THE MULTICOLOR FLUORESCENCE *IN SITU* HYBRIDIZATION (mFISH) HOMEPAGE

Liehr T*

***Corresponding Author:** Dr. Thomas Liehr, Institut für Humangenetik und Anthropologie, Kollegiengasse 10, D-07743 Jena, Germany; Tel.: +49-3641-935533; Fax: +49-3641-935582; E-mail: i8lith@mti.uni-jena.de

ABSTRACT

Multicolor fluorescence *in situ* hybridization (mFISH) assays are essential for a precise description of chromosomal rearrangements. Routine application of such techniques on human chromosomes started in 1996 with the simultaneous use of all 24 human whole chromosome painting probes in multiplex-FISH (M-FISH) and spectral karyotyping (SKY), even though the principle of mFISH was reported in 1989. Numerous approaches for chromosomal differentiation based on mFISH assays have been established, predominantly, to characterize marker chromosomes, but also in evolutionary biology, nuclear architecture, zoology and botany. The mFISH Homepage, which reviews all available literature at <http://www.med.uni-jena.de/fish/mFISH/mFISHlit.htm> will be introduced here.

Key words: Molecular cytogenetics; Multicolor fluorescence *in situ* hybridization (mFISH); Metaphase FISH; Marker chromosomes; FISH-banding.

INTRODUCTION

The discipline of cytogenetics went through many stages of development, each providing more possibility for characterization of normal and structurally abnormal chromosomes [1]. Characteriza-

tion of such chromosomes in humans is of high clinical importance and is the requisite condition for further molecular investigation aimed at identification of disease-related genes [2,3] or positional cloning strategies [4]. Also, cytogenetic studies are significant for understanding the evolution of different animal species including *Hominoids* [5]. In the last 25-30 years, studies were accomplished by molecular cytogenetics, *i.e.*, fluorescence *in situ* hybridization (FISH).

In 1969, Gall and Pardue [6], introduced *in situ* hybridization, which, for the first time, allowed nucleic acid sequences to be examined inside cells or on chromosomes. However, because radioactivity has well-known shortcomings [7], the radioactive probe for labeling was replaced by alternative labeling modalities or detection systems, *e.g.*, biotin detected by binding to avidin coupled to a fluorochrome [8]. Pinkel and coworkers [9] described the FISH technique that could be applied to human chromosomes and very soon thereafter the first two-color FISH study was published [10].

We have defined multicolor FISH (mFISH) as the simultaneous use of at least three different ligands or fluorochromes for the specific labeling of DNA (excluding the counter stain) [11]. According to this definition, the first successful mFISH experiments were those of Nederlof and coworkers [12] who visualized three differently-labeled nucleic acid sequences, simultaneously in blue [amino methyl coumarin acetic acid (AMCA)], in red [tetramethylrhodamine isothiocyanate (TRITC)] and in green [fluorescein isothiocyanate (FITC)].

* Institute of Human Genetics and Anthropology, Friedrich-Schiller-University Jena, Germany

Multicolor FISH assays have become indispensable for precise descriptions of chromosomal rearrangements, and studies including radiobiology, mutagenesis, inter-phase-architecture and chromosomal evolution. As the literature on this topic is huge, we created a regularly updated internet site in which all studies that apply mFISH are collected.

CONTENT

I have collected all the literature on mFISH and mFISH applications, and made the data available on the mFISH Homepage [11], which was created and has been available online since 2002. All literature that applies mFISH approaches according to our aforementioned definition is collected from publications available to the author and from PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>) and is arranged according to the scheme shown in Figure 1. There are nine main categories: 1) **Introduction** gives basic information on the page; 2) **Reviews** lists papers that provide an overview on mFISH techniques and their pitfalls; 3) **mFISH using wcp**s is subdivided into literature on M-FISH (multiplex-FISH)/ SKY (spectral

karyotyping) applied in human Clinical Cytogenetics, Tumor Cytogenetics and Others; 4) **Fish-banding** summarizes all available approaches using human probes to achieve an mFISH-based chromosome banding; 5) **CEP & SC-probes** reviews all papers on mFISH that use human centromeric and single copy probes; 6) **Additional mFISH** contains human mFISH probe sets not fitting any previous category; 7) **mFISH in animal** and 8) **mFISH in plant** are put together under these buttons. The corresponding sub-chapters can be seen in Figure 1. Under 9) **mFISH** probes available in cooperation and/or at cost price from the author of this article are listed.

The mFISH Homepage [11] can be used as a source of information in many ways. It can provide an overview into the importance of the mFISH field itself by taking note of the sheer volume of studies that apply multicolor molecular cytogenetics. It facilitates the search for mFISH reviews and basics. Also, it provides all relevant literature on already performed mFISH studies in clinical syndromes, special malignancies, mutagenesis, radiobiology, linkage/mapping, interphase-architecture and Zoo-FISH (Figure 2).

