TO: Board of Regents

FROM: Sharron Quisenberry, Ph.D.
Vice President for Research and Economic Development, Iowa State University

RE: FY2010 Grow Iowa Values Fund Appropriation

DATE: 15 July 2009

The Iowa State University's (ISU) request for the FY2010 Grow Iowa Values Fund Appropriation information is presented in this document. We propose to allocate the money consistent with the guidelines presented to the Board of Regents in FY2006. In summary, proposed projects are presented by category, with a brief description of the category following the list.

FY10 Funding Summary (for each year):

Infrastructure and Entrepreneurial Culture		$ 750,000
ISU Research Park	$200,000	
ISU PappaJohn Center	$200,000	
ISU Post-doc Entr. Program	$150,000	
IPRT	$100,000	
Vice Provost for Research	$100,000	
Commercialization Competitive Grants Program		$ 982,500
Annual Funding During This Period		**$1,732,500**

System for Innovation

The Iowa State University *System for Innovation* is focused on the transfer of university technologies into commercial applications in start up or existing companies. The *System* is coordinated by the Vice President for Research and Economic Development and has demonstrated significant success over the past 3 years. It recognizes that the "*fuel*" for a technology transfer/commercialization system is faculty and staff research.

Infrastructure and Entrepreneurial Culture

Funding for *Intrasructure and Entrepreneurial Culture* supports the people and activities required to grow, improve, and sustain the technology transfer/commercialization efforts at ISU. The staff and resources increase the capacity of the *System for Innovation* and fund resources and activities that improve performance. This progressive improvement is critical if the entrepreneurial culture at ISU and the *System for Innovation* is to achieve its full potential.

The *Post-doc Entrepreneurial Program* initiative was piloted in FY2008 with two students, and continued in FY2009 with two additional students. It is a year-long immersive program that takes science and engineering students who have just received their Ph.D. degrees and trains them in entrepreneurship. An expectation of the program is that the awardees will start a company during the year of training.

Commercialization Program

The purpose of the *Commericialization Program* is to build and sustain a larger pipeline of projects that will require enhanced visibility and funding. There is also a need to identify and foster new projects that have potential to contribute significantly to Iowa's economy. An RFP (Request for

Proposal) process is used to promote this campus-wide opportunity. The goal of the program is to obtain financial support for the development of innovations with commercial potential and to support the growth and expansion of existing companies. Funds not allocated in the RFP process are reserved for qualifying opportunities that arise during the year.

To date, 56 projects have been funded through the *Commercialization Program*, and seven (7) additional projects are recommended for funding in FY2010 (listed below, full proposals are located at http://www.industry.iastate.edu/GIVF/Funded/fundedFY10.htm). Forty-one of these projects are complete and many showed excellent progress in improving the competitiveness and profitability of the Iowa companies involved.

FY2010 Projects Recommended for Funding

PI	**Title**	**Amount Requested**
Byron Brehm-Stecher	Rapid Sequence-Based Detection of Human Pathogens: From Farm to Fork to Physician	$ 106,690
Jesse Goff	Use of Beta-Glucuronides of Vitamin D To Treat Inflammatory Bowel Disease	$ 89,657
Brad Bosworth	Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines	$ 146,610
David Grewell	Naturally Controlled Gelatinization of Corn Starch	$31,426
Bryony Bonning	Transgenic Plant Resistance to Invertebrate Pests	$ 107,680
Patrick Halbur	Development of a Novel Genetic Test for Inherited Bovine Diseases and its application to tissues and embryos	$ 69,500
Anumantha Kanthasamy	Testing of lead PK compounds in preclinical animal models of Parkinson's disease	$128,100
Atul Kelkar*	Waste Plastics, Crude Oil Sludge, and Tar Sand to Diesel – Capturing Energy from Waste	$ 9,337
Victor Lin*	Catalytic Production of 1,6-Hexanediol	$ 10,538
*Mike Kessler	Pultruded Window Frames from Agricultural Oils	$ 28,275
Mike Olsen*	Development of the Next Generation of Vortex Flow Meters for Engine Applications	$ 55,340
TOTAL**		**$ 783,153**

*In FY2009, the projects had already been selected before the 20% budget reversion. A 20% cut to these projects would have jeopardized their commercialization potential. We elected to cut the project budgets 7% and to partially fund some of the FY2009 projects during FY2010.

** The unallocated projects funds will be allocated to projects at the Vice President for Research and Economic Developments discretion, throughout FY2010, on projects consistent with the mission of funding guidelines.

Proposal for "Grow Iowa" Values Fund Grant Program

Use of Beta-Glucuronides of Vitamin D To Treat Inflammatory Bowel Disease

PI: Jesse P. Goff, BioMedical Sciences, Veterinary Medicine, ISU, jpgoff@iastate.edu ; Goff is a Professor in BioMedical Sciences 515-294-3719

Company Partner(s):
GlycoMyr, Inc., Dr. Jesse P. Goff & Dr. Ronald Horst, 3359 Stagecoach Road, Ames, IA 50010. jpgoff55@gmail.com 515-232-8468, 2 Employees. 1 year in business, Annual sales = $0.

Heartland Assays, Inc. Dr. Ronald Horst, 2325 N. Loop Drive, Suite 6300, Building 6, Ames, IA 50010 Ron.Horst@heartlandassays.com Phone: 515-520-1098, 3 Employees, In Business 3 years, Annual Sales = Confidential.

EXECUTIVE SUMMARY

Mounting evidence implicates vitamin D insufficiency as a factor contributing to autoimmune diseases, including inflammatory bowel disease. GlycoMyr was started by Dr. Jesse Goff and Dr. Ronald Horst. GlycoMyr to develop products to treat and prevent a number of human and animal diseases based on vitamin D compounds found in a plant of the *Solanaceae* family. These plant compounds are ß-glycosides of the hormonal form of vitamin D known as 1,25-dihydroxyvitamin D. They have unique activities affecting both calcium metabolism and cell growth and differentiation. GlycoMyr, working with Heartland Assays, has synthesized glucuronide analogs of these compounds, which are more stable than the native plant compounds, and has tested these compounds in rats and human cancer cell lines. In the course of these studies we discovered oral dosing of these compounds delivers the active vitamin D compounds almost exclusively to the lower intestine. By delivering the vitamin D compounds directly to the affected cells we can provide a therapeutic dose of vitamin D to the cells of the lower intestine involved in inflammatory bowel disease. **Because these glucuronide compounds are not available for absorption in the upper intestine, we also can reduce systemic absorption and development of high blood calcium, which precludes current use of the native hormone, 1,25-dihydroxyvitamin D.** GlycoMyr's competitive strength, at this point, is that we have synthesized glucuronide forms of vitamin D compounds with robust bioactivity that can be developed for a variety of applications and products. One goal is to test ß-glucuronides of 1,25-dihydroxyvitamin D as a treatment for inflammatory bowel disease. To date the compounds have been tested in rats and we know we can deliver high doses to the lower intestine with reduced systemic activity. Funding is sought to move this project to the next step – testing their effectiveness in animal models of inflammatory bowel disease.

Technical Objectives

Inflammatory Bowel Disease (**IBD**), which includes Crohn's disease and ulcerative colitis, affects ~ 1 in 1000 people in North America. IBD, particularly Crohn's disease, is an autoimmune disorder. The immune cells (Th1 lymphocytes) of the lower intestinal tract **inappropriately** secrete cytokines such as tumor necrosis factor (TNF)-α, and interferon (IFN)-γ in response to bacterial antigens. This induces excessive inflammation and destruction of normal gut tissues.

Vitamin D deficiency occurs in nearly 80% of Crohn's patients. Because Vitamin D absorption is sub-optimal in Crohn's patients, previous work in such patients focused on the role lack of the renal hormone, 1,25-dihydroxyvitamin D_3 (**1,25-D**), produced from vitamin D, played in calcium metabolism and bone health. Now studies suggest vitamin D deficiency may be a cause of IBD rather than the result of IBD! It is clear many tissues of the body, including immune cells and intestinal epithelial cells, can convert 25-hydroxyvitamin D (**25-D**), derived from vitamin D, to 1,25-D. Here it has an autocrine/paracrine role in regulating apoptosis and cell differentiation. In immune cells 1,25-D generally has an anti-proliferative effect and down-regulates production of Th1 cell inflammatory cytokines (Cantorna et al., 2004). Studies in mice also demonstrate 1,25-D acts within intestinal lining cells and is essential in maintaining the integrity of the intestinal mucosal barrier (Kong et al., 2008). In mouse models of induced IBD, mice that are vitamin D deficient or do not possess the vitamin D receptor (VDR) develop much more severe IBD. In wild type mice that are vitamin D replete, treatment with large doses of the hormone 1,25-D can reduce the symptoms and lesions of IBD in these models.

Three factors seem to combine to upset vitamin D metabolism during IBD, leading to inability of vitamin D to regulate the immune response in the lower gut properly.

1. **Low serum levels of 25-D.** This does not provide enough substrate for the 1-alpha hydroxylase enzyme that produces 1,25-D to produce the active compound within target cells. This could easily be fixed by increased dietary vitamin D.
2. **Loss of the 1-alpha hydroxylase enzyme that produces 1,25-D within these cells.** For some unknown reason prolonged vitamin D deficiency can result in loss of this enzyme activity so that even if we correct vitamin D deficiency with increased vitamin D in the diet- it does not cure the condition. In short term studies, treating mice with large doses of 1,25-D ameliorates IBD by delivering a therapeutic level of 1,25-D to the intestinal cells (Froicu and Cantorna, 2007). These doses overcome problems with synthesis and/or degradation of the hormone within the intestinal cells. *Unfortunately, prolonged use of 1,25-D at these doses in human IBD is precluded due to development of life threatening hypercalcemia (high blood calcium).*
3. **In patients with IBD the enzyme that destroys 1,25-D is up regulated (Liu et al., 2008).** This enzyme is known as vitamin D 24-hydroxylase. Because it is hyperactive it destroys any 1,25-D made in the cell or supplied pharmacologically nearly as fast as the cell can be treated. Slowing the enzyme would give the active 1,25-D compound more time to regulate cell function – which is what it does in normal cells.

A. Addressing the Problem

1. **Targeting 1,25-D to the affected tissues of the lower intestine.**

Conjugating glucuronic acid to drugs in a Beta (B)-linkage can prevent absorption of the drug until the drug is released following hydrolysis by B-glucuronidase. This enzyme is normally produced only by bacteria residing in the lower intestine. We have worked with Heartland Assays Inc, (Ames, IA) to synthesize several B-glucuronide conjugates of 1,25-D and 25-D. We have demonstrated that the glucuronide form of 1,25-D is stable in upper intestine duodenal contents but quickly hydrolyzed to glucuronic acid and 1,25-D in the ileum/colon. Oral administration of these compounds should allow us to deliver an effective dose of 1,25-D to ileum and colon cells to mitigate IBD. Because the glucuronide form is inactive and more water soluble, we also bypass the normal sites of 1,25-D absorption in the small intestine. This reduces entry of 1,25-D into the blood, reducing systemic effects, including hypercalcemia.

2. **Slow 1,25-D destruction with competitive inhibitors of 24-hydroxylase enzyme.**

Very high levels of 1,25-D have had to be used in previous studies to ameliorate IBD in mice (Froicu and Cantorna, 2007). This is, in part, due to the rapid destruction of 1,25-D as a result of increased levels of the vitamin D 24-hydroxylase enzyme (Liu et al., 2008). In vitro evidence demonstrates the effectiveness of 1,25-D can be enhanced within cells if the cells are also treated with a competitive inhibitor of the vitamin D 24-hydroxylase enzyme, such as 25-D (Reinhardt and Horst, 1989). We have also synthesized B-glucuronides of 25-D, which will be used to deliver high amounts of 25-D exclusively to the lower intestine with the goal of potentiating the action of 1,25-D on cells of the ileum and colon. This may allow us to get therapeutic effects with an even lower dose of 1,25-D, again reducing the risk of hypercalcemia.

Specific Aims and Tasks are as follows:

1. **To determine the maximal tolerable oral dose of the candidate B-glucuronide of 1,25-D over a prolonged period of time (4wks) in mice, with and without combined treatment with B-glucuronide of 25-OHD.**
2. **Test the hypothesis that administration of B-glucuronides of 1,25-D, alone or in combination with B-glucuronide of 25-D, can prevent or ameliorate symptoms of IBD in mouse models (feed dextran sodium sulfate water to induce IBD). Our treatment would supply 1,25-D to cells that may no longer make adequate amounts, and slow the rate of destruction of 1,25-D in those cells by providing 25-OHD in high enough doses to act as a competitive inhibitor of vitamin D 24-hydroxylase.**
3. **Pursue patents to cover use of glucuronides for treatment of IBD.**

Commercial Objectives

There is no cure for IBD such as Crohn's disease which affects 1 in 1000 Americans- therapy is based on reducing the inflammatory cell response using glucocorticoids and immunosuppressants such as cyclosporine and in many cases, surgical resection of the gut. Estimated costs are $18,022 per IBD patient/year. About 28% of this cost is related to doctor visits and prescription drugs (Yu et al., 2008). This means there are potentially 300,000 customers who have a record of spending about

$1.5 billion on palliative treatments for this disease each year.

