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From Bioenergy to Biodefense:
Bioscience Research at Sandia National Laboratories

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Sandia National Laboratories is a multimission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC., a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.

We have a history of developing μbioanalytical systems for national security applications

1990s

Chemical and Biological Detection

DOE CBNP Program

SNL LDRD

Chemical agent and Liquid-phase Explosives Detector

DOE CBNP

Liquid-phase Biotoxin Detector

2000s

Sample Prep, Detection and Diagnostics
Emphasis on Protein Assays

DHS, DoD, NIH

NIAID RapiDx Toxin Diagnostics

NIDCR Human Oral Microbiome

DHS CBNP

DHS BioBriefcase

Waters CRADA

Tenix CRADA

DoD TTP, DTO.50

2010s

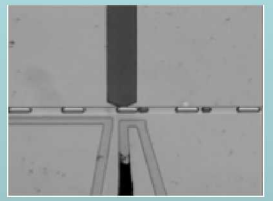
Sample Prep and Analysis, Detection and Diagnostics, Multi-omic, Multi-plexed

DHS, DoD, NIH, DOE

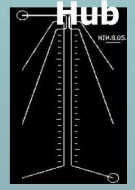
SpinDx

BaDx

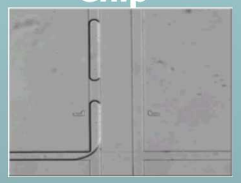
DMF Hub



Enzyme Chip



Sugar CE



SynBio Chip

Biosciences at Sandia National Laboratories

Sandia's Bioscience Research Foundation was created in response to emerging national needs in ***energy security*** (by reducing U.S. dependence on foreign oil) and ***homeland security*** (protecting our nation from biological threats).

We believe that a ***deep understanding of biological systems*** and the ability to predict their behaviors will play key roles in energy security, the protection of our environment, and our nation's ability to defend against biothreats.

Consequently, our biological scientists are conducting research in two strategic thrust areas—***biomass conversion*** and ***biodefense***—so that we can provide biological solutions to important problems in energy and homeland security.

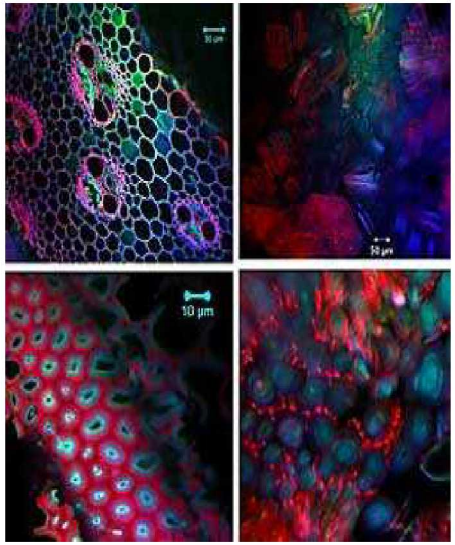
Cellulosic Biomass

Lignocellulosic biomass, or plant biomass, is an attractive source for biofuels. The public and researchers alike are intrigued by the idea of using *readily available "plant waste*," such as corn stover, sawdust, or other agricultural residue, to create carbon-neutral transportation fuels.

Creating alternative fuels from cellulosic biomass is *no easy task*. Biomass contains a large amount of energy but it is trapped in polysaccharides. Researchers must first find a way to *break down the polysaccharides into simple sugars*. These sugars must then be fermented and converted into the desired biofuels.

Main areas:

- Feedstocks;
- Deconstruction;
- Fuels Synthesis;
- Technologies



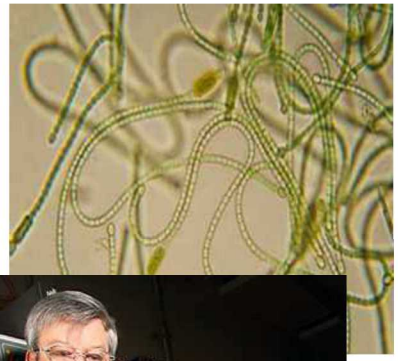
Autofluorescence to observe the breakdown of a switchgrass stem that was treated with ionic liquid. (See Singh et al., 2009, Biotechnology and Bioengineering vol. 104, pp 68–75.)

The Joint BioEnergy Institute (JBEI) Emeryville California.

Algal Biomass

Biofuels created from algae feedstock have tremendous potential to reduce U.S. dependence on imported oil. Algal biofuels could conceivably be produced in enough volume and represent the ultimate in *ecological sustainability* since *algae consumes CO_2 and can grow in brackish, or impaired, water*.

- repurposing currently unusable water supplies
- mitigate global-warming effects by capturing excess CO_2
- help solve the impending global energy crisis



Recent Algal Biofuels Projects at Sandia:

- Realizing algae's potential as a biomass feedstock
  - cultivating new algae strains
- Developing enhanced extraction and conversion technologies
  - decreasing the cost of producing fuels from algae
- Enhancing large-scale cultivation of algae biomass
  - understanding the challenges associated with large scale cultivation including differences in insolation (light levels), temperature, and predation/infection

Biodefense and Emerging Infectious Disease

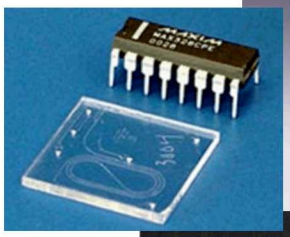
Biological weapons and emerging infectious diseases pose ***formidable and growing threats*** to our national security since pathogen-caused disease outbreaks could result in a devastatingly large number of casualties.

