Essentials of Cytogenetic and Molecular Cytogenetic Laboratory Testing

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By

Usha Dutta

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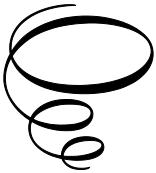

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Front figure description:
A picture of a human metaphase chromosome shows different techniques, with the top, left quadrant depicting G-banded chromosomes, top right FISH, bottom left microarray image and bottom-right SKY image.

This book is dedicated to innumerable patients and families
whom I had the pleasure of working with

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FOREWORD

Although high throughput sequencing technologies predominate the field, cytogenetics still is an indispensable tool in the human genetic laboratory. Chromosomal banding analyses allow fast and relatively inexpensive screening of the whole genome at a resolution of approximately 10 Mb. Many numerical and structural abnormalities can be easily identified. A much higher resolution can be achieved by molecular cytogenetic techniques, including molecular karyotyping using microarrays. India is the country with the second-largest number of residents worldwide and an unexplored genetic diversity. A high burden of genetic diseases, including chromosome abnormalities, may be prevalent in Indian populations. Consanguineous marriage practices increase the risk of autosomal-recessive disorders and congenital malformations. Despite a growing number of genetic laboratories, there is an even higher demand for cytogenetic and molecular testing. More laboratories with good practice in state-of-the-art cytogenetic and molecular technologies are needed.

The aim of this manual is to help geneticists and clinicians to establish "best practice" protocols, to set up and to gain experience in the laboratory to expand the future repertoire of available diagnostic services. For any genetic analysis, it is important to choose the appropriate sample collection tubes and to collect the required samples in the appropriate ways. In many instances, tissue culture is required. This manual does not only provide detailed protocols of the different techniques for cytogenetic and molecular analyses and reporting, but also valuable information on the basic laboratory equipment and safety regulations. The basic concepts of genetic laboratory analyses, including prenatal diagnostics, are well covered so that any scientist/geneticist with laboratory experience should be able to establish these protocols handily.

We recommend this manual for any genetic laboratory because of the simplicity of the language used and the way the author manages to convey essential aspects in detail. Dr Dutta has "hands-on" experience of 22 years in the field of Human Molecular Genetics and was instrumental in establishing the Molecular Cytogenetic Laboratory at the Diagnostics Division of Centre for DNA Fingerprinting and Diagnostics. In our opinion, the protocols described in this manual are a valuable addition to the existing

literature. They will be immensely helpful to set up new diagnostic laboratories or to establish additional testing methods in existing laboratories. We hope that this comprehensive manual will be used appropriately to improve genetic diagnostics in India and other countries.

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PREFACE

The field of “Genetics” has evolved drastically in the last decade due to increased knowledge of the human genome and the emergence of next-generation sequencing technology. The massively parallel sequencing helped in the identification of many disease-associated genes. Today, the targeted, clinical exome, exome, and genome sequencing are offered to many patients, but these tests are expensive. Even in this advanced genomic era, the field of “Cytogenetics” is still considered to be a gold standard because it gives a glimpse of the whole genome at a low resolution. This makes it necessary because of its deciding factor about the ordering of high throughput tests.

I work at the Centre for DNA Fingerprinting and Diagnostics, a premier research lab in India. The Diagnostics division of our centre offers genetic services for the public apart from actively being involved in the research. We provide diagnostic and prenatal services in chromosomal, molecular and biochemical genetics. Meanwhile, our study mainly focusses on establishing diagnostics tools for easy detection and maintaining the cost of the tests.

This book is a compilation of cytogenetic and molecular cytogenetic techniques that are routinely performed in a Molecular Cytogenetic laboratory. The cytogenetic protocols provide a summary of chromosomal disorders, mechanisms with illustrations and the actual laboratory procedures. Each protocol is followed by its principle, materials required, chemicals and equipment used for easy understanding for laboratory personnel.

Our Institute is also instrumental in organizing workshops which are always met with an overwhelming response. Many participants go back and introduce the learned techniques in their respective departments. Several people write to me requesting for protocols, student exchanges and invite me as a faculty or workshop faculty. Many people advised me to publish a manual, compiling the knowledge I have accumulated so far. This book titled” Essentials of Cytogenetic and Molecular Cytogenetic laboratory testing” is an outcome of these suggestions.

I hope this manual would be useful for aspiring diagnostics laboratories, scientists and clinical geneticists for a quick referral. I earnestly welcome the readers' suggestions for any further improvement.

ACKNOWLEDGEMENTS

I would like to thank the Department of Biotechnology, Government of India for the core and project support which helped us establish good laboratory techniques in the Diagnostics division of CDFD.

