



## Molecular Pathology - Printer Friendly Version

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### Fluorescent *in situ* Hybridization (FISH)

#### FISH-general - Molecular Pathology chapter

Used to visualize chromosomal deletions, amplifications, structural rearrangements and to identify whole chromosomes

**Advantages:** relative ease of sample preparation and analysis, high specificity, no requirement that the cells analyzed be actively dividing, can be performed on formalin-fixed, paraffin-embedded samples (allowing comparison of probe hybridization patterns to histology)

**Applications:** prenatal diagnosis and genetic counseling, oncology (especially chromosomal translocations), basic research, gene mapping, species identification of pathogens

**Specimen types:** solid tumors, bone marrow aspirates, peripheral blood (lymphocytes), amniotic fluid (amniocytes), skin (fibroblasts), chorionic villi

Uses interphase cells (non-cultured) or cultured cells in metaphase (culture + colcemid to block cells in metaphase)

**History:** FISH development followed development of cytogenetics

**1882** - Walter Flemming published the first illustrations of human chromosomes

**1956** - Identification of 46 human chromosomes

**1959** - Identification of trisomy 21 in Down's syndrome

**1959** - Identification of XXY in Klinefelter's Syndrome ([Nature 1959;183:302](#))

**1959** - Identification of XO in Turner's syndrome ([Lancet 1959;1\(7075\):711](#))

**1960** - Identification of Philadelphia Chromosome in chronic myelogenous leukemia ([J Clin Invest 2007;117:2033](#))

**1960's** - karyotype analysis now common, particularly with advent of dyes that reveal banding patterns specific to each chromosome pair

**Later** - radiolabeled probes used to bind to specific chromosomal sequences, leading to first molecular cytogenetic analyses of human tissue

FISH became common after development of fluorescent labeled nucleic acid probes

Currently 1 million+ cytogenetic analyses performed worldwide every year

FISH can be performed on tissue imprints, cytopreps, or bone marrow aspirate smears ([J Clin Pathol 2005;58:629](#))

Ultrasound decalcification may allow more successful FISH, PCR and RT-PCR ([AJSP 2006;30:892](#))

FISH can establish dizygotic origin of twin pregnancies if there are gender differences ([Hum Path 1995;26:1175](#))

#### FISH probes - Molecular Pathology chapter

Like most assays involving nucleic acid binding and detection, the specificity of FISH depends on the nucleotide sequence of the probe and the stringency of the annealing and washing conditions used

### **Nucleic acid types**

**Double stranded DNA probes:** more common; require denaturation prior to application and often reanneal to each other, lowering their effectiveness

#### **Single stranded DNA probes**

**Oligodeoxyribonucleotide probes:** usually short, up to 100 base pairs

**RNA probes:** usually single stranded complementary to target nucleic acid sequence; can be very specific but are easily degraded by mild alkaline conditions or Rnases, as are RNA target hybridization sequences

### **Detection types**

**Fluorescent probes:** most common, includes rhodamine, Texas red and fluorescein; used in most current assays; useful because require no secondary detection reagents, can be stored for relatively long periods, have strong signals with fluorescent excitation

**Enzymatic probes:** includes digoxigenin-antidigoxigenin, biotin-streptavidin; works well but often has high background signals; analysis is limited by short half lives of enzymatic activities

**Radioactive probes:** used in early studies, includes  $^3\text{H}$  and  $^{32}\text{P}$ ; cumbersome to use, take days-weeks to complete, require extra safety precautions and are limited by radioactive decay of probes

### **Target types**

**Bind specific sequences (or locus-specific FISH):** bind one nucleotide sequence found at one chromosomal region or specific to a few diseases; also used to identify deletions (with another probe used as a positive control) or amplifications of specific gene sequences; examples - BCR/ABL fusion transcript due to t(9;22)(q43;q11.2) in CML

**Bind unique sequences:** bind unique sequences found on different chromosomes, such as centromeric and subtelomeric regions; used to identify chromosomal changes in malignancy, birth defects or developmental delay

**Whole chromosome probes:** collections of many probes, each specific for different regions of each chromosome and each labeled with a different fluorophore, which together gives each chromosome a unique color; used to locate translocations difficult to otherwise identify

**Probe Length:** ranges from short oligonucleotides of 100 base pairs to nucleic acid sequences over 1 million base pairs; probe must be large enough to bind specifically to target, but not so large that its size interferes with efficient hybridization; optimal probe size depends on its target sequence - specific unique target sequences require shorter probe sequences but large target sequences (chromosomal deletions over large areas) often need larger probes

**Probe Sequence and Target Binding:** based on sequence from human genome project, grown in bacteria containing plasmids with specific sequences

## **FISH protocol - Molecular Pathology chapter**

Goal is to identify targets with high specificity, although research projects seeking related sequences intentionally use lower specificity

FISH employs three sequential steps: (a) sample fixation, (b) probe denaturation and hybridization, (c) several washing steps

**Fixation:** requires that sample is fixed to microscope slide so it does not wash off, but loose enough to allow probe-target hybridization

**Probe denaturation and hybridization:** usually marked excess of probe to target sequence is used to insure complete target binding; stringency of binding is manipulated using salt and detergent concentrations, temperature, and hybridization time; hybridization volumes are relatively low (10 to 20 ul), to minimize volume of expensive probes

Hybridization temperatures are typically 37° to 60° C in buffers containing 50% formamide and SSC buffer  
Tissue samples are usually pretreated with proteases, appropriate nucleases, and other reagents to remove sample constituents that might interfere with FISH (examples - RNase pretreatment to remove RNA from hybridization to DNA, proteolysis of DNases or RNases to avoid probe degradation, DNase pretreatment to remove DNA from reactions hybridizing probe to sample RNA)

## **FISH Probe patterns - Molecular Pathology chapter**

Following hybridization and washing, most probes are visualized by fluorescence when subjected to the proper excitation light wavelengths

**Absence of signal:** indicates deletion of the probe target (but need proper controls)

**Extra signals:** may indicate trisomy or other aneuploidy

**Fusion signal:** two different fluorophores in close proximity often emit a third color (example red and green together emit yellow)

**examples:** mantle cell lymphoma has diagnostic  $t(11;14)(q13;q32)$ , which juxtaposes the 11q13 BCL1 gene locus next to the 14q32 immunoglobulin sequence; if one locus is labeled green and the other red, a yellow signal indicates the translocation, but separate green and red signals means no translocation

**Split Signal Fluorescence:** differently labeled probes flank a region broken in a specific translocation; with red and green probes but no translocation, two yellow signals will be seen (unaffected chromosomes), but with the translocation, separate green and red signals will be seen

**Sub-Deletion Signal Fluorescence:** two differently labeled probes are used; red and green probes may be located flanking different possibly deleted areas; no deletion - 2 yellow signals; 1 deletion - one yellow signal and one red or green signal; examples include

Prader-Willi/Angelman, Cri-du-Chat, Williams, and Steroid Sulfatase Deficiency syndrome

## End of Molecular Pathology chapter

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