Approaches to biodefense challenges

Stevan Jovanovich, Ph.D.
Founder and Chief Technology Officer
IntegenX Overview

Founded in 2003, 129 employees, based in Pleasanton, Northern California

Growth-stage commercial life science tools company

• RapidHIT™ 200 System for rapid Human DNA Identification in less than 90 minutes at point of action
• Apollo 324™ Next-Generation Sequencing (NGS) library preparation system

Products covered by >500 proprietary and/or licensed patents

~$100M in private equity

$24M in grants and contracts: DARPA, DTRA, DHS, NIH

This is expensive work requiring access to Intellectual Property
Basic Biowatch needs

Rapid, accurate detection and identification of pathogens and toxins
High sensitivity and specificity with low false positives
Scalable, automated, and capable of widespread deployment
Low capital and operating costs
Rapidly re-configurable to detect new, emerging, or engineered threats
Autonomous field deployed systems complemented by lab-based systems with greater functionality
Integration of complete workflow into autonomous systems
- Reliable
- Sensitive
- Low false alarm rates: Example One, multi-dimensional assays
- Example Two, integration

Detection of known agents
- DNA/RNA assays
  - Real-time PCR
  - Isothermal assays
- Immunoassays
- Multi-dimensional assays: Example One

Detection of unknown agents—emerging agents and bioengineered
- Detection of cloning vectors, resistance genes, and toxin gene targets
- Nucleic acid probes
- Next generation sequencing: Example Three
Integration challenges

The hardest part

• Integrating the workflow: workflow integration and matching ‘impedances’ is key
• Retain maximal sensitivity by processing as much of the sample as possible
• Must match volumes and throughputs

Removal of background material

“Other threat considerations, such as means of agent dissemination, masking agents or interferents, are outside the scope of envisioned Gen-3 ADS operational capabilities.”
• Complex matrices present both chemical and physical challenges
• Confounds analysis
• Amount of biological material increases demands on system (particularly NGS)
• Solutions: filtration, immunomagnetic separations (IMS), size/charge methods

• Extremely low levels of target cells/DNA present hard challenges
  • DNA, cells, or toxins may stick to surfaces
  • Low signal-to-noise makes certainty difficult
Workflow integration and matching ‘impedances’ is key

Sample

1000 L to 1 mL

Aerosols
Swabs
DNA libraries
RNA
Cells
Tissue
Buccal cells
Blood
Forensics
samples
Food
Animal models
Biopsies

Filtration
IMS
Affinity capture
Cell lysis
DNA extraction and purification
Plasmid prep
Chromatography
Protein digestion
Laser capture

Immunohistochemistry
WGA
Enzymatic reactions
Cycle sequencing
RNA labeling
Protein labeling
Cell tagging

qPCR
Capillary array electrophoresis
Mass spec
Fluorescent readout
Flow cytometry
Microarray
Imager/Scanner
Single molecule

Genotype
DNA sequence
Diagnostics
Gene expression
Proteomics
Cell Biology
Workflow integration

Sample Collection → Extraction, Purification, Concentration → Quality Assessment → Molecular Biology (PCR, Amplify, Dilute) → Quantification → Detection → Database Query

- Sanger Sequencing Apollo 100/100xl
- Next Gen Sequencing Library Preparation: Apollo 324
- Integrated Sample-to-Sequence System
  Fully integrated NGS system for biodefense and molecular diagnostics
  -> RapidHIT™ 200 System
  Sample-to-answer rapid identification system

Apollo 100/100xl → Apollo 324

RapidHIT™ 200 System

Integrated Sample-to-Sequence System

integenX Proprietary | 7/1/2013
Example One: Integration, sensitivity, multidimensional assays