Our immediate commercial goal is to develop in vivo data to support a patent claim. The patent would cover use of glycosides/glucuronides to target delivery of 1,25-D and 25-D activity to the lower intestine site for the treatment/prevention of IBD. The studies outlined above use purified compounds for "treating" IBD patients. We also have the glycosides of 1,25-D isolated from plants, which we hope to develop as "preventative herbal supplements" for general public consumption.

We are already testing the glucuronides of vitamin D for anti-cancer activity thanks to a GIVF obtained last year. Our "targeted delivery" of vitamin D may prove useful for IBD and also prove beneficial to colon cancer. Development of herbal supplements for "prevention" rather than "treatment" of colon cancer will also be pursued.

The challenge for us is to develop the type of data in animal models of IBD to convince investors, such as pharmaceutical firms, that further development through human phase I and II clinical trials is warranted. GlycoMyr is a two-man operation now. However, once the patent is pending and the data on mouse studies is published the prospect of attracting investors to further fund this research company is greatly enhanced. Subsequent steps would involve hiring more specialized chemists and pharmacologists to help in the synthesis/isolation of the glucuronides of 1,25-D and other vitamin D compounds followed by larger scale manufacture of these compounds.

There are a number of possible pitfalls to our plan. Will it reduce clinical signs and lesions of inflammatory bowel disease without causing life threatening hypercalcemia? Our competitiors are focusing on designing new analogs of 1,25-D that are selectively able to turn on the vitamin D response elements responsible for "curing" inflammatory bowel disease, while not turning on the vitamin D response elements that initiate hypercalcemia. So far this has not been accomplished. Currently Schering – Plough and Roche have active teams working on these types of compounds. A number of start-up companies also claim to have the sought after non-hypercalcemic analog of vitamin D (see addendum document for "Novacea") and are garnering a great deal of financial backing. We feel making a non-hypercalcemic analog will be difficult and have focused instead on targeted delivery of 1,25-D and blocking 1,25-D destruction – ideas which seem not to have occurred to the big companies yet. Even if these other companies succeed they may still desire the "targeting" to cells the glucuronide technology developed by Goff and Horst of GlycoMyr can offer their compounds. ***Because the 1,25-$(OH)_2$D glucuronide delivers active compound only to the lower intestine, the problem of hypercalcemia developing in patients, which has greatly limited the use of 1,25-$(OH)_2$D to date, is reduced***.

Vitamin D therapy represents a new way to regulate activity of the immune cells. It is not traditional immunosuppressive therapy, which blocks all immune function-sometimes resorting to killing certain types of immune cells. This **restores normal function** to cells that have gone out of control. Iowa can be at the forefront of this new paradigm in treatment of conditions such as inflammatory bowel disease. With luck we may now have the basic drugs needed to start a profitable pharmaceutical company. GlycoMyr is also pursuing treatment of cancer, especially colon cancer, with this approach. Our approach is to apply this technology to as many of the vitamin D responsive diseases as possible. If just one disease responds to this "targeted delivery" of 1,25-D at levels that avoid hypercalcemia we will generate great investor interest.

Budget

CATEGORY	AMT REQUESTED	ISU COST-SHARE	GlycoMyr COST-SHARE	Heartland Assay COST-SHARE	TOTAL
Salaries	12,000		60,000	30,000	102,000
Benefits					
Personnel Sub-total	12,000		60,000	30,000	102,000
Equipment					
Lab Supplies				34,000	34,000
Field Supplies					
Other Supplies & Services	72,657			14,000	86,657
Travel	5,000				5,000
Publication					
Miscellaneous					
TOTAL	89,657		60,000	78,000	227,657

Budget Justification:
Grow Iowa Value Fund
1. Cost to establish mouse models of Inflammatory Bowel Disease at ISU Veterinary College Vivarium. =**$22,657.**
2. We will also need to synthesize another 10 mg of the 1,25-D glucuronide and 50 mg of the glucuronide of 25-D. The GIVF received last year for our cancer work allowed us to learn to synthesize these compounds for approximately half the cost of last year. Pre-cursors must be purchased from Sigma Synthesis Services. Heartland Assays performs final conjugation and purification and is offering these at cost. Cost of 10 mg of the 1,25-D and 50 mg of the 25-D glucuronides **-$50,000**
3.Technician 1/2 time – 1/4 of salary of person to help feed and care for mice and assist with tissue harvesting and preparation. $12,000.
4.Travel by Goff to scientific meetings - $5,000
Total GIVF= $89,657

Heartland Assay, Inc: "In Kind" Contribution
Dr. Horst will spend 10% of his time over next two years synthesizing and purifying ß-glucuronides of 1,25-$(OH)_2$D and 25-OH D **($30,000).**
Reagents, solvents, use of chromatography equipment provided by Heartland Assay for this project for determining levels ß-glucuronides of 1,25-$(OH)_2$D in tissues. **($ 34,000)**
Heartland Assay will contribute assays of 1,25-$(OH)_2$D and the glucuronides on normal and inflamed tissue of control and treated mice. **($ 14,000). Total = $78,000**

GlycoMyr, Inc: Cash. Will provide cash for Goff's salary (cash to ISU) over the summer months of 2010 **($48,000**) and will provide cash funds to cover ¼ time technical help to match that requested of GIVF **($12,000**). The technician may be a GlycoMyr employee, an ISU employee, or student labor, depending on funding from other sources that could perhaps move the position to a full time employee. **Total = $60,000**

Supporting documents

1. News Release on Novacea – start-up company that developed an analog of 1,25-D (non-hypercalcemic) and received financial backing from Schering-Plough
2. Letter from GlycoMyr pledging $60,000 cash toward this IBD project
3. Letter from Heartland Assays, Inc pledging $78,000 in kind toward this IBD project

Novacea Receives $60 Million Under Schering-Plough Agreement For Asentar

SOUTH SAN FRANCISCO, CA - July 10, 2007 — Novacea, Inc. (NASDAQ: NOVC), today announced that it has received $60 million from Schering-Plough Corporation (NYSE: SGP) under the terms of the previously announced worldwide development and commercialization agreement for Asentar™ (DN-101). The payment from the closing of this transaction follows the early termination by the United States Federal Trade Commission of the waiting period under the Hart-Scott-Rodino Antitrust Improvements Act of 1976.

Under the terms of the development and commercialization agreement, Novacea received an upfront payment of $60 million, including $35 million as reimbursement for past research and development expenses and a license fee of $25 million. In addition, pursuant to a related stock purchase agreement, Schering-Plough purchased $12 million of Novacea common stock. The development and commercialization agreement provides Novacea with potential pre-commercial milestone payments of up to $380 million, and royalties on worldwide sales of Asentar based on tiered royalty percentage rates.

Schering-Plough also will be responsible for all forward development costs in exploring indications for earlier stages of prostate cancer, such as androgen-dependent prostate cancer and adjuvant therapy and will lead all global commercialization efforts for Asentar. Novacea will provide medical support to Schering-Plough's commercial operations for Asentar in the United States, including deployment of their Medical Science Liaisons, which will be funded by Schering-Plough.

Asentar is essentially 1,25-dihydroxyvitamin D in a preparation for oral use. It is currently being developed as an oral treatment in combination with Taxotereâ (docetaxel) for the treatment of Androgen Independent Prostate Cancer. Prostate cancer is the second leading cause of cancer death in men with approximately 232,000 new cases and 30,000 deaths in the U.S. in 2005. AIPC is an advanced disease state of prostate cancer. Based on results of Novacea's completed Phase 2 clinical trial, known as ASCENT, the use of weekly Asentar in combination with weekly Taxotere may provide AIPC patients with an innovative cancer therapy that may prolong survival with the potential in reducing some of the toxicities and complications normally associated with chemotherapy. Novacea is now evaluating the benefits of Asentar in a 900-patient Phase 3 trial, known as ASCENT-2, which uses overall survival as the primary endpoint and compares weekly Asentar plus Taxotere to the current standard of care in the treatment of AIPC.

4.

GlycoMyr, Inc.

Developing Therapies Based on Plant Glycosides of Vitamin D

Glycomyr, Inc
3359 Stagecoach Rd
Ames, IA 50010

May 20, 2009

From: Jesse Goff DVM, PhD & Ronald Horst, Ph.D.
President and Vice President

Re: "Grow Iowa" Values Fund Grant Program

To Whom It May Concern:

Dr. Jesse Goff, currently a professor at Iowa State University, and Ronald Horst, currently President & CEO of Heartland Assays, Inc. formed a company called GlycoMyr. GlycoMyr was formed to develop novel therapies for cancer and auto immune disorders based on unique glycosides of 1,25-dihydroxyvitamin D Horst and Goff isolated from plants of the *Solanaceae* family some years ago. This joint venture, GlycoMyr, Inc. has data demonstrating these compounds have the unique ability to restrict proliferation of tumor cells and immune cells *in vitro*. The glucuronide technology is ideally suited for delivery of these compounds to tissues with high levels of glucuronidase. Cancer cells often express abnormally high levels of this enzyme and bacteria of the lower intestine produce large amounts of the enzyme. This allows us to target deliver otherwise toxic vitamin D compounds to just those tissues. We now know from studies done in recent months that we can deliver vitamin D compounds almost exclusively to the colon and ileum of the gastro-intestinal tract. In forming GlycoMyr, Jesse Goff and Ronald Horst have sold shares in the company and now have capital to invest into the project. GlycoMyr will pay Iowa State University Goff's summer salary ($48,000) in 2010 to allow him to work on commercial aspects of these projects – ie develop data to solidify a patent position and to entice further investment in GlycoMyr. Dr. Goff will work to develop the mouse models to test these compounds in *in vivo* models of inflammatory bowel disease. He will be assisted in these pursuits by Dr. Ronald Horst of Heartland Assays, Inc. In addition, GlycoMyr will pay for a 1/4 time technician ($12,000) to match the ¼ time technician pay asked for in the GIVF grant. This person will help perform the mouse experiments, which require a great deal of hand labor and observation. Altogether GlycoMyr pledges $60,000 cash toward this project.

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GIVF FY10-4

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May 26, 2009

From: Ronald Horst, Ph.D.
President and CEO Heartland Assays, Inc.

Re: "Grow Iowa" Values Fund Grant Program

To Whom It May Concern:

Heartland Assays, Inc. (HAI) is a dedicated research service laboratory, which performs assays of vitamin D and its metabolites for the medical research community. We have worked with Dr. Goff and I am personally in a joint venture with him to explore the therapeutic potential of vitamin D compounds conjugated to glucuronic acid. This joint venture is called GlycoMyr. Last year HAI worked with Goff and GlycoMyr to synthesize compounds that had some unique properties that allow targeted delivery of vitamin D hormones to cancerous cells in tissue culture. Work with that GIVF grant is continuing to progress and we are happy to take part in that project. Recently the possibility of using this same technology to treat inflammatory bowel disease arose. HAI will work with GlycoMyr to get these studies going and to aid in commercial development of the compounds.

In support of this project, HAI will pay my salary during the 10 % of my time that I will spend on this project, which I value at $30,000. I will prepare more of the vitamin D analogs and help with the synthesis of the glucuronide conjugates. I will perform High Performance Liquid Chromatography (HPLC) isolations of the 1,25-dihydroxyvitamin D glycosides and work out methods that will allow us to assay blood and intestinal tissue levels of the glucuronides. HAI will also contribute material and supplies necessary to do the isolations, including use of its HPLC System. The disposables we will contribute to the project will be worth approximately $34,000 over the next two years. In addition, HAI also will contribute assays of the 1,25-dihydroxyvitamin D concentration in sera and tissue of the experimental mice. If we charged our typical fee for this service it would amount to about $14,000.

In conclusion, HAI is willing to contribute a total of $78,000 toward this project.

We can provide a list of equipment, which is available for this project if the need arose. We feel this project has a good chance of succeeding and even if it does not the scientific knowledge to be gained is exciting and will be of great use.

Proposal for "Grow Iowa Values Fund" Grant Program

Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines

PI: Brad Bosworth, DVM, PhD
Dept of Animal Science, 11 Kildee Hall, 515-294-7250, brad1958@iastate.edu
Co-PI: Ryan Vander Veen, BS
Dept of VMPM, 14 Kildee Hall, 515-294-5589, ryanvv@iastate.edu

Company Partner: Harrisvaccines, Inc. d/b/a Sirrah Bios, 17 employees, incorporated 2005, $1M plus annual sales, Contact: Jerry McVicker or Joel Harris, 2325 N. Loop Drive, Ames, IA 50010 515-296-3984

EXECUTIVE SUMMARY

Harris Laboratory has had two Grow Iowa Values Fund projects funded recently. The first project demonstrated replicon particles (RP) could induce an immune response in swine using a human influenza vaccine prepared by Alphavax (AVX). These were the first studies determining the effect of RP in pigs. The second project showed that RP technology can be useful in making a vaccine for prevention of porcine reproductive and respiratory syndrome (PRRS). Both of these studies, plus exclusive licenses from ISURF and AVX, have helped to create Harrisvaccines, Inc. d/b/a Sirrah Bios, a profitable Iowa company which employs 17 individuals at ISU Research Park facilities.

