

2009 ANNUAL REPORT OF THE GLOBAL SCIENCE COPRS (GSC) MISSIONS TO THE UNIVERSITY OF BUEA, CAMEROON

Missions' periods: June 2009 and September 2009

Presented by: **Appolinaire Djikeng, Ph.D.**

I. Summary of Work Completed

The year 2009 was my second year of teaching at the University of Buea. Two courses were scheduled to cover my two annual trips to the University: Molecular Biotechnology and the integrated laboratory practicals. The two courses were part of the Master degree program in Biochemistry. The first course was conducted in two sessions. The first session was in June 2009 for a total of 40 classroom hours. During this session the following course outline was covered using a combination of lectures, students individual and group reading and classroom presentations.

Section I:	Fundamentals of Molecular Biotechnology
Chapter 1:	The Molecular Biotechnology Revolution
Chapter 2:	Molecular Biotechnology – Biological Systems
Chapter 3:	DNA, RNA and Protein Synthesis
Chapter 4:	Recombinant DNA Technology
Chapter 5:	Chemical Synthesis, Sequencing and Amplification of DNA
Chapter 6:	Manipulation of Gene Expression in Prokaryotes
Chapter 7:	Heterologous Protein Production in Eukaryotic Cells
Chapter 8:	Directed Mutagenesis and Protein Engineering

Section II:	Molecular Biotechnology of Microbial Systems
Chapter 9:	Molecular Diagnostics
Chapter 10:	Therapeutic Agents
Chapter 11:	Vaccines
Chapter 12:	Synthesis of Commercial Products by Recombinant Microorganisms
Chapter 13:	Bioremediation and Biomass Utilization
Chapter 14:	Plant Growth-Promoting Bacteria
Chapter 15:	Microbial Insecticides
Chapter 16:	Large-Scale Production of Proteins from Recombinant Microorganisms

Section III:	Eukaryotic Systems
Chapter 17:	Genetic Engineering of Plants: Methodology

Chapter 18:	Genetic Engineering of Plants: Applications
Chapter 19:	Transgenic Animals
Chapter 20:	Human Molecular Genetics

Section IV: Regulating and Patenting Molecular Biotechnology

Chapter 21:	Regulating the Use of Biotechnology
Chapter 22:	Patenting Biotechnology Inventions

After the course several exchanges continued by email to further discuss with students on selected topics and also supply them with additional reading material. In addition to teaching during this first trip, I took the opportunity to assess the needs for laboratory practicals as part of the second course. The needs were grouped in two categories: a) laboratories reagents, consumables and b) small laboratories instruments/equipments such as pipettes.

The second annual trip took place in August-September 2009. During this trip I held discussion sessions with students for the review of the course taught during the June teaching visit. In addition to teaching, during this trip I donated small laboratory equipments with a total value of ~ USD 7900. This decision to invest on the purchase of small lab equipments was in anticipation to the development of a modern laboratory teaching modules for MSC students as they prepare to enter the world of advanced scientific research. The donation of the lab equipments was very well received by the University authorities and was viewed as a major contribution to strengthening the research and teaching capacity at the University of Buea.

II. Outlook for 2010 and beyond

For the year 2010, three trips are planned for PhD and MSC courses. The first two visits will concentrate on classroom teaching for PhD and MSC courses. The third trip will focus (pending funding for laboratory reagents for a project outlined in Annex 1) on laboratory practicals for MSC and also PhD students. This will indeed be an opportunity to set the stage for my participation in MSC and PhD research projects supervision and co-supervision. Once implemented, the project/course outlined in Annex 1 (as is or in

various formulations but focusing on the same techniques) will be conducted during two additional years beyond 2010. Its relevance especially in the context of the “post-genomics era” is anticipated to achieve the following goals:

- 1- Introduce MSC students to a wide range of Molecular Biology and Biochemical techniques very early in their research career.
- 2- Provide a mechanism (in combination with my other courses in genomics, functional genomics and bioinformatics resources) for the identification of relevant MSC and PhD thesis research projects in areas including but not limited to functional characterization of parasites (Malaria, trypanosome, onchocerciasis, ...) genes as vaccines, diagnostics and drug target candidates.

III. Conclusion

As a remain affiliated with the JCVI (J Craig Venter Institute) in Rockville, Maryland, I have for the last 6 months been primarily working with the BecA-ILRI hub (Biosciences eastern and central Africa – International Livestock Research Institute) with responsibilities in scientific research, infrastructure management and setting up a genomics platform in support of research, capacity building and research-related services programs in animal, plants and microbial science. Leveraging these opportunities, my students at the University of Buea will continue to be exposed to biosciences research areas that are relevant to Africa’s needs especially in human and animal health, agricultural development and environmental studies.

The support from the University administration and the department of biochemistry has been outstanding. In my opinion the University of Buea has revolutionized university education in Cameroon in the relevance of the courses, thesis research projects, the quality of its graduates and the overall quest for excellence.

Annex 1: Pending Integrated lab course proposal (MSC and PhD).