NanoBioSentinel

Sample Capture and Purification Module

- Immuno-capture
- Lysis
- Nucleic Acid Purification

Toxins

Microchip-based sample processing

NanoBioProcessor Module

- \( \mu \)RT-PCR
- \( \mu \)CAE

Designed as an automated monitoring system for air-borne pathogens and toxins

RFP requested a sample preparation system for an unnamed detector

Danger!
The problem: sampling can result in significant losses

Approaches

Solid phase extraction on surfaces or membranes

Paramagnetic beads for nucleic acid and immunoassays

Integrated macro-to-micro sample volume transition for maximal sample detection efficiency. Collapse milliliters to microliters or smaller without sample loss\(^1\)

Magnetic beads can provide a ‘handle’ for precise manipulation of samples for process integration

IMS beads provide first dimension of specificity

Normalization of DNA extraction

Multiplexed assays are essential to prevent losses from sample splitting
Integrated multi-dimensional detection

Orthogonal analysis methods will decrease false alarm rates

Reagents

qPCR

CAE separation

IMS

Bead Input

Weir

Pump

Capture: IMS

Screen: qPCR

Confirm: Capillary array electrophoresis separation

Report

qPCR assay is excited by 488 nm laser. Real-Time PCR Reaction is monitored by an increase in FAM fluorescence

Capillary Separation of PCR products is monitored by TAMRA fluorescence
Overall process efficiencies and level of detection are dependent on multiple factors

- Bacteria Input Concentration
- Antibody Binding Avidity
- Bacteria Type/Quality
- qPCR primer/probe sensitivity
- Output sample volume
- Extremely Low Levels of Target
- Lysis Efficiencies
- Background Type and Sample Variation

*Front-end IMS capture efficiency is critical to overall yields; however the most consistent limiting step is nucleic acid purification at very low DNA levels.*
Integration example: RapidHIT™ 200 System

Usually 1-2 day process (a minimum of ~8 hours)

- Swabs
- Blood
- Semen
- Etc.

Sample in, profile out
Results in <90 minutes
Minimal training required
Point-of-action solution
Produces CODIS data and can query databases to give answers

Variable Number Tandem Repeats (VNTR) are found in bacterial and can be used to subtype microbes
Integration of sample preparation

**Cartridge**

- **Sample**: 4 Samples
- **Control**: 1 Sample, 1 Allelic Ladder, 1 Positive Control, 1 Negative Control

**Sample**

- **Process**
  - Lysis
  - Bead purification
  - 16-plex PCR amplification
  - Dilution in size standard
  - Capillary array electrophoresis
  - Answer

**EEPROM**

**Frame**

**Lysis chamber**

**Wash solutions**

**Beads**

**Lysis buffer/waste**

**Size standard**

**PCR premix**

**PCR reaction**

Patent pending
Capillary array electrophoresis of Allelic Ladder

16-plex PCR, all possible alleles. VNTR analysis would give similar profiles
RapidHIT 200 integrates automated processing of many sample types from raw sample input to database inquiry and can be run by anyone

- The sample is collapsed onto beads which are used to purify the DNA and to position the DNA for processing, helping integrate the workflow
- The process goes from milliliters of sample to microliters of lysate to nanoliters of beads to microliters

The discrimination power is $10^{17}$: multiple targets help increase discrimination

VNTR assays should directly drop into the RapidHIT system
Beyond current threats

Advent of genetic engineering and the capabilities created by synthetic biology presents a real danger of future threats having different signatures than expected.

Detection modalities must be enhanced

Next Generation Sequencing is a leading approach

- Targeted
  - Template capture
  - Amplicon sequencing
- Whole genome sequencing
Next Generation Sequencing Workflow

**NGS workflow to purify DNA, prepare and amplify libraries, and analyze on sequencers**

Sample in → **Sample Prep** → **Library Prep** → **PCR** → **PCR Clean-up** → Sequencing

- Sample Prep: Extracts, purifies, and fragments DNA from sample
- Library Prep: Optimizes fragments for specific sequencers
- PCR: Amplifies DNA fragments
- PCR Clean-up: Removes extraneous PCR materials
- Sequencing: MiSeq, HiSeq, PGM, 454, PacBio, etc.