To improve our nation's ability to ***anticipate and defend against biological threats***, our multidisciplinary research team is applying Sandia's traditional strengths in engineering and technology development to achieve the following goals:

- Discover and verify molecular and cellular disease signatures for pathogens that pose public health and biodefense concerns
- Develop assays, novel materials, and platforms to detect and diagnose traditional and unknown pathogens, as well as to discover novel therapeutic targets.
- Develop bioinformatics approaches for data integration, management, and knowledge extraction to inform effective public health responses to disease outbreaks

Pocket-sized anthrax detector aids global agriculture

µChemLab™



Lab-on-a-Chip Technology for Point-of-Need detection and diagnostics

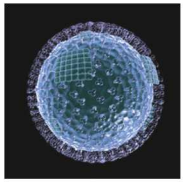
Novel Pathogens Arise Through Different Routes

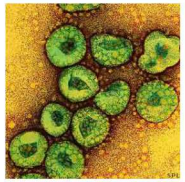
Naturally occurring: "Emerging"

- New variant of known pathogen (influenza)
- Pathogenic variant of otherwise non-pathogenic agent (SARS)
- Pathogen previously not known to science (Legionella)

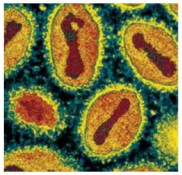
Synthetic: "Enhanced" & "Advanced"

- Pathogen modified or selected for greater virulence or to defeat countermeasures (antibiotic-resistant anthrax)
- Non-pathogenic agent modified for pathogenicity
- Pathogen synthesized *de novo*


Influenza


SARS


Legionella


Poxvirus


Anthrax

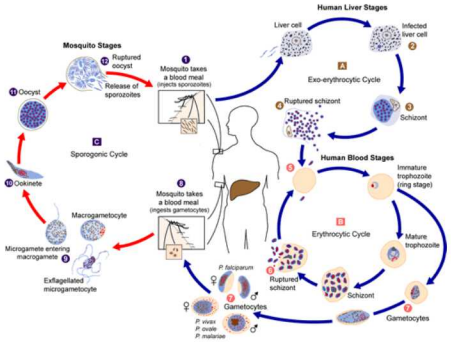
Novel pathogens increasingly threaten public health & biodefense

Natural emergence promoted by:

- Human encroachment on wildlife habitat
- Urbanization of agriculture
- Wildlife relocation due to climate change

Synthesis enabled by:

- Growing knowledge of pathogen & host biology
- Better & cheaper tools for agent engineering
- Global dispersion of biological materials, technology, knowledge & expertise

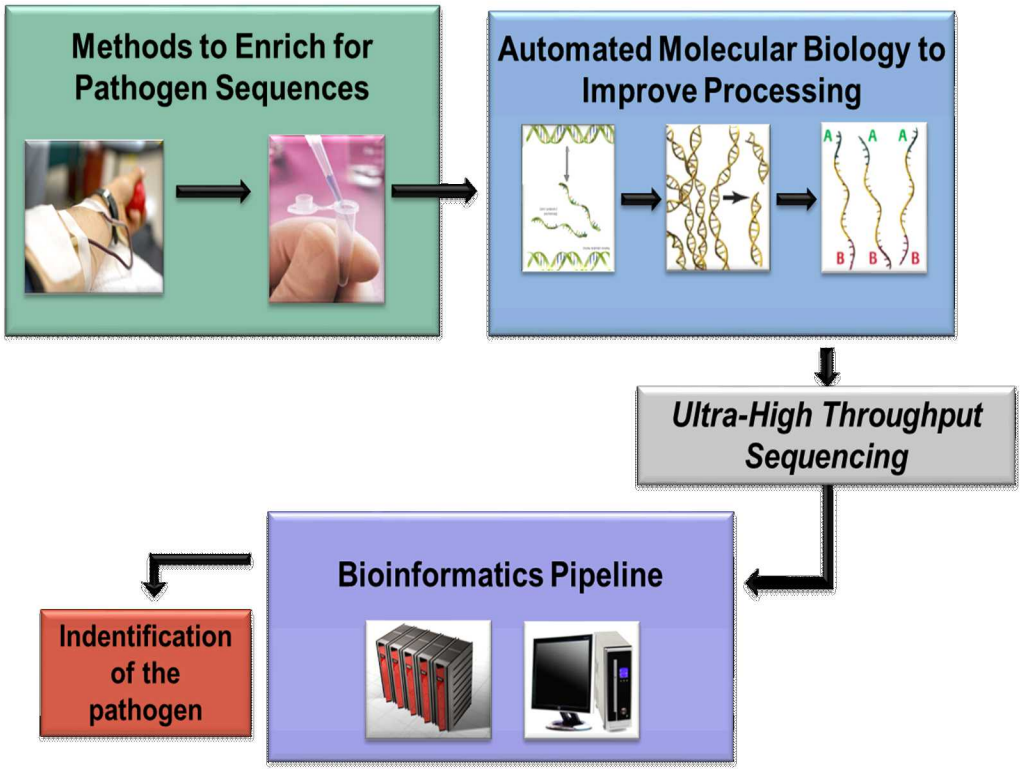


Outbreak spread enhanced by:

- Growing population density
- International trade & travel

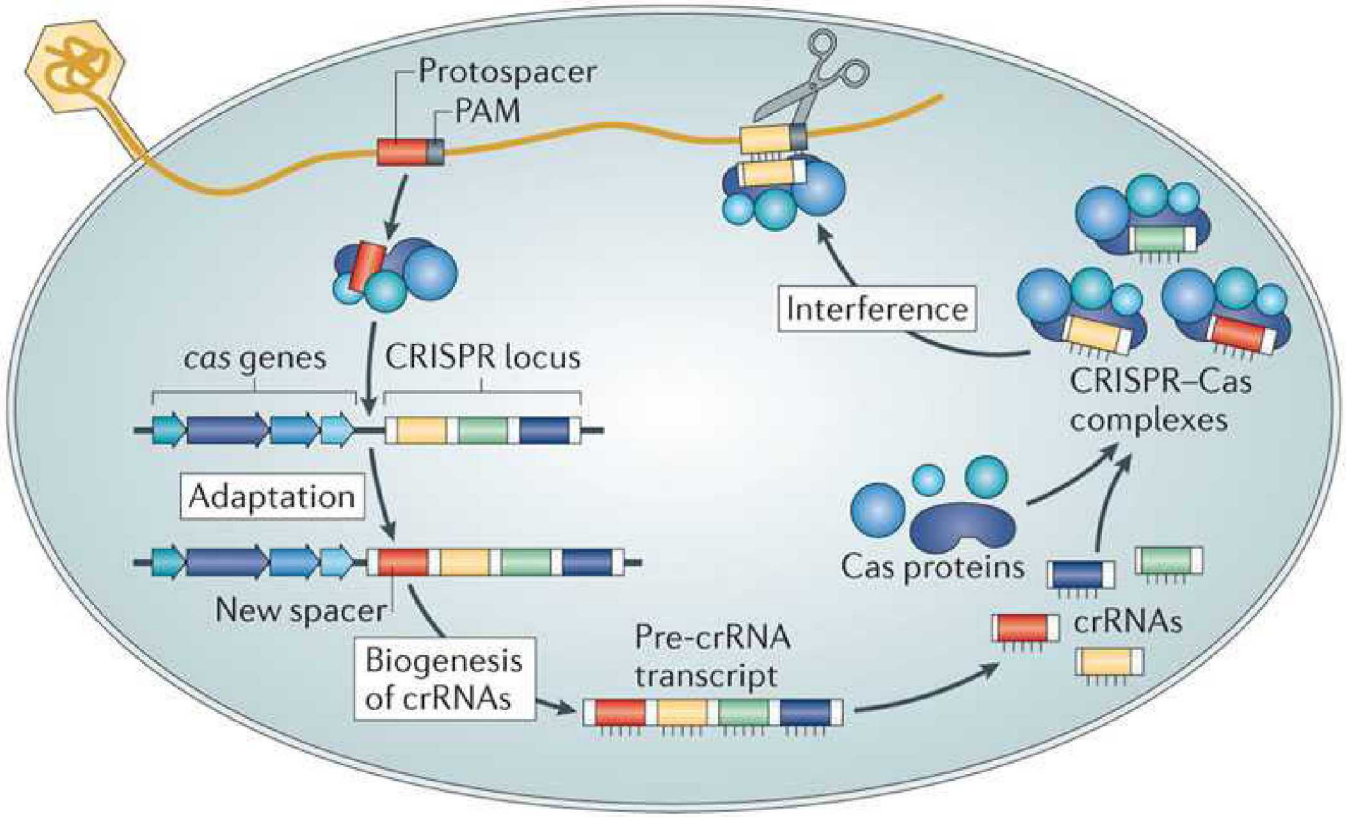
Biodefense Research: Rapid Threat Organism Recognition

Since the ***human genome project***, there have been dramatic ***advances in sequencing speed*** and cost. However, host DNA tends to overwhelm the sequencing capacity and "brute-force" sequencing yields few sequences from the pathogen. In order to speed the search for potentially harmful unknown pathogens, RapTOR developed methods to ***suppress host DNA***, ***automate the sequencing library preparation*** and create a ***bioinformatics pipeline*** to interpret the sequencing data.



CRISPR & Gene-editing: background and potential applications

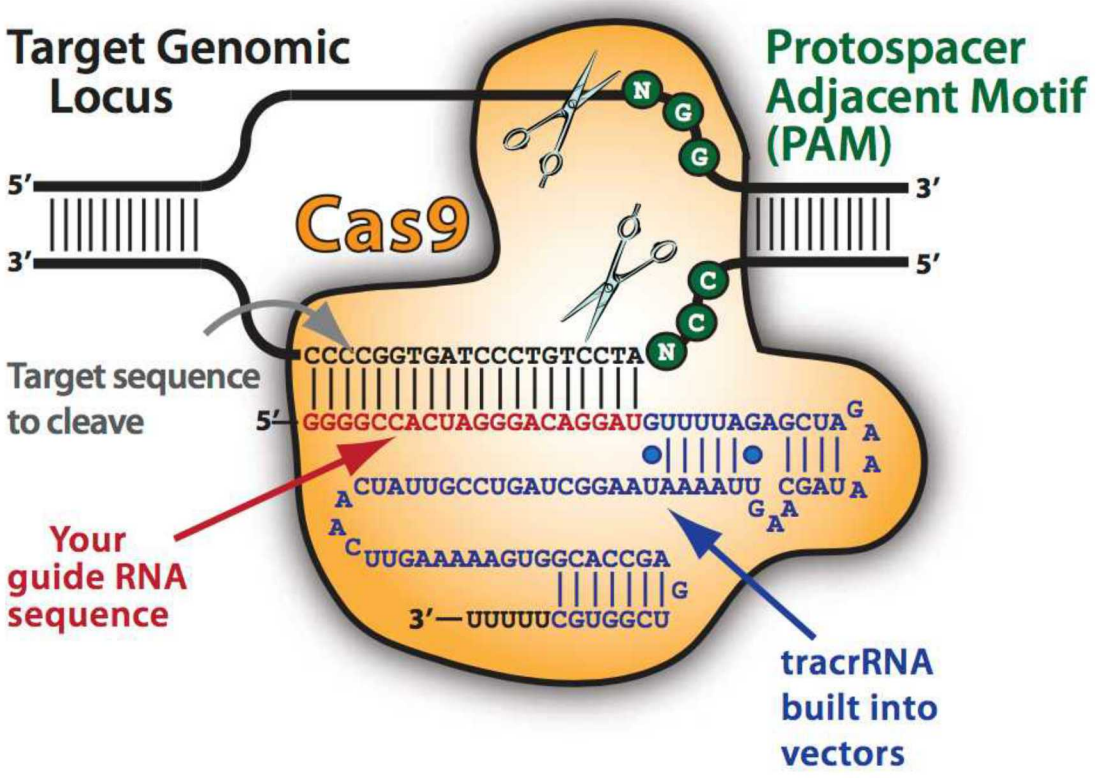
The CRISPR/Cas adaptive immune system



CRISPR: Clustered regularly interspaced short palindromic repeats
Cas: CRISPR-associated systems
Protospacer: DNA sequence targeted by CRISPR/Cas systems
PAM: Protospacer adjacent motif

