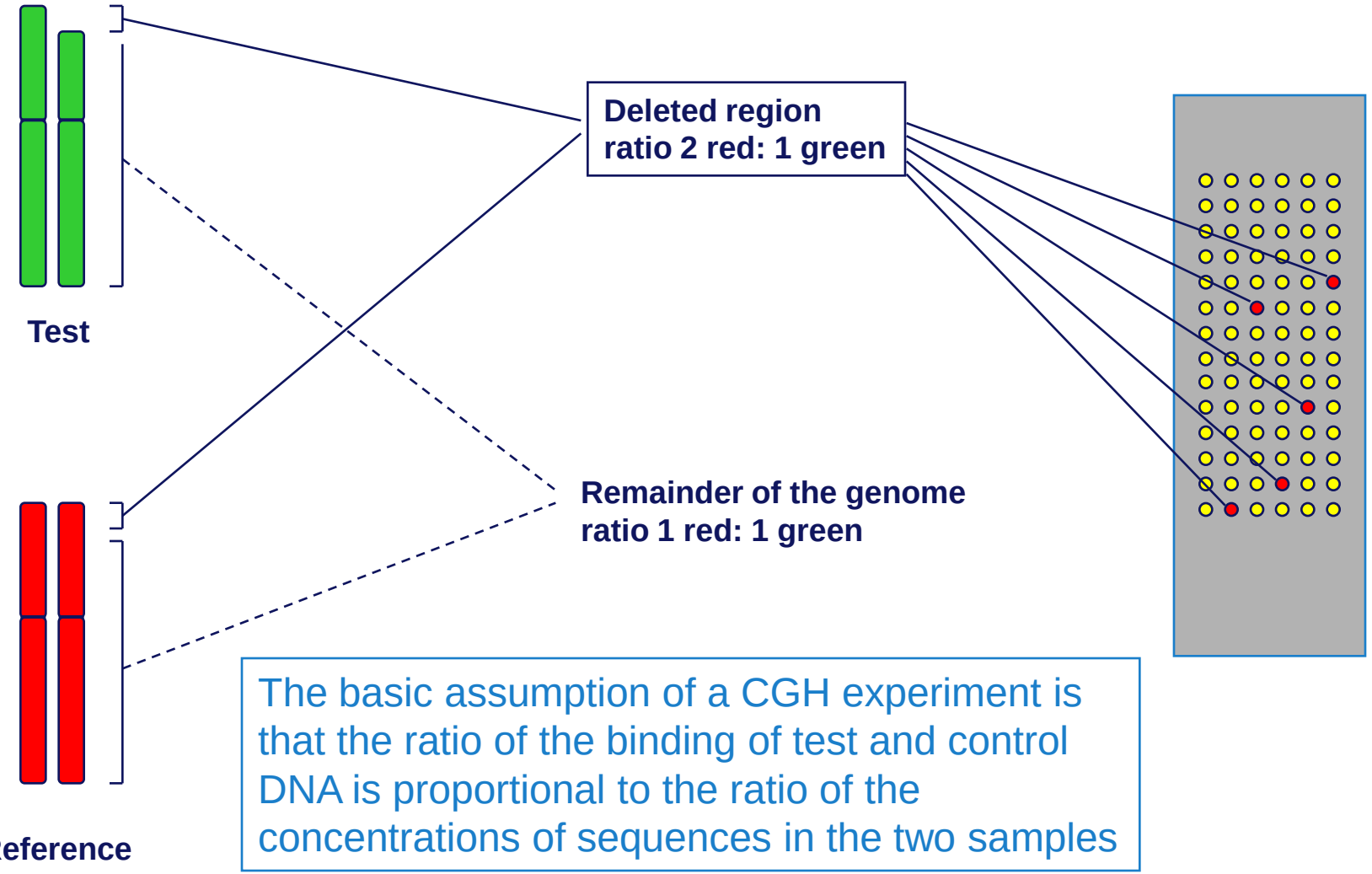
Principle of Array CGH



The basic assumption of a CGH experiment is that the ratio of the binding of test and control DNA is proportional to the ratio of the concentrations of sequences in the two samples

