Proposal for "Grow Iowa Values Fund" Grants Competition, Spring 2009

Project Title: "Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician"

Project Period: August 1st, 2009 – January 31st, 2011 (1.5 years)

PI: Byron Brehm-Stecher, Food Science & Human Nutrition, 2312 Food Sciences Building, byron@iastate.edu
Company Partner: Advanced Analytical, Inc., 2901 S. Loop Drive, Ames, IA 50010
Clinical Partner: Gary W. Procop, MD, Chairman, Dept. Clinical Pathology, Director, Molecular Microbiology, Mycology, and Parasitology, The Cleveland Clinic Foundation, 900 Euclid Ave., Cleveland, OH 44195

EXECUTIVE SUMMARY: The human bacterial pathogens *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia* and *Listeria* are responsible for over 4 million cases of foodborne illnesses annually, including more than 50% of all deaths caused by foodborne disease. An additional, emerging pathogen of concern is methicillin-resistant *Staphylococcus aureus* (MRSA). These bacteria may also infect food production animals, providing a possible route to human infection through consumption of contaminated foods such as milk, eggs, pork or beef. Complicating efforts at removing these contaminants from the food chain is the fact that these bacteria have the potential to become entrenched in and persist in agricultural production environments such as feedlots, hog containment buildings, poultry production facilities or soils, surface waters and fields adjacent to these areas. These pathogens are therefore a problem throughout the production-to-consumption-to-disease continuum – in other words, from "farm-to-fork-to-physician". In order to break this cycle of contamination and reduce the severe health and economic burdens levied by these human and animal pathogens, we need new rapid and powerful pathogen detection technologies that can be applied at any point within the chain – in the farm environment, in food processing facilities or in the clinic. Using patented technology licensed from Iowa State University (ISURF01604; Yeung et al., US 5,324,401; Yeung et al., US 5,498,324), Advanced Analytical has developed the DNA PROFiler system - an instrument capable of rapid and sensitive sequence-based detection of human pathogens. This home-grown Iowan technology could have a transformative impact on the environmental, food and clinical testing markets, allowing end users to not only quickly detect target pathogens, but also to characterize isolates and distinguish them based on minute genetic differences. These capabilities could have far-reaching implications for farmers, food processors, veterinarians or clinicians, as well as those involved in bio- or food defense. This project will facilitate collaborative work between Iowa State University, Advanced Analytical and the Cleveland Clinic Foundation, and will yield market-ready DNA PROFiler-based tests for use by environmental, food and clinical microbiologists.

Project Description: Although the areas of food, environmental and clinical microbiology parallel each other in terms of both their general goals and methods, they have traditionally been regarded as entirely separate disciplines. However, pathogens such as *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia*, *Listeria* or, more recently, MRSA are of concern throughout the production-to-consumption-to-disease continuum (from "farm-to-fork-to-physician"). Environmental microbiologists seek to follow their prevalence and distribution in the environment, food microbiologists seek to detect them to ensure the safety of the foods we eat, and medical professionals seek to detect them so that they may provide more timely disease intervention. "Classical" or culture-based methods for the detection of these pathogens are limited primarily by their heavy demands on time and labor. A wide variety of selective enrichment broths and agars have been developed to make identification of these pathogens easier in foods or clinical samples. Still, at least four sequential steps are generally required for cultural methods of detection: pre-enrichment, selective enrichment, selective plating and biochemical screening. As a result, positive detection of these pathogens in foods using cultural methods alone may take up to 5-7 days. Clearly, this timeframe is at odds with the rapid pace of today's food processing and distribution networks and with the need for timely diagnosis and intervention of disease. Additionally, new concerns regarding agricultural environments as reservoirs for the dissemination of antibiotic-resistant human pathogens mandate more timely surveillance of soils and surface waters for these pathogens, especially when animal facilities are in close proximity to crops destined for human consumption. Rapid methods for detection of these pathogens in the environment, in foods and in clinical samples would therefore be of great value to the agricultural production, food processing and health care sectors.