The AVX technology can be used to make both a replicon subunit (RS) and RP version of a vaccine. As a result Harrisvaccines operates as two entities. The Sirrah Bios division is currently selling a RS vaccine for PRRS under a Veterinary Client Patient (VCP) relationship (9 CFR §107.1) which does not require a USDA license, while the Harrisvaccines division is pursuing USDA licenses for RP vaccines for both PRRS and swine influenza virus (SIV) (H3).

Due to the occurrence of the 2009 Novel H1N1 influenza virus in humans and the numerous reassortant H1 viruses occurring frequently in swine, there is an urgent need for rapidly produced specific SIV vaccines which can protect against all these virus subtypes. The 2009 Novel H1N1 has caused over 15,000 infections and 99 deaths in humans thus far (May 29, 2009) and human to swine transmission has occurred in Canada. There are currently two additional H1 clusters of related SIV viruses occurring in swine in the U.S.: swH1β and swH1γ. Vaccination with one cluster subtype does not protect a pig against the other subtype. A vaccine prepared against the two current subtypes will not likely protect pigs against the 2009 Novel H1N1 and vice versa.

Currently available commercial swine vaccines do not protect against both the H1 subtypes and unlikely will protect against the 2009 Novel H1N1 virus. Because of the unique capability provided by AVX replicon technology, Sirrah Bios is in a unique position to quickly produce and sell RS vaccines for all subtypes of H1 and H3 influenza viruses. Simultaneously, the Harrisvaccines division can proceed with attaining USDA licensure for RP vaccines against all subtypes.

PROJECT DESCRIPTION

Technical Objectives

1. Create new RS and RP vaccines for 3 strains of SIV: 1) 2009 Novel H1N1 (A/California/04/2009), 2) swH1β, and 3) swH1γ
2. Evaluate immunogenicity of vaccines in pigs
3. Evaluate efficacy of vaccines in pigs in a vaccination-challenge model

Background and Technology

Swine influenza virus (SIV) is an RNA virus prone to mutation and recombination and thus is constantly changing (see Figure 1). Before 1997, there was only one subtype of SIV circulating in American swine herds, 'classical' H1N1. In 1997-98, there was a dramatic increase in seroprevalence and isolation of H3N2 SIV, leading to subsequent reassortant H1N1, H3N2, and H1N2 SIV subtypes. Currently, there are two clusters of H1 SIV circulating in U.S. swine: H1β (reassortant H1N1-like) and H1γ (H1N2-like). Current vaccines do not offer cross-cluster protection, and likely will not protect against the 2009 Novel H1N1 strain.

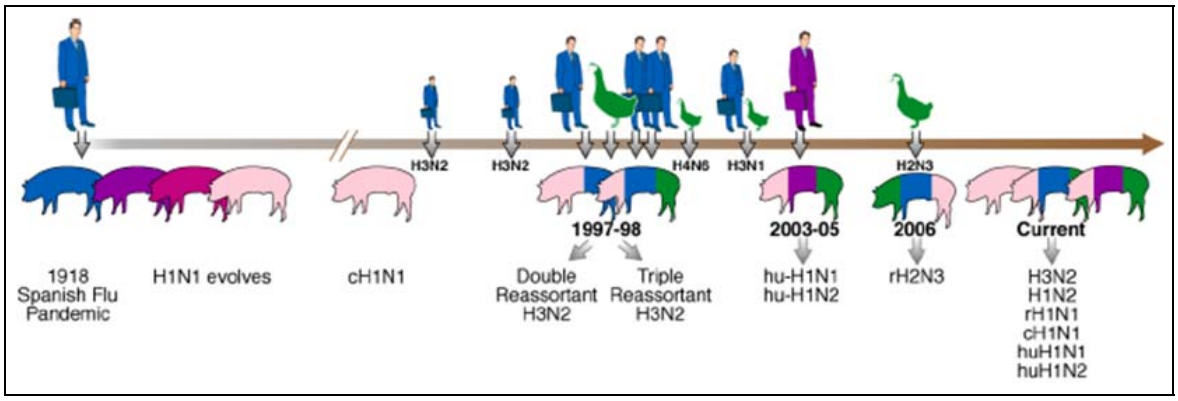


Figure 1. Epidemiology of SIVs in North America since 1918. Chronology of transmission events leading to reassortant viruses with genes from swine, human and avian influenza lineages. Swine virus lineage is color coded pink, avian lineage is coded green, human lineage is coded blue. Note, timeline not drawn to scale. *From:Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses: a North American perspective. Adv Virus Res. 2008;72:127-54.*

The AVX replicon technology uses the alphavirus Venezuelan Equine Encephalitis (VEE) virus as the basis for a vaccine vector. The technology involves the removal of components of the natural VEE virus, and replacement with an antigen from a targeted disease agent. Expression of this antigen induces an immune response against the disease of interest. The replicon technology platform merges subunit and live attenuated technologies providing a safe system of inducing protective immunity that mimics natural infection while producing numerous business advantages as well. Replicons can be created to express any antigen of interest (such as hemagglutinin (HA) from H1 or H3 viruses), thus making it an attractive candidate platform for influenza vaccination. With only a sequence of HA (rather than the entire virus), the HA gene can be synthesized in the laboratory. This gene is then inserted into the alphavirus replicon vector to produce

either RP or RS vaccines. This platform technology allows for rapid development and quicker turnaround time than traditional vaccines (weeks vs. months). Replicon particles express desired protein *in vivo* in the pig, whereas RS is made *in vitro* and adjuvanted protein is given as vaccine.

Our group has previous results demonstrating protection when pigs are vaccinated with the H3 RP. Vaccinated pigs had significantly lower viral loads detected in nasal swabs and BAL fluid than controls post-challenge. Gross lung lesions and body temperature were also lower in vaccinated pigs. Vaccinated pigs had high antibody titers as measured by HI assay, with mean titers peaking around 1:400. In a separate study, pigs vaccinated with a H3 RS vaccine developed HI titers ranging from 1:320 to 1:640 (≥1:40 is considered to be protective).

The importance of vaccination of swine against these endemic circulating SIV strains is highlighted by the recent outbreak of 2009 Novel H1N1 in humans worldwide. The studies proposed in this grant will provide evidence that both RP and RS vaccines are efficacious against circulating SIV strains, including the 2009 Novel H1N1

Work Plan

Replicons will be made expressing the HA genes from current H1 clusters currently circulating among US swine (H1β and H1γ) as well as 2009 Novel H1N1 strain. As stated, a replicon expressing triple-reassortant Clad IV H3 has already been made in our lab and shown to be protective as both a RP and RS vaccine. These replicons will first be evaluated for potential vaccine candidates based on an immunogenicity study. This first study will consist of 10 groups of 5 pigs each. Both RP and RS vaccine made from each replicon (H1β, H1γ, 2009 Novel H1N1, and H3) will be included, as well as commercial H1N1/H3N2 killed vaccine and negative control group. Immune response will be measured by the hemagglutinin inhibition (HI) assay, SIV ELISA, and western blot. For the HI assay, serum will be run against the other heterologous strains which were included in this study to see if there is cross-protection between H1 clusters and/or the 2009 Novel H1N1 strain. Titers obtained from this assay will also help determine challenge strain(s) to be used in the second study. If satisfactory results (HI titer ≥1:40) are seen the vaccines will be evaluated in a vaccination-challenge study.

Any challenge study using the A/California virus must be done in BL3 facilities, which are located at the National Animal Diseases Center in Ames, IA. All other challenge strains may be used in BL2 facilities located at Iowa State University. Assuming all vaccines induce significant HI titers, the challenge study design would be similar to the first immunogenicity study. For this study, however, there will be a total of 11 groups with 10 pigs each. Both RP and RS vaccine made from each replicon (H1β, H1γ, 2009 Novel H1N1, and H3) will be included, as well as commercial H1N1/ H3N2 killed vaccine, negative and positive control groups. Caesarean-derived, colostrum-deprived (CDCD) pigs will be used in both of these studies to assure freedom from influenza virus infection and to avoid maternal antibody interference. Vaccine efficacy in the challenge study will be measured by live virus titration and qPCR from nasal swabs and tracheal-bronchial wash, body temperature, HI and ELISA titers, western blots, and gross lung involvement.

Commercialization Plan

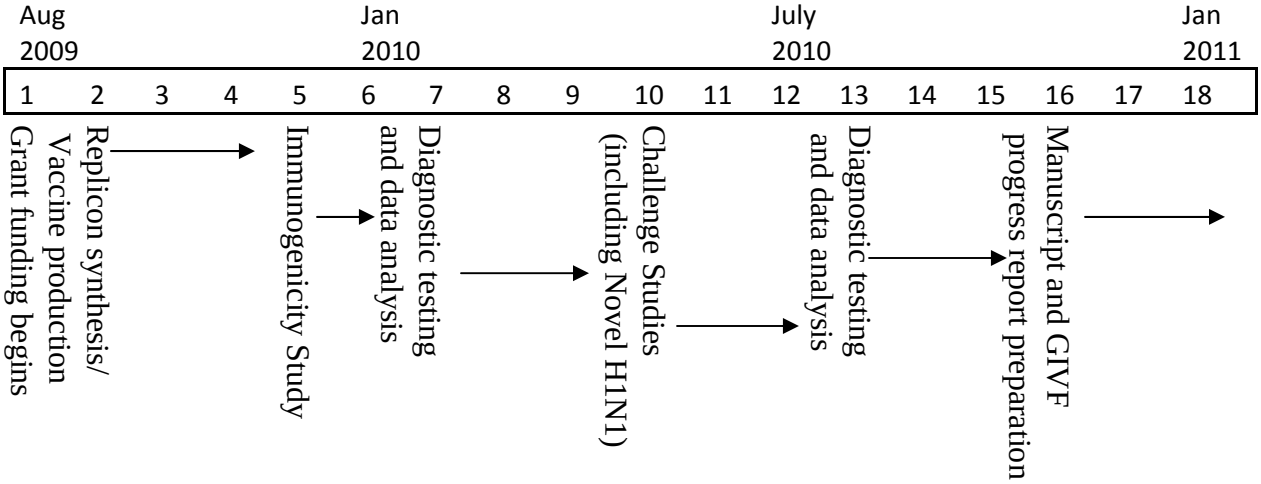
The global market for animal vaccines is $2.5 billion. The swine portion is $475 million (19%) and expected to grow to $730 million by 2013. The growth is due to a stronger focus on prevention, rather than treatment of disease. This is similar to what has already occurred in the poultry industry where vaccines represent about 80% of all poultry animal health sales. In North America, distribution is aided by a highly consolidated market where 34% of pig production is under control of the top 5 producers and 50% is under control of the top 20.

The current economic impact of SIV to the entire pork industry is estimated to be $200 million per year, creating a significant opportunity for an efficacious SIV vaccine. Pfizer, Intervet (Schering-Plough), Newport labs, Novartis, and MVP all have commercially available vaccine products for SIV. All of these vaccine products utilize traditional inactivated killed whole virus. None of these companies are in the process of manufacturing vaccines using a replicon technology. Existing commercial vaccines may offer protection against one but not all SIV strains that are effecting pork producers today and may take a year or more to bring to market. It is important to note that SIV has mutated into multiple strains and will continue to mutate. The ability to react quickly and produce new vaccines is very important. Harrisvaccines / Sirrah Bios will be able to successfully commercialize the SIV vaccine because of:

- The speed to market provided by the VCP (Veterinarian / Client / Patient) relationship. This strategy is not used by large producers because of their use of the traditional distribution channel.
- The speed to large scale production provided by the AVX replicon technology.
- The exclusive license to AVX replicon technology which allows for multiple strain coverage in a single dose, as well as a “combo vaccine” approach, which would reduce labor by providing multiple disease coverage in a single dose as well.
- Highly competitive cost of production for both RS and RP vaccine versions.

Currently Sirrah Bios employs 48 veterinarians licensed in 21 states, in addition to our staff of 17 at our Ames, IA headquarters. The veterinarians are a pathway for producers to seek out solutions to their PRRS and SIV problems. Sirrah Bios helps the veterinarians solve these problems. Harrisvaccines is in the process of USDA licensure for the RP technology to be used in Swine. SIV-RP will be the first product licensed, however, because of the multiple strains and the lack of cross protection, multiple products would derive from this licensure.

