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FINAL REPORT

INTERNATIONAL POSTGRADUATE COURSE THE POLYMERASE CHAIN REACTION IN RESEARCH, BIOMEDICAL DIAGNOSTICS AND ENVIRONMENTAL SURVEILLANCE

VENUE:

Center for Genetic Engineering and Biotechnology of Havana, Cuba (CIGBH).

HOST LABORATORY:

Molecular Biology Laboratory of Recombinant Antigens (MBLRA), Division of Immunotechnology and Diagnostics (DITD), CIGBH.

DATES:

December 5 to 15, 1994.

BACKGROUND:

Molecular techniques, such as the Polymerase Chain Reaction (PCR), can be applied to the diagnosis and epidemiology of infectious diseases. When appropriately implemented, these molecular techniques are more rapid, more specific, more sensitive, safer and less costly than existing methods. Here we proposed a hands-on course to introduce and implement PCR in order to facilitate diagnostics, epidemiology, and environmental surveillance of tropical infectious diseases and STDs in countries of limited resources. PCR is also a versatile technique used in basic science and we plan to address its utility for research purposes in the course as well. By this approach, we hoped to demonstrate the spectrum of applications of this new technique which has revolutionized molecular biology.

MOTIVATION. COURSE OBJECTIVES:

1. To provide participants with a theoretical insight of current molecular biology techniques, particularly PCR, cloning, and sequencing.
2. To provide participants with practical experience in the application of PCR to:
 - (a) diagnosis of tropical infectious diseases.
 - (b) environmental surveillance, with emphasis on the detection of pathogens in water sources.
 - (c) various uses in basic scientific research, such as: gene cloning and modification, sequence analysis, heteroduplex analysis.
3. To provide a milieu conducive to scientific interchange and network-building among participants from across Latin America and to foster scientific collaborations within Latin America as well as between the US and Latin America.

FACILITIES AND EXTERNAL SUPPORT:

The Center for Genetic Engineering and Biotechnology of Havana (CIGBH) is known in the region and around the world as a very prestigious scientific institution. The Center has modern laboratory

and lecture facilities. The Division of Immunotechnology and Diagnostics (DITD) is composed of a highly trained group of scientists with expertise in the field who could serve as professors and instructors in an international workshop. The DITD has previously acted as a host for international courses, trainings, and projects involving Latin American scientists.

External support was needed only to cover expenses involving travel and housing for students; travel, board and lodging of foreign invited professors; communications; reproduction of manuals; and the acquisition of some laboratory expendables and minor equipment. Such support was requested from the Pan American Health Organization (PAHO), and the Biotechnology Regional Program for Latinamerica and the Caribbean (BRPLC; UNDP/UNESCO/UNIDO). These institutions have previously sponsored courses and projects organized and executed by the CIGBH, and DITD.

The PAHO granted USD 10,000 that were used to cover expenses involving housing, travel, board and lodging of foreign invited professors, reproduction of manuals, and the acquisition of some laboratory expendables and minor equipment (APPENDIX 1). The BRPLC provided USD 12,500 that were used to cover expenses involving housing, travel, board and lodging of students, and the acquisition of some laboratory expendables and minor equipment (APPENDIX 2).

SELECTION OF STAFF:

The final Staff of the course was composed of 2 Cubans and 2 foreigners. The latter were selected on the basis of their knowledge in the field, and previous connections with the host laboratory. In addition, several resident scientists of the CIGBH were invited to present guest lectures on topics of their expertise (APPENDIX 3).

ANNOUNCEMENT:

Informative announcements were made using the channels of the Pan American Health Organization and directly by the CIGBH and by invited professors (APPENDIX 4). The announcement was also distributed by Dr. Rodolfo Quintero, Coordinator of the BRPLC.

STUDENTS AND SELECTION:

The students (to a maximum of 20) were to be selected from the Latin American region (Spanish and Portuguese-speaking: Dominican Republic, Cuba, Costa Rica, Guatemala, Honduras, Nicaragua, Mexico, Panama, Bolivia, Colombia, Ecuador, Peru, Venezuela, Argentina, Brazil, Uruguay, Paraguay, and Chile). The candidates should be at least university graduates, preferably with one to two years of laboratory practice in a related field, with some basic knowledge of molecular biology, and be able to read and interpret scientific articles in English. Selection of the students was to be done on the basis of the curriculum vitae and a letter of recommendation from their institution stating the foreseeable application of the techniques presented in this course.