<http://www.med.uni-jena.de/fish/mFISH/mFISHlit.htm>

Page about multicolor fluorescence in situ hybridization (mFISH) literature

1	Introduction	First successful mFISH experiment			
2	Reviews	Technique and applications			
3	mFISH using wcp	Pitfalls		About fluorochromes	
		Clinical Cytogenetics			
		Translocations	Cryptic rearrangements	Marker chromosomes	Pre-implantation
		Tumor Cytogenetics			
		Leukemia	Lymphomas	Solid tumors	Cell lines
		Others			
		Mutagenesis mFISH & immunohisto	Radiobiology Zoo-FISH	Linkage/ mapping FISH after midi	Interphase-architecture Alternatives to mFISH
4	FISH-banding	Available approaches			
5	CEP & SC-probes	Arm specific probes	mFISH and banding	chromosome bar code	Rx-FISH
		Spectral color banding (SCAN)		Multicolor banding (MCB)/ m-band	
		Available approaches			
6	additional mFISH	sub-telomeric probes tumor cytogenetics	"MultiFISH" assay mFISH for mapping	prenatal and PID mFISH using alphoid probes	clinical cytogenetics
		Available approaches			
7	mFISH in animal	"Four"-color CGH		Something Special	
8	mFISH in plant	mFISH in mice			
		mFISH with mouse wcp	mouse wcp & SC-probes	mFISH in animal apart from mice	
9		some mFISH probes are available at			

Figure 1. Interactive start page of the regularly updated mFISH Homepage. The figures 1-9 are not visible on the web page.

Figure 2. Number of published papers applying M-FISH/SKY in clinical genetics, tumor cytogenetics and other fields. Up to 04/2008 >700 publications used this mFISH approach, *i.e.*, FISH using all 24 human whole chromosome painting libraries as probes, simultaneously.

Figure 3. The FISH-banding approaches have been used in >200 research studies. The chromosome bar code (CBC), Rx-FISH and multicolor banding (MCB/m-band) are there most frequently applied.

It is always up-to-date on technical developments and new probe sets [11]. It provides information on mFISH combined with immunohistochemistry, microdissection after mFISH, and alternatives to mFISH approaches. For FISH-banding [13], the following probe sets can be used: arm specific probes, M-FISH combined with banding, the chromosome bar code, Rx-FISH (cross-species color

banding), spectral color banding (SCAN) and multicolor banding (MCB)/m-banding (Figure 3). Single copy probes and/or centromeric probes are applied in many different combinations and various applications; mFISH sets using sub-telomeric or microdeletion syndrome specific probes ("MultiFISH" assay) were created as well as for gene mapping, prenatal, preimplantation and clinical diagnostics

Figure 4. In animal and plants, mFISH is mainly applied in the mouse (*Mus musculus*).

or tumor cytogenetics. Furthermore, mFISH using alphoid probes, was developed for different applications, e.g., characterization of small supernumerary marker chromosomes [14].

While the aforementioned pages of the Homepage are mainly concentrated on human cytogenetics, mFISH applied in animals and plants are also included. Besides ~60 papers on mFISH using murine probes, also included are mFISH probe sets for *Nasonia* (Pteromalidae: Hymenoptera), chicken, rat, dog and horse (see FISH-banding) were reported in at least one publication, each. For plants only one mFISH application has been reported in *Acricotopus* (Figure 4). In summary, the mFISH Homepage is a valuable resource for all working in the diagnostic and research field of molecular cytogenetics.

ACKNOWLEDGMENTS

This study was supported in part by funds from the Stiftung Leukämie, Stefan-Morsch-Stiftung, DAAD 415-br-probal/po-D/07/09624 and D07/00070 and the DFG (LI820/11-1, LI820/17-1).

REFERENCES

1. Mitelman F. ISCN 1995: an international system for human cytogenetic nomenclature (1995):

recommendations of the International Standing Committee of Human Cytogenetic Nomenclature, Memphis, TN, October 1994. Basel: Karger. 1995; 1-4.

2. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973; 243(5405): 290-293.

3. Becher R, Korn WM, Prescher G. Use of fluorescence *in situ* hybridization and comparative genomic hybridization in the cytogenetic analysis of testicular germ cell tumors and uveal melanomas. *Cancer Genet Cytogenet* 1997; 93(1): 22-28.

4. Collins FS. Positional cloning moves from perdictional to traditional. *Nat Genet* 1995; 9(4): 347-350.

5. Yunis JJ, Prakash O. The origin of man: a chromosomal pictorial legacy. *Science* 1982; 215(4539): 1525-1530.

6. Gall G, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytogenetical preparations. *Proc Natl Acad Sci USA* 1969; 63(2): 378-381.

7. Luke S, Shepelsky M. FISH: recent advances and diagnostic aspects. *Cell Vis* 1998; 5(1): 49-53.

8. Langer PR, Waldrop AA, Ward DC. Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. *Proc Natl Acad Sci USA* 1981; 78(11): 6633-6637.

9. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 1986; 83(9): 2934-2938.
10. Hopman AH, Wiegant J, Raap AK, Landegent JE, van der Ploeg M, van Duijn P. Bi-color detection of two target DNAs by non-radioactive in situ hybridization. *Histochemistry* 1986; 85(1): 1-4.
11. Liehr T. mFISH Homepage at <http://www.med.uni-jena.de/fish/mFISH/mFISHlit.htm>.
12. Nederlof PM, Robinson D, Abuknesha R, Wiegant J, Hopman AH, Tanke HJ, Raap AK. Three-color fluorescence in situ hybridization for the simultaneous detection of multiple nucleic acid sequences. *Cytometry* 1989; 10(1): 20-27.
13. Liehr T, Starke H, Heller A, Kosyakova N, Mrasek K, Gross M, Karst C, Steinhäuser U, Hunstig F, Fickelscher I, Kuechler A, Trifonov V, Romanenko SA, Weise A. Multicolor fluorescence in situ hybridization (FISH) applied to FISH-banding. *Cytogenet Genome Res* 2006; 114(3-4): 240-244.
14. Liehr T. sSMC homepage at <http://www.markerchromosomes.ag.vu/> or <http://www.med.uni-jena.de/fish/sSMC/00START.htm>