16th Engineering Research and Development for Technology Conference

NANOBIOSENSORS FOR THE DETECTION OF PATHOGENS

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University of the Philippines Los Baños

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Heritage Hotel, Pasay City

Pathogen Detection

plays a vital role in biological threat surveillance, agricultural safety and medical diagnosis

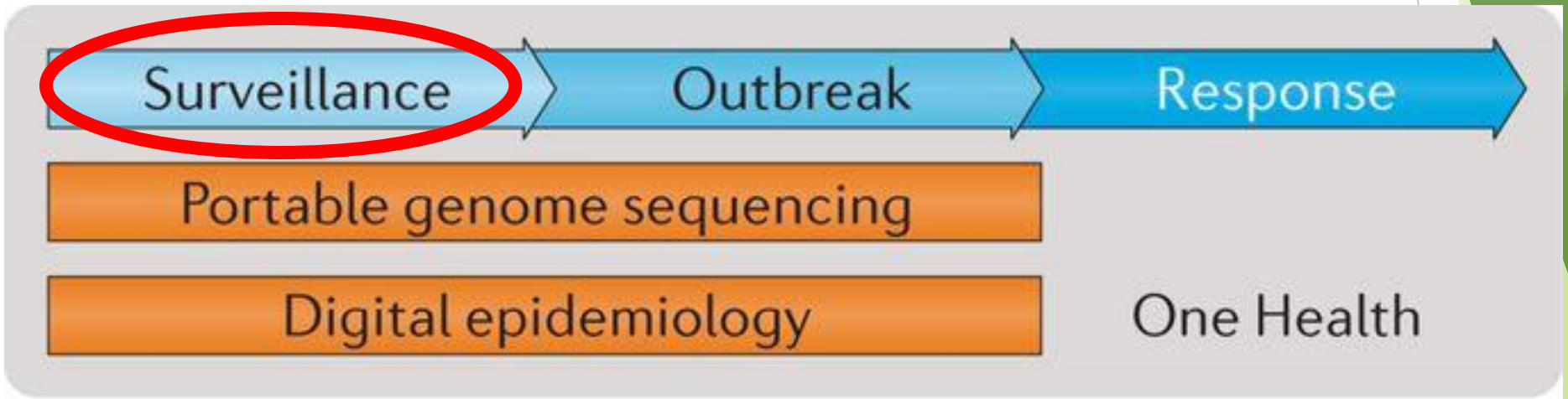
Challenge:

- there is a need for the continuous surveillance, rapid diagnosis and real-time tracking of pathogens.

There is a need for the development of new **rapid and sensitive** detection and identification technologies for microbial agents.

Important to scientists and to regulatory agencies since a systematic preventive approach to food safety is also promoted by the **Hazard Analysis and Critical Control Point (HACCP).**

OneHealth



Nature Reviews | Genetics

Gardy and Loman 2017

Limit of detection of common microbial diagnostic methods.

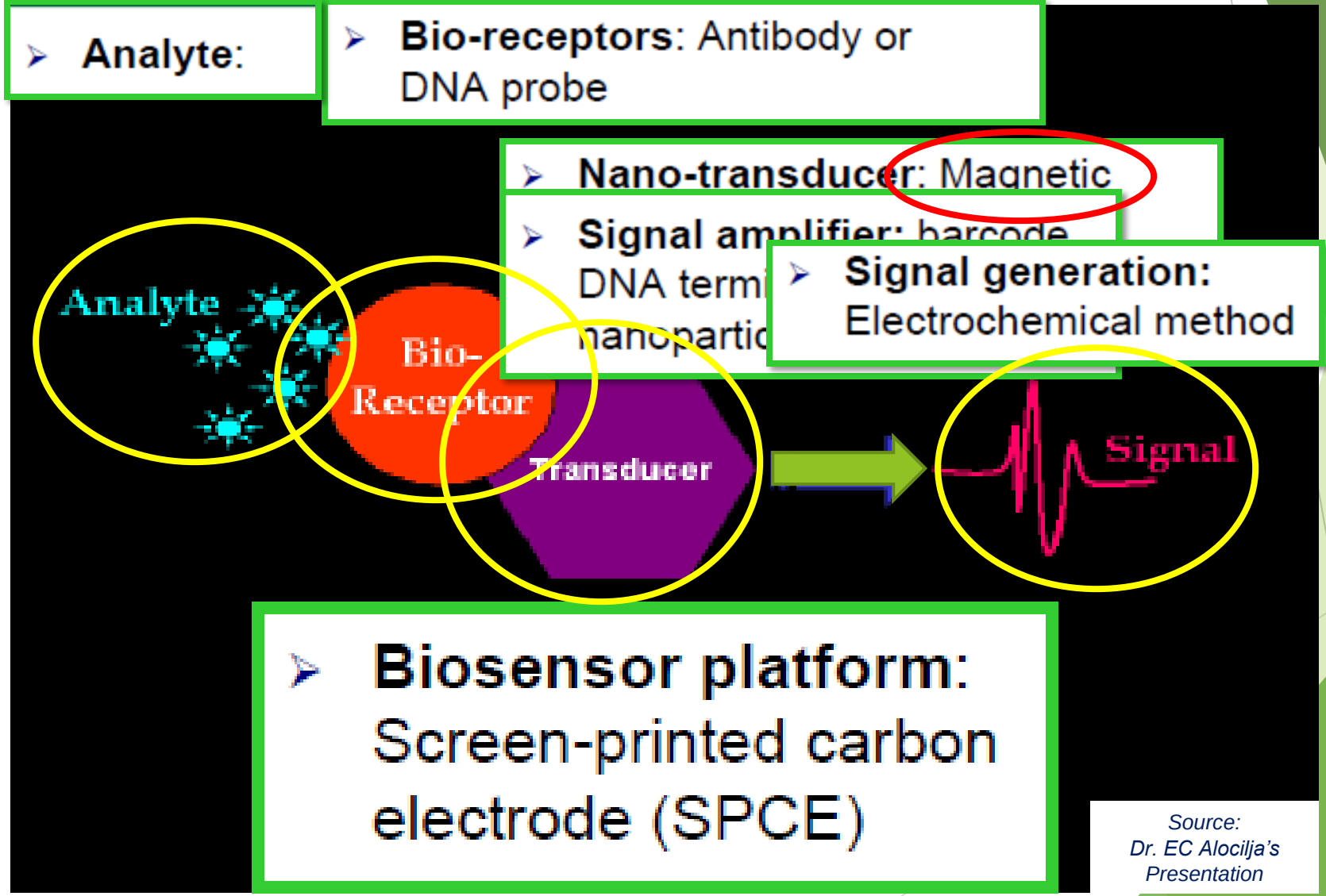
Method	**Limit of Detection**
Culture method	100-0.10 cfu/mL
PCR	ca. 100 cfu/mL
Nanobiosensor	fM concentration

Biosensor

- an analytical device that integrates a **biological sensing element** with a **transducer** to quantify a biological event into an **electrical output**

Biosensor Design

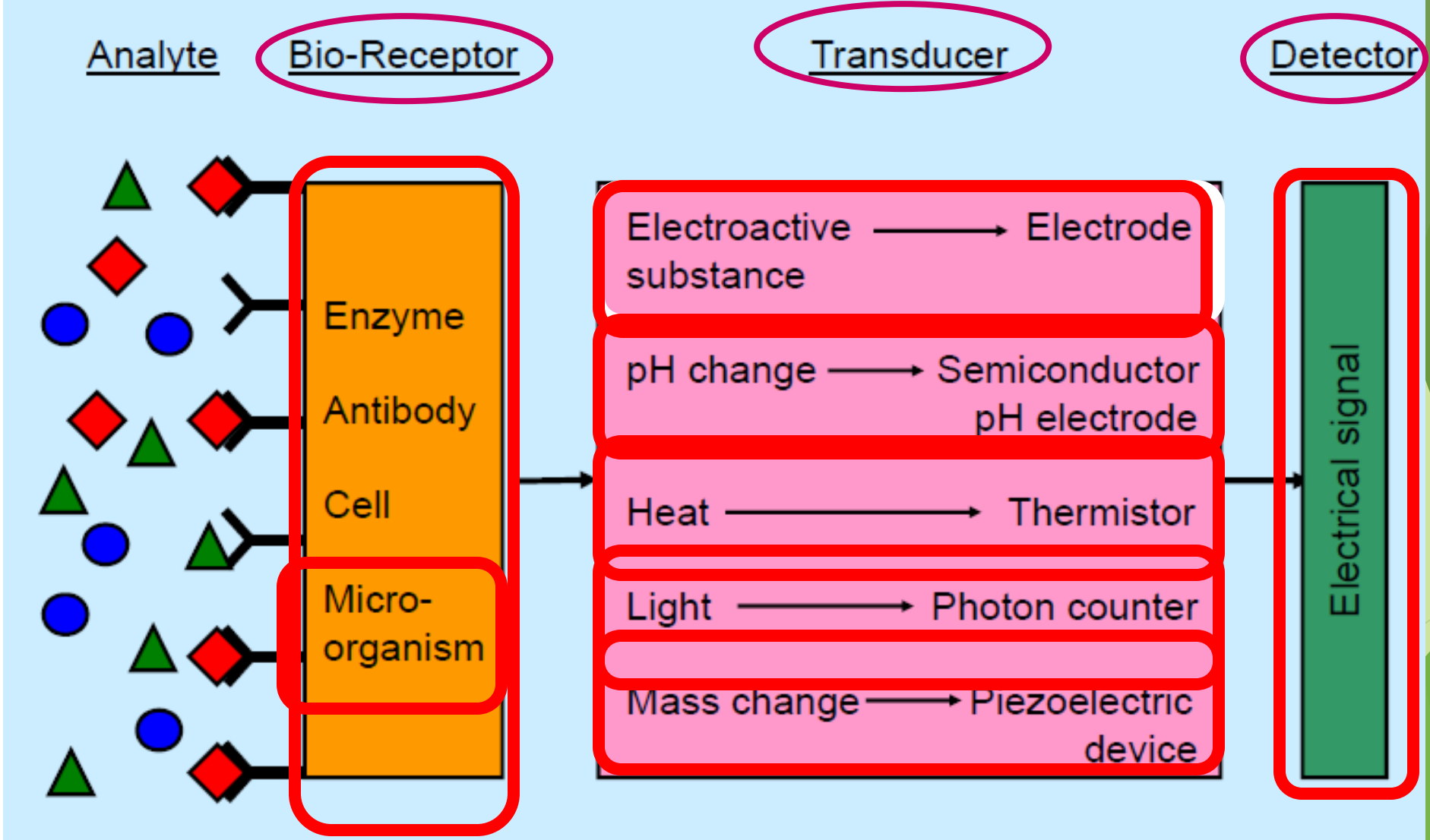
- **Analyte:**
- **Bio-receptors**: Antibody or DNA probe
- **Nano-transducer**: Magnetic
- **Signal amplifier**: barcode DNA
- **Signal generation:** Electrochemical method



- **Biosensor platform:** Screen-printed carbon electrode (SPCE)

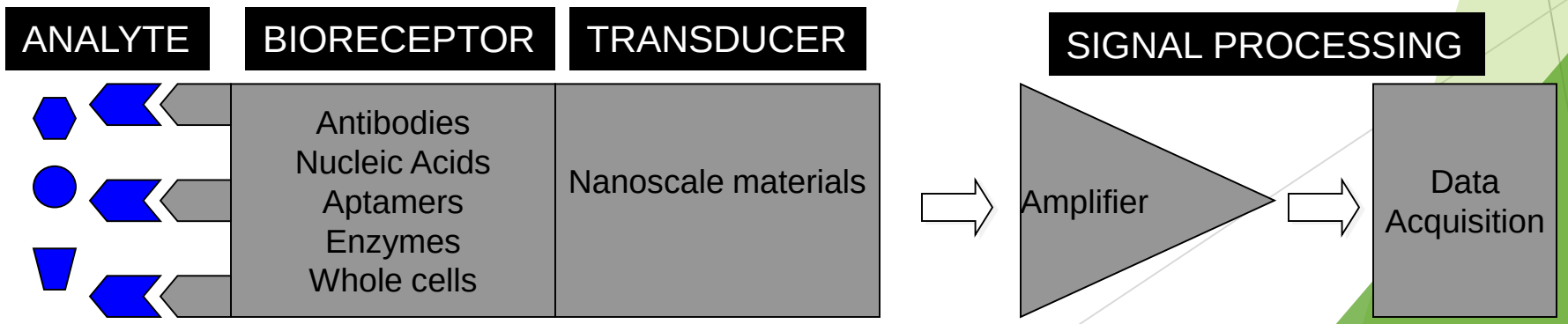
Source: Dr. EC Alocilja's Presentation

Elements of a Biosensor



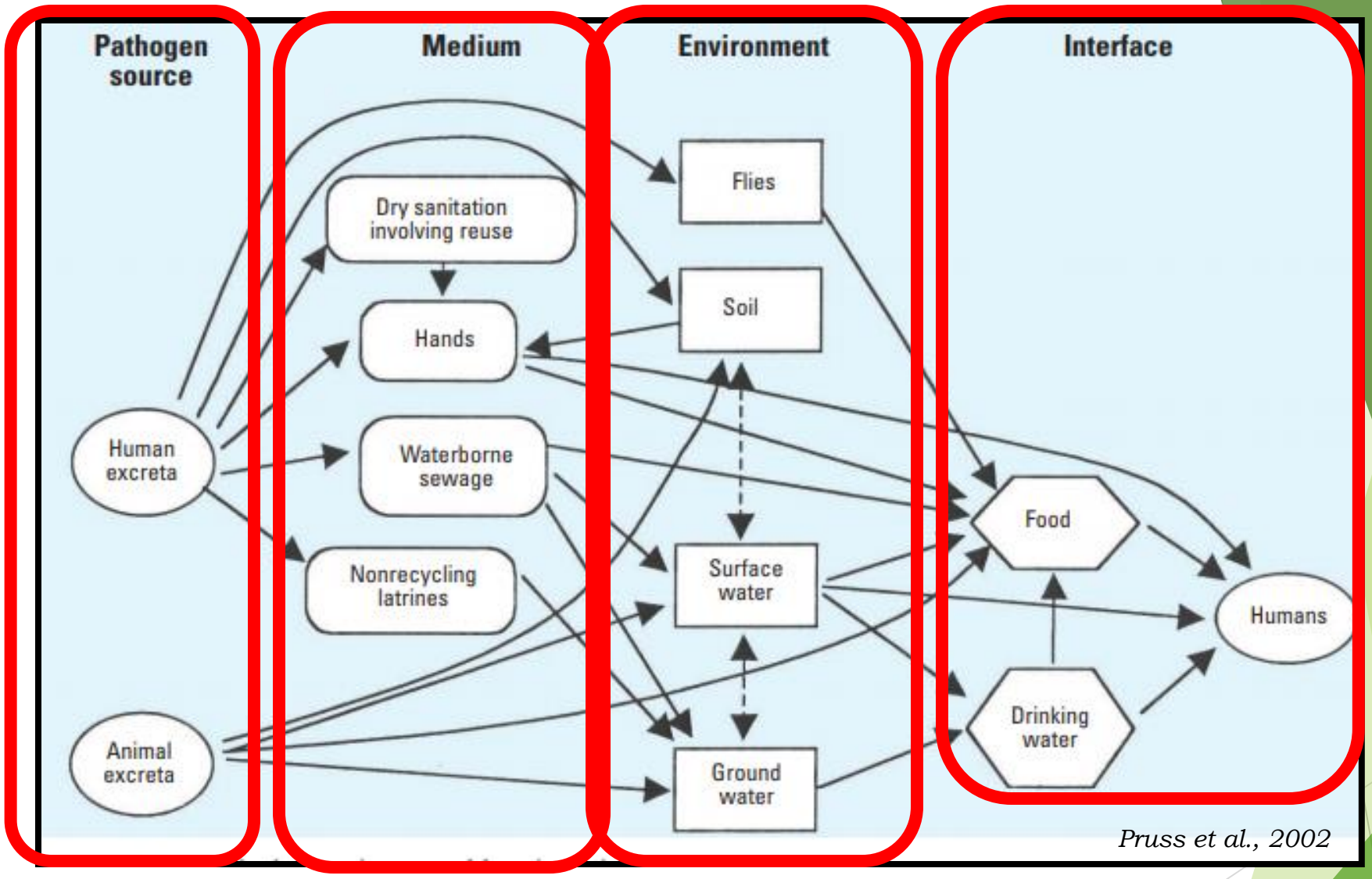
Advantages of a Nano-Biosensor

- ❖ Rapid detection time
- ❖ High sensitivity and specificity
- ❖ Compatible with data processing technologies



OneHealth

The areas of work in which a One Health approach is particularly relevant include **food safety**, the control of zoonoses (diseases that can spread between animals and humans, such as flu, rabies and Rift Valley Fever), and combatting antibiotic resistance



Transmission pathways of fecal–oral disease.

Global Occurrence of Disabilities due to Food Pathogens

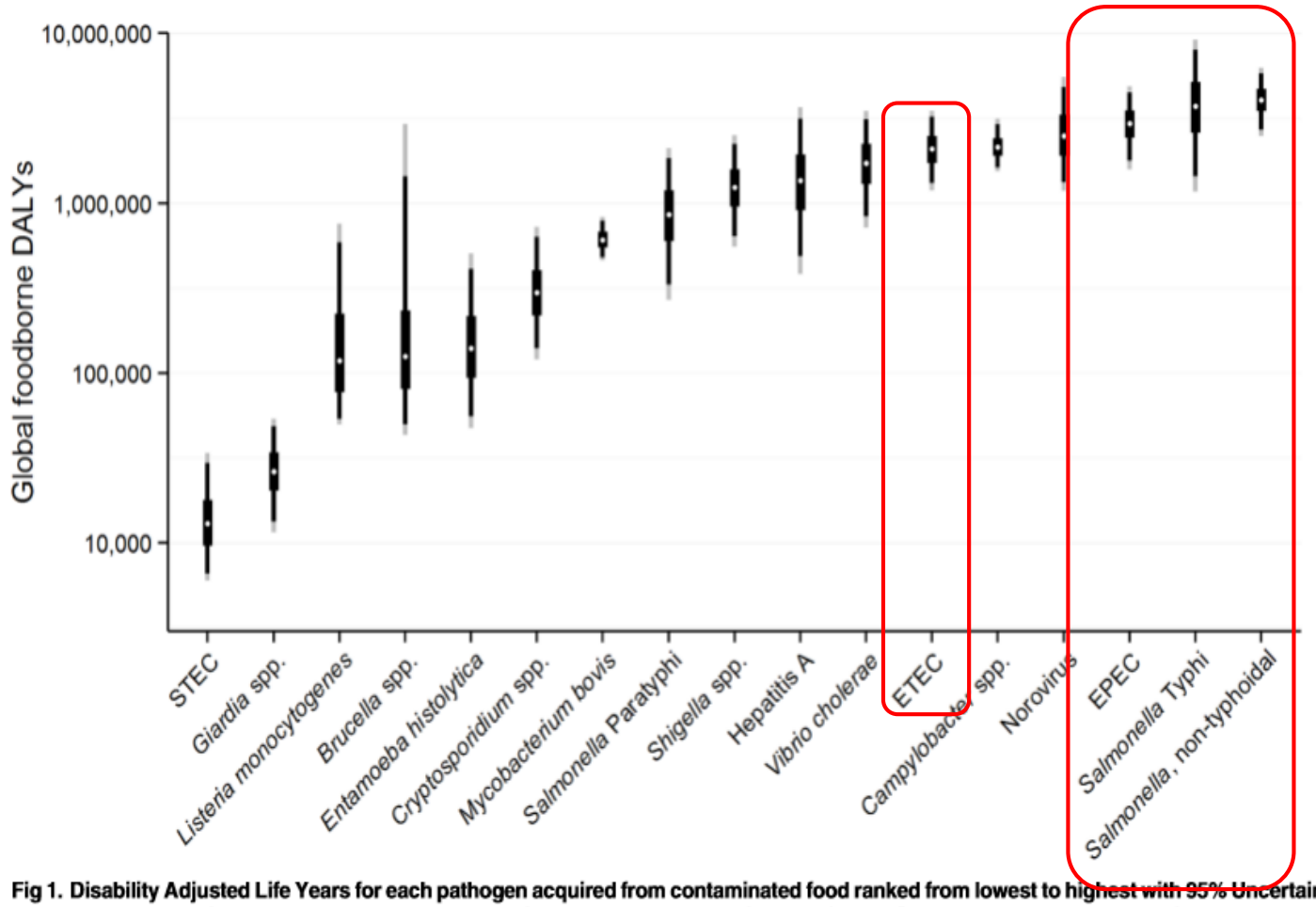
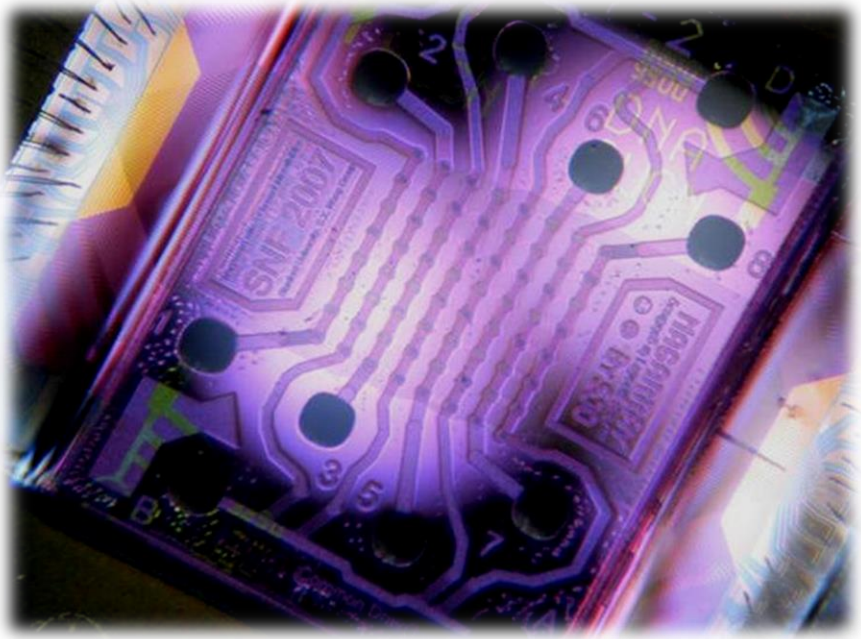


Fig 1. Disability Adjusted Life Years for each pathogen acquired from contaminated food ranked from lowest to highest with 95% Uncertainty Intervals, 2010. Note figure is on a logarithmic scale. The figure shows the median (white dot); Inter-Quartile Range = 50%UI = 25%/75% percentiles (thick black line); 90% UI = 5%/95% percentiles (thin black line); 95% UI = 2.5%/97.5% percentiles (thin grey line). Note, figure does not include four foodborne intoxications due to *Clostridium botulinum*, *C. perfringens*, *S. aureus*, and *Bacillus cereus* due to a lack of data for global estimation. In addition, data for non-

Food-borne illnesses

- 325,000 hospitalizations and 5,000 deaths each year
- 40% of the 50 million annual deaths worldwide
- common causative agents:
  - *Escherichia coli* O157:H7
  - *Salmonella enterica*

Diagnostics: Nano-Biosensors



Food-borne Pathogen

Salmonella

Republic of the Philippines
DEPARTMENT OF SCIENCE AND TECHNOLOGY
Philippine Council for Industry, Energy and Emerging Technology Research and Development (PCIEERD)

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Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Fluorescent bio-barcode DNA assay for the detection of *Salmonella enterica* serovar Enteritidis

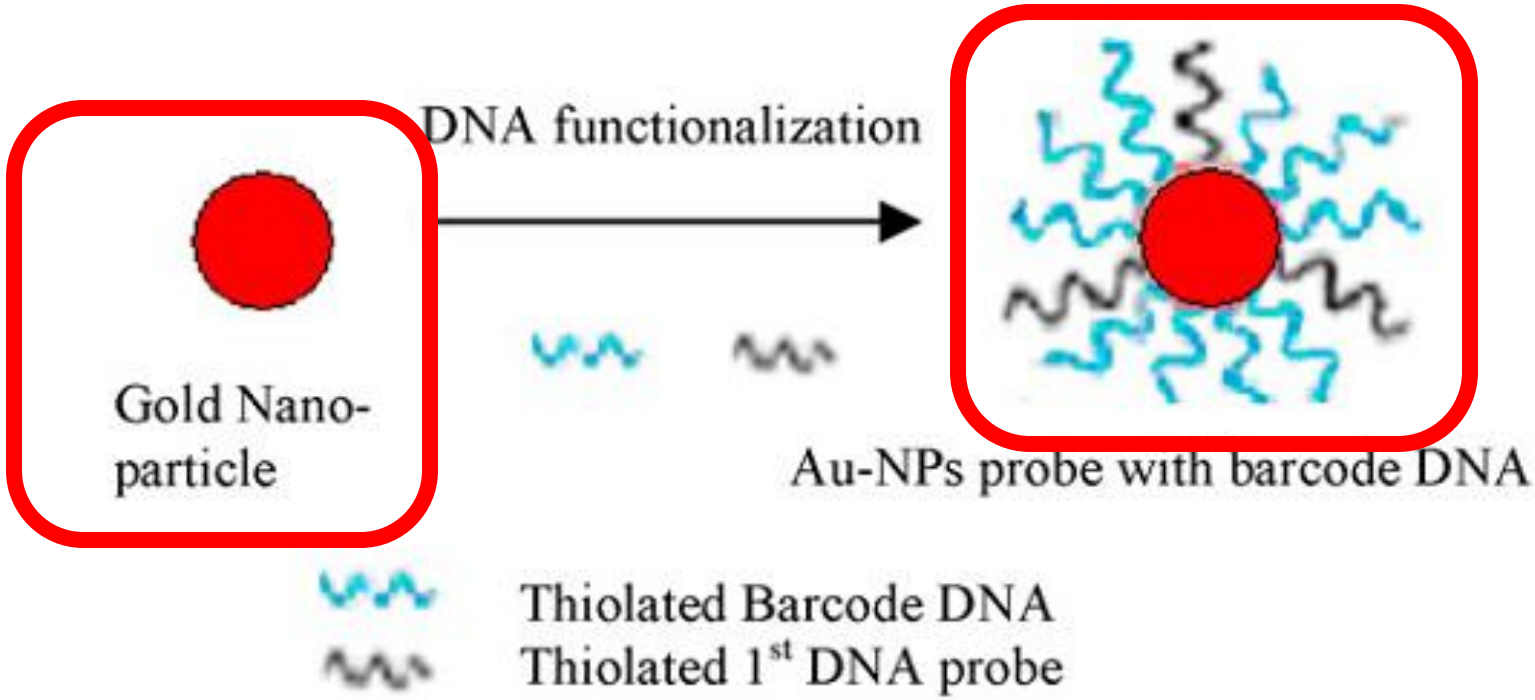
Deng Zhang, David J. Carr, Evangelyn C. Alocilja*

Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA

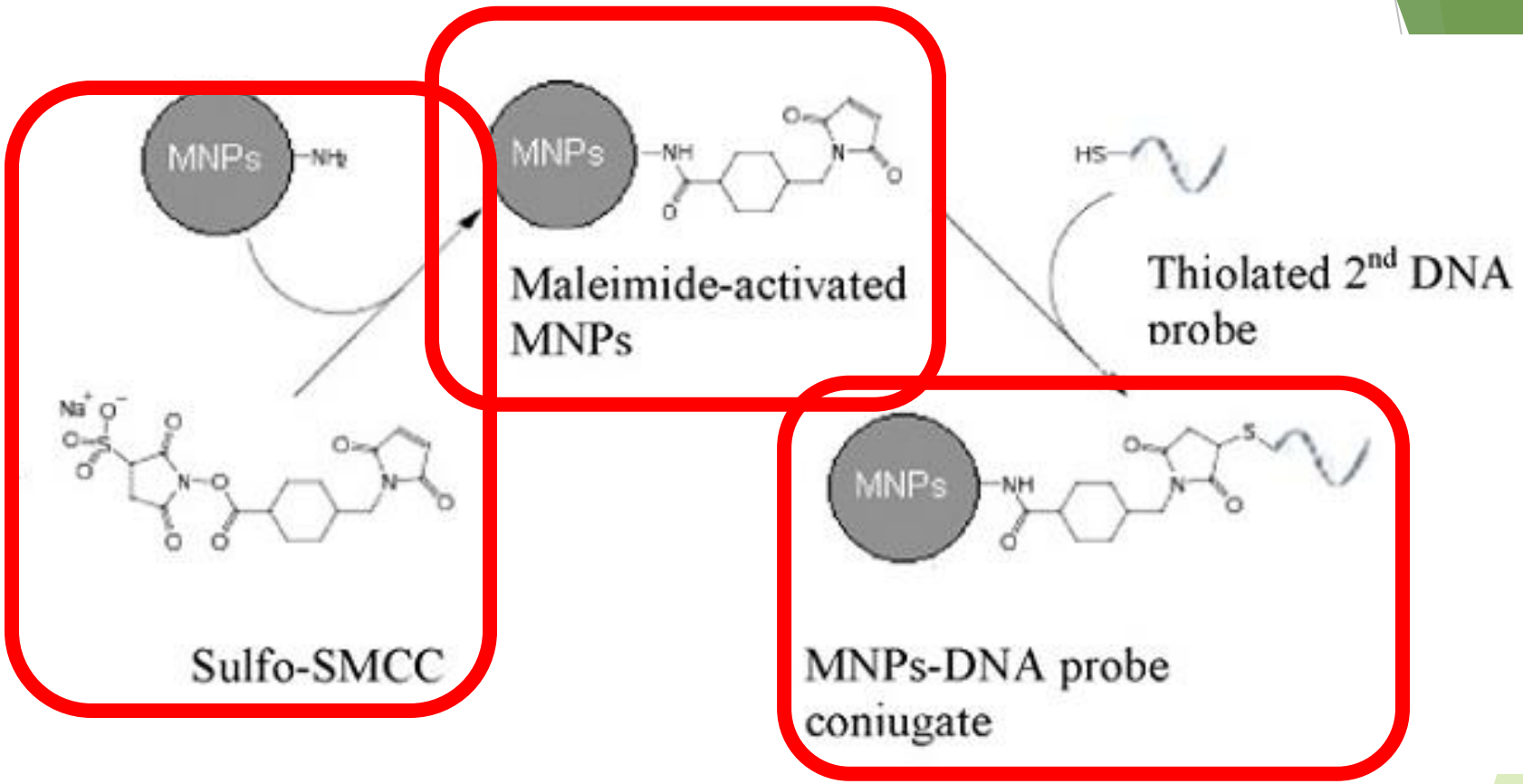
A highly amplified bio-barcode DNA assay for the rapid detection of the insertion element (Iel) gene of *Salmonella* Enteritidis.

Salmonella enterica serovar Enteritidis is one of the most frequently reported cause of foodborne illness.

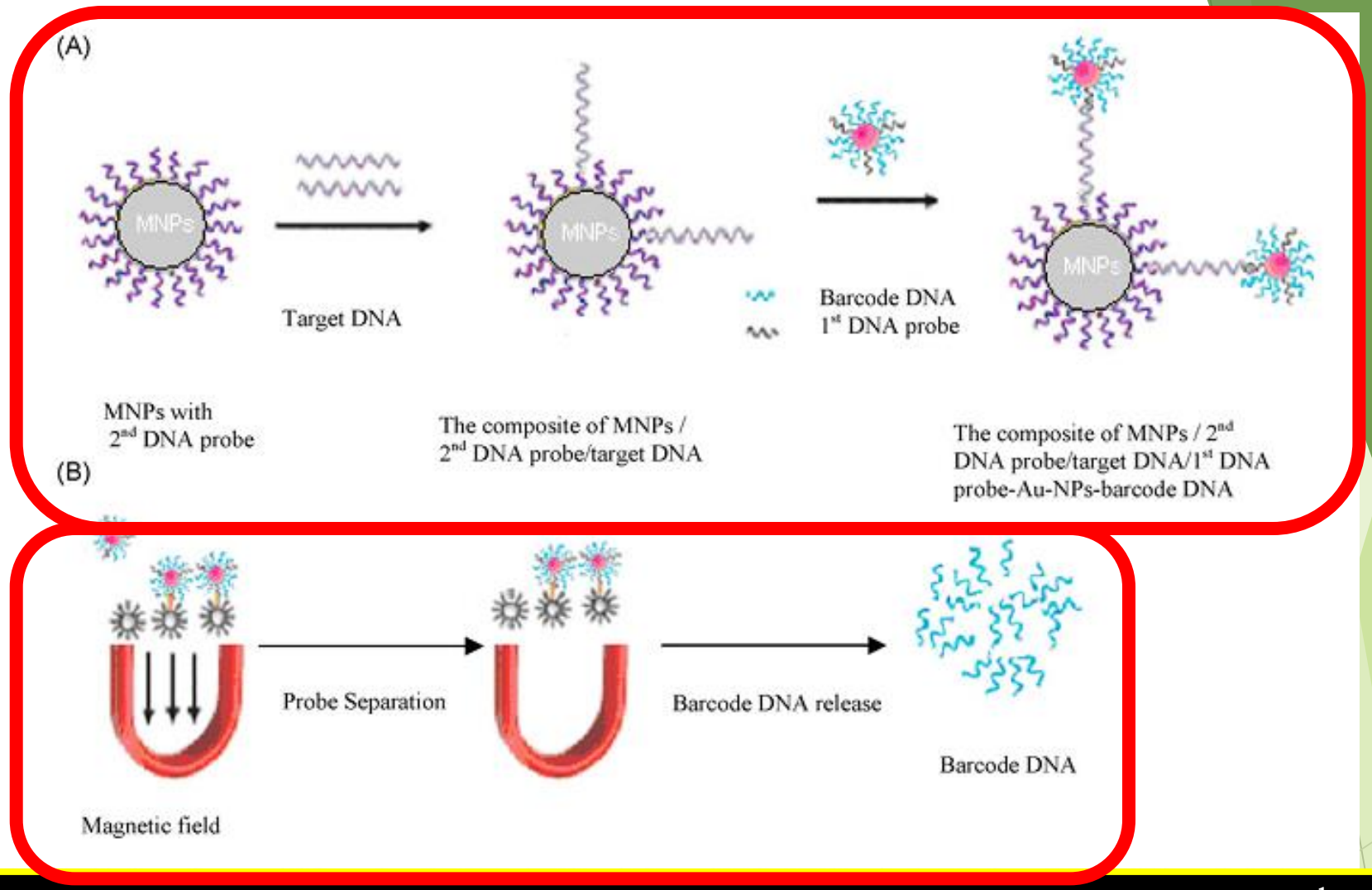
It is a major threat to the food safety chain and public health.



Schematic of the functionalization of AuNPs.



Schematic of the functionalization of MNPs.



Schematic of the bio-barcode assay: (A) formation of MNP-2nd DNA probe/target DNA/1st DNA probe-Au-NPs-barcode DNA; (B) barcode DNA separation and release.

The relationship between target DNA and the fluorescence signal of released barcode DNA

Sample concentration (ug/mL)	10	1	0.1	0.01	0.001	0
Fluorescence reading (counts)	5711	1440	777	426	270	117
3 x Standard deviation	380.00	209.16	18.52	67.26	28.84	12.01

The results show that the detection limit of this bio-barcoded DNA assay is 1 ng/mL (or 2.15×10^{-16} mol).

Food-borne Pathogen

Escherichia coli 0157:H7

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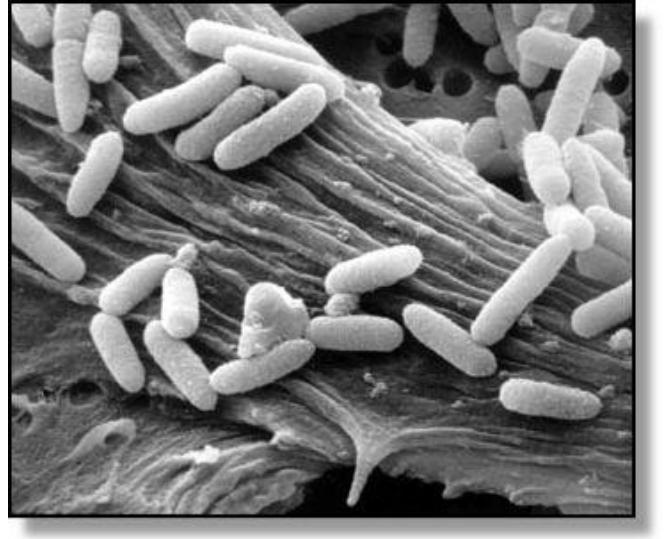
Escherichia coli

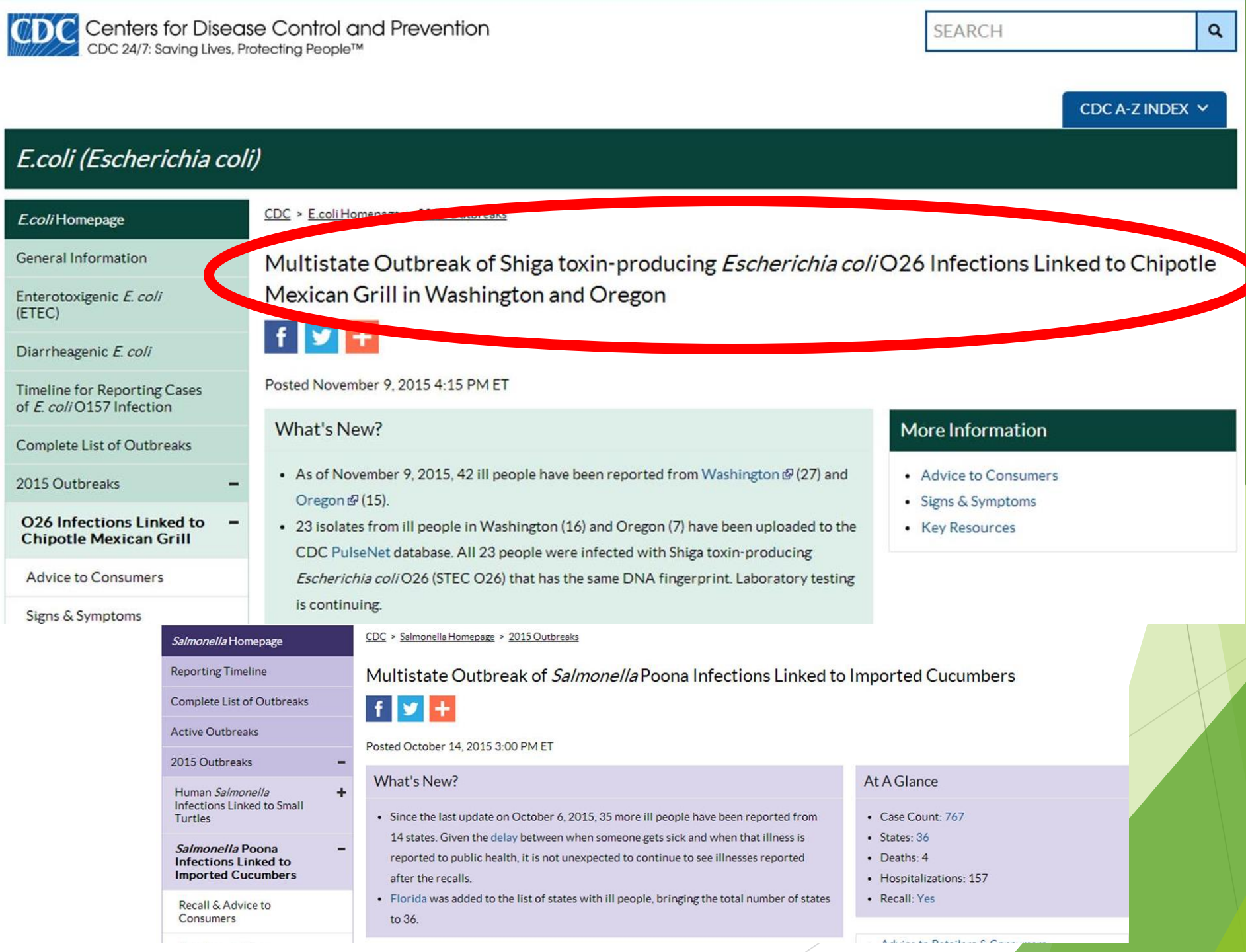


- serve as indicator of water quality and food contaminants by fecal matter.