Image from *Nature Rev. Microbio.* 11:675(2013)

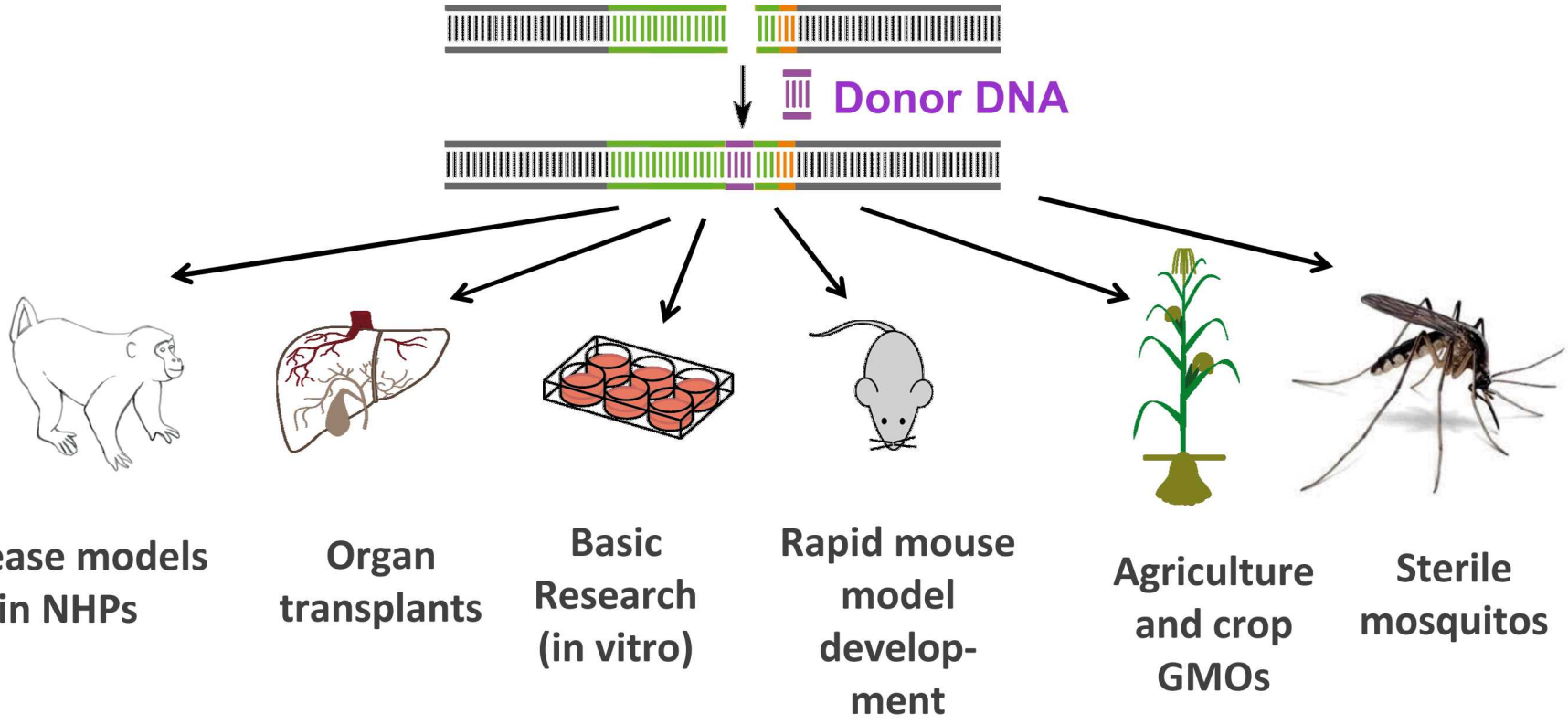
CRISPR technology: A two component system



Cas9: RNA-guided endonuclease
gRNA: Synthetic fusion of crRNA and tracrRNA

Image from systembio.com

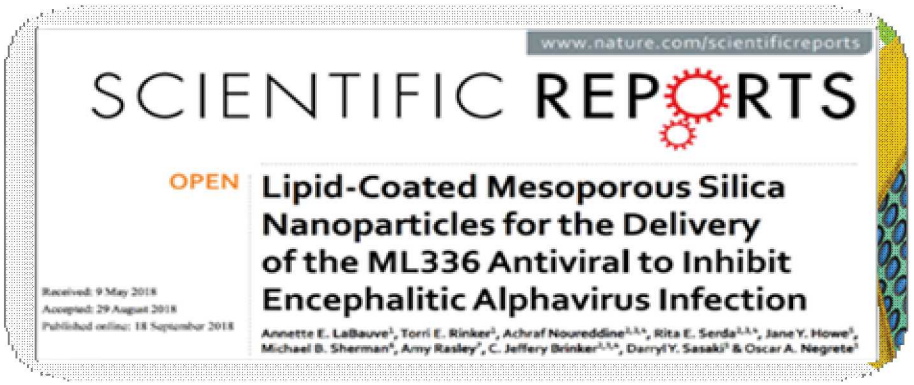
Genome-editing technology for Biomedical Applications

