Aptamer Selection Express: A Rapid Single-Step Selection of Double-stranded DNA Capture Elements (Briefing Charts)

Johnathan L. Kiel, Maomian Fan, Eric A. Holwitt, and Veronica K. Sorola
711th Human Performance Wing
Human Effectiveness Directorate
US Air Force Research Laboratory

Approved for Public Release: PA#09-406; 21 Aug 09
**Report Documentation Page**

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

<table>
<thead>
<tr>
<th>1. REPORT DATE</th>
<th>2. REPORT TYPE</th>
<th>3. DATES COVERED</th>
<th>4. TITLE AND SUBTITLE</th>
<th>5a. CONTRACT NUMBER</th>
<th>5b. GRANT NUMBER</th>
<th>5c. PROGRAM ELEMENT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 JUL 2009</td>
<td></td>
<td></td>
<td>Aptamer Selection Express: A Rapid Single-Step Selection of Double-stranded DNA Capture Elements (Briefing Charts)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. AUTHOR(S)</th>
<th>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</th>
<th>8. PERFORMING ORGANIZATION REPORT NUMBER</th>
<th>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</th>
<th>10. SPONSOR/MONITOR’S ACRONYM(S)</th>
<th>11. SPONSOR/MONITOR’S REPORT NUMBER(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conceptual Mind Works Inc., 9830 Colonnade Blvd. #377, San Antonio, TX, 78230</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved for public release; distribution unlimited.</td>
<td></td>
<td>Aptamer Selection Express: A Rapid Single-Step Selection of Double-stranded DNA Capture Elements (Briefing Charts)</td>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>a. REPORT</th>
<th>b. ABSTRACT</th>
<th>c. THIS PAGE</th>
<th>17. LIMITATION OF ABSTRACT</th>
<th>18. NUMBER OF PAGES</th>
<th>19a. NAME OF RESPONSIBLE PERSON</th>
</tr>
</thead>
<tbody>
<tr>
<td>unclassified</td>
<td>unclassified</td>
<td>unclassified</td>
<td></td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

*Standard Form 298 (Rev. 8-98) Prescribed by ANSI Std Z39-18*
Outline

• Advantages of aptamers
• ALISA, Where we came from
• Dot Blot Format, Other possibilities
• One step Quantum Dot De-quenching Assay
  – Why we need a double-stranded DNA aptamer
• Comparing SELEX to Aptamer Selection Express (ASExpP)
• Reagentless electronic sensors (RFIDs)
• Emerging disease agents and finding an unknown
  – Why we need a rapid technique for aptamer selection
• Summary
Advantages of Aptamers

- Aptamers are smaller than antibodies – ranging from 30 to 50 nucleotides
- Do not require either animals or tissue culture for production
- Can be synthesized chemically or by PCR
- Due to the nature of DNA, they are stable in harsh environments and do not require special storage conditions
- Offer additional chemistries and modalities for further stabilization (nuclease resistance) and assays

Approved for public release; distribution unlimited
SELEX: Selection of Aptamers

Selection begins with a library of ~10^{15} single strands of DNA. The target is bound to a filter, and a portion of the library binds to the target. The bound strands (+) are eluted from the target by heat and amplified using PCR, with the primer for the negative strand containing biotin at its 5’ end. After amplification, the DNA is denatured and the (-) strands separated from the (+) strands by passing the DNA over a streptavidin column, which retains the biotin containing (-) strands. Another round of selection is begun.
Where we came from: Tularemia in Houston: PCR and Immunoassays are not the last word


Francisella tularensis also discovered on Washington (DC) National Mall 24-25 Sept 2005; not reported until 1 Oct 2005

Approved for public release; distribution unlimited
Current Methods for Tularemia Diagnosis

Culture in cysteine enriched medium with glycerol (dangerous for lab personnel)
- 1-10 Organisms can cause infection
- Type A (most pathogenic)—glycerol catabolism positive (exceptions)
- Type B (self-limiting)—glycerol catabolism negative

CDC PCR method
- Smaller product—Type A
- Larger product (insertion)—Type B

Immuoassays (ELISA and Agglutination)
- For antibodies to F. tularensis in serum
- Can be adapted to find bacterial antigen
- Not type specific

Approved for public release; distribution unlimited
Tularemia in Houston: PCR is not always the last word
Tularemia in Houston: PCR is not always the last word
Sensitivity of Aptamers for Detecting *Bacillus thuringiensis* Spores and *Francisella tularensis*

**Figure 1** Sensitivity of anti-tularemia aptamer cocktail for *F. tularensis* subspecies *japonica* antigen and anti-tularemia antiserum as assessed by ALISA and ELISA. The assays were performed as described in ‘Materials and methods’. The data are presented as OD at 405 nm vs antigen quantity. Averages of four replication measurements are shown in the figure.