***E. coli* O157:H7** is a notorious pathogen confirmed in many outbreaks of food-borne illnesses.

- ✓ bloody diarrhea
- ✓ hemorrhagic colitis
- ✓ occasionally hemolytic uremic syndrome

Infection dose as low as ~100 cells can lead to disease or even death


CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™
SEARCH
CDC A-Z INDEX
E.coli (Escherichia coli)
E.coli Homepage
General Information
Enterotoxigenic E. coli (ETEC)
Diarrheagenic E. coli
Timeline for Reporting Cases of E. coli O157 Infection
Complete List of Outbreaks
2015 Outbreaks
O26 Infections Linked to Chipotle Mexican Grill
Advice to Consumers
Signs & Symptoms
CDC > E.coli Homepage
Multistate Outbreak of Shiga toxin-producing Escherichia coli O26 Infections Linked to Chipotle Mexican Grill in Washington and Oregon
Posted November 9, 2015 4:15 PM ET
What's New?
As of November 9, 2015, 42 ill people have been reported from Washington (27) and Oregon (15).
23 isolates from ill people in Washington (16) and Oregon (7) have been uploaded to the CDC PulseNet database. All 23 people were infected with Shiga toxin-producing Escherichia coli O26 (STEC O26) that has the same DNA fingerprint. Laboratory testing is continuing.
More Information
Advice to Consumers
Signs & Symptoms
Key Resources
Salmonella Homepage
Reporting Timeline
Complete List of Outbreaks
Active Outbreaks
2015 Outbreaks
Human Salmonella Infections Linked to Small Turtles
Salmonella Poona Infections Linked to Imported Cucumbers
Recall & Advice to Consumers
CDC > Salmonella Homepage > 2015 Outbreaks
Multistate Outbreak of Salmonella Poona Infections Linked to Imported Cucumbers
Posted October 14, 2015 3:00 PM ET
What's New?
Since the last update on October 6, 2015, 35 more ill people have been reported from 14 states. Given the delay between when someone gets sick and when that illness is reported to public health, it is not unexpected to continue to see illnesses reported after the recalls.
Florida was added to the list of states with ill people, bringing the total number of states to 36.
At A Glance
Case Count: 767
States: 36
Deaths: 4
Hospitalizations: 157
Recall: Yes

Results from Analysis of Raw Ground Beef and Raw Ground Beef Component Samples for *E. coli* O157:H7*

*Results are posted according to the sample analysis completion date.

Raw Ground Beef (RGB)						
Source	As of November 5, 2017			As of November 4, 2018		
	Number Analyzed	Number Positive	Percent Positive	Number Analyzed	Number Positive	Percent Positive
Federal Plants	**9,761**	**7**	**0.07**	**9,505**	**4**	**0.04**
Verification	9,612	6	0.06	9,408	4	0.04
Follow-up**	149	1	0.67	97	0	0.00
Retail Stores	**492**	**1**	**0.20**	**476**	**0**	**0.00**
Verification	491	1	0.20	476	0	0.00
Follow-up	1	0	0.00	0	0	0.00
Imports	**36**	**0**	**0.00**	**36**	**0**	**0.00**

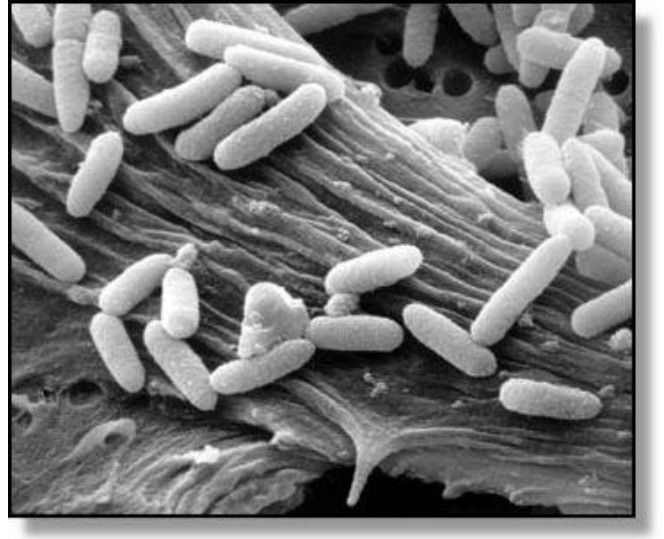
Raw Ground Beef Components (RGBC)						
Source	As of November 5, 2017			As of November 4, 2018		
	Number Analyzed	Number Positive	Percent Positive	Number Analyzed	Number Positive	Percent Positive
Federal Plants	**5,239**	**11**	**0.21**	**5,870**	**8**	**0.14**
Trim Verification***	3,029	6	0.20	3,238	6	0.19
Follow-up to RGB Positive	154	0	0.00	29	0	0.00
Follow-up to RGBC Positive	579	3	0.52	488	0	0.00
Other RGBC Verification	482	1	0.21	1,071	2	0.19
Bench Trim Verification	995	1	0.10	1,044	0	0.00
Import	**1,628**	**1**	**0.06**	**860**	**0**	**0.00**

*Results are posted according to the sample analysis completion date.

**Follow-up may also include non-routine testing.

Source: https://www.fsis.usda.gov/

Escherichia coli



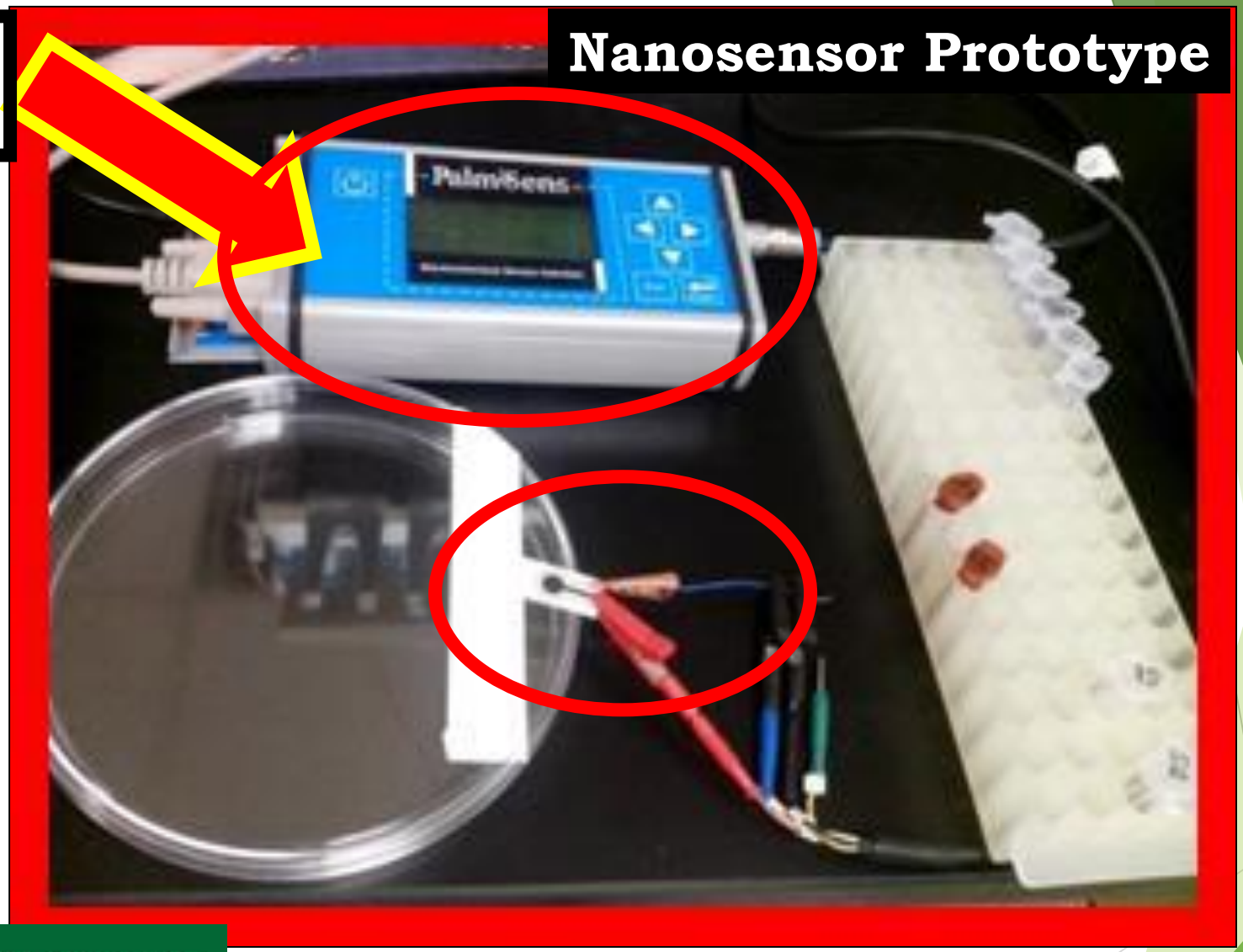
Typical detection methods:

- Culture techniques
- Enzyme-linked immunosorbent assays
- PCR

The **high cost**, **complicated processing steps** and the **requirement for specialized training** on the conventional detection methods for food-borne pathogens made it difficult on the side of health practitioners and monitoring agencies in the field.

Personal computer/ Laptop

Nanosensor Prototype



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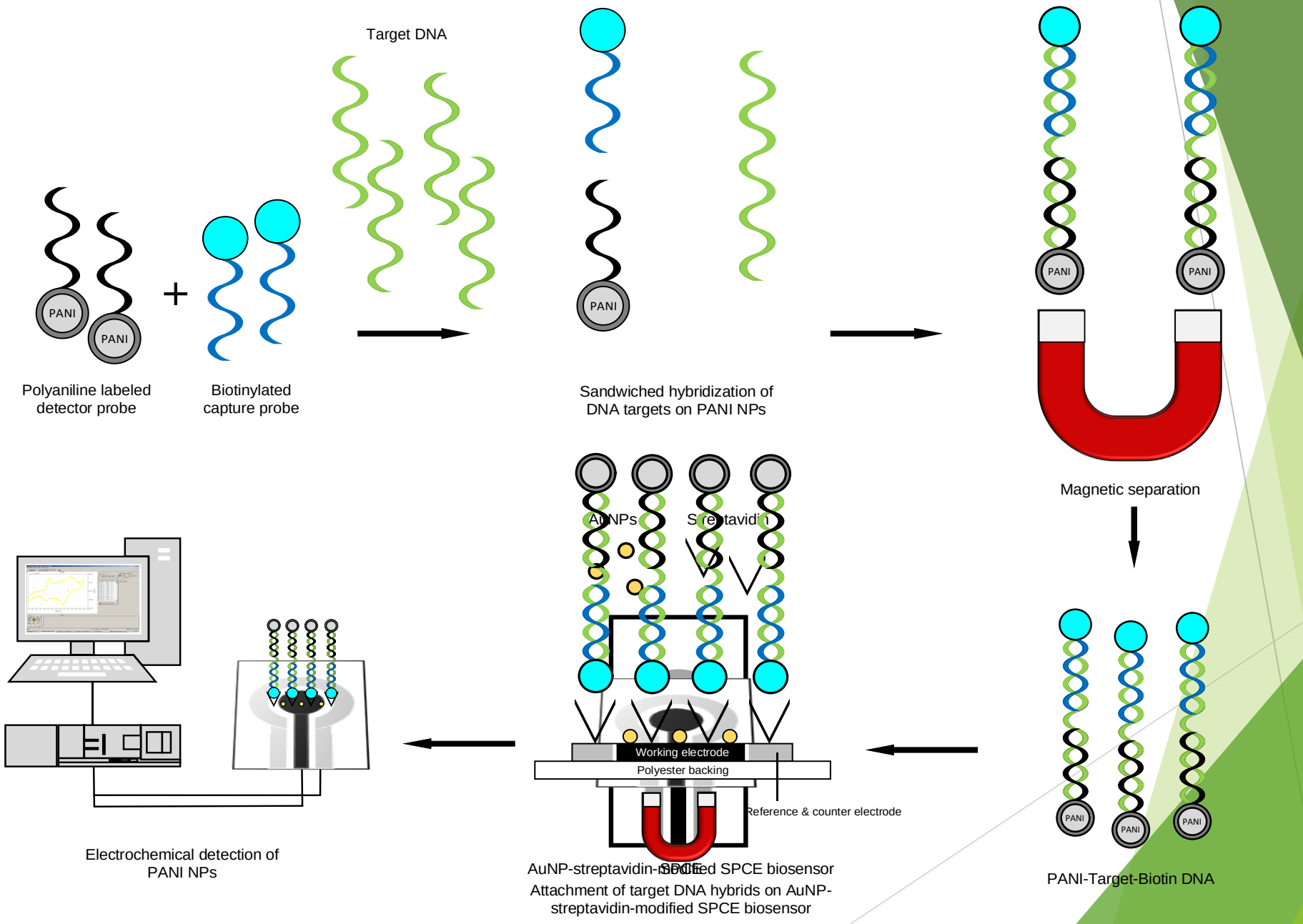
Sandwiched Hybridization

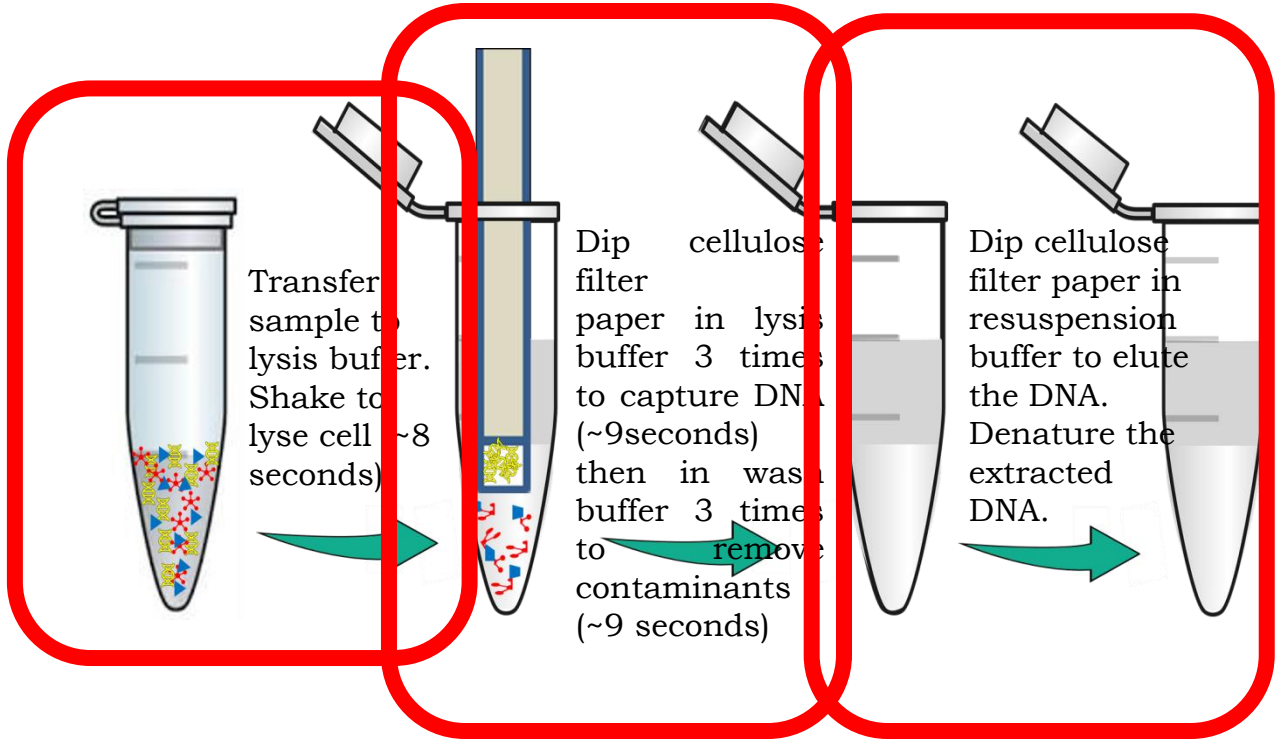
Created by: Matthew Vasher Edited by: Juan Miguel Parami



Nano-Biosensor System for **Food Safety** Applications

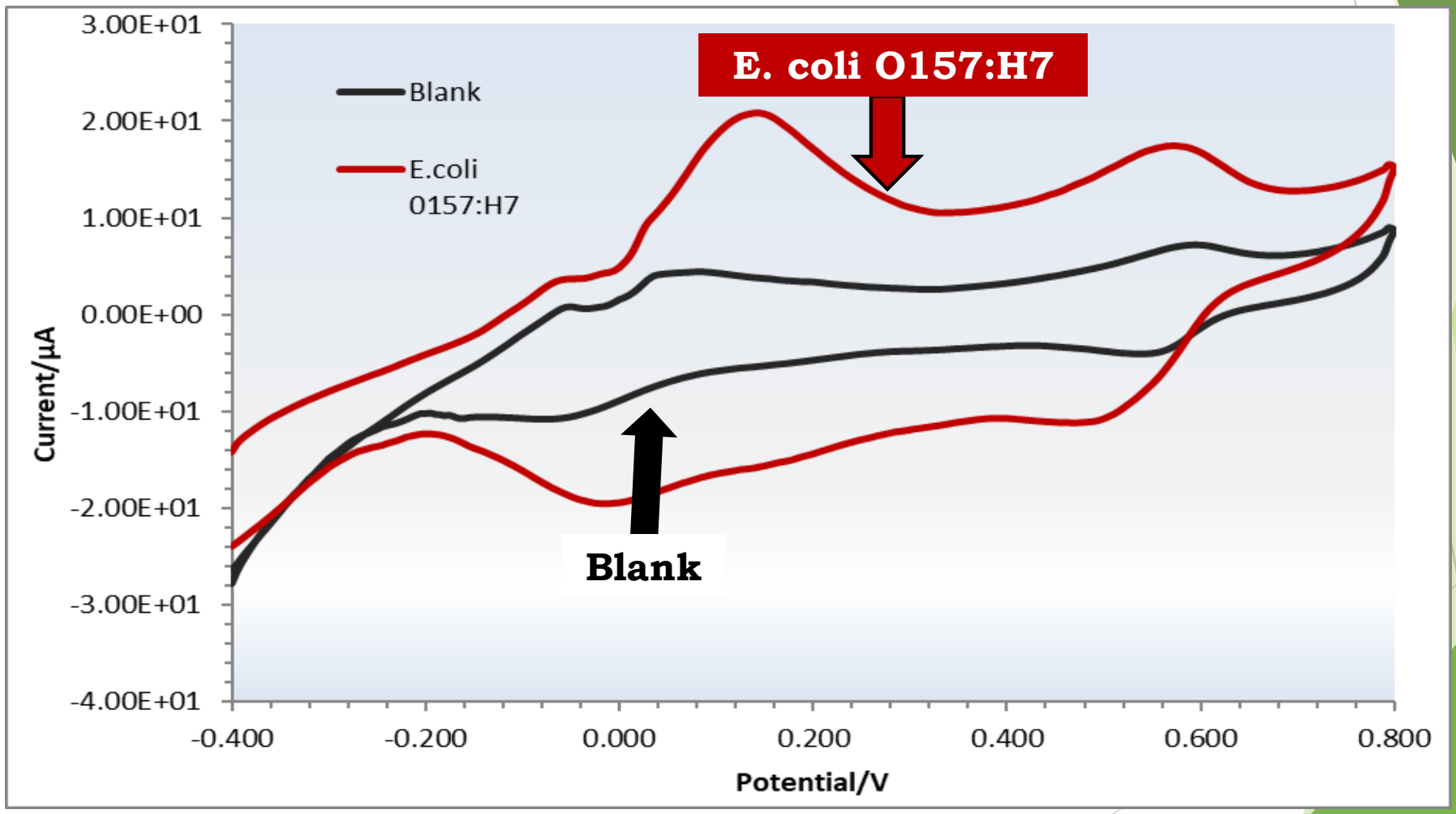
BIOTECH

30-second genomic DNA Extraction protocol

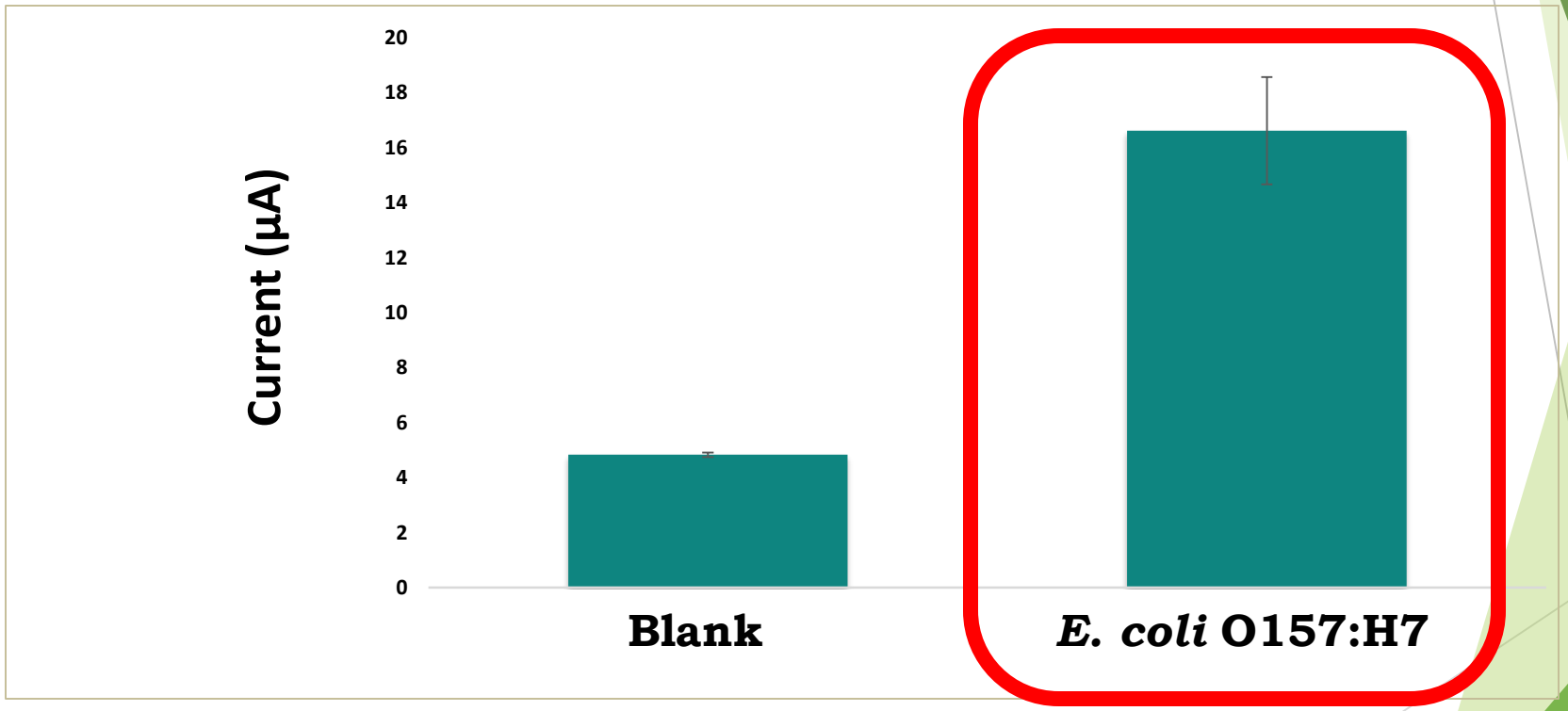


Adapted from *Zou et al, 2018*

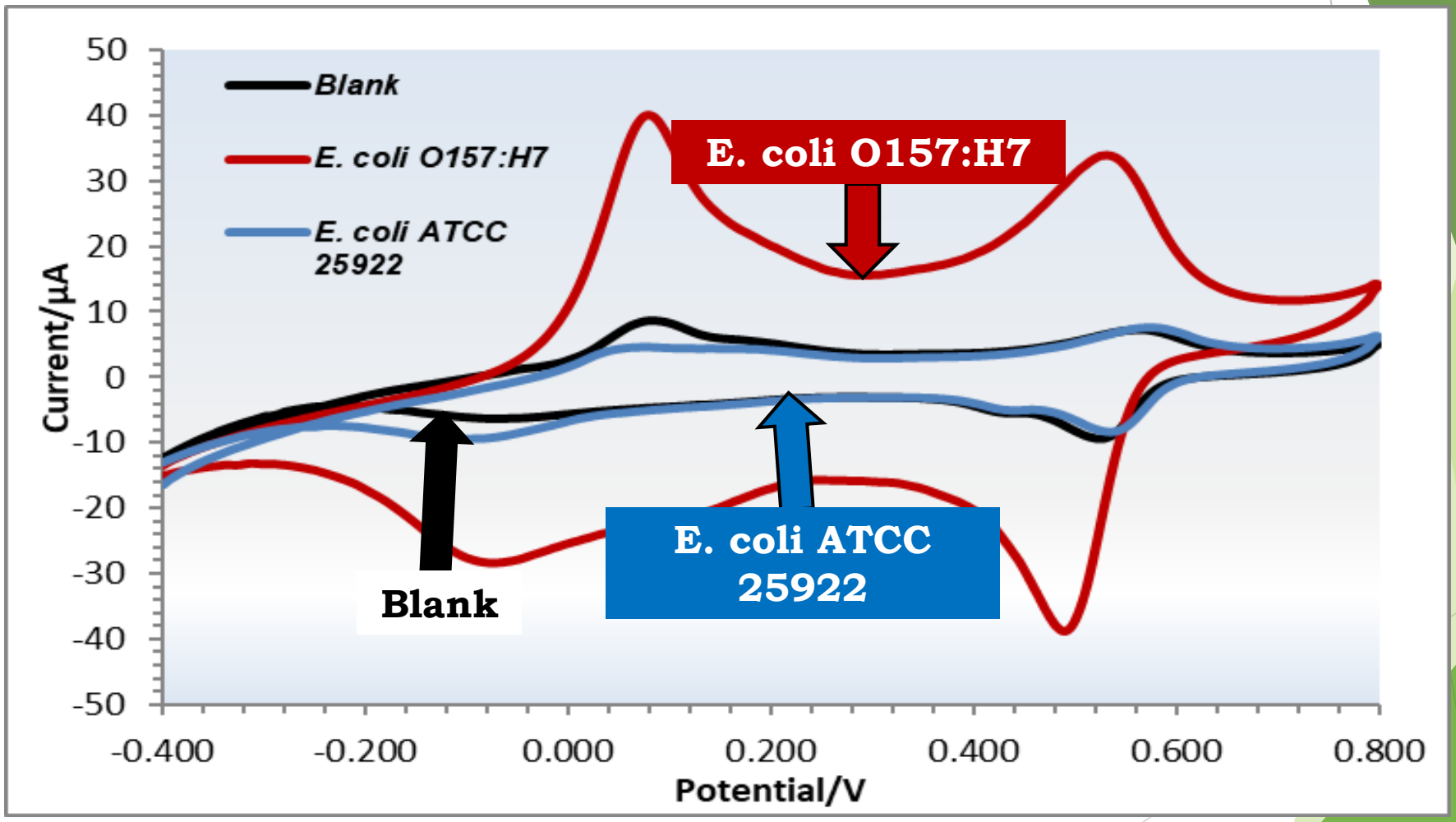
Detection of *E. coli* O157:H7 Using the 30-Second Extraction of Genomic DNA



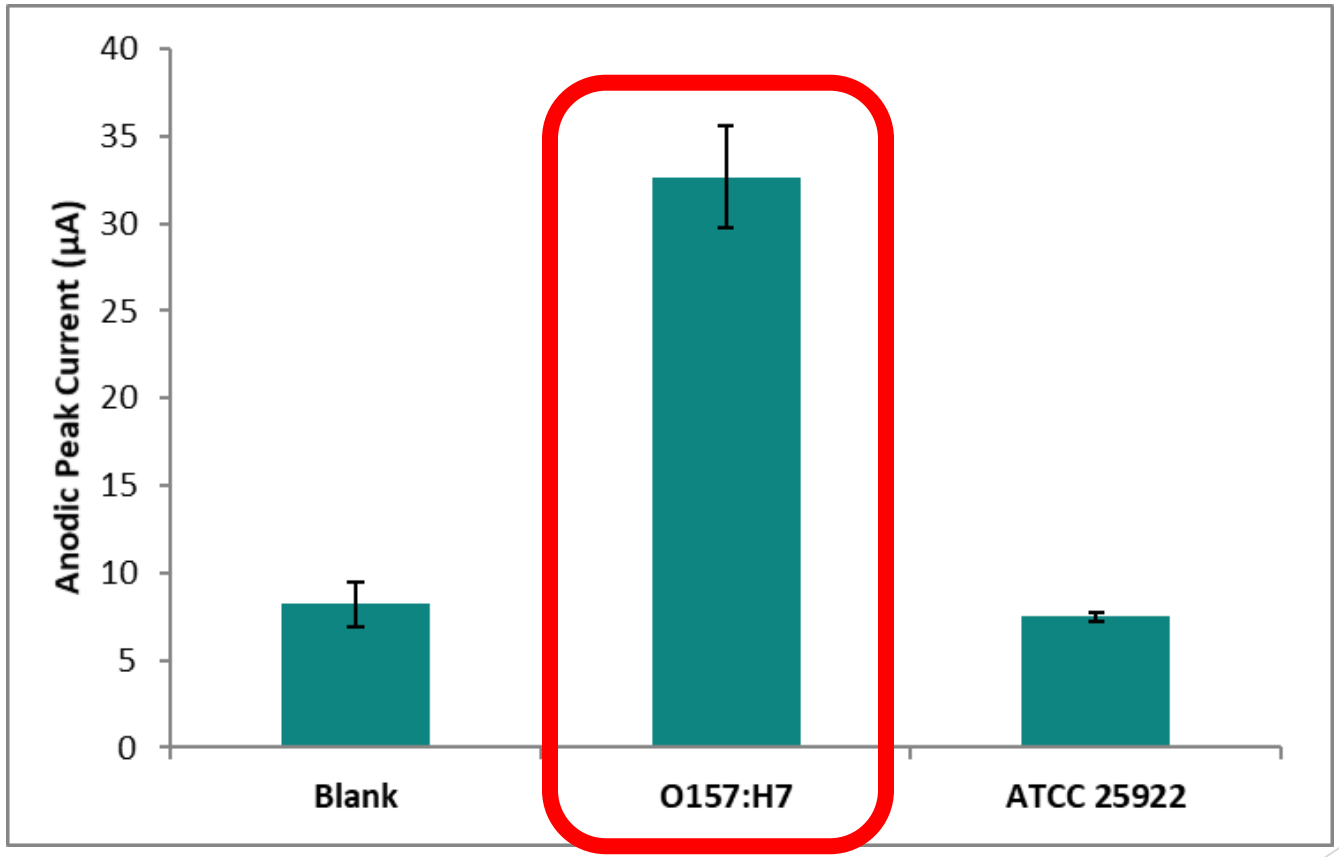
Detection of *E. coli* O157:H7 Using the 30-Second Extraction of Genomic DNA



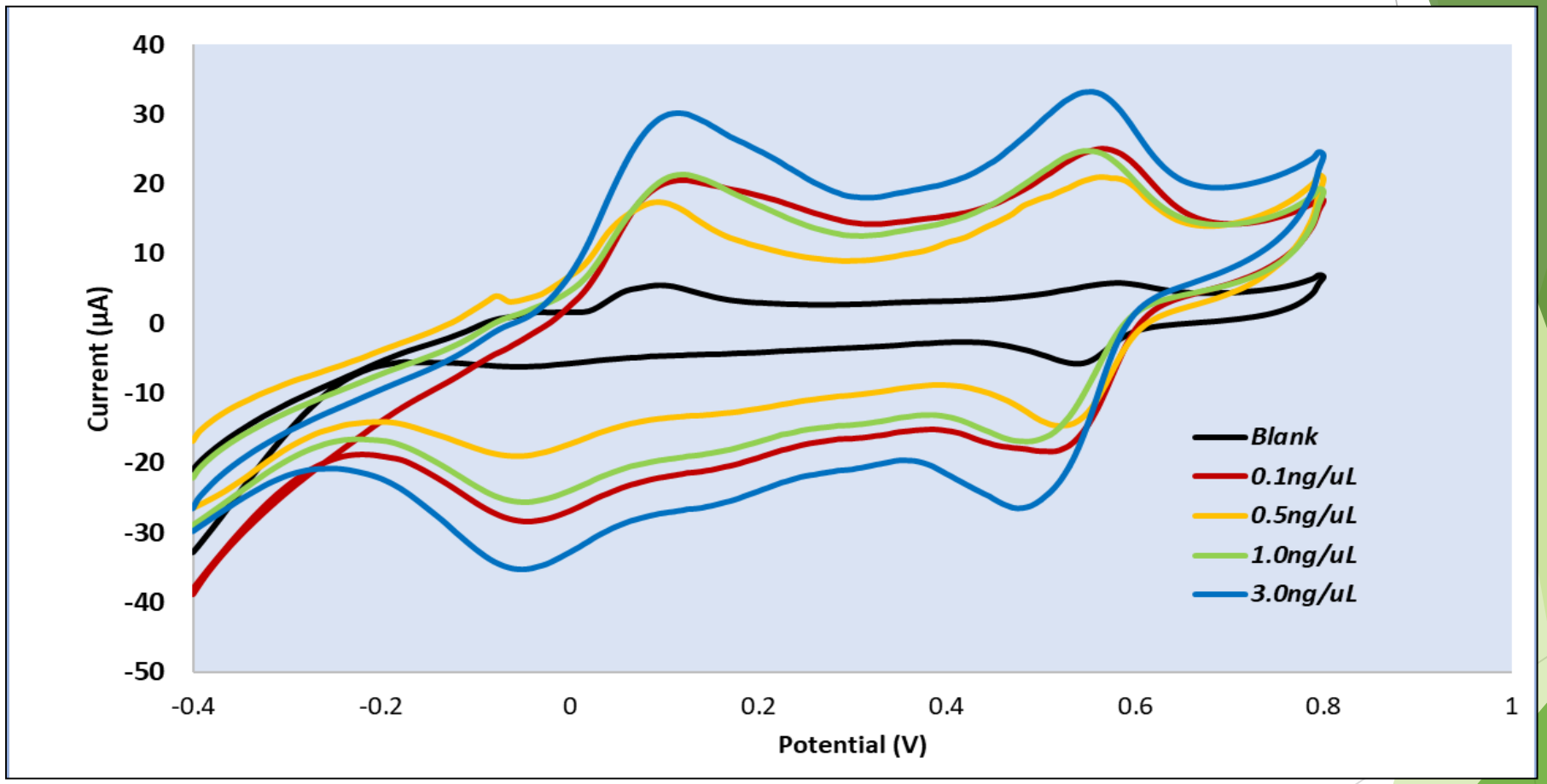
Specificity Test



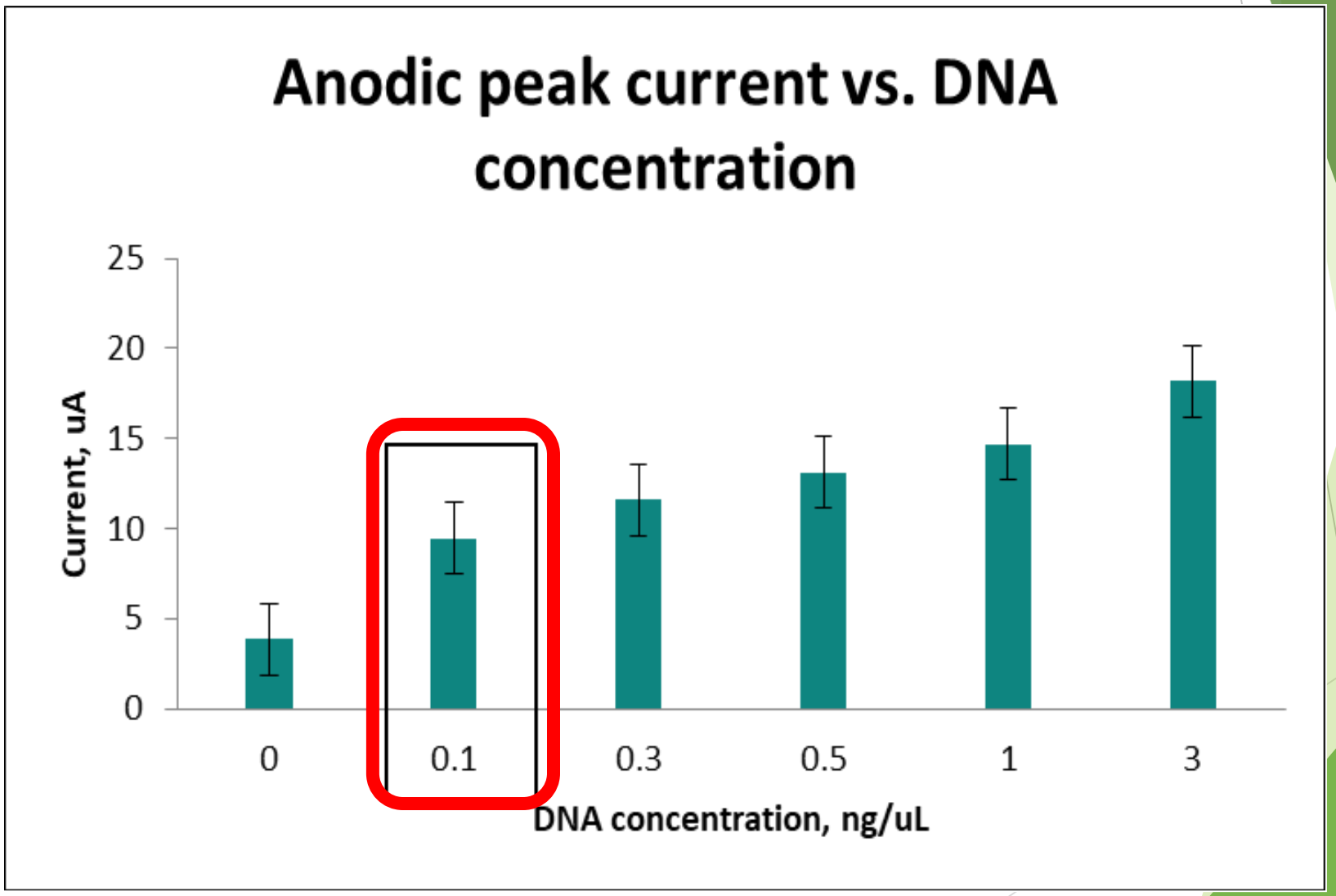
Specificity Test



Limit of Detection



Limit of Detection



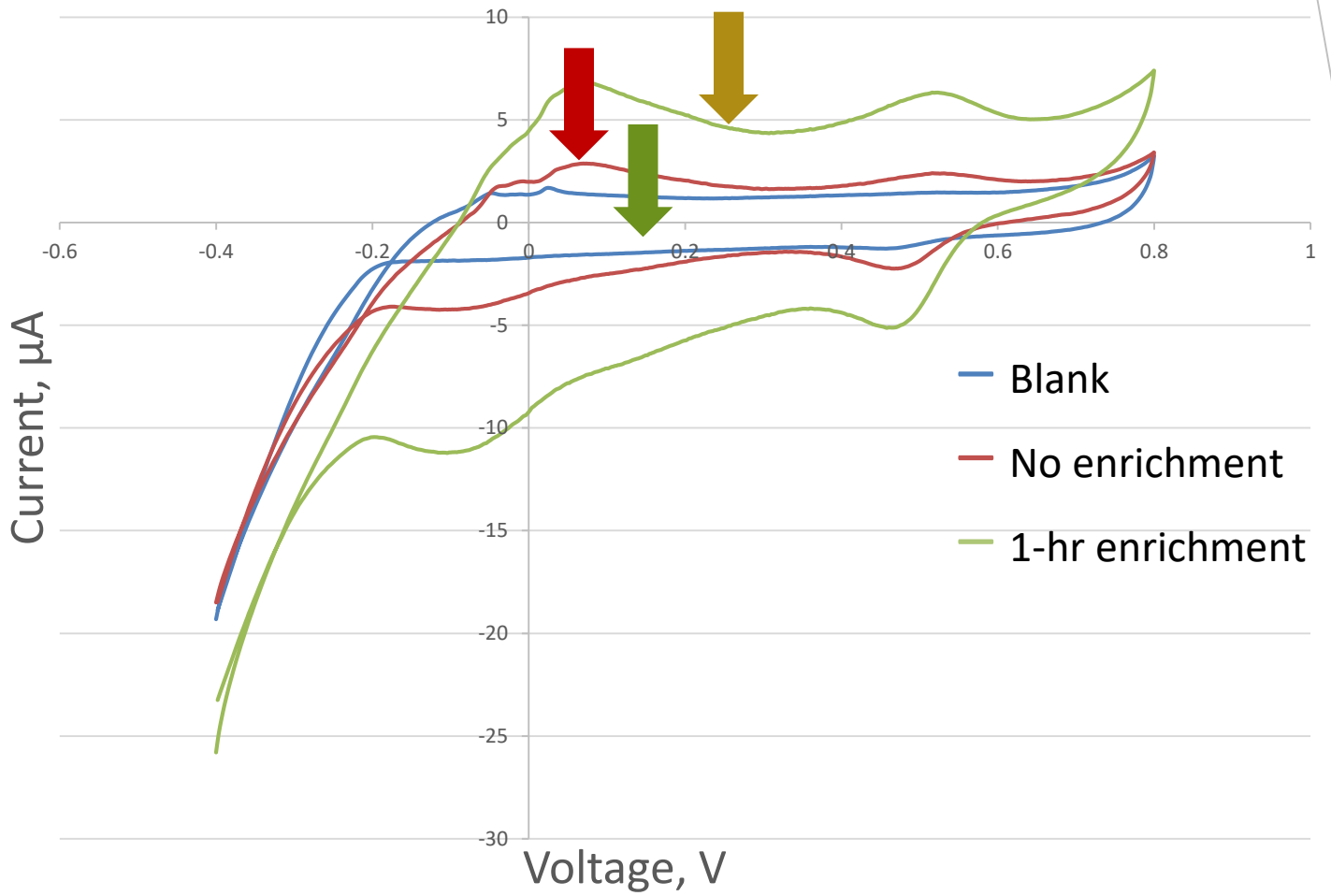
Detection of *E. coli* O157:H7 using the fabricated nanobiosensor