I extend my thanks to Dr Ashwin Dalal, Head, Diagnostics division, for his constant support and belief in me. My heartfelt thanks to Dr Nalini Mallikarjuna, Former Senior Scientist at ICRISAT for encouraging and helping me enhance my scientific career. I also thank Dr Madhumita Roy Chowdhury, Senior Scientist, Genetics Unit, All India Institute of Medical Sciences, New Delhi for continually encouraging me to turn my mere idea of writing a book into a reality.

All these years of my study and work have been a wonderful experience which has helped hone my intellectual and practical skills. I take this opportunity to thank all my former and present staff and students for their help through all these years.

I am deeply indebted to my mentor Prof. Dr Ingo Hansmann, for his constant help, encouragement and support. I would like to thank Dr Indrajit Nanda, Senior Scientist and Prof. Dr Thomas Haff, Director, Institute of Human Genetics, Julius- Maximilians-Universitaet Wuerzburg, Germany for their kind foreword.

Finally, I take this opportunity to thank my family – my father, mother, brother, for their persistent encouragement, love, support, and strong faith in my abilities which always kept me motivated. It is beyond words to express my gratitude to my husband, who stood as a pillar of support in developing my career. Lastly, I would like to thank my daughter Amora who never bothered me and also helped in edit this book.

My special thanks to my student Ms Laxmi Priyanka Posanapally, for helping in executing the cover page design.

I am grateful to DAAD for providing me with the re-invitation fellowship and SERB for the project support.

LIST OF ABBREVIATIONS

ALL= Acute lymphoblastic leukemia
AML= Acute myeloid leukemia
ACMG= American college of medical genetics
aCGH= array comparative genomic hybridization
bp= Base pairs
BrdU= Bromodeoxyuridine
C- banding = Centromere banding
CLL= Chronic lymphocytic leukemia
CML= Chronic myeloid leukemia
CVS= Chorionic villus sample
Cy3= Cyanine3
Cy5= Cyanine5
DGV= Databases of Genomic Variants
DECIPHER= DatabasE of Genomic variants and Phenotype in Humans Using Ensembl Resources
DNA= Deoxyribose nucleic acid
DAPI= 4′,6-diamidino-2-phenylindole
DMEM= Dulbecco's Modified Eagle *Medium*
EDTA= Ethylenediaminetetraacetic acid
FBS= Fetal bovine serum
FISH= Fluorescence *in situ* hybridization
FFPE= Formalin-fixed paraffin-embedded
GTG banding = G banding using Trypsin and Giemsa
GRCh37= The Genome Reference Consortium Human Build 37
HEPES= Hydroxy ethyl piperazin ethanesulfonic acid
ISCN= An International system for human cytogenomic nomenclature
ish= *in situ* hybridization
MLPA= Multiplex ligation-dependent probe amplification
MDS= Myelodysplastic syndromes
MMC= Mitomycin C
NGS= Next-generation sequencing
NOR= Nucleolar organizing regions
OMIM= Online Mendelian Inheritance in Man
p= short arm of a chromosome
Q-banding = banding with fluorescent quinacrine mustard stain
q= Long arm of a chromosome

R-banding = Reverse Giemsa banding
rpm= revolutions per minute
RPMI-1640= Roswell Park Memorial Institute
SSC= Saline-sodium citrate
SKY= Spectral karyotyping
WCP= whole chromosome paint
XIC= inactivation center on X chromosome

PART ONE:

BASIC CONCEPTS

CHAPTER 1

ESSENTIAL CELL COMPONENTS FOR CYTOGENETIC STUDY

All living beings are made up of tiny structures called cells. The cell is a basic unit of life that can divide to give rise to its own type. Unicellular organisms possess a single cell, and multicellular organisms are made up of many cells. The cell has different types of cellular components and varies significantly in size and type. The cell is enveloped by a cell membrane filled with cytoplasm, and in the center, a dense material called the nucleus is present. The cytoplasm contains many macromolecules, such as proteins, polysaccharides, lipids, minerals, and water, and cellular components, such as endoplasmic reticulum, Golgi complex, lysosomes, peroxisomes, mitochondria, ribosomes, and the nucleus (Fig. 1-1). The nucleus carries genetic material and plays a vital role in reproduction and repair.

