# FAZD Center Overview of Biological Systems Projects

3rd ANNUAL DHS UNIVERSITY NETWORK SUMMIT
Washington, DC
March 17-20, 2009

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FAZD CENTER

NATIONAL CENTER FOR FOREIGN ANIMAL AND ZOONOTIC DISEASE DEFENSE

TEXAS A&M UNIVERSITY 1876

U.S. DEPARTMENT OF HOMELAND SECURITY

UCDAVIS University of California, Davis

USC UNIVERSITY OF SOUTHERN CALIFORNIA

UTMB The University of Texas Medical Branch

# Core Partners

UC Davis — University of California, Davis

Texas A&M University

USC University of Southern California

UTMB — The University of Texas Medical Branch

# Associate Partners

University of Minnesota

Tuskegee University

University of Maryland

The University of Wisconsin–Madison

# National Partners

Los Alamos National Laboratory — Est. 1943

Lawrence Livermore National Laboratory — Science in the National Interest

Sandia National Laboratories

Pacific Northwest National Laboratory — ...delivering breakthrough science and technology

Plum Island Animal Disease Center

# State Partners

Texas Animal Health Commission — Serving Texas Animal Agriculture — Since 1893

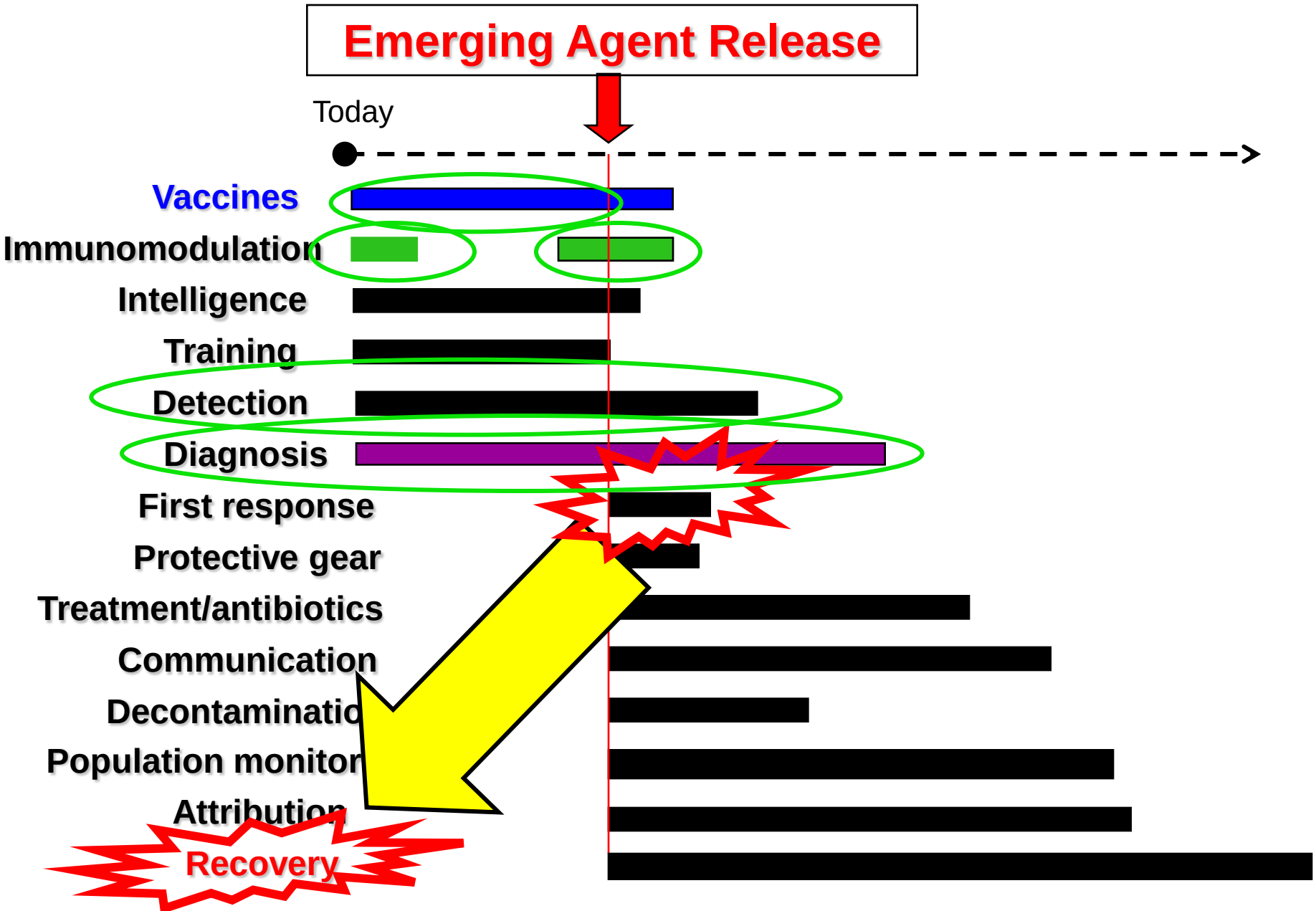
cdfa — California Department of Food & Agriculture

# Centers of Excellence

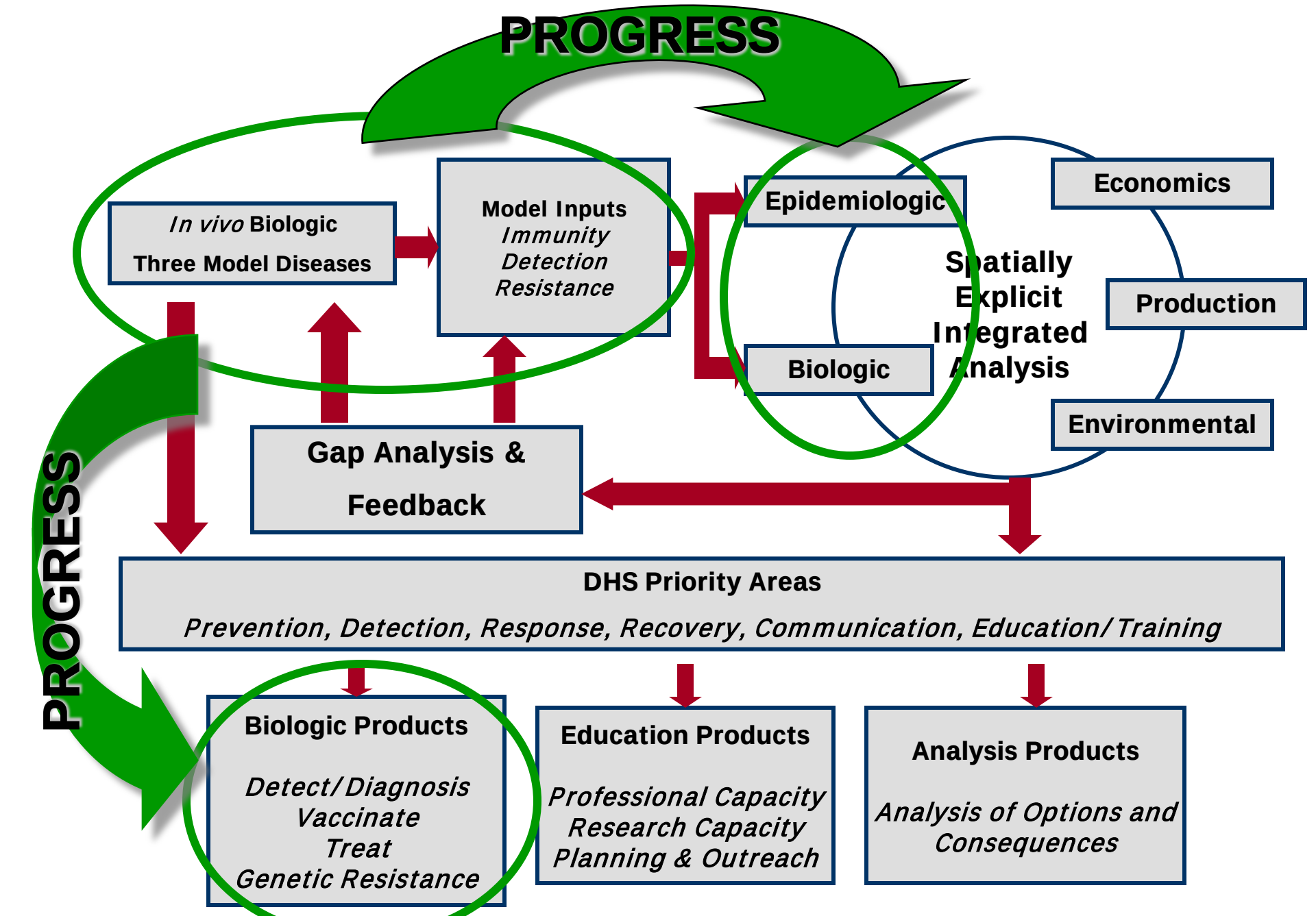
CREATE — Homeland Security Center

National Center for Food Protection and Defense — A Homeland Security Center of Excellence

# Biothreat BIOpreparedness Timeline



# Integrated Biological Systems Approach



# FAZD Biological Systems Research Teams

## Foot & Mouth Disease

Peter Mason
Mark Estes
Tilahun Yilma
Bill Golde
Luis Rodriquez

## Avian Influenza

Carol Cardona
Blanca Lupiani
Sanjay Reddy

## Vaccines

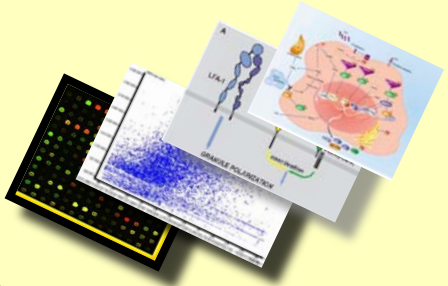
## Diagnostics

## Pathogenesis

## Rift Valley Fever

CJ Peters
Tilahun Yilma
Jim Womack
George Bettinger

## Cross-Cutting Platforms



Mark Estes
Skip Garner
Mary Lipton
Danny Rintoul

George Davidson
Stephen Johnston
Garry Adams

# Foot & Mouth Disease
## Progress toward Deliverables

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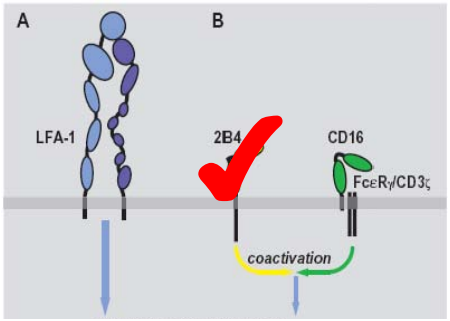
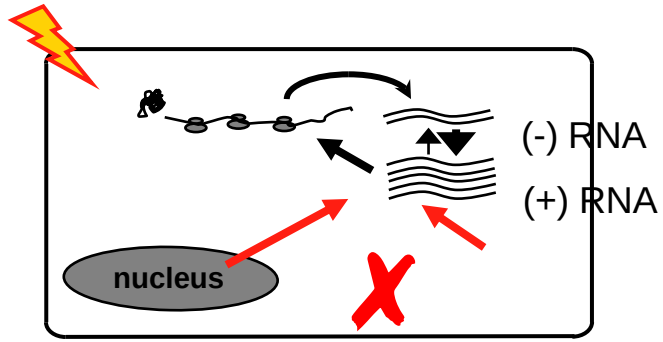
| Timelines of Progress | Y1 | Y2 | Y3 | Y4 | Y5 |
|---|---|---|---|---|---|

**Foot & Mouth Disease**

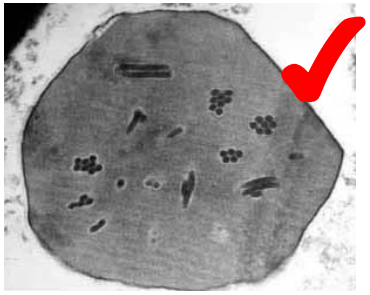
- Rapidly Acting FMDV Vaccines
- FMDV Antigen Chute-Side Test
- Replicon Based FMDV Diagnostic Test Antigens
- Mass Scale Replicon-based FMDV Robotic ELISA Test