BCH 613: INTEGRATED MSC LABS

Instructor Appolinaire Djikeng, Ph.D.
Assistant Professor
Infectious Diseases
The J Craig Venter Institute (JCVI)
Rockville, MD 20850, USA

Project Title Cloning and enzymatic characterization of the *E. coli* ribonuclease III (RNase III) enzyme.

This course is designed as part of the Institute of Advanced Study (IAS)-funded Global Science Corps (GSC) model for the University of Buea, Cameroon. The proposed course will be taught twice during the two remaining years of the GSC funding. It aims at providing a solid laboratory experience to graduate students embarking on research. Furthermore, this series of experiments will set the stage for the preparation of the University of Buea as a participating institution for future GSC-related activities and also for activities in the context of the African Renaissance Institute for Science and Technology (ARIST), which has been launched and will soon be extended to the University of Buea.

Course Objective

The overall objective of this course is to introduce MSC students to a wide range of biomedical research approaches and techniques including **scientific literature search** and **review, bioinformatics, molecular biology, functional genomics** and **enzymology**. As part of this course, students will actively participate in the amplification of the bacterial RNase III enzyme, its cloning into an expression vector, purification of the recombinant protein and testing of its activity on in vitro synthesized double stranded RNA. At the end of the course each student will possess the skills required to conduct experiments utilizing advanced molecular biology and genomics techniques. In addition, each student will acquire basic bioinformatics skills.

Overview of RNase III enzymes

Processes of maturation and degradation of RNA molecules are essential features in the context of gene expression, and provide the two main points for post-transcriptional regulation. Prokaryotic and eukaryotic cells employ a functionally diverse array of nucleases to carry out RNA maturation and turnover. RNases play an important role in the turnover of RNAs and particularly in the processing of precursor RNAs, creating the mature, functional RNAs. The ribonuclease III (RNase III) family currently is one of the most interesting families of endoribonucleases partly because they are found in every class of prokaryotes and eukaryotes. Viruses also employ cellular ribonucleases, or even use their own in their reproductive cycles. Recent studies of the regulation of gene expression have demonstrated that RNase III – like proteins are involved in the mechanism of RNA silencing and related processes. RNase III contains one of the most prominent protein domains used in RNA-protein recognition, the double-stranded RNA binding domain (dsRBD). The bacterial (*E. coli*) RNase III and other RNase III-like proteins use the double stranded RNA (dsRNA) as substrate which is degraded into short RNA fragments of sizes varying from ~ 10 to ~ 21 nucleotides.

Course content

The course will contain the following sections.

I. REVIEW OF THE LITERATURE ON RNASE III ENZYMES.

As part of this activity, students will learn how to critically review published literature, extract and summarize useful information. Each student or a group of 2 to 3 students will review a selected paper and make a presentation to the rest of the class in the form of journal club. Each presentation will last approximately 20 minutes followed by questions and answers and an overall discussion. An emphasis will be put on the methods used in the reviewed paper.

II. BIOINFORMATICS ANALYSIS (DATABASE SEARCH, DOWNLOADING AND ANALYSIS OF THE SEQUENCES OF THE *E. COLI* RNASE III ENZYME).

Students will be introduced to the NCBI database and other public domain integrated databases for scientific literature and genes/genomes sequences searches. Both protein and nucleic acid sequences of the *E. coli* RNase III will be downloaded to local computers for further analysis. The analysis will include the restriction enzyme mapping, the design of specific primers for amplification of the open reading frame (ORF) and choice of restriction enzymes for cloning into the expression vector. The primers for use to PCR amplify the RNase III protein-coding sequence will be designed based on the downloaded sequence.

III. AMPLIFICATION AND CLONING OF THE *E. COLI* COMPLETE OPEN READING FRAME INTO A PROTEIN EXPRESSION VECTOR.

E. coli genomic DNA will be prepared for use as template for the PCR amplification of the *E. coli* RNase III complete protein coding sequence. The PCR product will be analyzed by agarose gel electrophoresis. The PCR product and the plasmid vector will be digested with compatible restriction enzymes. The digested PCR product will be cloned into the digested vector to generate a recombinant plasmid containing the *E. coli* RNase III.

IV. EXPRESSION AND CHARACTERIZATION OF THE RECOMBINANT *E. COLI* RNASE III ENZYME.

The recombinant plasmid will be induced for protein expression followed by its purification by affinity chromatography. The activity of the purified recombinant protein will be tested on the dsRNA substrate. The dsRNA substrate will be prepared by *in vitro* transcription followed by annealing of two complementary single stranded RNAs.

V. EXPERIMENTAL PROCEDURES

V.1. Bioinformatics resources for nucleotide and amino acid sequences analysis (Database searches, identification and analysis of the *E. coli* RNase III genes)

Brief introduction to NCBI (Entrez and Medline)

Use of accessions numbers to query the NCBI database

Introduction to other databases (EMBL, etc ...)

Download the *E. coli* RNase III nucleotide and amino acid sequences.