Timeline (18 months)



Budget (18 months)

CATEGORY	AMT REQUESTED	ISU COST-SHARE	Harrisvaccines, Inc d/b/a Sirrah Bios	TOTAL
Salaries		36,139	28,500	64,639
Benefits		9,830	6,270	16,100
Tuition			5,000	5,000
Personnel Sub-total		**45,969**	**39,770**	**85,739**
CDCD Pigs	96,466			96,466
Pig Per Diem	32,144			32,144
Lab Supplies	8,000		33,000	42,000
Diagnostic Services	10,000	31,200		39,250
Supplies/Services Subtotal	**146,610**	**31,200**	**33,000**	**210,810**
TOTAL	**146,610**	**77,169 (cash)**	**72,770 (cash)**	**296,549**

Budget Justification

Salaries	**Requested**	**ISU**	**Harrisvaccines**
Hank Harris (10% salary – 18 months)		22,647	
Brad Bosworth (75% salary – 18 months		13,492	
Ryan Vander Veen (50% salary – 18 months)			28,500
Benefits			
Hank Harris (27.2%)		6,160	
Brad Bosworth (27.2%)		3,670	
Ryan Vander Veen (22%)			6,270
Tuition			
Ryan Vander Veen			5,000
CDCD Pigs (cost of sows, surgical derivation, and rearing until 6 weeks of age-see quote from Struve Laboratory)	96,466		
Pig Per Diem (FY 2010)			
160 pigs x $4.10/day x 49 days	32,144		

Lab Supplies			
Replicon synthesis: $6,000 x 3 (gene synthesis, lab consumables)			18,000
RP and RS Vaccine Production (media, roller bottles, sterility tests, potency assays)			15,000
Misc. Lab Supplies (syringes, needles, blood tubes, pipettes, tips, euthanasia drugs)	8,000		
Diagnostic Services			
HI assay: 160 pigs x 4 viruses x 5 bleedings/pigs x $8		25,600	
SIV ELISA: 160 pigs x 5 bleedings/pig x $5		4000	
Histopathology: 160 pigs x $5/slide x 2 slides		1600	
In-house assays: (Live virus titrations, qPCR, western blots)	10,000		
TOTALS	**146,610**	**77,169**	**72,770**
TOTAL COST-SHARE (CASH)			**$149,939**
TOTAL AMOUNT REQUESTED	**$146,610**		

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FY10-5

1603 Enterprise Street
Manning
IOWA 51455

Date: 26 May, 2009

Estimate

Ryan VanderVeen

Sows and C-Sections	$ 36,618.00
Pigs to 6 wks of age	$ 57,760.00
Study Events	$ 1,584.00
Delivery	$ 503.74

Subtotal	$ 96,465.74
Tax	
Total	$ 96,465.74

Remittance Advice

Struve Laboratories
Attn: Jen
1603 Enterprise Street
Manning
IOWA 51455
(712)653-2125

Debtor:

Amount: $ 96,465.74

harrisvaccines

5/28/2009

Dr.Brad Bosworth
Iowa State University
Dept of Animal Science
11 Kildee Hall
Ames, IA 50014

Dear Dr. Bosworth,

We are excited for this opportunity to evaluate the efficacy of vaccines for swine influenza virus as described in your grant proposal **"Prevention of Swine Influenza: Commercialization of Replicon Particle and Replicon Subunit Vaccines".**

Harrisvaccines, Inc. agrees to supply in kind the replicon subunit (RS) and replicon particle (RP) vaccines as well as laboratory and technical support. The information gained from this project is crucial for further development of vaccines for prevention of respiratory diseases in swine.

We believe that the providing of efficacious vaccines for swine influenza virus will decrease the likelihood of pandemic influenza virus infections in humans.

Sincerely,

Jerry McVicker, PhD
COO
Harrisvaccines, Inc.

Joel Harris
COO
Sirrah Bios

6-1-09 **Proposal for "Grow Iowa Values Fund" Grants Program**

Transgenic Plant Resistance to Invertebrate Pests

PI: Bryony C. Bonning, Department of Entomology, 418 Science II Hall

Tel: 294-1989, Email: bbonning@iastate.edu

Co-PI: W. Allen Miller, Department of Plant Pathology, 351 Bessey Hall

Tel: 294-2436, E-mail: wamiller@iastate.edu

Company Partners: Pioneer Hi-Bred International, a DuPont Company, with headquarters in Johnston, Iowa: Pioneer has been providing growers with leading seed products for over 80 years. With annual sales of more than $4 billion dollars and over 8,000 people, Pioneer is a worldwide leader in discovering and commercializing novel genetic solutions for growers and users of seeds and grain products worldwide. Pioneer brand corn and soybeans are the market leaders in North America and Pioneer has a significant and rapidly expanding international seed business, providing services to customers in nearly 70 countries.

Contact: Dr. Gusui Wu, Research Director, Plant Protection, Pioneer Hi-Bred International, Inc.
7250 NW 62nd Avenue, P.O. Box 552, Johnston, IA50131 Johnston, IA 50131
Tel.: 515-270-3163
Fax: 515-270-3924
E-mail: GUSUI.WU@pioneer.com

EXECUTIVE SUMMARY

Invertebrate pests (insects and nematodes) cause devastating agricultural losses worldwide and are estimated to consume 15% of total agricultural output. Overall yield losses to food and fiber crops resulting from invertebrate pest damage in the U.S. alone are well over a billion dollars per year. Current management relies primarily on the use of chemical pesticides, the efficacy of which can be short-lived as pesticide resistance develops. Hence new tools for invertebrate pest management are essential for sustained and environmentally sound agricultural productivity. A single technology effective for control against multiple pests would have significant value when introduced into leading germplasm that has other major traits and attributes demanded by growers and the marketplace. We have developed a novel transgenic technology for plant resistance to aphids, and propose to test this technology for efficacy against a broad range of invertebrate pests, including economically important plant bugs, moth pests, and nematodes. This technology consists of a plant virus coat protein fused to a toxin that acts within the body cavity. The coat protein enters the insect body cavity, thereby delivering the toxin to its target site. We expect to demonstrate resistance to a broad range of invertebrate pests, thereby providing an environmentally benign alternative technology to chemical pesticides. The development of transgenic plants resistant to a broad range of invertebrate pests would contribute to environmentally sustainable pest management with reduced yield losses and chemical inputs for sustainable food and biomass production. This technology would confer significant economic benefit both to Iowa and to U.S. agriculture as a whole.

PROJECT DESCRIPTION

Technical Objectives We have developed a new transgenic technology for management of aphid pests (U.S. Patent 7,312,080. Plant Resistance to Insect Pests Mediated by Viral Proteins. W.A. Miller and B.C. Bonning). While this in itself is a significant achievement, this technology may have broader commercial application for other economically important pests including plant bugs and nematodes. The objectives of this proposal for research at ISU are to (1) test the resistance technology against a broad range of invertebrate pests, and (2) construct transgenic plants and determine the extent of pest resistance. Attainment of these goals will demonstrate the breadth of application of this new technology for invertebrate pest management. Our industrial partner, Pioneer Hi-Bred International, Inc., a DuPont Company, will then proceed with construction of transgenic soybean for commercialization.

Scope of the Work: Sap-sucking insects (order Hemiptera) including the pervasive aphids and plant hoppers, cause significant economic loss on a wide range of agricultural and horticultural crops. Current management of such insect populations depends primarily on application of classical chemical insecticides, with relatively few resistance genes available to plant breeders. We have developed a novel transgenic means for hemipteran pest control: we showed that the coat protein (CP) of an aphid-vectored plant virus, *Pea enation mosaic virus* (a luteovirus), can deliver an insect-specific toxin (ω-atractoxin-Hv1a) that acts within the aphid body cavity (hemocoel) (Miller and Bonning 2007). Aphid feeding on the recombinant CP-P-Hv1a fusion, either in membrane feeding assays or via transiently transfected plants (*Nicotinia benthamiana*), resulted in significant mortality of the pea aphid, *Acyrthosiphon pisum* and the green peach aphid, *Myzus persicae* (Fig. 1). While this strategy shows great potential for management of aphid pests, there are several questions that need to be addressed to clearly define the commercial prospects for this new technology. Specifically, the mechanism of uptake of CP into the aphid hemocoel is poorly understood, and hence it is unknown whether the CP-P-Hv1a fusion protein would also be effective against other invertebrate pests. It has been postulated that glycan (D-mannose) binding plays a role in plant virus uptake into the aphid hemocoel, in which case other invertebrates with D-mannose in the gut may be susceptible to CP-P-Hv1a. In addition, the toxin Hv1a alone has been shown to be orally active against moth pests and arachnids (spiders, mites) (Khan, Zafar et al. 2006; Mukherjee, Sollod et al. 2006). We propose to explore the extent to which CP-P-Hv1a can be applied for pest control, by testing the range of pests susceptible to the recombinant fusion protein, CP-P-Hv1a, and by constructing transgenic *Arabidopsis thaliana* for *in planta* bioassays.

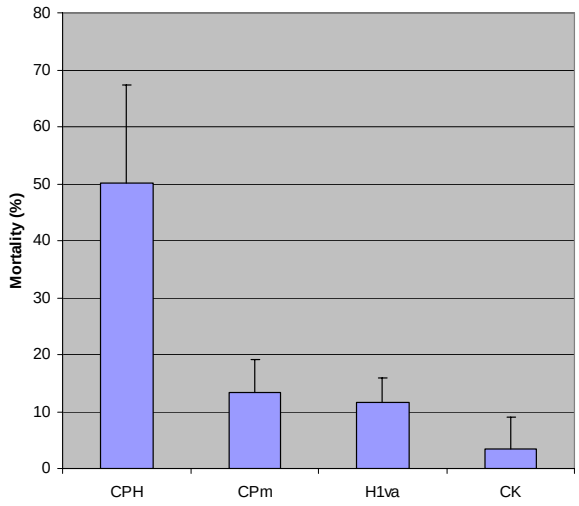

Fig. 1. Bioassay of *E. coli*-produced fusion proteins against the pea aphid by membrane feeding. Mortality (%) is shown for CP-P-Hv1a (CPH); CP-P-Hv1am, a fusion with a modified, inactive toxin (CPm); toxin alone (Hv1a), and 25% sucrose alone (CK).

Objective 1 ***Test efficacy of CP-P-Hv1a against invertebrate plant pests*** We will produce and purify recombinant CP-P-Hv1a in *E. coli* using the pGEX expression system (GE Life Sciences). We will conduct bioassays to assess the efficacy of CP-P-Hv1a against key invertebrate pests of significant economic importance, including the cotton aphid (*Aphis gossypi*), soybean aphid (*Aphis glycines*), plant bug (*Lygus spp.*), and tomato hornworm (*Manduca sexta*).

Objective 2 ***Assess resistance of transgenic Arabidopsis to invertebrate pests*** We will construct transgenic *Arabidopsis* expressing CP-P-Hv1a and test for resistance to pests that readily feed on this plant, including the green peach aphid (*Myzus persicae*), the tobacco hornworm (*M. sexta*), and soybean cyst nematode (*Heterodera glycines*).

Project Deliverables: Upon completion of these studies, we expect to have (1) more clearly defined the range of invertebrate pests that are susceptible to CP-P-Hv1a, and (2) demonstrated the efficacy of CP-P-Hv1a against invertebrate pests when delivered from transgenic plants. This research will provide the foundation for development of agronomically relevant transgenic plants (soybean) for agricultural use by our industry partner.

Benefits and Sustainability: Invertebrate resistant transgenic plants will benefit U.S. agriculture by enhancing agricultural productivity for both food and biofuels, and will reduce environmental contamination resulting from use of classical chemical insecticides for pest management. This technology would confer additional economic and environmental benefits as follows: (1) The proposed technology is less likely to impact nontarget organisms than use of chemical insecticides, which deleteriously affect all beneficial insects including insect natural enemies (e.g., lacewings and ladybugs that help reduce pest aphid populations), bees, and butterflies. Many chemical sprays can also impact vertebrate populations (Flickinger, Juenger et al. 1991). (2) Costs to the grower associated with application of chemical insecticides for protection against pests would also be avoided. (3) The use of fossil fuels required for transportation and aerial or ground spray application of chemical insecticides will be mitigated. (4) The efficacy of chemical insecticides can be short-lived with the rapid evolution of insecticide resistance (Devonshire 1989). In the event that insect resistance arises to the toxin, an alternative toxin with a different target site could be employed to overcome the resistance. The invertebrate resistance technology will provide a useful alternative tool to growers for pest management.

References

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Davis, E. L. and G. L. Tylka. (2000). "Soybean Cyst Nematode Disease." The Plant Health Instructor DOI 10.1094/PHI-I-2000-0725-01, from http://www.apsnet.org/education/LessonsPlantPath/SoyCystNema/.
Devonshire, A. L. (1989). Resistance of aphids to insecticides. Aphids, their biology, natural enemies and control. A. K. Minks and P. Harrewijn. Amsterdam, Elsevier. **C.:** 123-139.
Flickinger, E. L., G. Juenger, et al. (1991). "Poisoning of Canada geese in Texas by parathion sprayed for control of Russian wheat aphid." J. Wildl. Dis. **27**: 265-268.
Khan, S. A., Y. Zafar, et al. (2006). "Spider venom toxin protects plants from insect attack." Transgenic Res **15**(3): 349-57.
Marking, S. (2000). "New aphid attack." Soybean Digest: 12-13.
Miller, W. A. and B. C. Bonning (2007). Plant Resistance to Insect Pests Mediated by Viral Proteins. U.S. Patent 7,312,080.
Mukherjee, A. K., B. L. Sollod, et al. (2006). "Orally active acaricidal peptide toxins from spider venom." Toxicon **47**(2): 182-7.