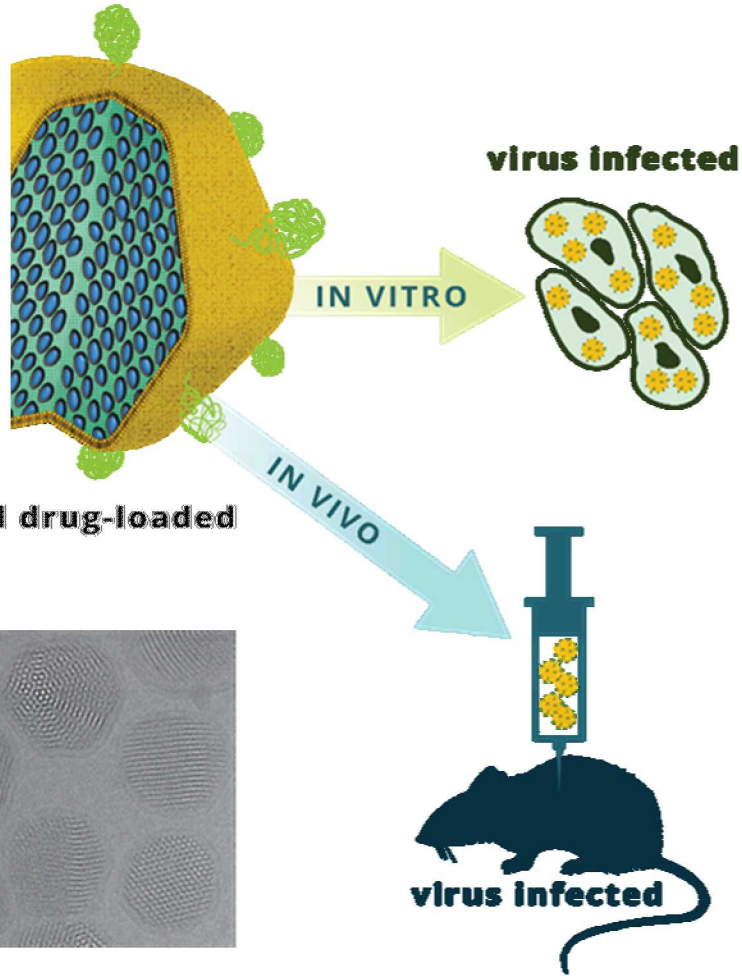


CRISPR activities at Sandia

- Identified Cas9 homolog – rCas9 –that targets RNA in sequence-specific manner – allows direct targeting and editing of viral RNA genomes and host transcripts
- High-throughput CRISPR screens to identify therapeutic targets for pathogens of biodefense concern
- Cell-based therapeutics enabled by CRISPR
- Nanoparticle vehicles for targeted delivery of CRISPR therapeutics
- Detection tools for Cas9 *activity*
- Tools for controlling Cas9 activity