**Figure 2** Tularemia bacterial antigen binding to anti-tularemia aptamer cocktail and anti-tularemia polyclonal antibodies as assessed by ALISA and ELISA using HRP activity. The assays were performed as described in ‘Materials and methods’. The bacterial antigen used in the binding assay was prepared from *F. tularensis* subspecies *holarctica* (live vaccine strain). The data are plotted as OD at 405 nm vs number of bacteria/ml. Averages of triplicate measurements are shown in the figure.

DOI 10.1007/s10895-007-0158-4

---

Laboratory Investigation advance online publication, 20 March 2006; doi:10.1038/labinvest.3700417

Approved for public release; distribution unlimited
Quantum Dots

- Quantum Dots
  - Very bright
  - Resistant to photo-bleaching
  - One excitation wave length
- Vendors
  - Evident Technologies: T1 (block co-polymer) and T2 (lipid)
  - Invitrogen (polymer)
ALISA approach: Quantum Dot DCE Assay for Shiga Toxin Compared to FITC Antibody Assay

(Kiel et al, SPIE 5617: 382-387, 2004)

DCE=DNA Capture Element aptamer

Approved for public release; distribution unlimited
Immobilization of Aptamer/Qdot

Synthesis of reverse system

Approved for public release; distribution unlimited
Immobilization of Aptamer/Qdot

Approved for public release; distribution unlimited
Quenching/Dequenching

Aptamer Complementary Strand with Quencher

Target

Quantum Dot

Complementary Strand with Quencher

Quenched Dequenched

Approved for public release; distribution unlimited
Possible type of Assays

ELISA-like Assay

Competitive type Assay

Approved for public release; distribution unlimited
Microtiter Demonstration (Macro Visualization) of De-quenching of Quantum Dots (Positive Control)

Dr Eric Holwitt and Ms Veronica (Franz) Sorola

Approved for public release; distribution unlimited
Microtiter Demonstration (Macro Visualization) of De-quenching of Quantum Dots Specific for Shiga Toxin with Shiga Toxin

No internal standard

DNAase

Buffer

Quenched

0.5 to 12 nanomoles of Shiga toxin added

Internal standard (another quantum dot added without aptamer)

Dr Eric Holwitt and Ms Veronica Sorola

DNAase positive control

Note that the de-quenching with Shiga toxin with various amounts reached a maximum and then declined somewhat; this was a result of cross-linking of excess Shiga toxin, causing precipitation

Approved for public release; distribution unlimited
Aptamer/Quenched Quantum Dots Response to Shiga Toxin or Ovalbumin using SELEX DNA Aptamers


![Graph showing response to Shiga toxin and Ovalbumin with Microliters of quantum dot-aptamer complex added to well]
Nanoparticles and Nanocrystals Attached to Anthrax Spores: Contact Detection/Identification and Collection
Paramagnetic Particles with DNA Capture Element (DNA Aptamers) and Quantum Dots (QD) Attached

Bright Field | UV Excitation
--- | ---
Control: No specifically bound Dots

Anthrax spores linked to DCE, QD and paramagnetic particles

Bacillus atropheus (globigii) spores linked to DCE, QD and paramagnetic particles for anthrax spores

Approved for public release; distribution unlimited
Aptamer Based Agent Detection
GOAL: Man portable detection of biological agents in the field

The Portable Test Laboratory has been flown on the International Space Station

Identifies positive sample in field allowing for further analysis in a controlled location!

The Portable Test Laboratory has been tested in conditions of extreme heat and cold

Approved for public release; distribution unlimited
AFRL/CRL Device

- Cartridge provides ability to retrieve samples for identification and analysis of substance
- Device tests and identifies the contained specimen
- Current system measures fluorescence
- 9.25x4.625x2.50 inches
- Battery Operation for 4 hrs
- ~ 2 lbs

Currently Marketed Portable Test Lab

RFU as Function of Concentration

Adenosine

Series1
Series2
Log. (Series1)
Linear (Series2)

Approved for public release; distribution unlimited
Magnetic Nanoparticle or Microparticle and Quantum Dot Separation

Internal Standard Subtractive Ratio Assay Method

Quantum Dot is displaced by Target: Increase in fluorescence in downstream optical window