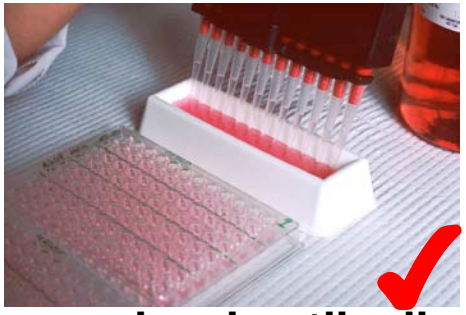

enhanced vaccines



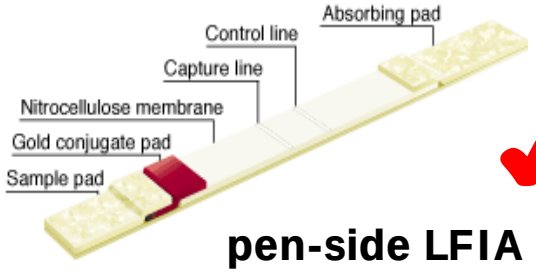

replicon antigens



baculovirus antigens



monoclonal antibodies




pen-side LFIA test

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# Enhanced FMDV Immune Modulation

## Targeting activating NK receptors via vaccine constructs which modulate IFNγ production and cytotoxicity

**Activating cell surface receptors:**

***CD16 (ITAM mediated signaling)**

**KIR: Killer cell immunoglobulin-like receptor**

**Natural cytotoxicity receptors:**

**NKG2D**

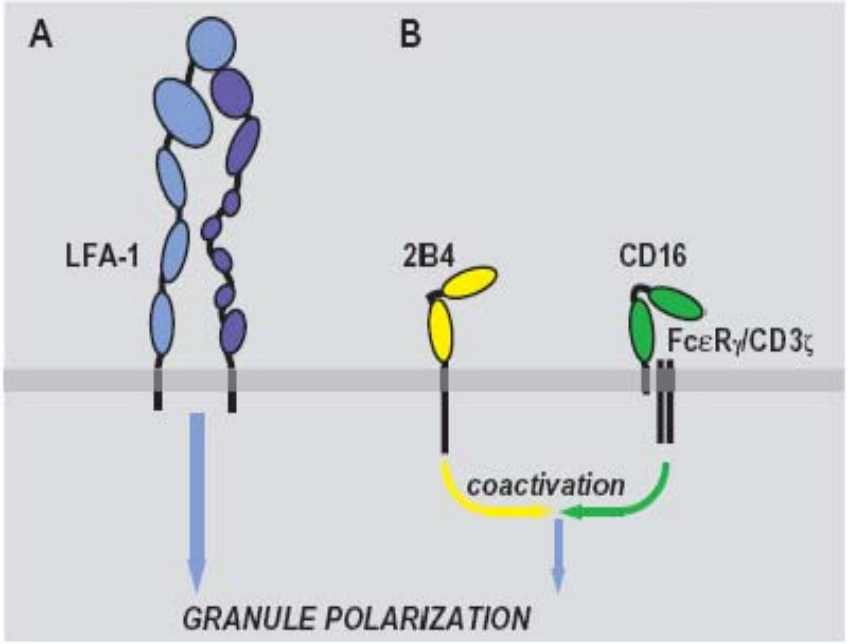
**NKp30**

**NKp44**

***NKp46 (non-ITAM mediated signaling) NKRP1**

***2B4 [CD244]-(non-ITAM-mediated signaling)**

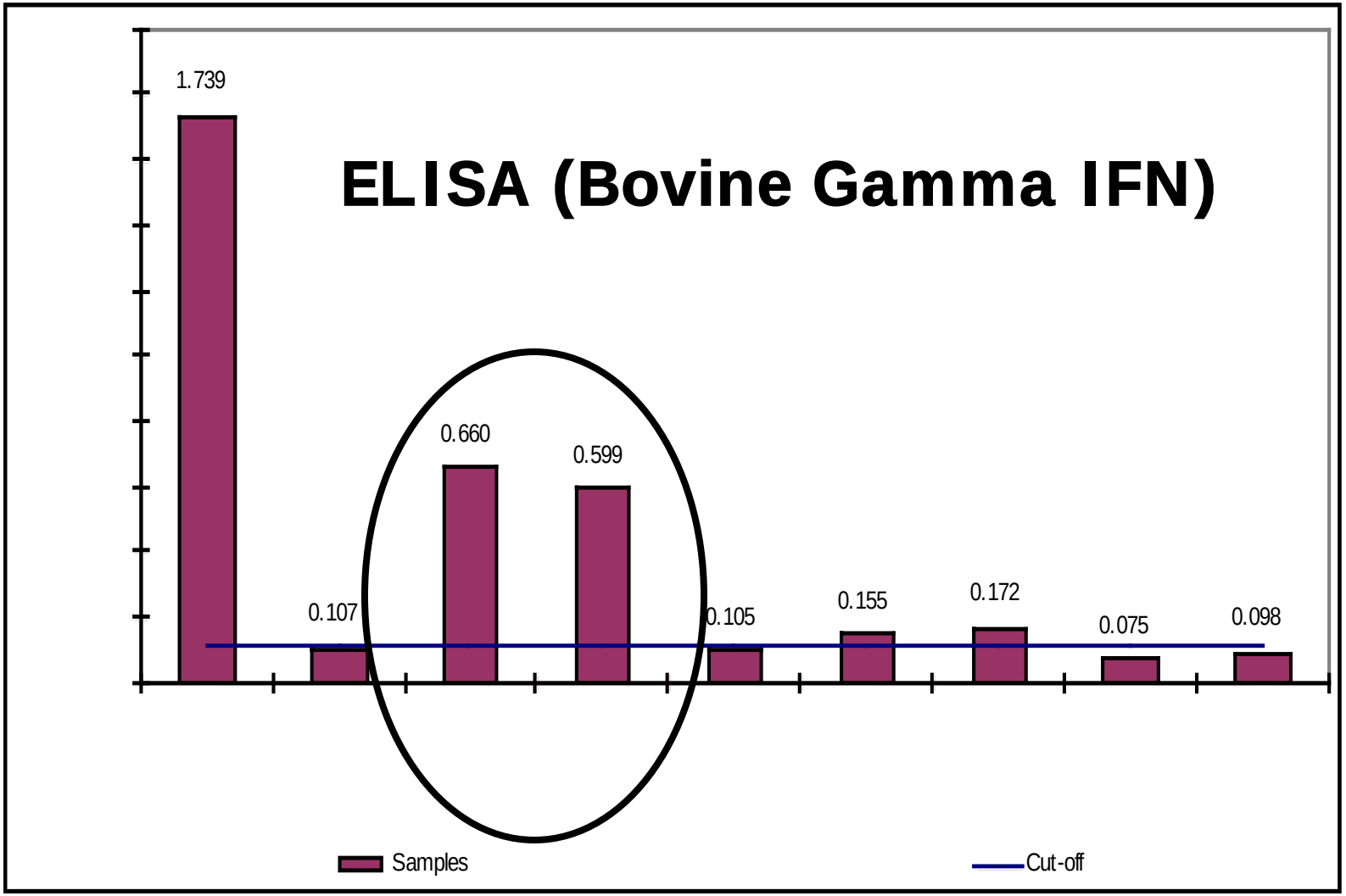
***current modulation targets for vaccination**




*Immunological Reviews 2006*
*Vol. 214: 73–91*

# Enhanced Protective Immunity FMD Vaccination

Secreted IFN-gamma levels following antibody and/or cytokine treatment



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# Development of FMD New Diagnostics under BSL2 protocols

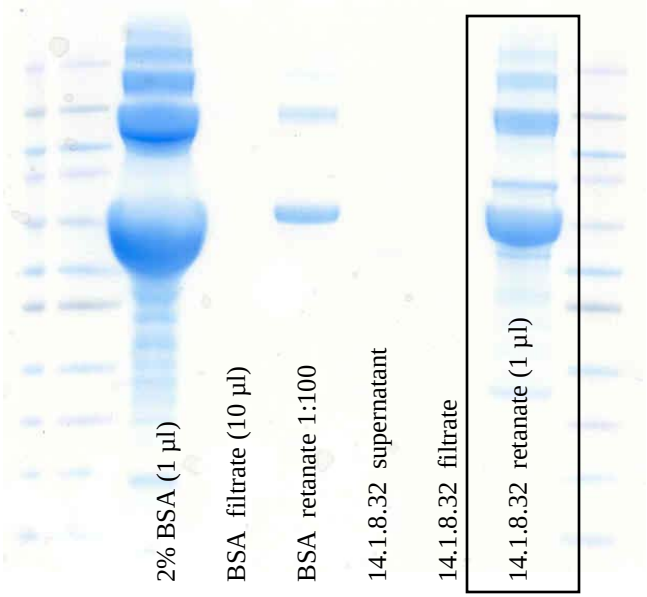
- ✓ **Develop FMDV replicon-expressing eukaryotic cells as antigen source to detect antibodies in robotic ELISA**
  - First-generation antigens produced from replicon-expressing cell lines
  - FMDV replicons shown to have BSL2 mass-scale antigen potential
  - Cross-reactivity profile of replicon-derived antigens to be determined with a PIADC serum panel
  - Optimized antigen preparations for robotic ELISA testing HUB labs

- ✓ **Express FMDV P3D gene in baculovirus vector to purify & characterize protein antigens**
  - P3 gene sequenced, plasmid vector constructed & FMDV P3D expressed in baculovirus as antigen for producing Mabs

- ✓ **Development of the lateral flow *antigen* detection strip test**
  - FMDV P3D specific Mabs ready to be tested PIADC in pen-side lateral flow immunoassay *antigen* detection assay

***Peter Mason – UTMB & Tilahun Yilma – UC Davis***

# Evaluation of Mab anti-FMDV P3D

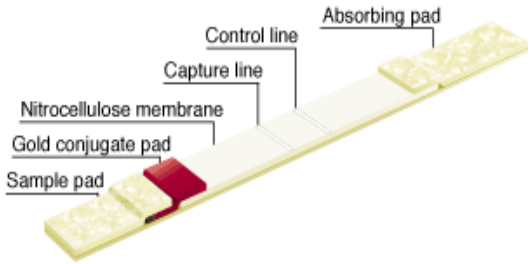



Coomassie blue stain of MAb # 14.1.8.32 (inside rectangle) after 100 fold concentration using Centricon concentration devices.

The sum of the bands represents ~10 µg of monoclonal antibody in 10 µl of retanate.

Lateral flow devices require 1-5 µg of antibody at the test line.

- 18 MAb's with IgG isotype developed
- Amplify & purify before conjugation with horseradish peroxidase
- MAbs targeting different epitopes of P3D conjugated with colloidal gold & tested at different concentrations on the strip test prototypes
- Evaluate FMDV-P3D capture assay in a LFIA test in the spring of 2009 in US negative & positive cohorts at Plum Island.



