

Advanced Molecular Cytogenetic Methodologies

REGISTRATION FORM

This course consists of 3 modules/20 hours of study. Each module includes questions to be submitted to the instructor for review. A strong background in cytogenetics and/or clinical genetics is recommended.

COURSE FEES:

BCSLS Members **\$195.00**

Non-members **\$260.00**

Online registration or Mail registration and payment to:

BC Society of Laboratory Science
720 - 999 West Broadway Avenue
Vancouver, BC

V5Z 1K5

Tel: (604) 714-1760

1-800-304-0033

Fax: (604) 738-4080

Registrant: _____

BCSLS MEMBER?: Yes ___ No ___

Address: _____

Phone : _____

E-mail: _____

Amount Submitted: \$ _____ + 5% GST _____

Total \$ _____

www.bcsls.net - bcsls@telus.net

Last printed 22/01/2010 14:15:00



presents

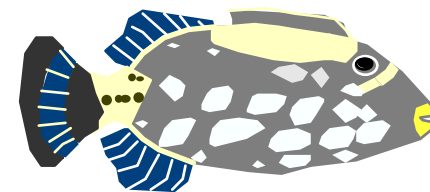
Advanced Molecular Cytogenetic Methodologies:

A Guide to the Principles and Practice

YEAR 2002

TOPICS INCLUDE:

Comparative Genomic Hybridization
24-colour Karyotyping (MFISH, SKY)
High Resolution Multi-colour Banding (Mband)
DNA Microarray Technology



The BCSLS is pleased to present this sequel to our popular correspondence course *FISH: A Practical Approach*.

Advanced Molecular Cytogenetics Methodologies: A Guide to the Principles and Practice

Course Instructor

Brenda Lomax, BSc, RT (Cg)

Course Objectives

This correspondence course is offered by the BCSLS to provide you with:

a colourful, well-illustrated course describing the advanced molecular cytogenetic methods that have been developed in the past decade. Students are provided the appropriate theory and practical knowledge to introduce these technologies into clinical cytogenetic practice.

Module One: Comparative Genomic Hybridization

Comparative genomic hybridization (CGH) is a FISH-based technique that can detect chromosomal imbalances. Module One outlines the principles of each step in the CGH procedure and provides detailed protocols, including: DNA extraction and quantification, DNA labelling by nick translation, probe preparation, hybridization, CGH analysis and interpretation, ISCN nomenclature, troubleshooting. Applications of CGH are described using examples of numerous real clinical cases.

Module Two: 24-colour Karyotyping

24-colour karyotyping labels each of the human chromosomes with a uniquely distinctive colour. This FISH-based approach to

karyotyping readily identifies both simple and complex chromosomal rearrangements. While this module describes both SKY (spectral karyotyping) and MFISH (multi-colour FISH) approaches, the major focus is on the use of commercially available MFISH assays (*Vysis, Metasystems*). Topics include: principles, analysis and interpretation, limitations, clinical applications, troubleshooting. The use of cross-species colour banding and high resolution multi-colour banding (Mband) is also described.

Module Three: DNA Microarray

This third module provides an overview of the emerging DNA microarray technology with an emphasis on its significance to the clinical cytogenetic laboratory.

Course Authors

Brenda L. Lomax, BSc, RT (Cg)
BC Women's and Children's Hospital, Vancouver, BC

Valia S. Lestou, PhD
British Columbia Cancer Agency, Vancouver, BC

This course is being submitted to the CSMLS for review to establish eligibility for credits under the Continuing Professional Studies (CPS) program and competency assurance credits under the CSMLS Professional Enhancement Program. This course provides theoretical information only and is not intended to replace practical training in these areas.



Advanced Molecular Cytogenetic Methodologies: A Guide to the Principles and Practice

INSTRUCTIONS

- 1) The course manual is 92 pages long, containing 3 modules. You may keep the course manual for reference. Please remember this material is copyrighted and **do not copy or reproduce all or part of the manual without our permission.**
- 2) Read each module and answer the corresponding questions/assignments. The questions can be answered on the booklet page, photocopy or on a separate piece of paper at your preference.
- 3) You have two months to review all of the course material.
 - a) After you have reviewed the course material, please complete the assignments.
 - b) If you require assistance with the course material, please contact the BCSLS office stating your name, evening telephone number, and the topic you wish to discuss.
 - c) Our instructor will return your call.
- 4) Please complete the course evaluation. This information will assist us in improving our future courses.
- 5) Mail the **assignments** and **course evaluation** to:

BCSLS
#720 - 999 West Broadway Avenue
Vancouver, BC V5Z 1K5

Your marks and "certificate of participation" will be mailed to you within 6 weeks after we receive your questions/assignments.

British Columbia Society of Laboratory Science
#720 – 999 West Broadway Ave
Vancouver, BC V5Z 1K5 Canada
tel: (604) 714-1760 or 1-800-304-0033
fax: (604) 738-4080
email: bcsls@bcsls.net
www.bcsls.net