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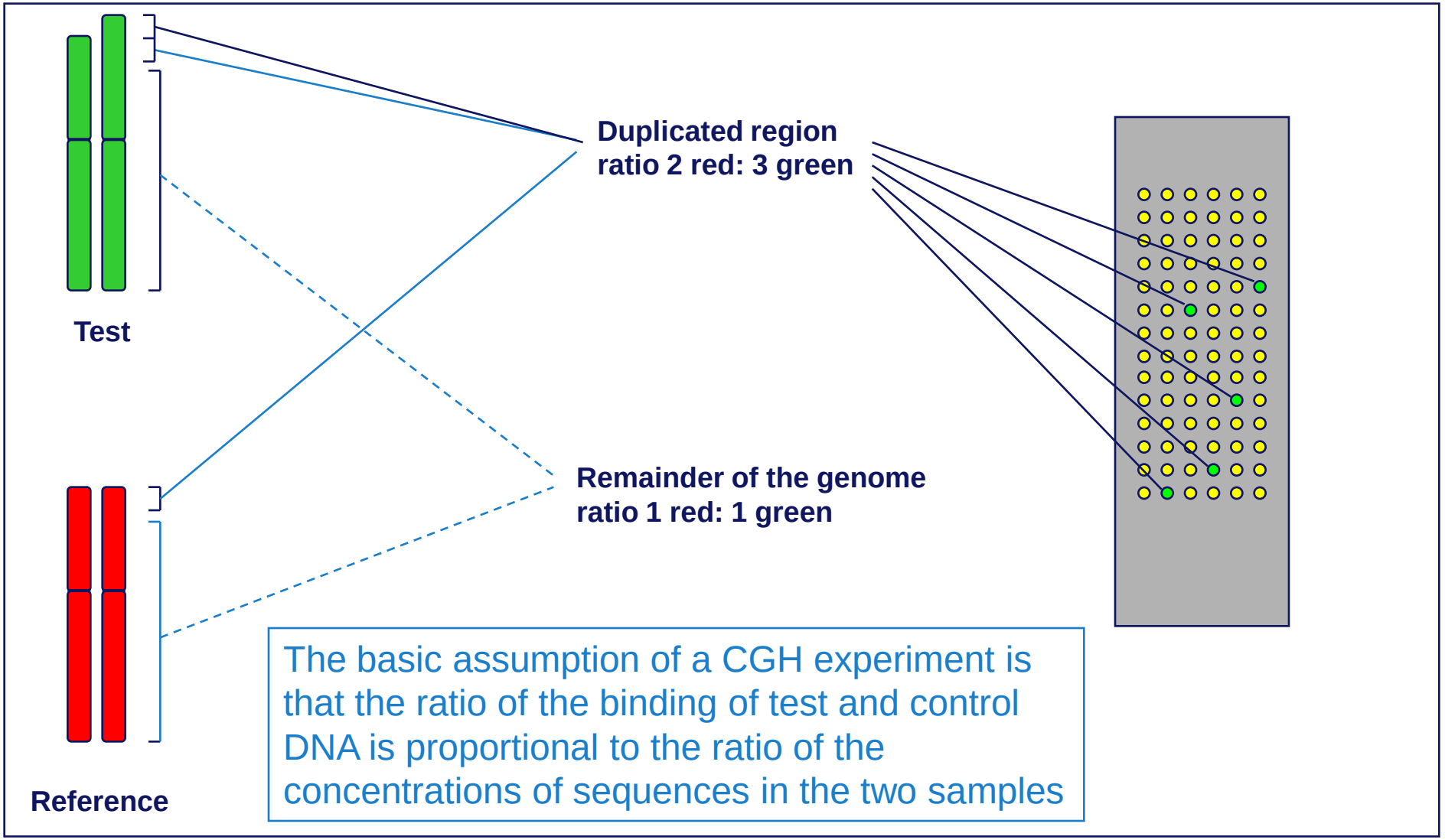
Principle of Array CGH



QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.

