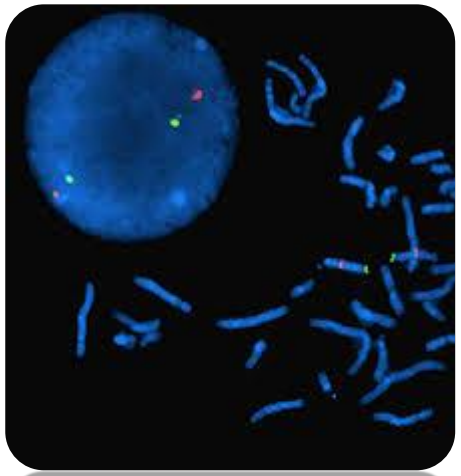


Practical Aspects of FISH

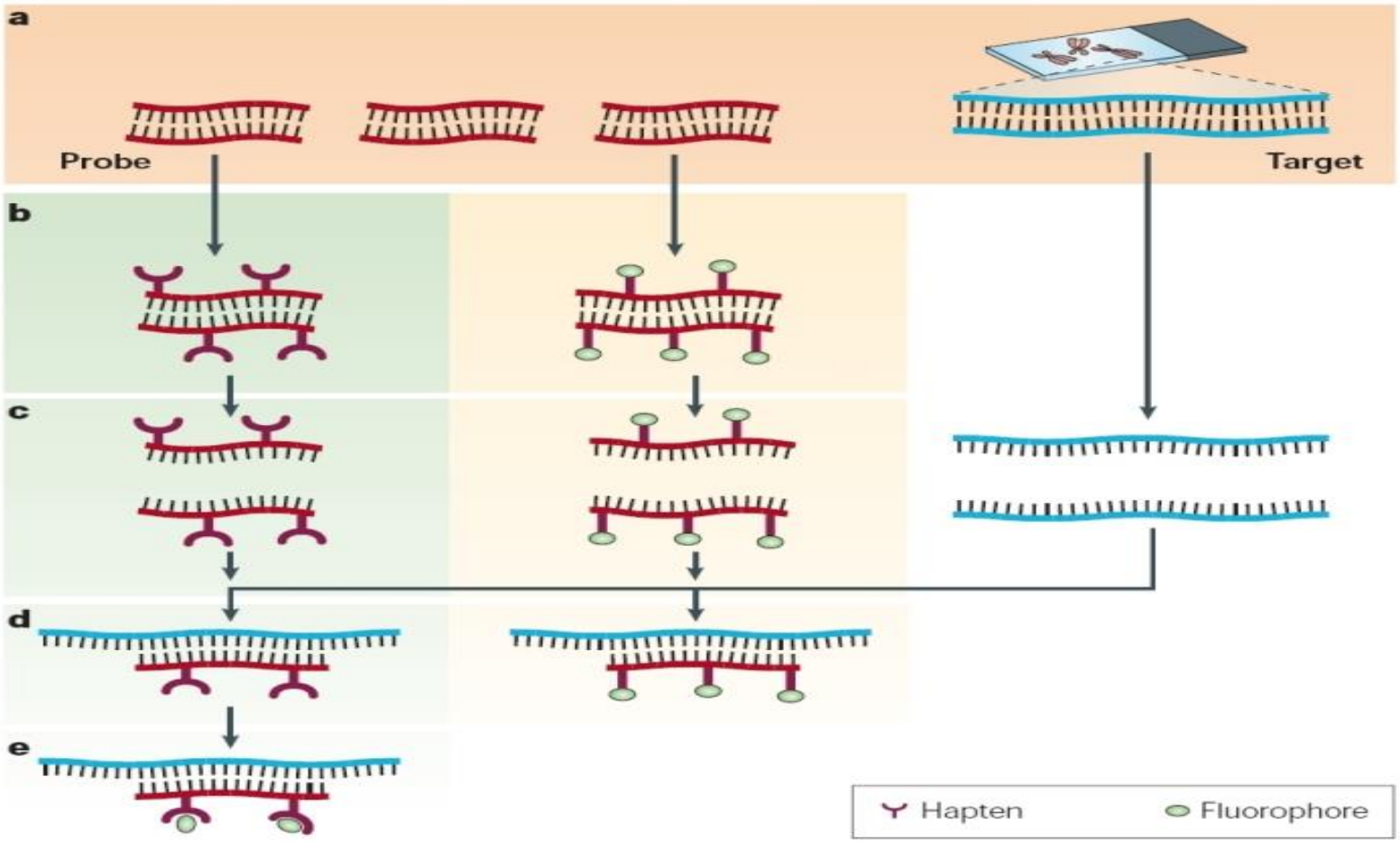
NAHLA BAKHAMIS

Outline

- What is FISH
- Types of probes
- Overview of the procedure
- Advantages and disadvantages of FISH
- Some FISH applications

What is FISH?