Commercialization Plan

Business case for the project Based on preliminary technical data and the economic importance of invertebrate pests, this project warrants further development: Economic losses resulting from insect pest damage are second only to losses resulting from natural disasters. Among the most economically important invertebrate pests are aphids, plant bugs, and soybean cyst nematodes. Aphids affect almost all agricultural crops and invasive species such as the recently introduced soybean aphid have had a particularly severe impact on Iowa agriculture. In 2003, the soybean aphid *Aphis glycines* exceeded 3,000 aphids per plant, and cost farmers an estimated $80 million in Minnesota alone (Marking 2000; Associated-Press 2003). In Iowa, soybean yields were reduced by 32% compared to the 2002 season. In 2007, an estimated 50% of the nearly 8.5 million acres of soybean grown in Iowa was treated with a foliar insecticide targeting the soybean aphid at a cost of $68 million. Sap-sucking plant bugs within the genus *Lygus* (Miridae) are also significant agricultural pests. The tarnished plant bug for example feeds on more than half of all commercially grown crop plants. The soybean cyst nematode is a devastating pest of soybean worldwide. This nematode has resulted in annual losses of at least $500 million and has reduced yields by as much as 75% (Davis and Tylka 2000). Clearly, a plant resistance technology that confers resistance to multiple invertebrate pests would be of significant commercial and economic value for protection of agricultural yields.

Given that *there are currently no competing technologies for management of sap-sucking insect*s, this technology could result in Pioneer developing and commercializing new products that bring value to customers worldwide, thereby boosting the Iowa economy and benefiting both national and international agriculture. Pioneer is interested in licensing the patent for this technology, U.S. Patent 7,312,080 Plant Resistance to Insect Pests Mediated by Viral Proteins, W.A. Miller and B.C. Bonning, which is available for licensing, along with others that may result from the proposed research. The potential impact of this technology is considerable. We envisage the success of this technology to be comparable to that of transgenic plants expressing herbicide resistance (Roundup Ready) that are now widely used for management of weeds within the United States. In 2008, 92% of the soybean acres planted in the United States was transgenic. With a $10 technology fee per bag of soybean seed for the herbicide resistance trait, this amounts to generation of over $680 million per year for soybean seed companies in the United States. If the CP-P-Hv1a trait controls aphids and soybean cyst nematodes, a trait value similar to Roundup Ready is expected. As an additional benefit, reduced insecticide use will reduce environmental impacts and producer contact with potentially harmful chemicals.

ISU research capabilities: In the event that the fusion protein is not toxic to any of the invertebrates tested, funding will be sought by Bonning for directed modification of the fusion protein to broaden the host range based on the physiological basis for the lack of toxicity. Following development of agriculturally relevant transgenic plants (soybean) by Pioneer, Bonning will apply for funding from the USDA Biotechnology Risk Assessment Research Grants Program to examine the potential impact of the fusion protein on non-target, beneficial invertebrates such as aphid natural enemies (ladybugs, parasitoids, lacewing larvae). Such data would facilitate EPA registration of products carrying the toxin fusion system.

List of competitors Competitors for production of transgenic pest resistance in crops include Bayer CropScience, Dow Agrosciences, Monsanto, and Syngenta.

Budget

CATEGORY	AMT REQUESTED	ISU cost share (in kind)	Pioneer cost share (cash)	USDA cost share (in kind)	TOTAL
Salaries	45,675	6,360	45,675		97,710
Benefits	10,505	1,730	10,505		22,740
Graduate student stipends				19,570	19,570
Benefits				2,270	2,270
Tuition				4,466	4,466
Undergraduates					
Personnel Sub-total	$56,180	$8,090	$56,180	$26,306	146,756
Equipment	25,000		9,200		34,200
Lab Supplies	18,000		25,000		43,000
Field Supplies					
Other Supplies & Services	5,000		5,000		10,000
Travel	2,500		2,500		5,000
Publication	1,000				1,000
Miscellaneous			2,120		2,120
TOTAL	$107,680	$8,090	$100,000	$26,306	$242,076

Budget Justification

Personnel: Funds are requested for a postdoctoral research associate for a period of 24 months (benefits 23%, 3% pay increase for Yr 2). A two year funding period is requested due to the time taken for production of transgenic *Arabidopsis*.

Equipment: Funding is requested for a Percival PGC15.5 plant growth chamber. This estimate includes the 10% discount provided by Percival to ISU.

ISU Cost Share (in kind): Bonning will spend 6% time on this project (benefits 27.2%).

Pioneer Hi-Bred International Cost Share (cash): Funding provided by industry (subject to conditions specified in the supporting letter) will also include purchase of an Eppendorf refrigerated microcentrifuge (5417R: $8,000) and associated rotor ($1,200).

USDA NRI Cost Share (in kind): USDA NRI grant “Aphid-luteovirus interaction” B.C. Bonning and S. Liu (3-1-09 to 2-28-12).

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GIVF FY10-9

DuPont Agriculture & Nutrition
Pioneer Darwin Building
7100 N.W. 62nd Ave.
P.O. Box 1000
Johnston, IA 50131-1000
(515) 270 3200 Tel

May 22, 2009

Dr. Bryony C. Bonning
Department of Entomology
Iowa State University
418 Science II
Ames, Iowa 50011-3222 USA

Dear Dr. Bonning:

We understand that you are submitting a grant proposal to the Grow Iowa Values Fund ("GIVF") program entitled *Transgenic Plant Resistance to Invertebrate Pests*. Because Pioneer believes this subject has scientific, economic, and commercial merit, Pioneer is interested in providing scientific and monetary support should your grant be funded by GIVF.

Pioneer Hi-Bred International, Inc., a DuPont Company, headquartered in Johnston, Iowa, is a worldwide leader in discovering, developing and commercializing novel genetic solutions for growers and users of seeds and grain products worldwide. Pioneer's annual revenues exceed $4 billion dollars and Pioneer brand corn and soybeans are the market leaders in the North America. We have a significant and rapidly expanding international seed business.

Pioneer encourages the discovery and development of innovative science and technology throughout the global scientific community for crop yield improvement, crop protection against weeds, insects and environmental stresses, and crop utilization for industrial applications. Technical success in your project of *Transgenic Plant Resistance to Invertebrate Pests* would not only be of interest to Pioneer, but when combined with leading germplasm containing a suite of important traits, would also have significant positive economic and societal impact throughout the world.

Pioneer is willing to provide financial support for the proposed project in the form of a matching fund of US$100,000 for two year, subject to: (1) your success in obtaining funding from the GIVF program; (2) execution of an exclusive commercial license to Pioneer for U.S. Patent 7,312,080; and (3) execution of a research collaboration agreement between Pioneer and the ISU Research Foundation. Pioneer wishes you success in obtaining funding from the GIVF program and in conducting a successful project.

Sincerely,

Robert T. Giaquinta
Director, Technology
Transfer and Licensing

Proposal for "Grow Iowa" Values Fund Grant Program

Development of a Novel Genetic Test for Inherited Bovine Diseases and its application to tissues and embryos

PI: Patrick G. Halbur DVM, MS, PhD
VDPAM
2203 Lloyd Vet Med
(515) 294-6970
pghalbur@iastate.edu

Co-Investigators:
James K. West, DVM, MS
VDPAM-Embryo Transfer Unit
2416 Lloyd Vet Med 4-3837
jkwest@iastate.edu

Paul Plummer, DVM; DACVIM
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2426 Lloyd Vet Med 4-3837
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2630E Vet Med 4-1950
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Marianna Jahnke, MS
VDPAM- Embryo Transfer Unit
2428 Lloyd Vet Med 4-3837
marianna@iastate.edu

Company Partner:
Ames Center for Genetic Technologies
2711 South Loop Drive; Suite 4400
Ames, Iowa 50010
Contact: Melissa Madsen
Phone: 515-230-3947
mlmadsen@iastate.edu

Executive Summary

In recent years, the increasing awareness of the role of genetics in improving livestock efficiency and eliminating disease has resulted in a marked increase in demand for genetic information on breeding stock. Traditionally genetic testing has been conducted on samples from live animals (hair, blood, etc.) and has been performed for single genes of interest. This testing strategy is expensive for livestock producers because it requires both the cost of pregnancy (testing is done on live offspring) and the cost of running multiple tests. Current tests have limited capacity for multiplexing to decrease cost. Advances in molecular techniques may now allow for the development of next generation genetic testing modalities that address both of these issues by reducing the sample size necessary for analysis (i.e. embryo biopsies) and allowing for a multiplexed genetic testing platform that is easily and quickly customizable at a fraction of the cost. This proposal focuses on an innovative collaborative effort between the Ames Center for Genetic Technologies, Inc. (ACGT), the ISU College of Veterinary Medicine Embryo Transfer Unit (ISU ET), and the ISU Veterinary Diagnostic Laboratory (ISU VDL) to develop, validate and apply this novel testing platform for animal genetics. *Our goal with this project is to validate and scale-up the genetic testing platform (for production efficiency and disease traits) on clinically derived animal tissues and embryo biopsy sections and commercialize it through the ISU VDL.*

There will be two concurrent studies to achieve the outcomes: (1) demonstrate application of novel test platform on clinically derived livestock tissues (blood, hair, semen, etc.) and (2) adapt embryo biopsy techniques and validate efficacy for use in genetic testing and subsequent freezing of the biopsied embryo. These two aims are complementary and sufficient for full realization of a comprehensive genetic testing platform; however, the outcome of each aim is independently useful for commercialization of the technology. The completion of these 2 aims will fulfill the goals of the project to support ACGT, ISU ET unit, ISU VDL and Iowa cattlemen.

Research Plan and Management

Ames Center for Genetic Technologies, Inc. (ACGT), the ISU CVM Embryo Transfer (ISU ET) unit, and the ISU Veterinary Diagnostic Laboratory (ISU VDL) have partnered to validate and apply this novel testing platform for animal genetics. ACGT is a start-up company resulting from the ISU Entrepreneurial Program, focusing on genetic testing methods and their commercialization. They have **a unique testing platform that allows for rapid, cost-effective, and flexible panels for genetic tests** that can be applied to both genetic diseases and animal production efficiency genes. **It requires very small amounts of DNA making testing of embryo biopsies a realistic possibility.** The ISU ET unit provides comprehensive embryo transfer services that currently include embryo sexing technology done with the use of polymerase chain reaction. **This unit was responsible for 58% of all the transfers of biopsied embryos reported nationwide** in the most recent survey published by the AETA. While the majority of these transfers were on fresh (un-frozen) embryos the ISU ET unit has preliminary results suggesting that freezing biopsied embryos can provide pregnancy rates of 50% which is acceptable for commercialization of the technology. The final member of the team, the ISU VDL will be the distributor of the testing services. The ISU VDL is one of the highest volume veterinary diagnostic laboratories in North America. It currently provides timely, accurate, broad scope (bacteriology, virology, pathology, toxicology, and serology) diagnostic services in animal diagnostic testing on approximately 45,000 case submissions per year that come to the ISU VDL from Iowa and 14 other states, Canada, Mexico and Australia. The ISU VDL is the only laboratory in Iowa that is fully accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and it is also one of 12 core laboratories in the National Animal Health Laboratory Network (NAHLN).

Aim 1 – Tissue and genetic testing platform

The Ames Center for Genetic Technologies Inc. (ACGT) is a genetic testing laboratory focused on improving current technologies in genetic identification. By employing proven new chemistries and analyses, the ACGT is able to profile genetic samples more rapidly at a reduced cost, enhancing competitiveness and market share. Current industry methods for genetic identification use gel-based separation techniques that require extensive maintenance and are limited in sample size and speed of analysis. The solid phase platform employs novel intellectual property, some of which has been licensed from Iowa DNA Technologies (IDT) of Coralville, Iowa to enhance accuracy, reduce maintenance costs, increase speed of analysis and provide an overall enhancement in detection limits. The later is particularly important with samples of limited quantity or with degraded samples. Unlike current technologies that depend on relative measurements, this technology uses a unique “binary” or “on/off” signal that discriminates genetic profiles in extremely small quantities of DNA, a significant advancement in the field.
ACGT has chosen to initially deploy their genetic testing technology in the field of animal genetics, focusing on bovine genetic diseases including Bovine Leukocyte Adhesion Deficiency (BLAD), Complex Vertebral Malformation (CVM), the newly identified Arthrogryposis complex, and sex determination. Significant difficulties with development of this platform are not anticipated since this platform has already been validated by ACGT using human SNPs. Development of the kit, which is the goal of Aim 1, will include design of unique PCR primers and oligonucleotides necessary to correctly genotype each of the genetic traits of interest to cattle

producers, validation of the multiplexed assay conditions, and testing of the assay sensitivity and specificity based on blood, hair, semen and embryo samples from various cattle lines. This technology will be designed as a multiplexed system that provides a flexible platform to accommodate customers' needs. This custom genetic testing allows the ISU VDL to meet the consumers' specific needs without providing unnecessary information they must pay for. Additionally the flexibility of this genetic testing platform will allow the ISU VDL to quickly and inexpensively add markers as new genetic diseases are identified.

Aim 2 – Genetic testing of embryos and subsequent freezing

The embryo biopsy technique will be adapted and validated to achieve acceptable pregnancy rates following post-biopsy genetic testing and freezing. In addition to providing the needed tissues to validate the genetic testing platform on embryonic tissue, this component will provide sufficient data to demonstrate that samples for genetic testing can be safely derived from embryonic tissue without significantly altering the viability and pregnancy rate of the biopsied embryos.