Analysis of the *E. coli* RNase III protein sequences (Multiple alignments, phylogenetic analysis, and identification of protein domains)

Analysis of the *E. coli* RNase III nucleotide sequence (restrictions enzymes mapping, identification of restriction enzymes with and without sites in the nucleotide sequence, design of forward and reverse primers for the amplification of the protein coding region).

Reagents needed, cost and availability:

Computers with connection to the internet (Available)

DNA analysis software: DNASTAR (Available)

V.2. Amplification and purification of the *E. coli* RNase III gene

Preparation of *E. coli* total genomic DNA using the QIAGEN DNeasy kit.

Amplification of the *E. coli* RNase III complete ORF using the primers designed in section A.

Reagents needed, cost and availability:

0.5 ml eppendorf tubes (Available)

1.5 ml eppendorf tubes (Available)

Agarose (Available)

Tris Borate EDTA (TBE) buffer (Available)

Tris Acetate EDTA (TAE) buffer (Available)

Ethidium bromide (Available)

DNA loading dye (Available)

Purification of the PCR products (QIAGEN, PCR purification kit. Cat. # 28104; USD 96.00)

Genomic DNA purification Kit (QIAGEN; DNeasy kit Cat. # 69504; USD 133.00)

Oligonucleotides: (Invitrogen; custom made; already available)

BR3-FB: 5' CGC GGA TCC AAC CCC ATC GTA ATT AAT CGG CTT CA 3'

BR3-R: 5' GAC GTC CGA CGA TGG CAA TAA ATC CG 3'

BR3-RX: 5' CCG CTC GAG GAC GTC CGA CGA TGG CAA TAA ATC CG 3'

Nuclease free molecular biology grade water (Available)

DNA amplification system (Pfx50™ DNA Polymerase; Invitrogen, Cat. # 12355-012; 100 reactions; USD 127.00).

DNA molecular weight markers (Invitrogen, USD 155.00)

V.3 Cloning of the RNase III into the pGEX expression system

Preparation of the pGEX-2T and pGEX-4T1 cloning vectors as follows:

Digestion of pGEX-2T with *Bam*HI + *Sma*I

Digestion of pGEX-4T1 with *Bam*HI + *Xho*I .

Digestion of the PCR product with *Bam*HI and *Hind*III.

Gel purification of digested vector and insert.

Ligation of the insert to the digested vector.

Transform competent *E. coli* DH5a cells.

Screening of recombinant clones.

Purification of recombinant plasmid DNA

Reagents needed, cost and availability:

pGEX-2T (GE Healthcare; Cat. # 27-4801-01; USD 473.00)

pGEX-4T1 (GE Healthcare; Cat. # 27-4580-01; USD 473.00)

*Bam*HI (NEB; Cat. # R0136S; USD 53.00)

*Sma*I (NEB; Cat. # R0141S; USD 56.00)

*Xho*I (NEB; Cat. # R0146S; USD 63.00)

*Hind*III (NEB; Cat. # R0104T; USD 53.00)

Rapid DNA ligation kit (Roche; Cat. # 1 635 379; USD 306.00)

Competent DH5a cells (Invitrogen; Cat. # 18258-012 USD 350.00)

Plasmid DNA preparation kit (QIAGEN; Cat. # 12123; USD 166.00)

Gel purification kit (QIAGEN, Cat. # 28704; USD 96.00)

LB broth premix (Fischer Scientific, USD 200.00)

LB Agar premix (Fischer Scientific, USD 200.00)

Ampicillin (Available)

Transformation tubes (Available)

100% Ethanol molecular biology grade (Available)

V.4 Expression, purification and functional characterization of the recombinant *E. coli* RNase III enzyme

Transform competent BL21 *E. coli* cells.

Induce recombinant BL21 *E. coli* cells for protein expression.

Purify the recombinant protein by affinity chromatography.

Acrylamide gel electrophoresis for the analysis of the recombinant protein.

Preparation of the dsRNA substrate:

- Synthesize both sense and antisense ssRNAs using the T7 RNA polymerase.
- Purify the ssRNA by size selection chromatography.
- Anneal sense and antisense RNA to generate dsRNA

dsRNA degradation reaction using the recombinant *E. coli* RNase III.

Acrylamide and/or agarose gel electrophoresis for the analysis of the degraded dsRNA.

Reagents needed, cost and availability:

BL21 *E. coli* cells (GE Healthcare; Cat. # 27-1542-01; USD 47.00)

Protein staining system (Available)

IPTG (Available)

GST SpinTrap Purification Module (GE Healthcare; Cat. # 27-4570-03; USD 392.00)

Acrylamide gels (Available)

Protein markers (Available)

T7 transcription kit (Epicentre Tech, T7 Ampliscribe Cat. # AS2607; USD 155.00)

P-6 column (BioRad Cat. # 732-6222, USD 200.00).

V.5 Data analysis and student report

Each student will write the experiment report in the form of a short manuscript for publication with relevant references. Each student will choose the format of illustration such as figures, tables and graphs.

Budget

Laboratory Consumables:

USD 3794.00

Cost of shipping and handling: It could cost as much as USD 5000.00 to ship these products to the University of Buea, Cameroon.