Detection of O157:H7 from farm produce samples

Lettuce

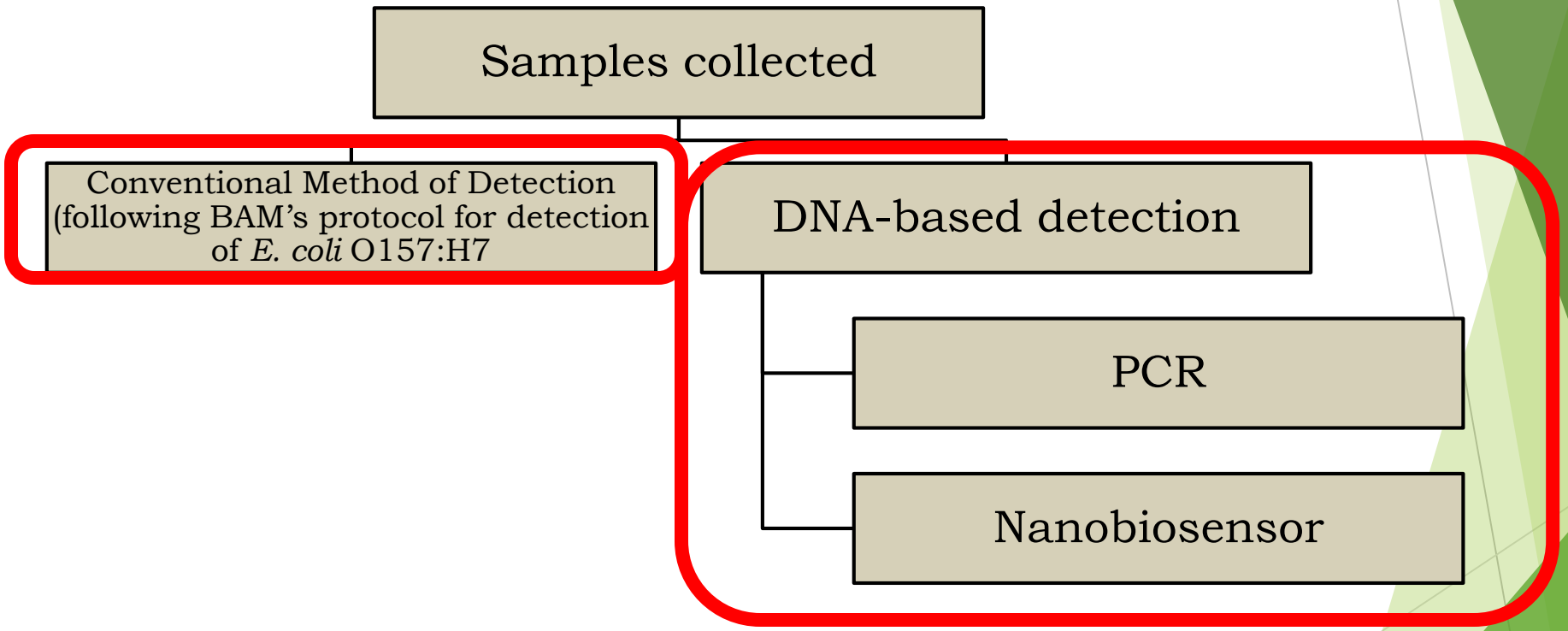
Celery

Paco

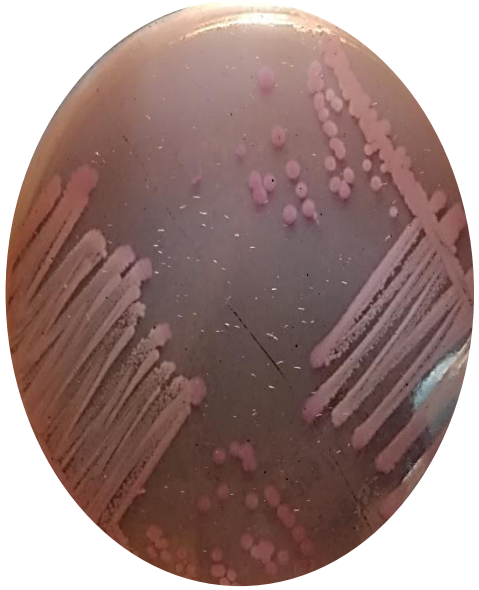


Cyclic voltammograms of samples at 50 mV/s.

Detection of O157:H7 from farm produce samples

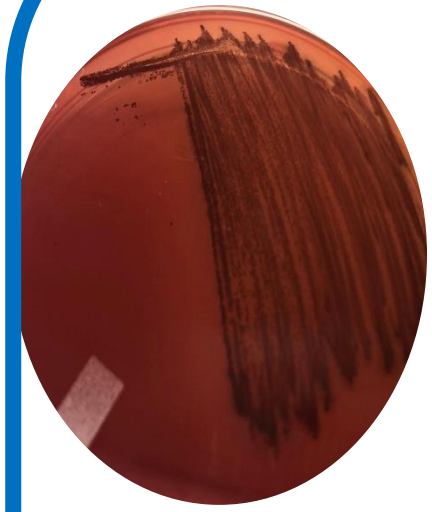


CONVENTIONAL METHOD OF DETECTION (CULTURE PLATING)



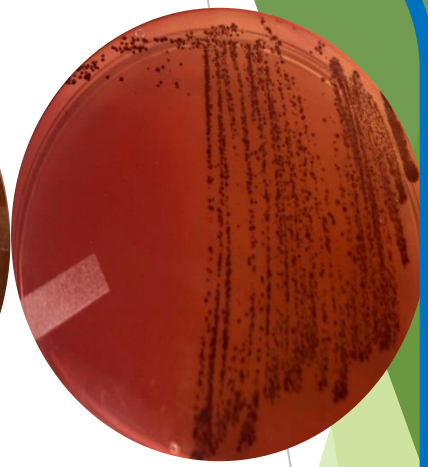
Typical O157:H7 in TC SMAC

Colorless or neutral gray with smoky center and 1-2mm diameter



Lettuce

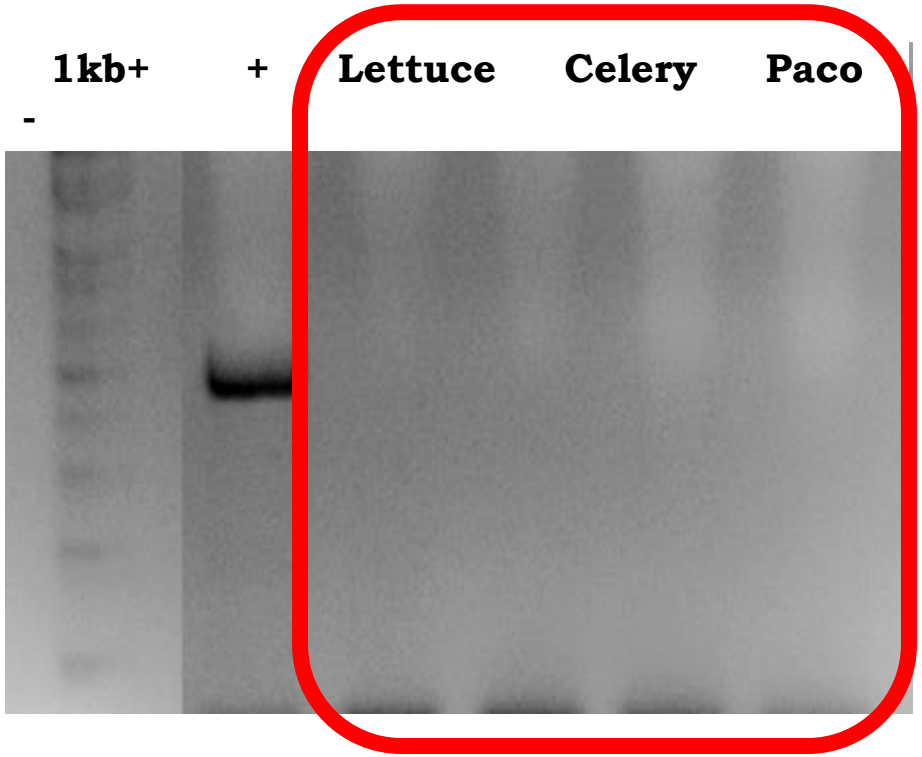
Celery



Paco

Plated lettuce, celery and paco samples in TC SMAC resulted in dark-colored colonies, different from expected typical O157:H7 in TC SMAC

Polymerase Chain Reaction



Amplification of stx 1 gene of *E. coli* O157:H7, resolved in 2% agarose gel and viewed under gel photodocumentation system. The DNA size marker is an Invitrogen™ Life Technologies, Inc, 1kbp DNA Ladder.

Plant Pathogen

Phytopthora palmivora

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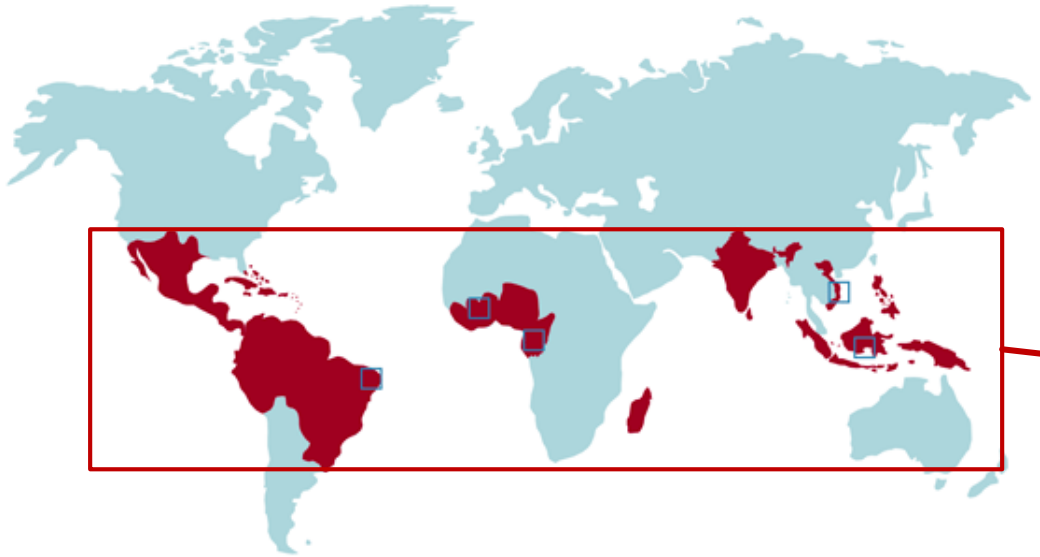
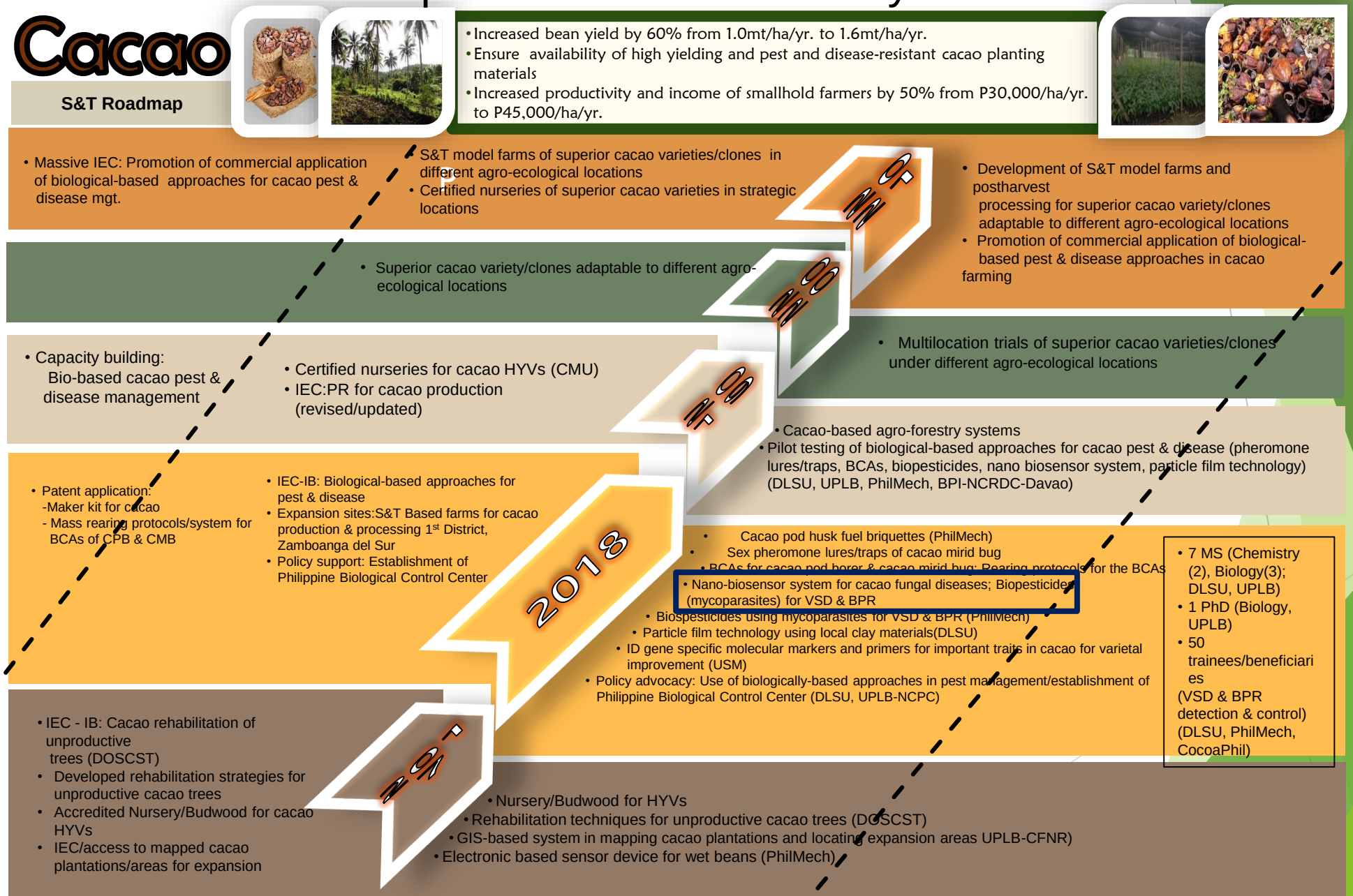
Cacao



Grown along the “Cacao Belt”.

Cultivated mainly for its beans

The Philippine government exerts effort to boost cacao production in the country.

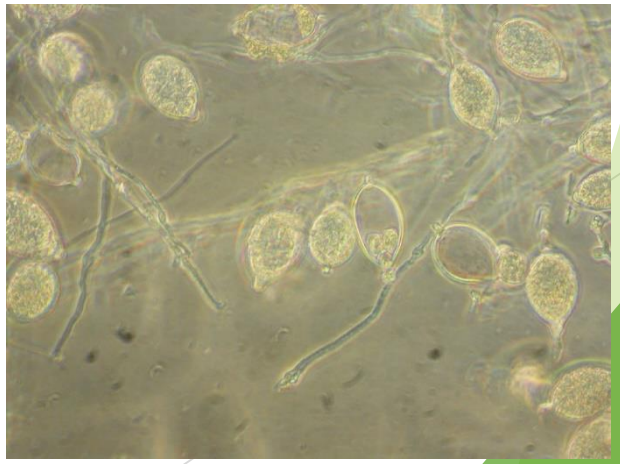


Black Pod Rot of Cacao

Symptoms:

- Brown to black necrotic lesions
- Mummification of pods
- Stem cankers in cases of severe infection

Primarily caused by *Phytophthora palmivora* in SEA



Screening:
Triage Technique

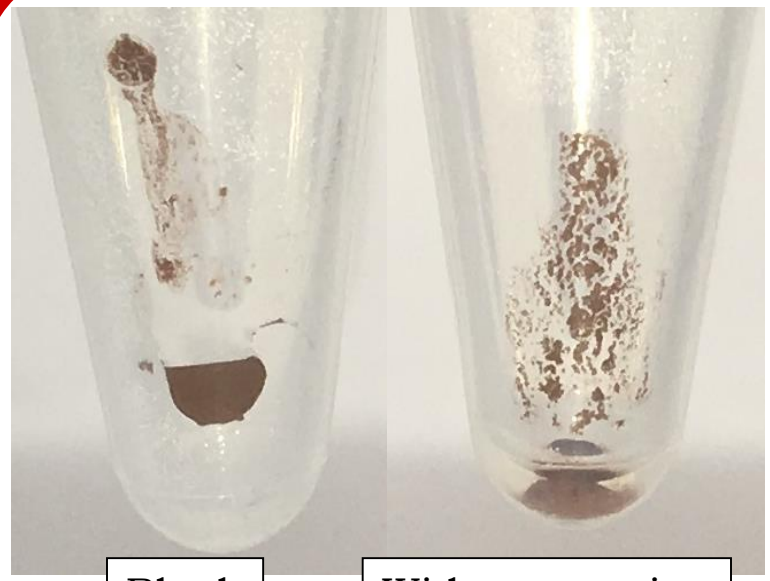
"Matting" Technique Using Magnetic Nanoparticles

Positive detection of *Phytopthora palmivora*, causal organism of Black Pod Rot in cacao

Matting using MNPs in Dengue virus (Dr. Alocilja research result)

Image analysis of magnetic nanoparticle matting

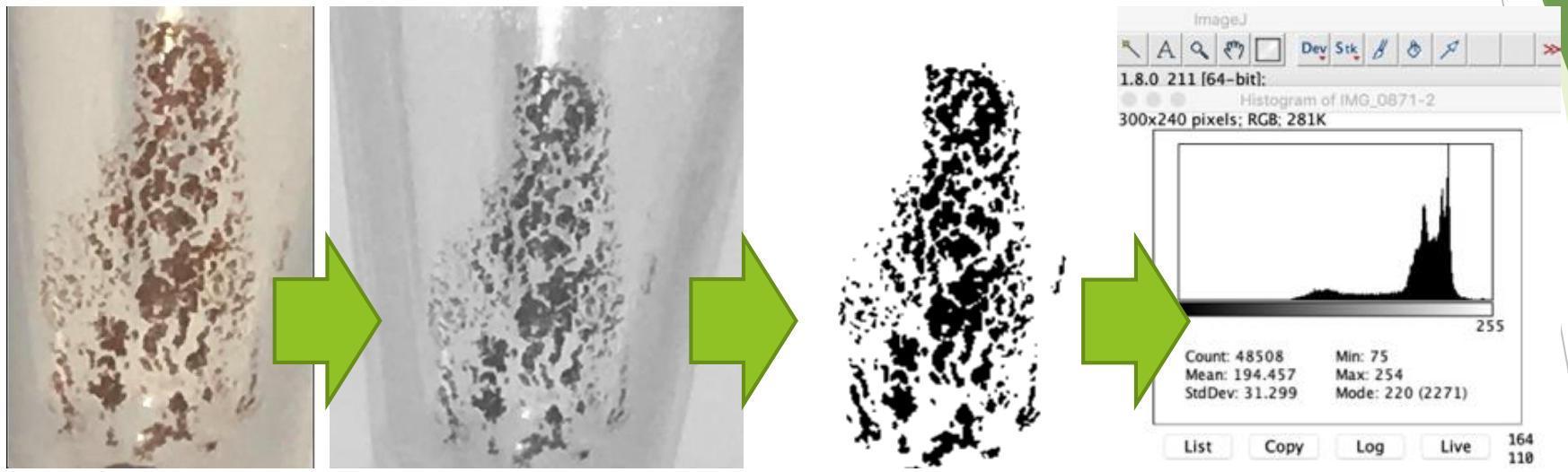
Sample: *Lasiodiplodia theobromae* spores



- Matting was observed on the positive control (suspension).
- Image analysis may help in providing more sensitive measure of some parameters.

Image analysis of magnetic nanoparticle matting

Sample: *Lasiodiplodia theobromae* spores



Original | Gray Scale/8-bit | B&W/Binary | Calculation

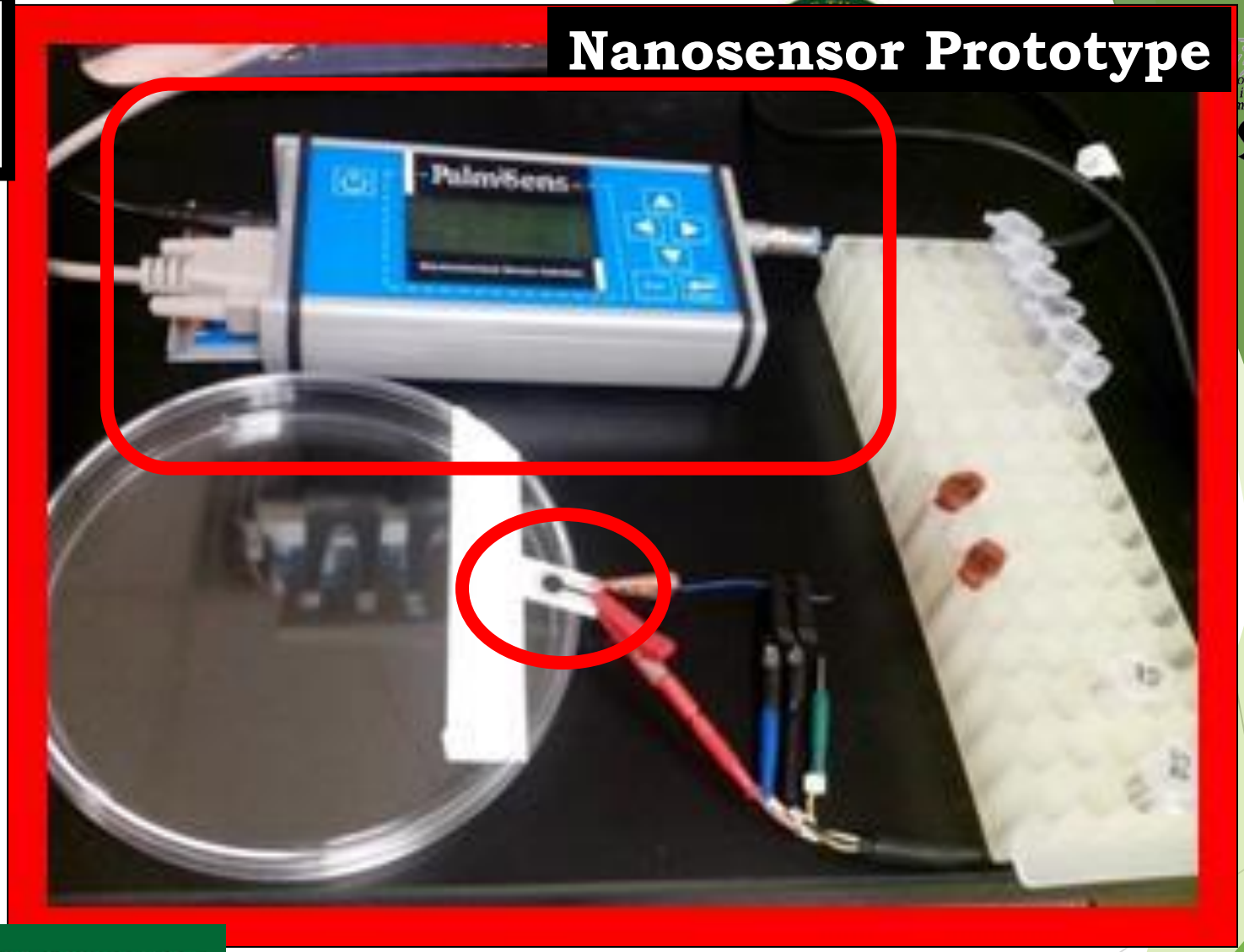
Sample	Mean threshold intensity
Blank	171.167
With spore suspension	194.457

The intensity of matting in the presence of the spores was calculated to be greater than that of the blank.

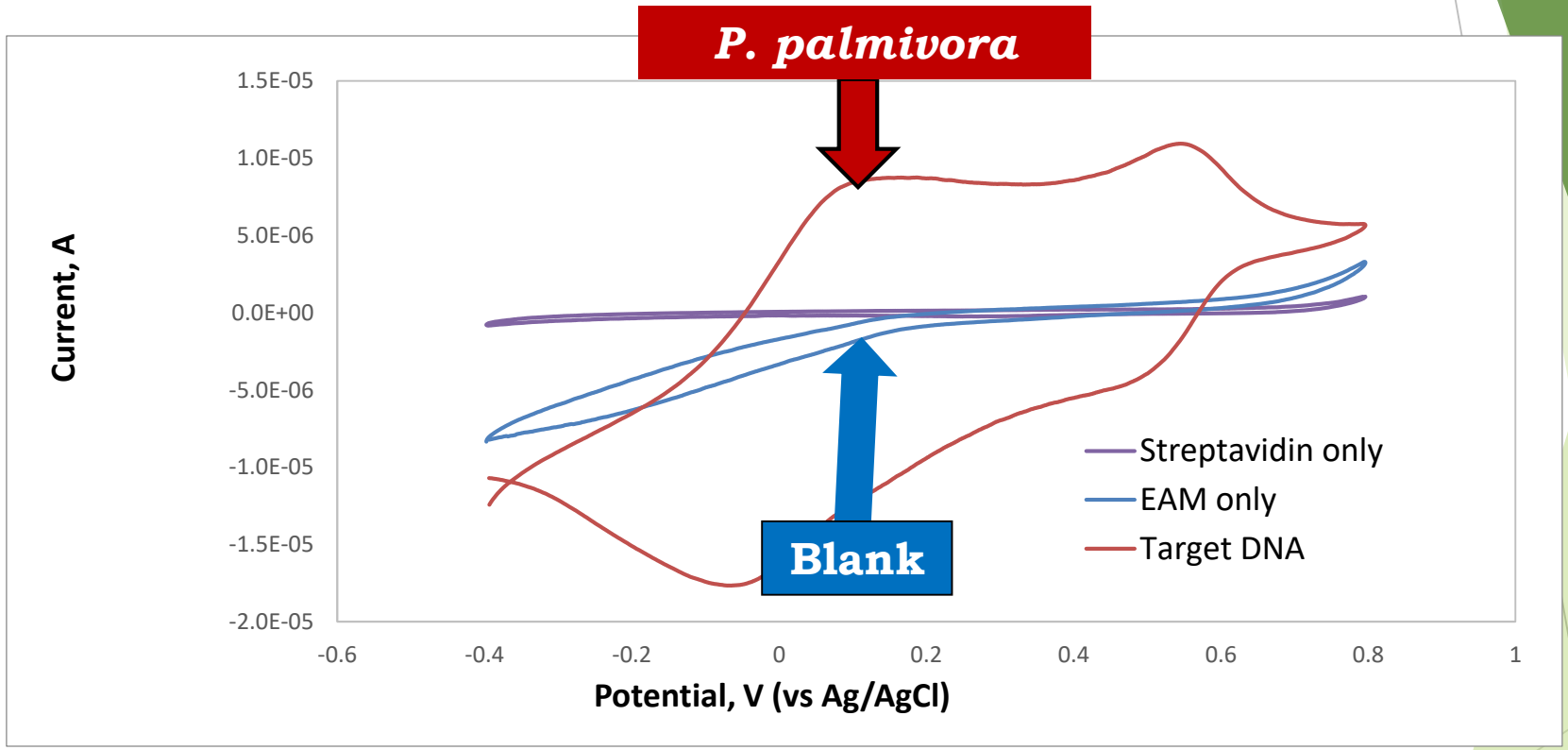
Diagnosis: Specific Detection

Cellphone/ Personal computer/ Laptop

Nanosensor Prototype

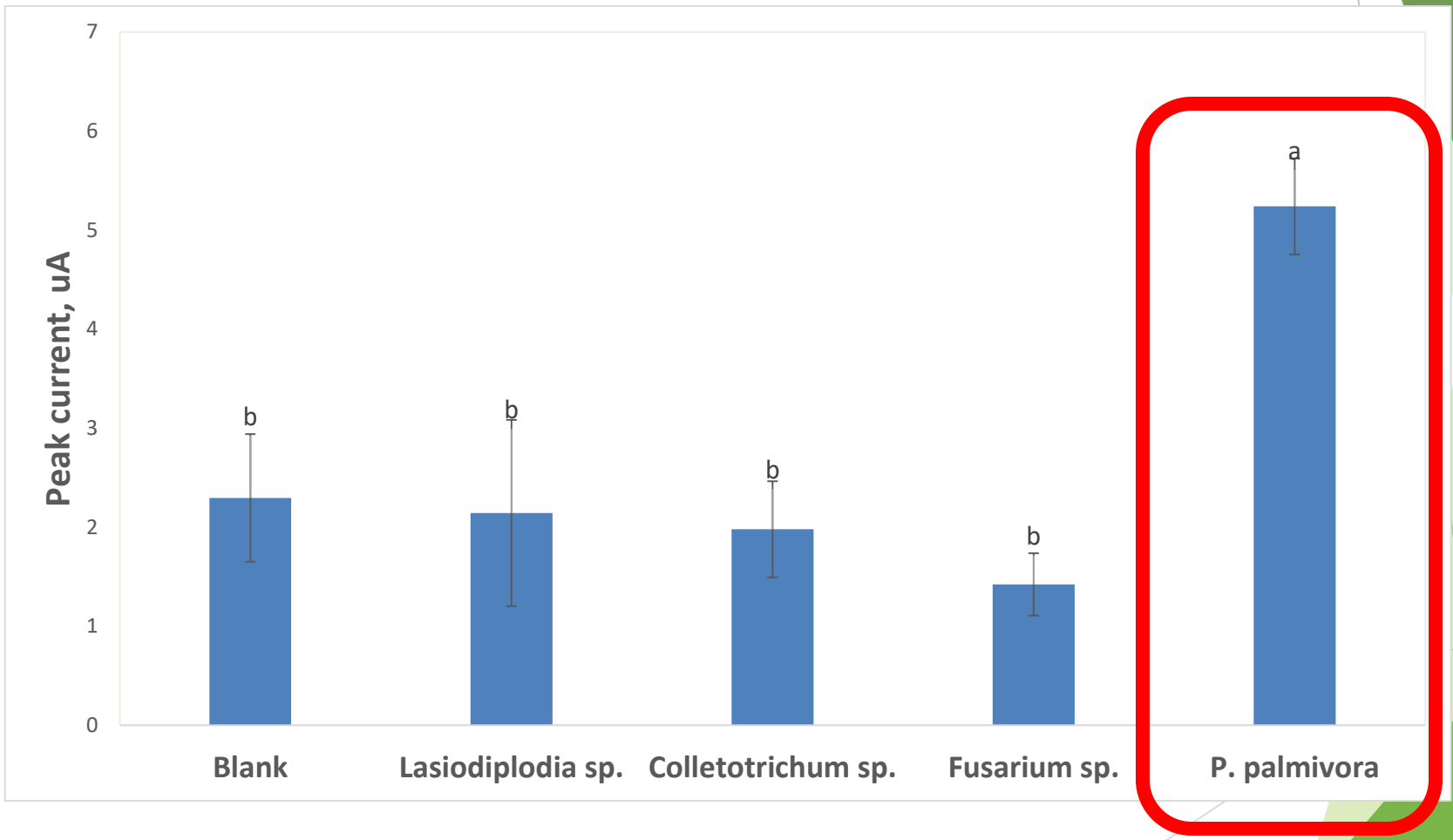


Dual hybridization and electrochemical detection

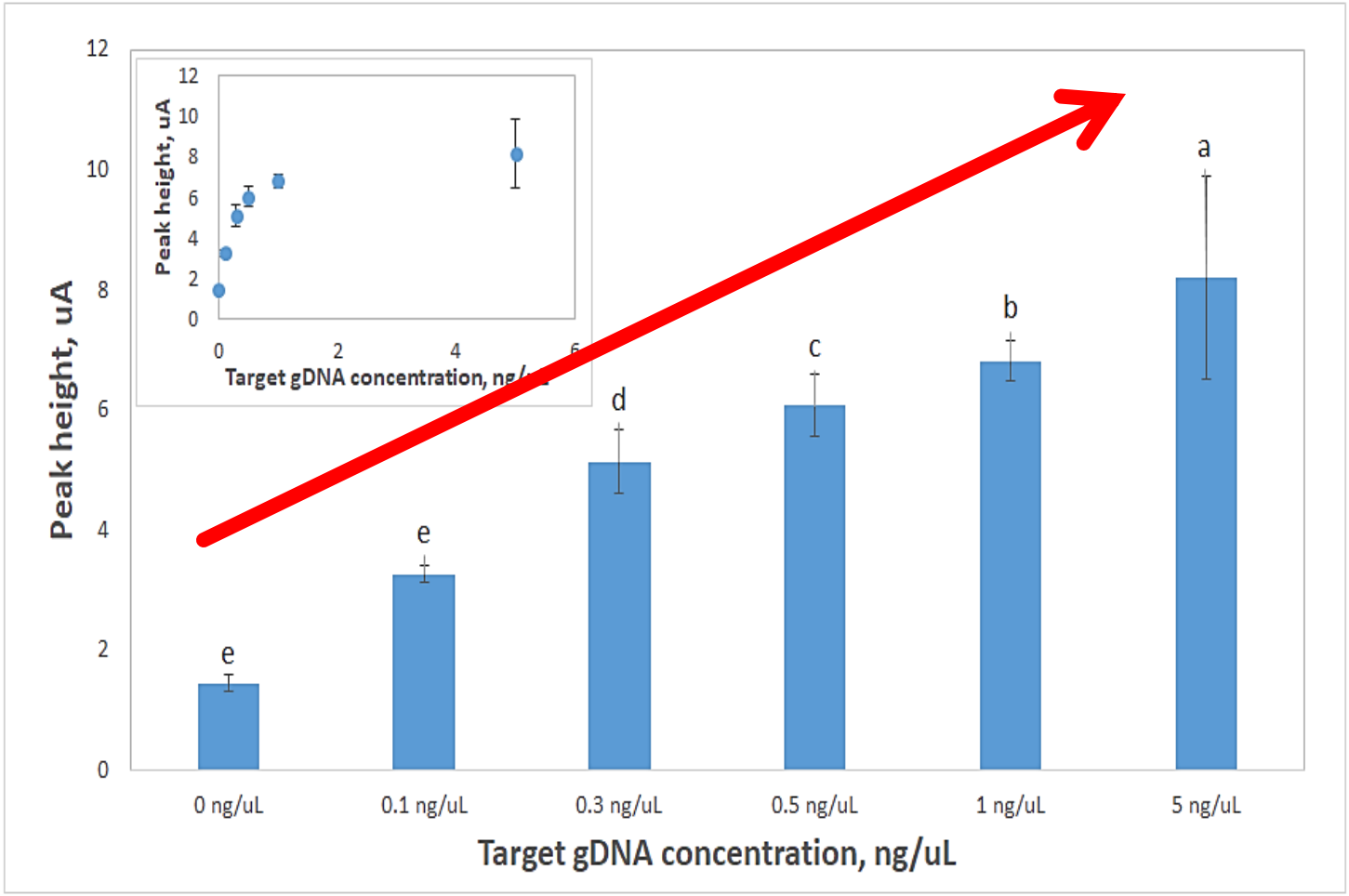


Cyclic voltammograms of Streptavidin – modified electrode, EAM added electrode, and *P. palmivora* genomic DNA hybrids on the electrode in 0.1 M HCl at 50 mV/s.