**End repair** → **Bead** → **A-Tail** → **Bead** → **Adaptor Ligation** → **Bead size selection**
Walk-Away, User-Friendly Library Automation

70 min automated library preparation
1-48 samples

Intuitive user interface with step-by-step instructions

Robotic arm with eight-nozzle pipette head and built-in magnetic particle separation

Two 96-well Peltier temperature control units

Reagent plate, racks for disposable tips and waste bin

Optimized, simple-to-use reagent kits for DNA, RNA and ChIP-Seq
The ISS System was designed:

- As an automated sample-to-answer NGS platform to detect emerging and genetically engineered biothreats
- To produce sample-to-sequence data in a few hours by a non-scientist
- Ultrahigh throughput DNA sequencing
- Ability to “look” deep into environmental samples down to 10 organisms per mL
- Screen for known pathogens and target sequences including toxin genes, drug resistance, and plasmids
- BLAST against known sequences and look for clusters to find evidence of bioengineering

IntegenX and partners demonstrated proof-of-concept of an integrated workflow process.
Integrated workflow and modules

**Samples**

- Bead-based lysis
- DNA purification
- WGA

**Integrated Consumables Cartridge**

**Sample Processing Module**

- Fractionation
  - End repair
  - BeadX™
  - A-Tail
  - BeadX™
  - Adaptor Ligation
  - BeadX™ size selection
  - Normalization

**Library Construction Module**

**Sequencing Module**

- Bridge amplification
- Sequence-by-synthesis

**Flow cell & cartridge**

**Integrated workflow and modules**
Integration: Sample Processing Module

- Accepts 0.3-1.5 mL samples
- Mechanical lysis
- Magnetic bead DNA purification
- Lysis integrated with DNA purification demonstrated.

Cartridge with integrated lysis device can effectively lyse and purify spores

![Diagram of sample processing module with labels for lysis, purification, WGA, fragmentation, library prep., cluster generation, and next gen. sequencing.](image-url)
Integrated Library Construction

Cartridge-based library preparation works well

- Lysis
- Purification
- WGA
- Fragmentation
- Library Prep.
- Cluster Generation
- Next Gen. Sequencing

- Reaction Compartment
- Waste
- Input/Output Chamber
- Reagents input

End repair
- Bead
- A-Tail
- Bead
- Adaptor Ligation
- Bead size selection

- % of Reads Mapped (Confident Mappings)
- % of Genome Covered (Confident Mappings)

Cartridge-Based Experiments
Manual Experiments (protocol matched to experiments)
Manual Experiments (standard protocol)
Integrated Next Gen Sequencing System Summary

A sample-to-answer fully integrated NGS system can provide unprecedented sensitivity and throughput for detection of engineered or emerging pathogens

IntegenX and collaborators developed the foundation for an Integrated Sample-to-Sequence System in a few cubic feet

- Processing units for cell/spore lysis and DNA purification
- NGS library preparation
- Integrated sample-to-answer next generation sequencing workflow
- The workflow was able to correctly sequence blind samples with as low as 8 bacteria/mL

With the right team, a first generation Biowatch sample-to-answer NGS system should be achievable in 2-3 years for ~$15m/effect
Summary and Recommendations

Build on a modular system based upon current technologies

• Set standards for fluidic interfaces
• Front end sample preparation is similar for many detections modalities

Defense in depth

• Environmental sampling with Biowatch
• Diagnostic capabilities at hospitals and clinics
• LRN
• Multidimensional assays
• NGS as it is integrated

Focus next gen systems on current and emerging threats

• Get first systems out for known threats
• Add suite of assays onto platform
• Bioengineered threats need to be addressed
• NGS system should include participation of system provider
Summary and Recommendations

Funding

- Target systems on the ‘doable’ not the possible— BAND: 2 cu ft., $25k….
- No ‘moonshots’ needed
- Short start times after long delays do not match well with industry planning and resource allocation
- Encourage consolidation of best performers into teams

Fund at levels that can support full system development

- Multiyear commitments improve predictability
- Gaps in funding can lead to team dismantlement
- Fund three systems through prototypes TRL 7 (System prototyping demonstration in an operational environment)