The **DNA PROFiler** system from Advanced Analytical leverages ISU-based intellectual property to enable sequence-based detection and characterization of microbial pathogens, including those problematic in agricultural environments, in foods and in clinical specimens. We propose development of DNA PROFiler-based assays for detection of human and animal pathogens that are problematic in the production-to-consumption-to-disease continuum – in other words, from "farm-to-fork-to-physician". These assays will incorporate all necessary steps, from cell separation and concentration from environmental, food or clinical matrices, to the matrix-specific sample preparation methods needed to ensure successful polymerase chain reaction (PCR) amplification prior to DNA PROFiler-based analyses. We will work closely with our colleagues at Advanced Analytical and the Cleveland Clinic Foundation to ensure that a core set of useful, marketable and cross-disciplinary assays for select microbial pathogens is developed. Additionally, we will ensure that these assays enable detection of target pathogens regardless of the initial sample source – whether it is environmental, food or clinical in nature. These assays will incorporate recent advances made in Dr. Brehm-Stecher's lab and elsewhere for rapid separation and concentration of microbial cells from complex sample matrices, with concomitant removal of substances inhibitory to downstream PCR. Our approach will include circulating Immunomagnetic separation, which is well developed for food applications, but which has not been explored to the same extent in environmental or clinical applications. **The proposed research addresses the "Grow Iowa Values Fund" objectives to expand the commercialization of ISU technology, to help ISU technology reach the marketplace, to help the growth of existing Iowa companies, to develop collaborative research with a company having an Iowa presence, and to increase sales and/or profitability of Iowa companies through provision of new market opportunities.** Letters of support for this work, provided by Advanced Analytical, The Iowa Farm Bureau and Dr. Gary Procop of the Cleveland Clinic Foundation are attached.

Summary of Technical Tasks

1. Develop and apply novel methods for pre-PCR sample preparation to food & environmental samples. Pathogens of interest to include *Salmonella* spp., *Campylobacter* spp., and methicillin-resistant *Staphylococcus aureus* (MRSA).
2. Optimize combination of new sample preparation methods with PCR to provide suitable input for sequence-based analysis via the DNA PROFiler system. Finalize "soup to nuts" assay flow from sample preparation to final PROFiler results.
3. Transfer most promising assays to colleagues at Cleveland Clinic and provide technical assistance/advice as they apply them for PROFiler-based detection of target pathogens in clinical samples.
4. Publish results in high-visibility peer-reviewed journal(s).

Schedule (Technical Tasks)

Task	Year 1				Year 2	
	Q1	Q2	Q3	Q4	Q1	Q2
Task 1						
Task 2						
Task 3						
Task 4						

Competitive Environment: Progressive clinical investigators (such as Dr. Procop) are leading the diagnostics field by applying new sequencing tools to the identification of clinical isolates. However, current state of the art sequencing tools are designed for genome-scale analyses, are prohibitively expensive and require the additional equipment and personnel support typical of dedicated "core" sequencing labs. As such, they are within reach of only a few specialized centers and are not appropriate for routine and widespread testing in food, environmental or clinical labs. Additionally, these sequencing platforms (e.g. 454 Life Science's instrument, Applied Biosystems' SOLID system, etc.) are simply too powerful to use in routine testing – the molecular diagnostic equivalent of killing a flea with a sledgehammer. **Limitations of Traditional PCR and Sequencing Approaches:** Although it was first introduced in 1983, the polymerase chain reaction (PCR) has only recently become a widely used molecular tool for detecting pathogens in foods. Factors responsible for easing PCR further into the mainstream testing environment include the commercial availability of pre-packaged reagents and advances in automated detection of PCR products. Still, traditional PCR provides only "yes/no" answers – either a band indicating the presence of target DNA is generated, or it is not. PCR alone does not enable the detection of subtle differences in nucleic acid sequence that may be diagnostic for different strains of a given pathogen, or that may signal antibiotic resistance. "Cycle" sequencing, an outgrowth of the human genome project, is widely used in academic labs, but is time-intensive. First, a PCR reaction is performed to generate enough material to be sequenced. Second, another (long) PCR-like reaction is carried out to generate a series of fluorescently labeled DNA fragments. These must then be purified to remove unincorporated reaction products and then sent to a dedicated "core" lab to be analyzed using a "slab" gel or in a specialized capillary electrophoresis machine (a DNA sequencer). Results are typically received back in the lab within 2-3 days – clearly *not* within the timeframe demanded of rapid diagnostic tools upon which time-sensitive decisions, such as food recall or antimicrobial therapeutic decisions may depend. **The DNA PROFiler System:** Advanced Analytical's DNA PROFiler system, based on patented ISU technology, combines a series of reagent, hardware and software solutions to provide information similar to that obtained with traditional DNA sequencing, but with results that are obtained within ***hours***, instead of ***days***. A powerful aspect of using the DNA PROFiler system is its