Selectivity



Limit of Detection

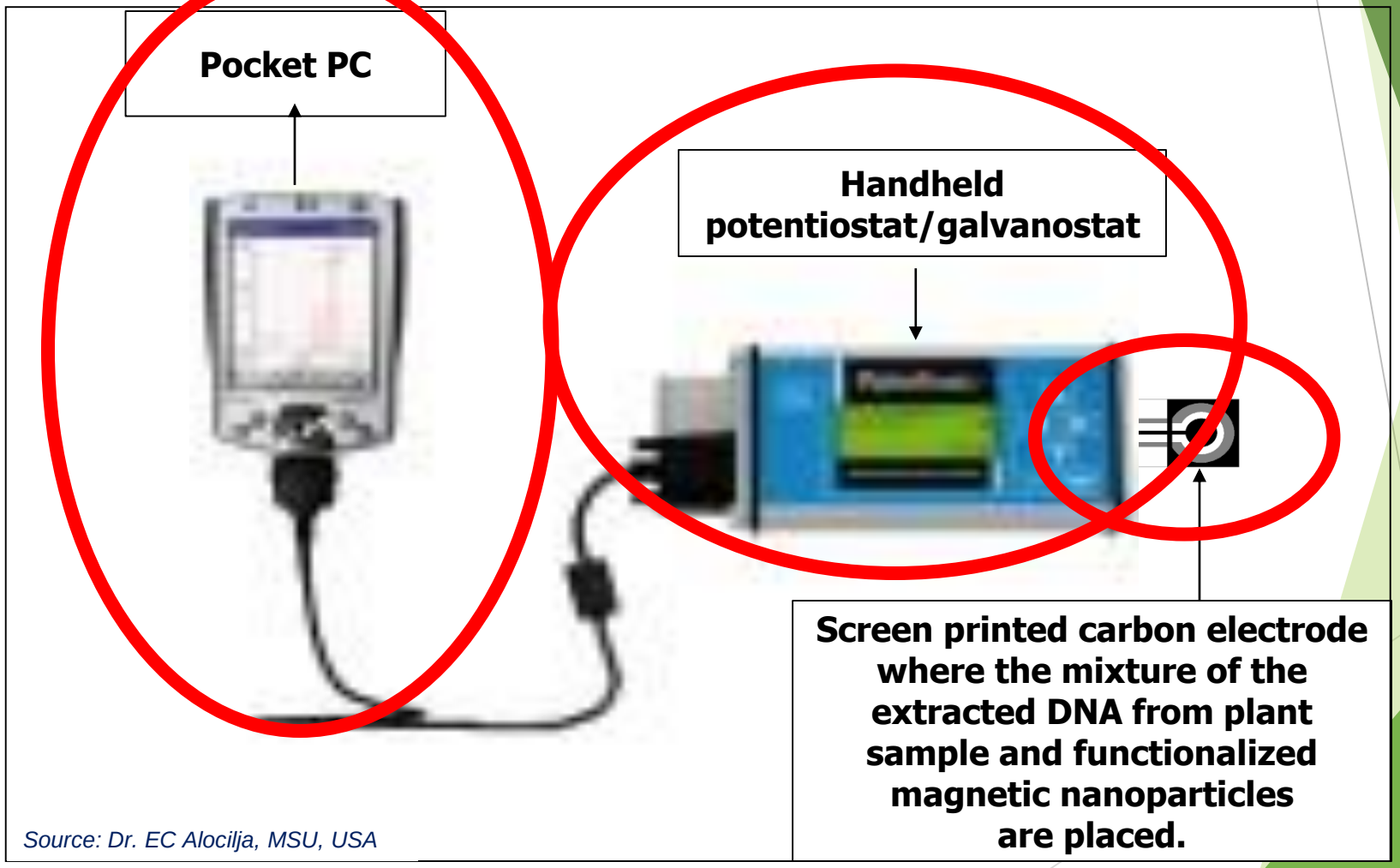


High Performing Nano-Diagnostic Kit for Crop Diseases

Site selection for field evaluation of nano-biosensor technology for the different commodities

Commodity	Disease	Causal Organism	Site selection
Banana	Panama Disease/Fusarium Wilt Bunchy Top	*Fusarium oxysporum f. sp. cubense* (Foc TR4) Banana Bunchy Top Virus (BBTV) vectored by aphids, *Pentalonia nigronrvosa*	Region XI (Davao) World's largest contiguous banana plantation with 432,000MT in 2017
White Potato	Late Blight Rugose Mosaic Virus	*Phytophtora infestans* Potato Virus Y (PVY) vectored by aphids, *Mysuz persicae*	DA-CAR (Benguet) 110,752 MT; 85% of the total production
Tomato	Bacterial Wilt	*Ralstonia* (*Pseudomonas*) *solanocearum*	DA-Region I (Pangasinan) Leading tomato producing province with 16% total area
Mungbean	Yellow Mosaic	Mungbean Yellow Mosaic Virus (MYMV) vectored by whitefly, *Bemicia tabaci*	DA-Region II (Cagayan Valley) Shared 30.2% of the total production second to Ilocos Region with 39.6% of the total production
Peanut	Cercospora Leaf Spot	*Cercospora araahidicola* ?	DA-Region I (Ilocos Norte) 35% of the total production

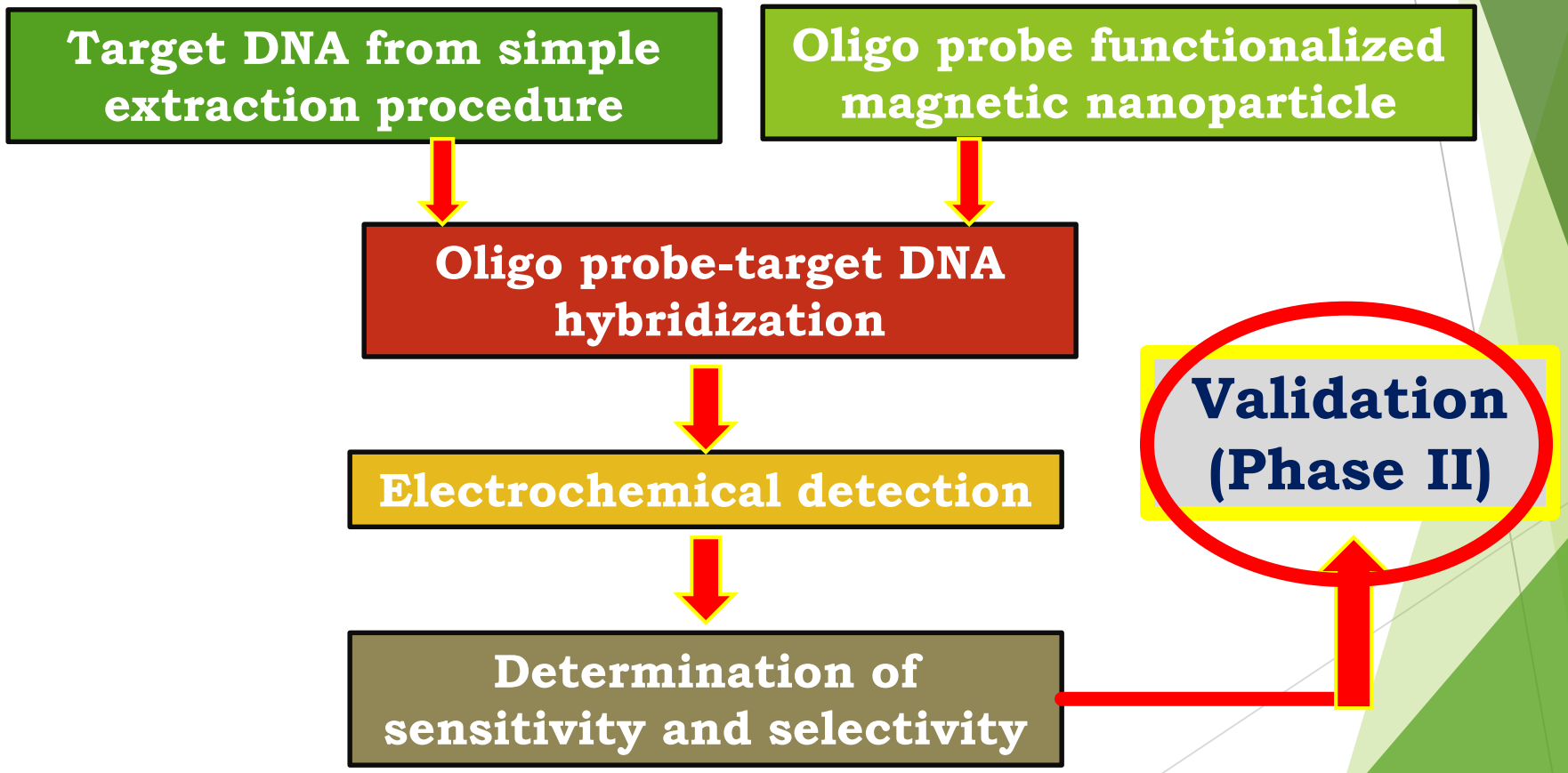
Schematic of the proposed handheld barcode DNA diagnostic system adopted from Michigan State University.



Source: Dr. EC Alocilja, MSU, USA

Methodology

Overall schematic diagram :



Detection of Dengue and Japanese Encephaitis Virus in Mosquitoes

RAPPLER News Video Business Newsbreak MovePH Views Life & Style Entertainment Sports Tech BrandRap

PHILIPPINES

DOH declares national dengue epidemic

The Department of Health reports a total of 146,062 dengue cases from January to July 2019

Janella Paris

Published 6:06 PM, August 06, 2019
Updated 12:39 AM, August 07, 2019

Facebook
Twitter
Reddit
Email

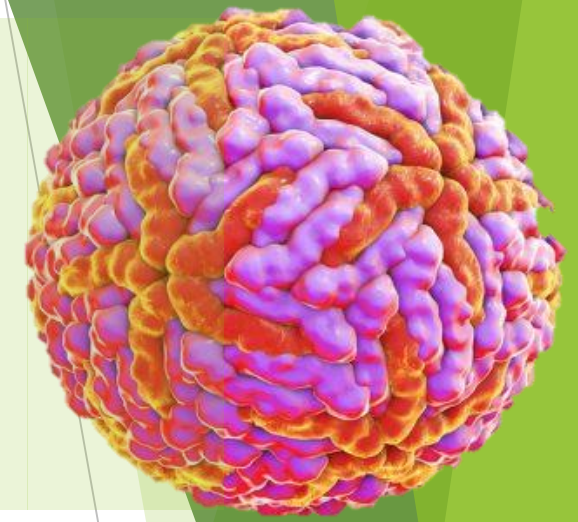
Microsoft

Table 1. Reported AMES Cases and Deaths by Region (N=1,664)
Philippines, January 1-May 25, 2019 vs 2018

Region	2019 Cases	2019 Deaths	2018 Cases	2018 Deaths	% Change
PHILIPPINES	**1,664**	**99**	**1,787**	**78**	↓7
I	168	12	223	8	↓25
II	183	5	94	4	↑95
III	245	3	315	12	↓22
IV-A	133	5	124	6	↑7
MIMAROPA	25	1	18	1	↑39
V	67	3	118	6	↓43
VI	146	3	164	7	↓11
VII	124	15	113	7	↑10
VIII	5	0	19	4	↓74
IX	31	2	29	4	↑7
X	41	1	105	2	↓61
XI	97	3	89	1	↑9
XII	52	4	39	1	↑33
ARMM	87	14	51	2	↑71
CAR	44	1	54	2	↓19
CARAGA	38	1	40	1	↓5
NCR	178	26	192	10	↓7

Dengue Virus (DENV)

- Causes dengue fever which can lead to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)

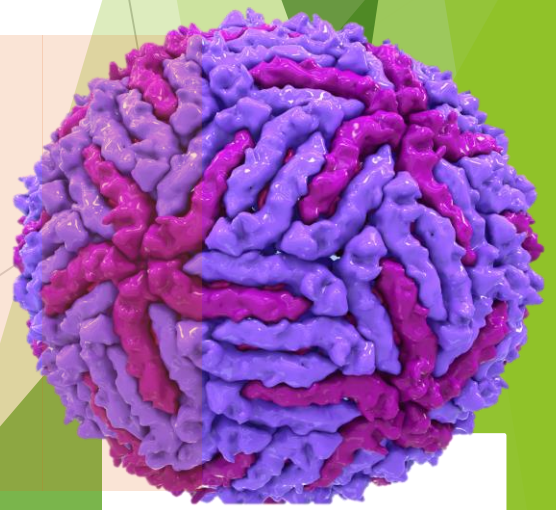


Pictures from thenativeantigencompany.com

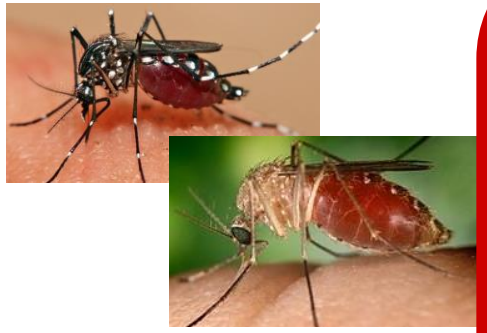
BACKGROUND

Japanese Encephalitis Virus (JEV)

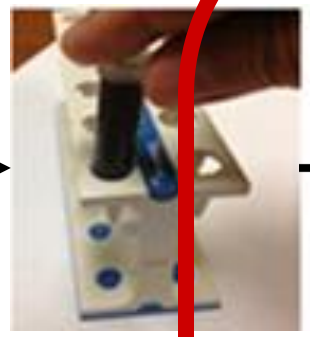
- Main cause of viral encephalitis (inflammation of the brain)



Nanodiagnostics

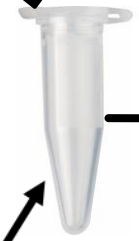


Mosquito sample
(*Aedes or Culex)*



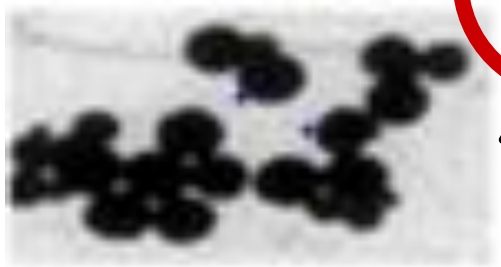
Virus extraction and
Concentration
using a simple
magnetic rack

Matting: infected (left)
Non-infected (right)
Completed in 10 mins

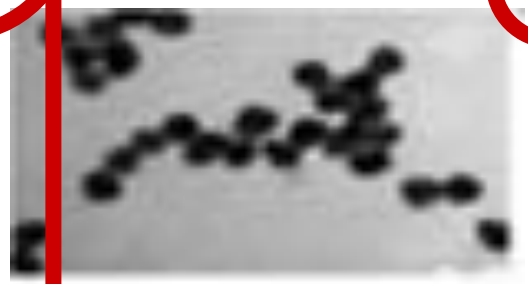


RNA Extraction:
RNA/DNA
transcription
& Au Probe
hybridization

PCR-Less genomic
detection
Completed in 2h



Functionalized Magnetic
Nano-Particles (MNPs)



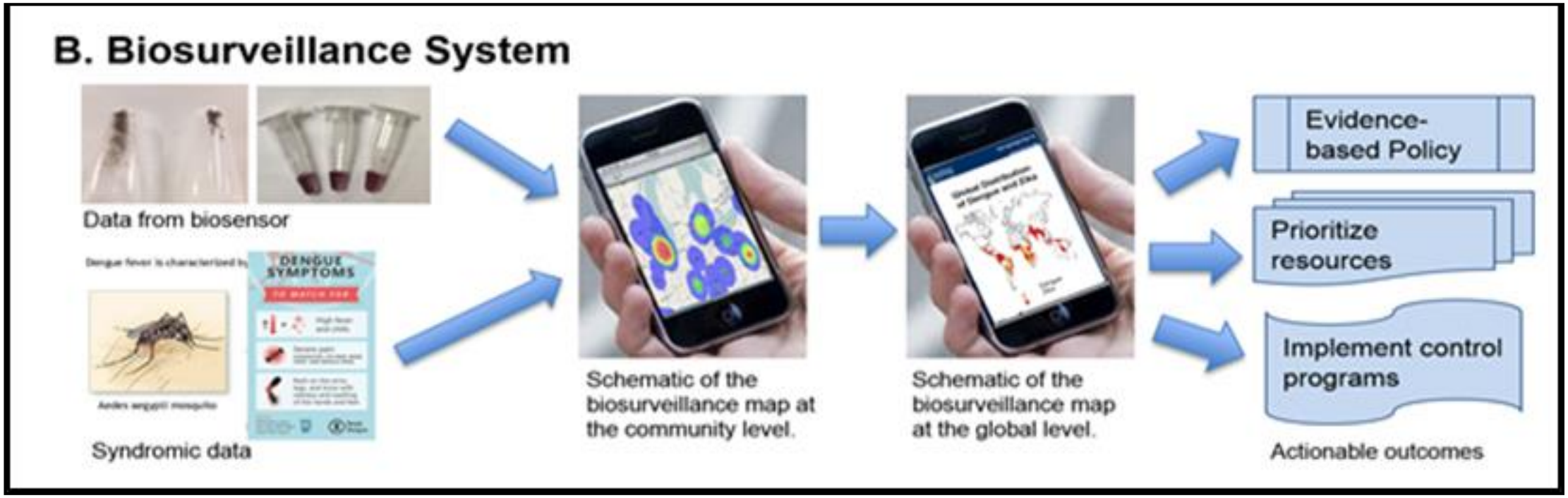
Gold Nanoparticle with
Specific RNA/DNA probe
(Au probe)

TRIAGE TECHNOLOGY

SPECIFIC DIAGNOSTIC TECHNOLOGY

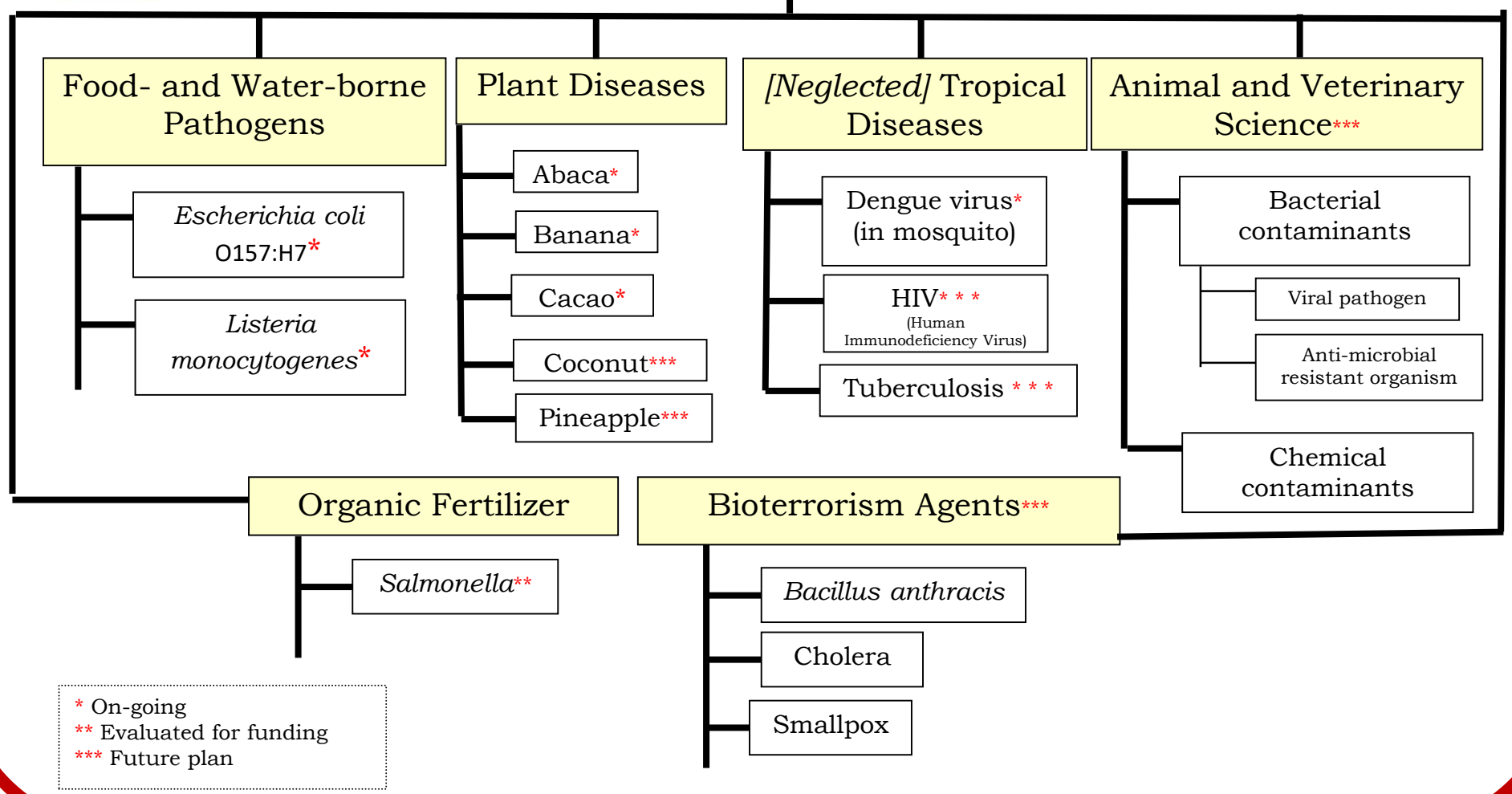
Nanodiagnostics

Technologies Comprising the Global Biosensing Network (GBN)



The Global Bio-Sensing Network with two novel technologies: Bio Surveillance System (Alocilja, 2018)

Nano-Biosensors Program



* On-going
** Evaluated for funding
*** Future plan

Acknowledgement

The NanoBIOTECH Team


Thank
you!

16th Engineering Research and Development for Technology Conference

NANOBIOSENSORS FOR THE RAPID AND EARLY DETECTION OF PATHOGENS

25 October 2019
Heritage Hotel, Pasay City

16th Engineering Research and Development for Technology Conference

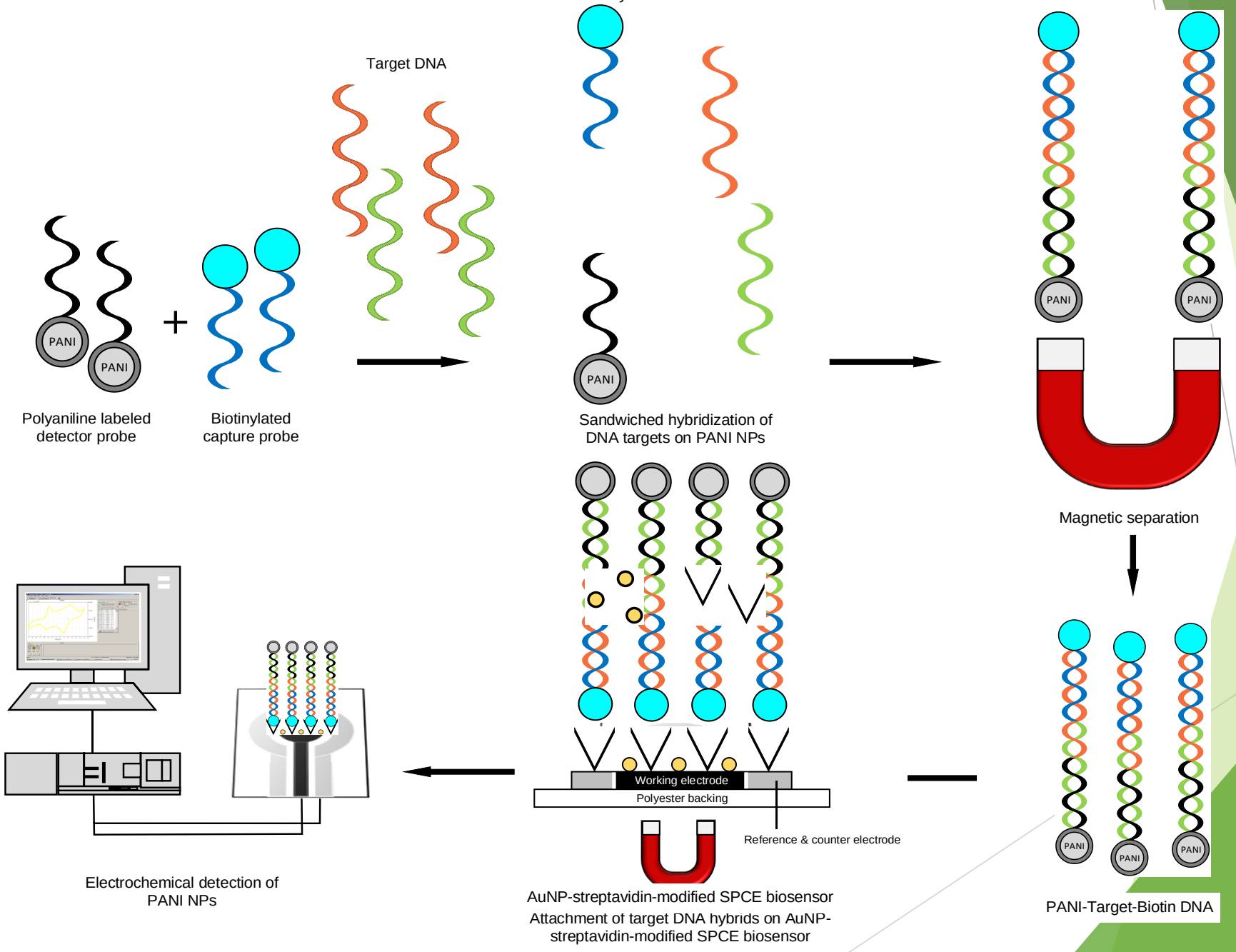
NANOBIOSENSORS FOR THE RAPID AND EARLY DETECTION OF PATHOGENS

Lilia M. Fernando, ***David Joram R. Mendoza, Ma. Kimberly C. Suministrado, Ida Allen P. Lopez, Ma. Theresa Jonna A. Atienza, Anthony James DM. Franco, Jomari R. Noe, Maria Teresa M. Perez, Lorele C. Trinidad, Evangelyn C. Alocilja, Edna A. Aguilar, Divina M. Amalin, Francisco B. Elegado***

25 October 2019
Heritage Hotel, Pasay City

Sandwiched Hybridization

Created by: Matthew Vasher



Economic Analysis

Cost-Benefit Analysis: Three-step analysis

Step 1 - Identify all the potential benefits of the technology

Step 2: Identify all the relevant costs of the technology

Step 3: Compare the costs to the benefits estimated in steps 1 and 2

Estimated Cost for Detection of Crop Diseases using "CROPBIOQUEST " High Performing Nano-Diagnostic Kit for Crop Diseases per test /sample

Test	Component	Amount/test	Cost/test (in PhP)
Triage	MNP Other materials (Eppendorf tube, extraction solution, magnetic rack, dropper)	0.025mg	2.55 ($0.05) 7.45 ($0.15)
Total Cost			**10.00 ($0.20)**
Diagnosis	MNP AuNP Other materials	0.5mg 30µL	50.00 ($1.00) 10.20 ($0.20) 40.00 ($0.80)
Total Cost			**100.00 ($2.00)**

One Nanobiosensor Kit with 1000mg MNP approximately will cost Php 3,000 good for 40,000 samples for triaging; Additional 10ml AuNP for Php 3,300 good for 350 samples for diagnosis. Approximately, the total cost for 1 kit will be Php 6,000-6.500

Biosensor : Market Drivers & Challenges

Source: http://www.strategyr.com/MarketResearch/Biosensors_Market_Trends.asp

Sample Preparation

Transfer samples into a modified buffered peptone water (mBPW) for short-time enrichment

↓

Obtain one (1) mL sample and transfer to 1mL lysis buffer

↓

Shake to lyse the cells and to released DNA into the medium

↓

Capture DNA by dipping the filter paper in the lysis buffer

↓

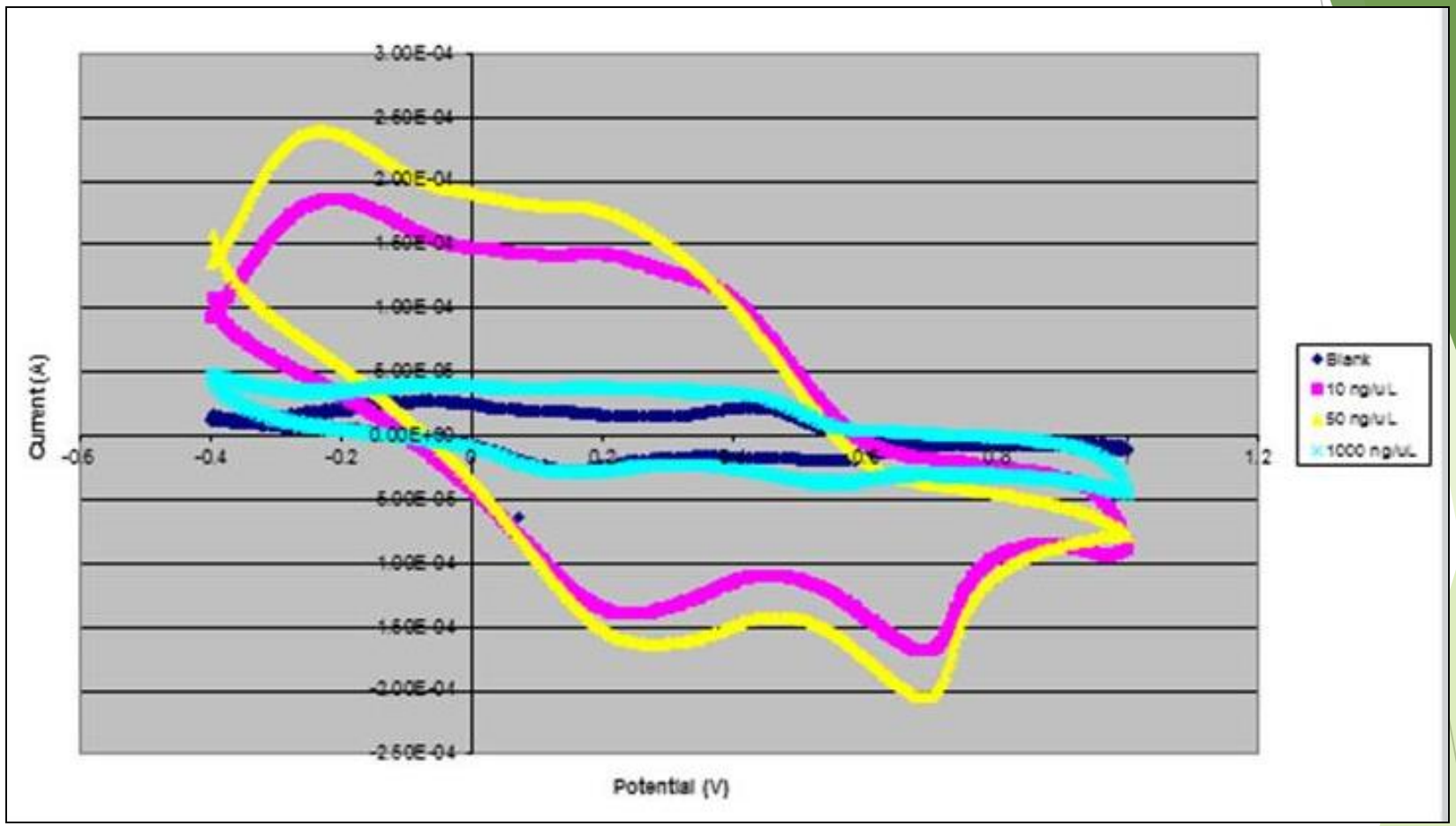
Wash non-target molecules by dipping the filter paper into wash buffer

↓

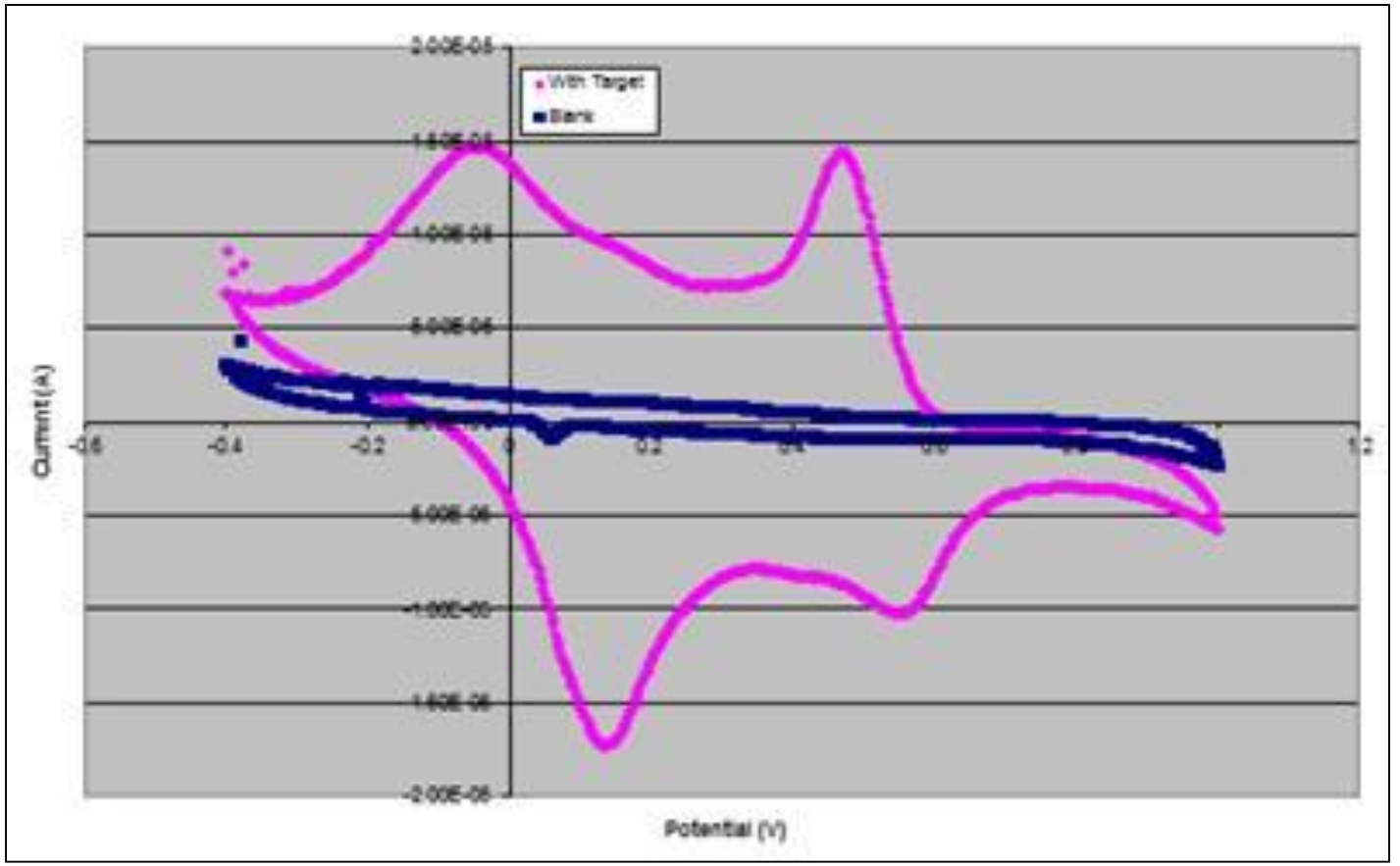
Elute the DNA by dipping the filter paper into a resuspension buffer or ultra distillied water

↓

Detect extracted genomic DNA using DNA-based nanobiosensor



Electrochemical response of the *Listeria monocytogenes* nanobiosensor for the detection of the lis O gene at different target concentrations.



Electrochemical response of the nanobiosensor for the detection of listeriolysin O gene (2 nmols) in *L. monocytogenes*

Table A2
Percent Positive *Salmonella* Tests in the PR/HACCP Verification Testing Program
Aggregated Results by Product Class, 1998 – 2014

All Years 1998 - 2014										
	Large		Small		Very Small		Unknown		All Sizes	
Product	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos	# Samp	% Pos
Broilers	98,033	7.5%	35,449	11.9%	5,276	19.9%	54	7.4%	138,812	9.1%
Market Hogs	17,055	2.4%	21,523	5.0%	35,940	3.3%	108	0.9%	74,626	3.6%
Cows/Bulls	3,451	0.2%	19,111	1.2%	8,621	1.9%	0		31,183	1.3%
Steers/Heifers	14,105	0.1%	14,346	0.5%	16,520	0.4%	99	0.0%	45,070	0.3%
Ground Beef	17,950	3.6%	178,446	2.6%	106,717	1.6%	429	2.8%	303,542	2.3%
Ground Chicken	1,490	26.2%	4,270	24.1%	623	31.9%	106	11.3%	6,489	25.2%
Ground Turkey	9,914	21.8%	2,756	16.9%	528	12.1%	66	6.1%	13,264	20.3%
Turkeys	10,101	4.0%	5,269	3.6%	180	7.8%	32	0.0%	15,582	3.9%

NOTE: HACCP classifications for a plant can change over time. This table reflects the sum of the individual tables, i.e. HACCP size at the time the samples were taken.

ttps://www.fsis.usda.gov

Comparison of Nanobiosensor and Commercially Available Kits

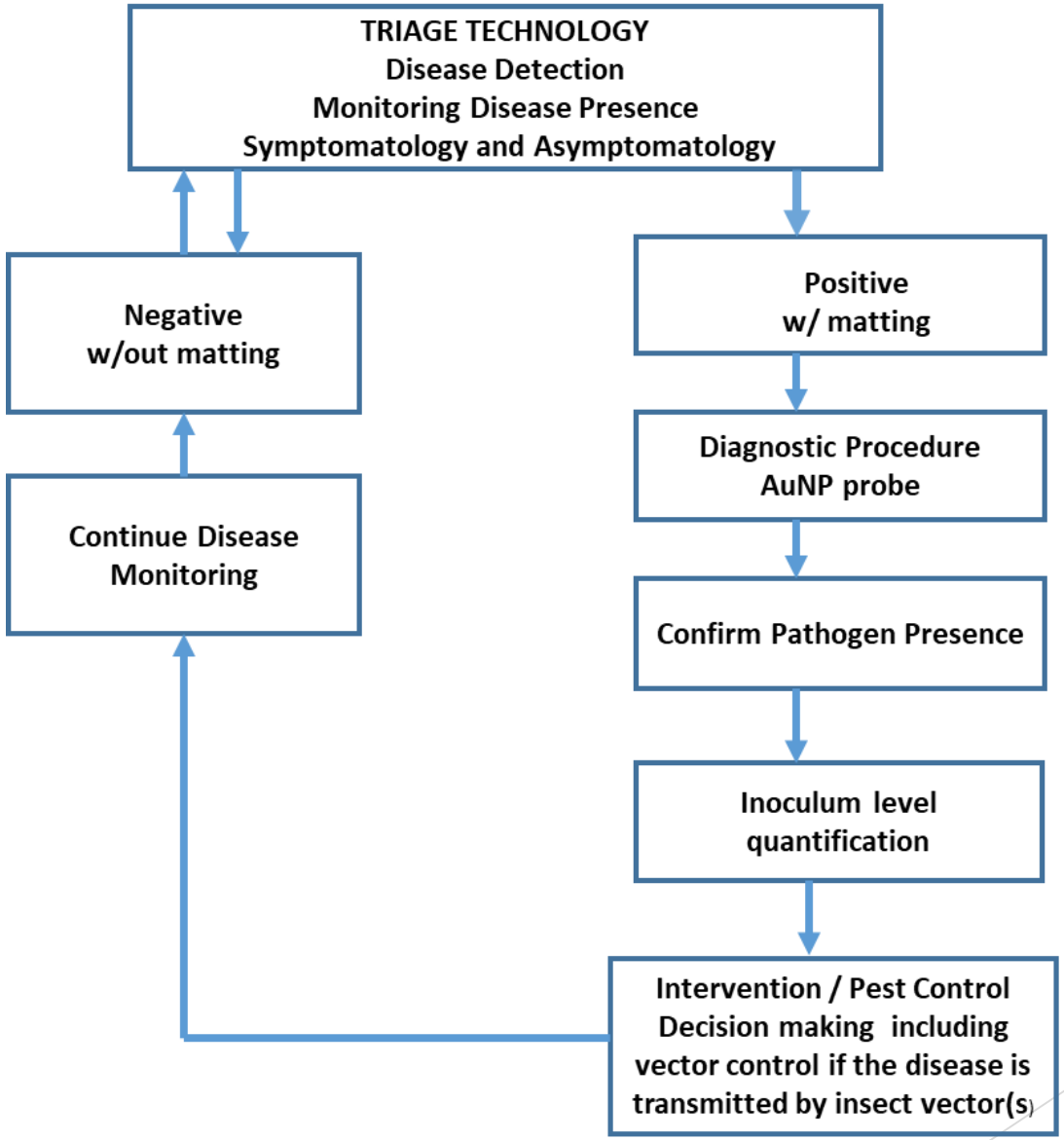
PARAMETER		Conventional Plating Method	Leading Molecular–based Detection System in the local market	Leading Antibody-based Detection System in the local market
MECHANISM	DNA-based	Cell-based	DNA-based	ELISA-based
OUTPUT	Quantitative (Cell Population)	Qualitative (Presence or Absence)	Qualitative (Presence or Absence)	Qualitative (Presence or Absence)
DETECTION TIME	5 hours	1-2 weeks	24-30 hours	32-44 hours
SENSITIVITY	10^2 bacterial cells	10^4 bacterial cells	10^3 bacterial cells	Not documented
PORTABILITY	Portable	Laboratory Use Only	Laboratory Use Only	Laboratory Use Only
ESTIMATED UNIT COST (Equipment)	₱ 350,000.00	₱ 900,000.00	₱ 850,000.00	₱ 650,000.00
ESTIMATED UNIT COST (Consumables)	₱ 600.00	₱ 800.00-1000.00	₱ 625.00	₱ 1,200.00

Validation sites for Nanobiosensor

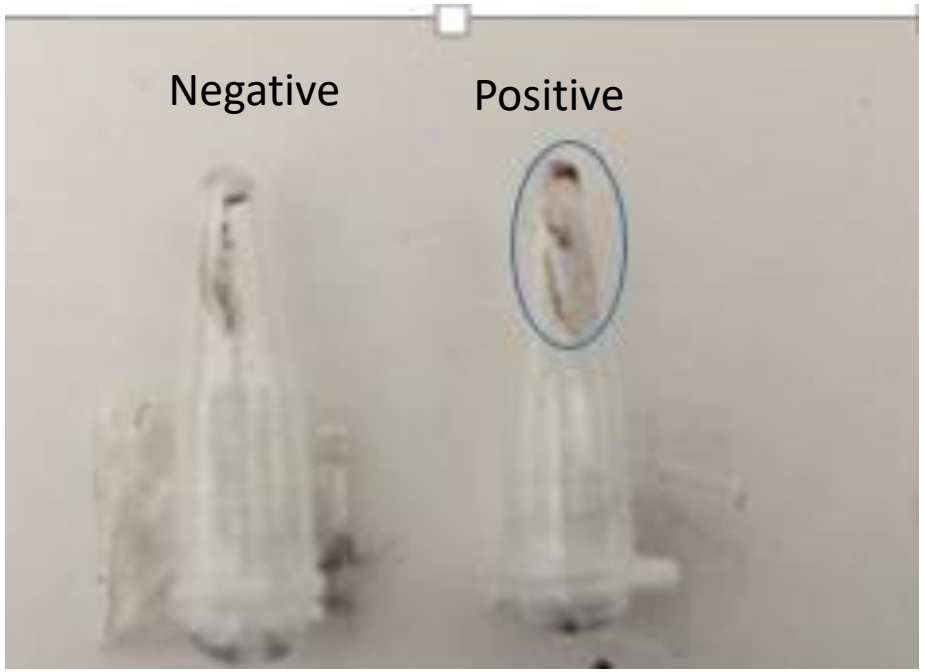
BIOTECH-UPLB has negotiated with or entered into agreements with the following companies and/or agencies:

- Agrichexers Corporation (Bulacan)
- St. Ambrose Farm (Laguna)
- ASTS Laboratory (Gen. Santos)
- La Filipina Uy Gongco Corp. (Iloilo)
- Vitarich Corporation (Bulacan)
- Pilmico Corporation (Tarlac)
- National Meat Inspection

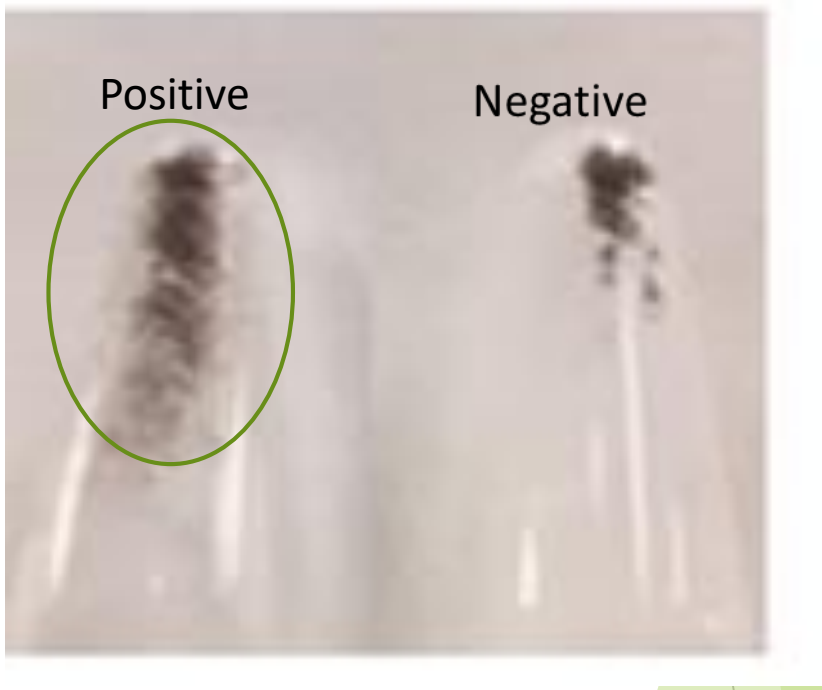
Conceptual Framework



Proof of Concept for Agricultural use



Positive detection of *Phytopthora palmivora*, causal organism of Black Pod Rot in cacao



Matting using MNPs in Dengue virus (Dr. Alocilja research result)

Economic Viability

Example of demonstrating the economic benefits of crops grown under IPM with monitoring system versus conventional “calendar spraying” programs.

