scFISH: A Primer

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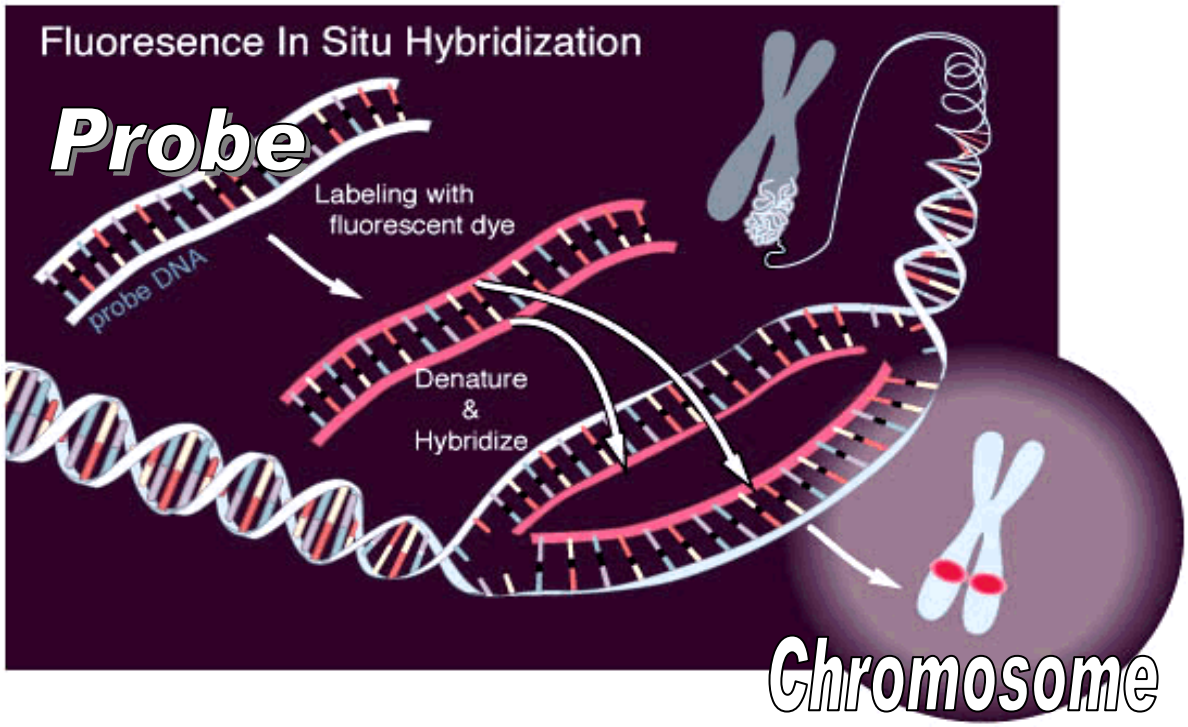
FISH: A Molecular Cytogenetic Test



Complementary nucleic acid and chromosomal target DNA bind noncovalently; binding detected by fluorescence.

This test is used in the clinical cytogenetics laboratory to diagnose chromosome abnormalities.

Applications of FISH

- **Clinical:** detection of chromosome imbalances in inherited (such as Down syndrome) and acquired disorders (such as leukemia)