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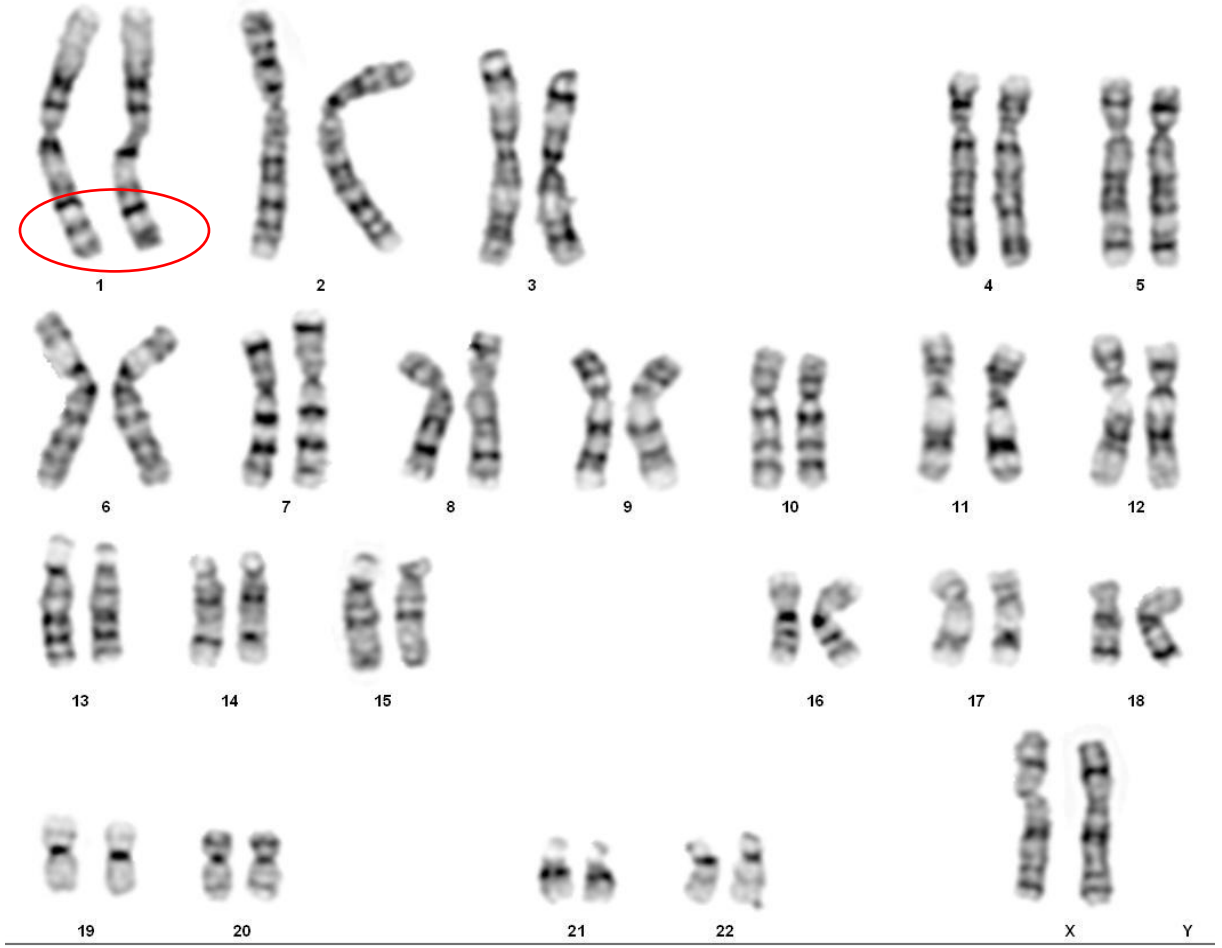
Array CGH in the Diagnostic Lab

- Improved resolution over conventional cytogenetics for the detection of copy number changes
  - Confirmation of cytogenetically visible abnormality
  - Elucidation of cytogenetically visible copy number change
  - Detection of Microscopically Invisible Copy Number Change
- Patients with developmental delay + dysmorphism
- Initially used in retrospective cases with normal conventional cytogenetics
- Increasingly used as a front line test

QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.