	Weekly costs to farmers for pest management activities (php / hectare)	
	Non-IPM Calendar Spray Program (No monitoring)	IPM with monitoring system using nanobiosensor kit
Week 1	1,000 php for fungicide spray	500 php for disease monitoring
Week 2	1,000 php for fungicide spray	500 php for disease monitoring
Week 3	1,000 php for fungicide spray	500 php for disease monitoring
Week 4	1,000 php for fungicide spray	1,000 php for fungicide spray
Total cost/month	**5,000 php total cost for calendar spraying**	**2,500 php total cost under IPM**

Enterohemorrhagic *Escherichia coli* (EHEC), a subset of Shiga toxin-producing *E. coli* (STEC) strains, is a leading cause of bacterial enteric infections in the United States and worldwide.

EC causes ~100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States alone.

Most of these illnesses have been linked to consumption of foods derived from animal products and, recently, organically grown vegetables.

Although O157:H7 is currently the predominant serotype and accounts for ~75% of EHEC infections worldwide, several non-O157 EHEC serotypes are also emerging as serious concerns for foodborne illnesses.

❖ In the United States, a group often referred to as the **"Big 6" (O111, O26, O121, O103, O145, and O45)** accounts for the majority of the non-O157:H7 serotypes isolated from clinical infections and, therefore, is also a focus of concern.

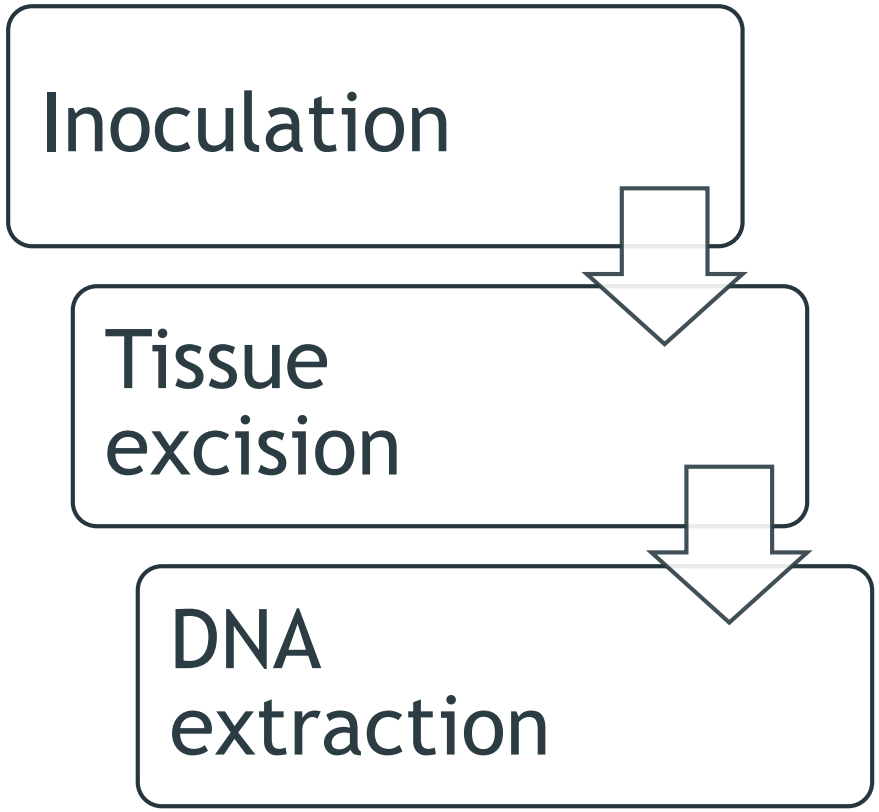
(www.cdc.gov/foodsafety/pdfs/foodbornediseaseoutbreaks-annual-report-2013-508c.pdf).

Development of an Electrochemical DNA – Based nanobiosensor for the Detection of *Phytophthora palmivora* (Butler) Butler in Soil and in *Theobroma cacao* L. Pods

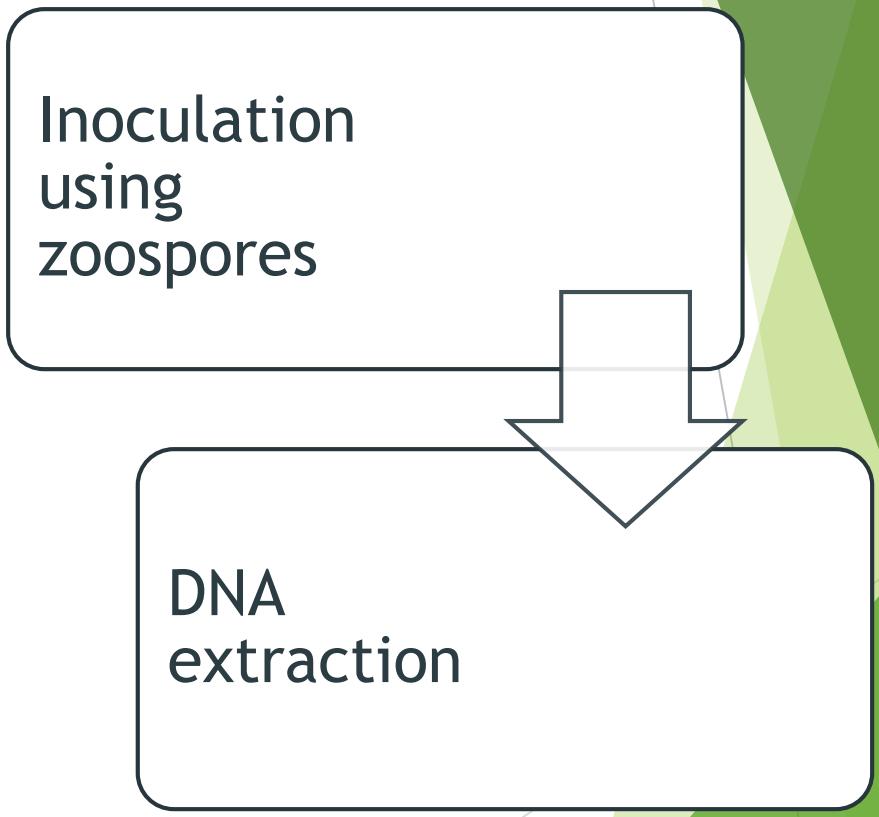
Anthony James del Mundo Franco
MS Agricultural Chemistry

Preparation of samples

Plant samples



Soil samples



DNA extraction

Pure mycelia

- ZR Fungal/Bacterial DNA MiniPrep™ (Catalog No. D6005)

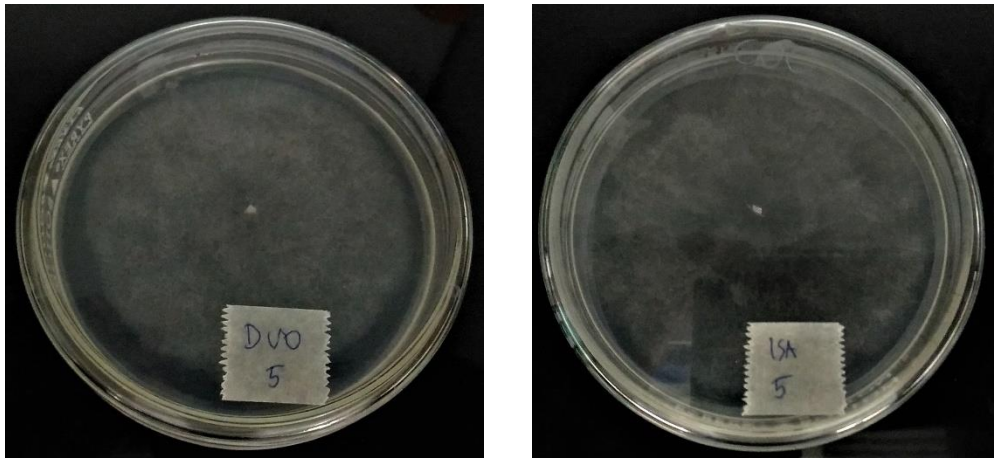
Soil samples

- ZR Soil Microbe DNA MiniPrep™ (Catalog No. D6001)

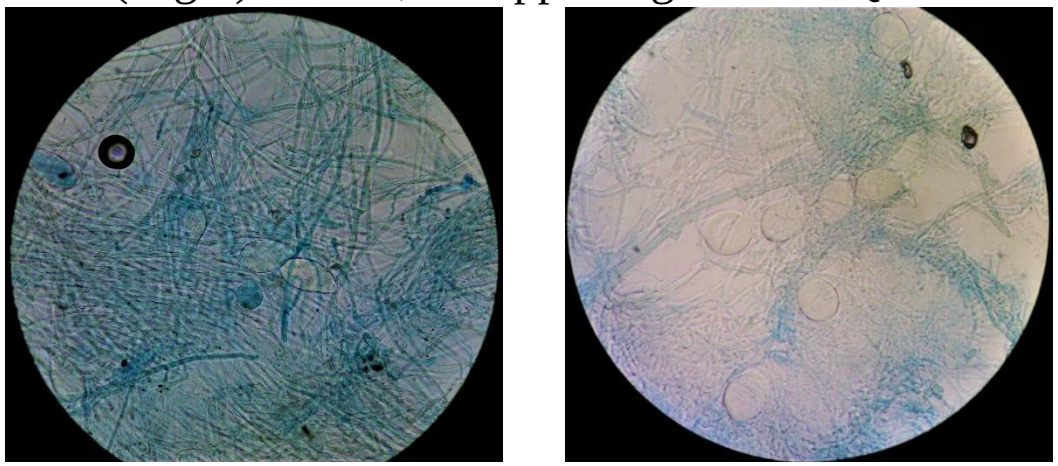
Plant samples

- Quick-DNA™ Plant/Seed Miniprep Kit (Catalog No. D6020)

Isolation of *P. palmivora*



Purified *P. palmivora* isolates obtained from (Left) Davao and (Right) Isabela, Philippines grown on QS – PDA



Optical micrograph of the *P.palmivora* isolates obtained from (Left) Davao and (Right)Isabela, Philippines (40X Magnification)

- Presence of papillate sporangia suggests that the isolate is of genus *Phytophthora*.
- Contamination of cultures were eliminated by successive hyphal tip isolation.
- Cultures were maintained on slants to minimize contamination.

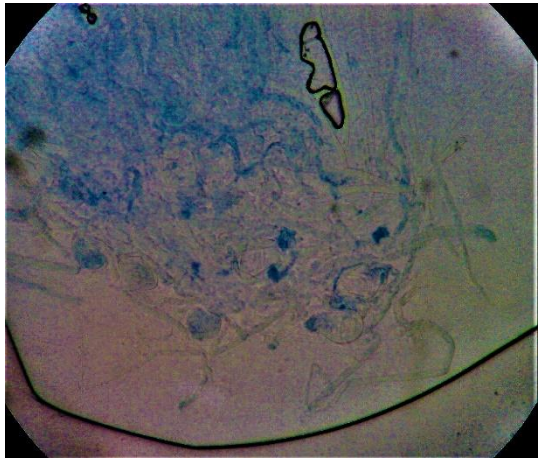
Pathogenicity tests

Moist chamber culture set – up containing the inoculated cacao pods

- Lesions on all inoculated pods were observed at 3 DAI.
- At 5 DAI, mycelial growth on pod surface is observable

Pathogenicity tests

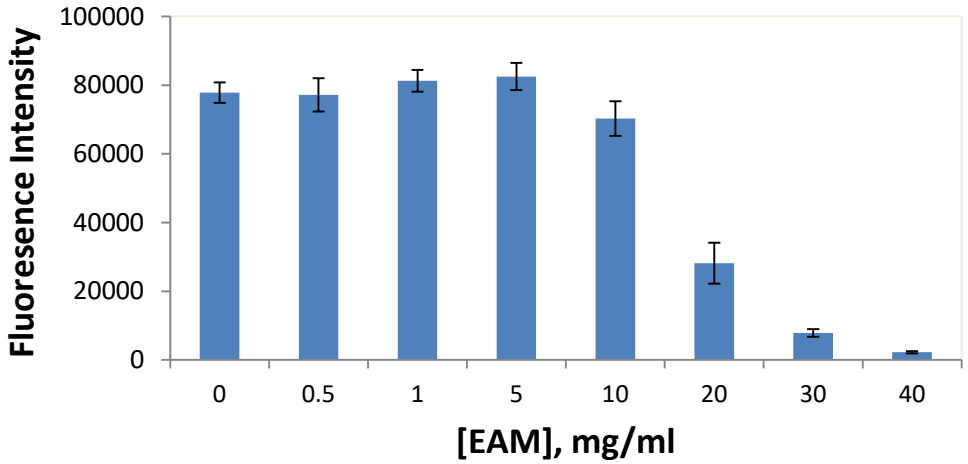
Cacao leaves inoculated with *P. palmivora* showing dark necrotic lesions 3 days after inoculation



Cacao leaves inoculated with *P. palmivora* showing dark necrotic lesions 3 days after inoculation

Conjugation of EAM and detector probe

EAM – probe conjugation

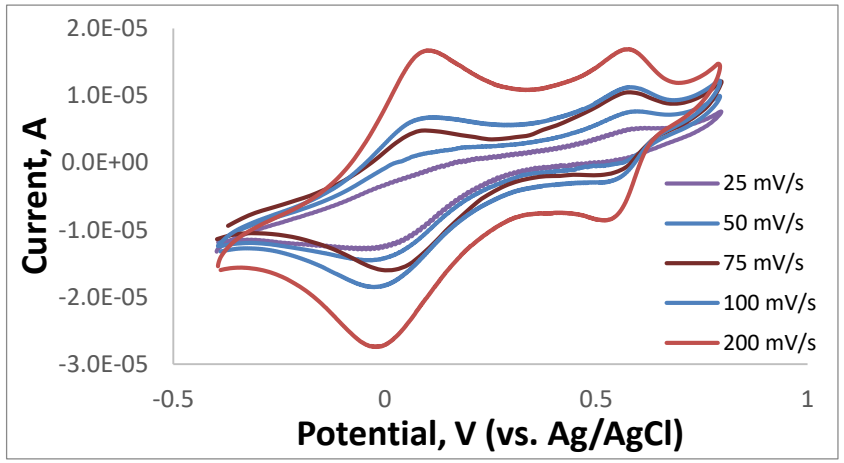


Reduction of fluorescence intensity of 6-Carboxyfluorescein versus increasing concentration of EAM

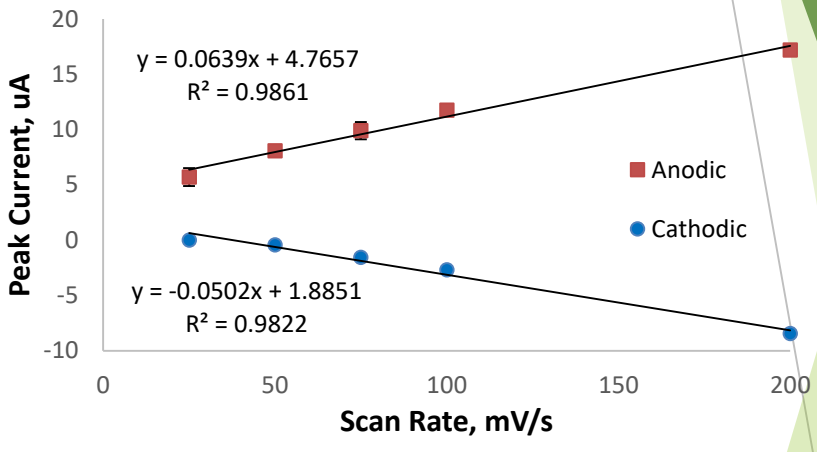
- Used 3'-carboxyfluorescein labelled detector probe to determine the concentration of EAM that will be completely saturated with the detector probe.
- It was found that at **40 mg EAM/mL**, the EAM is completely saturated with the probe.

Dual hybridization and electrochemical detection

Effect of scan rate



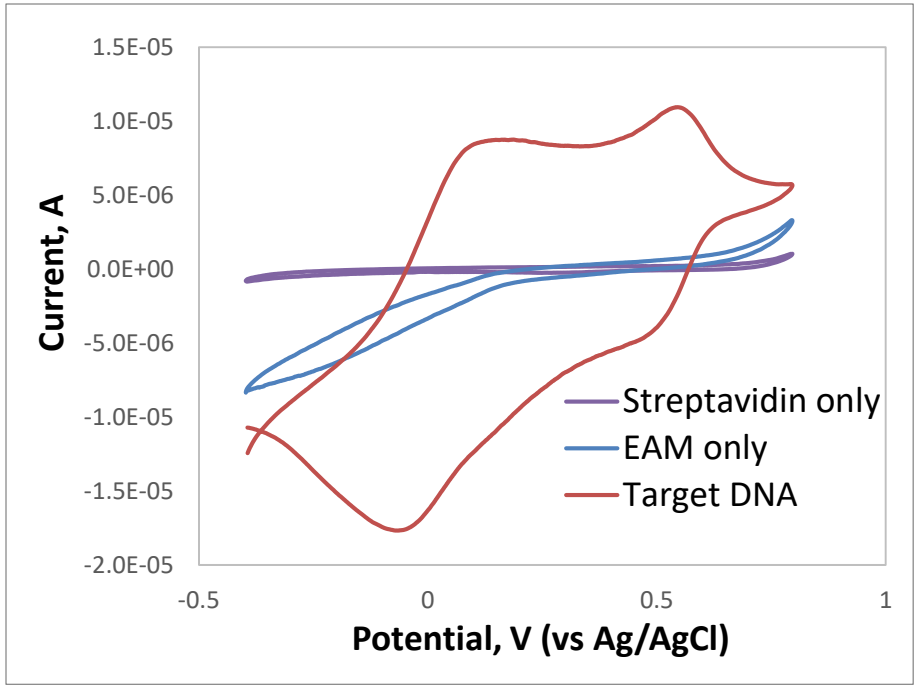
Electrochemical response of EAM in 0.1 M HCl with increasing scan rate



Anodic and cathodic peak current of EAM in 0.1 M HCl at different scan rates

Electrochemical response increases linearly as scan rate increases. This implies that the redox process on the electrode is **diffusion – controlled**. This suggests that the **current measured is proportional to the concentration of the transducer**.

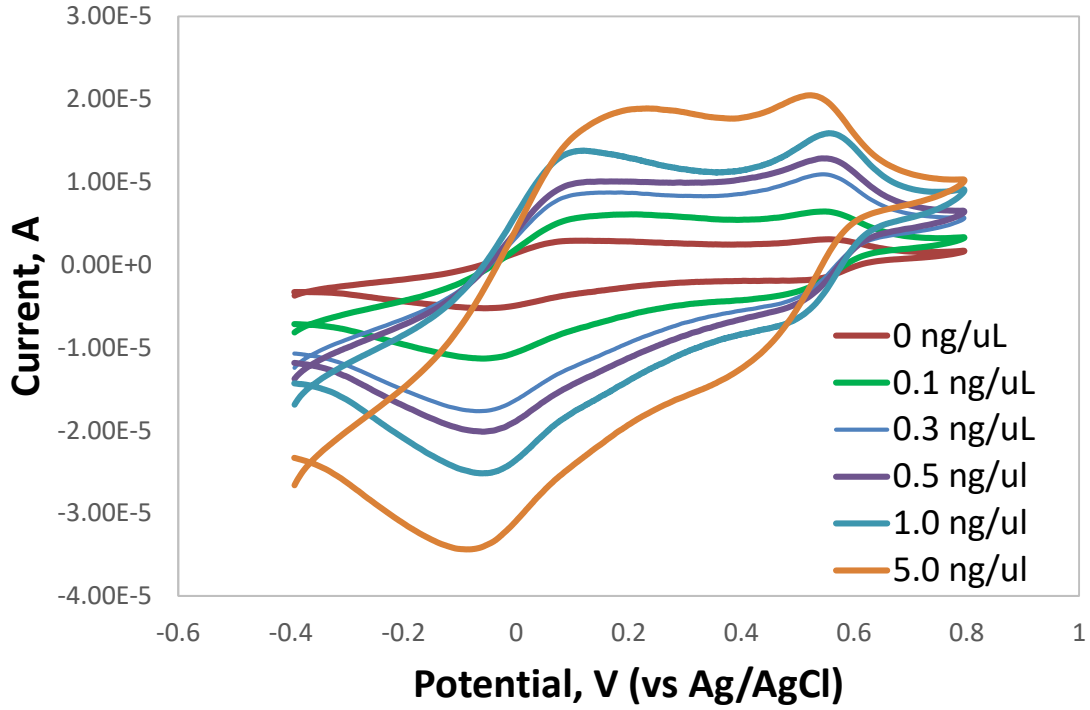
Dual hybridization and electrochemical detection



Cyclic voltammograms of Streptavidin – modified electrode, EAM added electrode, and *P. palmivora* genomic DNA hybrids on the electrode in 0.1 M HCl at 50 mV/s.

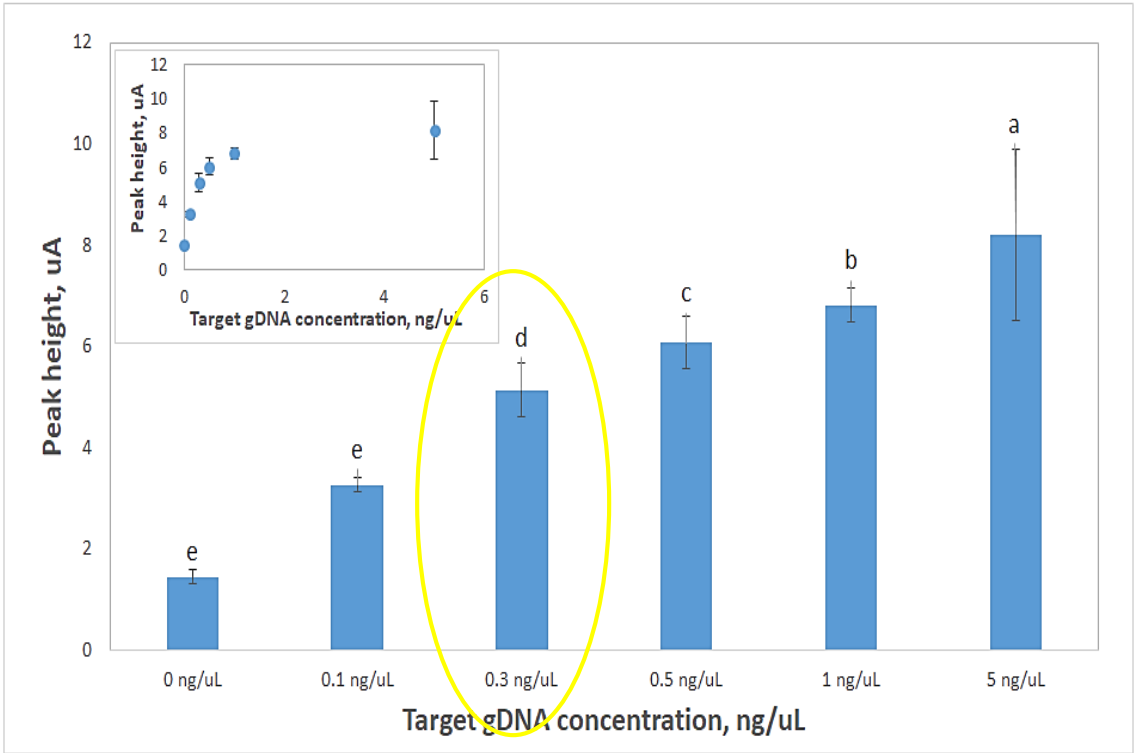
- Electrochemical detection of the formed hybrids was successfully demonstrated

Limit of Detection



Electrochemical response of the biosensor on *P. palmivora* genomic DNA concentrations ranging from 0 to 5.0 ng/µL in 0.1 M HCl at 50 mV/s

Limit of Detection



Anodic peak height at different *P. palmivora* genomic DNA concentrations ranging from 0 to 5 ng/µL in 0.1 M HCl at 50 mV/s (Mean ± SD; n = 3).Statistical analysis through Tukey's Honest Significant Difference Test was performed at 95% confidence. Inset: Plot of anodic peak height versus *P. palmivora* genomic DNA concentration.

- Statistical analysis showed that the limit of detection is at 0.30 ng/µL
- This limit was verified by calculating S/N:

$$S/N_{@0.3} = \frac{5.138\mu A}{1.450\mu A} = 3.544$$

- Since the calculated value is greater than 3, then the limit of detection is determined to be at 0.3 ng/µL

Precision

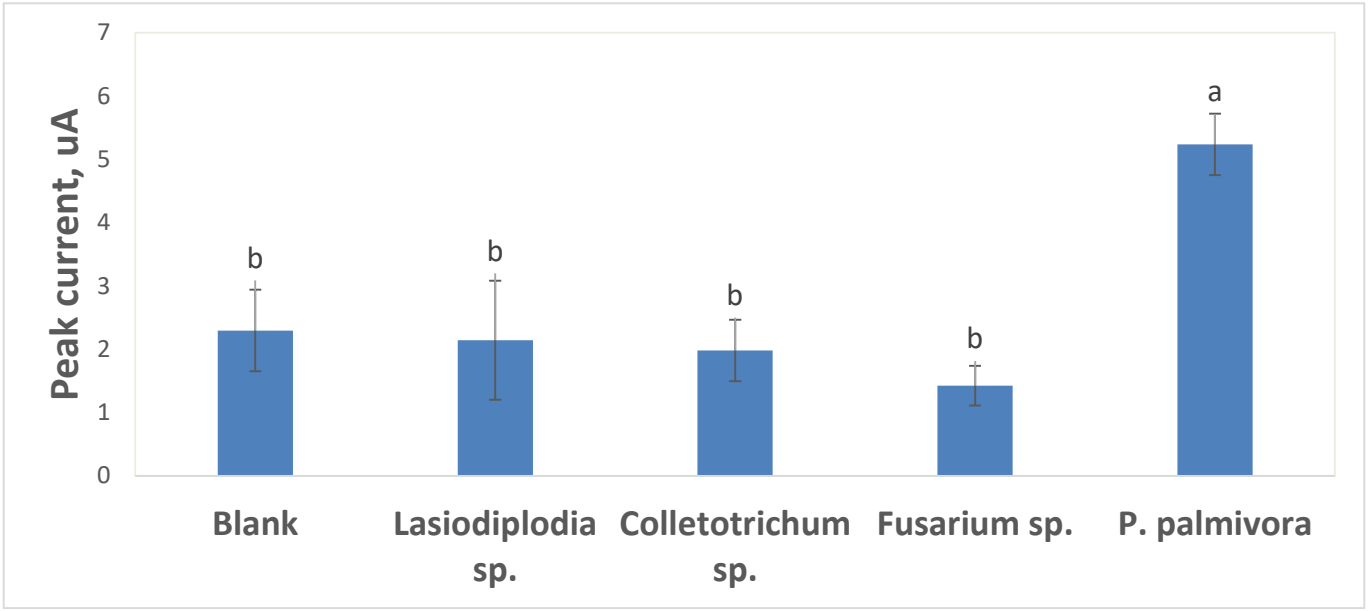
DNA extract sources and the corresponding average RSD of anodic peak heights

DNA extract source	Average RSD
Pure mycelia	22.16
Plant samples	36.36
Soil samples	40.03
Other fungi	30.17

- All the values are greater than 10% which suggests that the repeatability of the detection is unsatisfactory
- The variation could be attributed to:
  - EAM aggregation
  - Washing step at the electrode

  Which could be addressed by using less EAM
- Although unsatisfactory, the precision is enough to establish statistical significance of results.

Selectivity

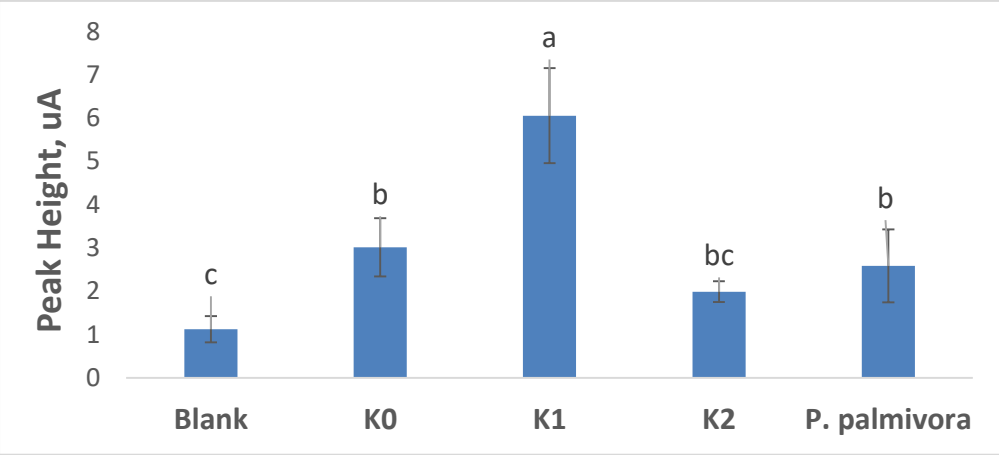


Anodic peak height of cacao – associated fungal species (Mean ± SD; n = 3). Statistical analysis through Tukey's Honest Significant Difference Test was performed at 95% confidence.

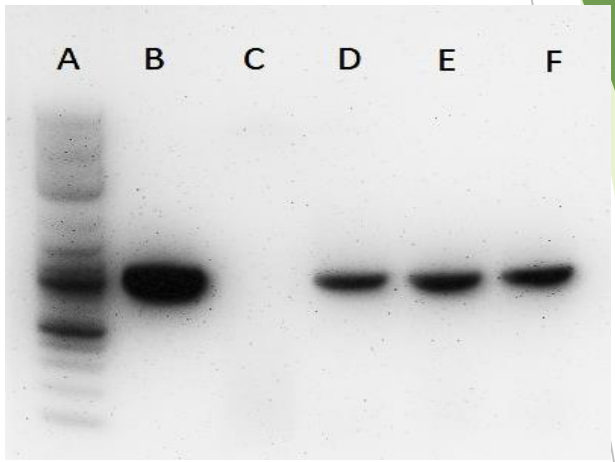
Result suggests that the nanobiosensor can distinguish *P. palmivora* among other cacao – associated fungal species.

Detection on Plant Samples

Leaf sample



Anodic peak height of inoculated cacao leaf tissue samples obtained at different distances from the lesion margin (Mean ± SD; n = 3). Statistical analysis through Tukey's Honest Significant Difference Test was performed at 95% confidence.



Agarose gel electrophoretogram of the PCR products from the inoculated cacao leaf samples. Lanes: A – 1 kb Ladder; B – DNA extract from pure *P. palmivora* mycelia; C – No template control; D – Excised leaf tissue at position K0; E – Excised leaf tissue at position K1; F – Excised leaf tissue at position K2

Detection was successful at positions K0 and K1 but not at K2. This suggests that positive **detection is possible in plant samples with moderate to severe infection**.

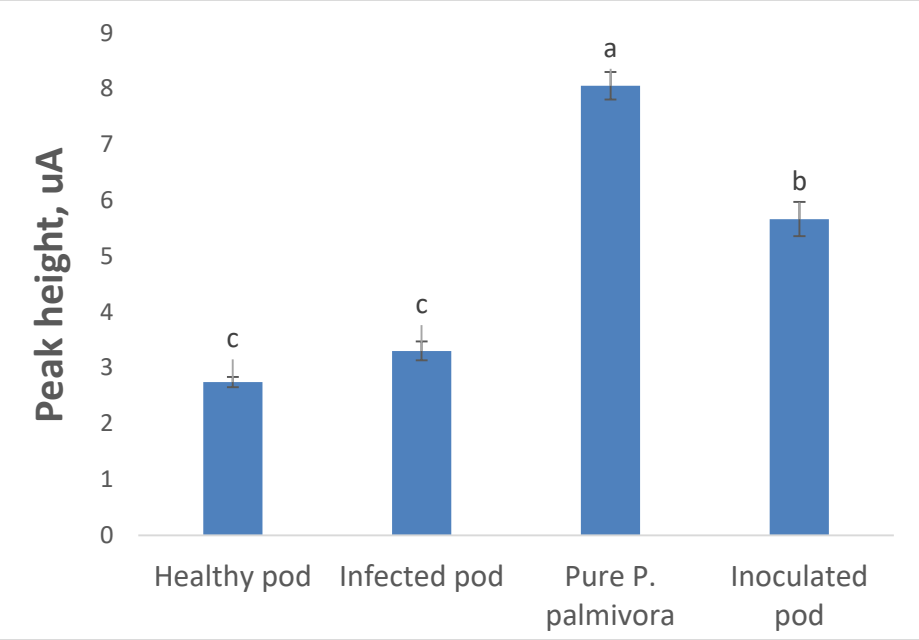
Detection on Plant Samples

Pod samples

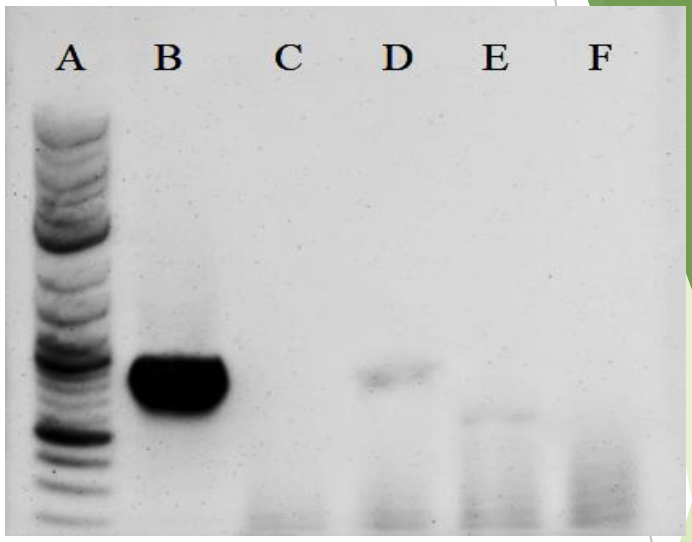
Image of (a) inoculated cacao pod 3 DAI and (b) obtained infected cacao pods

Detection on Plant Samples

Pod samples



Anodic peak height of cacao pod samples (Mean ± SD; n = 3). Statistical analysis through Tukey's Honest Significant Difference Test was performed at 95% confidence

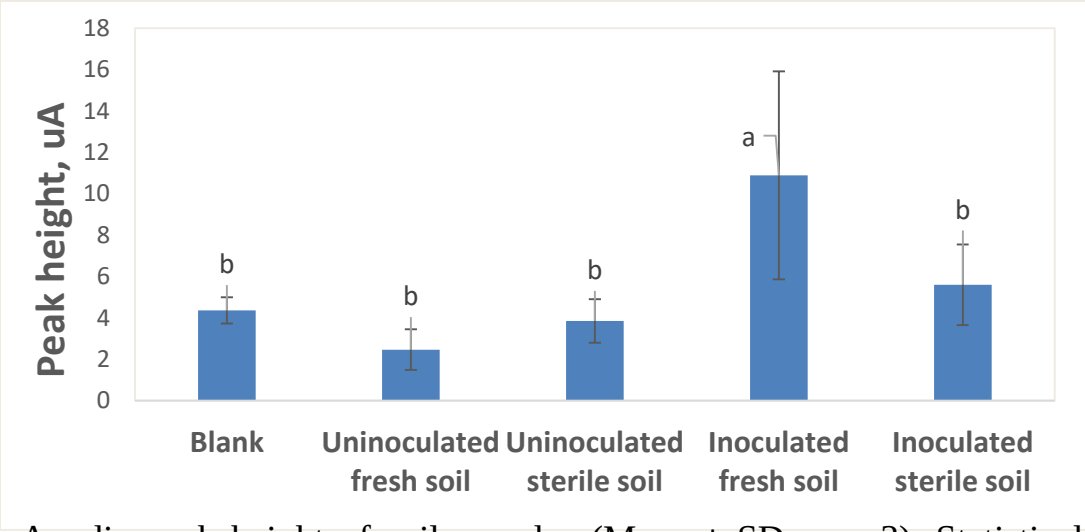


Agarose gel electrophoretogram of the PCR products from cacao pod samples. Lanes: A – 1 kb Ladder; B – DNA extract from pure *P. palmivora* mycelia; C – Healthy pods; D – Inoculated pod with *P. palmivora*; E – Cacao pod sample showing lesions; F – No template control.