- Fluorescent In Situ Hybridization
- confirmatory for karyotype result
- Paint chromosomes with fluorescent molecule
- DNA probe bind to complementary sequence
- Hybridization on Metaphase or Interphase



FISH principle: Right panel is direct label. Left panel is indirect label (extra step is required)

Beginnings of FISH

- 70s & 80s -- radioactive DNA and RNA
- evolved into fluorescent chromosomes (powerful)

FISH probes

- for almost all human DNA sequence
- better resolution than conventional karyotype

  (target from 1kb can be detected)
- Longer probes less specific than short ones
- 3 types:

  - whole chromosome paint

  - locus specific

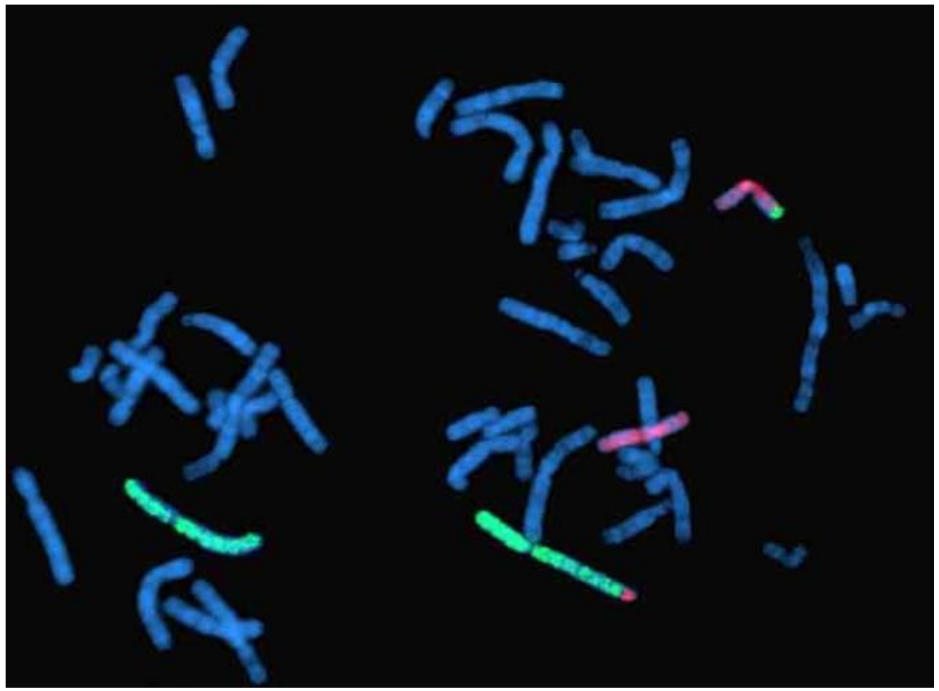
  - repetitive DNA sequence

Whole chromosome paint (WCP)

- derived from each specific chromosomes
- paint chromosomes of interest
- uses:

  - structural abnormalities to confirm material exchange bet. Chromosomes
- Limitation: metaphase chromosomes

Whole chromosome paint



46,XY,t(2;11)

- Spectrum Green: chromosome 2
- Spectrum orange: chromosome 11

DNA sequence probes

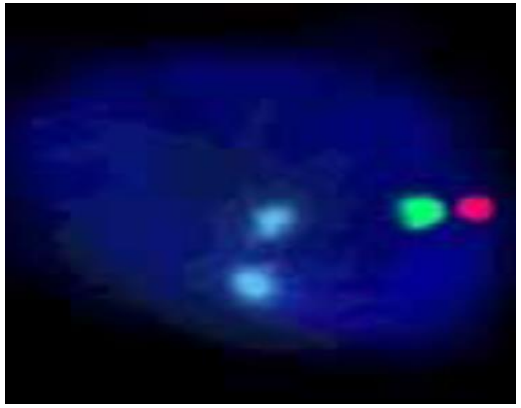
- labelled α or β satellites DNA
- specific to each chromosome except 13/21 & 14/22
- rapid hybridization
- compact, specific signal
- uses:
    - both metaphase and interphase
    - aneuploidy detection
- Limitation: cross hybridisation