Approximately 30 donor cows will undergo embryo recovery by conventional technique until 120 embryos grade 1 and 2 are obtained. A biopsy will be taken from bovine compacted morula and blastocysts, using a micromanipulation technique which does minimal damage to the inner cell mass. Soon after the micromanipulation all embryos will be frozen. In order to evaluate the viability of the genotyped frozen-thawed embryos, all embryos will be transferred to recipients. All biopsies will be provided for samples to the ACGT to validate the test platform on embryonic tissue. We expect to achieve pregnancy rates greater than 50% and that genetic testing of the embryonic tissue would match the genetic tests of the calves. **The ultimate goal of this project is to demonstrate acceptable performance and genetic testing on embryos resulting in significant cost savings for cattle producers of Iowa.**

Commercialization

Market Size: Available data suggest that significant commercial markets exist for genetic testing of both live animals and embryos (the two markets targeted by this proposal). Live animal numbers in Iowa alone are currently estimated at 216,000 dairy cows housed on 2000 dairy farms and an additional 1,070,000 beef cows housed on 25,000 farms. Furthermore, given that the novel testing platform proposed will significantly reduce the cost of testing by as much as 5-10-fold compared to other commercially available tests, we anticipate that a large amount of additional testing could be attracted from out-of-state test submissions. This would result in increased cash flow into the Iowa economy. Equally exciting are the potential markets on the embryo testing side of the proposal. Currently embryo testing is not commercially available and given the high cost of the embryo transfer procedure and the marketing advantage associated with knowing the genotype of embryos, it is expected that a significant portion of the industry would be interested in genetic testing of embryos. Data collected in 2007 and presented by the American Embryo Transfer Association indicated that in the North Central region of the United States (including Iowa) there were a total of 19,361 cows undergoing ET in that single year with collection of 117,634 transferable quality embryos (TQEs). Nationally the data indicated that 54,080 cows, both beef and dairy, underwent the procedure in 2007 with a total of 332,496 TQEs.

Industry Impact: The Iowa dairy industry provides more than 26,000 jobs and annually contributes in an excess of $1.5 billion to Iowa's economy. Iowa has 12,866 jobs directly related to the beef cattle industry with cash receipts from cattle and calves in 2005 accounting for $2.425 billion in the state economy. These genetic tests will significantly benefit all Iowa cattle producers and the state's cattle industry by decreasing costs associated with maintaining the pregnancies of genetically diseased animals and allowing for selection of genetically superior seed stock.

Expected consumers and benefit: ET consumers utilize the technology to increase their marketing options for the offspring of highly desirable cattle. This is achieved by either directly selling the frozen embryos or by using ET to increase the potential number of live offspring that can be marketed. As the number of genetic diseases increases rapidly (3 new diseases characterized in the last 6 months) many of these valuable cattle may be identified as "carriers" for one or more of these diseases. This results in the situation where the offspring are at high risk of being either carriers or affected in which case their value is significantly diminished (carriers) or completely lost (affected) as breeding stock. The ability to genotype embryos removes any potential risk to a buyer purchasing frozen embryos and leads to significant savings to the seedstock producer who no longer needs to invest time and money into implanting diseased embryos into cattle only to discover the condition nine months later after the calf is born.

Expected Market Growth: Growth in the overall genetic testing market is approximately 15-20% annually. The animal testing market is newly emerging so initial growth should be much higher. Since this is a new field, there are no standard targets in the animal market and our competitors are small, focused laboratories. The work described in this proposal would greatly enhance our marketing strategy since it would demonstrate a "one of a kind" service not available using other testing platforms that require larger DNA samples. Basic genetic testing in the food animal market currently cost $40-$150 for single markers. Our platform will be for multiple markers and we expect to test 5 to 10 markers per sample. We expect our testing charges to be approximately $5 per marker depending on the volume and the number of markers analyzed. With these highly competitive prices both the ISU VDL and ACGT should quickly gain a substantial amount of the market share. The genetic testing services would likely become a separate section within the VDL and employ 6-12 upper level technicians and research associates. To supply the ISU VDL with the necessary kits to meet demand, ACGT would likely need to employ 6-12 people in scientific development and production.

Within the state of Iowa and the Midwest region, the ISU VDL has potential to be a leader in the genetic diagnostic testing market because of well established, long term relationships with livestock producers and the veterinarians who serve them. The ISU VDL receives an average of 7,500 case submissions per year from 14,500 cattle producers for diagnosis and surveillance of diseases and toxicoses. Approximately 75% of the bovine submissions are associated with beef cow-calf breeding herds or dairy operations. Of these, the majority of the dairy operations and approximately 50% of the beef operations utilize advanced reproductive techniques such as artificial insemination or ET and thus are likely customers. Additional labs in the genetic testing business include Pfizer Animal Health and Merck-Meriel Animal Health Labs (which hold the largest market share), Agrigenomics, DDC Animal DNA Services and SGS Brookings.

Budget

CATEGORY	AMT REQUESTED	ISU COST-SHARE	ACGT COST-SHARE	INDUSTRY 'Y' COST-SHARE	TOTAL
Salaries		18,000	20,000		38,000
Benefits		5,360			5,360
Graduate student stipends					
Benefits					
Tuition					
Undergraduates					
Personnel Sub-total					
Equipment					
Lab Supplies	34,500				34,500
Field Supplies including donor and recipient cow boarding	24,000	16,000			40,000
ISU VDL payment to ACGT for platform assay development		20,000			20,000
Travel	6,000				6,000
Publication					
Marketing	5,000				5,000
Miscellaneous					
Armbrust Endowment		10,000			10,000
TOTAL	69,500	69,360	20,000		$158,860

Budget Justification:

ISU and ACGT Cost Share

- ISU salary and benefit cost share is $15,360 for Dr. West and Marianna Jahnke.
- ACGT salary and benefit cost share is $20,000 for Drs. Madson and Gunning.
- Approximately 50% of the proceeds from the Armbrust Professorship Endowment for 2010 ($10,000) will be applied to this project for recipient care.
- Up to $20,000 from ISU ET unit service account will be applied towards costs of boarding of donor and recipient cows.
- $20,000 cash from ISU VDL to ACGT for assay development.

Requested from Grow Iowa Values Fund

- $34,500 for lab supplies for embryo recoveries, biopsy, freezing and transfer
- $6000 for travel to recover and transfer embryos
- $5000 for marketing of the new services through the ISU VDL
- $20,000 for donor cow care and boarding and other expenses associated with embryo flushing and biopsy

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Paradocs
embryo transfer, inc.
121 Packerland Drive • Green Bay, WI 54303
Email: pdocset@sbcglobal.net

AMERICAN EMBRYO TRANSFER ASSOCIATION CERTIFIED

Scott W. Armbrust, D.V.M.
Phone (920) 498-8262
Phone/Fax (920) 498-8181
Mobile: (920) 639-0405

June 1, 2009

To Whom It May Concern:

The recent mapping of the bovine genome has identified many important cattle breeding genetic markers. Identifying these markers has given the cattle/agricultural industry the ability to consistently and accurately test for recessives, production, type, as well as health and fitness trait genetics.

A.I. companies will be able to utilize this new genetic tool to look beyond traditional pedigree selection and double their accuracy in sourcing young bulls that enter sire testing programs. Ultimately, genomic predictions will improve a dairy/beef herd's genetic worth.

The accessibility and use of genomic testing is crucial to U.S cattle breeders, buyers and research facilities allowing for the rapid progressive improvement of genetic intervals.

A biopsy of embryonic cells will give the cattle breeder a look into the future and increase the probability of success. A laboratory's ability to genomic test an embryo for recessives and animal production traits before implanting to carry a nine month pregnancy, is the key to insuring the shortest generation interval in elite cattle genetics .

Sincerely,

Scott W. Armbrust, DVM

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AMES CENTER FOR GENETIC
TECHNOLOGIES, INC.

2711 South Loop Drive
Suite 4400
Ames, Iowa 50010

T 515-230-3947

June 1, 2009
Dr. Patrick Halbur
Professor and Chair, Department of Veterinary Diagnostic and Production Animal Medicine
Executive Director, ISU Veterinary Diagnostic Laboratory
Iowa State University College of Veterinary Medicine
1600 S. 16th Street
Ames, Iowa 50011

RE: Development of a Novel Genetic Test for Inherited Bovine Diseases and its application to tissues and embryos

Dr. Halbur:

I am writing to express my strong support for the research program that the Iowa State University Veterinary Diagnostic Laboratory and Embryo Transfer Group is submitting to the "Grow Iowa" Values Fund Grant Program for support.

Ames Center for Genetic Technologies, Inc., has a continuing interest in the successful implementation of a novel, cost effective genetic testing platform for the cattle industry that will be marketed through the Iowa State University Veterinary Diagnostic Laboratory. Additionally, our genetic testing platform will complement the embryo transfer technology being developed and validated through this project. Expanding cost-effective genetic testing options to prescreen embryos prior to transfer represents an important step forward in the difficult business of increasing margins in a tight production animal market.

We would like to offer you an in-kind support of scientist salaries (Drs. Gunning and Madsen) of $20,000 over the course of the grant period.

I look forward to hearing that you have been successful in obtaining support.

Sincerely,

Melissa L. Madsen, President

Proposal for "Grow Iowa Values Fund" Grant Program
Naturally Controlled Gelatinization of Corn Starch

PI: David Grewell, Agricultural and Biosystem Engineering, Iowa State University, dgrewell@iastate.edu

Company Partners
($5,000 cash, $25,000 in-kind):

Grain Processing Corporation
$5,000 cash, $20,000 in-kind
Muscatine, IA
Perminus Mungara
(perminus_mungara@grainprocessing.com)

Emerson Electric Corporation
$5,000 in-kind
Danbury, CT
Jon Piasecki
(Jon.Piasecki@emerson.com)

Has this or a similar proposal been submitted in previous years to the GIVF competition?
NO

EXECUTIVE SUMMARY

The main thrust of the proposed work is to characterize, demonstrate, and scale-up the use of high powered ultrasonics to partially and controllably gelatinize corn starch for an application. Grain Processing Corporation (GPC), in collaboration with Dr. Grewell's laboratory, has produced bench-scale data that show ultrasonics can be used to partially gelatinize corn starch. This new processing method would allow GPC to market a new product with estimated annual sales of $10 million. This is based on the fact that this product would be made without the use of aggressive chemistries and sold as a "natural" product. In recent years, there has been a push by consumers for food or food ingredients made without use of synthetic chemicals. These foods or ingredients with the "natural" label have been the fastest growing product segment in the food industry with an estimated annual growth of 20%. These pre-cooked starches are used in food products such as gravies, soups, fruit pie fillings, puddings, dressings, dips, and pizza toppings, among others.

Annual production is estimated to be 15 million lb/year, requiring 20 continuous flow ultrasonic systems from Emerson Electric Co. (EMR). The combined sales of the new starch and ultrasonic systems represent a multi-million dollar sales increase in the state of Iowa. In this project, ISU researchers will work closely with GPC to optimize processing conditions. In more detail, ISU will treat samples at various conditions and GPC will characterize the effects giving ISU researchers direct and immediate feedback. Based on optimized conditions, ISU, GPC and EMR will work together to design and implement this technology into GPC's production facilities.

It is important to note that the PI has been successful with previous commercialization projects, including projects with Creative Composites (Ankeny IA-letter attached) and SoyWorks (IL).

Technical Objectives

This work will develop, demonstrate, and scale-up a novel, efficient technology for partially and controllably gelatinizing No. 2 yellow dent corn starch for food applications. In recent years, there has been a push by consumers for food or food ingredients made without use of synthetic chemicals. These foods or ingredients with the "natural" label have been the fastest growing product segment in the food industry with estimated annual growth of 20%. Natural instant starch (pre-cooked) is one such example of an important food ingredient. Instant starch is a cold water thickening starch that has many applications in food processing. These pre-cooked starches are used in food products such as gravies, soups, fruit pie fillings, puddings, dressings, dips, and pizza toppings, among others. In these applications, instant starch is preferred over conventional cooked starch because it delivers more stable products and increases efficiency during food processing.

Currently, methods for producing instant starches either involve use of harsh chemicals or energy intensive processes. For instance, in some methods native starch is modified with synthetic chemicals, such as propylene oxide, in order lower viscosity and achieve lower gelatinization temperatures. Other conventional methods for producing instant starches, such as extrusion and drum drying, are energy intensive. The proposed method will allow for convenient partial gelatinization of corn starch without application of harsh chemicals and using low energy intensity. This chemical-free method will allow the subsequently made product to be marketed as "natural." Researchers at GPC and Iowa State University (ISU) have shown that partial bulk (not limited to surface gelatinization) gelatinization is possible with high ultrasonic fields in a liquid medium (water). The work has been demonstrated with both batch and continuous flow systems. The partially gelatinized starch does not build viscosity and remains a pumpable fluid at room temperature. In addition, this product has the potential of being turned into a "natural" instant starch. Based on the existing market size, GPC projects $10 million of new annual sales for this Iowa-based company.

Ultrasound is sound waves at a frequency above the normal hearing range of humans (> 15-20 kHz). When the ultrasound wave propagates in a medium such as a liquid or slurry, it produces cavitation [1,2] and acoustic streaming [3]. The cavitation generates powerful hydro-mechanical shear forces in the bulk liquid [4], which disintegrates nearby particles by extreme shear forces. The main benefit of streaming in corn slurry processing is mixing, which facilitates the uniform distribution of ultrasound energy within the slurry mass, convection of the liquid, and dissipation of any heating that occurs.