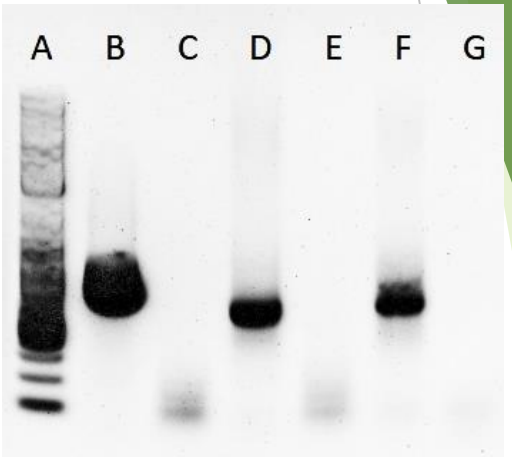
Detection was **successful on the inoculated pod**; the infected pod was apparently infected by a different pathogen as indicated by the faint band with shorter amplicon size and the result of the nanobiosensor. This suggests the potential of the nanobiosensor to discern the infection caused by *P. palmivora*.

Detection on Plant Samples

Soil samples



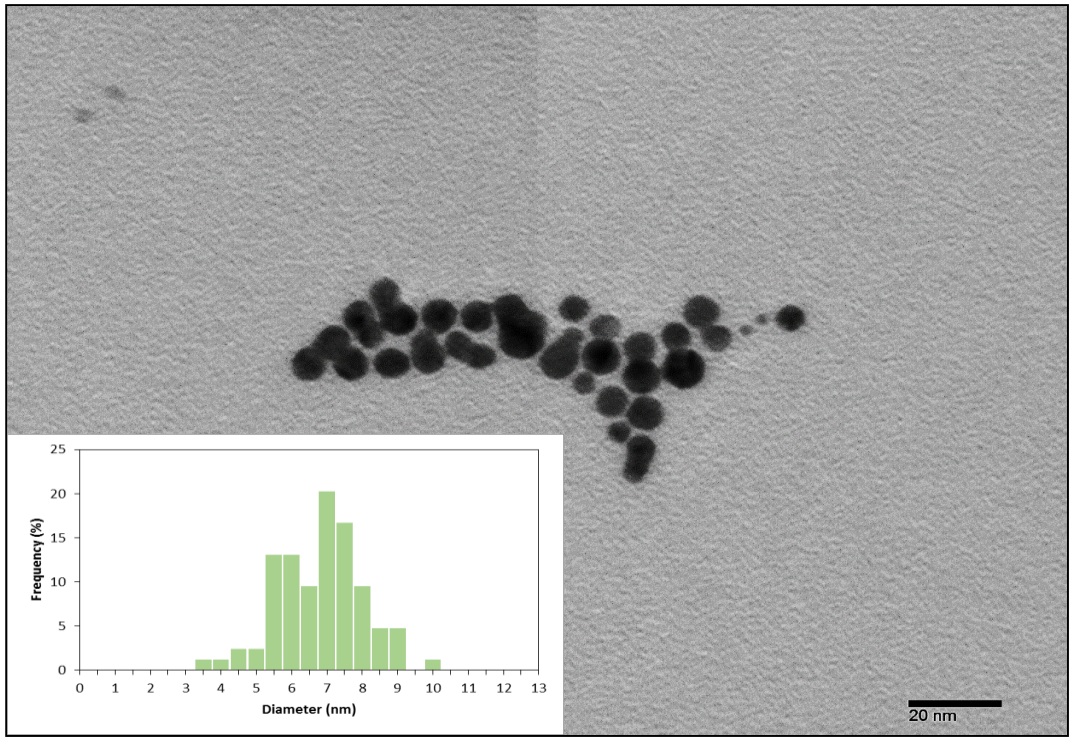
Anodic peak height of soil samples (Mean ± SD; n = 3). Statistical analysis through Tukey's Honest Significant Difference Test was performed at 95% confidence.



Agarose gel electrophoretogram of the PCR products from the soil samples. Lanes: A – 1 kb Ladder; B – DNA extract from pure *P. palmivora* mycelia; C – Uninoculated sterilized soil; D – Uninoculated fresh soil; E – Inoculated sterilized soil; F – Inoculated fresh soil; G – No template control

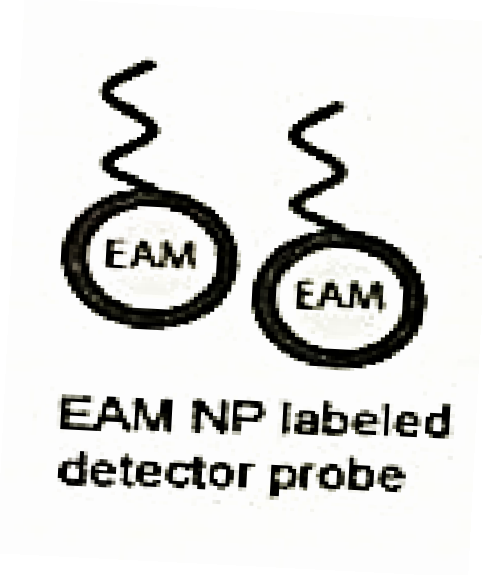
Detection on the inoculated fresh soil was successful; the other soil samples tested negative to the nanobiosensor. The nanobiosensor was able to detect the presence of P. palmivora even when DNA from other fungal species are present.

Characterization of AuNPs



TEM image of dextrin-capped gold nanoparticles (AuNPs). Inset shows the size distribution.

Functionalization of Electrically Active Magnetic Nanoparticles (EAM NPs)

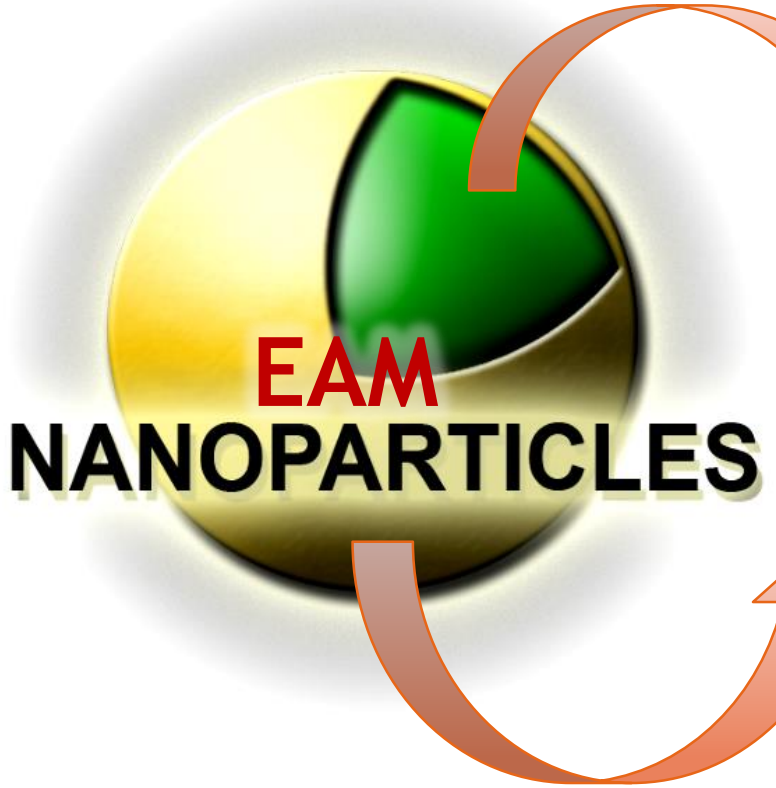


- EAM NPs - core: Fe_2O_3, coat: polyaniline has high electrical and magnetic properties

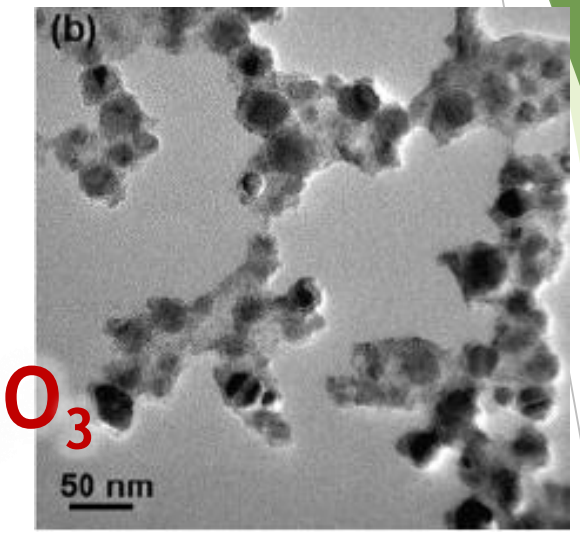
- Detector Probe (Ph-PRO) - Oligonucleotide phosphorylated and labelled with fluorescent [6-fluorescein (FAM)] dye

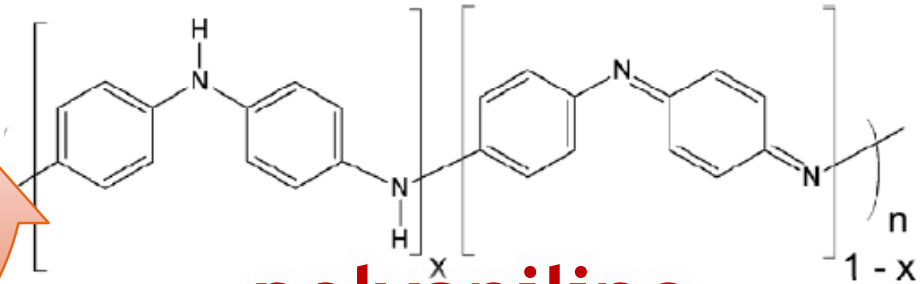
5’-Ph-TCCACTCTGGGGGCAATTCTGATGCGCAGAACTATTAGCAGTTGAGGGGG-FAM-3’

Electrically-active Magnetic Nanoparticles (EAM NPs)



γ-Fe_2O_3

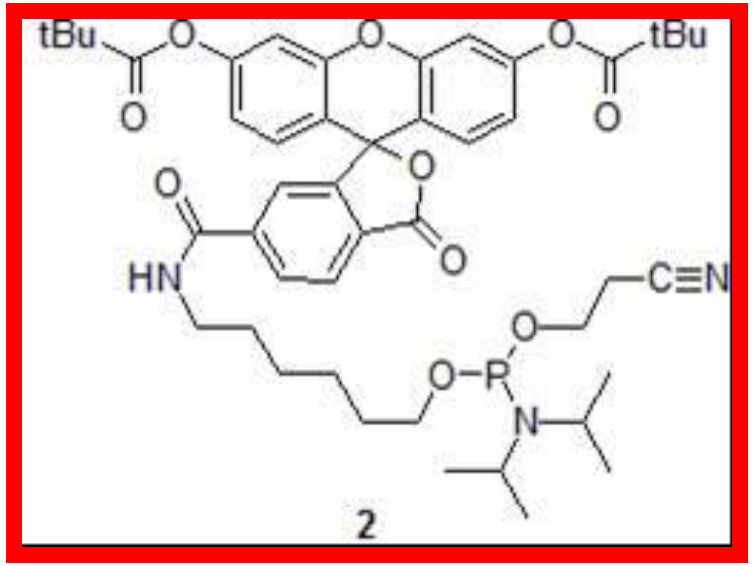




polyaniline

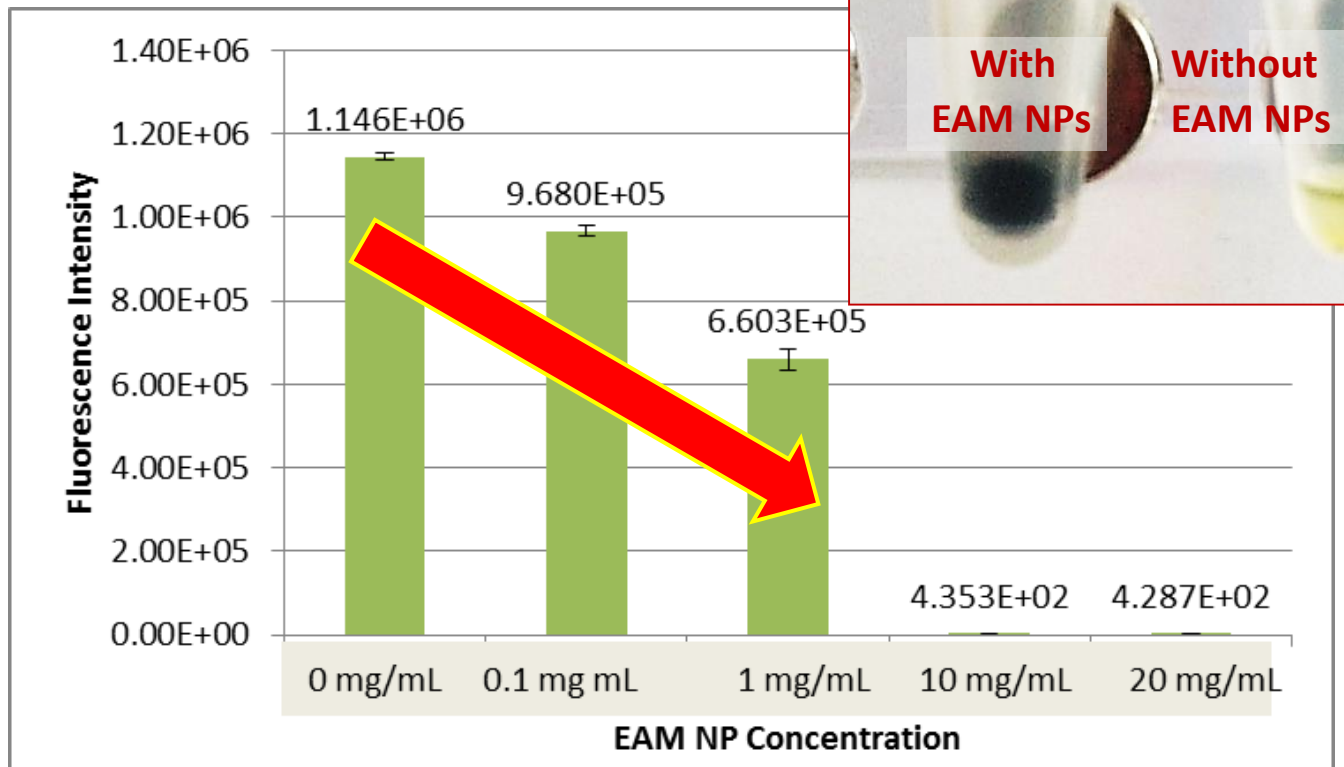
Determination of Conjugation Efficiency

FAM-labeled oligonucleotide probe



Emmision λ: 520 nm

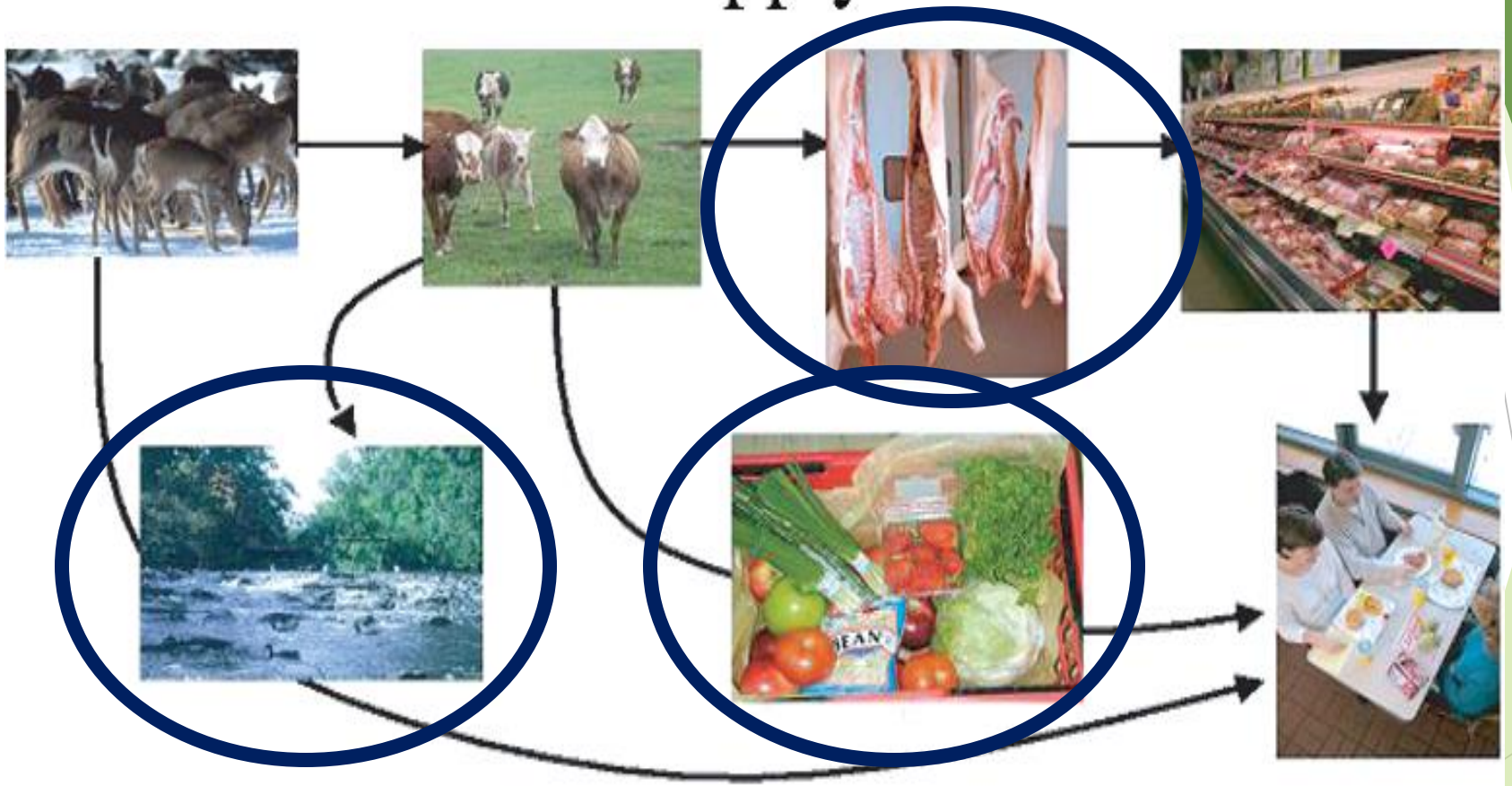
Functionalization of Electrically Active Magnetic Nanoparticles (EAM NPs)



10 mg/mL EAM NPs was chosen since efficient conjugation was observed based on fluorescence measurements.

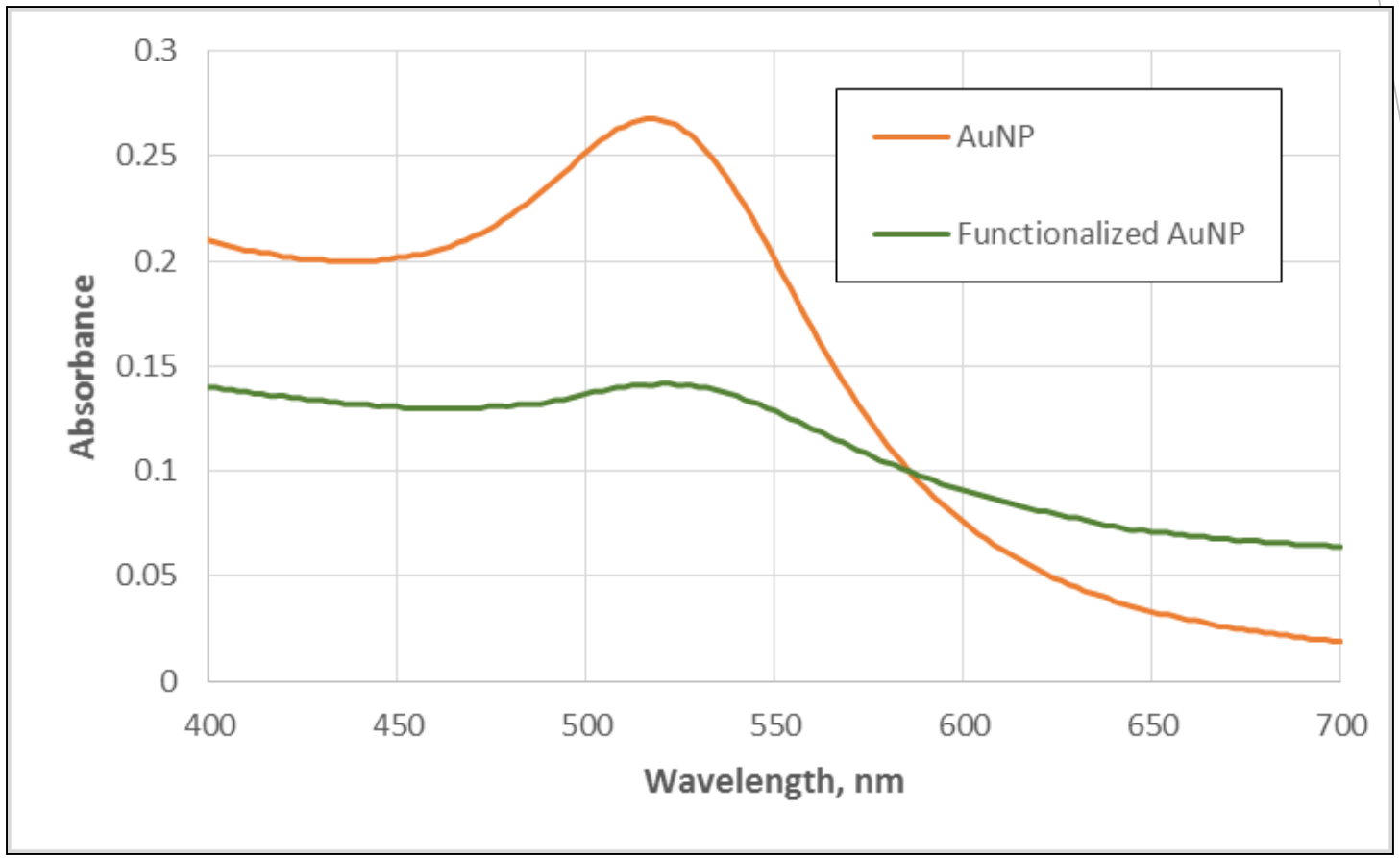
NanoBIOTECHnology Laboratory

Food Supply Chain



Zuorob et al., 2005

Characterization of AuNPs



Absorbance spectra of unmodified and functionalized gold nanoparticles.

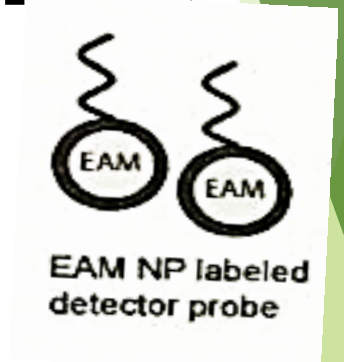
Determination of Conjugation Efficiency

Functionalization of Electrically Active Magnetic Nanoparticles (EAM NPs)

Pal and Alocilja, 2010

- **Fabrication of EAM NPs labeled detector probe**

EAM + **Ph-PRO** —**EDC**→ **EAM- Ph-PRO**



Conjugation Efficiency

Read Fluorescence Intensity
(0.1 s FAM)
($\lambda_{excitation}$ = 485 nm, $\lambda_{emission}$ = 535 nm)

Pal and Alocilja, 2010

Nanobiosensor Design (Pal and Alocilja 2010):

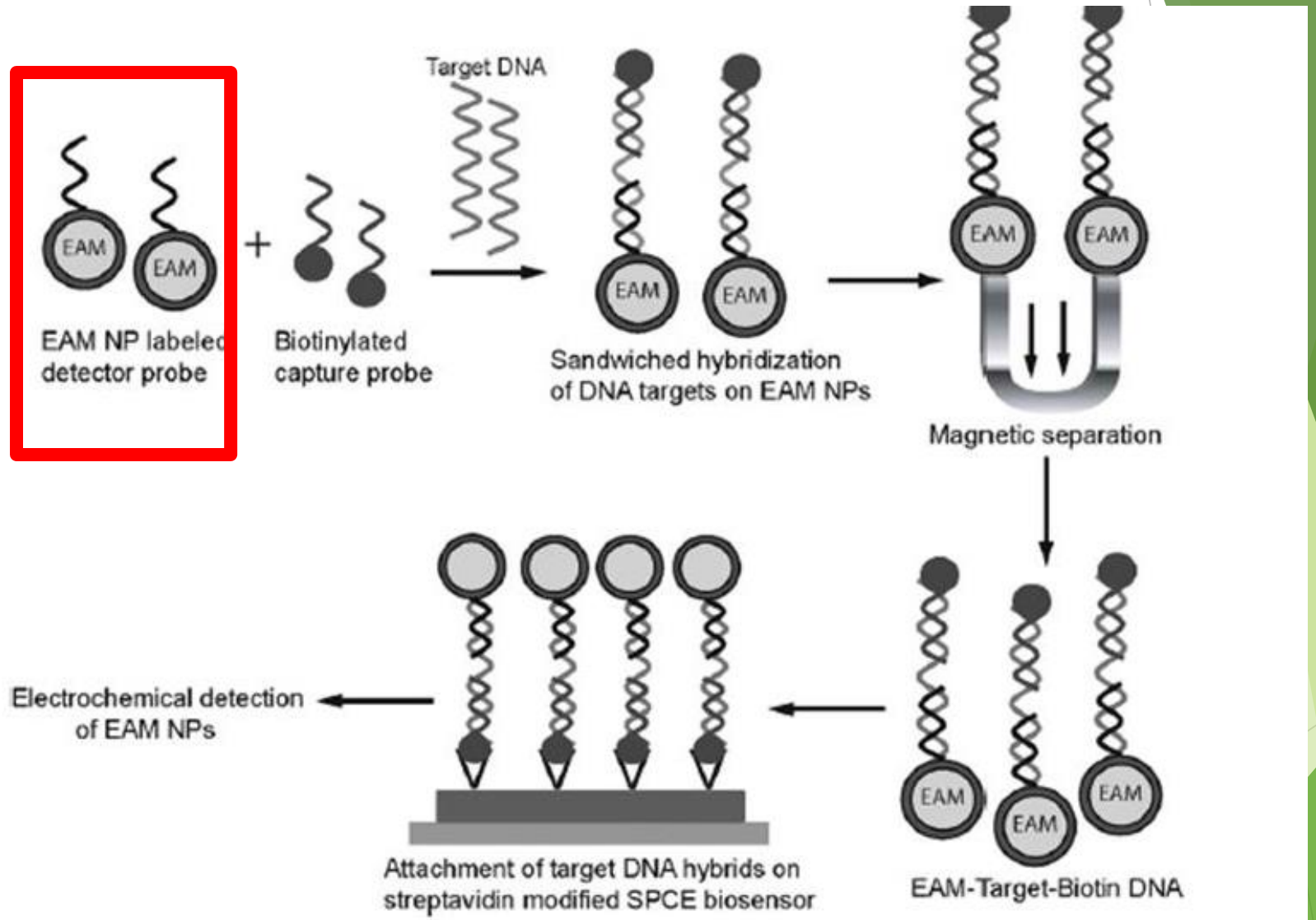
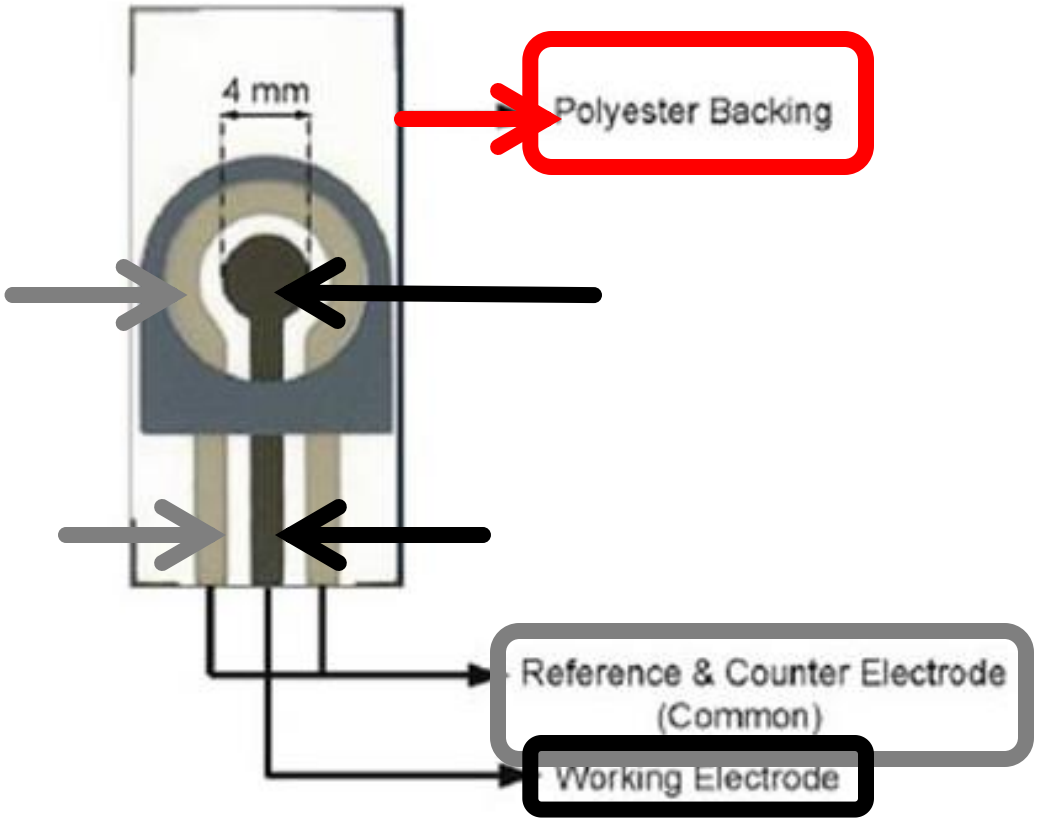
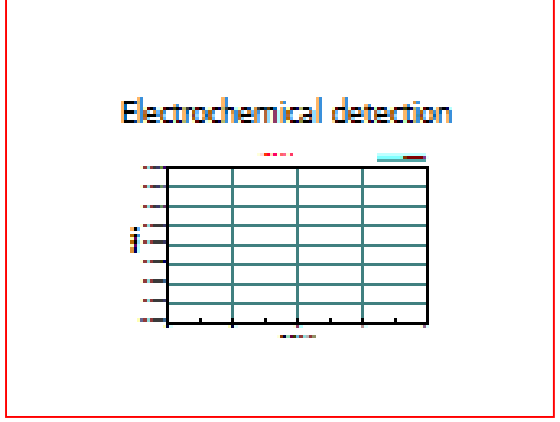


Fig. 1. Schematic illustration of (a) screen printed three-electrode sensor and (b) EAM based electrochemical DNA biosensor detection principle

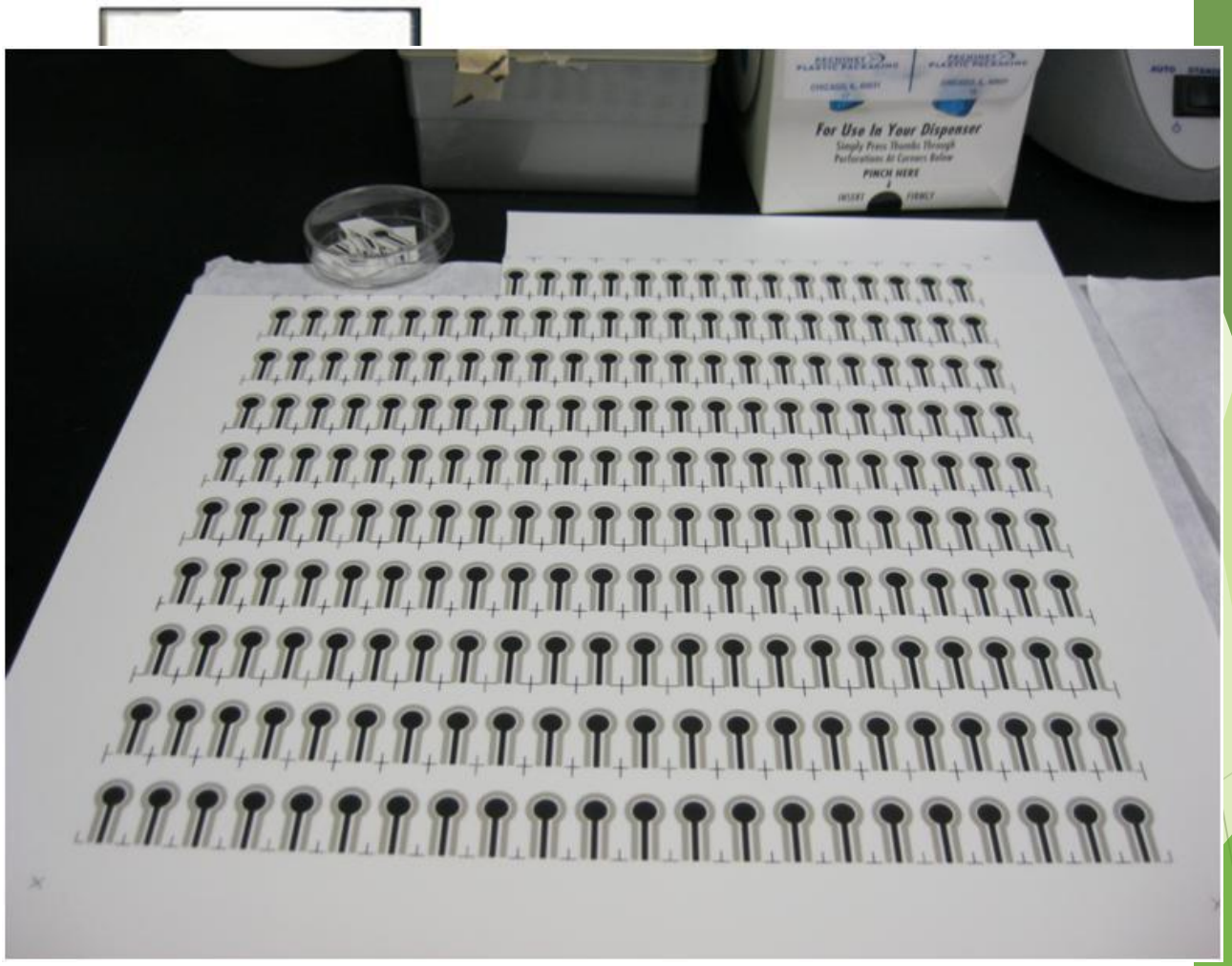
Screen printed carbon electrode (SPCE)



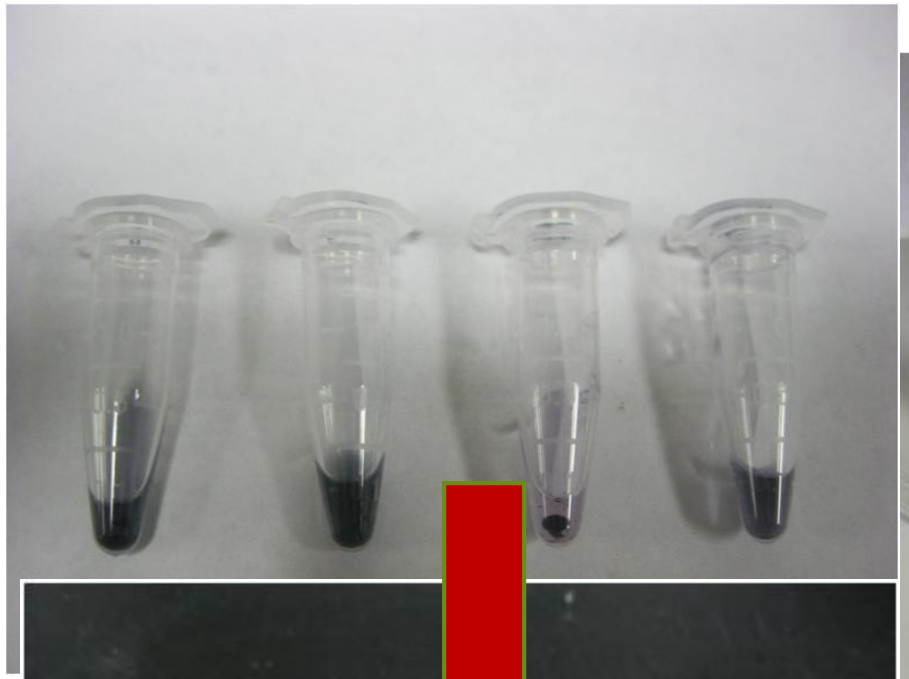


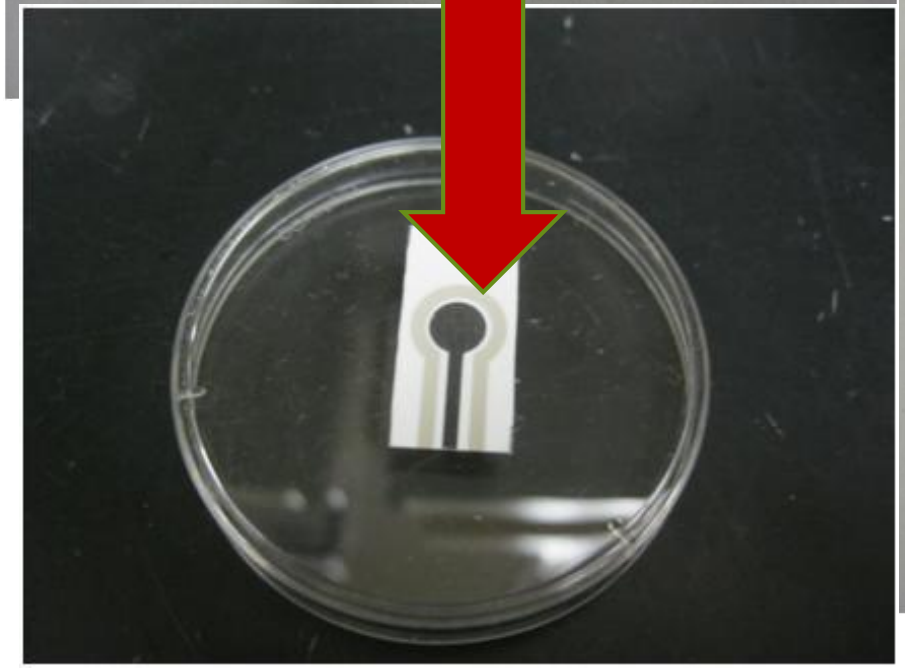
Electrochemical detection

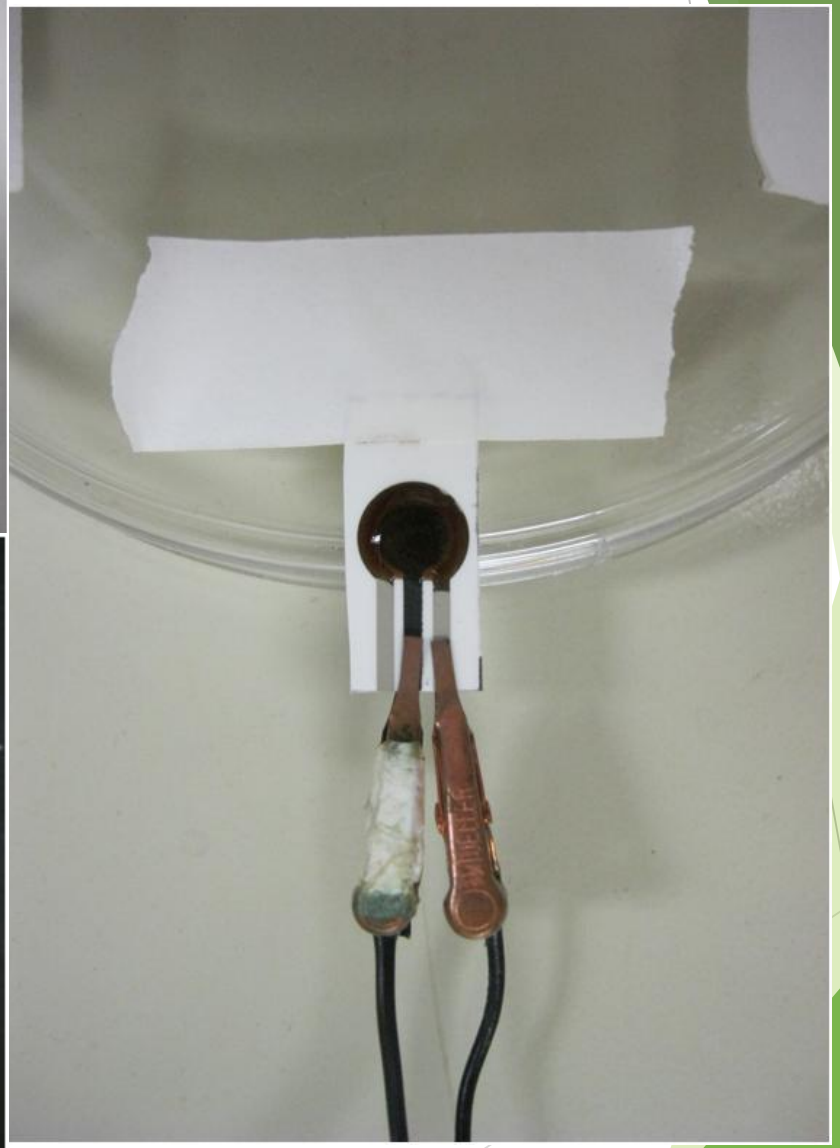
Screen Printed Carbon Electrode (SPCE)