# Rift Valley Fever
# Progress toward Deliverables

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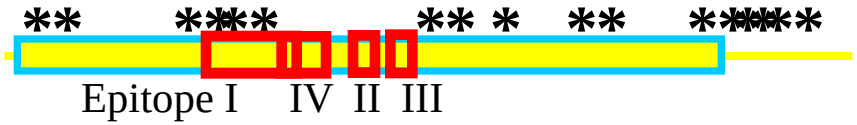
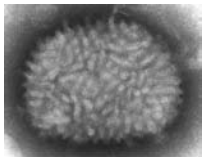



**Vaccinia Vectored Vaccines**

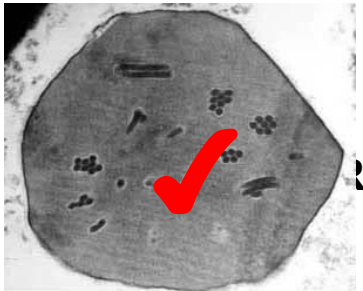


**MP-12 Vaccine**

| Timelines of Progress | Y1 | Y2 | Y3 | Y4 | Y5 |
|---|---|---|---|---|---|

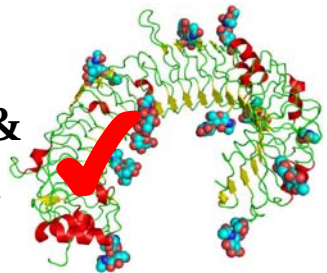
**Rift Valley Fever**

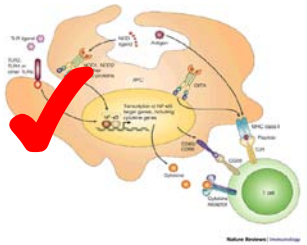
- Attenuated RVF Vaccine
- Vaccinia Virus RVF Vaccine
- Anitbody Response & Safety of RFV Vaccines in Sheep
- RVF Diagnostic Test
- Innate Genetic Resistance to RVF



**Baculovirus MP-12 RVF diagnostic test antigens**

**Toll-like Receptor genes mapped in rats & SNPs identified in cattle**





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# Progress on Rift Valley Fever Vaccine Development

- ✓ **Vaccinia vectored G1/G2 genes**
  - RVFV G1/G2 genes expressed in vaccinia vector for safety test in sheep in ‘09
- ✓ **Reverse genetics of RVFV developed for manipulating the genome to produce vaccines**
  - Successful rescue of MP-12 vaccine strain from DNA has been achieved
  - Scale up production being implemented for safety test in sheep in ‘09
  - Technology transfer developed

***CJ Peters – UTMB & T. Yilma - UC Davis***

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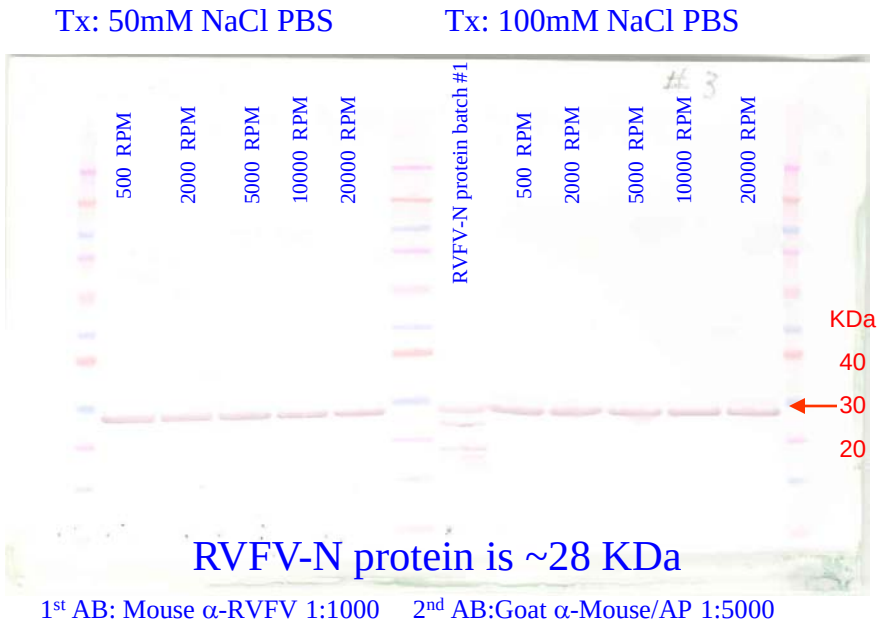
# Progress on Rift Valley Fever Diagnostic Test Development

- ✓ **BSL2 Baculovirus expressed RVFV N protein based - IgM IgG ELISA assay**
  - RVFV G1/G2 genes expressed in baculovirus for ELISA testing at USAMRIID or PIADC
- ✓ **BSL2 production of inactivated MP-12 RVFV based - IgM IgG ELISA assay**
  - Propagated RVFV MP-12 in serum-free media up to 2-4 x $10^8$ PFU/ml
  - Studies of MP-12 inactivation by binary ethylene imines, beta propriolactone & gamma irradiation underway for ELISA testing at USAMRIID or PIADC

# bRVFV-N ELISA antigen development

**RVFV-N recombinant protein detected with sera from mice vaccinated with MP12**




The gene for the RVFV-N protein was subcloned into a baculovirus system and expressed in Sf9 insect cells. The authenticity of the recombinant RVFV-N protein confirmed by western blot analysis using sera from MP12 vaccinated mice.

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# Innate Resistance against Select Agent Rift Valley Fever

- ✓ **Map bovine innate resistance gene candidates - Toll-like Receptor (TLR) & Nucleotide binding/Oligomerization Domain (NOD)**
  - 10 TLR & NOD1 genes mapped to bovine chromosomes
- ✓ **Sequence bovine TLR and NOD genes**
  - Bovine TLR7 and TLR10 sequenced, exons of all bovine TLR and NOD genes sequenced
- ✓ **Conduct Single Nucleotide Polymorphism (SNP) analysis of bovine TLR and NOD genes**
  - 29 SNPs identified in bovine TLR genes so far
- ✓ **Identify & map rat innate RVFV resistance genes**
  - Recombinant inbred LEW strains bred for RVF challenge

***James Womack - TAMU***

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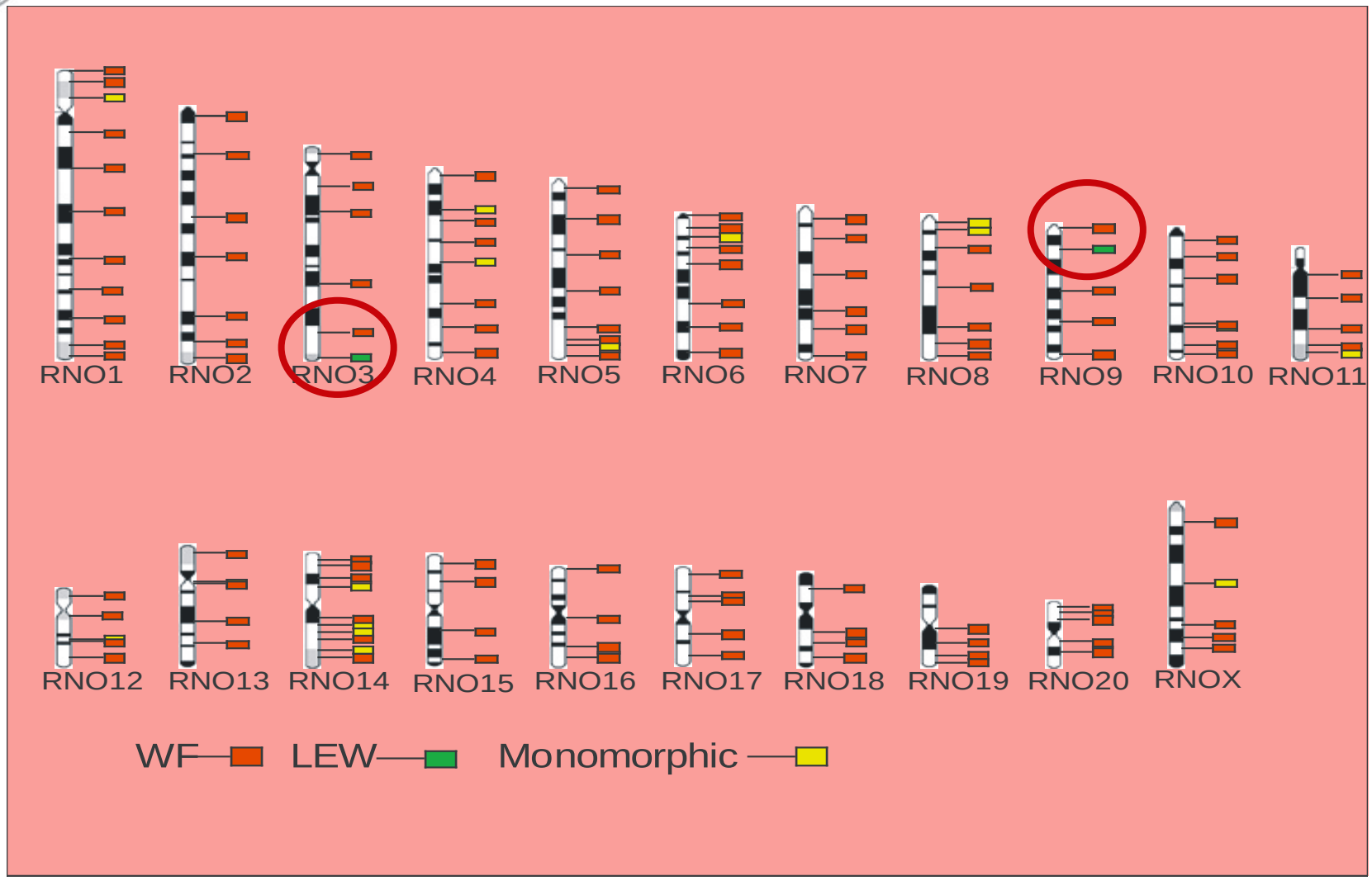
# Genome scan for congenic segments of LEW rat strain carrying resistance to RVFV



# Avian Influenza
## Current Timeline toward Deliverables

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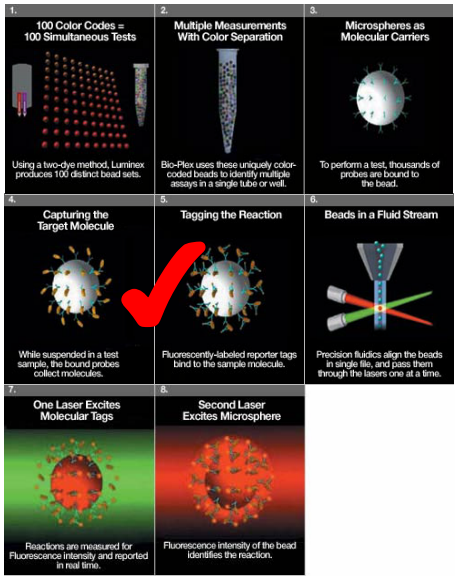


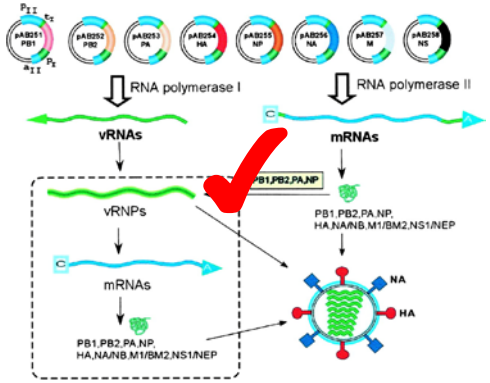


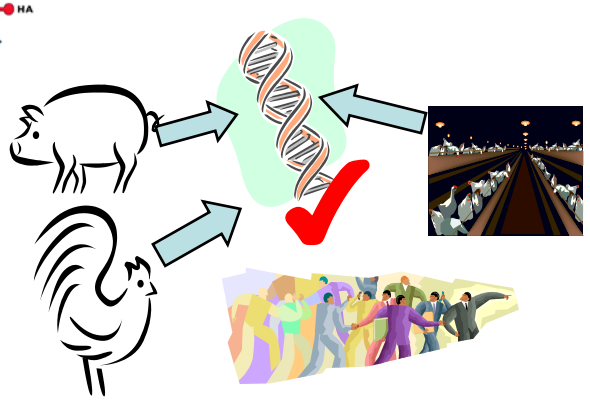

| Timelines of Progress | Y1 | Y2 | Y3 | Y4 | Y5 |
|---|---|---|---|---|---|
| **Avian Influenza** | | | | | |
| Chicken & Turkey H5 ELISA & Luminex AI Test | → | → | → | → | |
| Recombinant AI DIVA Vaccine | | → | → | → | |
| B Cell Epitope Mapping of AI Virus | | | | → | |
| AI Test for Free-Flying Birds | | | | → | |
| AI Genomic & Proteomic Biosignatures | | | | → | |