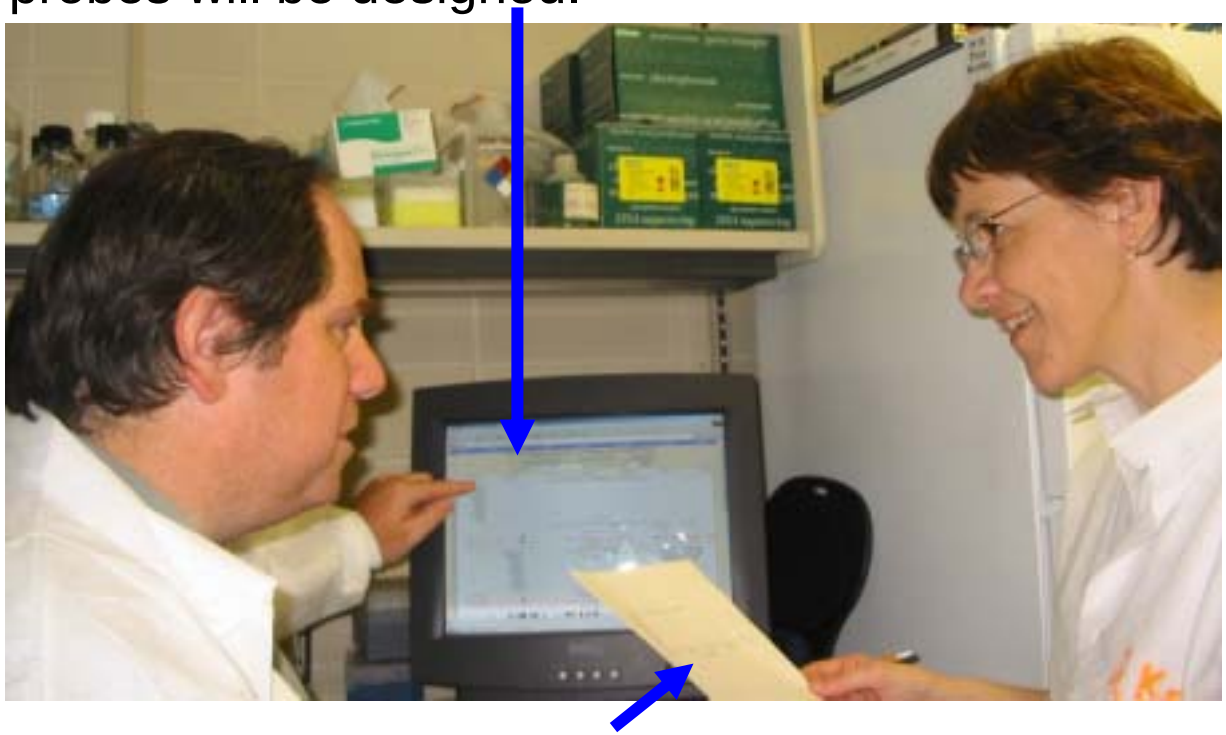
- **Research:** gene mapping, chromatin structure and organization

Production of Research-Grade scFISH Probes

- Human genome sequence provided raw data for DNA probe development.
- Software was developed to design probes.
- Probe synthesis was automated and optimized.
- Probes were validated primarily on chromosomes of normal individuals and a few patients with chromosome abnormalities.

scFISH Probe Design

1. Using the human genome sequence, we determine a chromosomal interval from which probes will be designed.



2. Our software then analyzes this interval, indicates the locations of potential probes, and selects sequences for generating these probes.