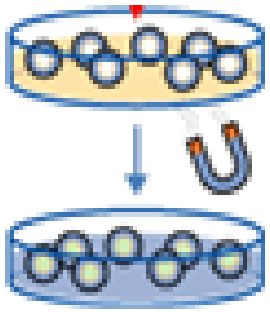
Pal and Alocilja, 2010



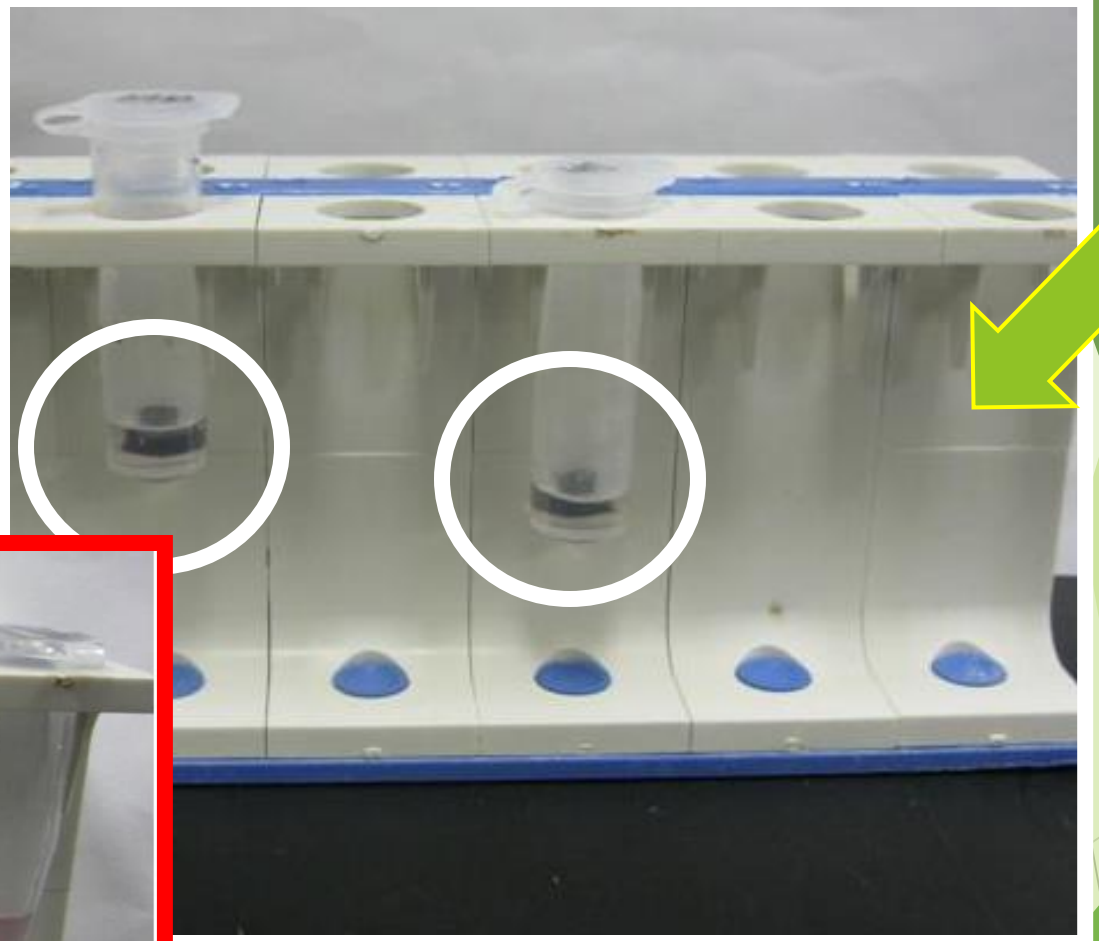




Magnetic separation

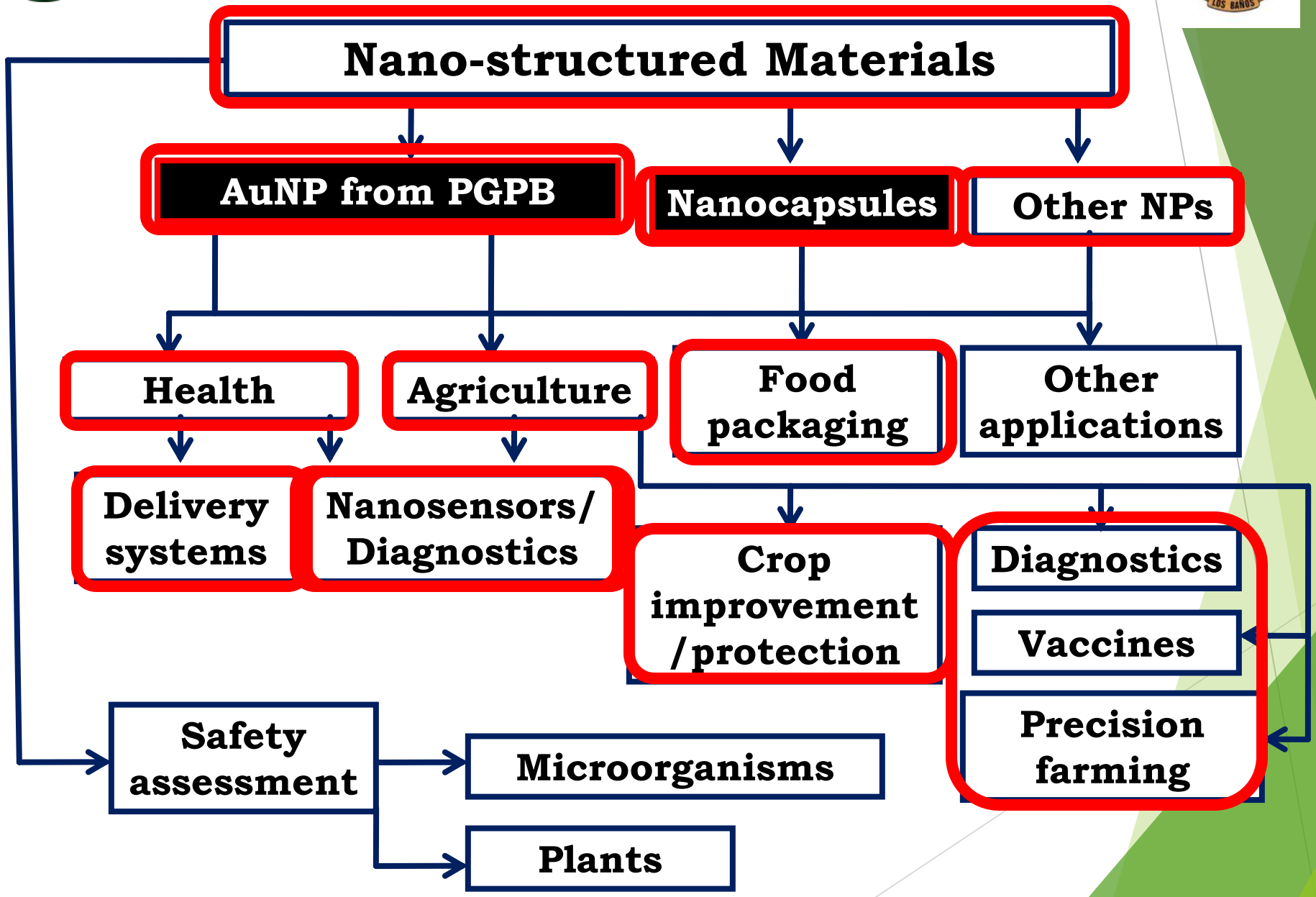


Magnetic separation



Laboratory

The NanoBIOTECH Laboratory

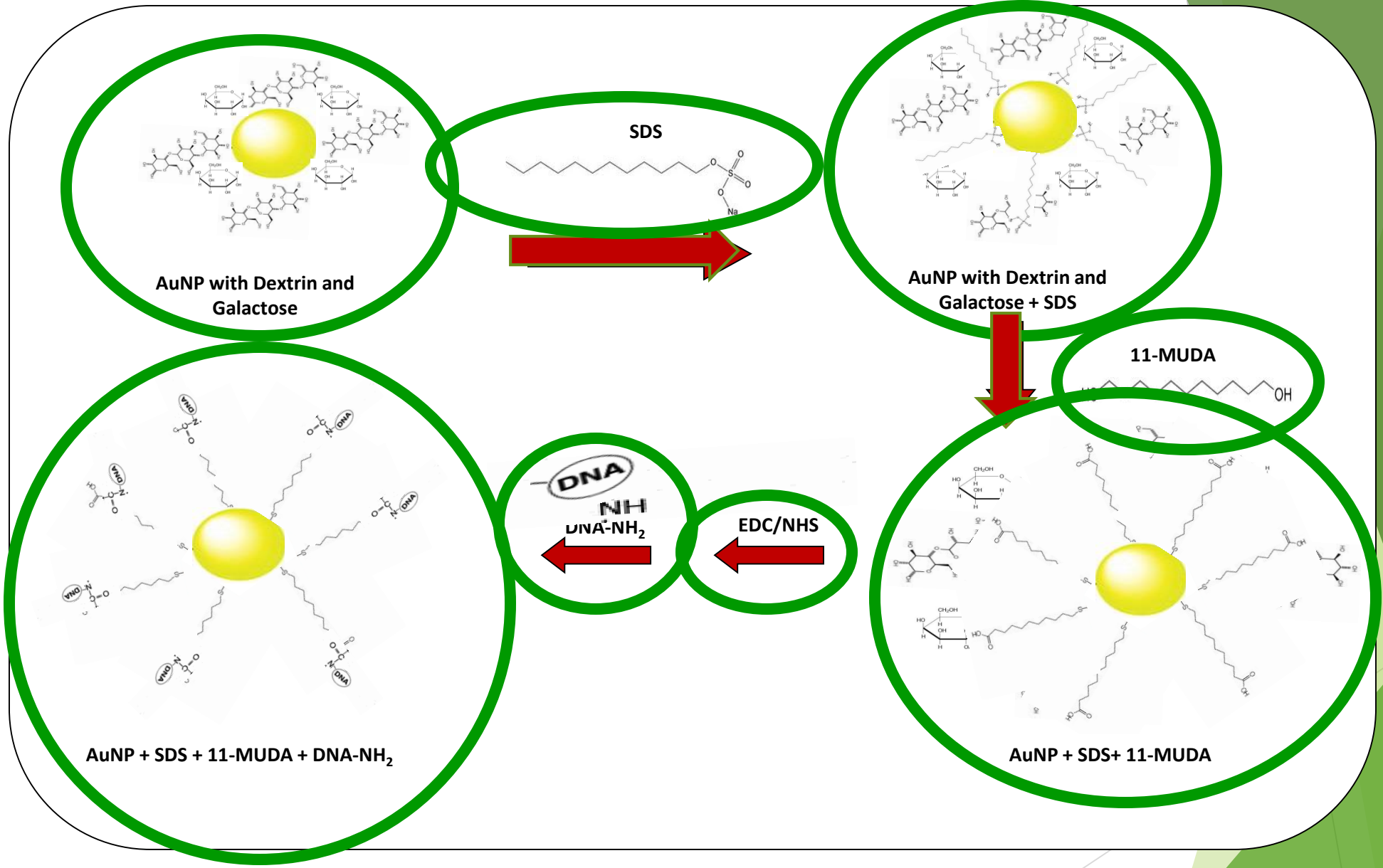


The NanoBIOTECH Laboratory

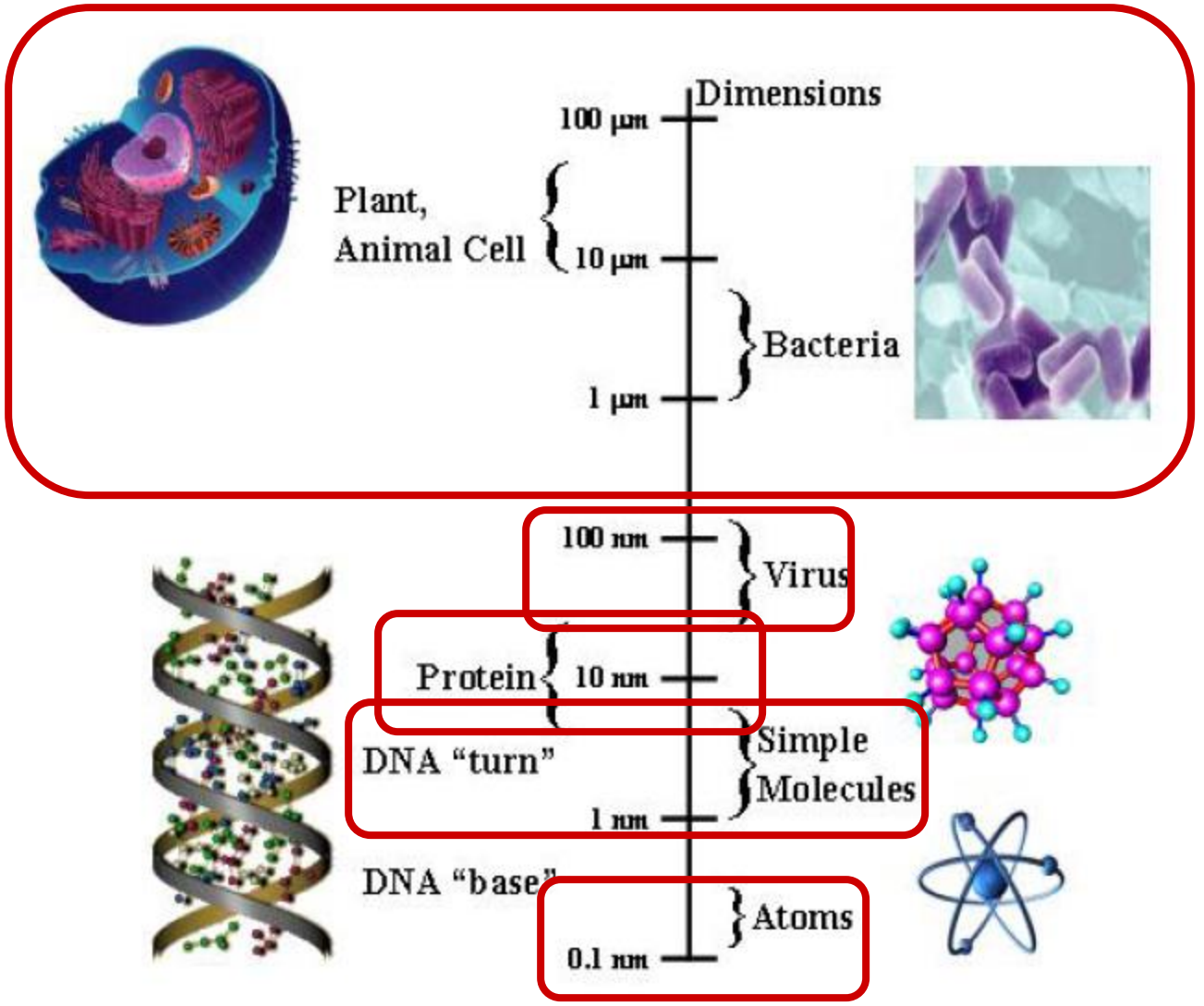
Nano-Biosensors Program

- Food- and Water-borne Pathogens
  - *Escherichia coli* O157:H7*
  - *Listeria monocytogenes**
- Plant Diseases
  - Abaca*
  - Banana*
  - Coconut***
  - Pineapple***
- [Neglected] Tropical Diseases
  - Dengue virus* (in mosquito)
  - HIV* (Human Immunodeficiency Virus)
  - Tuberculosis*
- **Animal and Veterinary Science*****
  - **Bacterial contaminants**
    - **Viral pathogen**
    - Anti-microbial resistant organism
  - Chemical contaminants
- Organic Fertilizer
  - *Salmonella***
- Bioterrorism Agents***
  - *Bacillus anthracis*
  - Cholera
  - Smallpox

* On-going
** Evaluated for funding
*** Future plan

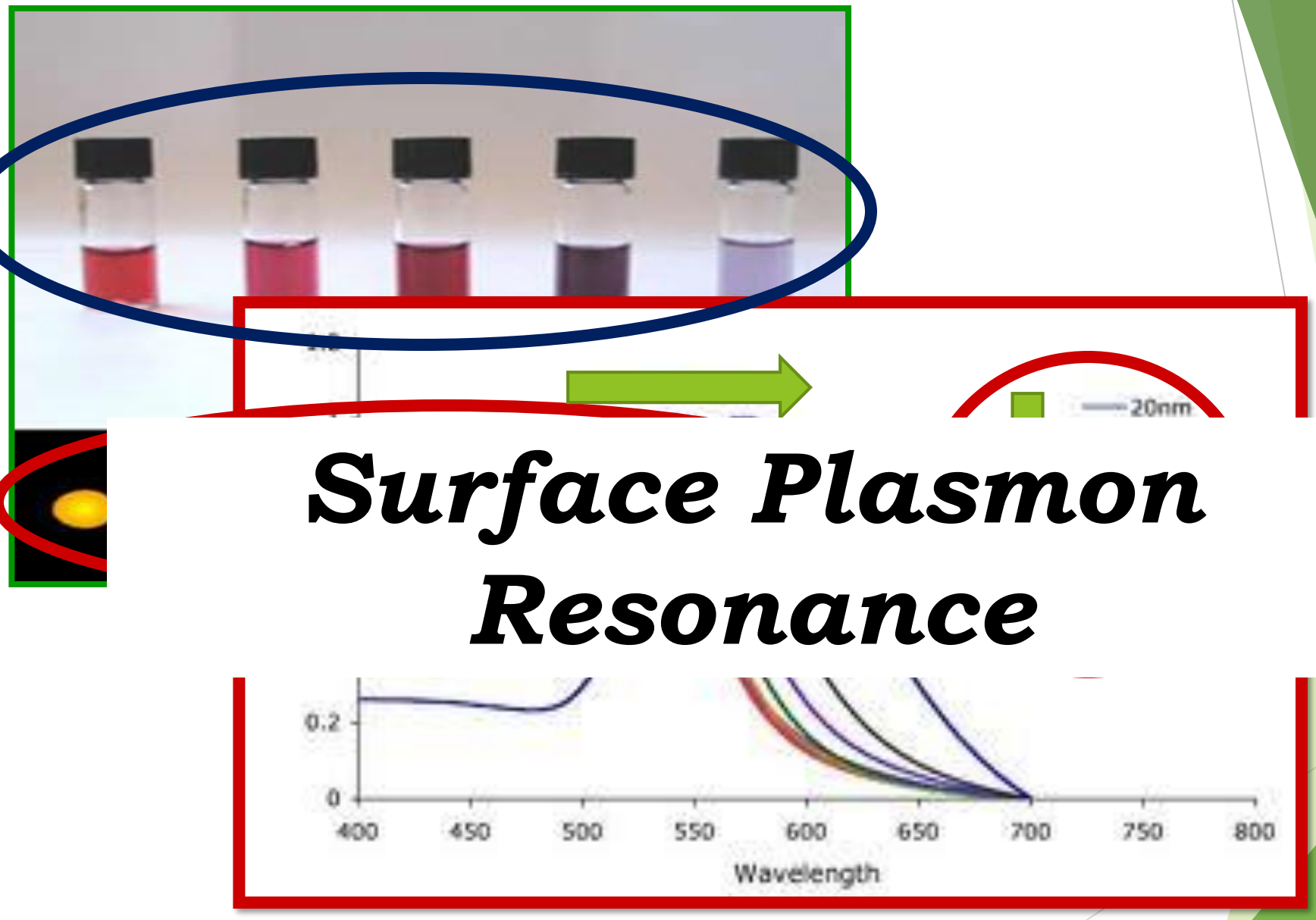


Schematic for the One-Pot Functionalization of AuNP



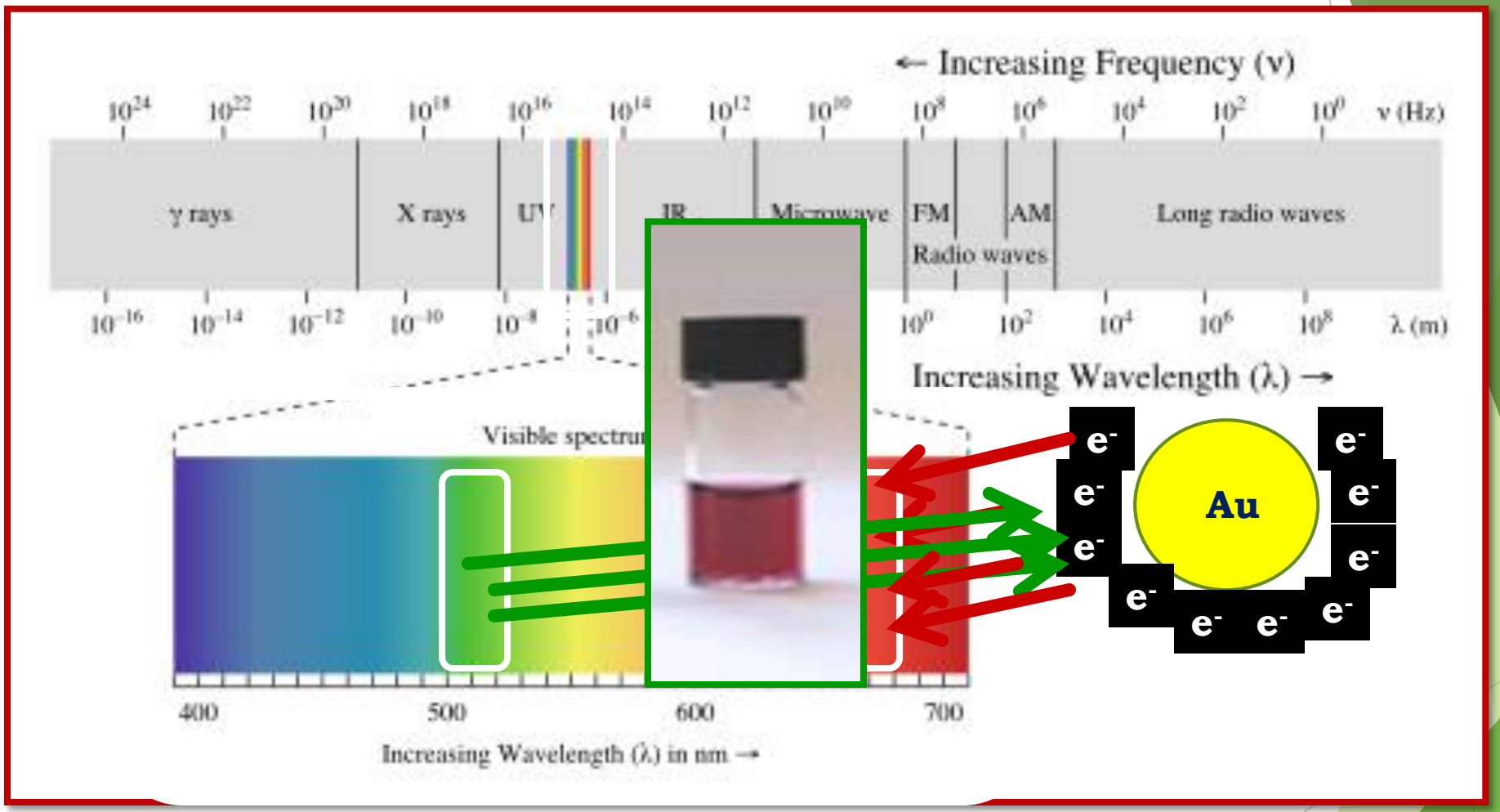
Scale showing the dimensions of different nanometric and micrometric object

Warad and Dutta



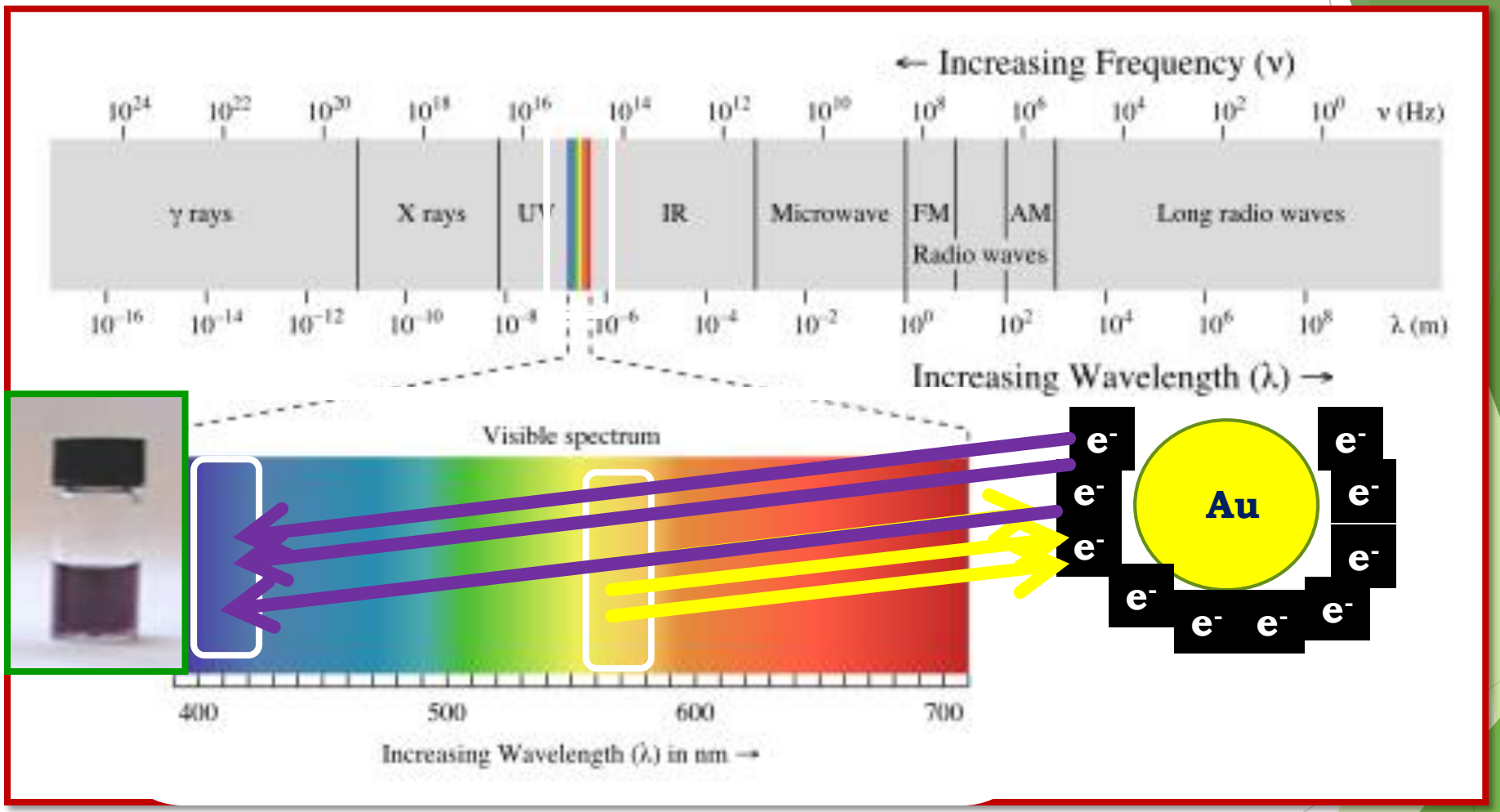
Surface Plasmon Resonance

Surface Plasmon Resonance



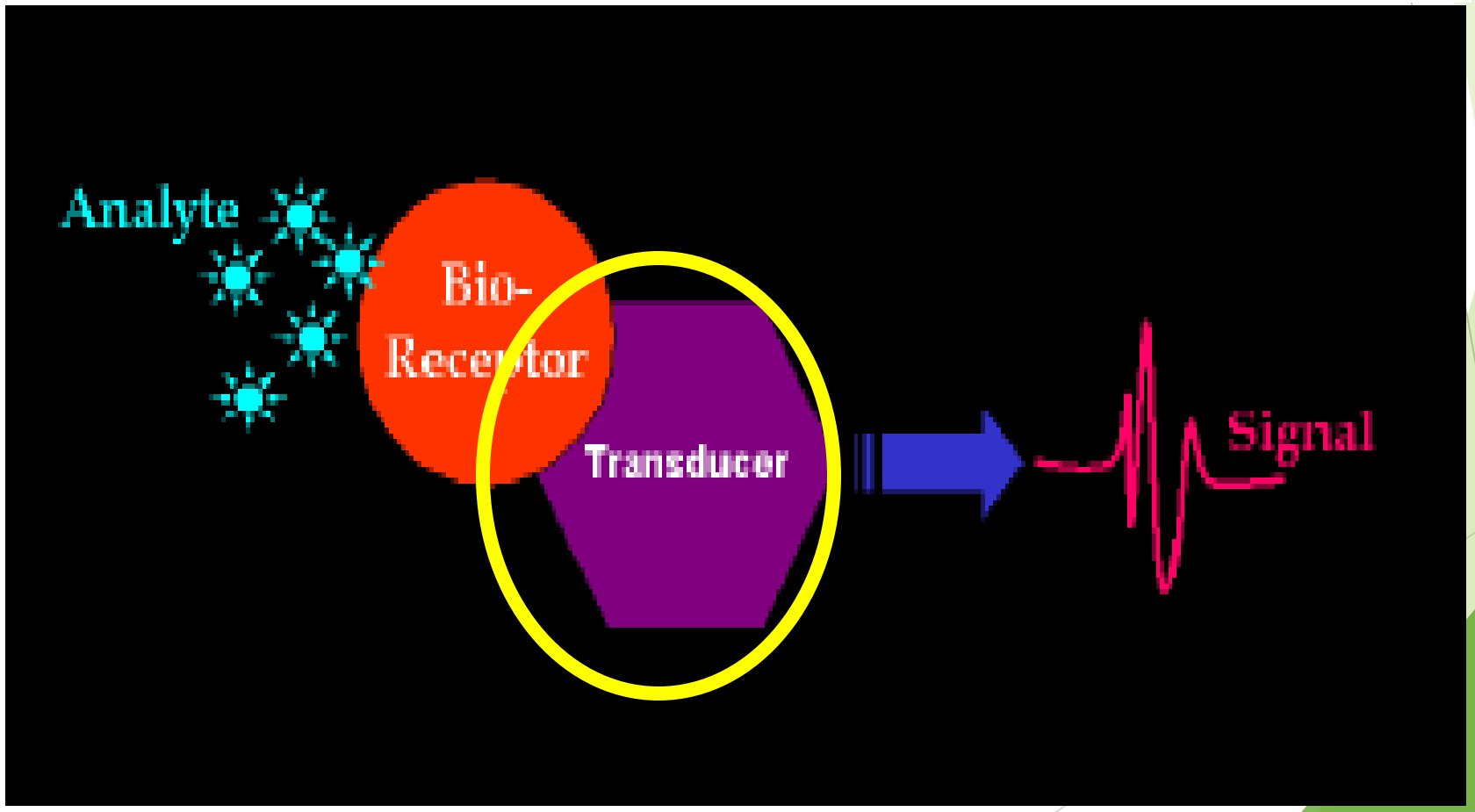
The electromagnetic spectrum and the classification of the spectral region

Surface Plasmon Resonance



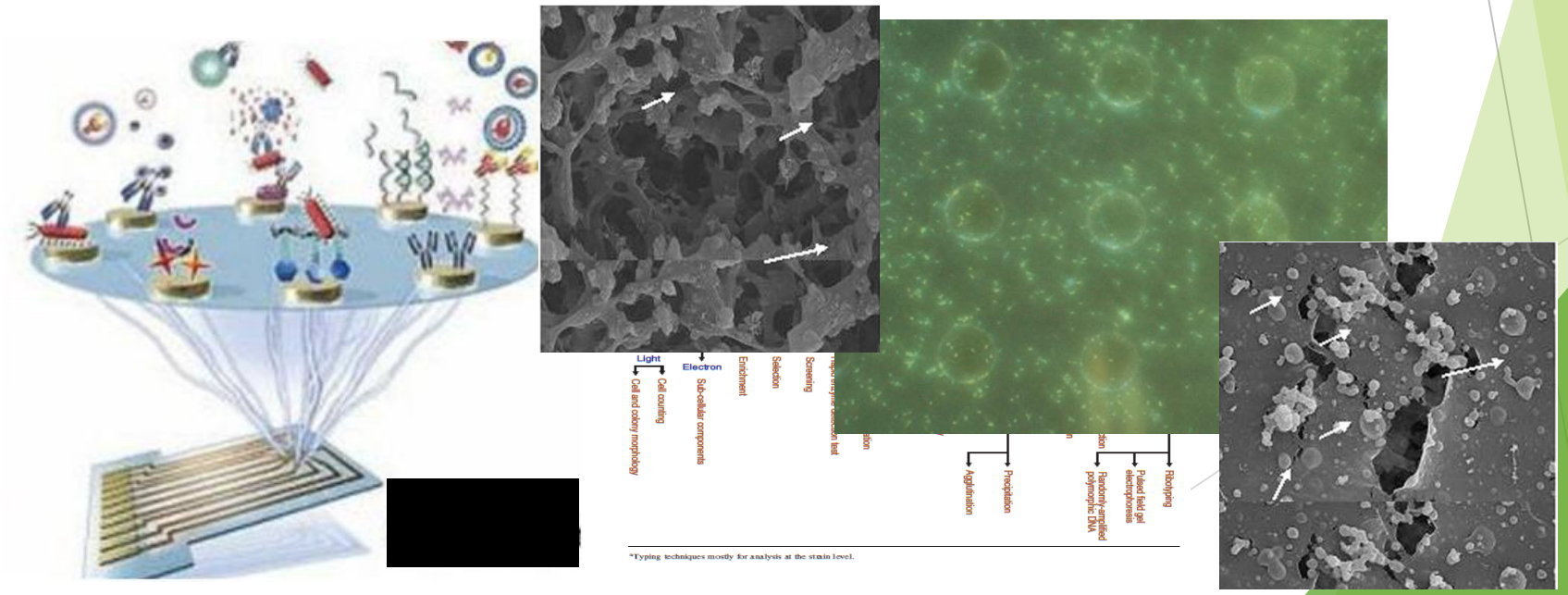
The electromagnetic spectrum and the classification of the spectral region

Application of AuNP in Biosensor



Application of Nano-Biosensor

Pathogen Detection



MP-Oligo Probe+ Target DNA

Suspension of magnetic iron oxide particles coated to provide primary amine groups

Characteristics

Mean Diameter:	~1.5µm
Particle Concentration:	50 mg/mL

netic particle (MP) + Oligo Probe

G – 3’

