INSTITUT SENEGALAIS DE RECHERCHES AGRICOLES

UNITE DE PRODUCTION DES VACCINS (UPV)

LABORATOIRE NATIONAL DE L'ELEVAGE ET DES RECHERCHES VETERINAIRES – BP 2057 – DAKAR-HANN

COMPTE RENDU DE MISSION

A **l'ELS** de San **francisco** et à l'Université de Californie, Davis, CA, USA
dans le cadre de transfert technologique en recombinaison génétique
pour la mise au point de vaccins recombinants
du 03 avril au 04 août 1999

par M. KONTE
chef de l'Unité de Production de Vaccins

Réf. 0131 Kotto-Ame

Réf. UPV/Septembre 1999

COMPTE RENDU DE MISSION

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IDENTITE DU MISSIONNAIRE :

Prénom : Mamady
Nom : KONTE

OBJET DE LA MISSION :

Formation dans les nouvelles méthodes de Biologie moléculaire, des gènes recombinants en particulier et transfert de technologies en matière de mise au point de vaccins de type recombinant. La formation scientifique et technique est précédée d'une participation à un cours intensif d'anglais pour une remise à niveau linguistique.

PAYS ET LIEUX DE FORMATION

Etats-Unis d'Amérique (USA) : 4 mois : du 03 avril au 4 août 1999 :
Cours intensif d'anglais : ELS Language Centers de San Francisco en Californie : du 05 au 30 avril 1999.
Biologie moléculaire : Laboratoire international de Biologie moléculaire (ILMB), Université de Californie, Davis : du 03 mai au 03 août 1999

ORGANISATION/PERSONNALITES RENCONTREES

L'organisation des cours intensif d'anglais est parfaite et efficiente.

La formation scientifique et technique a été réalisé à travers l'exécution conjointe de quatre projets de recherche en attente, préalablement élaborés par les chercheurs du l'ILMB. L'exécution de chaque projet est dirigé par le tuteur scientifique qui en est l'auteur. La réalisation de l'ensemble des projets offre l'opportunité de pratiquer toutes les techniques de biologie moléculaire dans leurs conceptions les plus récentes.

FINANCEMENT

Bourse AIEA, référence C6/SEN/98006R

NATURE DE LA MISSION

Voyage d'étude et de formation spécialisées

POINTS ESSENTIELS

Le financement AIEA est consenti dans l'optique d'un transfert de technologies des pays développés aux pays en développement d'Afrique, en particulier, en application d'un principe voulu, exprimé et constamment soutenu par le Directeur de l'ILMB, le Professeur T. YILMA. Le choix de ce site de formation par l'AIEA est des plus judicieux.

Les compétences acquises dans le domaine du génie génétique autorise la conception et la réalisation d'outils biotechnologiques dans le domaine du diagnostic des maladies et l'identification des agents microbiens ainsi que dans le domaine des vaccins nouveaux de type recombinant au LNERV/ISRA.

RAPPORT DE MISSION

Deux rapports détaillés, un à mi-parcours et un autre en fin de formation, ont été produits et communiqués aux organismes de supervision de la formation (AIEA à Vienne, NRC à Washington, et ILMB de U C Davis) : copies jointes ci-après.

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FIRST TECHNICAL REPORT

Dr. Mamady KONTE
C6/SEN/98006R
Mail : mkonte@ucdavis.edu

FIRST TECHNICAL REPORT

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FELLOW NAME : Dr, Mamady KONTE

FELLOWSHIP NUMBER : C6/SEN/98006R

ADRESS IN USA : Office : c/o Dr. Tilahun YILMA, UCD-ILMB-PMI-VM
2079 Haring Hall, Davis, CA 95616

Home : 717 Adeline Place, Davis, C!A 95616

TRAINING BEGINNING DATE : May 03, 1999

TRAINING EXPECTED END DATE : August 03, 1999

TRAINING PLACE : International Laboratory of Molecular Biology
Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine-University of California, Davis
2079 Haring Hall, Davis, CA 956 16 - USA

TRAINING SUPERVISOR : Tilahun YILMA, DVM-PhD, Director and Professor
Training tutors : Shabbir AHMAD, DVM-PhD, Assistant Professor (Research)
Paulo VERARDI, PhD, Post-graduate Researcher
Zhiyun MA, MS
David WADDELL

TRAINING SCHEDULE :

1- PRELIMINARY WORK :

May 03 through May 14, 1999 : Administration aid, Housing, Documentation

May 17 through 21, 1999 : Polymerase Chain Reaction (PCR) technics and attendance course on Biosafety (may 18 at 1138 Meyer Hall, UC Davis)

Objective: Diagnostic applications of PCR

PCR performing : Preparation (extraction) of DNA from cells samples
DNA polymerization by PCR
DNA gel electrophoresis

Tutor : David WADDELL

May 24 through May 28, 1999 : PCR technics (cont'd)

Tutor : David WADDELL

II – FIRST AREA OF CONCENTRATION : Sub-cloning a gene

Project 1: Sub-cloning of IFN-gamma in a retrovirus.
Tutor : Zhiyun MA

Objective : Development of live attenuated vaccine by deletion mutation and insertion of human interferon gamma gene.