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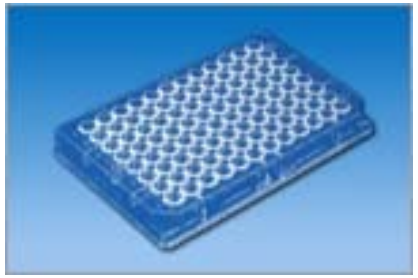
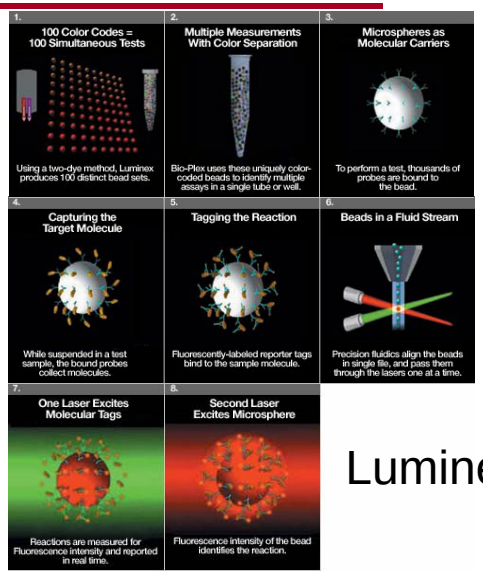
# Development of diagnostic tests

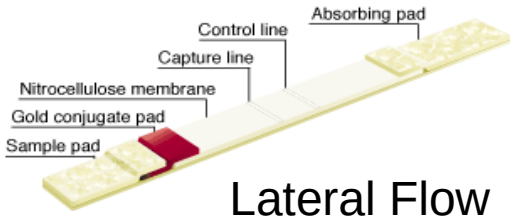
- Diagnostic tests:
  - *Antibody detection*:
    - ELISA
    - Luminex
  - *Antigen detection*:
    - Luminex
    - Lateral flow immunoassay



ELISA




Luminex




Lateral Flow

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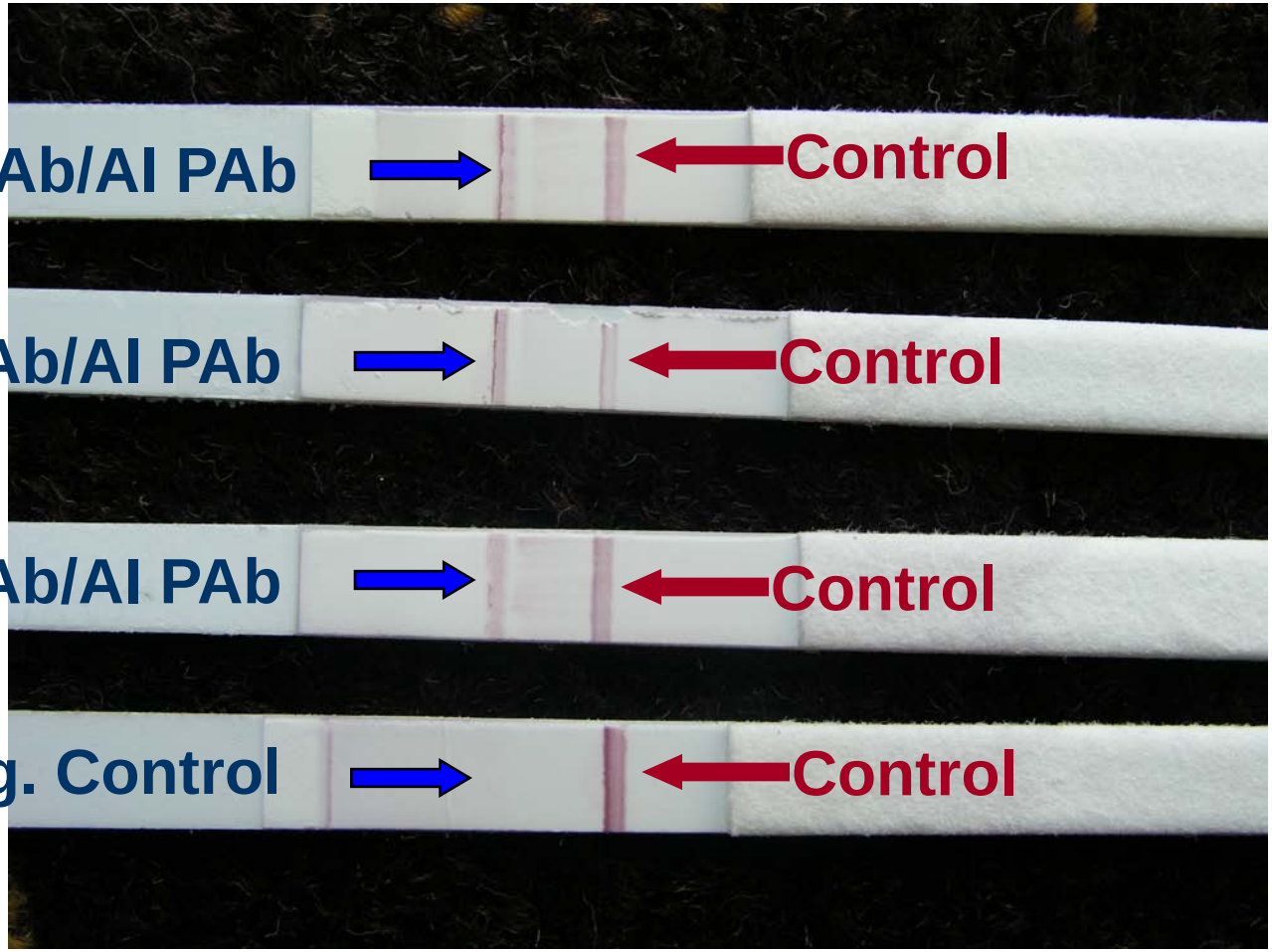
# AI Lateral Flow Immunoassay




*J. El-Attrache - TAMU*

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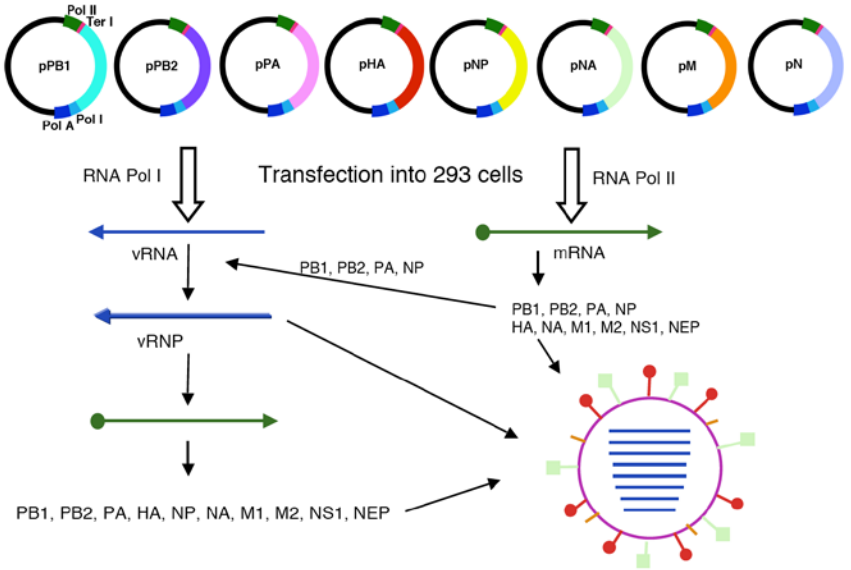
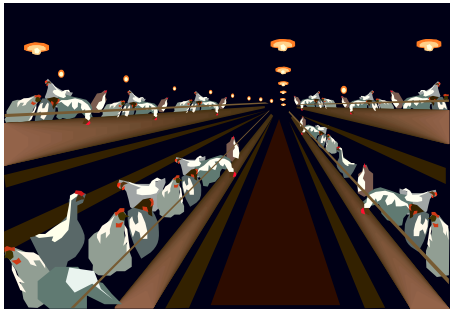
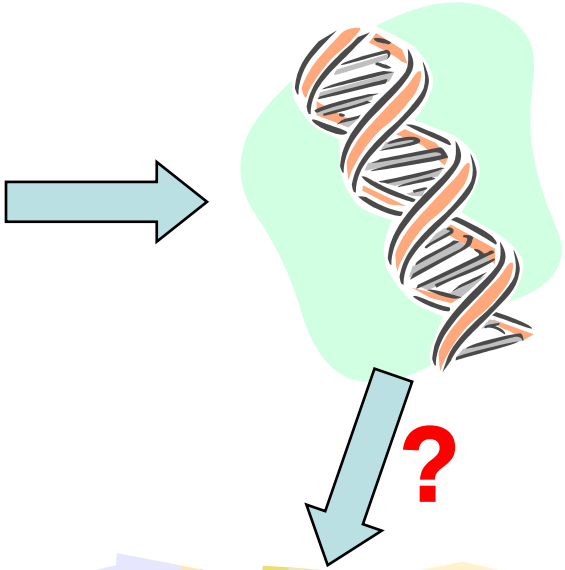
# Avian Influenza Vaccine Developments

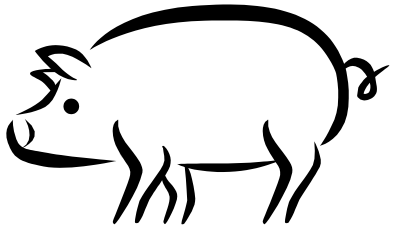
- **DIVA Strategies**
  - Vaccines and diagnostics
    - Multiple uses needed
    - Multiple formats
  - Reverse genetics
  - Developed as a package



# Avian Influenza U.S. Live Bird Market Epidemiology





*Carol Cardona – UC Davis*

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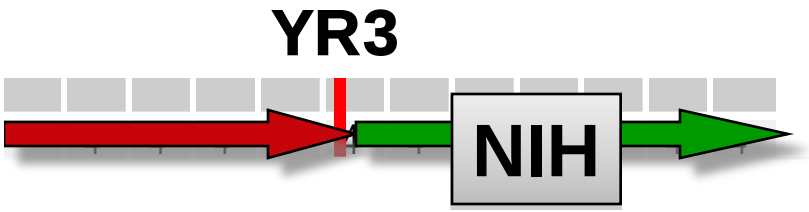
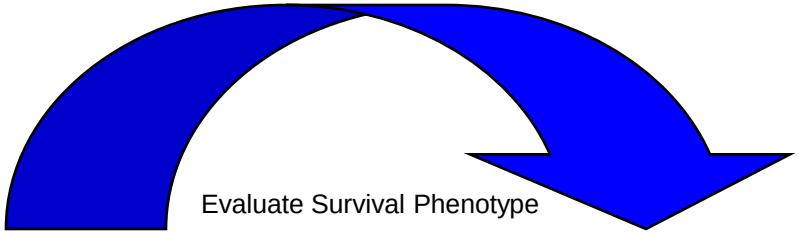
# Brucellosis