Twenty-three applications were received: 1 from Argentina, 3 from Brazil, 5 from Mexico, 1 from Ecuador, 5 from Bolivia, 2 from Colombia, 1 from Peru, and 2 from Venezuela, 1 from Nicaragua, 1 from Guatemala, 1 from Costa Rica. Two more applications (1 from Venezuela and 1 from Colombia) were received during the "Biotecnologia 94" Conference held at the CIGBH prior to the course

The eligibility criteria mentioned were strictly followed. As stated in the announcement, an initial selection was made immediately after September 15. A final list was prepared after receiving confirmation of assistance. This included: 1 from Brazil, 1 from Ecuador, 5 from Mexico, 1 from Bolivia, 3 from Colombia, 2 from Venezuela, 1 from Nicaragua, 1 from Costa Rica, and 1 from Guatemala.

All students were informed in September regarding their acceptance. Additional information concerning support, visa arrangements, deadlines for confirmation and flight schedule, etc. were included. The organizers of the course provided assistance with visa arrangements, whenever the information was sent before the stated deadline. Two weeks before the onset of the course, some changes had to be done due to last minute financial problems of several foreign students. We included a new student from Bolivia and increased the financial support of another foreign student. A detailed list of the 16 Latin American and 6 Cuban participants is presented in APPENDIX 4.

HOUSING AND TRAVEL:

A special fund of USD 7,750.00 was designated from the BRPLC contribution to partially support expenses for 20 foreign students. These funds were delivered directly to the students by the local UNDP office at their arrival to Havana. USD 400 of financial assistance were awarded to 10 participants to reimburse airfare, and the other 10 students received USD 375 for room and board. Students were lodged in the hotel BioCaribe, located one block from the CIGBH. A low-price "package" was prepared, with double rooms. From Monday to Friday, meals were provided at the courtesy of the CIGBH. Lunch on Saturday was also de provided by the CIGBH.

PROGRAM:

The program was developed with theoretical lectures during the first hour, followed by daily practical laboratory sessions from Monday to Friday, including Saturday morning as well. The contents were the following:

1. Theory - Molecular biology: fundamentals of molecular biology; cloning; sequencing, applications in research and clinical settings; transfer of molecular technology. PCR: fundamentals of the Polymerase Chain Reaction; applications of PCR to diagnostics and basic research, state of the art technology. HIV: molecular biology, sequence variation, heteroduplex analysis.

2. Practical -

(a) In research: Amplification of variable regions of HIV gp120 from infected human genomic DNA, introduction of restriction sites, cloning, Plasmid purification, restriction analysis. Cycle-sequencing, sequence analysis and alignment with the use of software. Heteroduplex analysis of HIV sequence variability (heteroduplex formation, polyacrylamide gel electrophoresis, visualization and analysis).

(b) In diagnostics: Detection of Mycobacterium tuberculosis in sputum samples. Identification and typing of dengue virus. Detection of PCR products by agarose gel electrophoresis.

(c) In environmental surveillance: screening of water sources potentially contaminated with Vibrio cholerae. Manual cycling and detection of PCR products by electrophoresis in agarose gels.

These practical demonstrations were divided in operative blocks, under the guidance of staff professors, and projected to be developed in 10 working days. Free time (e.g. during PCR amplification) allowed the opportunity for study, interaction between students and professors.

planning of collaborative projects, and interaction with other aspects of the work of the Division, as per individual interests of the students. A detailed practical manual (APPENDIX 6) was prepared, with selected references illustrative of the techniques in use.

Four days were devoted to exploring the application of PCR to detection of infectious pathogens, and six days to the use of PCR in basic research. PCR techniques commonly used in investigation were introduced using as a vehicle a project involving analysis of HIV sequence variability in perinatal transmission. Thus, course participants carried out a number of different applications of PCR, in addition to the indispensable process of cloning and sequencing, all within the context of a single research project. Sequence variability was assessed by cloning and sequencing as well as by the new technique of heteroduplex analysis, and the two processes, and the information generated by each, were compared and discussed. 10 hours of lectures were dedicated to theory, such that participants were provided with a theoretical basis for each technique executed in the laboratory. The rest of each day was spent in the practical sessions.

Students were divided into 4 groups of 5, each led by an instructor, and the experiments were carried out by each of the working groups. Due to this organization, all students participated directly in the experiments. A detailed description of the Final Program can be found in the first pages of the Manual (APPENDIX 6). A total of 85 hours of laboratory activities were carried out.

At the end of the course, a general discussion of the workshop was conducted, where participants voiced their impressions and comments regarding the course. In addition, a written course evaluation was completed by all participants and is included in APPENDIX 7. All course participants (students and professors) were provided with a Certificate of completion (APPENDIX 8).