Sulfo-NHS ester

MP-NH$_2$

DNA-NH$_2$

R' disperse

prepared

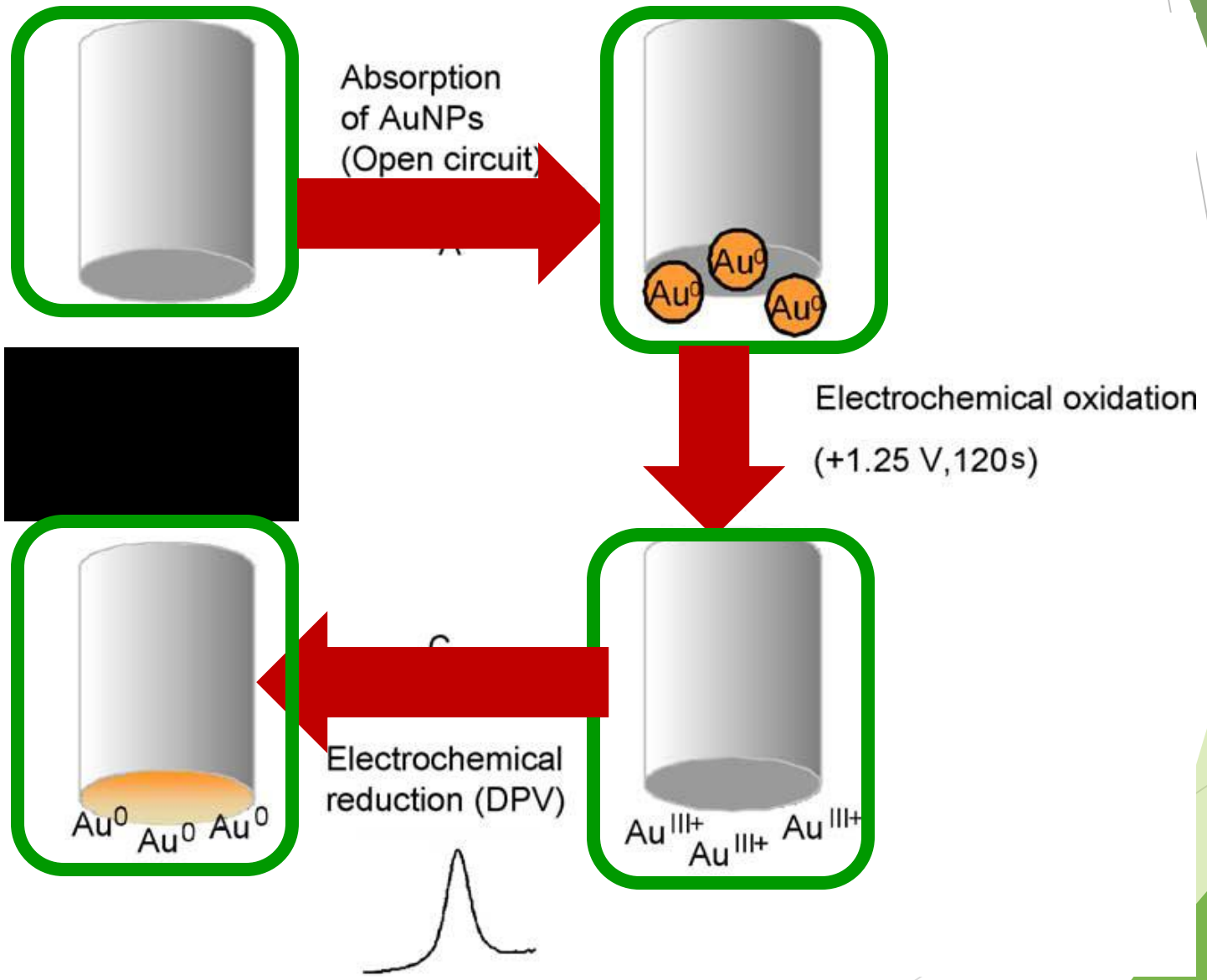
Amide derivative

DNA-Phos

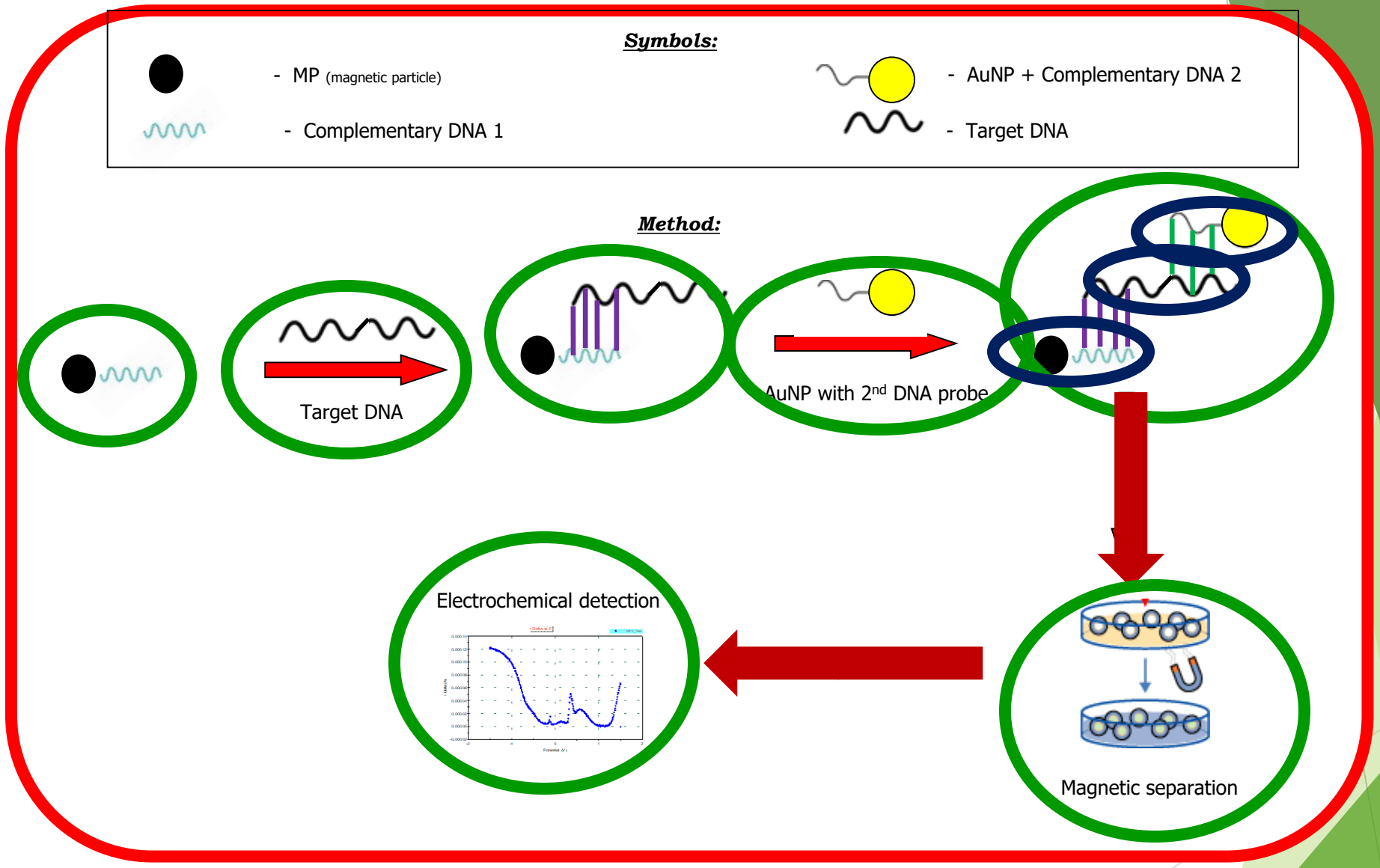
o-Acylis
reactive

8. Take o
nanopa
9. Leave t
up to st
10. Wrap ir
11. Add 3µ
12. Wrap ir
13. Spin fo
14. Resusp

Differential Pulse Voltammetry (DPV)



Pumera et al., 2005

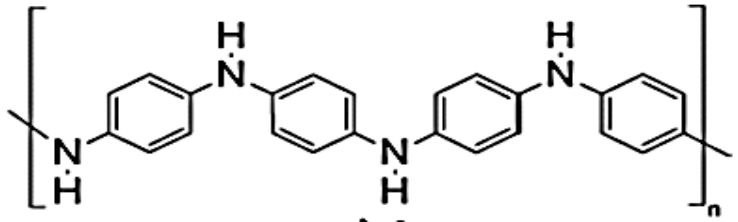


Schematic Diagram of the Target DNA Detection System


uniscan instruments
PG581

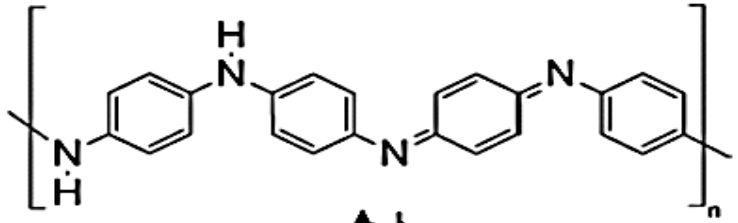
CONDUCTIVE FORMS OF POLYANILINE

Leucoemeraldine Base



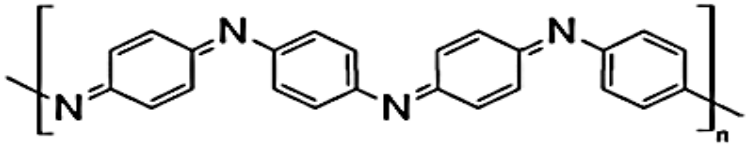
Red ⇅ Ox

Emeraldine Base



Red ⇅ Ox

Pernigraniline Base



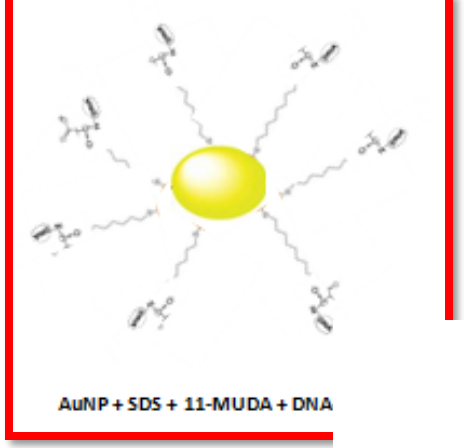
Cyclic voltammetric analysis of polyaniline-coated EAM NPs:

Two redox peaks:
1. **+0.118 V**
2. **+0.600 V**

Scan rate: 20 mV/s
Potential applied: -0.4 V to +1.0 V

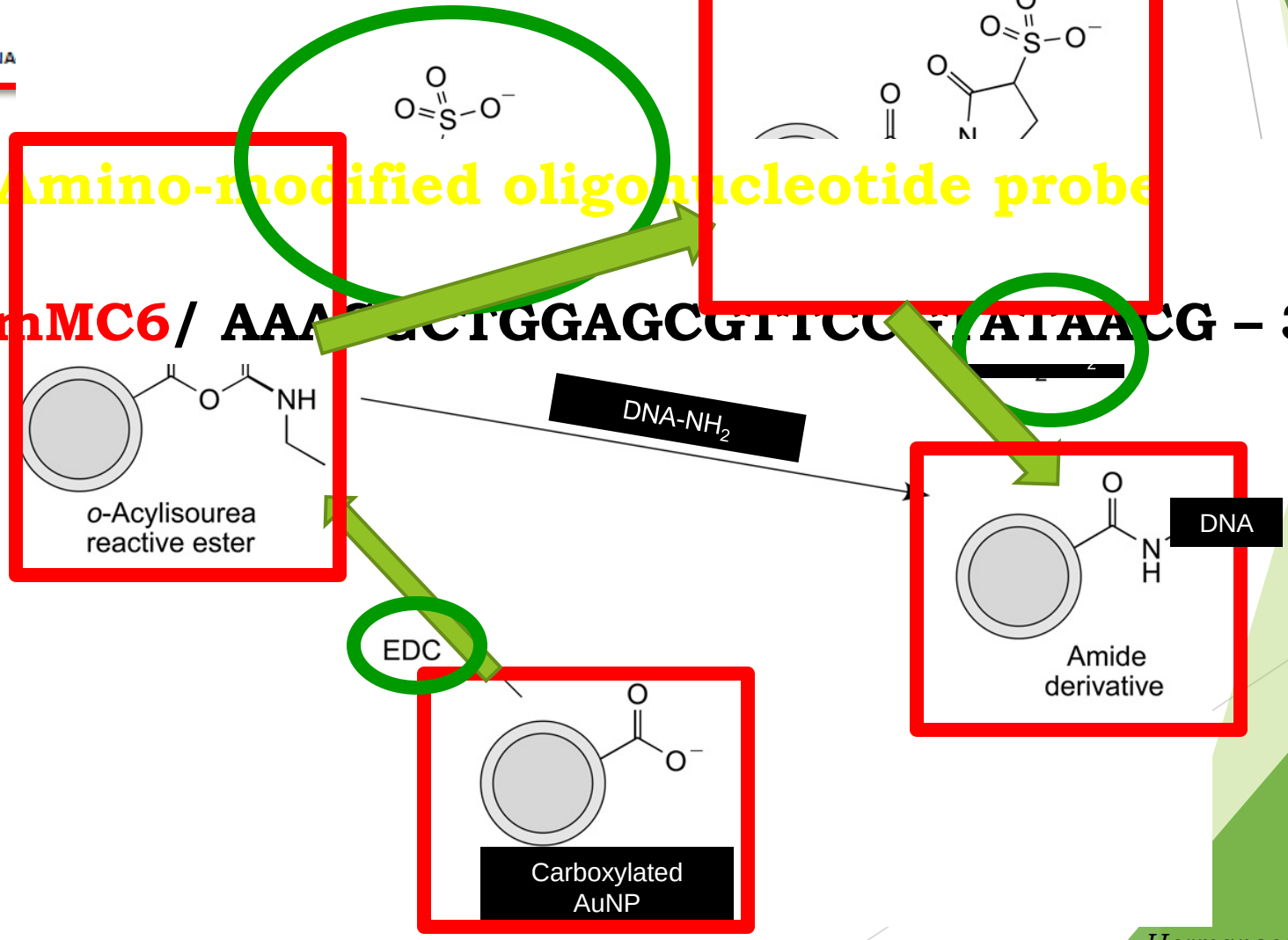
Source: Pal and Alocilja, 2010

AuNP + SDS + 11-MUDA + DNA-NH_2



Amino-modified oligonucleotide probe

(5'- 5AmMC6/ AAA[illegible]GCTGGAGCGTTCC[illegible]ATAACG – 3')

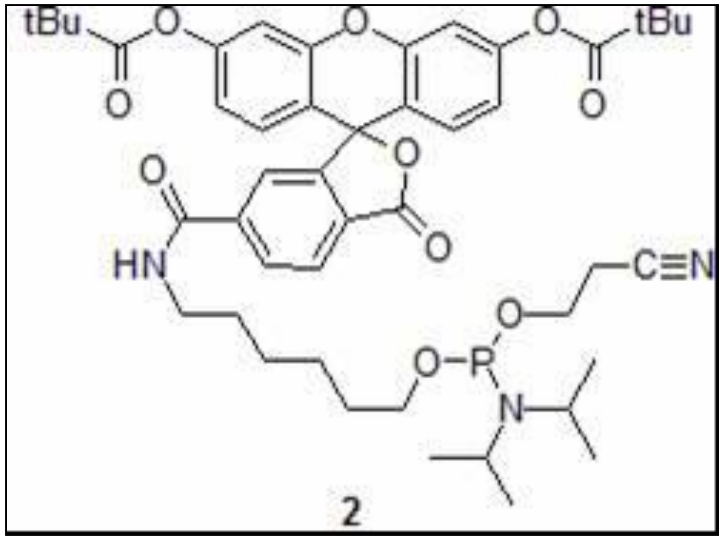


Hermanson, 2008

Determination of Conjugation Efficiency

FAM-labeled oligonucleotide probe

(5’- /56-FAM/ AAACGCTGGAGCGTTCCGTATAACG /3AmMO/ – 3’)



Emmision λ: 520 nm

DTT
(1, 4-dithio-DL-threitol)

Development of a Portable Nanodiagnostic Kit for the Detection of the Dengue and Japanese Encephalitis Virus in Mosquitoes

Project Leader: Divina M. Amalin, PhD

Implementing Agency: De La Salle University, Manila

PROJECT 1

Mosquitoes of Makiling Forest Reserve Areas with Characteristic Land Use and Survey of their Arbovirus Diversity through Vector-enabled Virome Sequencing (VEVS)

Dr. Ma. Anita M. Bautista – NIMBB, UP Diliman)

PROJECT 2

Detection and Characterization of Arboviruses from Mosquito Samples

Dr. Mark Pierre S. Dimamay – St. Luke's Medical Center

PROJECT 3

Development of a Portable Nanodiagnostic Kit for the Detection of the Dengue and Japanese Encephalitis Virus in Mosquitoes

Dr. Divina M. Amalin and Dr. Lilia M. Fernando- De La Salle University, Manila

Program: **Land Use Change in Makiling Forest Reserve and Its Impact on**

Dengue Transmission in the Area

Program Leader: Ma. Anita M. Bautista, PhD

PROGRAM INTEGRATION

Project 1
A Study of Mosquitoes of Makiling Forest Reserve Areas with Characteristic Land Use and Survey of their Arbovirus Diversity through Vector-enabled Virome Sequencing (VEVS)

- Collect mosquitoes and other arthropods potentially carrying arbovirus(es)
- Conduct molecular identification of mosquitoes
- Perform taxonomic and phylogenetic analysis of virus sequences
- perform taxonomic and phylogenetic analysis of virus sequences

- **Provide Project 2 and 3 with mosquitoes and sequences of dengue (and other arboviruses) resulting from virome sequencing**

Project 2
Detection and characterization of arboviruses from mosquito samples

- detect and isolate Dengue, Zika, Japanese Encephalitis (JE), and Chikungunya viruses from mosquitoes collected through Project 1 of the program

- **Conduct next generation sequencing of genes for characterization of viral isolates**
- **Provide Project 3 isolates of dengue and Japanese encephalitis virus for the validation of Au probes**

Project 3
Development of a Portable Nano Diagnostic Kit for the Detection of the Dengue and Japanese Encephalitis Virus in Arthropods

- develop a portable nanopartIcle-based sensor for the detection of the dengue and Japanese encephalitis virus in arthropods, which includes triage technology and diagnosis technology
- Primers to be provided by Projects 1 & 2

BIOSURVEILLANCE DATA (alarm indicator along with meteorological data for early warning system)

- Implement Control Programs
- Prioritize Resources
- Evidence-Based Policy

COOPERATING AGENCIES

1) Nanobiotechnology Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH), UP Los Baños
2) Philippine Genome Center (PGC)
3) National Institute of Molecular Biology and Biotechnology (NIMBB), UP Diliman
4) St. Luke's Medical Center
5) Research Institute for Tropical Medicine (RITM)
6) Nanobiosensors Laboratory, Michigan State University
7) DOH- Epidemiology Bureau and Disease Prevention and Control Bureau (DPCB)

DURATION

24 months

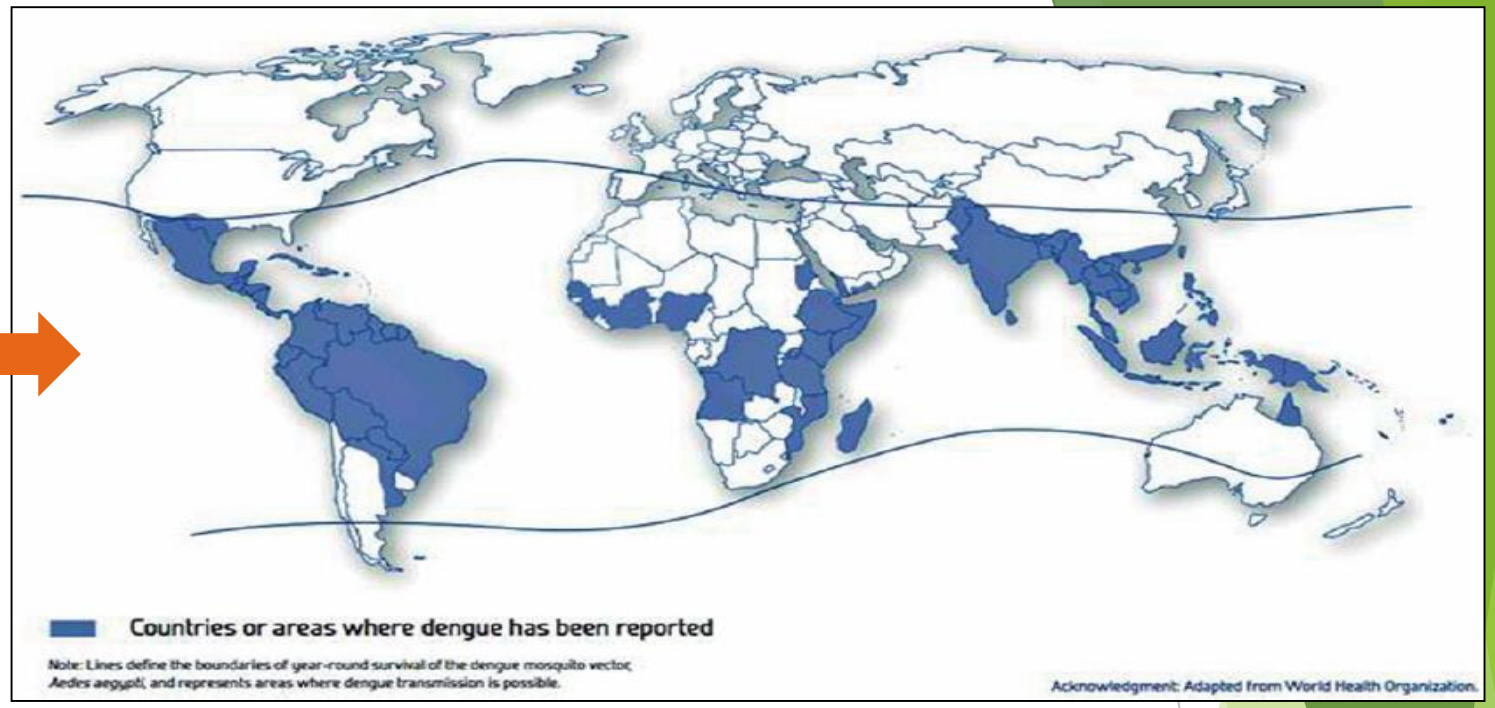
FUNDING AGENCY

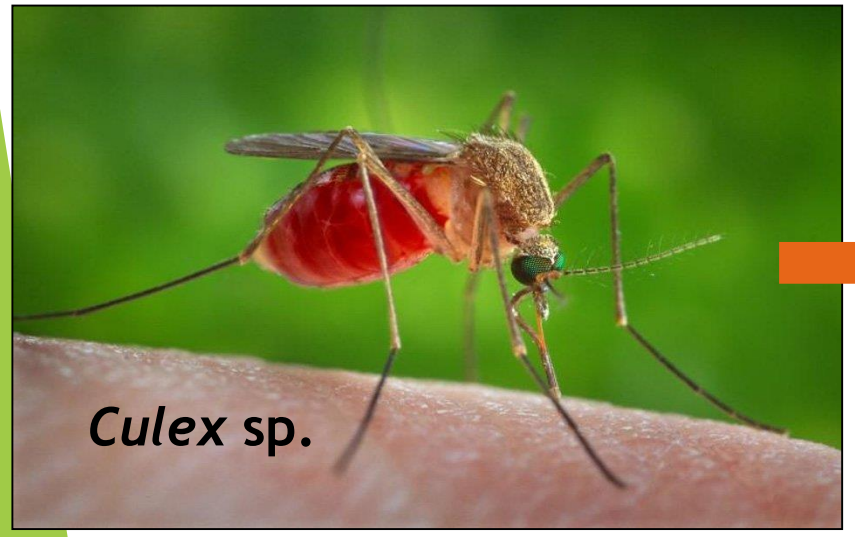
Department of Science and Technology (DOST)

PROJECT 3- Development of a Portable Nanodiagnostic Kit for the Detection of the

Dengue and Japanese Encephalitis Virus in Mosquitoes


Aedes aegypti


Countries or areas where dengue has been reported
Note: Lines define the boundaries of year-round survival of the dengue mosquito vector, *Aedes aegypti*, and represents areas where dengue transmission is possible.
Acknowledgment: Adapted from World Health Organization.


Culex sp.


Russia
Mongolia
China
North Korea
South Korea
Japan
Afghanistan
Pakistan
Nepal
Bhutan
India
Bangladesh
Burma
Laos
Vietnam
Thailand
Taiwan
Saipan
Guam
Philippines
Cambodia
Brunei
Malaysia
Singapore
Sri Lanka
Papua New Guinea
Indonesia
Timor-Leste
Australia
Arabian Sea
Bay of Bengal
Indian Ocean
South China Sea
Philippine Sea
Japanese Encephalitis Risk Areas
Areas with No Known Japanese Encephalitis Risk

Vector control

WHO to lead global vector control response

WHO has a new global strategy to reduce the burden and threat of vector-borne diseases through effective, locally adapted sustainable vector control. The *Global vector control response 2017–2030* outlines a broad approach aligned with the *2030 Agenda for Sustainable Development*. Strengthened political and financial commitments are required, along with new vector control tools and approaches.

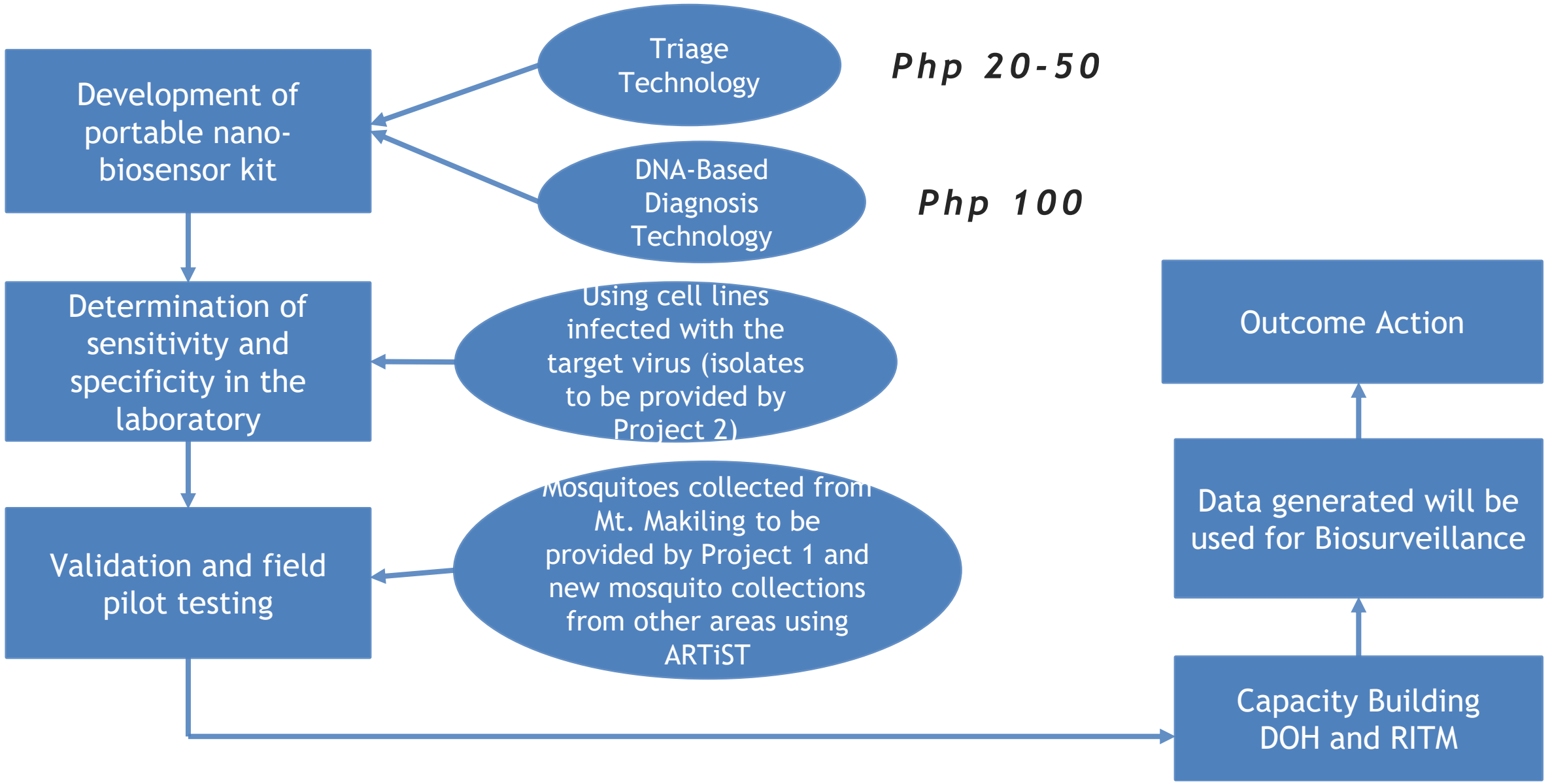
GLOBAL VECTOR CONTROL RESPONSE 2017–2030

World Health Organization

TDR For research on diseases of poverty
UNICEF • UNDP • World Bank • WHO

CKGROUN

PROJECT 3 framework



STRATEGY

		END-USERS
INPUT	**Nano-biosensor kit for dengue and Japanese encephalitis in mosquito vectors** **PCR-less diagnostic filed kit for dengue and Japanese encephalitis**	**DOH Epidemiology Bureau** • Public Health Surveillance System • Integrated Vector Management Program **DOH Regional Offices** **RITM Entomology Department**
OUTPUT	**Monitor alert signals through mosquito vectors** **Surveillance system- viral surveillance** **Contribute to Integrated Vector Management; training for health worker**	**DOH Epidemiology Bureau** • Public Health Surveillance System • Integrated Vector Management Program **DOH Regional Offices** **RITM Entomology Department**
OUTCOME	**Effective Outbreak Management**	**Community**

General objective

Develop a portable nanoparticle-based sensor for the detection of dengue (all 4 serotypes) and Japanese encephalitis virus in mosquitoes, which includes triage technology and diagnosis technology

SPECIFIC OBJECTIVES

Design oligonucleotide probes for the detection of the dengue and Japanese encephalitis virus in mosquitoes

Functionalize metallic nanoparticles with the designed probes for the detection of the dengue and Japanese encephalitis virus through matting

Perform electrochemical detection of the dengue and Japanese encephalitis virus using the oligo probe functionalized nanoparticle

General objective

Develop a portable nanoparticle-based sensor for the detection of the dengue (all 4 serotypes) and Japanese encephalitis virus in mosquitoes, which includes triage technology and diagnosis technology

SPECIFIC OBJECTIVES

Determine the sensitivity and specificity of the fabricated nanosensor

Develop a forecasting model based on input from nanosensor data, weather data, environmental data for predicting potential disease outbreak

Nanodiagnostic

Designing of probes specific for the DENV and JEV

Functionalization of metallic nanoparticles with the oligonucleotide probes

- Preparation, synthesis and characterization of metallic nanoparticle
- Conjugation and Optimization

Fabrication of nanosensors

Hybridization and electrochemical detection of the viruses

Determination of limit of detection, sensitivity and selectivity of the fabricated sensor

ARTiST

Oviposition attractant experiments

1) Releases of mosquitoes (sequential experimental design)
2) Identification of the most effective attractant and determination of its chemical profile
3) Biological assay using olfactometer

Designing and implementation of two types of trapping system

Field testing

Building **ARTiST**: Automatic Real Time Surveillance and Trapping of Adult Mosquitoes for Entomologic Surveillance

ARTiST

Trap's Features

a) Wingbeat Sensing
b) Wingbeat Frequency Determination
c) System Implementation
d) Sensor Chamber Segment Design
e) Detection Electronics
f) Microcontroller
g) Code
h) Data Storage

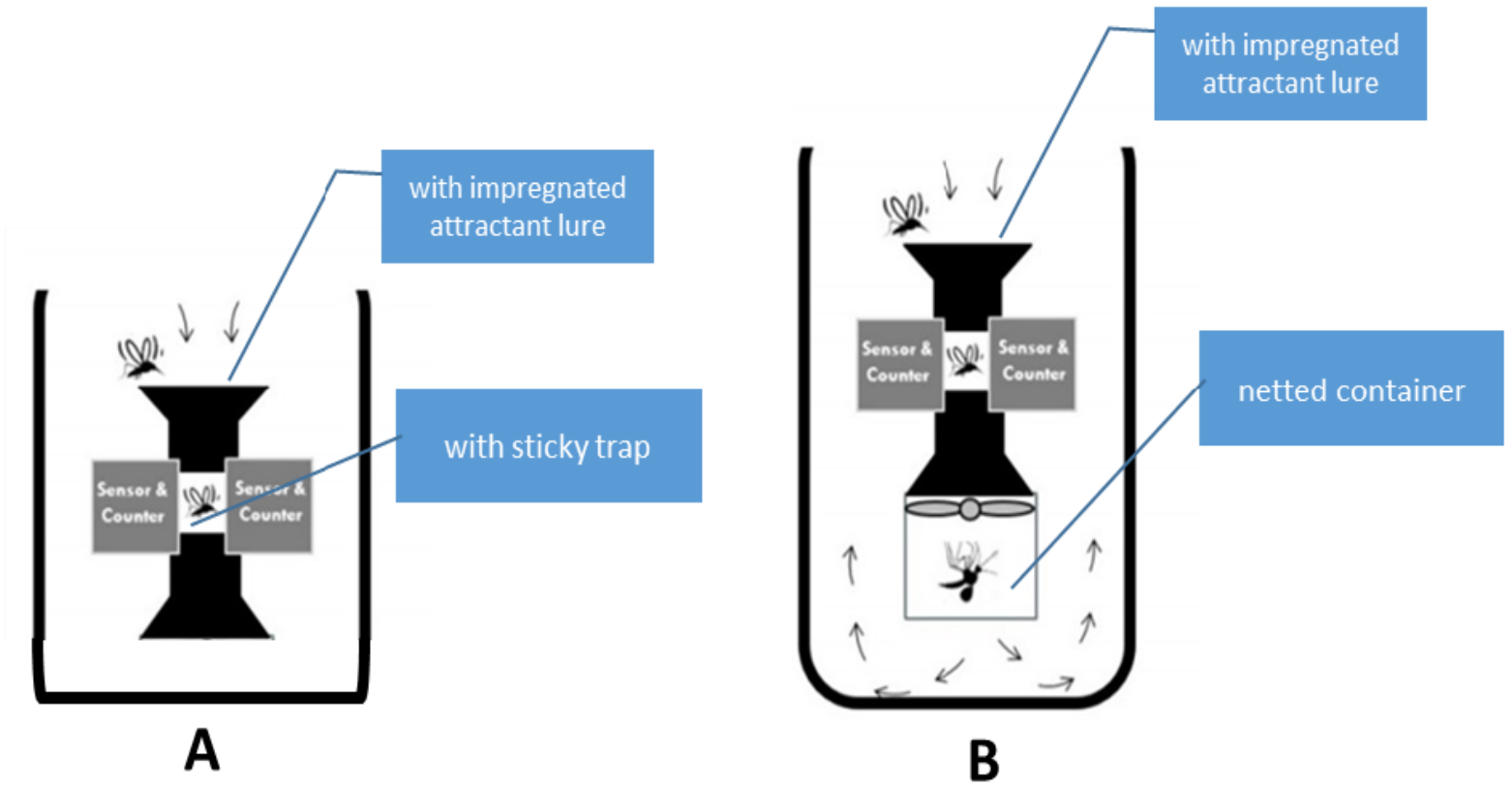


Figure 5. Potential designs for the trap,(A) with impregnated lure and sticky trap to trap and kill mosquitoes and (B) with impregnated lure and suction mechanism to trap and kill mosquitoes.

ARTiST

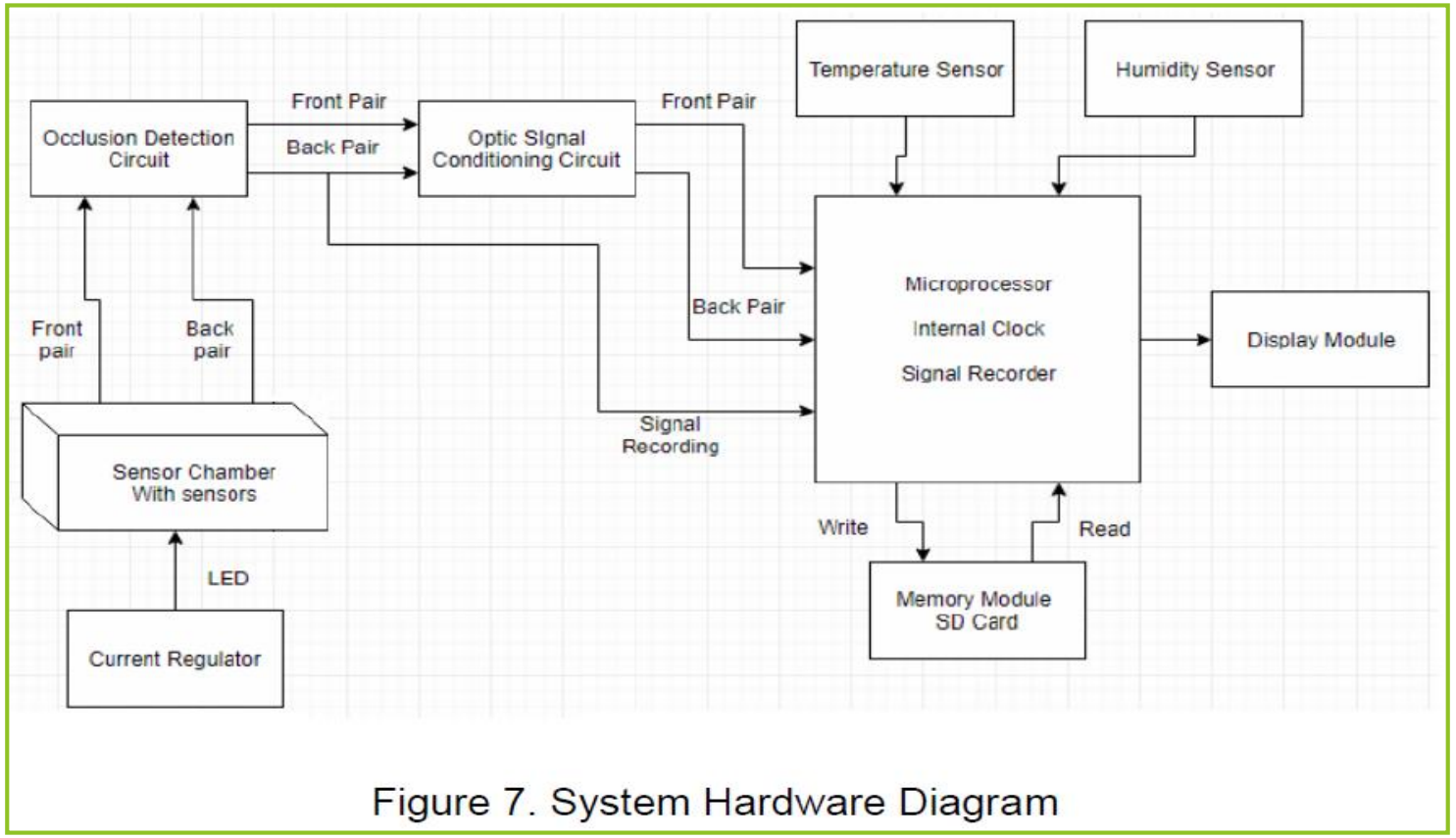


Figure 7. System Hardware Diagram

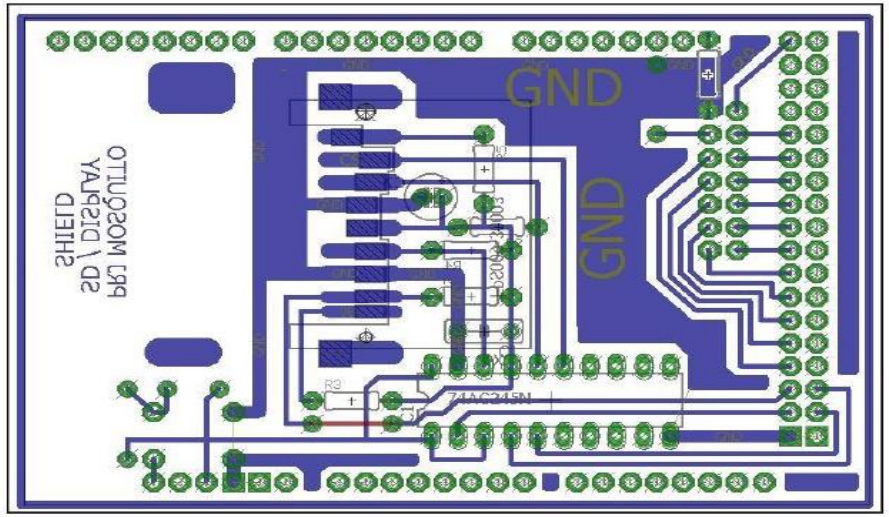


Figure 9. SD Card and LCD Arduino Shield

Gaps to address:

1. ‘’…ovicyte and larval indices offer little value with respect to surveillance because of the low survival rates of eggs and larvae (DOH Probing Mosquito Upsurge).”
2. Conventional traps cannot automatically count and identify trapped mosquitoes to give real-time count data for entomologic surveillance.
3. Expensiveness- ARTiST will be cheaper since local materials will be used.

NANODIAGNOSTIC

Construction and development of nano-biosensor kit

Validation and field testing of in hotspot areas

Development of disease modeling and forecasting technology for predicting potential disease outbreak

Capacity building and technology transfer

Economic Analysis

ARTiST

Oviposition attractant experiments

1) Releases of mosquitoes (sequential experimental design)
2) Identification of the most effective attractant and determination of its chemical profile
3) Biological assay using olfactometer

Designing and implementation of two types of trapping system

Field testing

NANODIAGNOSTIC

Mechanism of nanobiosensor

MNP + Gold Nanoparticles