flexibility - scientists can select the PCR primers needed to generate pathogen or application-specific PCR products for his/her application from the vast body of public domain sequences available in the scientific literature. A similar wealth of information exists for pre-PCR analytical methods, such as cell separation from complex matrices and sample preparation for removal of PCR inhibitors. The applications development work to be performed by Drs. Brehm-Stecher and Procop will be informed by these existing bodies of work and by recent experience obtained in the Brehm-Stecher lab, and is therefore accomplishable within the relatively short time period of this grant. **Anticipated Market:** Overall, the global DNA sequencing market is estimated at **$1.7 billion**. The DNA PROFiler system and the applications development proposed here are expected to be welcome additions to a diagnostics market that has no "middle ground" between the rapid, but limited detection afforded by PCR and the high-end sequence-based characterization characteristic of high-end genome-scale sequencing systems. It is expected that use of the DNA PROFiler system will place rapid, sequence-based detection and characterization of microbial pathogens within the reach of midsize and larger environmental testing labs; food companies and heath care facilities. Apart from microbial applications, the DNA PROFiler may also find additional use in genetic testing, forensics, paternity testing, biodefense or plant breeding uses.

Summary of Commercial Tasks

1. Identify microbial tests having greatest market potential and for which DNA PROFiler-based analyses may provide advantages not available with existing methods.
2. Develop and optimize pathogen and matrix-specific tests for the DNA PROFiler system in environmental, food and clinical samples (overlap with technical tasks, above).
3. Raise awareness of this technology and its potential through high-profile presentation and publication of research results at national conferences and in leading peer-reviewed journals.

Schedule (Commercial Tasks)

Task	Year 1				Year 2	
	Q1	Q2	Q3	Q4	Q1	Q2
Task 1						
Task 2						
Task 3						

Why Grow Iowa Values Fund Support is Critical: We believe that this proposal is an excellent fit for the GIVF program and addresses or satisfies key criteria unique to this funding program, as described above at the bottom of page 2 of this proposal. A challenge to prospective investigators is that GIVF is an accelerated program, with a very short timeframe between submission to funding and only 18 months from funding to completion. It is important to note that key personnel for the proposed work are **already in place** in the Brehm-Stecher Rapid Microbial Detection and Control Laboratory. These include Dr. Bledar Bisha and Ms. Brittany Porter. Dr. Bisha graduated from Dr. Brehm-Stecher's lab in May 2009 with a thesis focused on rapid molecular detection of pathogens in complex food matrices. Ms. Porter is an MS candidate in Dr. Brehm-Stecher's lab and is supported until May 2010 on a DNAPROfiler-based project entitled "New CE tools for rapid sequence based detection and characterization of dairy pathogens", funded by the Midwest Dairy Association (MDA). If the current proposal is funded by GIVF, we will be able to start work immediately (August 1, 2009) with a knowledgeable team, and with the ability to leverage both funding and experience gained from the MDA work, which has already resulted in a poster presentation at a national meeting (B. Porter et al., "DNA PROFiling for characterization of *Salmonella* spp.", American Society for Microbiology General Meeting, 2009). The synergistic overlap between these two complementary proposals will result in enhanced student learning opportunities and will provide

critical supply, travel and publication support cut from the MDA project last year due to budget limitations.

