RapTOR: Rapid Threat Organism Recognition

Sandia National Laboratories

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LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Problem

RapTOR addresses a critical national security need: Detection and Characterization of Unknown Biological Threats

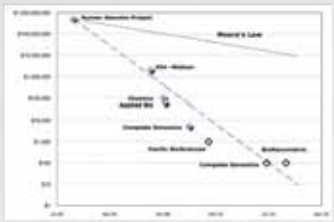
The nature of the unknown threat is varied and unpredictable:

Emerging pathogens
- SARS, WNV, Hantavirus
- Many emerging pathogens were not known to science prior to an outbreak

Enhanced pathogens
- Can be as simple as adding a single gene to a traditional agent (IL-4 added to mousepox or antibiotic resistance added to anthrax)

Advanced pathogens
- Can take the form of multiple gene additions to de novo synthesized agents
- The nature of these threats can not be predicted or known until the agent is deployed

Approach



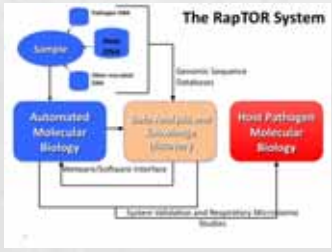
Sharp reductions in cost (and time) for sequencing DNA provide a powerful tool: UHTS

Host background can overwhelm pathogen signal in clinical samples:
>100,000 transcripts per hit on the pathogen genome

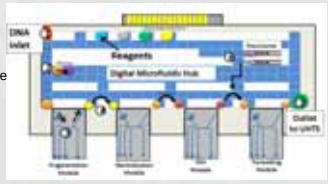
There are processes that can be employed to allow us to discard or ignore the host nucleic acids with varying levels of stringency according to the needs of the system
- Column extraction
- Differential degradation of high abundance NAs
- Suppression of amplification of host NAs
- Using control host DNA as a driver

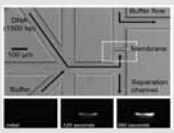
Suppression can reduce the background from the host and microbiome leaving a less complex and more informative derivative.

The **(RapTOR) Grand Challenge** will develop a novel automated molecular biology approach combined with data analysis tools and an informatics architecture to create a new capability based on Ultra High Throughput Sequencing (UHTS) to detect and characterize unknown bioagents rapidly enough for decision-makers to effectively respond to an attack or outbreak

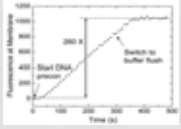


1. **Fragmentation**
   - dsDNA will be cut to appropriate length fragments
   - Oligonucleotide "handles" ligated onto the ends
2. **Normalization**
   - Preferential destruction of numerically abundant sequences, thus relatively increasing the rare sequences
3. **Subtractive suppressive hybridization (SSH)**
   - Subtraction of host or background host DNA through selective hybridization
4. **Formatting**
   - Prepare DNA for direct input into UHTS systems

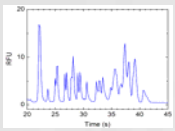




Images illustrating concentration of dye-labeled dsDNA at a nanoporous polyacrylamide membrane. Field can also be cycled to achieve mixing.

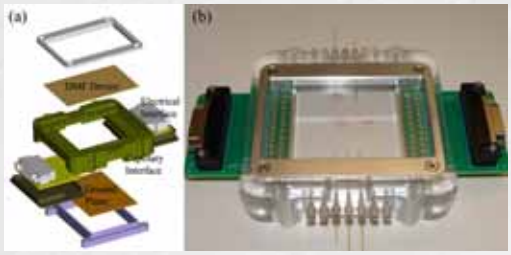


Significant concentration of DNA can be achieved in a matter of minutes, without apparent dropoff in concentration rate.

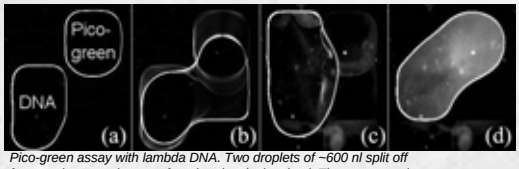


Chip electrophoretic separation of DNA ladder following membrane concentration

Results



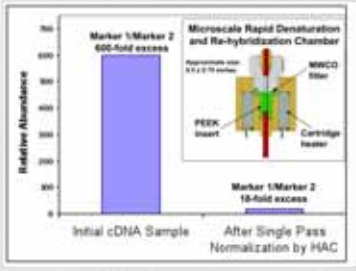
(a) Schematic and (b) photo of the automatic molecular biology platform (acrylic) with an ITO-based DMF device. Capillary tubes interface with the DMF device to transport sample to and from the surface-bound droplet. Printed circuit boards with spring-loaded pogo pins make electrical connections to the ground plane and DMF device.



Pico-green assay with lambda DNA. Two droplets of ~600 nl split off from each reservoirs were fused and actively mixed. The concentration of double-stranded DNA can be quantified by the fluorescence intensity as shown in (d). (a) and (b): Dark field only, (c) and (d):Dark field and fluorescence with a GFP filter set.

"Normalization" is a hybridization-based process resulting in the preferential destruction of numerically abundant sequences thus increasing the relative abundance of rare sequences.

RapTOR uses hydroxyapatite capillary-based chromatography (HAC) to achieve normalization. Shown is an example of HAC normalization of a simplified sample. Before normalization (left) ratio of background (marker 1) to target (marker 2) was 600 fold. After normalization (right) that ratio is reduced to 18 fold. Upper right is a schematic of the RapTOR normalization module.



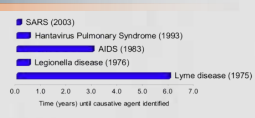
RapTOR Integrated Relational Database Management System and bioinformatics pipeline. Sequence reads will be processed, analyzed and stored in the interoperable, modular pipeline and relational database management system (RDMS). Derived results will be stored in the RapTOR Knowledge Base. Our own implementation of open-source tools will be used to build the RDMS and bioinformatics pipeline. Custom BioPerl scripts will be developed to integrate workflows and RDMS components.

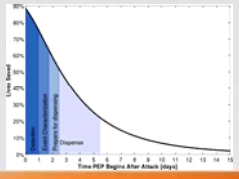
Significance

It is increasingly likely that our population will face devastating disease outbreaks caused by unknown pathogens introduced naturally, accidentally, or intentionally into the population

Methods to identify *unknown* pathogens are currently very slow (weeks to years) — these delays will be costly in terms of casualties (>100 K) and economic damage ($B)

We will close this S&T gap by ***developing, validating, and optimizing RapTOR: a unique approach to identify and characterize unknown pathogens present in clinical samples and provide actionable information to decision-makers***





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