Ultrasonication has been applied widely in various biological and chemical processes. The use of high-powered ultrasound has been used to enhance starch-protein separation in a wet-milling operation. Ebringerová et al. [5] used ultrasound to aid in the extraction of active xylan and heteroxylan from corn cobs and corn hulls, respectively. Wood et al. [6] studied the effects of ultrasonic treatment on ethanol fermentation from mixed office paper. The authors demonstrated that sonication of recycled paper increased ethanol production by as much as 20%. In addition, Dr. Grewell's laboratory has shown that starch granules for both no. 2 yellow dent and sugary-2 corn can be gelatinized at room temperature [7].

In order to employ this technology commercially, characterization and optimization are needed, including tool design and parameters. The goal of this project is to work with GPC and EMR to realize this technology's potential by optimizing and characterizing the technology, performing continuous flow studies (scale-up) and implementing the technology in GPC production facilities. Once the process is optimized, the PI and key personnel from EMR will assist GPC with full-scale equipment design, selection, and setup. This will occur through communications as well as plant visits to selected plant locations. It is important to note that EMR offers a commercially available ultrasonic system that has standard piping flanges for attachment. This assures that existing GPC

facilities can be retrofitted with ultrasonic systems. In addition, these systems have been proven in other industries, such as the municipal waste treatment industry.

Scope of Work

The work will be divided into three tasks: 1) optimization of bench-scale systems, 2) scale-up and continuous flow characterization, and 3) commercialization of the technology. These tasks are detailed below:

TASK 1: Bench-scale Optimization of Controlled Gelatinization of Corn Starch

In the initial phase, preliminary results from GPC and ISU will be further characterized to develop empirical models between processing parameters, such as energy density and amplitude to gelatinization. The degree of gelatinization will be characterized with cross polarization microscopy and thermal analysis methods, such as differential scanning calorimetry (DSC). GPC will conduct the characterization studies. Samples will be shipped to GPC (overnight) for studies and GPC will provide the results directly to ISU.

TASK 2: Scale-up of Controlled Gelatinization of Corn Starch

In order to gain insight into the scale-up, tests will be conducted in a continuous flow sonication chamber. A single and multiple donut horn system with a 3.0 kW (per horn), 20 kHz power supply will be employed. The reaction mixture will be pumped at various pressures and flow rates (1, 5, 7, 10, and 15 gallon/min). The parameters to be studied include:

- Amplitude: A (5 levels) 0, 3, 6, 9, and 12 μm_{p-p}
- Pressure: P (5 levels) 5, 20, 40, 60, and 80 psi
- Flow rate: Q (5 levels) 1, 5, 7, 10, and 15 gallon/min

Again, GPC will characterize the effects of the ultrasonic treatment and give ISU direct feedback.

TASK 3: Commercialization of Controlled Gelatinization of Corn Starch

Once the optimum conditions are identified, the PI and key personnel from EMR will work with GPC to design a full-scale demonstration plant. This will occur through communications and consultations as well as production plant visits. The PI and key personnel will make at least 2 trips to a plant within the United States for 3 days/visit. In addition, EMR will assist with these installations at their own expenses.

Materials

GPC will supply all required feedstocks, including starch, for the entire duration of the project.

Characterization

GPC will characterize the processed feedstocks and provide direct feedback to the PI on the processes, modeling, and optimization. This will include, but not be limited to, differential scanning calorimetric (DSC), polarized optical microscopy and spray drying results.

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1 Suslick, K. (1990). Sonochemistry. *Science*, **247**, 1439-45.

2 Flint, E. B., and Suslick, K. S. (1991). The temperature of cavitation, *Science*, 253, 1397-1399.

3 Faraday, M. (1831). On peculiar class of acoustical figures; and on certain form assumed by groups of particles upon vibrating elastic surfaces. *Phil. Trans. Roy Soc*. London, 121, 299.

4 Kuttruff, H. (1991). Ultrasonics Fundamentals and Applications. Elsevier Science Publishers Ltd., Essex, England.

5 Ebringerová, Z., Hromádková, J. H., Alföldi, and Ibalová, V. (1998). The immunologically active xylan from ultrasound-treated corn cobs: Extractability, structure and properties. *Carbohydr. Polym.*, **37**, 231-239.

6 Wood, B. E., Aldrich, H. C., and Ingam, L. O. (1997). Ultrasound stimulates ethanol production during the simultaneous saccharification and fermentation of mixed waste office paper. *Biotechnol. Prog*., **13 (13)**, 323-327.

7 S. Khanal, M. Montalbo, J. van Leeuwen, G. Srinivasan, D. Grewell, "Ultrasound Enhanced Glucose Release from Corn in Ethanol Plants", *Journal of Environmental Science and Technology*, *Biotechnology and Bioengineering*, 98(5) 978-985

Commercialization Plan

The commercial objective of this work is to develop a new product for a medium-sized Iowa-based company (GPC) with a global reach. The new processing method developed uses ultrasonics to partially gelatinize corn starch and allows GPC to market a new product with estimated annual sales of $10 million (GPC estimate). This is based on the fact that this product would be made without the use of aggressive chemistries and would be sold as a "natural" product.

Because preliminary data at ISU has demonstrated that 15 gal/min of corn starch slurry can be treated with a single ultrasonic system; therefore, the projected annual sales of 15 million lbs with an assumed 30% wt. total solids, would require 20 units. This generates a new market for EMR. With an estimated cost of $10,000/ultrasonic unit, the GPC's payback period on the investment will be less than 12 months. New sales of 15 million lbs of starch annually equates to new utilization of approximately 370,000 bushels of corn annually. The corn has a value of about $1.4 million/year at today's price of $3.95/bushel and creates a new market for Iowa corn producers.

Project Deliverables

1. Develop a novel method, with accurate control, to partially gelatinize corn starch.
2. Commercialize resulting novel processing technologies and increase sales.

Budget

Category	Requested amount	ISU Cost Share	GPC	EMR	Total
Faculty	$3,206	$4,778	$1,572		$9,556
Scientist, Post doc	$21,828				$21,828
Salaries & Wages Total	$25,034	$4,778	$1,572		$31,384
Fringe Benefits Total	$5,892	$1,300	$428		$7,620
Equipment	$0				$0
Travel	$0		$3,000		$3,000
Materials & Supplies	$500				$500
In-kind (Productization)			$20,000	$5,000	$25,000
Total Cost	**$31,426**	**$6,078**	**$25,000**	**$5,000**	**$67,504**

Budget Justification

Salaries and Wages:

Principal Investigator: 50% salary support for one month during the summer. Postdoctoral Research Associate: Hired for 12 months (50%) at a cost of $21,828.

Benefits: Fringe benefits are estimated as 27.2% and 23% of salary for the PI and the postdoctoral research associate, respectively.

Materials and Supplies: All materials and chemistries will be provided by GPC. The project will be charged $500 for overnight shipping of materials.

Travel: Trips to visit collaborating companies will be charged to the project at a cost of $3,000. All travel by corporate sponsors will be paid directly by the companies.

Cost Share: Attached are selected letters of support detailing the industrial contribution. Cash cost share includes: $5,000 from GPC. In-kind cost share includes: $5,000 from EMR, $20,000 from GPC, and $6,078 from ISU as one month of cost share for one month of salary for the PI. These in-kind cost share amounts will be used for technical support, chemicals, and feedstocks (namely starch), product development, and commercialization of the proposed products.

Schedule This project will have a total duration of approximately 12 months as detailed below:

Task	Q1	Q2	Q3	Q4
1) Bench-scale Optimization				
2) Continuous Flow Optimization				
3) Production Demonstration				

Where Innovation Comes Naturally
GPC

May 27, 2009

David Grewell, PhD
Agricultural and Biosystem Engineering
100 Davidson Hall
Iowa State University
Ames, IA 50011
515-294-2036
FAX 515-294-2255

Dear Dr. Grewell,

On behalf of Grain Processing Corporation, We are highly supportive of the Grow Iowa Values Fund proposal by Dr. Grewell entitled "Natural Controlled Gelatinization of Corn Starch."

Grain Processing Corporation (GPC) is a major supplier of carbohydrate ingredients for the food industry and sees the tremendous economic potential of supplying "Natural" line of starches. The consumer-driven natural line of food ingredients is the fastest growing throughout the world and GPC is well placed to become a major player in this field by developing high quality products that GPC is known for.

In the last few months, GPC had an opportunity to work in your lab and use ultrasonication equipment to test the concept of controllably gelatinizing native starch to a level that is amenable to post treatment processing. This preliminary work showed tremendous potential for being adaptable and scaleable for industrial production of partially gelatinized starches. However, the work also revealed a need for process optimization and product characterization. These results will give GPC a better position in an industrial set-up. GPC is fully committed to working with you to realize successful industrial adaptation of this processing method. GPC agrees to support with $5,000 in cash and $20,000 of in-kind support in terms of technician and laboratory support.

Additionally, successful development of natural instant starches resulting from this unique technique has ability to generate over $10M/year in sales to this Iowa based company.

ISU and GIVF will be instrumental in helping develop this processing method. Also, a successful commercial launch of a product resulting from this technique fulfills the mandate of GIVF. This proposal is an excellent example of how academic-industrial collaborations can be leveraged with State Funds in order to create novel methods or new products that benefit the State of Iowa. In addition, this project will help satisfy the consumer need for "naturally" processed food ingredients.

GPC looks forward to the opportunity of working with you.

Sincerely,

Frank Barresi, PhD, Senior VP, Research & Development

Perminus Mungara, PhD, Research Scientist, Research & Development

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GIVF FY10-26

May 31, 2009

Dr. David Grewell
Agricultural and Bio-Systems Engineering
Iowa State University
100 Davidson Hall
Ames, IA 50011-3232

Subject: Letter of support for the project on "Bio-fuels Unit Operations Course Development"

Dear Dr. Grewell,

It is my great pleasure to participate in "**Naturally Controlled Gelatinization of Corn Starch**" for Grow Iowa Value Funds. As you know Branson Ultrasonics is a division of Emerson Electric which has great interest in developing technologies relating to sono-chemistry and in particular to the food processing industry. We feel that this is a large market opportunity for Emerson's core technologies and help us remain competitive in a struggling economy.

Branson will happily provide technical support during installation and equipment design/selection up to $5,000. With success of the research, Branson would like to help commercialize this growing market.

I strongly support the proposed research under "**Naturally Controlled Gelatinization of Corn Starch**". If you need any further information, please feel free to contact me.

Yours Sincerely,

Peter J. Kelch
Director of Advanced Engineering
Branson Ultrasonics

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Aron Fleischmann
Engineer
Creative Composites
1451 NE 69th Place
Ankeny, IA 50021

June 1, 2009

Dr. David Grewell
Agricultural and Bio-Systems Engineering
Iowa State University
100 Davidson Hall
Ames, IA 50011-3232

Subject: Letter of support

To Whom It May Concern,

As you know we were part of an ongoing GIFV project and are very encouraged by the results of this work and are currently testing commercial designs for future products. Dr Grewell has been very helpful in formulation development, testing and product design. He and his group are always very responsive and willing to respond at a moments notice. They have supplied us invaluable information on our current product as well as the new proto-types allowing us to make engineering and marketing plans regarding product design. These new designs will give us a competitive edge relative to our competitors' products. Dr. Grewell's team has lead us through these new formulations and designs and it has been a great pleasure working with them.

Please feel free to contact me directly for any further details.

Yours Sincerely,

Aron Fleischmann

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.

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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

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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

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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
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procopg@ccf.org

Proposal for "Grow Iowa Values Fund" Grant Program

Title: Testing of lead PK compounds in preclinical animal models of Parkinson's disease.

Principal Investigator: **Anumantha Kanthasamy, Ph.D. Distinguished Professor, BMS, ISU, IA 50011, 515-294-2516; akanthas@iastate.edu**

Collaborator: **George A Kraus, Ph.D. Director, IPRT Administration, ISU, Ames, IA 50011. 515-294-8902; gakraus@iastate.edu**

Company partners: **PK Biosciences Corporation (since 2006; 4 employees)**
Vellareddy Anantharam, Ph.D., Vice President
2501 N. Loop Drive, Suite 1600, Ames, IA 50010,
515 450-4836; info@pkbio.com

EXECUTIVE SUMMARY

Parkinson's disease (PD) is a major debilitating neurodegenerative disorder characterized by a selective degeneration of nigral dopaminergic neurons in the brain. PD affects more than 1.5 million people in the U.S. with over $2.5 billion spent annually for pharmaceuticals. The existing treatment approach for PD is symptomatic and fails to prevent the progression of the disease. Development of neuroprotective agents to delay progression of the disease is the subject of active research, patent applications and clinical trials. Lack of understanding of the molecular mechanisms underlying the disease severely hinders the development of neuroprotective drugs for PD. Fortunately, we have identified a key apoptotic cell death pathway in PD cell culture and animal models that involves aberrant activation of a novel protein kinase-C, namely PKCδ, and inhibition of the kinase protects the dopaminergic neurons, suggesting that PKCδ is a promising therapeutic target for PD. This work led to the filing of two U.S. patent applications in 2005 (patent pending: SN: 11/262,677 and 11/479,173) with ISURF. Additionally, we established the biopharmaceutical company PK Biosciences Corp., in Ames to further develop novel neuroprotective strategies for treatment of PD. In 2008, PK Biosciences, in collaboration with Dr. Kraus' organic synthesis group, secured SBIR phase-1 funding from NIH to synthesize and characterize a library of PKCδ small molecule inhibitors (PK analogs). This led to the identification of three lead PK compounds with excellent neuroprotective activity in PD cell culture models. The next logical step of this very promising project is to test the lead PK analogs in well-established preclinical animal models of PD to determine *in vivo* neuroprotective efficacy. Funding from GIVF will be used to demonstrate the feasibility of the study in PD animal models, and will provide preliminary data for a SBIR-Phase-II application. Thus, the requested GIVF funding will support this promising drug discovery project at its critical juncture to transition from SBIR-phase-I to SBIR-phase-II in developing an effective neuroprotective drug for treatment of PD. The ultimate success of the proposed neuroprotective technology can be expected to create many new opportunities in Iowa, including economic and job growth and a viable biotechnology industry.