Budget

Category				Requested Amount	ISU Cost Share	Company Cost Share	TOTAL
Faculty				$18,382	$6,204		$24,586
Faculty Benefits (27.2%)				$5,000	$1,687		$6,687
Postdoc				$48,000			$48,000
Postdoc Benefits (23%)				$11,040			$11,040
Grad Student				$15,059	$14,621		$29,680
Grad Student Benefits (13.2%)				$1,988	$1,930		$3,918
Tuition				$2,721	$3,356		$6,077
Salaries & Wages Subtotal				*$81,441*	*$20,825*		*$102,266*
Fringe Benefits & Tuition Subtotal				*$20,749*	*$6,973*		*$27,722*
Equipment						$100,000	$100,000
Lab Supplies (cash support)						$20,000	$20,000
Travel				$3,000			$3,000
Publication				$1,500			$1,500
Total Cost				**$106,690**	**$27,798**	**$120,000**	**$254,488**

Budget Justification (Request):

Salary: Support (**$48,000**) for a postdoc (Bledar Bisha, DVM, PhD) is requested for the duration of the 1.5 year project at an annual salary of $32,000. Support (**$15,059**) is requested for Brittany Porter, MS candidate, for a 9-month period (May 1st, 2010 – Jan 31st, 2011) at the FY '10 stipend rate of $20,079. **This project will leverage existing funding for a complementary DNA PROFiler project from the Midwest Dairy Association to cover the first 9 months of Ms. Porter's stipend, benefits and tuition** (see cost share below). Two months of summer salary ($18,382) is requested for Dr. Brehm-Stecher over the duration of the 1.5-year project. Dr. Brehm-Stecher is on a 9-month appointment at a projected annual rate of $82,719 (current rate of $81,497 + 1.5% increase). **Benefits:** Benefits are 23% for postdocs, therefore, **$11,040** is requested. Benefits for graduate students are 13.2%, therefore**, $1,988** is requested. Benefits are 27.2% for faculty, therefore, **$5,000** is requested. **Tuition: $2,721** is requested (Summer/Fall, 2010). **Travel:** Funds are requested for travel to one national meeting for Dr. Bisha and Ms. Porter ($3,000). **Publication:** $1,500 is requested for publishing the results of our studies. **Explanation of Cost Share:** Advanced Analytical will supply **$20,000 in cash** support for the lab supplies needed to carry out this work. The company will also donate a DNA PROFiler instrument to Dr. Brehm-Stecher's lab, valued at **$100,000**. Together, this represents a cost share commitment of $120,000. **Salary:** The first 9 months (08/01/09-04/30/10) of Ms. Porter's stipend (**$14,621**) and benefits (**$1,930**) will be paid from a current DNA PROFiler-based project funded by the Midwest Dairy Association, based on the current annual stipend rate of $19,494. Tuition for Fall 2009 and Spring 2010 **($3,356**) will also be paid from this grant. Dr. Brehm-Stecher will devote 5% of his time to this project during its 1.5-year duration. Dr. Brehm-Stecher is on a 9-month appointment at a projected annual rate of $82,719. This represents a cost-share commitment of **$6,204**. The cost share of benefits related to this amount is **$1,687,** for a total of **$7,891** in faculty salary and benefits cost share.

CONFIDENTIAL
GIVF FY10-28

Steven J. Lasky, PhD
President and CEO
Advanced Analytical, Inc.
2901 S. Loop Drive
Ames, IA 50010
April 17, 2008

Office of the Vice Provost for Research
& Economic Development
Iowa State University
Ames, IA 50011
Attn: Grow Iowa Values Fund Grants Competition

To Whom It May Concern:

I am writing this letter in support of Dr. Byron Brehm-Stecher's application for funding from the Grow Iowa Values Fund. The work proposed by Dr. Brehm-Stecher, "Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician", will leverage the diagnostic power of our new DNA PROFiler system for detection of microbial threats occurring in the farm environment, in foods and in clinical specimens. I expect that the outcome from this work will be of general value to farmers, food processors and veterinarians or clinicians, and of special value to my company as we launch this new instrument into these key markets.