DNA sequence probes

- Spectrum Green: chromosome X
- Spectrum Orange: chromosome Y
- Spectrum Aqua: chromosome 18

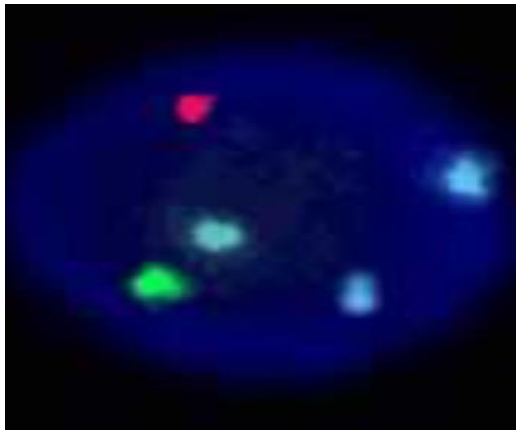
2x18
1xX
1xY

Normal Male



3x18
1xX
1xY

Male with trisomy 18



Locus specific probes

1.Subtelomeric probes:

Telometric sequence – common to all chromosomes

Subtelomeric seq: unique to each Chromosomes & specific arm of it

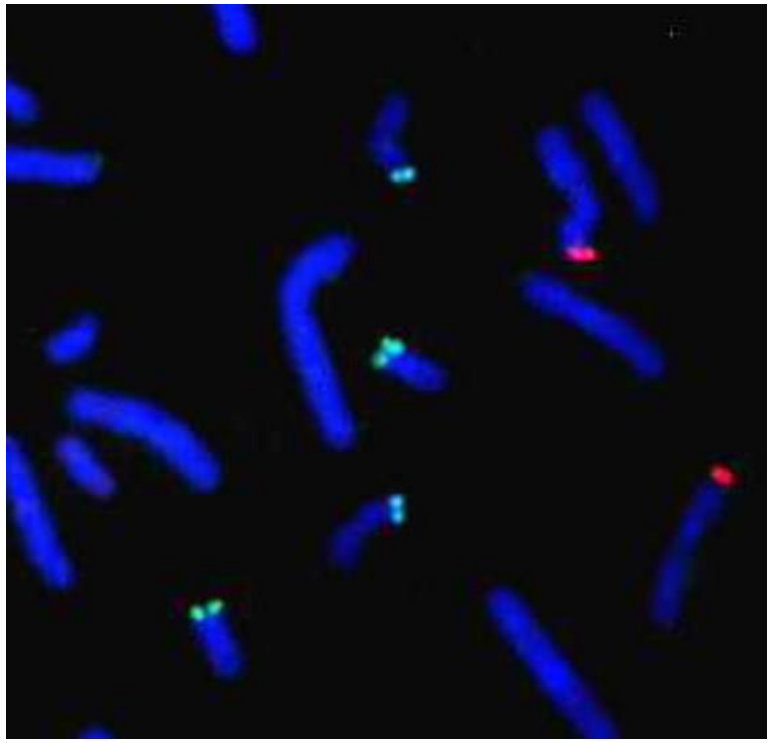
Uses:

- metaphase & interphase
- subtelomeric chromosomes rearrangements

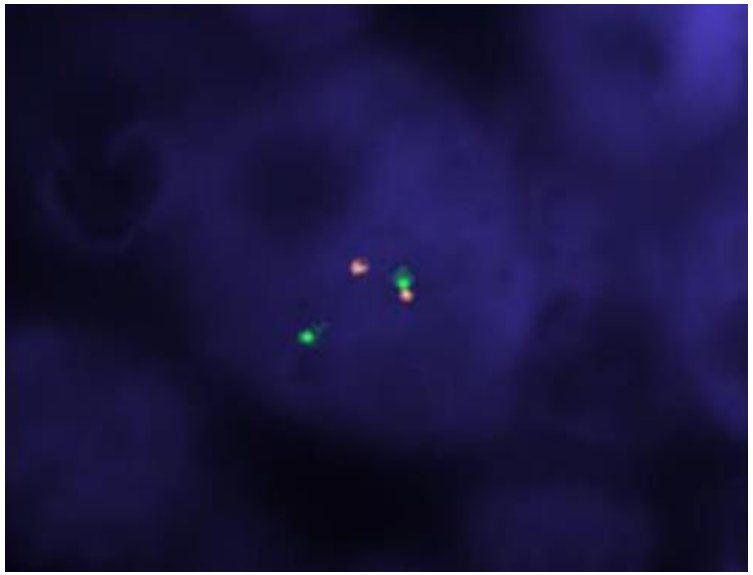
Locus specific probes

2: LSI probes: unique sequences

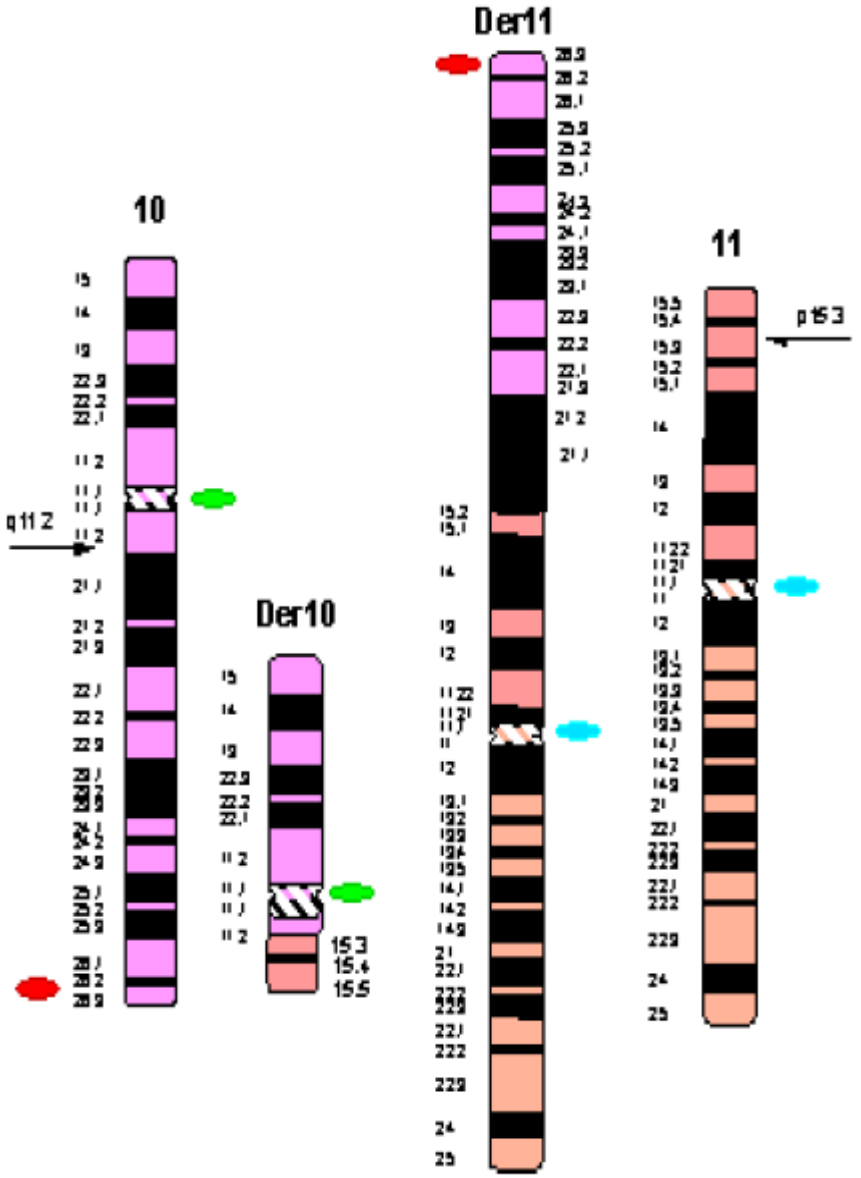
- highly specific to each site on chromosome
- uses:
  - both metaphase & interphase
  - Microdeletion, insertion and duplication
  - specific rearrangement (malignancies)
- Limitations: limited fluorescent colour, longer hybridization time and smaller signals

