In Situ Hybridization

Laboratory Companion

Edited by
Melody Clark

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Forward

In situ hybridization as a technique has evolved greatly since the first published results of Pardue and Gall in 1969. Then the ability to localise crude satellite DNA preparations to nuclei was regarded as revolutionary, but now, just over 25 years later, the multiple localisation of single copy sequences onto extended nuclear fibres is routine. During this period, what was initially seen as a minor extension to traditional cytogenetics has evolved into a multidisciplinary technique providing a link between many different areas of biological research.

As an example, interphase cytogenetics using genomic *in situ* hybridization and chromosomal *in situ* supression (see chapters 4 and 1) has impacted heavily on the field of cancer genetics. Diagnosis of the progression of a disease is no longer reliant upon obtaining metaphase chromosome spreads, chromosome paints can highlight individual chromosomes within the nucleus, defining any genome changes which have taken place. Combining this with immunocytochemistry (chapter 4) enables such genome changes to be precisely correlated with cell type, allowing a further refining of diagnosis and ultimately leading to more accurate and successful treatments.

The use of genomic *in situ* hybridization is not solely restricted to this one field, it can also reveal important clues as to evolutionary relationships between species and the spatial organisation and behaviour of chromosomes within the nucleus. All of which are ably demonstrated in chapter 1 with its agricultural bias. At the opposite end of the molecular scale, multiple single copy clones can now be routinely mapped onto extended nuclear fibres at resolutions of up to 5Kb (chapter 2). Thus providing a valuable link between all the sequencing, cloning and genetic linkage data generated by the Human Genome Project.

PCR, that universal tool of molecular biology has also been adopted by *in situ* hybridization. As with molecular biology, it can be used as an alternative to traditional cloning and labelling strategies. Chapter 3 extols the virtues of such an adaptable technique.

It is important to realise that *in situ* hybridization is not only limited to chromatin. Another expanding field is that of developmental genetics which is now heavily reliant upon this technique as a means for localising expression of genes, both at the organism and organ level (chapter 5). It is providing answers to not only gene product localisa-

tion, but also in tandem with studies of transgenics, answers as to the control elements necessary for that expression in the first place.
Finally we cover the least publisized level of the technique: the EM level. It must be remembered that not all answers are gained with bright multicoloured painted chromosomes or embryos, the ultimate definition of DNA and RNA localisation with respect to the ultrastructure of the cell is only obtained at the EM level, a field which has been comparatively neglected, but which shows great potential.
So the aim of this manual was not necessarily to catalogue the latest *in situ* hybridization techniques, but to put together a whole range of techniques which reflect major areas of current research and interdisciplinary approaches. All have proven reproducibility with immediate and practical applications. Detailed methods are supplemented with helpful hints on technique and rounded off by comprehensive troubleshooting sections (although it is hoped that this final section is not needed!) to ensure that the novice researcher gets the best introduction to this field. One should be aware that *in situ* hybridization is a rapidly evolving field (with an equally rapidly expanding number of acronyms!) and that this manual can only hope to provide a sound basis for interesting developments to come.

Good Luck!

February 1996 Melody Clark

A remark on the layout of this book:

In order to facilitate the use of this book as a methodological source for your bench work, a wide page format has been chosen.
Due to its proper, durable binding the book has the advantage of lying flat on your bench top for convenient use. In addition, a wide margin leaves room for your own notes and provides some key notes and pictograms that shall assist you in finding the relevant information:

a pipette symbol marks the start of a step by step protocol section, a grey bar follows the whole protocol section

this symbol draws your attention to potential hazards and safety suggestions

comments on the key steps in methodology are highlighted by a key symbol

a "good idea" symbol marks useful hints for optimization of methodology

this pictrogram indicates discussions of alternative approaches

the tool indicates troubleshooting guides that shall help you in finding out what could or did go wrong and in solving and avoiding problems

suggestions for monitoring quality and reliability of the experimental procedure are highlighted by the magnifying glass

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Abbreviations

The following is a list of the most common abbreviations used throughout the book.

AMCA	7-amino-4-methylcoumarin-3-acetic acid-N-hydroxy-succinamide ester
AP	alkaline phosphatase
ATP	adenosine triphosphate
BCIP	5-bromo-4-chloro-3-indolyl-phosphate, also known as X-phosphate
bp	base pairs
BSA	bovine serum albumin
cDNA	complementary DNA
CGH	comparative genomic hybridization
CHAPS	3-[(chloramidopropyl)-dimethylammonio]-1-propane-sulphonate)
CISS	chromosomal *in situ* suppression
CTP	cytidine triphosphate
Cy3	surprisingly this is a chemical name, not an abbrevi-ation
DAB	diaminobenzidine
DABCO	1,4-diazabicyclo [2.2.2] octane
DAPI	4,6-diamidino-2-phenylindole
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
ddATP	dideoxy adenosine triphosphate
ddCTP	dideoxy cytidine triphosphate
ddGTP	dideoxy guanidine triphosphate
ddTTP	dideoxy thymidine triphosphate
DEPC	diethylpyrocarbonate
dGTP	deoxyguanidine triphosphate
DIG	digoxigenin

DIRVISH	direct visual *in situ* hybridization
DMF	dimethyl formamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
dUTP	deoxyuridine triphosphate
EDTA	ethylene diaminotetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EM	electron microscope/electron microscopic
FACS	fluorescence activated cell sorting
FISH	fluorescent *in situ* hybridization
FITC	fluoroscene isothiocyanate
GTP	guanidine triphosphate
Kb	kilobase pairs
mRNA	messenger ribonucleic acid
N-As-Mx	napthol As-Mx phosphate
NBT	4-nitro blue tetrazolium chloride
PBS	phosphate buffered saline
PCR	polymerase chain reaction
POD	horseradish peroxidase
POX	peroxidase
PVP	polyvinylpyrrolidone
rDNA	ribosomal deoxyribonucleic acid
RNA	ribonucleic acid
rt/RT	room temperature
SDS	sodium dodecyl sulphate
SDW	sterile distilled water
SSC	standard saline citrate
TESPA	3-aminopropyltriethoxysilane
tRNA	transfer ribonucleic acid
UTP	uridine triphosphate
UV	ultraviolet
YAC	yeast artificial chromosome