Example 1

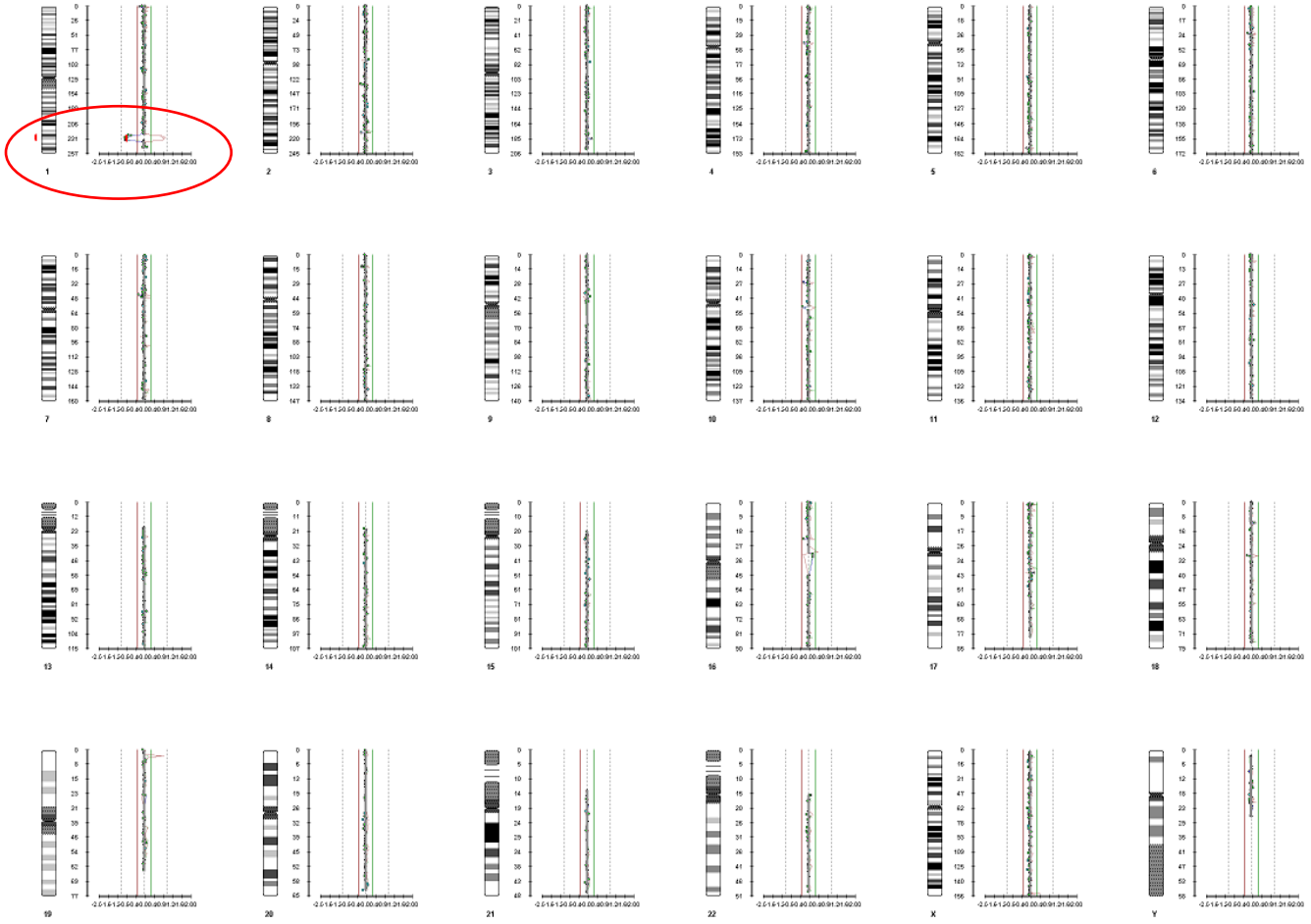
- **Case 1: Confirmation of cytogenetically visible abnormality**



QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.