Probe Synthesis

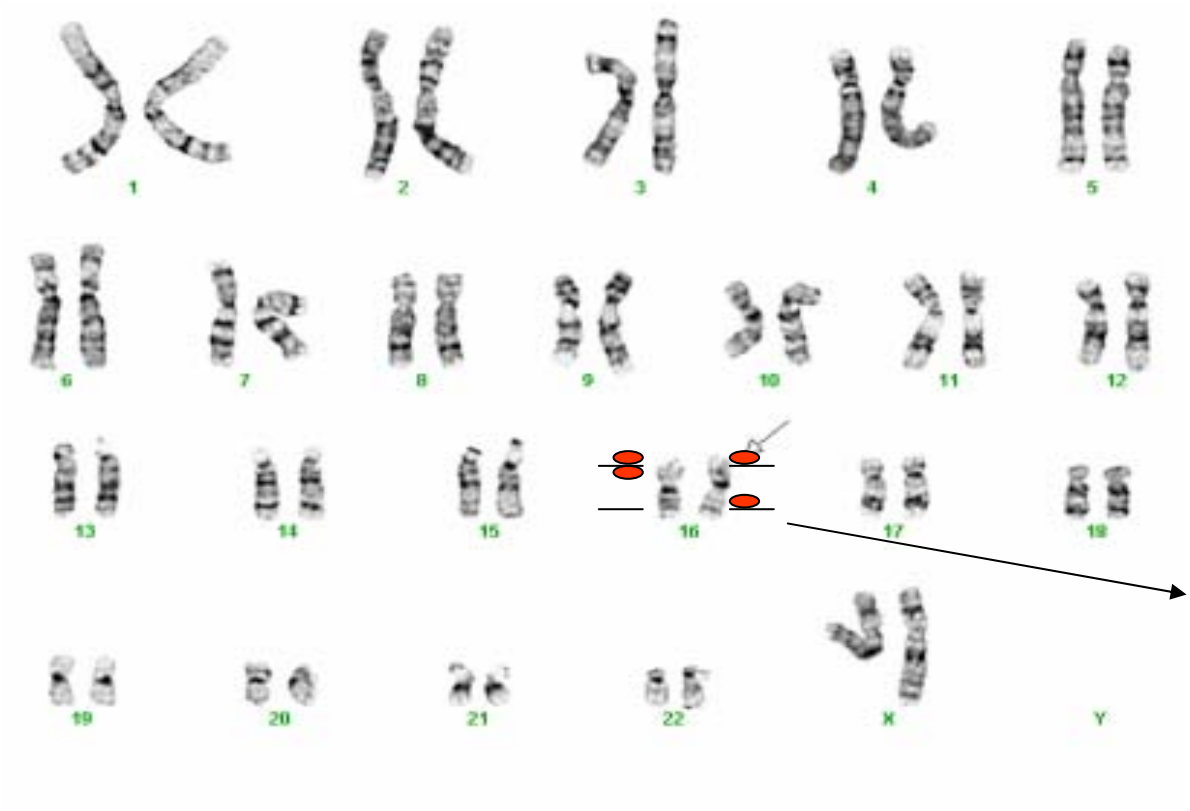
The laboratory robot sets up reactions for DNA probe synthesis.

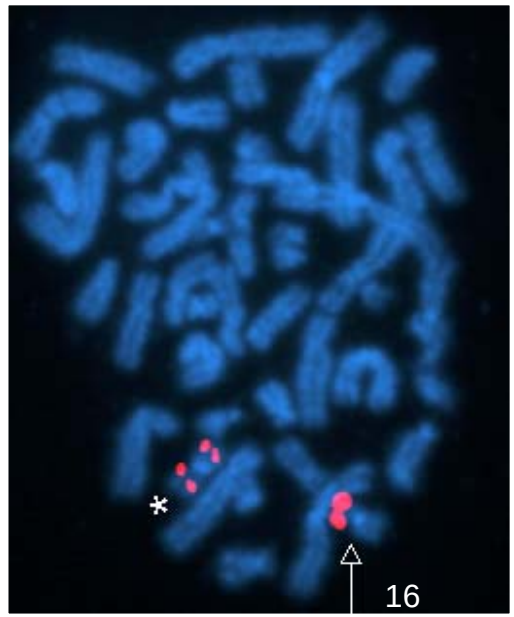
Probes are synthesized with a thermal cycler.

Analyzing Chromosome Hybridizations

The fluorescence microscope is used to examine probes bound to chromosomes to identify abnormalities in patient specimens.

Acute Myelogenous Leukemia (AML M4): Detection of inversion of chromosome 16 by scFISH probe.





Note: Inv (16) often difficult to detect by routine cytogenetic analysis; presence of inv(16) confers good prognosis.