## Progress toward Deliverables

**Protection against Brucella**






Transposon

Brucella Genome

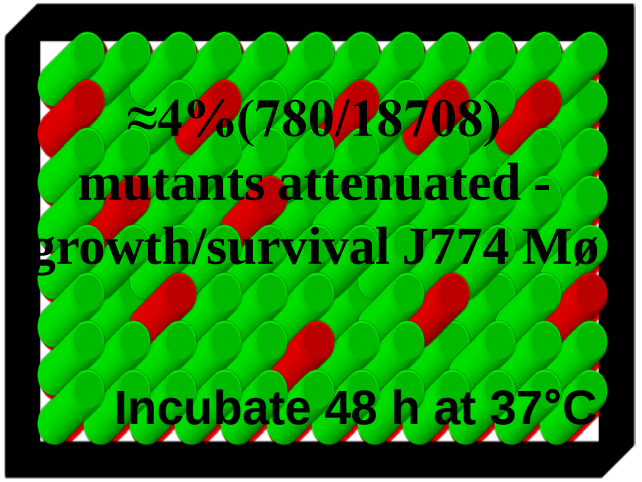
1 2 3 4 5 6 7 8 9

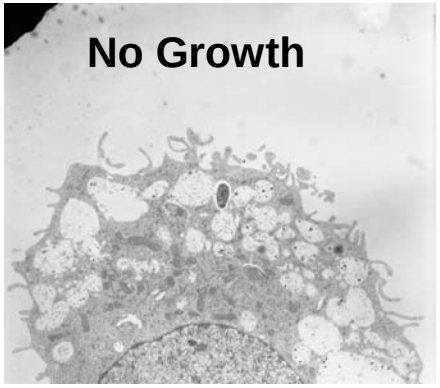
Select for kan$^R$ growth

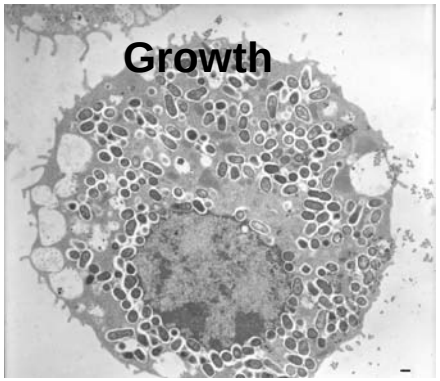
Kan$^R$

1 2 3 4 5 6 7 8 9

Sequence Interrupted Gene



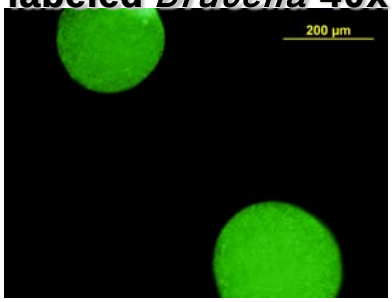

*Tom Ficht, Allison Ficht & Garry Adams - TAMU*

# Microcapsulation enhances *Brucella* vaccine protective immunity efficacy

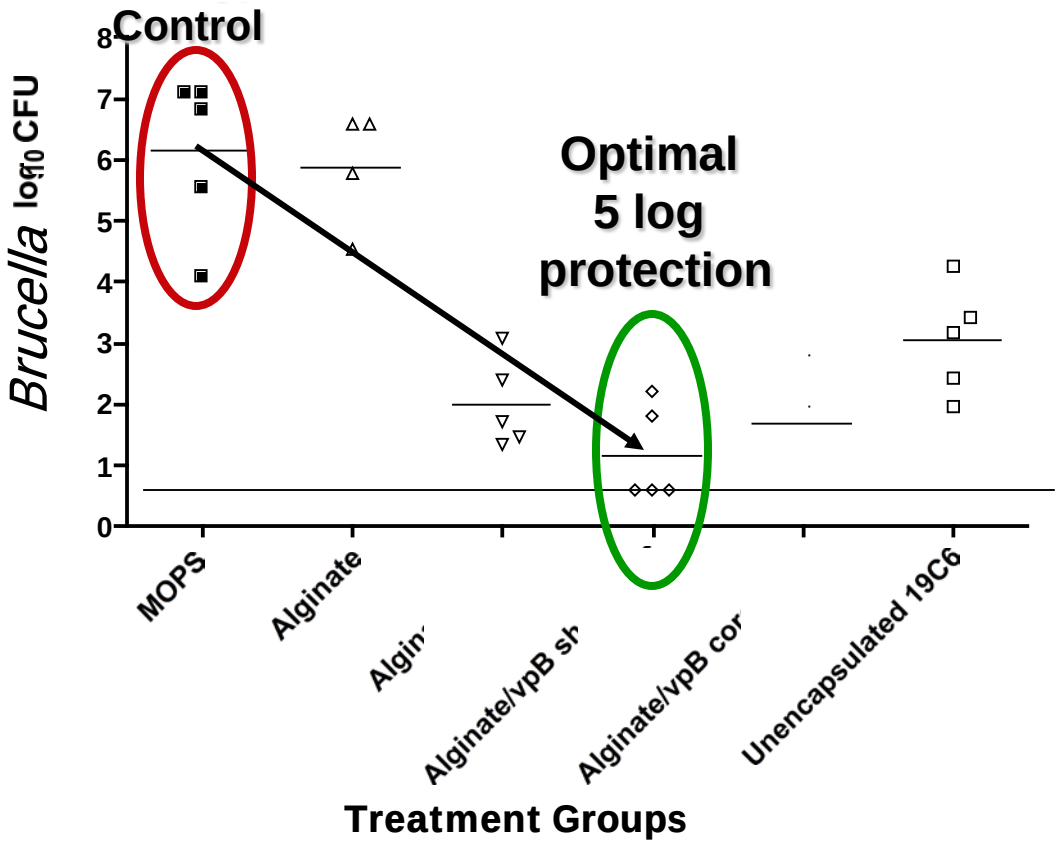
Encapsulation provides:

- Controlled release of attenuated mutants
  - Oral delivery
  - One-time dosing
  - Extend and enhance immune response
  - Prolonged storage at ambient temperatures

**Encapsulated labeled *Brucella* 40x**




**Vaccine Efficacy [32 wk post-vaccination]**




**Vaccines proven protective in mice & goats will be tested in Non-human Primates.**

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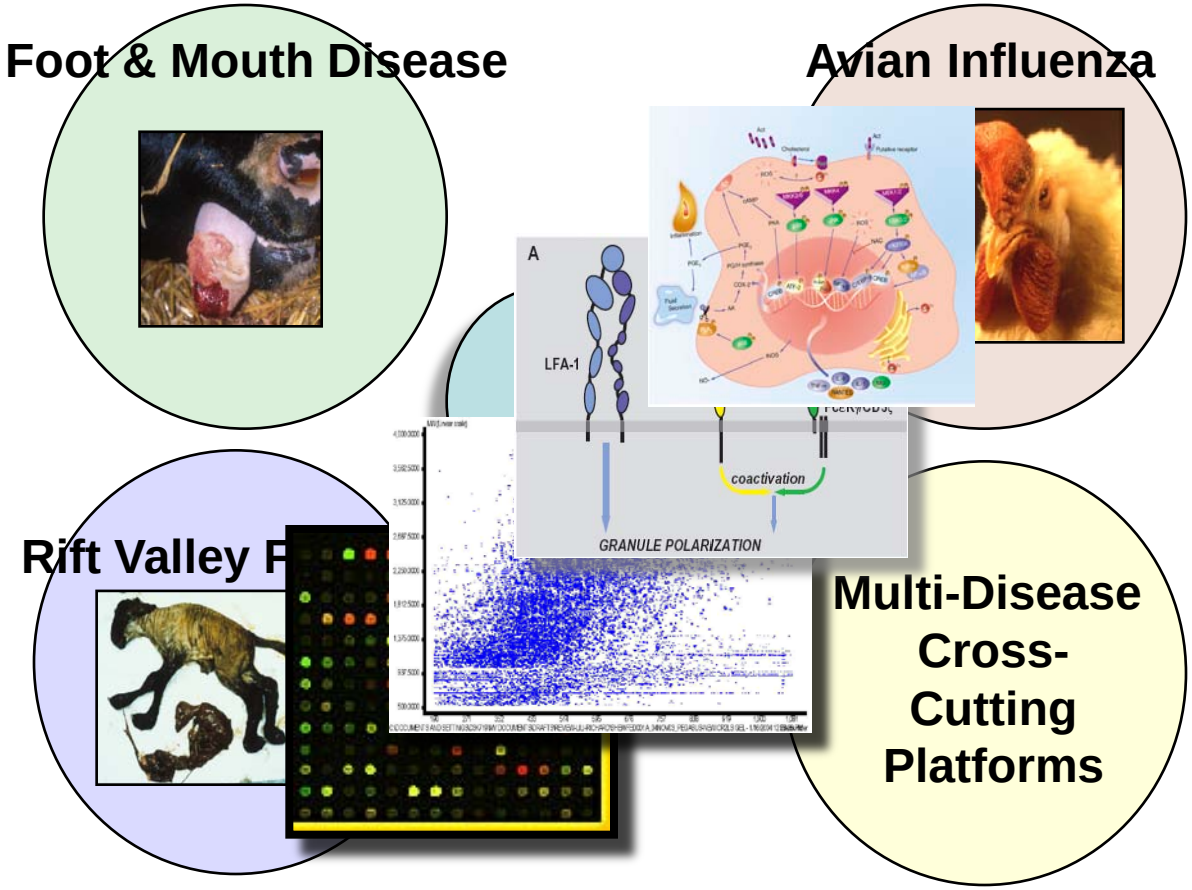
# Cross-Cutting Technology Platforms
## Progress toward Deliverables




| Timelines of Progress | Y1 | Y2 | Y3 | Y4 | Y5 |
|---|---|---|---|---|---|
| Cross-Cutting Technology | | | | | |
| Rapid Antibody & NK Cell Vaccine Response to Biothreats | | | | → | |
| Integrated Multi-Agent Detection Platform | | | | → | |