TABLE OF CONTENTS

| | |
|---|-----------|
| INTRODUCTION..... | 1 |
| MODULE ONE: Comparative Genomic Hybridization | 3 |
| 1.0 Introduction to Comparative Genomic Hybridization (CGH)..... | 4 |
| 2.0 Extraction of Total Genomic DNA | 6 |
| 2.0.1 Storage and Handling of Stock DNA..... | 7 |
| 2.0.2 Determination of DNA Quantity and Purity | 7 |
| 2.1 DNA Labelling by Nick Translation | 8 |
| 2.2 Precipitation of DNA..... | 10 |
| 2.3 Preparation of Target Metaphases Slides | 10 |
| 2.4 Denaturation, Hybridization and Post-hybridization Wash..... | 11 |
| 3.0 Capturing Digital Images..... | 11 |
| 4.0 CGH Analysis | 12 |
| 5.0 Interpretation of CGH Profiles | 14 |
| 5.1 Limitations..... | 16 |
| 6.0 Applications of CGH..... | 18 |
| 7.0 Reporting CGH Results | 23 |
| 8.0 Conclusion | 25 |
| APPENDIX I: High-Salt DNA Extraction Method | 26 |
| APPENDIX II: Determination of DNA concentration and purity by spectrophotometry..... | 29 |
| APPENDIX III: Estimating DNA Molecular Weight by Agarose Gel Electrophoresis..... | 30 |

| | |
|---|-----------|
| APPENDIX IV: Nick Translation Labelling Protocol (direct labelled fluorochromes) | 31 |
| APPENDIX V: Probe Precipitation..... | 32 |
| APPENDIX VI: Target Metaphase Pretreatment..... | 33 |
| APPENDIX VII: Comparative Genomic Hybridization Protocol | 34 |
| MODULE ONE REFERENCES | 36 |
| QUESTIONS FOR MODULE ONE | 36 |
| | |
| MODULE TWO: 24-Colour Karyotyping..... | 41 |
| 1.0 Introduction to 24-Colour Karyotyping | 42 |
| 1.1 MFISH..... | 45 |
| 1.2 Spectral Karyotyping (SKY) | 47 |
| 1.3 MFISH versus SKY | 49 |
| 2.0 MFISH Methodology..... | 50 |
| 2.1 MetaSystems 24 Xcyte Assay | 50 |
| 2.2 VYSIS SpectraVysion™ Assay..... | 53 |
| 3.0 MFISH Analysis and Interpretation..... | 57 |
| 4.0 Limitations | 59 |
| 5.0 Clinical Applications..... | 59 |
| 6.0 Related Methodologies..... | 61 |
| 6.1 Cross-species colour banding technique..... | 61 |
| 6.2 High resolution multi-colour banding (Mband) | 62 |
| | |
| APPENDIX I: Summary of the 24 Xcyte MetaSystems Assay Procedure..... | 66 |

| | |
|--|-----------|
| APPENDIX II: Summary of the SpectraVysion™ Assay Procedure .. | 70 |
| MODULE TWO REFERENCES | 73 |
| QUESTIONS FOR MODULE TWO..... | 74 |
| MODULE THREE: DNA Microarrays | 81 |
| 1.0 Introduction to DNA Microarray Technology..... | 82 |
| 2.0 The Principle..... | 82 |
| 3.0 Arrays..... | 83 |
| 4.0 DNA Microarray Procedure | 83 |
| 5.0 Applications..... | 84 |
| 5.1 Identification of sequence (gene/gene mutation)..... | 84 |
| 5.2 Determination of expression level of genes | 84 |
| 5.3 Detection of genomic gains and losses (array CGH)..... | 87 |
| 6.0 Conclusion..... | 89 |
| MODULE THREE REFERENCE AND RELATED READING | 90 |
| QUESTIONS FOR MODULE 3 | 91 |
| AFTERWORD | 92 |

LIST OF FIGURES

MODULE ONE

| | |
|---|----|
| Figure 1: Comparative genomic hybridization methodology | 4 |
| Figure 2: Principle of CGH analysis..... | 5 |
| Figure 3: Evaluation of nick translation products by gel Electrophoresis | 9 |
| Figure 4: Digital image acquisition | 12 |
| Figure 5: CGH metaphase and karyotype | 13 |
| Figure 6: Generation of relative fluorescence intensity profile..... | 14 |
| Figure 7: CGH fluorescence intensity profile: 46,XY..... | 15 |
| Figure 8: CGH fluorescence intensity profile: 46,XY, iso8q | 17 |
| Figure 9: G-banded 17p+ and corresponding CGH profile..... | 19 |
| Figure 10: G-banded 4q+ and corresponding CGH and FISH Analysis | 20 |
| Figure 11: G-banded 12q+ and corresponding CGH and FISH Analysis | 21 |
| Figure 12: CGH analysis offers advantages for analysis of spontaneous abortions..... | 22 |

MODULE TWO

| | |
|---|----|
| Figure 1: G-banded karyotype of normal human male (46,XY) | 42 |
| Figure 2: 46,XY SKY karyotype | 43 |
| Figure 3: 46,XY MFISH karyotype (Metasystems)..... | 44 |
| Figure 4: Combinatorial labeling..... | 45 |
| Figure 5: Epi-fluorescence microscope | 46 |
| Figure 6: MFISH digital imaging..... | 47 |
| Figure 7: SKY imaging | 48 |
| Figure 8: Metasystems 24 Xcyte colour labelling scheme | 51 |
| Figure 9: Six digital images comprise a MFISH composite image... | 52 |
| Figure 10: An abnormal MFISH karyotype and corresponding inverted-DAPI karyotype | 53 |
| Figure 11: Vysis SpectraVysion™ colour labelling scheme..... | 54 |
| Figure 12: FISH/CGH/MFISH workstation (Vysis) | 55 |
| Figure 13: Overview of MFISH analysis..... | 56 |
| Figure 14: Fluorescence blending | 57 |
| Figure 15: Translocation demonstrated by MFISH..... | 58 |
| Figure 16: Marker chromosomes identified by MFISH..... | 60 |
| Figure 17: Cross-species colour banding..... | 62 |
| Figure 18: MFISH and Mband analysis of a lymphoma..... | 63 |
| Figure 19: Mband karyotype | 67 |
| Figure 20: Mband analysis of chromosome 1..... | 65 |

MODULE THREE

Figure 1: Expression chips86

Figure 2: Chromosomal and Array CGH88