Example 1

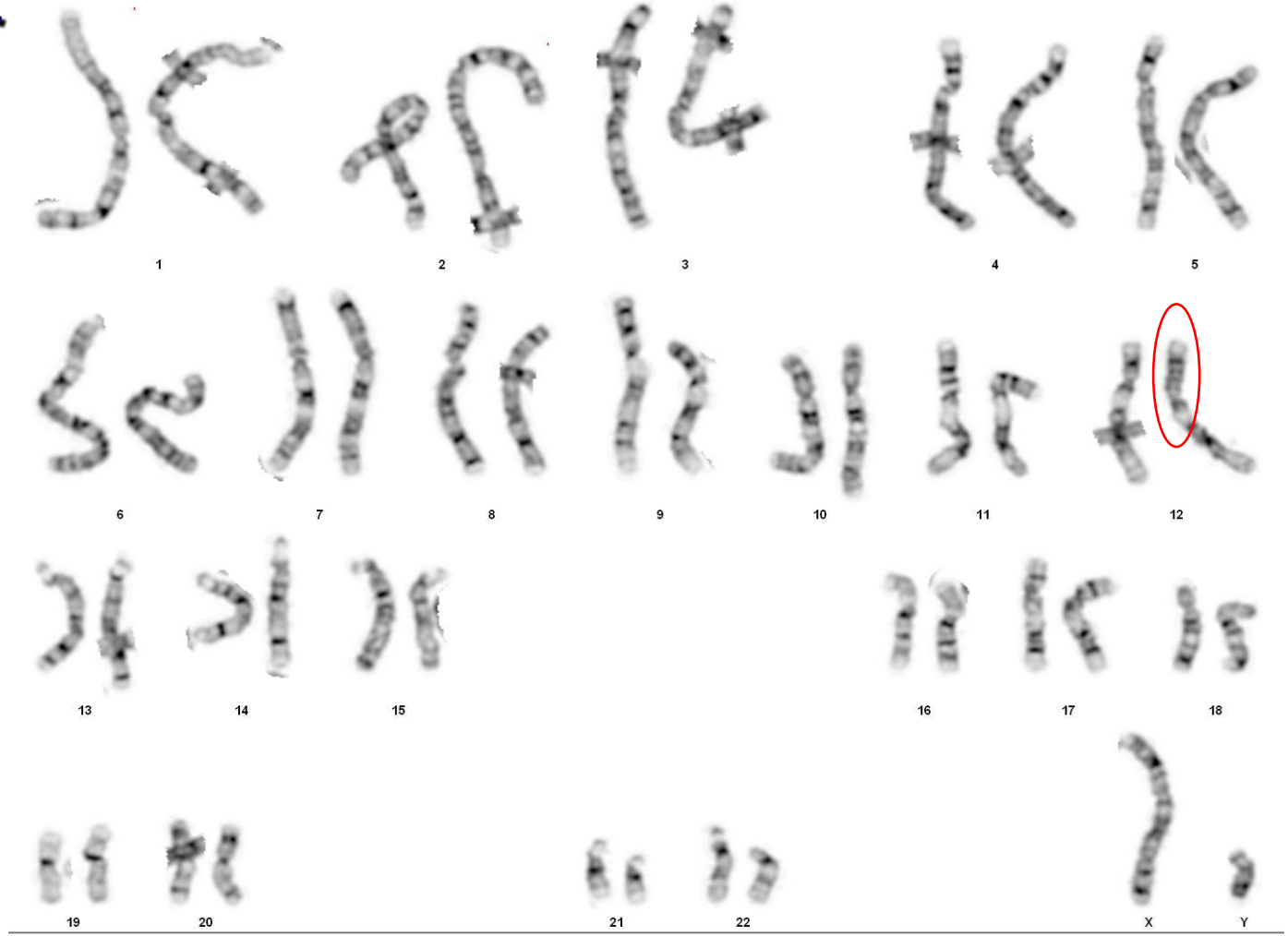
- **Case 1: Confirmation of cytogenetically visible abnormality**