The students had access to the library of the CIGBH, and were provided with free-of-charge photocopy service.

One special session, conducted by the General Director of the CIGBH, Dr. Manuel Limenta, was devoted to discuss the history, structure, goals, and accomplishments of the Center, and the role of Biotechnology in Cuba and Latinamerica.

CONCLUSIONS:

The shared contribution from PAHO and UNIDO allowed us to organize an ambitious course, in terms of its postgraduate level, the high number of participants, and complexity of the program. Due to the way in which the Theory and Practical Programs were interlaced, direct participation by all of the students in each of the experiments was maximized. The organization of the workshop was highly praised by course participants, and enabled an enormous amount of theoretical information and technical knowledge to be disseminated in a short period of time.

The group of students was excellent, and warm contacts were established with the staff of the host laboratory and the invited professors, as well as among themselves. In addition, of their own initiative, course participants formed a network for mutual support and the advancement of Biotechnology in Latin America. A number of collaborations were initiated including the

organization of future courses in other Latin American countries, the definition of projects involving implementation of molecular techniques by workshop participants with course professors as consultants, and collaborations between laboratories in the US and Latin America to work together in the analysis of specified sample sets, combining expertise and resources from both sides.

Taking into account the overall evaluation of the students (APPENDIX 8), and the opinions gathered from students and foreign professors, we can conclude that the course was extremely successful and that its objectives were amply achieved.

COURSE DIRECTOR:

Jorge V. Gavilondo, Ph.D, Head, Division of Immunotechnology and Diagnostics, Center for Genetic Engineering and Biotechnology, P.O.Box 6162, La Habana 10600, Cuba. Fax: 53-7-218070, 336008, e-mail: inmdiag@ingen.cigb.edu.cu

APPENDIX 1: Expenses covered with PAHO funds.

A total of USD 10,000 were awarded to the CIGBH.

The funds were used to cover:

| | |
|--|-------------|
| 1. Prof. Christine Rousseau, air ticket: | USD 940.78 |
| 2. Prof. Rousseau board and lodging: | USD 400.00 |
| 3. General overhead expenses (facilities) | USD 300.00 |
| 4. Expenses in meals provided at the CIGBH from Monday to Friday. (USD 20.00/day for 16 students, 10 days) | USD 3200.00 |
| 5. Minor Laboratory Equipment and Reagents: | |
| Camera Polaroid FB PDC 34: | USD 362.88 |
| Camera Chamber FB PDH 1314: | USD 68.40 |
| Films 667 (16 Boxes) | USD 227.88 |
| Freezer | USD 230.00 |
| Microwave Oven | USD 190.00 |
| Electrophoretic Chamber | USD 610.00 |
| Electrophoresis Chamber (Gel apparatus) | USD 330.00 |
| Electrophoresis Power Supply | USD 1500.00 |
| Semi/Dry Transfer | USD 1300.00 |
| Boheringer Manheim restriction enzymes | USD 113.00 |
| Millipore Filters/manifolds | USD 108.25 |
| BioRad DNA Markers | USD 99.75 |
| Total: | USD 9980.16 |

APPENDIX 2: Expenses covered with BRPLC funds

A total of USD 12,500 were awarded for the course.

USD 7750.00 were assigned to the UNITED NATIONS DEVELOPMENT PROGRAMME Field Office in CUBA, for travel subsidy and allowance for participants. This amount was distributed directly to each foreign participant in Havana.

The USD 4750.00 awarded to the CIGBH were used to cover:

| | |
|---|--------------|
| 1. Prof. Eva Harris board and lodging | USD 400.00 |
| 2. Promotion, photocopies and printed material: | USD 1,498.00 |
| 3. Communication expenses: | USD 1,000.00 |
| 4. Minor Laboratory Equipment and Reagents: | |
| Computer, IBM compatible | USD 1775.00 |
| NEB Enzymes | USD 76.21 |

Totals: USD 4749.21

APPENDIX 3: Composition of the Staff, including guest lecturers.

STAFF

Eva Harris, Ph.D., Intercampus Program in Molecular Parasitology, University of California at San Francisco, 3333 California St. Suite 150, San Francisco, CA 94118, USA. Fax: (415) 476-0664, Tel.: (415) 476-6850, E-mail: eharris@cgl.ucsf.edu

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Christine Rousseau, M.Sc., Mary Claire King Laboratory, School of Public Health, 140 Warren Hall, University of California at Berkeley, Berkeley, CA 94720, USA. Fax: (510) 642-0687, Tel. (510) 642-0285, E-mail: rousseau@mendel.berkeley.edu.