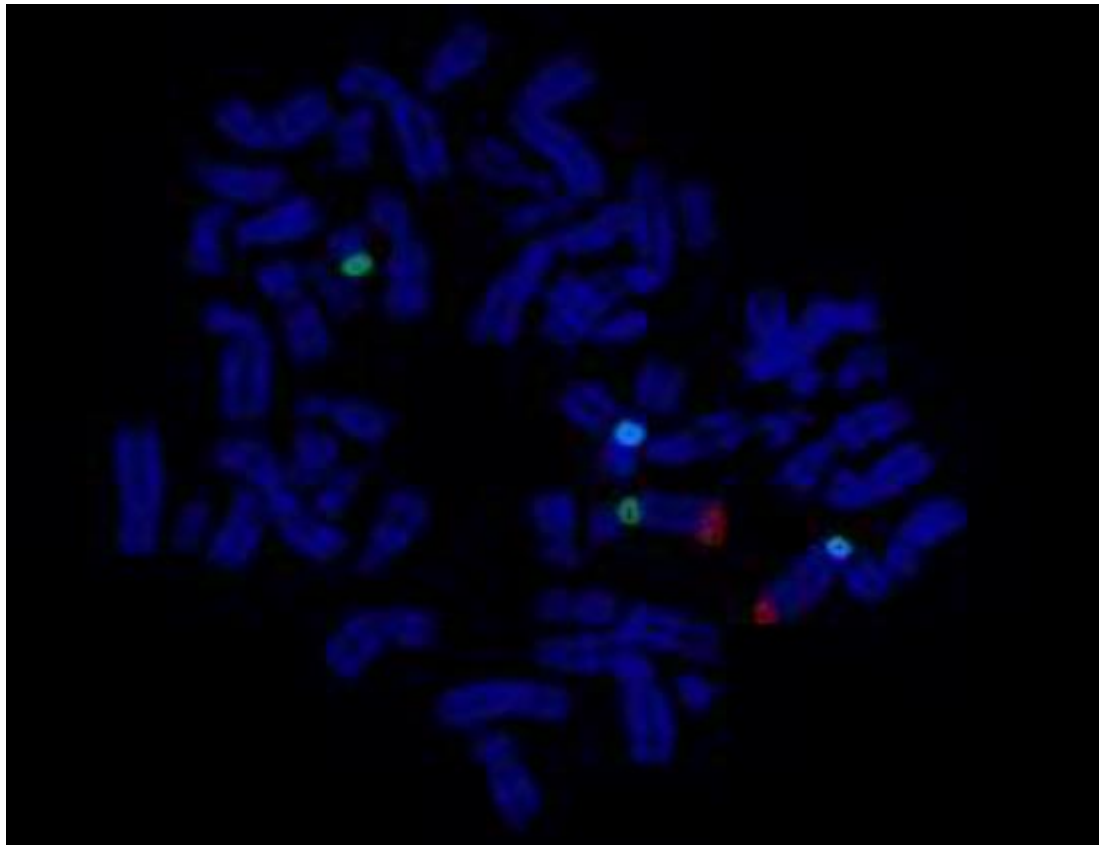


Subtelomeric probes



LSI probes





46,XX,t(10;11)(q11.2;p15.3)

FISH procedure

1. slide preparation:

- nearly all human cell types (lymphocytes, buccal cells ..etc)
- different sample may require different solution

2. digestion:

get rid of cytoplasmic residues

3. Fixation

FISH procedure

4. Denaturation
5. Hybridization
6. Post hybridization wash
7. Mounting
8. Signal observation
9. Reporting

FISH procedure

Denaturation:

- Use heat to break hydrogen bond of dsDNA (both Chro &probes)
- 73 – 75 C in the dark
- 2 types:

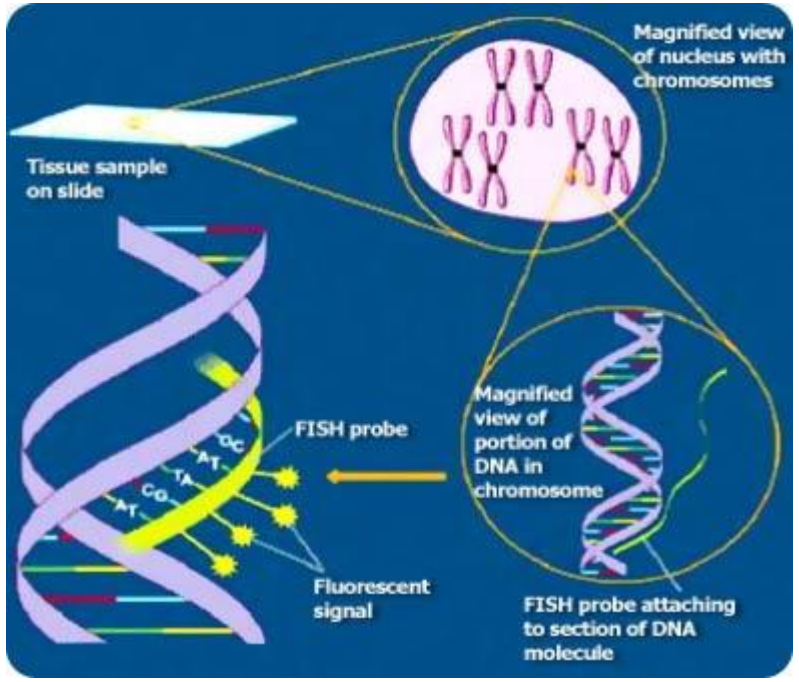
  - co denaturation

  - separate denaturation

FISH procedure

Hybridization:

- allow probe bind to target sequence
- 37 C for 1 hr depending on the probe
- presence of formamide



FISH procedure

Post hybridization wash

- clean slide & remove excess, unbound or loosely bound probe
- 2 types;

    - formamide 50-70%

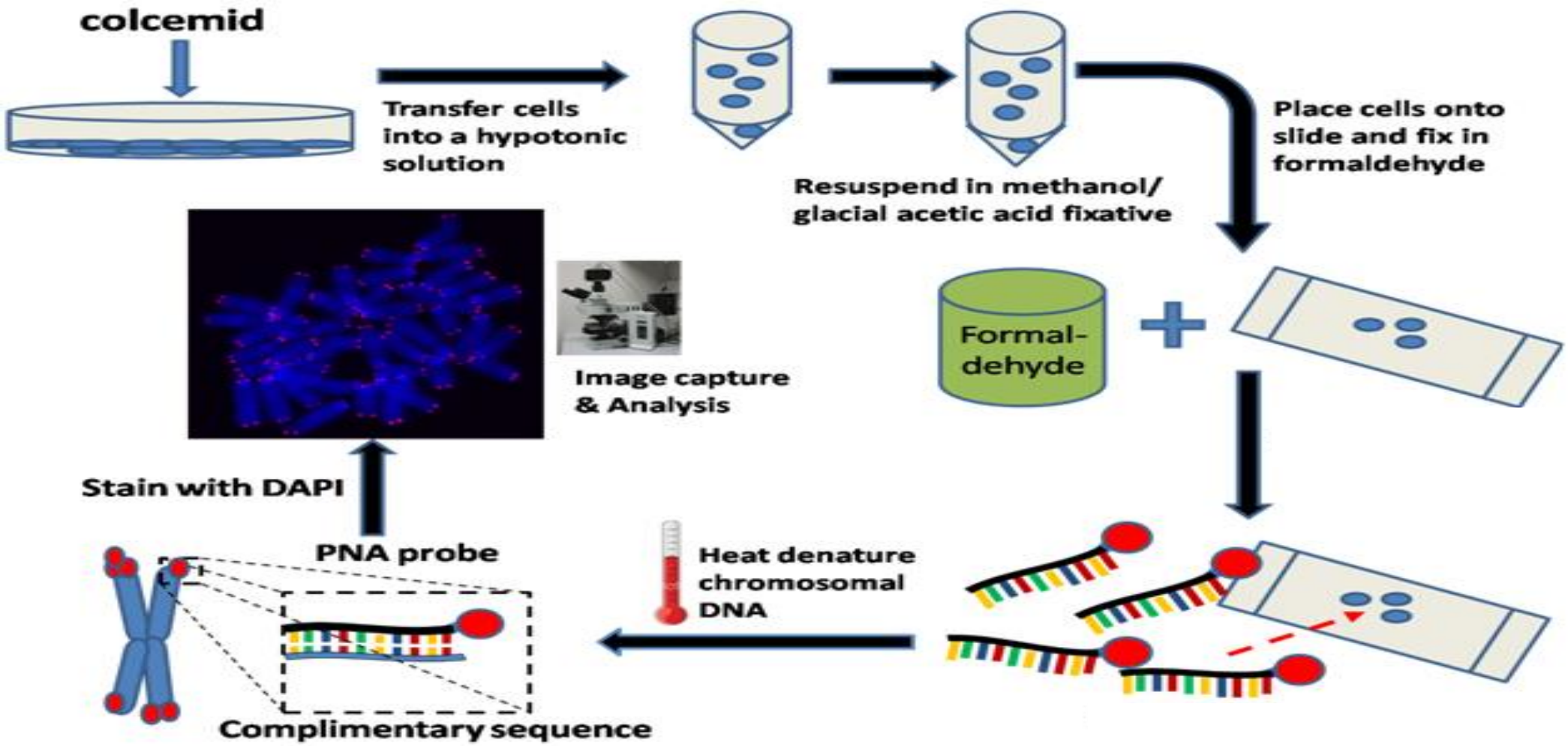
    - NP40 (tergitol-type NP-40) which is nonyl phenoxypolythoxylethanol