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Example 2

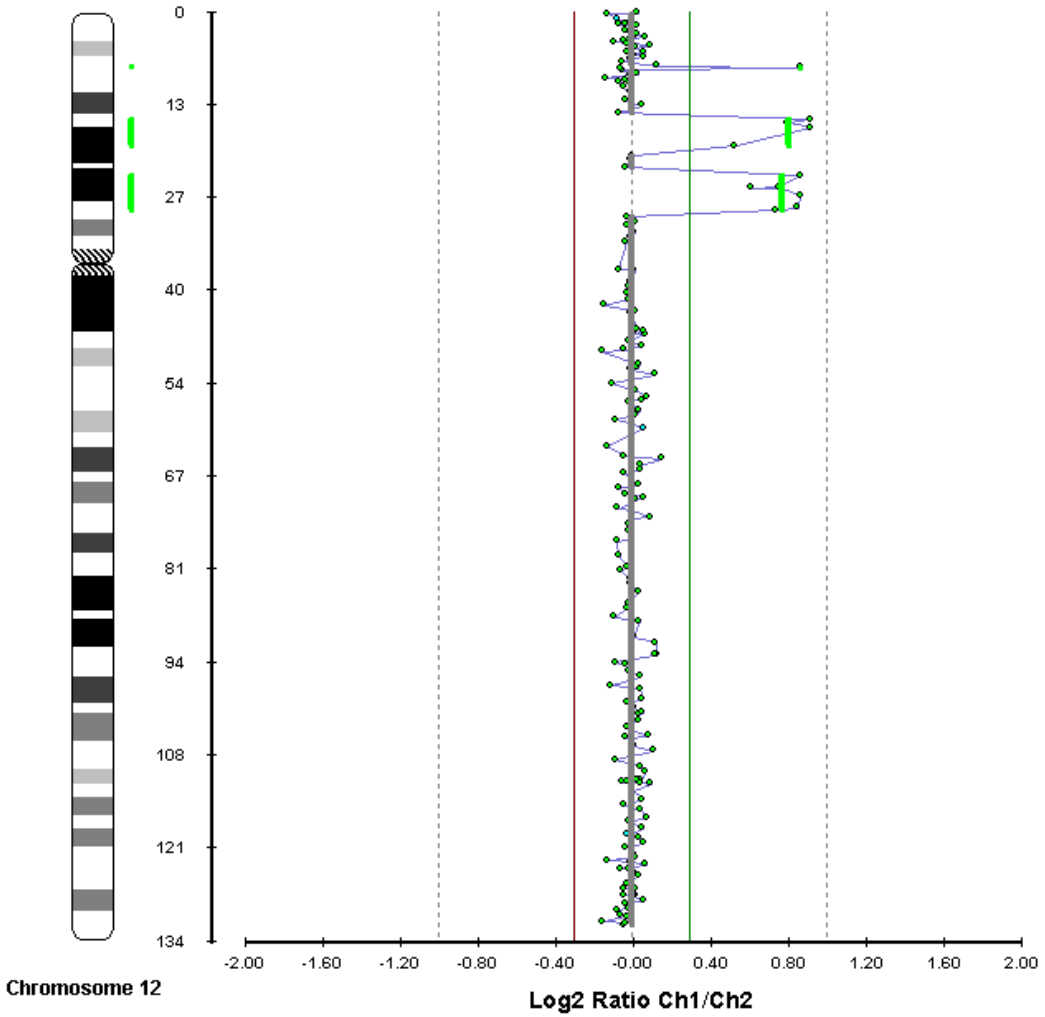
- **Case 2: Elucidation of cytogenetically visible copy number**



QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.