FAZD Biological Systems Research Teams

# Cross-Cutting Transcendi[illegible]s

Foot & Mouth Disease

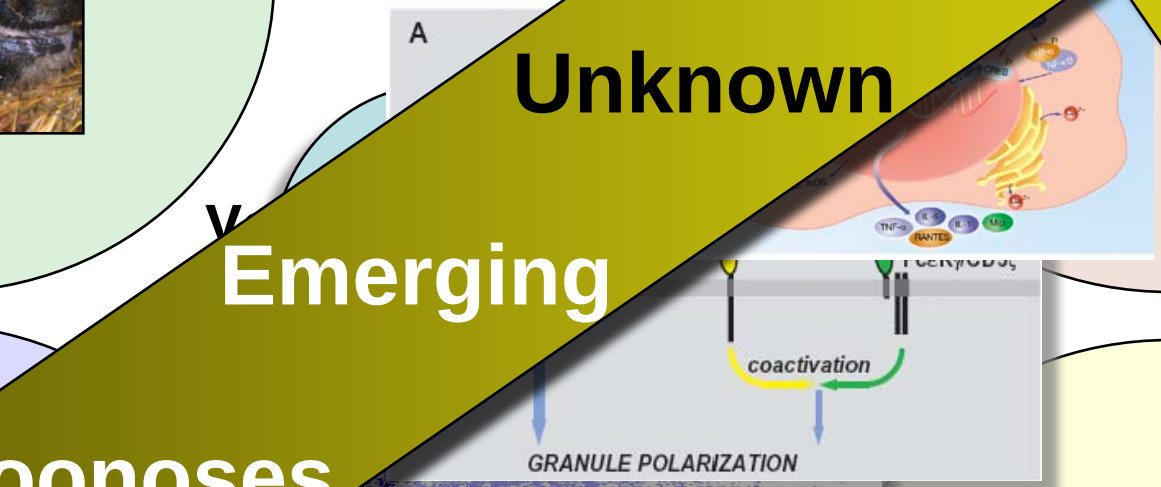
Engineered

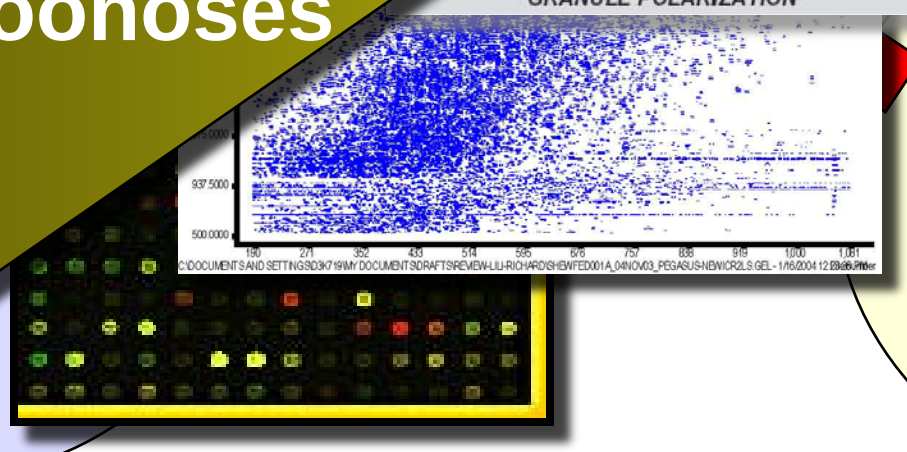
Unknown

Emerging

Zoonoses

Known

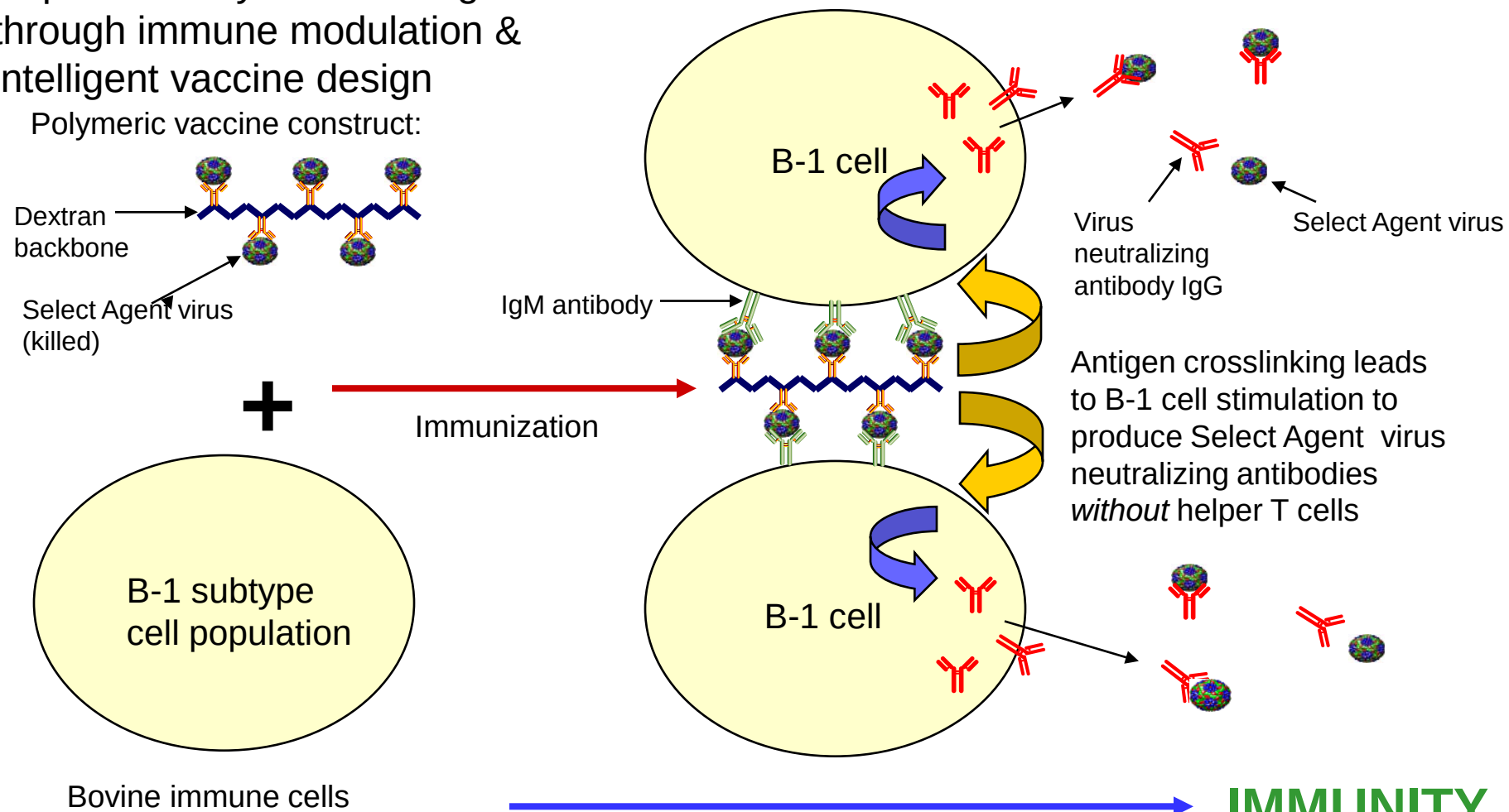


Multi-Disease Cross-Cutting Platforms

# Transcending Vaccine Strategy to Induce Rapid Serum Neutralizing Antibody Responses to Select Viral Agents

Rapid antibody to Select Agent through immune modulation & intelligent vaccine design




***Mark Estes – UTMB, Bill Golde & Luis Rodriquez - PIADC***

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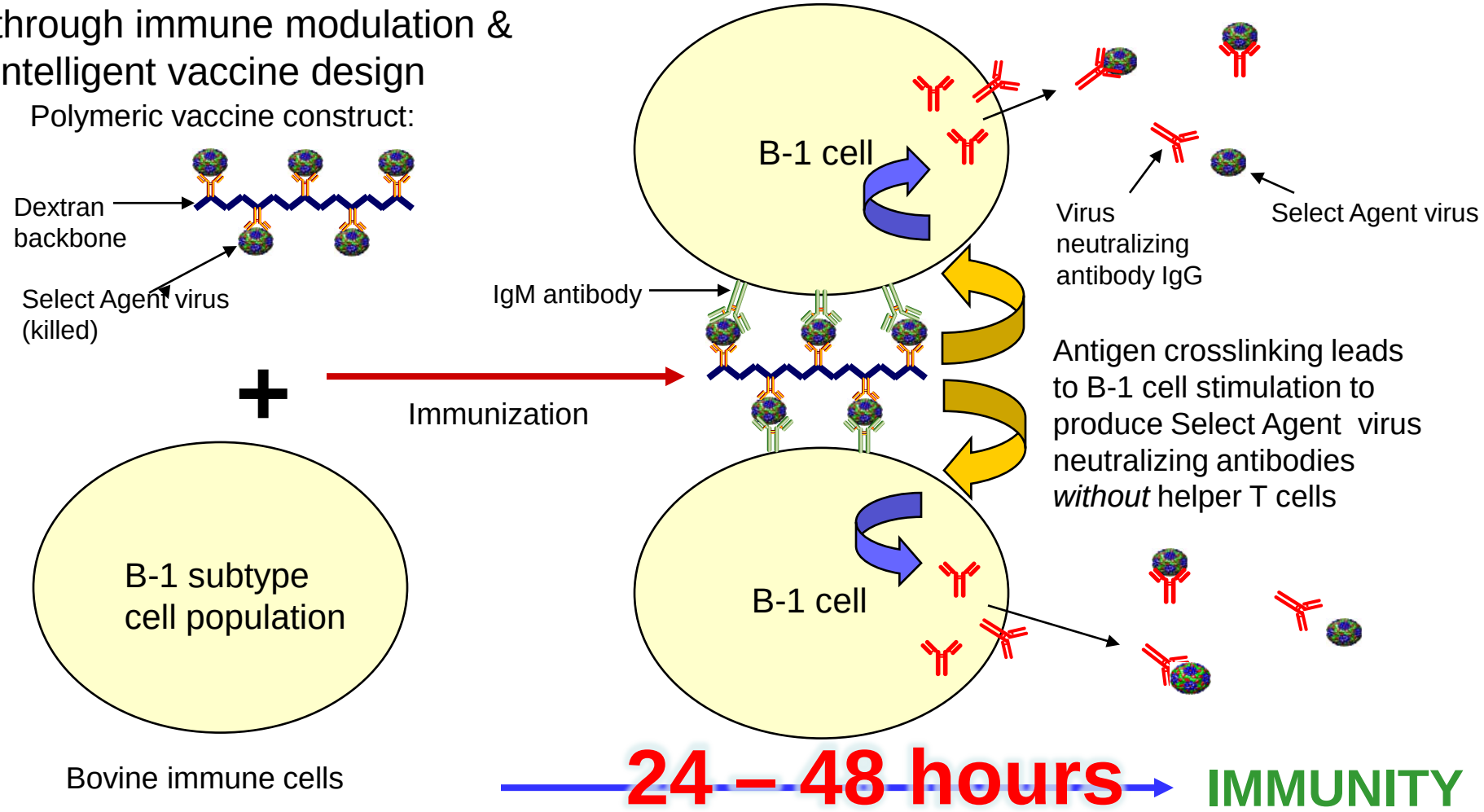
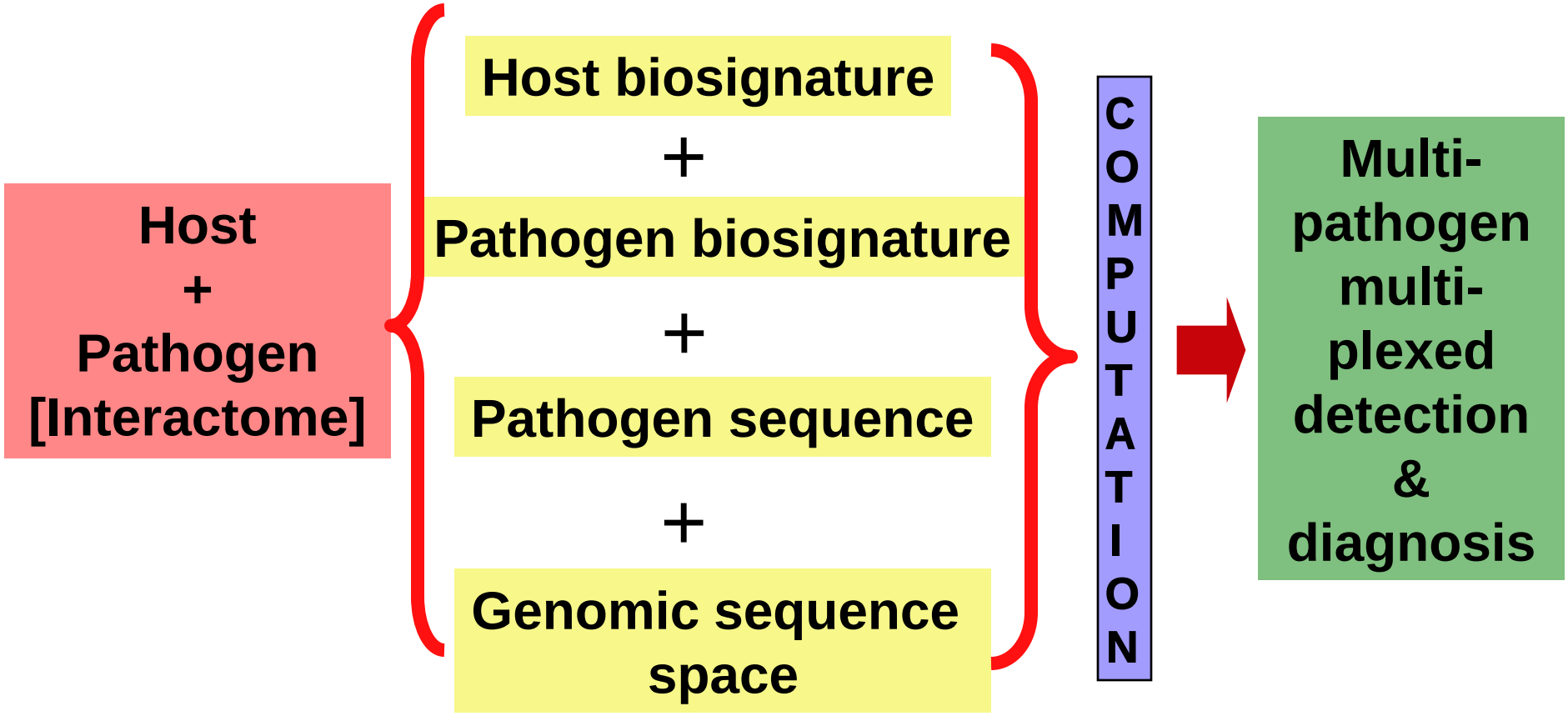