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LABORATORY TECHNICIANS

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GUEST LECTURERS

Carlos Duarte, M.Sc. Head of the AIDS vaccine department. Vaccines division. Center for Genetic Engineering and Biotechnology. P.O.Box 6162, La Habana 10600, Cuba. Fax: 53-7-218070, 336008

Julio Fernandez, M.Sc. Molecular Biology Laboratory. Pharmaceutical Division. Center for Genetic Engineering and Biotechnology. P.O.Box 6162, La Habana 10600, Cuba. Fax: 53-7-218070, 336008

Verena Muzio, MD. Cloning and Expression in Yeast Laboratory. Vaccines Division. Fax: 336008

APPENDIX 4: Course Announcement

CURSO TEORICO-PRACTICO INTERNACIONAL DE POSGRADO

La Reacción en Cadena de la Polimerasa en la Investigación, el Diagnóstico Biomédico, y el Control Ambiental

La Habana, Cuba. Diciembre 5 al 15, 1994

Organiza:

Centro de Ingeniería Genética y Biotecnología (CIGB), La Habana, Cuba.

Auspician:

- Programa Regional de Biotecnología PNUD/UNESCO/ONUDI para Latinoamérica
- Organización Panamericana de la Salud/OMS

Lugar:

División de Inmunotecnología y Diagnóstico, CIGB, Ave. 31 entre 158 y 190,
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Coordinador:

Jorge V. Gavilondo, Ph.D. Jefe, División de Inmunotecnología y Diagnóstico, CIGB.

Profesores Principales:

1. Eva Harris, Ph.D., Intercampus Program in Molecular Parasitology, University of California at San Francisco, USA. Fax: (415) 476-0664. E-mail: eharris@cgl.ucsf.edu.
2. Marcelo Nazábal, M.Sc., Laboratorio de Antígenos Recombinantes, Depto. de Diagnóstico, División de Inmunotecnología y Diagnóstico, CIGB.

Alcance y Objetivos:

Especialmente desde de la aparición de la Reacción en Cadena de la Polimerasa (PCR), las técnicas moleculares se han convertido en una herramienta indispensable para el diagnóstico y los estudios epidemiológicos de las enfermedades infecciosas. Cuando se implementan apropiadamente, estas técnicas son mas rápidas, específicas, sensibles, seguras y económicas que otros métodos existentes. Este curso se propone dar a los participantes una visión teórica de técnicas en biología molecular, y en particular de la PCR, así como una experiencia práctica elemental en la aplicación de la PCR para: (a) el diagnóstico de enfermedades infecciosas, (b) el control ambiental, con énfasis en la detección de patógenos en agua y (c) la investigación básica.

Contenido:

Teoría- Nociones elementales de biología molecular. Clonación y expresión de genes. Secuenciación de ADN PCR: origen, desarrollo y aplicaciones. Uso de sondas de ADN. Información básica sobre los agentes patógenos a detectar.

Práctica- Control ambiental: detección de *Vibrio cholera* en muestras de agua. Investigación diagnóstica: Tipificación de virus Dengue. Detección de *Mycobacterium tuberculosis* en muestras clínicas. Investigación básica: Clonación de genes de secuencia desconocida. Introducción de sitios de restricción. Construcción de genes artificiales. Introducción de epítopes antigénicos. Generales: PCR en condiciones de laboratorio avanzado. PCR en condiciones de laboratorios con bajos recursos.

Participantes y Requisitos:

El curso tendrá cupo para un máximo de 20 estudiantes, provenientes de países de habla hispana y portuguesa de Latinoamérica. Los estudiantes deben ser graduados universitarios, preferiblemente con uno o dos años de trabajo de laboratorio, conocimientos básicos de biología molecular, y capaces de leer y entender inglés.

Admisión y Apoyo:

El staff de profesores seleccionará a los participantes sobre la base de documentos de su institución que certifiquen el nivel de posgrado, sus actividades investigativas actuales y experiencia práctica, y las razones que motivan su interés en el curso. La selección tendrá en cuenta también el balance de asistencia entre los diferentes países y la fecha de llegada de las solicitudes. Se debe emplear fax, correo DHL o correo electrónico para hacer llegar la información a la dirección del curso. Existirá un fondo de ayuda económica para un número limitado de los participantes, que cubrirá : (a) USD 400.00 del costo del pasaje, (b) alojamiento y alimentación por los días del curso. Los candidatos deben argumentar la necesidad de apoyo financiero. La fecha límite de recepción de las solicitudes es el **15 de septiembre**.