Catalog of Selected Disorders Detected by scFISH

Monosomy 1p36	Chrom. 1
Wolf-Hirschorn Sx	4
Cri-du-Chat Sx	5
Myelodysplastic Sx	5
Williams Sx	7
Langer-Giedeon Sx	8
CML (Chronic Myelogenous Leukemia)	9;22
ALL (Acute Lymphocytic Leukemia)	12;21
Trisomy 13 (*ZIC2*)	13
Prader-Willi/Angelman Sx	15
Inverted Duplication 15 Sx	15
AML-M4 (Acute Myelogenous leukemia -M4)	16
Rubenstein-Taybi Sx	16
Charcot-Marie-Tooth Disease Type 1A	17
Smith- Magenis Sx	17
Miller-Dieker Sx	17
Trisomy 18 (*GALR1*)	18
Alagille Sx	20
Down Sx	21
DiGeorge/ VCFS Sx	22
Kallman Sx/ Turner Sx	X/Xp21.1

Peer-Reviewed Publications

- Knoll JHM, Rogan PK: Sequence-based, *in situ* detection of chromosomal abnormalities at high resolution, American Journal of Medical Genetics Part A, DOI: 10.1002/ajmg.a.20123, 2003.

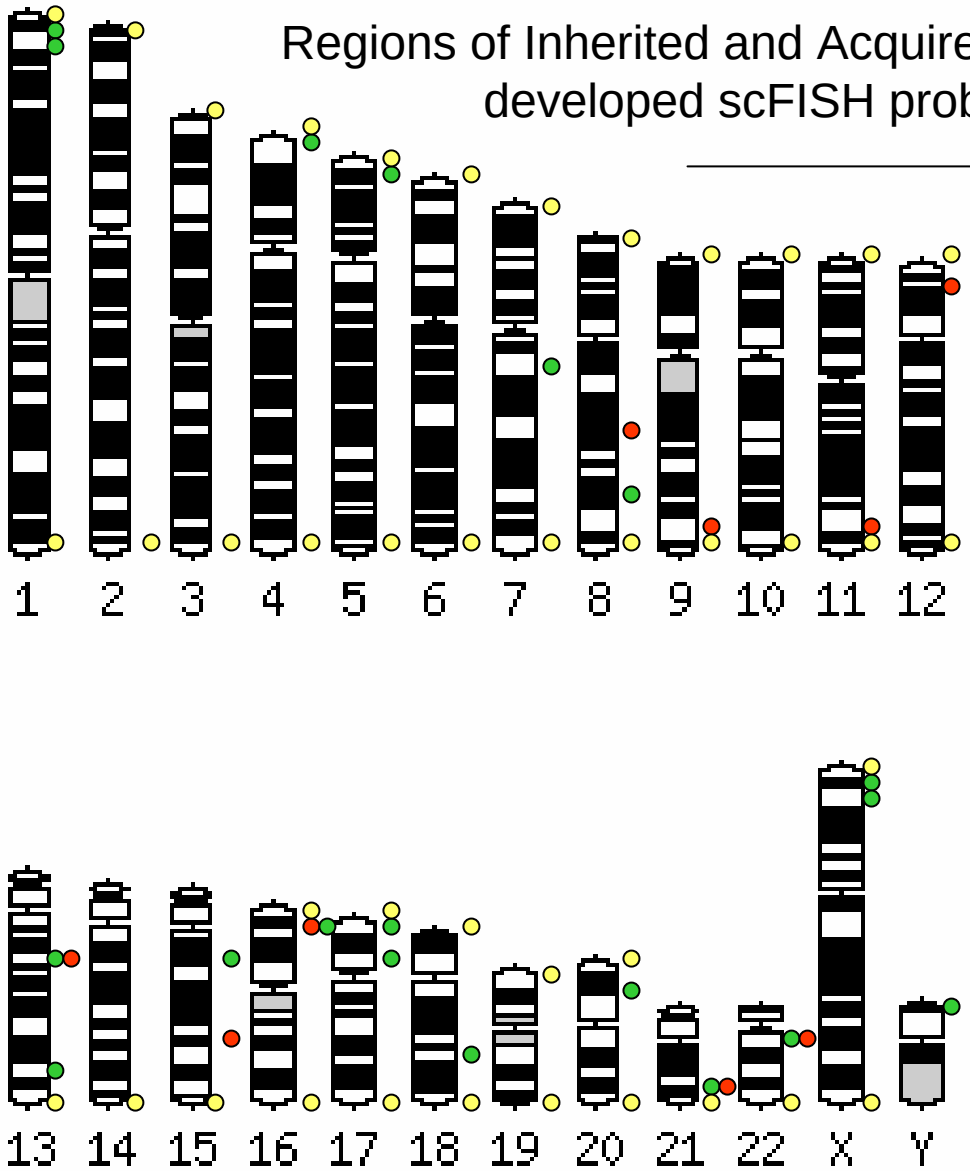
- Rogan PK, Cazcarro PM, Knoll JHM: Sequence-based design of single copy genomic DNA probes for fluorescence in situ hybridization, Genome Research, *11*: 1086-1094, 2001.