# Transcending Diagnostics Platform Concept




*Skip Garner – UTSWMedCtr & Garry Adams – TAMU*

# Pathomics Infection Biology

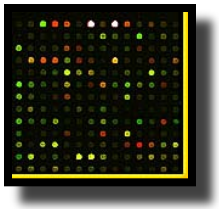
## Deliverables

Bovine Genome

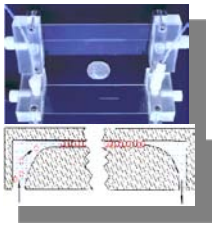
*In vitro/in vivo* infection

**the interactome**

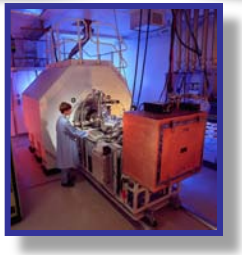
Pathogen Genome



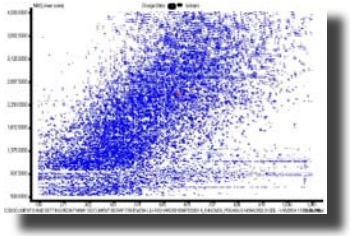
Bovine microarrays



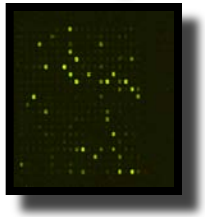
MPSS



*HF Fourier Transform Ion Cyclotron Resonance Mass Spectrometry*



Proteome



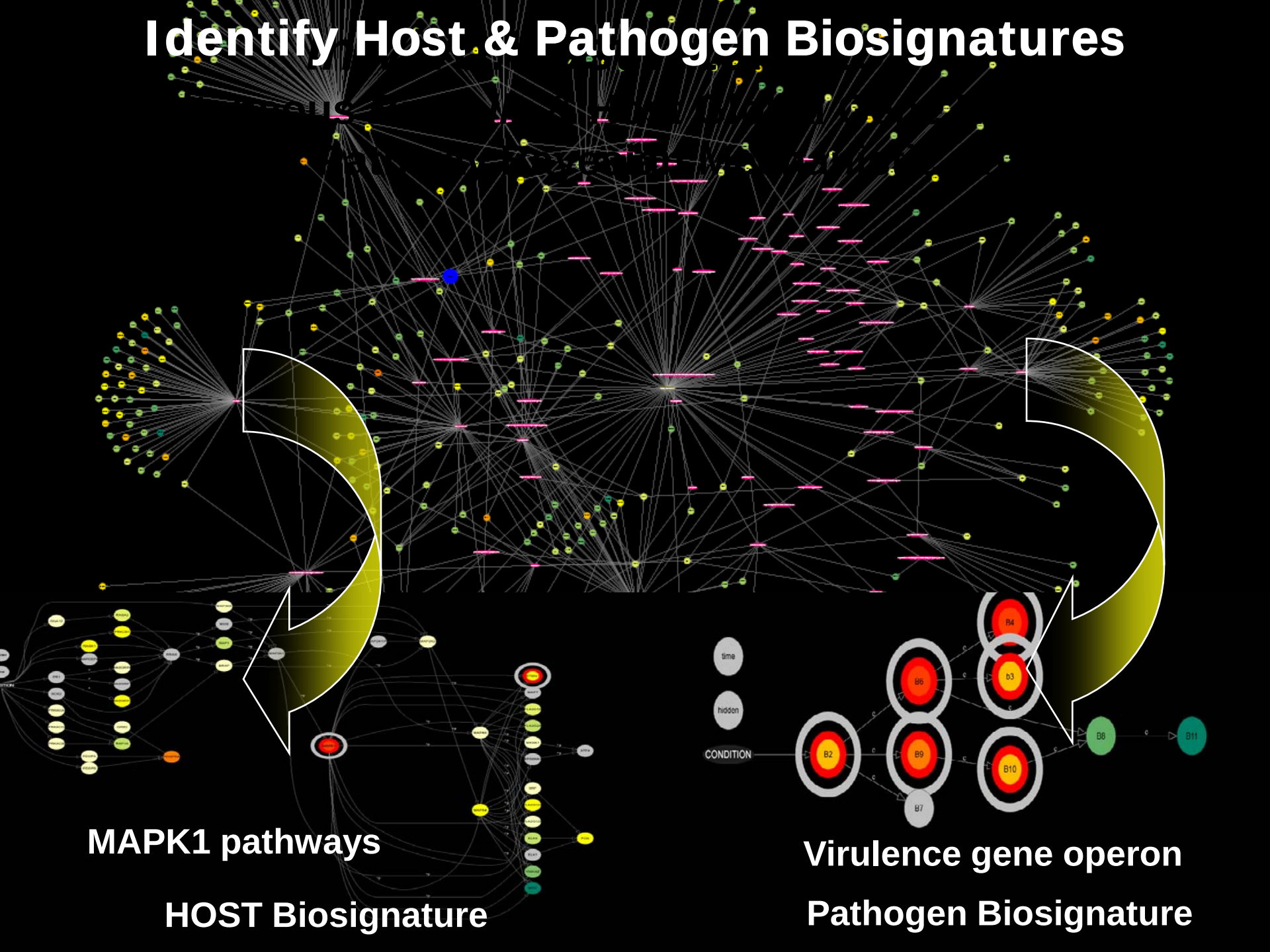
Pathogen microarrays

host & pathogen *interactome*
[transcriptome & proteome]
data warehousing, data management

informatics, computational biology & modeling

**infection biology data** → **data fusion** → **model** → **knowledge discovery infer** → **validate** → **in vivo animal model** → **biological actionable intelligence**

# Identify Host & Pathogen Biosignatures




MAPK1 pathways

HOST Biosignature

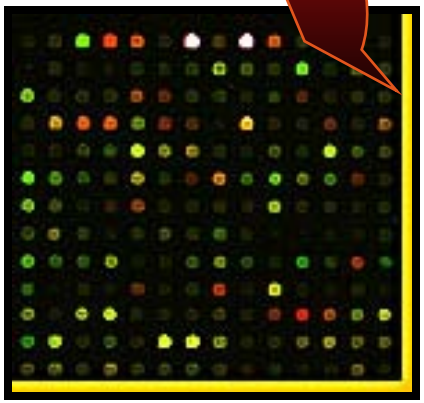
Virulence gene operon

Pathogen Biosignature

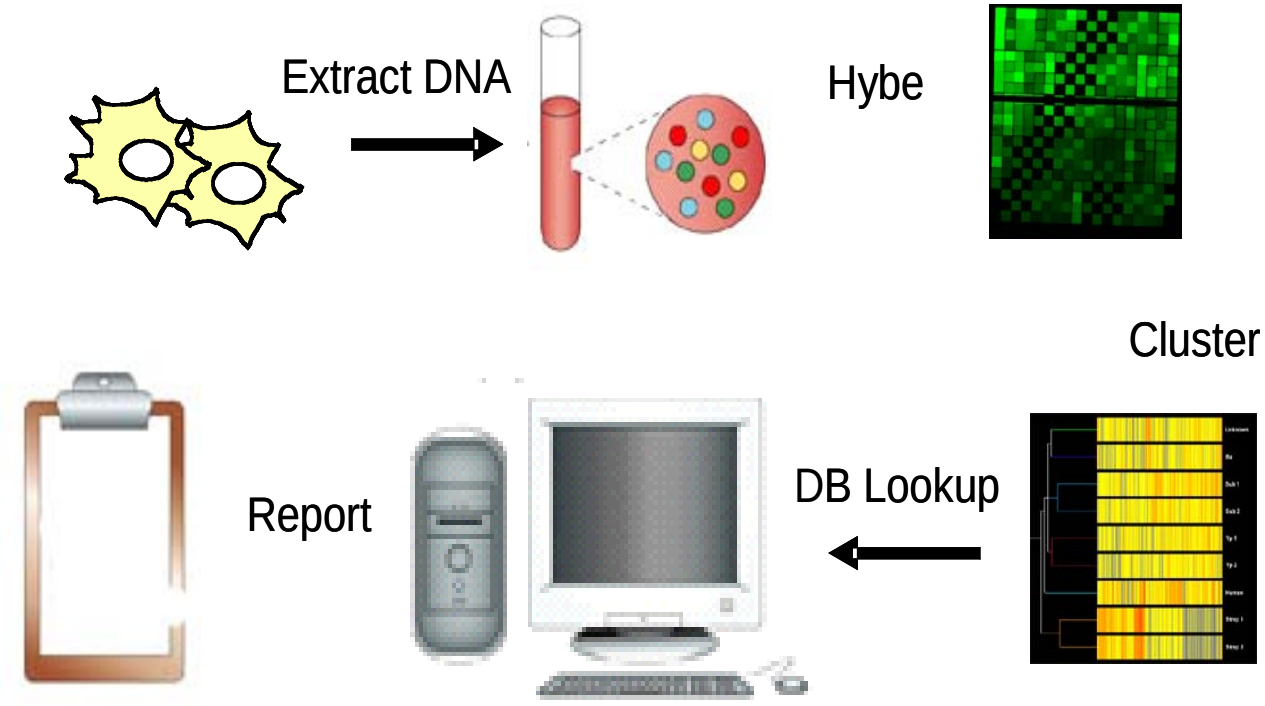
# Multi-Select Agent Arrays

African horse sickness virus
African swine fever virus
Akabane virus
HP Avian influenza virus
Bluetongue virus (Exotic)
Bovine spongiform encephalopathy
Camel pox virus
Classical swine fever virus
Cowdria ruminantium (Heartwater)
Monkeypox virus
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
Mycoplasma capricolum
Mycoplasma mycoides mycoides
Newcastle disease virus (velogenic)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis viruS
Yersinia pestis
Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei
Burkholderia pseudomallei
Coccidioides immitis
Coxiella burnetii
Eastern Equine Encephalitis virus
Francisella tularensis
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

- **Probe design using computational biology bioinformatics tools**
- **Consensus sequence probes for all known pathogens**
- **Produce multipathogen arrays**

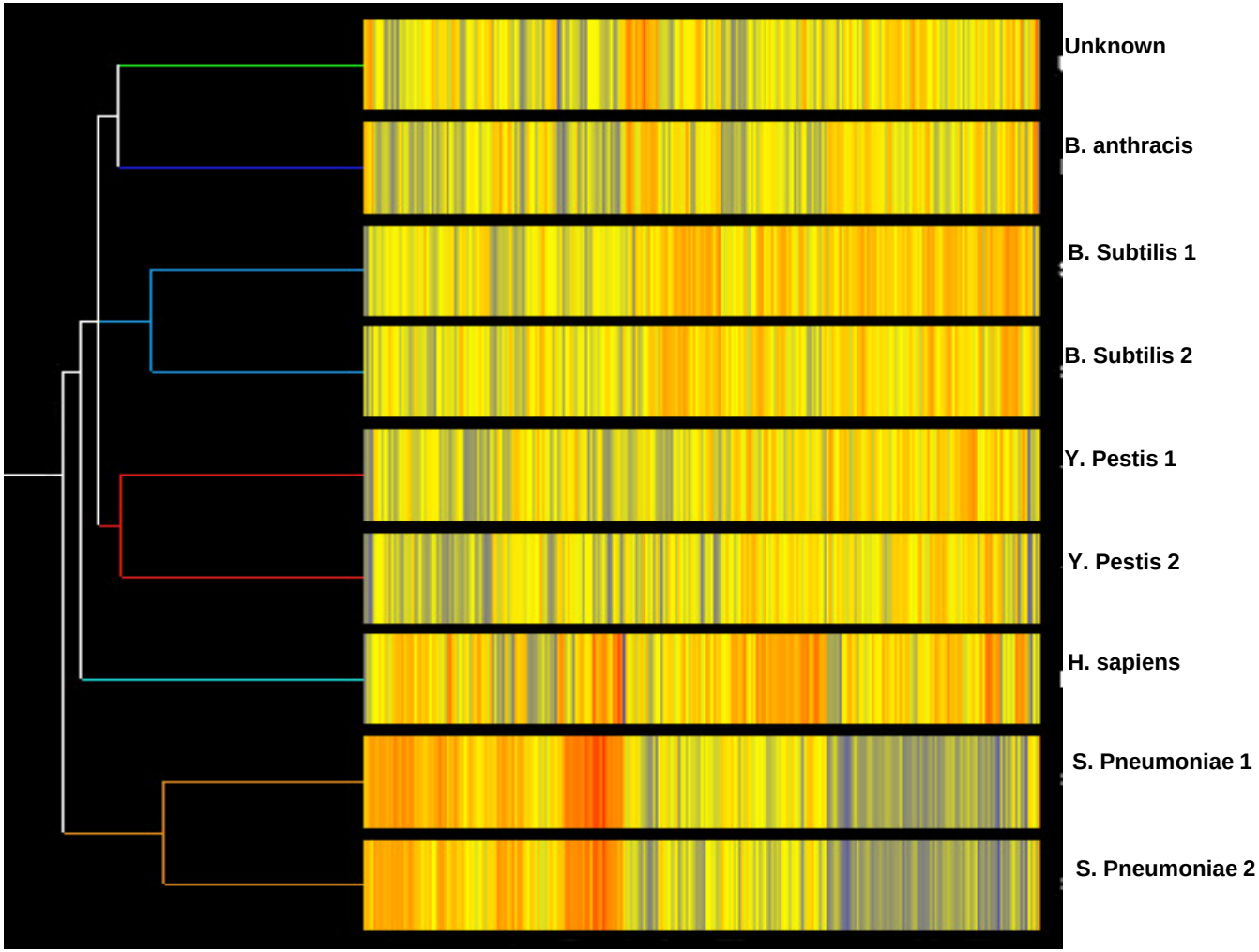


# Universal Biosignature Detection Array




- **Probes based on genomic space**
- **Array design virtually complete**
- **Whole genome amplification method in testing**
- **Array hybridizations and data analysis in progress**