Evaluación:

Se realizará una evaluación final sobre teoría y práctica. Se emitirá un certificado de asistencia y constancia de evaluación.

Nota:

El curso se celebrará la semana siguiente al evento Internacional Biotecnología Habana'94 (noviembre 28 a diciembre 3). Los participantes al curso pueden aprovechar la oportunidad para asistir a esta importante reunión científica. Para más información respecto al evento, contacte al Comité Organizador a través de los mismos números de fax ya mencionados, y el correo electrónico biot94@ingen.cigb.edu.cu.

APPENDIX 5: List of Course Participants

1. Betzabe Rodriguez. Ministerio de Salud de Nicaragua. C.N.D.R., Managua. 505-2-97723
2. Jorge A. Alvarado A. Universidad de Costa Rica 506-224-6730
3. Blanca E. Rivera Chavira. Facultad de Ciencias Químicas, UACH, Chihuahua, Chihuahua, México.13-7462
4. Maria del C. Gonzalez Horta. Facultad de Ciencias Químicas, UACH, Chihuahua, idem ant.
5. Isidro Contreras Morales. Facultad de Ciencias Químicas, UACH, Chihuahua, idem ant.
6. Amelia Valdez Aguirre. Facultad de Ciencias Químicas, UACH, Chihuahua, idem. ant.
7. Ruth M. Valbuena Serrato. Corporacion Colombiana de Investigación Agropecuaria (CORPOICA), Santafé de Bogota, Colombia. 2445460, 2697587
8. Elizabete José Vicente. Universidad de Sao Paulo, Instituto de ciencias Biomedicas, Caixa Postal 66208-CEP 053889-970, Sao Paulo, Brasil.55-11-8187420, 55-11-8187350
9. Fernando Martinez Morales Ceingebi, Cuernavaca, México. e-mail: ferm@pbr322.ceingebi.unam.mx
10. Mauricio Espinel. Instituto Juan Cesar Garcia, Ecuador. e-mail: mespinel@ijuga.ecx.ec
11. Rolando G. Aguilera Mejia. Universidad San Carlos, Fac. Agronomia. Ciudad Universitaria Zona 12. Guatemala, Guatemala. e-mail: cberges@nicarao.apc.org. 502-2-769770
12. Edgar Orlay Valbuena Ussa. Avenida 81, No. 50-54, apto 313, Universidad Pedagógica Nacional, calle 73, no.11-95, A.A. 75144, Santafé de Bogotá, Colombia. 2111293, 2866269, 2352044 ext. 177
13. Nirsa Teresa Quintero. Av. El Parque, Edf, Leonardo PB. Apt. 1 "Las Acacias", Caracas, D.F., Venezuela. 814800 casa:02817147, Trab:02838330
14. Jorge Olivares Plaza. Universidad Mayor de San Andrés, Instituto de Genética, La paz, Bolivia. 591-2-359593, 591-2-359613, casa:791310
15. Patricia Olaya Garcia. Laboratorio de Diagnostico Bioenner. Transv 27, no.: 123-59, Bogota, Colombia. 6122161, 213863
16. Nola Montiel. Universidad de Zulia, Facultad experimental de Ciencias. Av. Universidad Edif. Grano de Oro, Division de Posgrado. Maracaibo, Venezuela. PO. Box: 526.58-51-524310, 58-51-529432
17. Maida Candelario Frontela. Center for Genetic Engineering and Biotechnology. P.O.Box 6162, La Habana 10600, Cuba.53-7-218070
18. Raul Poutou. Center for Genetic Engineering and Biotechnology. P.O.Box 6162, La Habana 10600, Cuba. 53-7-218070
19. Minerva Yaniz. Center for Genetic Engineering and Biotechnology. P.O.Box 6162, La Habana 10600, Cuba. 53-7-218070
20. Lazaro Miranda Piñeiro. Laboratorio Nacional del SIDA, Habana, Cuba. Aptdo: 23031.53-7-331682, 53-7-787981
21. Liatsy Lauzan Perez. Instituto de Medicina Tropical " Pedro Kouri". La Habana, Cuba. PO. Box: 601. e-mail: kouri@ceniai.cu. 53-7-336051, 53-7-204911
22. Antonio Miranda Cruz. Centro de Ingenieria Genetica y Biotecnologia Camagüey 1. Camagüey, Cuba. PO. Box: 387. e-mail: dir@cigbcam.cigb.edu.cu53-7-218070, 53-322-61587

APPENDIX 6: Course Schedule and Manual.