Target can be specific to live only marker, dead and/or live marker, and metallic marker in three separate channels of microfluidics cassette

\[ 1 - \frac{R_0}{R_1} = \text{target to sensor ratio} \]
ASExpP

1. Library of ss-DNA
2. PCR
3. Library of ds-DNA
4. Target
5. Wash with small amount TE buffer
6. Supernatant containing small amount ds- and ss-DNA
7. PCR
8. Ethanol/NaAc
9. ds-DNA
10. Wash with H$_2$O
11. PCR
12. Clone
13. Sequence

Approved for public release; distribution unlimited
Bot Tox Aptamers: **SELEX** and **ASExpP**

<table>
<thead>
<tr>
<th><strong>Selected by ASExpP against BoTox, type A-light chain (for DCE-1)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1(+) AgTCTAgAgggCCCCAgAATACACCCgACAACCTAgAT</td>
</tr>
<tr>
<td>ACCCATCAAAAAGTCACCAACAgATAgCAggggT</td>
</tr>
<tr>
<td>1(-) ACCCCTgCATCCTTTgCTggACTTTTTgATgggTATCTA</td>
</tr>
<tr>
<td>gTTgTCgggTgTATTCTggCCCTCTAgACT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Selected by ASExpP against BoTox, type B-light chain (for DCE-2)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2(+) AgTCTAgAgggCCCCAgAATTATCCACTAgCgggAATC</td>
</tr>
<tr>
<td>gTACATCTCACCCAgCAAAggATgCAggggT</td>
</tr>
<tr>
<td>2(-) ACCCCTgCATCCTTTgCTgggTgAgTgTACTACTTCC</td>
</tr>
<tr>
<td>CgCTAgTgggAATATTCTggCCCTCTAgACT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Selected by SELEX against BoTox, type A-light chain (for DCE-3)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>3(+) CATCCgtTCACACCTgTCTCTgAgggATAgTTggTgTTggCT</td>
</tr>
<tr>
<td>ACCCTATTCAAgggCgAATTCT</td>
</tr>
<tr>
<td>3(-) gTAggCAgTgTggACgAgACCCTACACACACCACACCACAC</td>
</tr>
<tr>
<td>gAgggCATAgTTCCCCgCTTAAgA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Selected by SELEX against BoTox Holotoxin (for DCE-4)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>4(+) CATCCgtTCACACCTgTCTCTgATCACTACAgCCTgCTg</td>
</tr>
<tr>
<td>AAgTggTgTTggCTCCATgATA</td>
</tr>
<tr>
<td>4(-) gTAggCAgTgTggACgAgATAgTgTACgACAGCCgACTTC</td>
</tr>
<tr>
<td>ACCACACCgAgggCATAgT</td>
</tr>
</tbody>
</table>

Approved for public release; distribution unlimited
Double-Strand DNA Response De-quenching Using ASExpP vs. SELEX Aptamers against Bot Tox A

**Figure A.** Fluorescence change resulting from the interactions of DCE-1 (made from aptamer against BoTox, type A-light chain by ASExpP process) with different types of BoTox.

**Figure B.** Fluorescence change resulting from the interactions of DCE-3 (made from aptamer against BoTox, type A-light chain by SELEX process) with different types of BoTox.

GE GRC Concepts: Complementary Sensing Structures

Detection of changes in capacitance and resistance of sensing gap between electrodes provides improved sensitivity and stability and rejects interferences.


Approved for public release; distribution unlimited
### GE GRC: Two Designs of RFID Sensing Electrodes

<table>
<thead>
<tr>
<th>Full antenna sensing structure</th>
<th>Complementary sensing structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFID sensor antenna</td>
<td>RFID sensor antenna</td>
</tr>
<tr>
<td>IC memory chip</td>
<td>IC memory chip</td>
</tr>
<tr>
<td>Sensing material</td>
<td>Sensing material</td>
</tr>
<tr>
<td>Complementary sensing region</td>
<td>Complementary sensing region</td>
</tr>
</tbody>
</table>

#### Pros
- Simple design
- Ease of fabrication
- Smaller sensing area
- Ease to deposit sensing films
- Highest sensitivity

#### Cons
- Reagent cost
- Medium fabrication difficulty
GE GRC: Analysis of $K_d$ for Thrombin Aptamer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Aptamer use</th>
<th>Sensing format</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee, M.; Walt, D. R., Anal. Biochem. 2000, 282, (1), 142.</td>
<td>5’-NH-C6 on silica beads</td>
<td>Competition, fluorescence</td>
<td>300nM</td>
</tr>
<tr>
<td>GE GRC (THIS WORK)</td>
<td>Functionalized aptamers on glass slide</td>
<td>Fluorescence</td>
<td>10-100nM</td>
</tr>
</tbody>
</table>


Approved for public release; distribution unlimited
GE GRC: Spore detection: Characterization of 2-D Bare Nanogap FIB-fabricated Electrodes

