PROJECT PROPOSAL

I. PROJECT PROFILE	
A. Title of the Project	Development of Portable Detection Kit for Fusarium Ear Rot Infections in Corn Phase I
B. Proponent	University of the Philippines (UPLB) Romblon State University (RSU)
C. Project Cooperators	OPAG, DENR
D. Project Duration	1 year project implementation 2 years monitoring of outcomes
E. Total Project Cost	**PhP 3,167,850.00** DOST MIMAROPA - PhP 1,061,850.00 RSU-CAS (researchers salary&facility)- PhP 1,226,000.00 UPLB-IPB (researchers salary&facility)- PhP 880,000.00
II. PROJECT PROPOSAL	
A. Rationale	In the advent of various diseases associated with unsafe food and improper food handling, more and more people are becoming keen to the food they eat. Little did we know that even what we perceived as fresh and natural food could become a source of mycotoxin that is very dangerous to health when accumulated in the body. Corn as one is the second most important staple in the Philippines and considered a banner commodity in Romblon. It is widely used as food and feed, and major raw material in most processed foods production such as cereals, chips, bread, and pastries. However, corn when not properly handled could pose health risk to humans and animals because of a common type of fungus that specifically infest corn kernels. This fungus called Fusarium Ear Rot (FER) in infected corns produces Fumonisin that was reported to cause esophageal cancer and neural tube birth defects in humans, and related diseases in animals like porcine pulmonary edema and equine leukoencephalomalacia. Several reports on the occurrence of FER that caused spoilage in many other crops such as rice, sorghum, millet, citrus, asparagus, yams, bananas and pineapples are also recorded. However, there is still no available rapid detection kit for Fumonisin in the market. This project recognizes the importance of early detection of FER in pre and post harvest conditions of corn to minimize losses and avert harmful effects of this fungi. UPLB and RSU are on its heels to address the above concern and to create something that is of value and to be relevant and responsive to contribute to community particularly to food and feed safety.
B. Project Description	This project would attempt to develop a portable detection kit for fumonisin genes using LAMP-PCR optimized protocol that was already developed by UPLB-IPB researchers. Since the procedure was already established, the UPLB will capacitate RSU in its validation before the development of kit. The kit would be similar to the concept of lab-in-a-mug technology for dengue detection using LAMP-PCR method. It comes with specific primers/markers for fumunisin gene. It will also utilize Hydroxynaphtol blue dye to

	facilitate visual detection. The RSU through its College of Arts and Sciences, and College of Engineering and Technology with supervision from UPLB-IPB will design and develop the kit. The target output/final product is expected to create big impact for food and feed processors in the assurance of quality and safe products.
C. Objectives	The general objective of this research is to validate fumonisin gene markers and develop a portable detection kit for Fusarium Ear Rot (FER) in corn using UPLB-IPB LAMP-PCR developed procedure. Specifically, it intends to: 1. validate the identified FER primers/markers, and the optimized protocol; 2. develop a portable detection kit for FER within the 1st-3rd year of project implemenation; 3. test the performance of the kit on laboratory and field; 4. collaborate with UPLB scientists/researchers in the development of kit and knowledge transfer; 5. conduct capability enhancement for the RSU researchers; 6. engage corn farmers and agricultural technicians during field testing; 7. generate other DOST 5PS for research project (people, partnership and places, protection, publication and policy); and 8. ensure safe and quality corn for food and feed industry.
D. Methodology	While the project proposal is subjected to Regional Technical Evaluation Committee (RTEC) for review, the UPLB-IPB and RSU researchers will discuss the terms of the project. Once the project is approved and funds are allotted, MOA/MOU will be crafted. The DOST-MIMAROPA with the help of the PSTC would facilitate the purchase of proposed equipment and reagents for the research. Meanwhile, related activities such as arrangement of knowledge transfer from UPLB to RSU, capability enhancement, and preparation of facilities would be done after or simultaneously with the purchase of equipment. A total of 1 lead scientist, 3 researchers from UPLB, and at least 2 researchers from the RSU will be engaged in the project. These numbers on the other hand are not restricted as declared to accommodate other contingencies during project implementation. Validation of UPLB-IPB LAMP-PCR developed procedure will be done at the RSU facility. After validation, development of the portable detection kit will follow. The prototype portable detection kit will be verified at the UPLB-IPB facility.