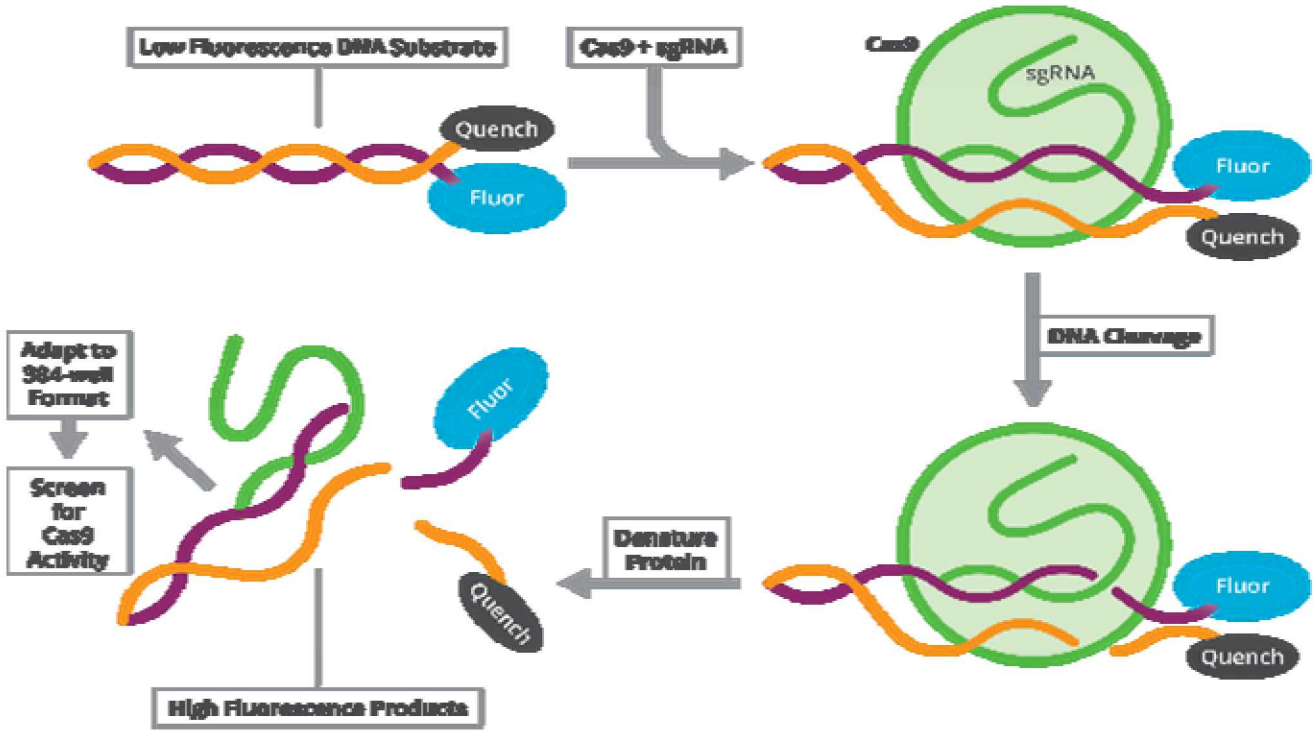
Targeting Gene Editing for Therapeutics





NanoCRISPR Grand Challenge

Assaying for Gene Editing Activity



analytical chemistry

Article

Cite This: *Anal. Chem.* 2018, 90, 6913−6921

pubs.acs.org/ac

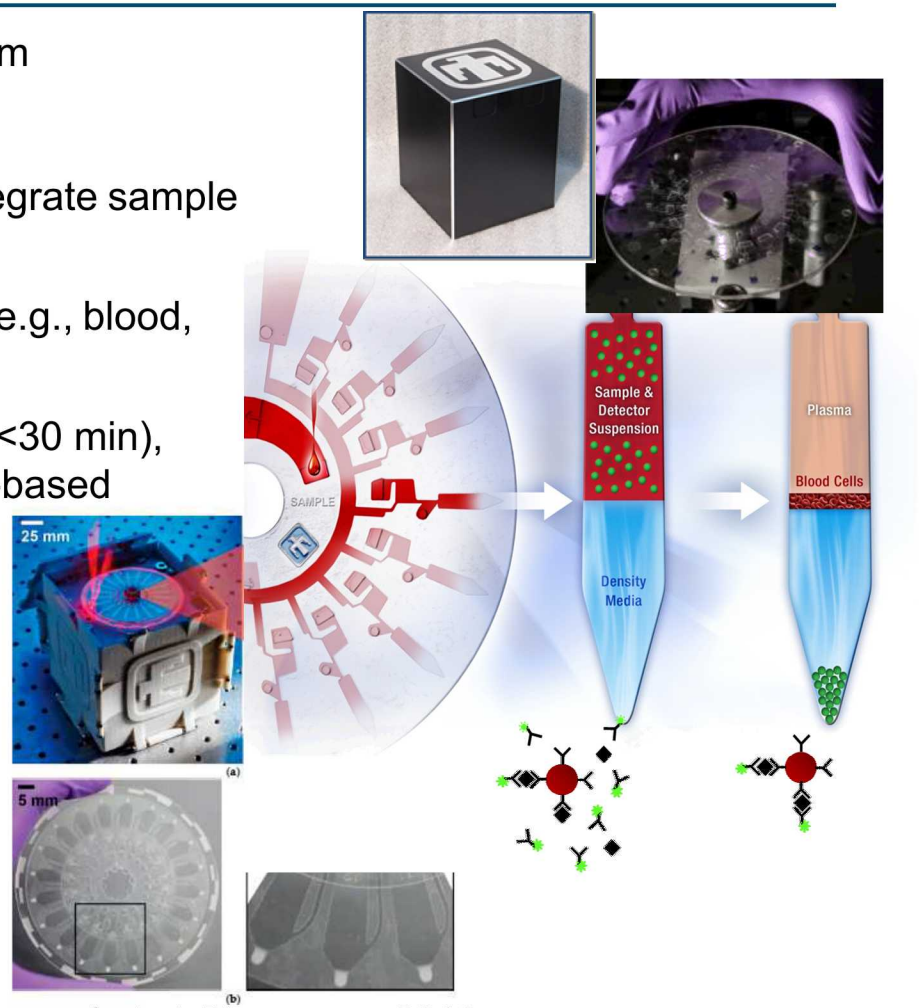
Versatile High-Throughput Fluorescence Assay for Monitoring Cas9 Activity

Kyle J. Seamon, Yooli K. Light, Edwin A. Saada, Joseph S. Schoeniger, and Brooke Harmon*

SpinDx™

SpinDx platform overview

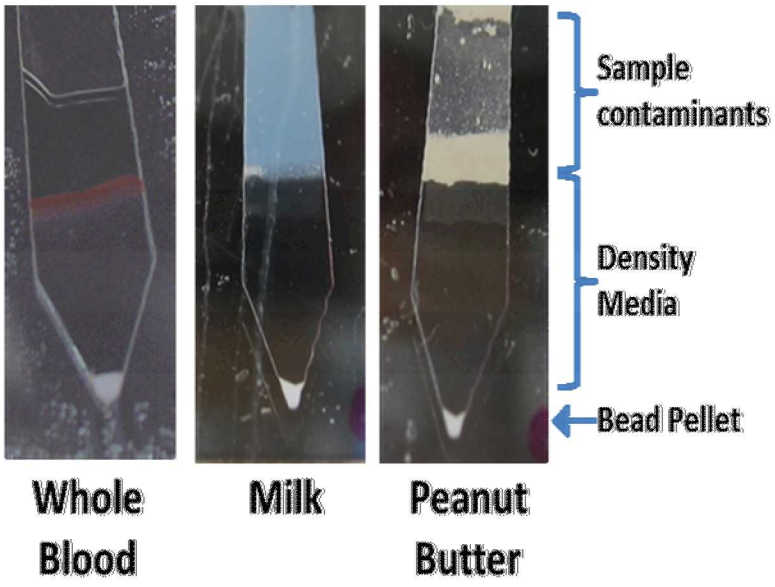
- Multiplexed, centrifugal microfluidic platform
- Detection of proteins and nucleic acids
- Patented sedimentation-based assays integrate sample prep into disposable plastic disk
- Compatible with clinical sample matrices (e.g., blood, serum, saliva, urine, swab eluate, stool)
- Typically more sensitive (<pg/mL), faster (<30 min), easier to use (single step) than laboratory-based methods
- New assays can be adapted to the platform readily with no change to overall platform architecture
- Commodity plastics, amenable to high volume manufacturing techniques (e.g., injection molding), largely COTS components – potential for affordable scale up