FISH procedure

Mounting

Mounting media is used to allow nuclei visualization

DAPI (4’,6-diamidino-2-phenylindole)



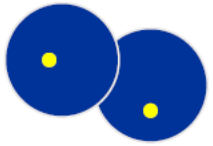
FISH procedure

Signal interpretation

Two signals

Two signals –
one split signal

Three signals



Binucleate

Two signals
(one diffused)

Two signals
With back-
ground

Reporting; ISCN

Abbreviation	Meaning
–	Absent from a specific chromosome
+	Present on a specific chromosome
++	Duplication of a signal
x	Multiple, must be followed by the number of signals seen
.	Separates cytogenetics observation from FISH
;	Separates probes on different chromosomes
del	deletion
ish	In situ hybridisation
nuc ish	Nuclear or interphase ish
wcp	Whole chromosome paint

Reporting eg

- Metaphase ish

  46,XY.ish 22q11.2(D22S75x2)

Normal male karyotype & FISH using a probe in (D22S75) region with 2 signals on

Chromosome 22q11.2 (normal)

- Metaphase ish

  46,XY.ish del(22)(q11.2q11.2)(D22S75-)

Normal male karyotype, with absence of signal on chromosome 22q11.2, DiGeorge syndrome confirmed by FISH

Reporting eg

- Interphase ish

  nuc ish 21q22(D21S65x2)

In situ hybridisation on interphase nuclei 2 copies of locus DS21S65

- Interphase ish

  nuc ish 21q22(D21S65x3)

In situ hybridisation on interphase nuclei 2 copies of locus DS21S65

Advantages

- Sensitivity
- Specificity; target sequence <1kb

  probe size 1-5kb
- Resolution; v.good resolution
- Availability; widely available probes, some in more than one colour
- Multiplicity; combine different colours/round

  more than 1 round of hybridisation

Limitations

- Sensitivity;

  background or noise

- Specificity;

  Cross hybridization, polymorphisms

- Availability;

  some specific regions may not be available and/or in specific colour

- Multiplicity;