The following activities will be performed:

1. LAMP assay will be performed in detecting fumonisin gene with DNA as the template.

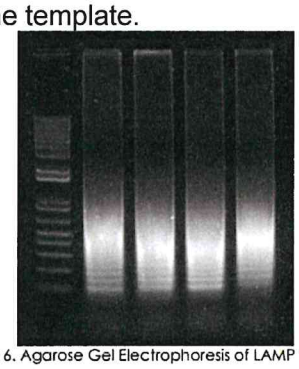

Figure 16. Agarose Gel Electrophoresis of LAMP Products

2. Primer specificity using other DNA will be tested.

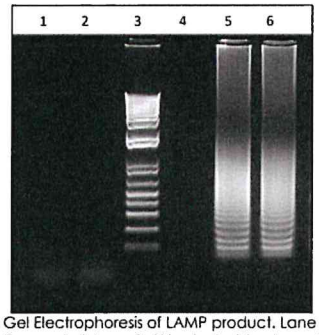

Figure 17. Agarose Gel Electrophoresis of LAMP product. Lane 1-Downy mildew of corn, Lane 2-negative control, Lane 3- 1kb plus ladder, Lane 4- blank lane, Lane 5&6- Infected corn detected with fumonisin gene.

3. Successful extraction of corn samples in kernel and pellet form will be done by adopting extraction protocol of Nick Talbot with some modification.

Figure 18. Crude DNA extracted from infected kernels and pellets.

4. Procedure tested on laboratory and post harvest facilities.

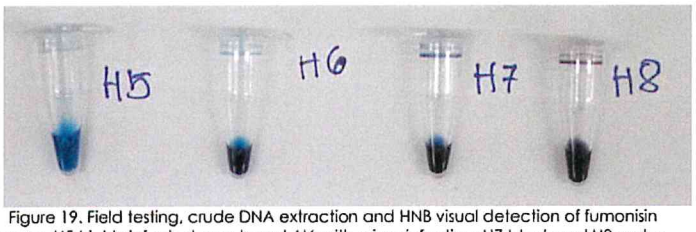

Figure 19. Field testing, crude DNA extraction and HNB visual detection of fumonisin gene. H5-highly infected corn kernel; H6-with minor infection; H7-blank and H8-water (negative control).

5. Confirmation and quantification of the procedure using ELAISA kit.

Figure 20. Quantification of H5-highly infected kernels using Agraquant ELISA Fumonsin kit

6. Development of similar prototype - portable detection kit for FER

(lab-in-a-mug detection kit, Biotek-M)

Once the technology is protected through patents, spin-off and/or commercialization will commence. Mass production of portable detection kit and production of customer's instruction manual would be done. Application of the DOST's other 5Ps would also be done. When the portable detection kit was successfully developed, its validation, mass production, and market application would be done in the 2nd phase of the project.

F. Activity Schedule

The following timetable will be followed:

Activity	2020	2021			
	3-4Q	1Q	2Q	3Q	4Q
Consultation and meeting	X				
Project proposal preparation	X				
Project proposal review	X				
Revision, approval and MOA signing	X	X			
Fund release		X			
Procurement of equipment & supplies			X		
Implementation			X		
Completion and report preparation			X	X	X
Report presentation					X

Monitoring and evaluation would be made by the PSTC-Romblon so that the performance objectives and other deliverables will be attained.

G. Budget Breakdown

The following line-item-budget would be followed for the project:

ITEM	DOST-GIA (P)	RSU (P)	UPLB (P)
Personal Services		576,000.00	180,000.00
MOOE			
Traveling Expenses - local	44,000.00		
Training Expenses			
Other Professional Services	41,600.00		
Printing and Publication	250.00		
Representation	15,000.00		
Rents-Motor Vehicles	25,000.00		
Supplies and Materials Expenses			
Office Supplies	8,800.00		
Laboratory Supplies	300,000.00		
*breakdown of supplies			
Communication Expenses	1,200.00		
Telephone Expenses			
Other Professional Services	42,000.00		
Scientist @ 4,200/mox10mos	84,000.00		
3 Researchers @ 2,800/mox10mos			
Equipment Outlay			
PCR Machine, conventional. Cfx touch 96 wells	310,500.00		
Gel Electrophoresis set-up, horizontal, with extra trays	69,500.00		
Lab in a mug @ 60,000x2	120,000.00		
Operating Funds		150,000.00	
Building and Facility		500,000.00	700,000.00
Total	**1,061,850.00**	**1,226,000.00**	**880,000.00**

H. Project Management

The project will be managed by the DOST PSTC Romblon with close collaboration with UPLB scientist and researchers, and RSU researchers. Financial matters will be handled by the DOST-MIMAROPA regional office with the assistance of RSU Administrative and Finance Services office.