# Universal Biosignature Detection Array (UBDA)



# Integrate BSBA, MSAA and UBDA into a single orthogonal array-based detection and/or confirmatory diagnostic platform

- ✓ Optimize sample preparation
- ✓ Nimblegen/Affimetrix array technology
- ✓ Seralogix Bayesian data mining
- ✓ Construct database of patterns for select agents
- ✓ Transfer optimized array designs and protocols to commercial manufacturers and vendors

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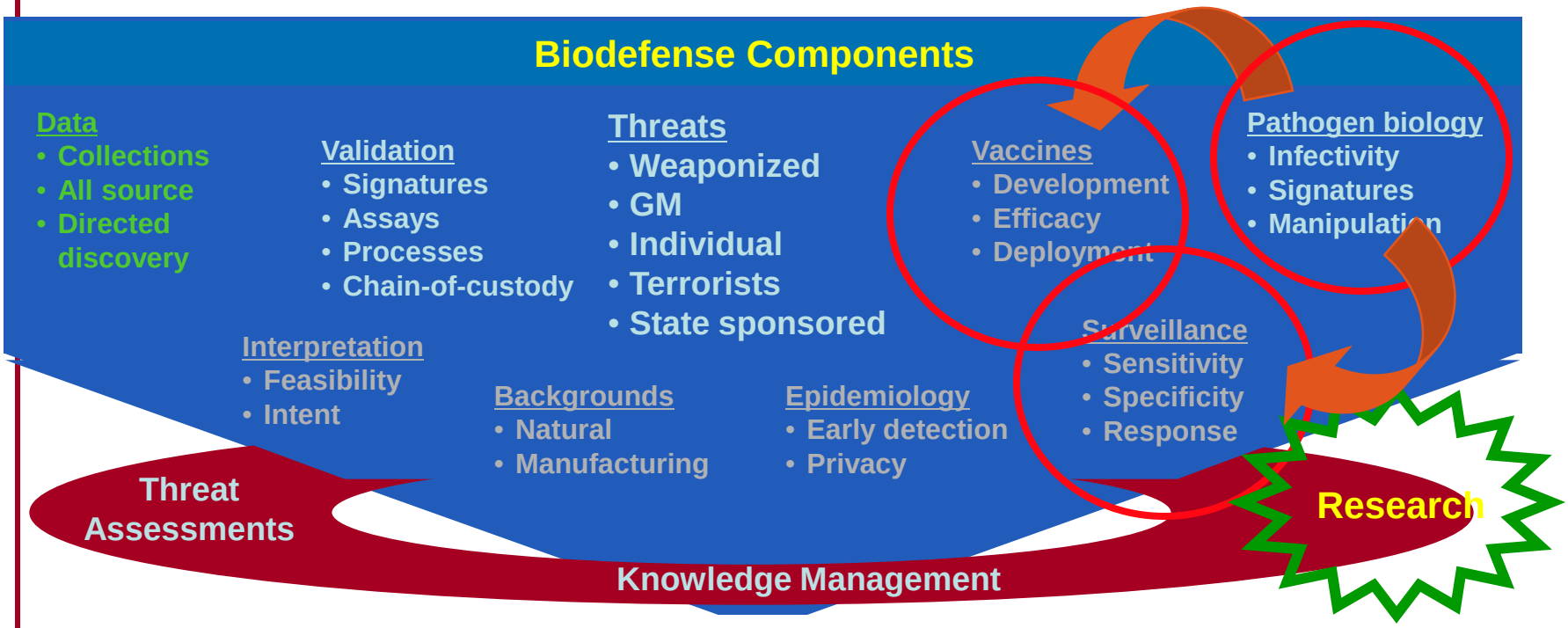
# Biological Systems

## Biodefense research is a multifaceted problem that requires *integrating* many *disparate* components



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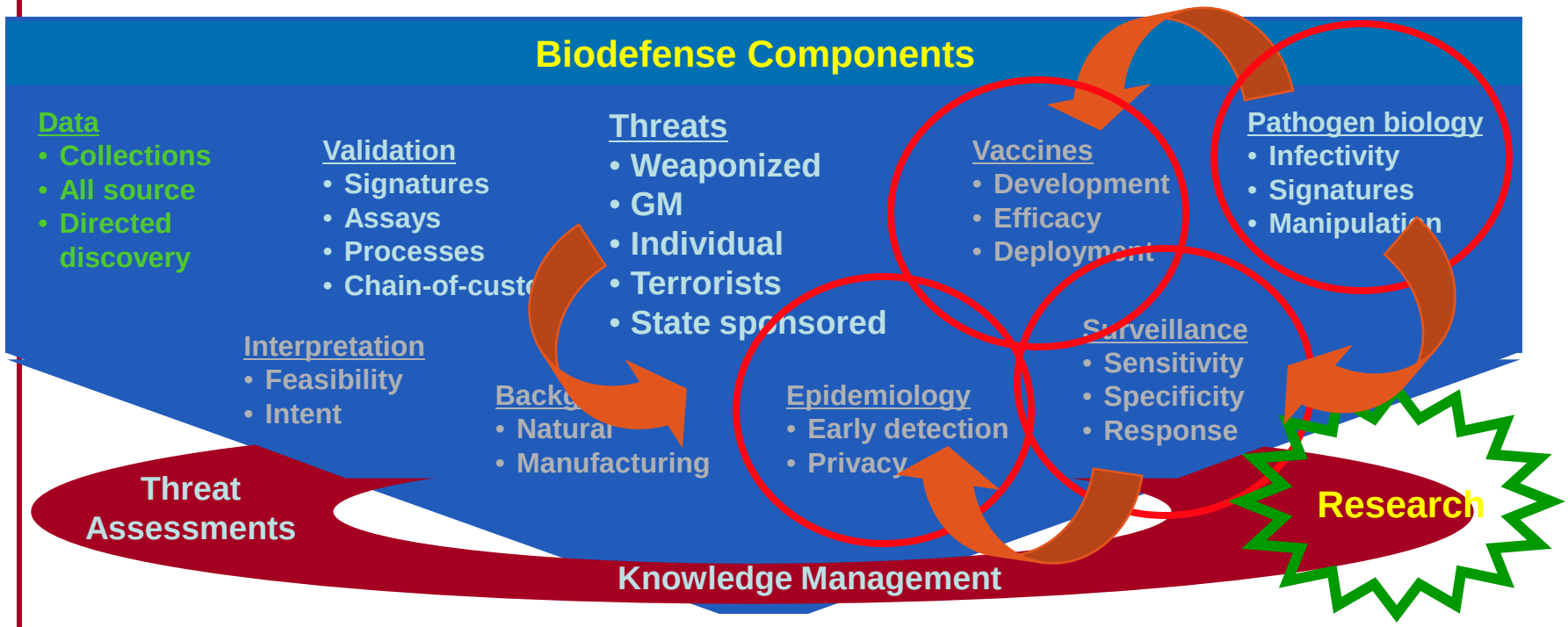

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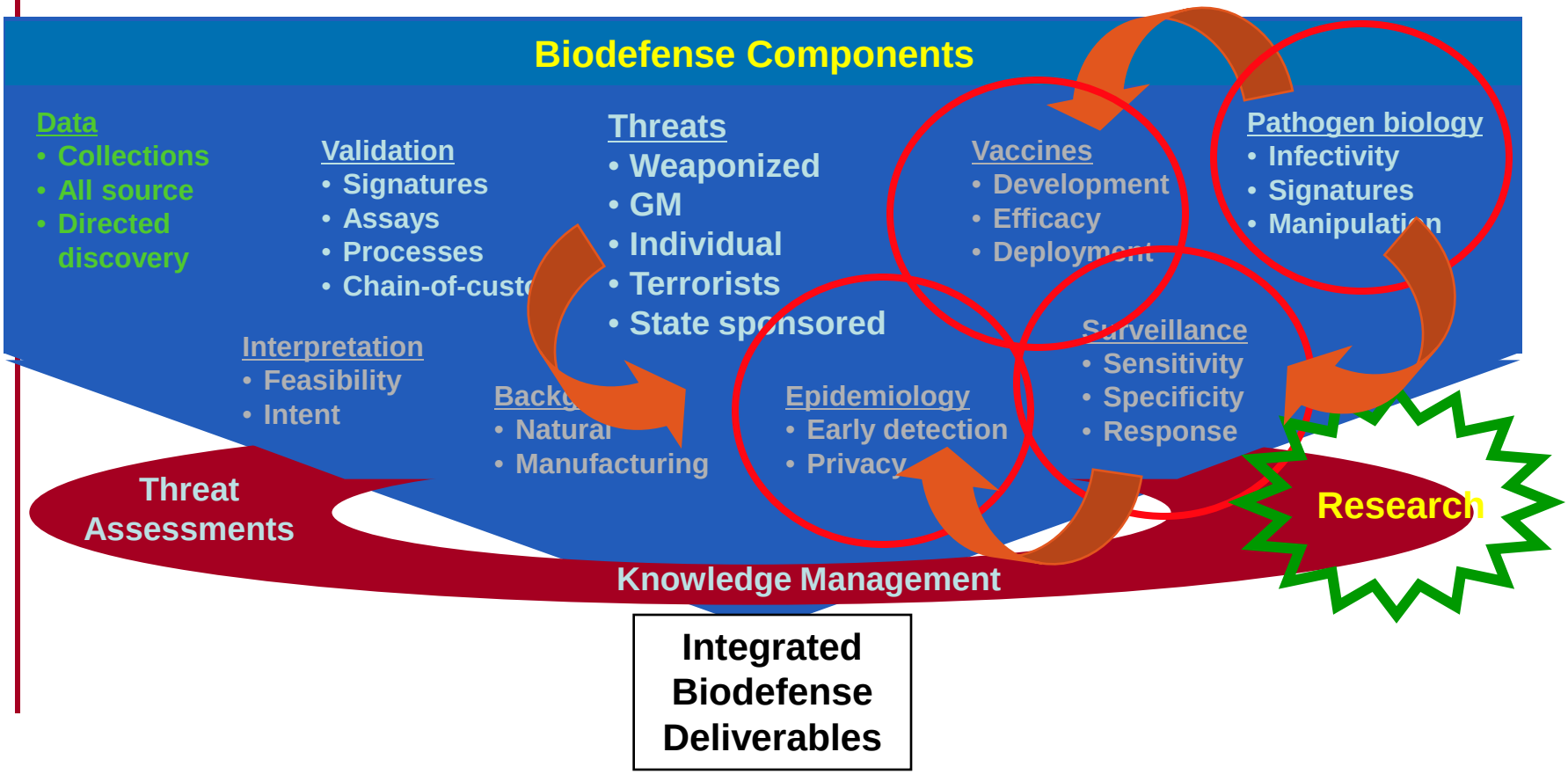


**Scalable knowledge management *processing* & linking all components into an *integrated* national *animal* biodefense system.**

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Homeland Security