Over the past decade, Advanced Analytical has emerged as a leader in the field of applied microbial cytometry. With the recent merger of Advanced Analytical and CombiSep, we have acquired exciting new capabilities in capillary electrophoresis (CE) technology. Through the introduction of our CE-based DNA PROFiler system, based on patents licensed from Iowa State University, we seek to gain additional presence and market share in the microbial detection arena. Receipt of this Grow Iowa Values Fund grant will facilitate collaborative research between ISU, Advanced Analytical and the Cleveland Clinic. This collaborative work will be key to further development, validation and successful launching of our DNA PROFiler system into the food, environmental and clinical testing fields. The research team we have assembled for this project brings together the combined experience in food, environmental and clinical microbiology needed to launch the PROFiler system into these lucrative testing markets.

Based on preliminary data from both Dr. Brehm-Stecher's lab and from the Cleveland Clinic, I feel that this work stands a high chance for success. I anticipate that this work will enhance our company's ability to bring novel and beneficial products and applications to market and I strongly recommend that it be funded.

Sincerely,

Steven J. Lasky, PhD
President and CEO

CONFIDENTIAL
GIVF FY10-28

IOWA FARM BUREAU
PEOPLE. PROGRESS. PRIDE.

April 16, 2008

Office of the Vice Provost for Research
& Economic Development
Iowa State University
Ames, IA 50011
Attn: Grow Iowa Values Fund Grants Competition

To Whom It May Concern:

I am writing this letter in support of Dr. Byron Brehm-Stecher's recent application for funding from the Grow Iowa Values Fund grants program. The work proposed by Dr. Brehm-Stecher, "Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician", will provide improved pathogen testing based on Advanced Analytical's new DNA PROFiler system. The resulting tests will enable rapid detection and sequence-based identification of human pathogens in samples from various sources key to Iowa's agricultural economy, including raw agricultural products and dairy, beef, hog or poultry farm environments.

The health and well being of Iowa's farmers and production animals forms the backbone of our farm economy. Dr. Brehm-Stecher's team will also adapt the sampling and DNA PROFiler-based tests they develop for food and environmental work to enable rapid detection and identification of bacterial pathogens in human or veterinary clinical samples.

Iowa leads the nation in corn, soy, hog and egg production. The safe production and processing of these agricultural products is key to maintaining Iowa's cornerstone position in the nation's farm economy. I believe that the approach proposed by Dr. Brehm-Stecher and his colleagues at Advanced Analytical will provide the advances in rapid microbiological testing needed to ensure the safety and quality of Iowan agricultural products as well as the health of Iowa's farming workforce.

Sincerely,

David Lyons
Chief Business Development Officer

5400 University Avenue, West Des Moines, IA 50266-5997 / (515) 225-5400

CONFIDENTIAL
GIVF FY10-28

Cleveland Clinic

Gary W. Procop, MD, Chairman
Department of Clinical Pathology
Director, Molecular Microbiology,
Mycology, and Parasitology

April 17, 2008

Office of the Vice Provost for Research
& Economic Development
Iowa State University
Ames, IA 50011
Attn: Grow Iowa Values Fund Grants Competition

To Whom It May Concern:

I am writing this letter in support of Dr. Byron Brehm-Stecher's application for funding from the Grow Iowa Values Fund program. Dr. Brehm-Stecher's submission, "Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician", seeks to bridge the needs of food, environmental and clinical microbiologists by creating sequence-based pathogen detection assays for use at all points across the "consumption to disease continuum".

Specifically, Dr. Brehm-Stecher's work will combine novel approaches for microbial cell separation and concentration from complex sample matrices with rapid detection using Advanced Analytical's DNA PROFiler system, now under development. Matrices to be examined will include raw agricultural products or processed foods and samples from farm environments, such as feedlot soils or adjacent surface waters. My laboratory at the Cleveland Clinic will apply the methods for sample preparation developed by Dr. Brehm-Stecher to clinical samples, followed by analysis using the DNA PROFiler system.