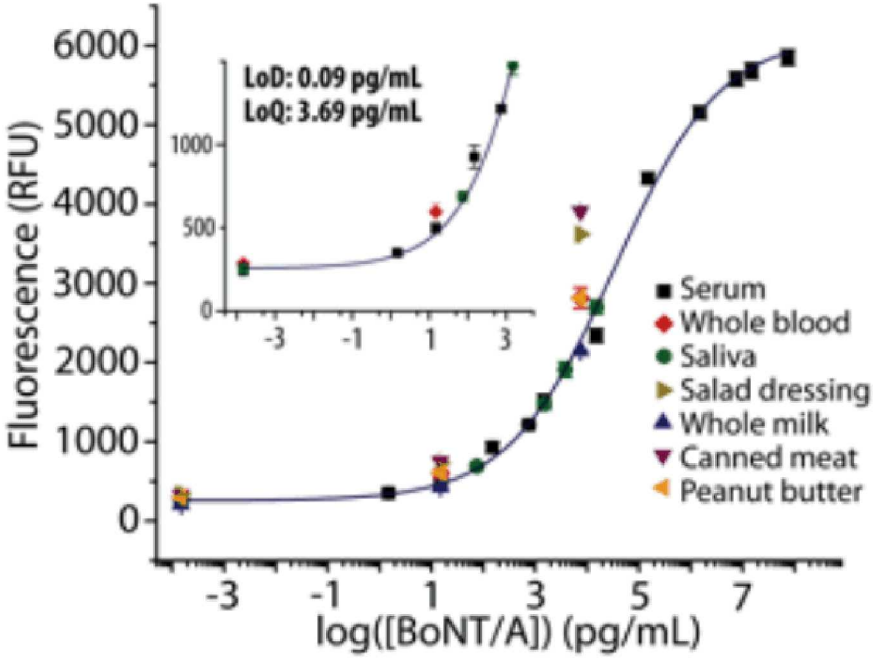


Phaneuf *et al*, Biosensors 2016

Key Advantage: Essentially No Sample Prep

- Density-based sedimentation
  - analysis of complex sample matrices
  - sample matrix is completely separated from beads before detection)
- Compatible with wide variety of sample types
  - Clinical (whole blood, serum, saliva)
  - environmental
  - food samples

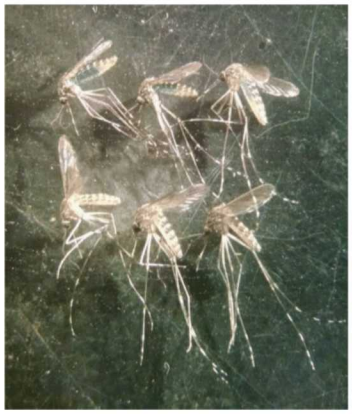




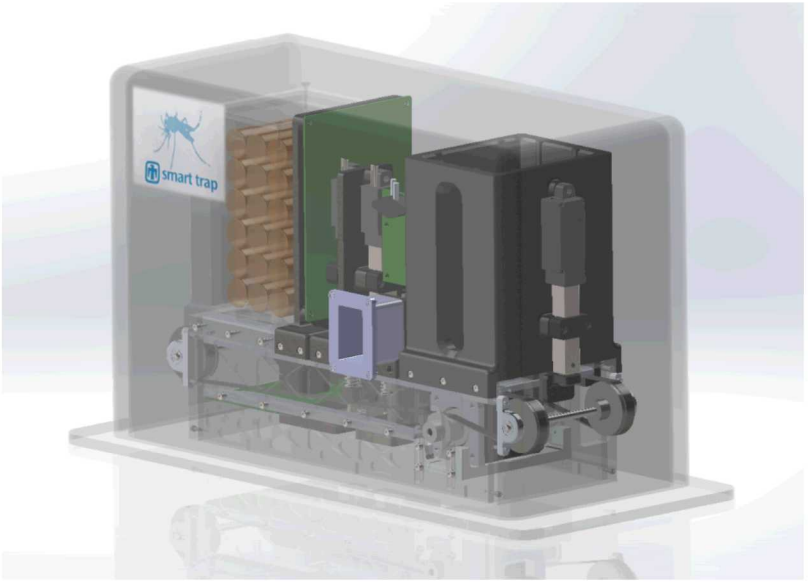
Smart Trap and Smart LAMP

SMART Trap System

Sugar baits – "the Bait Cave"



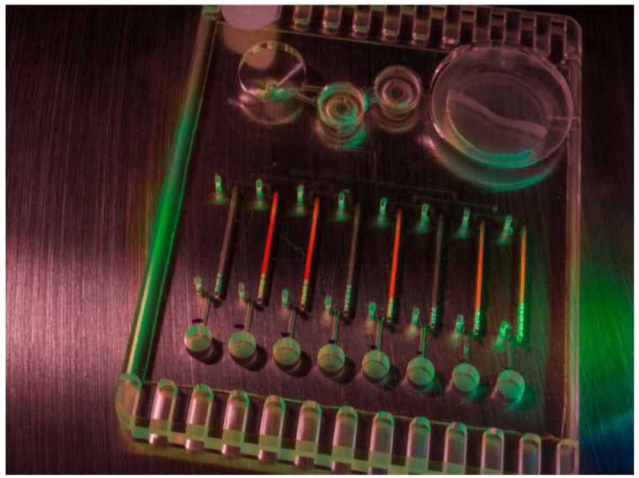
Scented, polymer-modified spun sugar as a stable attractive bait for mosquitoes