June 01 through June 11.1999 :

Preparation of the insert : pLG-Hu-IFN-gamma
Preparation of the vector: pBRp429-3'Nef/NotI
Set up the ligation vector/insert (V-I)
Transformation of E. coli by the V-I
Selection of recombinant colony and extraction plasmid DNA
Recombination check by plasmid and insert isolation

III – SECOND AREA OF CONCENTRATION : Vaceinia Virus as an Expression Veetor

Project 2 : New selection Systems for Vaccinia Virus (Zeocin and Blasticidin)

Objective : To test the feasibility of using two new antibiotics for selection of vaccinia virus recombinants in eukaryotic cells. Vaccinia virus recombinants expressing the small zeocin (375 bp) or blasticidin (398 bp) resistance genes under the p7.5 promoter will be developed. Future studies will test their potential use as a fast and reliable selection method for recombinant vaccinia viruses.

Tutor : Paulo VERARDI

3.1 Project 2 Preliminary work : June 14 through July 02, 1999 :

A)- Design primers for PCR cloning of the zeocin and blasticidin resistance genes with engineered *Xma* I (blasticidin) or Pin AI (zeocin) sites. Order primers.

B)- Perform Vent DNA Polymerase PCRs. Clean up PCRs for cloning.

3.2.Project 2 performing Week 1 : July 06 through iulv 09

C)- Cloning of zeocin and blasticidin resistance genes (PCR products) into the vaccinia virus transfer vectors pSC 11 (*Xma* I site, under p7.5). Sequencing of inserts.

3.3. Project 2 performing week 2 : July 19 through July 23, 1999 :

D)- Transfection of BS-C-l cells infected with VV WR with `pSC11Zeo' and `pSC11Bsd' transfer vectors.

E)- Recombinant vaccinia virus plaque-purification (plaque assay #1) of `vWRTK-Zeo' and `vWRTK-Bsd' with TK selection and beta-gal screening.

3.4. Project 2 performing week 3 : July 26 through July 30. 1999 :

F)- Recombinant vaccinia virus plaque-purification (plaque assay#2) of `vWRTK-Zeo' and `vWRTK-Bsd' with TK selection and beta-gai screening.

G)- Recombinant vaccinia virus characterization (histochimical staining, DNA analysis, zeocin and blasticidin resistance gene expression).

IV – THIRD AREA OF CONCENTRATION : Isolating a gene of interest by PCR

July 12 through 161999

Project 3 : Isolating and cloning of vif gene of a retrovirus in a plasmid (pVL1393) to construct pVL1393vif

Objective : Vaccine and pathogenesis studies

Tutor : Shabbir Ahmad & Zhiyun MA

V – FOURTH AREA OF CONCENTRATION : Bacnlovirus

Poject 4 : Construction, isolation, and production of a reeombinant baculovirus expressing a gene of interest

Tutor : Shabbir AHMAD

June 14 throueh Julv 30, 1999

A)- Insect cell culture

B)- Molecular cloning of gene of interest into transfer plasmid (From step IV)

C)- Transfection of insect cells with recombinant plasmid (pVL1393vif) and linear DNA of baculovirus.

D)- Identification and plaque purification of recombinant baculovirus

E)- Molecular analysis of baculovirus expressing the recombinant protein.

F)- Production and partial purification of antigen in insect cells and larvae.

G)- Development of ELISA

Last three steps will be performed if time allows.

FACILITIES AND MAIN LAB EQUIPMENT

Electric Freezer, refrigerator
Centrifuges-microfuge-cent.refr.
Spectrophometer
Water bath
Hot shaker
Magnetic stirrer
Powerpac 3000 with probe
Bio-Dot Apparatus
Rack LN storage system
Integrated speed vac
Sequencing gel
Sonic dismembratr
Maxima vac pump
New Brunswick sci. incubator shaker
Sterilization equipement
Biological Safety cabinet w/3 petcocks

Vortex-genie mixer
Napco vacuum oven
Revco Upright 20.2 CU. Ft. freezer
Napco sterilizer/autoclave w/dryer
Incubator
Thermocycler
Ultra violet transilluminator
Electrophoresis material
Electrophoresis photo material
Pipets
Ice machine
Pro-pipets
Others lab material
Chemical products and enzymes supplies
ELISA computer system Iecturer
Computers

THE FELLOW

Dr. M. KONTE

THE TRAINING SUPERVISOR

Pr. T. YILMA

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FINAL TECHNICAL REPORT

Dr. Mamady KONTE
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FINAL TECHNICAL REPORT

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FELLOW NAME : Dr. Mamady KONTE

FELLOW NUMBER : C6/SEN/98006R

ADRESS IN SENEGAL : ISRA/UPV
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Tel. : 221 832 27 62 ; Fax : 221 832 21 18
Mail : konte@elsmail.com.