In my practice as a physician, I have been a leader in the early adoption of technologies that show clear potential for enabling both cost savings and improved patient outcome. In my estimation, Advanced Analytical's DNA PROFiler system shows exceptional promise as an emerging diagnostic tool. I have been involved in the development of the PROFiler from the clinical side and look forward to working with Dr. Brehm-Stecher to improve sample preparation methods and to harmonize use of this unique detection platform across the disciplines of food, environmental and clinical microbiology.

Sincerely,

Gary W. Procop, M.D.

The Cleveland Clinic Foundation
Pathology and Laboratory
Medicine Institute

9500 Euclid Avenue / L-11
Cleveland, Ohio 44195

Tel 216 444-5879
Fax 216 444-4414
procopg@ccf.org

GROW IOWA VALUES FUND GRANT COMPETITION
COLLEGE REVIEW FORM

PI Name: Byron Brehm-Stecher

Project Title: Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician

College Ranking _3 of 6__

Recommendation: Fund _X__ Fund if Possible ___ Do Not Fund ___

SECTION I: TECHNICAL MERIT (60% of recommendation)

4.5

Considerations:

- What is the scientific merit of the proposed project?
- Is the project technically feasible to accomplish in the listed time frame?
- Does the budget seem reasonable?
- Does the PI/team of researchers have the qualifications necessary to carry out the work?

Justification:

High scientific importance with a highly qualified team of researchers. A bit high budget for a 1.5-yr project.

SECTION II: BROADER IMPACTS (30% of recommendation)

4.5

Considerations:

- What is the probability that this project could be used to leverage future funding from non-ISU sources?
- What is the potential to increase ISU's research capabilities or capacity?
- What is the potential to enhance learning opportunities for students?

Justification:

Strong industry connection. Clear leveraging of industry-sponsored research. Unclear connection with graduate students.

SECTION III: COMMERCIAL POTENTIAL (10% of recommendation)

4.5

Considerations:

- What is the likelihood that new intellectual property will be generated?
- What is the likelihood of eventual commercial success?

Justification:

Unclear if project would lead to new IP and eventual commercial success.

Proposal Number: FY10-28

PI Name: Byron Brehm-Stecher

Project Title: Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician

College Ranking:

College Rating:

Commercial Recommendation: **Fund**

Commercial Potential:

1) Will this project lead to a new Iowa business/company, or increase the sales/profitability of a recently started Iowa company?

   Yes

   Please comment on the above:

   New assays should make the DNA PROFiler a widely used instrument in multiple industries.

2) Will this project increase the sales or profitability of an existing Iowa business/company ?

   Yes

   What is the probability of commercial success:

   This project has the potential to increase the sales of Advanced Analytical through the development of PCR-based diagnostic assays for food and environmental pathogens that are optimized for the company's DNA PROfiler system.

3) Are competitors identified, is the advantage of the proposed technology clear.

   Yes

   Please comment on the above:

   While the proposal was quite well written, the discussion on competition was limited in that it was focused on conventional microbiology and PCR techniques but overlooks other recent advances and approaches. The advantages of the proposed approach could have been made more compelling if it had been compared to more contemporary approaches or specific competitors, or if cost advantages had been quantified.

4) Is there a clear strategy for entry to the market, start up or existing Iowa business/company.

   Yes

   Please comment on the above:

   The strategy for entry to market is to validate assays in a clinical setting at the Cleveland Clinic, and then commercialize them through Advanced Analytical.

5) Please make any other comments related to the commercial potential of this proposal:

Good team and partners. Excellent cost-share/match. Good description of why the technology is needed. Some information on market segmentation (for example, food pathogen testing) would have enhanced the proposal.

Intellectual Property Evaluation

1. Have any current ISURF invention disclosures been identified as background IP? No

ISURF technology ISURF#01604 developed by Dr. Yeung is licensed to Advanced Analytical (DNA PROFiler)

If Yes:

a) What is/are the ISURF number(s)?

b) What is the IP/patent status?

c) What is the licensing status?

2. Does this project have the potential to generate new intellectual property? Yes/No

(please explain)

Not sure

3. Based on your current knowledge, without having the opportunity to receive input from the principle investigator or to conduct a market or technology assessment, what will be the barriers to commercializing this technology?

Competition from alternative technologies