Detection limit of BG spores = 35 spores
Most techniques except for culture (1 spore) detect a minimum of 100-100,000 spores

Potyrailo et al, 2009

Approved for public release; distribution unlimited
Emerging Exotic Pathogens: Heartwater and Viper Plague

- **Heartwater**
  - Tick-borne disease: *Amblyomma variegatum, A. hebraeum, A. lepidum, A. maculatum*, other Amblyomma tick carriers
  - Causal agent: *Cowdria ruminantium*, now *Ehrlichia ruminantium*

- **Imminent threat to Western Hemisphere**
  - Mortality in cattle and other ruminants: excess of 70%
  - Has been found in African spurred tortoises (*Geochelone sulcata*) and leopard tortoises (*Geochelone pardalis*)
  - Is now in Caribbean Islands
    - Antigua
    - Guadeloupe
    - Marie Galante
    - Perhaps Cuba

- **Viper Plague**, a mimic of heartwater, and associated ticks entered the USA in 2002
  - VP rickettsia was isolated in viper cells and propagated in turtle cells, but also infects bovine endothelial cells, and human cells (HeLa)


Approved for public release; distribution unlimited
**Molecular Biology Confusion (Standard Diagnostic PCR) Between Heartwater and Viper Plague**


---

PCR products sent for sequencing:

- **PCR pCS20F-HpCS20R**: 750pb instead of 1100pb
- **Nested pCS20**: 280pb like *Ehrlichia ruminantium* (heartwater agent)

**VSW and BSE ER**: Viper spleen and Bovine endothelial cells infected

**VSWC & BSEC**: uninfected cells

**T+** = positive control DNA from *Ehrlichia ruminantium*

**MW** = ladder 100pb

Approved for public release; distribution unlimited
Centrifuged Bovine Endothelial Cell Supernatant Showing Rickettsia (requires many large culture flasks to accumulate this number of rickettsia)
Bovine Endothelial Cells: Infected with VP Showing a Hidden Type D Immunosuppressive Retrovirus associated with the Disease and Compared to Human Type D Retrovirus

ROBERT C. BOHANNON, LAWRENCE A. DONEhower, AND RICHARD J. FORD
Division of Molecular Virology, Baylor College of Medicine, and Department of Molecular Pathology, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Received 29 November 1990/Accepted 23 July 1991

JOURNAL OF VIROLOGY, Nov. 1991, p. 5663-5672
Copyright © 1991, American Society for Microbiology

Isolation of a Type D Retrovirus from B-Cell Lymphomas of a Patient with AIDS
ROBERT C. BOHANNON, LAWRENCE A. DONEhower, AND RICHARD J. FORD
Division of Molecular Virology, Baylor College of Medicine, and Department of Molecular Pathology, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
Received 29 November 1990/Accepted 23 July 1991

JOURNAL OF VIROLOGY, Nov. 1991, p. 5663-5672
Copyright © 1991, American Society for Microbiology

Johnathan L. Kiel, Yvette Gonzalez, Ishmael I. Rosas and David F. Vela,
Out of Africa: Do Viruses Play a Role in the Emergence of New Rickettsial Diseases?
Presentation at the 5th International Meeting on Rickettsiae and Rickettsial Diseases. Marseille, France. May 2008

Approved for public release; distribution unlimited
New Retrovirus Infects a Wider Host Range than VSW Virus

General Spotted Fever or Typhus targeting by anti-OX-19 Antigen Aptamer-coated Particles (Dr Fan by ASExpP)

Visible and UV Light Photomicrographs after OX-19 Fluorescent Antibody Treatment

Control VH2 Cells

RLO Infected VH2 Cells

Suspected RLO attached by OX-19 aptamer to nanocrystal of iron oxide on micro mag bead

Facilitated Uptake of RLO Bound to Beads (to bursting of cells)

I. Rosas and D. Vela

Summary

• Aptamers need to be selected under the conditions in which they are going to be used
  – **SELEX** aptamers sometime work as double-stranded contact reporting aptamers, but many times do not in spite of very low Kds
  – **ASExpP** fulfills the above criteria
• **SELEX**, by its very nature and mass action, selects for aptamers against the most abundant ligand not necessarily the most specific
  – **ASExpP**, because of its low cycle number and initial stringent conditions, selects for the highest affinity aptamer to the rarest target
• Several photochemical and electronic options exist for sensing platforms for aptamers
• Rapidity of aptamer selection in general allows for fast response to new emerging agents
• Finally, the double-stranded DNA capture elements allow for detection, identification and non-destructive safe collection for further orthogonal analysis in the lab

Approved for public release; distribution unlimited