TRAINING BEGINNING DATE : May 03, 1999

TRAINING END DATE : August 03, 1999

TRAINING PLACE : International Laboratory of Molecular Biology
Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine, University of California, Davis
2079 Haring Hall, Davis, CA 95616, USA

TRAINING SCHEDULE :

- 1. Diagnostic applications of PCR
- 2. Sub-cloning of IFN-gamma in a retrovirus
- 3. Vaccinia virus as an expression vector : new selection systems for Vaccinia virus (Zeocin and Blasticidin)
- 4. Isolating a gene of interest by PCR.
- 5. Construction, isolating and production of a recombinant baculovirus expressing a gene of interest.

SCHEDULE IMPLEMENTATION :

Project # 1 : 1 had the oppotunity to adapt my knowledge (since 1994) to the new approach of PCR performing and the new equipment actually in use (new thermocycler in particular).

Project # 2 : The objective was the development of a live attenuated vaccine against HIV-2 by deletion mutation and insertion of human interferon gamma gene.
The technology has been completely perfonned but we doesn't get the expected result because both Xhol and KpnI enzymes haven't allowed to isolate the insert (pLG-Hu-IFN-gamma). So, research will continue to fmd the appropriated restrictions enzymes.
In conclusion, I can say that 1 possess now this technology and I can do it again completely by myself. That's positive. However, the designing technic of appropriated primers and restriction enzymes must be repetead with trainers.

Project # 3 : Obective : to test the feasibility of using two new antibiotics for selection of vaccinia virus recombinants in eukaryiotic cells and after, to study their potential use as a fast and reliable selection method for recombinant vaccinia virus.
This technic has been performed, since ptimers designing to first plaque assays. The short time allowed have not permit to perform eompletely the necessary others plaque assays and the next stape which is the study of potential use as selection method for recombinant vaccinia virus.

Project # 4 : Objective : isolating (by PCR) and cloning of Vif gene of SIV in a plasmid (pVL1393) to construct pVL1393 for vaccine and pathogenesis studies.
The technic have been completely perfonned but no expected pVL1393vif after three remakes with different protocols. **In conclusion,** the test must be done again using others plasmid and restriction enzymes. However, 1 possess the technology and I can do it again by myself

Projeet # 5 : We have only start this project with an experimental infection of an insect (Spodoptera frugiperda) larvaes which are inoculated, with different recombinant baculovirus, part by injection with serynge, part by infected food. The dead larvaes proteins were then extracted and stored at –70°C (done) for further analysis by 1 0%PAGE/western blot (not done).
Because of the absence of the training tutor on vacation, we have not been able to perform this project which is important for us as it is the basic technic of recombinant vaccine production.

General conclusion : To master these basic molecular biology technologies, more time is necessary. So 1 wish and ask for one more or one and half more month in the next year (2000) to finalize the training schedule, important for me and my work

COURSE ATTENDANCE :

1 have completed a course of health and safety instruction entitled : comprehensive Biology and Medical Waste, on may 18, 1999 (1138 Meyer Hall, UC Davis) (enclosed certificate).

PERSONAL APPRECIATION :

Training quality : excellent ; that gives to Senegal LNERV full proficiency for its molecular biology laboratory in the areas of disease diagnostic and recombinant vaccine production, also to Dakar university (Veterinary School) and Nouakchott (Mauritania) university (science school) where 1 am in charge of molecular biology course since 1995.

MY ACTUAL DUTIES :

1. Head of Microbiology laboratory of ISRA/LNERV : laboratory management, research and diagnosis of animal diseases agents ; also quality control of veterinary vaccines producted by ISRA/UPV.
2. Director of the vaccines production unit of ISRA (ISRA/UPV) : unit management : vaccines production , administration, marketing and comptability
3. Trainer : molecular biology course for Veterinary school students of Dakar university and for biology science students of Nouakchoot university in Mauritania, since 1995.

OBSERVATIONS/SUGGESTIONS :

1. Excellent choice for the training place
2. Training schedule and trainers qualities: perfect
3. Qualities and suitabilities of equipments : perfect
4. Living conditions : acceptable ; lodging conditions to improve
5. Luggage excess weight and books allowances must be increased
6. Authorities help : correct.

Dakar, september 09, 1999

Dr. M. KONTE