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
VDPAM
2426 Lloyd Vet Med 4-3837
pplummer@iastate.edu

Rodger Main, DVM, PhD
V DL
2630E Vet Med 4-1950
rmain@iastate.edu

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

CONFIDENTIAL
GIVF FY10-18

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

CONFIDENTIAL
GIVF FY10-18

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

GROW IOWA VALUES FUND GRANT COMPETITION
COLLEGE REVIEW FORM

PI Name: Patrick Halbur

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

College Ranking **2 of 8**

Recommendation: **Fund X** **Fund if Possible ___** **Do Not Fund ___**

SECTION I: TECHNICAL MERIT (60% of recommendation)

Low 1 2 3 4 5 High

Considerations:

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

Justification:

REVIEWER 1. It was not made clear why the genetic testing would not be simply performed on the dam/cow and sire/bull as opposed to performing the testing on the embryo. Would the embryo testing be used as a "confirmation" of the breeding fidelity? It would have been useful had the authors provide an example or two of the genetic disease(s) that would be targeted or that may have recently been detected. While a minor point, there was no discussion as to how long the embryos would be frozen.

It would also have been useful to include some blinded samples from animals with a known genetic defect in order to assess the sensitivity and reliability of the technology.

The research team is well qualified to complete the tasks described in the time frame allotted.

Budget seems appropriate.

REVIEWER 2. Proposal has merit- will need to acquire adequate case numbers; have embryos

- Merit is smaller testing sample versus DNA and more tests
- Key will be how testing affects success of birth rate
- Advantage is access to testing material at ISU versus other companies that perform testing
- The PI (Halbur) has no background in genetic testing. The PI should be Dr. West unless there is COI. VDL (PI) role is marketing.
- Budget- No role is given in budget justification for salaries: Madson (Madsen- president?) and Gunning; what is their role and need? Budget overall reasonable

SECTION II: BROADER IMPACTS (30% of recommendation)

Low 1 2 3 4 5 High

Considerations:

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

Justification:

REVIEWER 1. There would seem to be good potential for the ISU VDL to partner with the AGCT. Should this technology be developed, there would seem to be a market for other animal breeders such as those with the AKC. There seems to be a real problem with genetic defects in many highly inbred dogs.

Considering the demands on agriculture to meet the food needs of consumers, it will be important to maintain healthy food and fiber animals especially as the population grows.

It was not clear how ISU students would be engaged in this project.

Other than potentially applying for an SBIR/STTR, it was not clear to what federal funding agencies the authors would apply for grants.

REVIEWER 2. What are PI and CO-PIs relation to this company?

- If the PI is involved with the company that is partnering, ISU makes sure they have a COI management plan in place before they receive the funding and will identify an alternate ISU PI to provide oversight for the project.
- Less likely future federal funding; proposal will lead to revenue center for VDL and possibly the company; depends on MOU
- Leverage for federal funding not addressed
- Impact high for ISU-VDL reputation if successful.
- Minimal student impact; impact highest for producers

SECTION III: COMMERCIAL POTENTIAL (10% of recommendation)

Low 1 2 3 4 5 High

Considerations:

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

Justification:

REVIEWER 1. Based on the information provided and the credentials of the individuals involved, it would seem that the commercialization of this genetic testing approach is fairly high. It would seem that the application of new IP would derive from the team making use of published findings by others looking to identify novel genetic traits.

REVIEWER 2. Information scant on what company is and does- Ames Center for Genetic Technologies. Web search – minimal information. Appears they have testing platform

- Company credentials not stated but web states 2 employees and annual sales of $76,000 which seems minimal. Can this company sustain this research? Role?
- Can have large impact if primary source of cattle genetic testing; this should be further researched http://www.igenity.com/
- Per above, what will Merial's role be?
- Capturing markets beyond Iowa will be key to success since cattle numbers high in regional states – Beef/Nebraska; Dairy/Wisconsin
- Testing may be more expensive than proposed unless the State of Iowa via VDL will subsidize. Platform proposed may keep cost down. Cost/benefit key in this industry

Proposal Number: FY10-18

PI Name: Patrick Halbur

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

College Ranking:

College Rating:

Commercial Recommendation: **Fund If Possible**

Commercial Potential:

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

Yes

Please comment on the above:

Ames Center for Genetic Technologies, Inc.

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

No

What is the probability of commercial success:

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

Yes

Please comment on the above:

Pfizer Animal Health, Merck-Meriel Animal Health Labs, Agrigenomics, DDC Animal DNA Services and SCS Brookings. The ISU VDL would receive the diagnostic kits from ACGT. This project is unique in that it will benefit a recent Ames startup (ACGT), and also an ISU unit which sells service to cattle producers.

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

Yes

Please comment on the above:

The private marketplace will shift to take advantage of a new demand and the opportunity may be short lived. A department at ISU will gain new competitive advantage.

Commercialization will involve both a private entity which will utilize an ISU Dept. for initial distribution. A question about exclusivity, or a period of exclusivity, is not clear.

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

Intellectual Property Evaluation

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

   If Yes:

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

   b) What is the IP/patent status?

   c) What is the licensing status?

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

   (please explain)

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