  some probes may not work in combination with others

Limitations

- need to know exactly what you are looking for before doing the test

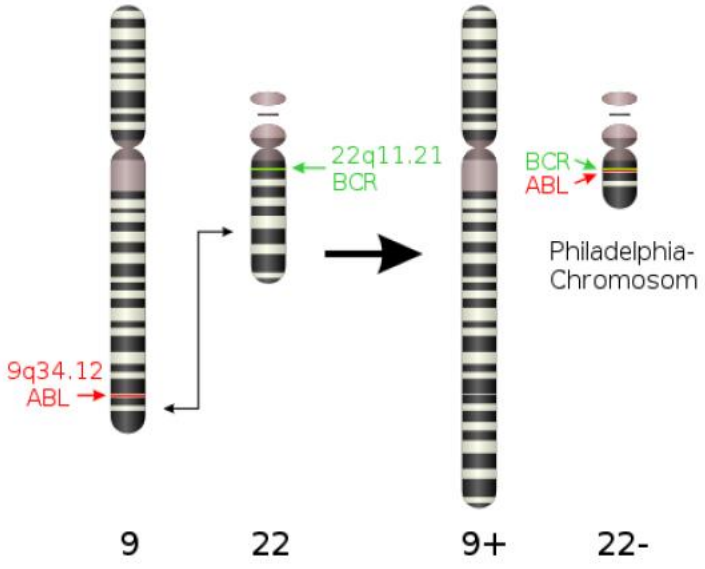
Applications of FISH

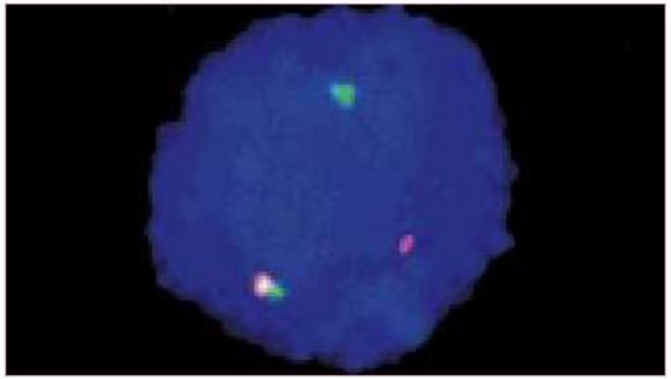
- widely used in clinical & research
- eg; PGD, cancer (late 80s) and gene mapping
- detection of numerical & structural abnormalities in prenatal diagnosis & PGD
- first applied in detection of trisomy 21 then 18
- At present more numerical and structural rearrangements can be detected

FISH in cancer

Philadelphia chromosome

95% of patients with CML reciprocal translocation bet chromosome 9&22

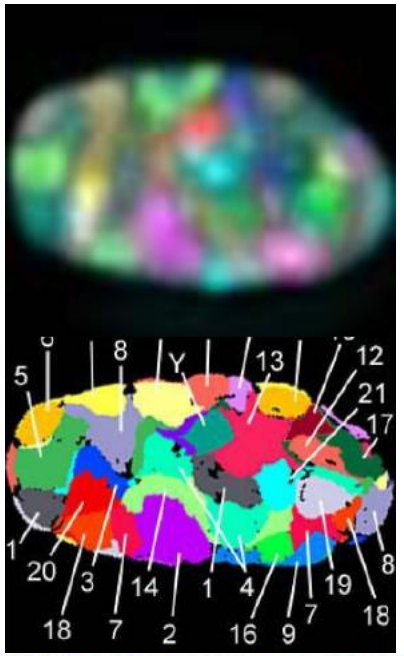


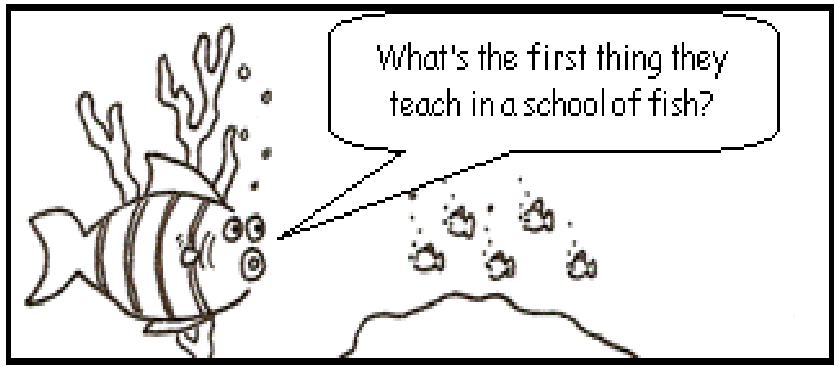


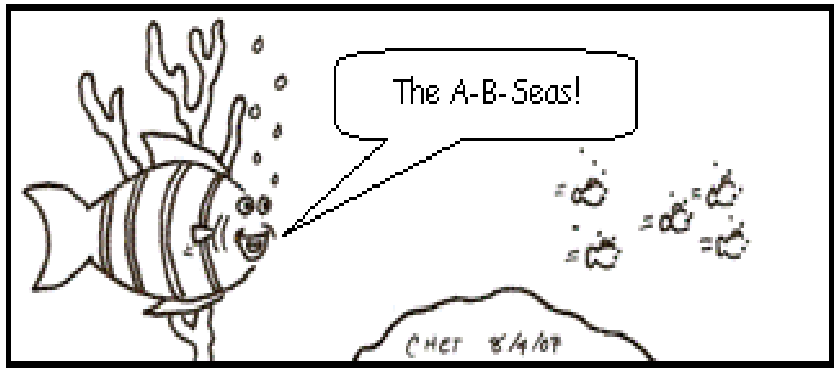
Ph (or Ph') chromosome t(9;22)(q34.1;q11.2).

3D FISH

- 3D maps of all chromosomes in pro-metaphase human nuclei
- specific paints for each chromosome combining with different fluorochromes
- help ID non-random arrangements of gene-denes chromosomes territories toward the centre of the nucleus




What's the first thing they teach in a school of fish?


The A-B-Seas!

Thank you


Any
Questions