Example 2

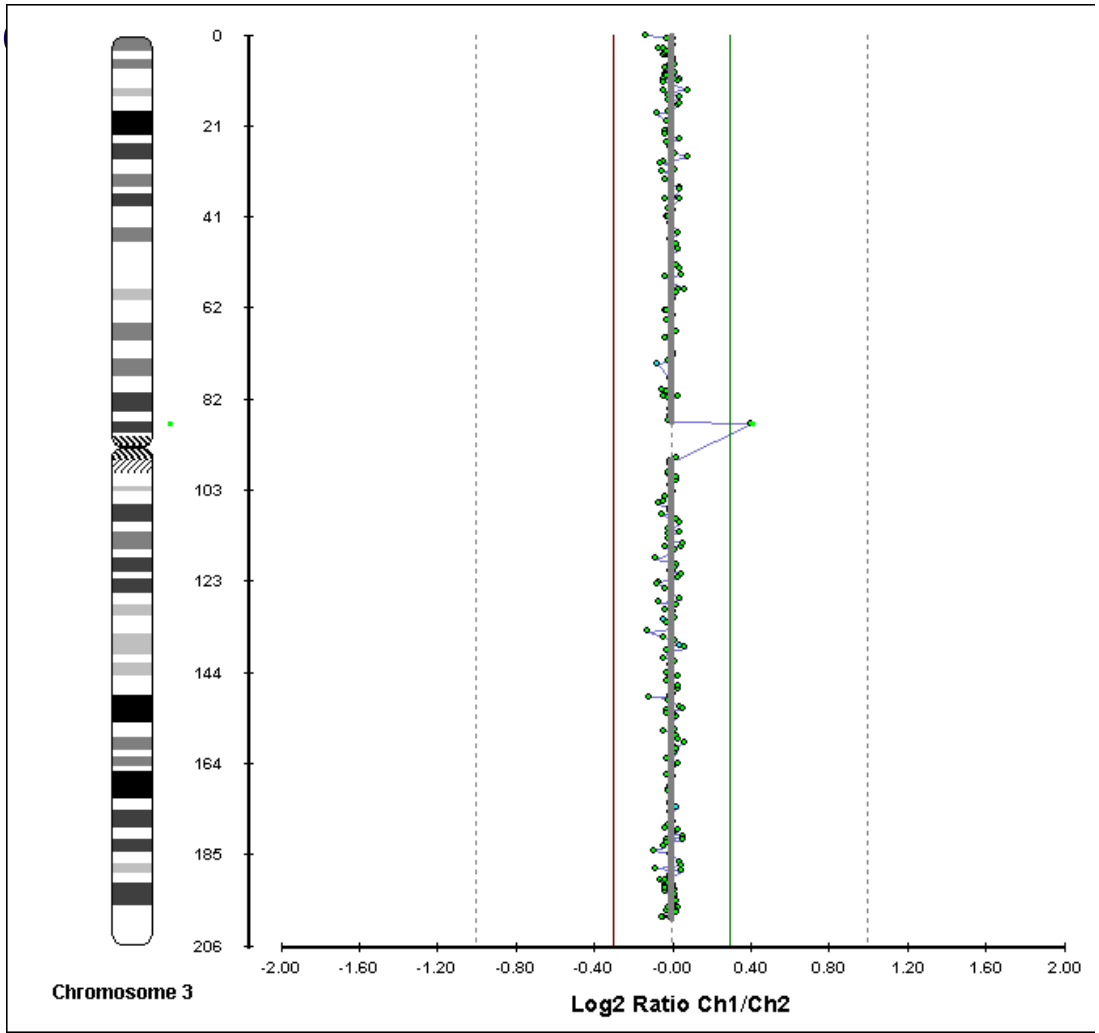
- **Case 2: Elucidation of cytogenetically visible copy number change**



QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.

Example 3

- **Case 3: Detection of a microscopically invisible copy number**



QuickTime™ and a TIFF (LZW) decompressor needed to see this picture.