Background and Rationale: Parkinson's disease (PD) is a major progressive neurodegenerative disorder characterized by the cardinal motor symptoms of rigidity, bradykinesia, tremors, and postural instability. A pathological hallmark of PD includes a significant loss of dopaminergic neurons in the substantia nigra leading to a dramatic depletion of dopamine in the striatum. The etiology of PD cases has been estimated to be >95% sporadic and <5% familial. PD incidence increases with age, with the mean age of onset around 55 years. Early onset PD cases have been linked significantly to genetic factors. The degenerative process of PD has been proposed to be influenced by genetic susceptibility, environmental neurotoxin exposure, mitochondrial respiratory failure, excessive free radical injury, excitotoxicity, and aging [1-9]. **Neuroprotection and PD**: The existing treatment approach (primarily levodopa and dopamine receptor agonists) for PD aims to control symptoms but fails to prevent the progression of the neurodegenerative process and produces severe side effects including dyskinesia (the inability to control muscles). The discovery of levodopa for the treatment of PD represents one of the most remarkable success stories in the history of medicine. However, the drug confers only symptomatic relief of what remains an inexorably progressive neurodegenerative disorder. Hence, novel neuroprotective agents designed to interfere with the basic pathogenic mechanism of cell death in PD are clearly needed. Several lead compounds representing different classes of pharmacological agents have been explored for neuroprotective potential in PD but the clinical trials have not been encouraging. We have in the past five years performed translational research based on solid mechanistic studies aimed at identifying a small molecule kinase inhibitor of PKCδ with greater neuroprotective efficacy and a high therapeutic index for PD. Scheme-1 summarizes the overall progress to date and the long term goal of our economic development in the neuropharmacology area.

Scheme 1:Time-line

2000-2004
TRANSLATIONAL RESEARCH
Discovery of a novel therapeutic target for Parkinson's disease

2005
Invention disclosure to ISURF

2006
1. OIPTT files patent application (SN: 11/262,677 and 11/479,173)
2. Birth of PK Biosciences Corp. in Ames, IA

2007
PK Bio submits SBIR phase 1 grant
PK Bio affiliates with ISU Research Park

2008
1. PK Bio awarded NIH SBIR Phase I for testing PK analogs in cell culture PD models.
2. ISU Technology commercialization fellowship awarded to one of the PKBio founder

2009
1. Identification of lead PK analogs for testing in a preclinical animal PD models.
2. Business plan completed.
3. Negotiating licensing option for ISURF patents.

2009-2011
1. Preclinical animal studies
2. Generate funding from GIVF, SBIR phase 2 & MJ Fox foundations

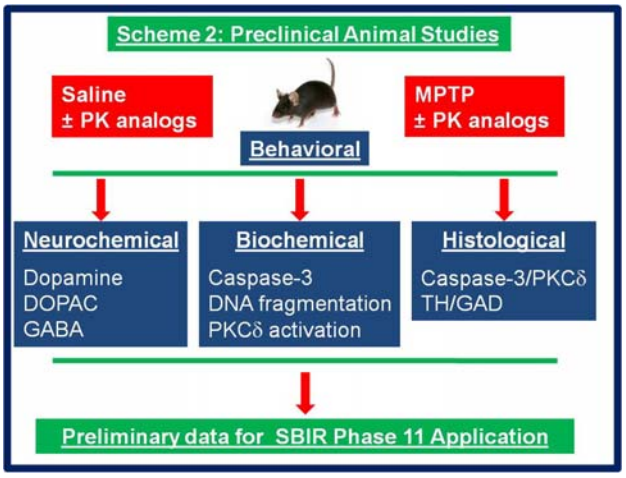
2010 & beyond
1. Raise $$$ Venture Capitalists & Angel Investors
2. Explore biopharmaceutical partners to promote human clinical studies

Research Design: *Hypothesis: Novel PK analogs will be effective in preventing neurodegenerative processes in a preclinical model of Parkinson's disease*

Specific Aim: To determine the potential neuroprotective effects of the lead PK analogs in the MPTP-induced preclinical animal models of Parkinson's disease Using SBIR phase-I funding, we screened our PK chemical library consisting of first, second and third generation PK analogs in the well-characterized cell culture model of PD known as mesencephalic dopaminergic neuronal cells (N27 cells) [10-17]. Among the first generation analogs tested, analog PK08102 most effectively protects against MPP^+-induced apoptotic cell death. Results obtained with subsequent screening of the second and third generation analogs

suggested that PK08202 and PK09301 most effectively prevent MPP^+-induced neurotoxicity. Verification of the neuroprotective effect of selective and potent PK compounds in a whole animal model of PD is the next logical step. From the results obtained from cell culture studies, we propose to test the potential neuroprotective effect of PK compounds PK08102 and PK09301 in the well-characterized C57 black mouse model of MPTP dopaminergic neurotoxicity. The overall plan is outlined in the Scheme 2. The following section describes the experimental design and methods for the proposed studies.

Characterization of the neuroprotective efficacy of the lead novel compounds in the MPTP treated murine model of PD.



C57 black mice, at postnatal week 12-14 (approx. 25 g), will be arranged by weight and randomized into control, MPTP and MPTP plus PK analog treated groups. Each group will have 20 animals (10 animals for neurochemical/biochemical and 10 animals for immunohistochemical studies). We will test two doses of each analogs and use total of 200 animals for the studies. Animals will be injected with MPTP at a dose of 25 mg/kg (i.p.) once a day for five days to induce Parkinsonism. The drug treated groups will receive once daily 1, 3 or 10 mg/kg (p.o.) of each PK compound (PK08102 and PK09301). The PK compound treatment will begin along with the MPTP treatment and will continue for six days post MPTP-treatment. Seven days post-MPTP treatment, animals will be subjected to behavioral, neurochemical and biochemical studies.

***Behavioral testing of animals*:** Since chemical-induced neurological deficits have traditionally been diagnosed by clinical behavioral evaluation, we propose to evaluate motor signs in MPTP-treated mice that reflect difficulty in initiation of ambulatory activity and changes in ambulatory activity patterns by measuring the locomotor activity with a Versamax computerized activity monitoring system (Accusan, Columbus, OH). Following the behavior measurements, animals will be sacrificed and neurochemical/biochemical and histopathological changes in the brain will be assessed.

***Neurochemical/biochemical measurements*:** Brains from animals designated for neurochemical/biochemical measurements will be removed and then striatum and nigral tissues will be separated. We will measure dopamine and its metabolites (DOPAC, HVA) from one half of the tissue using an HPLC-electrochemical procedure, and the other half will be used for biochemical analyses. These include immunoprecipitation PKCδ kinase assays and Western blot analyses of cleaved caspase-3.

Immunohistological analysis of nigral brain tissue: The second set of animals will be used for immunohistological studies as described previously in our publication [19]. After the treatments, animals will be perfused transcardially with 4% paraformaldehyde and 40 micron brain sections will be processed for immunohistochemical studies and evaluated for TH, GAD, microglia and astroglial activation, and caspase-3 cleavage. All methods will be performed as described previously in our lab [11, 14, 16-21].

Time line and extramural funding plan: We request 18 months of funding for the proposed studies. The proposed animal studies are time consuming and labor intensive. The 18 month time window will allow generation of sufficient preliminary data for submission of a strong SBIR phase-II application in order to secure the funding. In phase-II, the focus will be on optimization of a lead PK drug candidate, efficacy studies in a primate model and preclinical toxicological evaluation.

Commercialization Plan and Potential Economic Impact of the Proposal: Parkinson's disease (PD), a devastating neurodegenerative disorder affecting several million people worldwide, inflicts a tremendous social and economic burden on modern society. In 2004, the global neurological degenerative disease (NDD) pharmaceutical market was valued at $5.7 billion, of which PD treatments accounted for $2.5 billion. The total NDD market achieved a compound annual growth rate of 23% in 2000-2004.[1] Recent studies estimated that Parkinson's disease costs approximately $5 billion in the U.S. alone, and over $25 billion per year globally [22-25]. The existing treatment approach (levadopa and dopamine receptor agonists) for PD is symptomatic not only fails to prevent the progression of the neurodegenerative process but it also produces severe side effects including dyskinesia. Quite simply, no drugs are currently available to effectively treat progressive neuronal cell death in PD. A neuroprotective drug for PD would have a huge market value. The average medication cost per patient is estimated at $2,500 a year, and surgical procedures typically cost up to $100,000 per patient. The current worldwide market for the treatment of Parkinson's disease is estimated to be approximately $1.5 billion. This proposal represents translational research based on solid mechanistic studies aimed at identifying a small molecule kinase inhibitor of PKCδ with greater neuroprotective efficacy and high therapeutic index in animal models of PD. At the end of the project, we will have generated animal data required for a collaborative partnership between Iowa State and PK Biosciences Corp,. The company aims to translate mechanistic studies of key protein kinases (PK) into therapeutic strategies for treatment of neurodegenerative disorders. The company was awarded the SBIR phase-I award to test novel PK analogs in cell culture models of PD. The results from the SBIR-I study warrant extension of study to preclinical animal models. The company is currently negotiating a licensing option with the PKCδ-based neuroprotection technology (U.S. patent pending: serial numbers 11/262,677 and 11/479,173) from OIPTT at Iowa State. The GIVF will also enhance the value of IP. PK Biosciences received GIVF two years ago to develop a PKCδ peptide based gene therapy technology for PD. We reported encouraging results from the study and will pursue this technology further once PK Bioscience completes the licensing agreement with OIPTT. The ultimate success of the proposed neuroprotective strategy can be expected to create many new opportunities in Iowa, including economic and job growth and a viable biotechnology industry.

Our PK neuroprotective technology was ranked as one of the top neuroprotective approaches by a Foresight Science & Technology™ Company's review, which was initiated by NIH. According to the review, there is only one competitor Teva Pharmaceutical Industries, which recently markets Azilect (rasagiline) for PD and generates annual sales of $1 billion. Azilect is not a mechanism-based drug and it shows only moderate activity in slowing the disease's progression. The long term effect of the drug is not known. We expect to capture similar sales of our drug when finally approved by FDA.

CATEGORY	AMT REQUESTED	ISU COST-SHARE	PK Bio COST-SHARE	TOTAL
Salaries Vellareddy Anantharam (Investigator) (10%) $12K/yr Research Assistant	9,000 30,000	9,000 VA salary (NIH) 30,000 AGK salary		18,000 30,000
Benefits	8,900	8,900 AGK salary		17,800
Graduate Student stipends	22,000	22,000 GAK salary		
Personnel Sub-total	**69,900**	**69,900**		**139,800**
Equipment Refrigerated centrifuge /Incubators	8,000	2,000 Drklab	6,000 PKBio	16,000
Supplies & Service Animals and Animal care:	15,000	15,000 AGK salary		30,000
HPLC-Neurochemical analysis	9,000	9,000 Drklab		18,000
Brain Histology and Immunocytochemical studies	9,000	7,000 Drklab	2,000 PKBio	18,000
Chemicals for synthesis	5,000	5,000 Dr GAK salary		10,000
Biochemical reagents	10,000	8,000 Drklab	2,000 PKBio	20,000
Travel	1,200	1,200 AGK salary		2,400
Publication	1,000	1,000 AGK salary		2,000
Miscellaneous				
TOTAL	**128,100**	**118,100**	**10,000**	**256,200**

Budget Justification: Please detail the planned expenditures and indicate the nature of sources of cost-shared funds (i.e. cash or in-kind).
Cost-shared Funds: The equal matching fund will be used from

AGK: Prof Anumantha G Kanthasamy salary savings (34% 56,100)
GAK: Prof George A Kraus's salary savings (15%, $27,000).
VA: Dr. Vellareddy Anantharam's salary savings (10% $9,000)
PKB: PK Biosciences facilities usage ($10,000)
Drklab: Dr. Kanthasamy's lab facilities usage ($26,000)

Equipment: A refrigerated centrifuge and incubator is requested for brain tissue samples.
Chemicals, Biochemicals and Radioligands: A number of biological reagents and other normal lab reagents are required for the project.
Histology and immunohistochemistry supplies: Slides, coverslips, Permount, slide boxes, Tissue-tek, Histo Freeze, paraformaldehyde, 1^o antibodies, 2^o antibodies, ABC complex, etc..
Animal studies A significant amount of the budget requested is for animal purchase, maintenance and animal studies.

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