Presentations at Regional, National and International Meetings

1. Knoll, JHM, Angell PS, Marion AM, Walters PJ, Rogan PK: High resolution detection of chromosomal rearrangements using molecular cytogenetics and the human genome sequence. **XIX International Congress of Genetics**, July 2003
2. Knoll JHM, Rogan PK: Detection of Chromosomal Rearrangements with Single Copy FISH. **2nd Annual Kansas City Area Life Sciences Day**, March 2003.
3. Knoll JHM (Invited Speaker): Sequence-based *in situ* Detection of Chromosomal Abnormalities at High Resolution – Probing the Genome with ScFISH. **KUMC Cancer Institute**, May 2003.
4. Knoll JHM, Angell P, Rogan PK: Detection of chromosomal rearrangements with single copy FISH probe arrays. **American Society of Human Genetics**, October 2002.
5. Knoll JHM, Rogan PK: Detection of chromosomal rearrangements with sequence-defined, single copy hybridization probes. **Second International NCI-EORTC Meeting on Cancer Diagnostics**: From Discovery to Clinical Practice. June, 2002.
6. Knoll JHM (Invited, Grand Rounds): A New Approach to Detection of Chromosome Abnormalities. Health Science Centre, **University of Manitoba**, May 2002.
7. Cazcarro P, Rogan PK, Knoll JHM: Rapid sequence-based definition of chromosomal abnormalities. **American Society of Human Genetics**, October 2001.
8. Rogan PK, Cazcarro P, Knoll JHM: Novel features of genome organization revealed by single copy FISH. **American Society of Human Genetics**, October 2001.
9. Rogan PK, Cazcarro P, Knoll JHM: Single copy hybridization probes for detection of chromosome rearrangements derived by genomic sequence analysis. **10th International Congress of Human Genetics** (platform), May 2001.
10. Rogan PK, Cazcarro P, Knoll JHM: Single copy hybridization probes for detection of chromosome rearrangements derived by genomic sequence analysis. **American Association for Cancer Research**, March 2001.
11. Rogan PK, Cazcarro P, Knoll JHM: Single copy hybridization probes derived by genomic sequence analysis. **American Society of Human Genetics**, October 2000.
12. Knoll JHM, Cazcarro P, Rogan PK: Clinical application of sequence-based single copy probes for FISH, **American Society of Human Genetics**, October 2000.

Human Chromosome Ideogram and Probe Intervals:

Regions of Inherited and Acquired abnormalities for which we have developed scFISH probes & chromosomal ends



Chromosome ends are generally light staining. As a result, exchanges between ends is not detected by conventional methods.

- 42 ends are chromosome specific (ie. most chromosomes have two specific ends)

- inherited
- acquired
- ends or subtelomeres

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