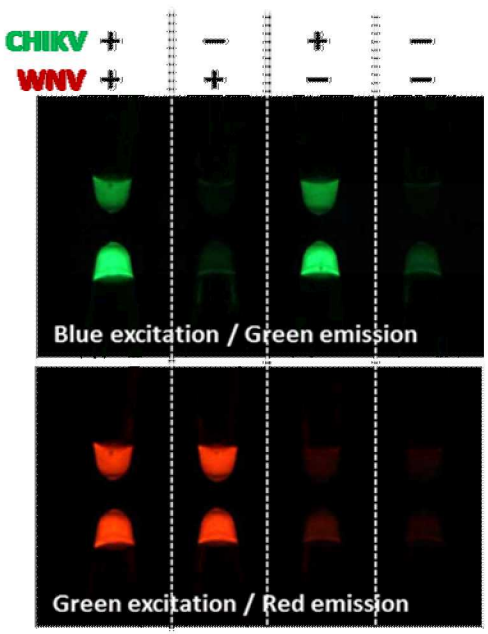


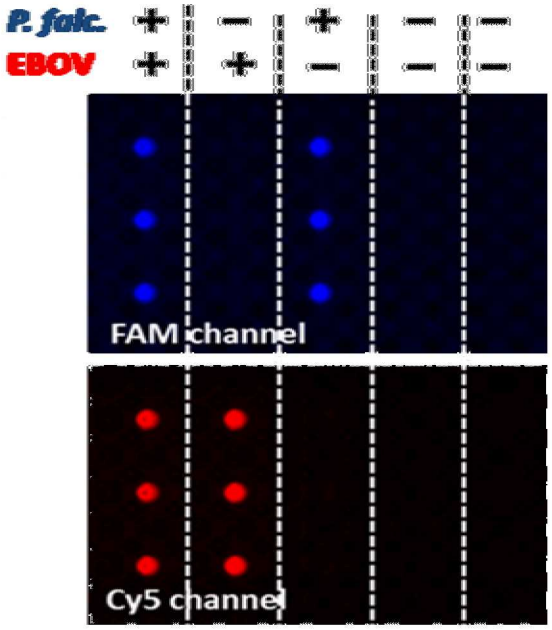
The Smart Trap automates sugar baiting and molecular assay for viral RNA

Viral Assays in the SMART Trap System

- Novel detection chemistry “QUASR” gives bright and distinctive fluorescence endpoint signal, with multiplexing capability and reduced false positives compared to “traditional” LAMP
- RT-LAMP quantitative precision and sensitivity is usually less than qRT-PCR

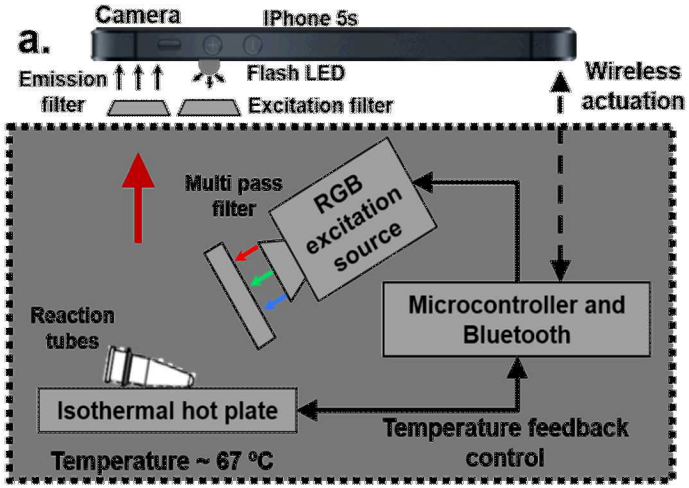


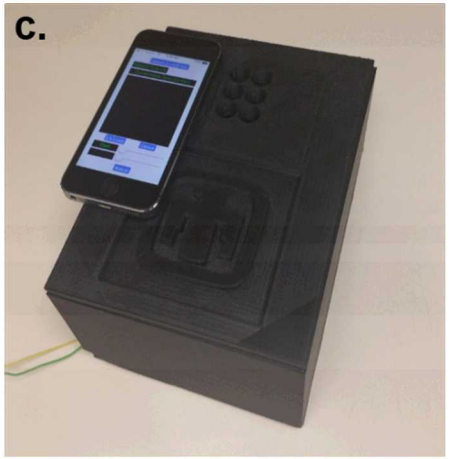




Smart Phone platform overview

- Handheld box contains heater, optics, and Bluetooth enabled microcontroller
- Compatible with iPhone and Android phones (Lamp To Go app)
- Smart phone app “front end” controls heater, timing, and fluorescence image acquisition
- Heater accepts a variety of formats: PCR tubes, microwells, or planar chips
- Hardware costs: about $50 with plastic filters; $500 with high quality coated glass filters, plus phone
  - Compare to $18-20k for portable isothermal fluorimeter such as Genie III.

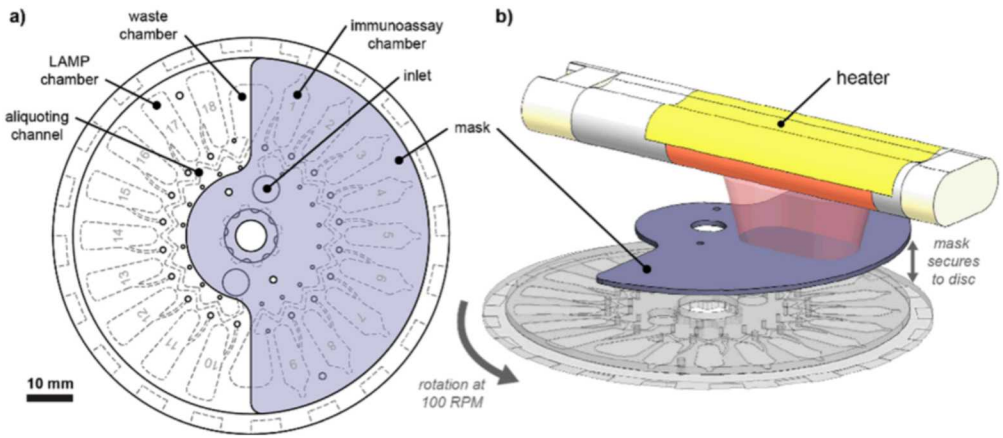




Priye *et al*, *Scientific Reports* (2017), **7**, Article Number: 44778

Next Gen SpinDx Functionality: Integrated LAMP and immunoassay

The ability of our technology to detect multiple analyte types in parallel at the point-of-care can serve a variety of needs, from routine patient care to outbreak triage, in a variety of settings to reduce disease impact and expedite effective treatment.



Phaneuf, C. et al., Biosensors and Bioelectronics 120 (2018) 93–101

Questions?