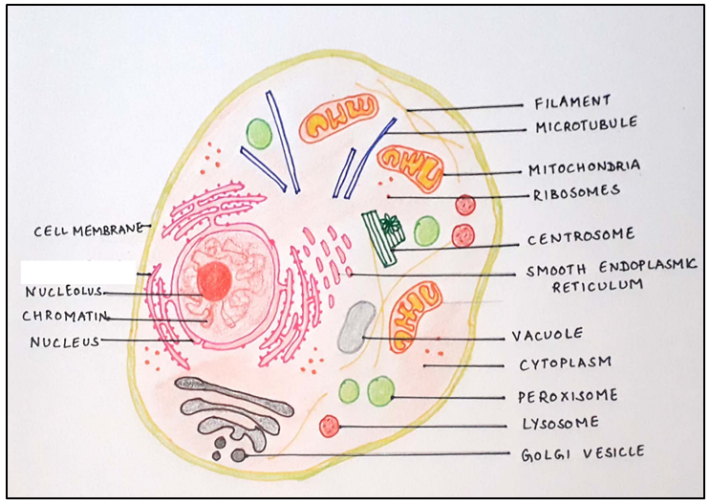

Figure 1-1: Schematic representation of a cell and cell components.

It is essential to know the cell membrane's contents and a few cell components. It helps us to understand the chemicals/reagents' action on the cell membrane for our subsequent staining and hybridization experiments. The cell components that are involved in the cytogenetic study are highlighted for easy readership.

1.1 Cell membrane

A cell's cytoplasm is enclosed in a membrane called the cell membrane, which distinguishes one cell from another. Every cell has a cytoplasm and nucleus, except red blood cells, which are enucleated. The cell membrane is made with phospholipids and proteins. The phospholipids form a bimolecular layer with the hydrophilic ends facing outside and the hydrophobic ends facing inside. The proteins are distributed throughout the membrane. The nonpolar amino acids are positioned inside, and the polar amino acids are exposed on the outside. This polarity is crucial as it determines how the interactions with different chemicals occur.

1.1.1 Functions

1.1.1.1 The cell membrane is selectively permeable as it can regulate the movement of the substances from inside to outside and vice versa, as it has a regulatory barrier. There are two mechanisms: diffusion and exocytosis (outside the cell) or endocytosis (inside the cell). Since water can move freely inside and outside, it allows the hypotonic solution (less concentration) to enter the inside of the cells, creates swelling in the mitotic metaphase stage, and helps spread chromosomes for cytogenetic study during hypotonic treatment (Arsham, Barch, and Lawce, 2017).

1.1.1.2 Glycoproteins are the proteins that contain sugar molecules in between the protein-lipid layer and project out of the membrane on both sides of the cells. These help the adhesion to both the outside of cell culture flasks and the inside of the cells. Trypsin, a protease enzyme used during the harvesting of cells, digests these glycoproteins, thereby freeing the cells from their attachment to the flasks.

Other functions of glycoproteins:

1.1.1.3 Cell to cell contact: For example, if lymphocytes loose

glycoproteins, they are no longer present in the lymph nodes.

1.1.1.4 Antigenic nature: Glycoproteins are antigenic. For example, in red blood corpuscles, they determine the blood type.

1.1.1.5 Contact inhibition: A process in which the cells stop dividing due to confluent growth. In tumor cells, this property is lost.

1.2 Centrioles

Centrioles are small sets of microtubules present in the cytoplasm. There are nine groups of microtubules that are found next to each other in a perpendicular way. This is called a centrosome, and it is involved in organizing microtubules in the cytoplasm, which serve as the cells' skeletal system. During the G1/S phase, the centrioles self-duplicate and migrate to opposite poles forming spindle fibers—this helps separate the chromosomes from their respective daughter cells in cell division. Many proteins interact and regulate chromosomes. For example, mutations in the *TP53* gene leads to extra copies of centrosome formation, which causes changes in the spindle apparatus, aneuploidy, and tumor formation (Pelengaris and Khan, 2006).

In the cytogenetic lab, colchicine inhibits the cells from completing mitosis. The chromosomes try to remain in the metaphase stage and do not enter the anaphase. Colchicine binds to the monomer tubulin, blocking the polymeric spindle fibers. It is a spindle poison, and hence chromosomes cannot separate.

1.3 Nucleus

The nucleus is an essential component of the cell as it maintains the genetic material of the daughter cells. The nucleus in the interphase (non-dividing phase) and the mitotic phase (dividing) is different. During the interphase stage, the nucleus forms a network called chromatin. This chromatin is divided into two: the chromatin, which lightly stained, is called euchromatin, and the highly-condensed chromatin is called heterochromatin. During cell division, the chromatin condenses into rod-like structures called chromosomes (chromo=color; soma=body). The chromosome number differs for different species, and telomeres cap them to maintain integrity.

1.3.1. Euchromatin: This is decondensed during the interphase and is present in the interior region of the nucleoplasm. This structure does not change throughout the cell cycle.