I. Expected Output	*Products.* The products that would be developed out of this research is the prototype portable detection kit for FER in corn. *People*. One master's degree graduate will be considered for this research. This will come from a faculty co-operator of this research. This will also open opportunities for farmers and agricultural farm technicians to ensure quality and safe corn for consumers. Proponent will also be provided with at least 2 capability enhancement trainings such as but not limited to Training on Gene Marker Identification and Training on the Production of FER Testing Kit. At least 6 participants all in all are targeted for these trainings. *Places and partnerships*. Partnerships with the corn and other grains industry sector will be made. Partnerships with multinational companies that engage in agricultural businesses will also be explored. *Publication.* At least one paper for publication in a Scopus- indexed journal will be considered. Another knowledge product that could be copyrighted is the documented process for the research. *Protection.* An application for patent, utility model or industrial design, whichever is applicable, for the protection of intellectual property will be made. *Policy*. Once implemented and spun off, a policy for the application of the research results, outputs, products, and processes will be lobbied in the local governance.
J. Monitoring and Evaluation	Monitoring and evaluation will be made by the DOST MIMAROPA through its PSTC Romblon. Monitoring and evaluation will be centered on project deliverables as listed in the expected output.

*Breakdown of Laboratory Supplies

Technical Description	UOM	QTY	Unit Cost	Estimated Budget
MOLECULAR BIOLOGY REAGENTS/ LAB CHEMICALS				
BST DNA Polymerase; without Endonuclease and Exonuclease activity;2000u/ml	kit	4	15,000.00	60,000.00
Fumonisain-ELISA kit	kit	1	35,000.00	35,000.00
Taq DNA Polymerase System/ Kit; 500 units; with DNTPs and buffers	kit	2	4,500.00	9,000.00
1kb + Molecular weight DNA ladder 250ug	kit	1	17,000.00	17,000.00
2-propanol,(isopropanol) 2.5L	bottle	1	1,500.00	1,500.00
Absolute Ethanol,molecular grade;2.5L	bottle	1	1,900.00	1,900.00
Betaine,> 98% purity,50g	bottle	2	6,000.00	12,000.00
Custom Oligos	bases	800	25.00	20,000.00
Sodium EDTA, 500 g	bottle	1	6,000.00	6,000.00
Magnesium sulfate, heptahydrate, analytical reagent,ACS,USP and BP,500g	bottle	1	8,000.00	8,000.00
Potassium Chloride,analytical reagent ;99-100% purity,500g	bottle	1	3,600.00	3,600.00
Hydroxynaphthol blue (dye)	bottle	3	5,400.00	16,200.00
DNA gel stain 400ul	bottle	2	6,500.00	13,000.00

LAB SUPPLIES/ SMALL DEVICES				
Face mask;3-ply ear loop (50pcs/box)	box	5	270.00	1,350.00
Glass media/reagent bottles,with screw caps 1 liter (borosilicate, with markings for volume)	bottle	2	500.00	1,000.00
Glass media/reagent bottles,with screw caps 250 ml (borosilicate, with markings for volume)	bottle	2	500.00	1,000.00
Glass media/reagent bottles,with screw caps 100 ml (borosilicate, with markings for volume)	bottle	2	500.00	1,000.00
Latex gloves,powder free;small(100pcs/box)	box	4	500.00	2,000.00
Latex gloves,powder free;medium (100pcs/box)	box	4	500.00	2,000.00
Microcentrifuge tubes;1.5ml;(500pcs/pack)	pack	3	750.00	2,250.00
PCR tubes 0.2ml hinged- flat tops thin- wall polypropylene,Dnase/Rnase-free	pack	8	2,200.00	17,600.00
Pipet tips;100-1000ul,1000pcs/pack; universal fit	pack	6	1,200.00	7,200.00
Pipet tips;sterile,5-200ul,1000pcs/pack:universal fit	pack	6	800.00	4,800.00
Pipet tips;sterile,ultra-long;0.1-10ul;1000pcs/pack; universal fit	pack	6	800.00	4,800.00
Tissue paper;2-ply (12roll/pack)	roll	20	30.00	600.00
Delicate task wipers;280 sheets(4.4x8.4in/11x21cm)	box	3	400.00	1,200.00
Pipettors, starter set of 3 pipettors plus one carousel/ pipette stand; volume ranges of 2-20µl, 20-200µl,and 100-1000µl	set	2	25,000.00	50,000.00
TOTAL			**300,000.00**	

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