Development of a Rapid and Portable Beet Curly Top Virus Detection System for Enhanced Agricultural Disease Management

California Department of Food and Agriculture Grant Proposal

A. Cover Sheet

1	Project Title	Development of a Rapid and Portable Beet Curly Top Virus Detection System for Enhanced Agricultural Disease Management
2	Organization	Alveo Technologies
3	Type of Business	Small Start Up Business
4	Project Leader	Slava Elagin 1000 Atlantic Ave, Suite 114, Alameda, CA 94501 Cell: (513) 504-0993 Email: slavae@alveotechnologies.com
5	Organization	Alveo Technologies
6	Duration	8-12 months
7	Estimated Cost	CFDA (BCTVCF): $150,000 Alveo: $350,000
8	Agreement Manager	Rixun Fang 1000 Atlantic Ave, Suite 114, Alameda, CA 94501 Cell: (650) 380-3908 Email: Rixun.fang@alveotechnologies.com

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B. Executive Summary (not to exceed two pages):

Alveo Technologies, a biotech company specializing in point-of-care molecular diagnostics, is seeking a grant from the California Agricultural Department to support the research and advancement of the portable microfluidic device developed by Alveo Technologies, with the aim of transforming it into a rapid and easy-to-use tool for the detection of Beet Curly Top Virus (BCTV) in agricultural settings.

BCTV is a pathogenic plant virus of the family *Geminiviridae*, containing a single-stranded circular DNA. It is known to have caused serious disease in crops such as sugar beets (*Beta vulgaris*), tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), and beans (*Phaseolus vulgaris*), leading to significant yield losses and economic damage (1,2,3). The current detection methods, such as PCR-based technologies, are time-consuming, expensive, and impractical for on-site monitoring due to the requirement of specialized equipment and trained personnel (3,5). Furthermore, these methods rely on DNA purification using sophisticated extraction techniques, which adds complexity and cost to the detection process. They also lack sensitivity to detect different BCTV strains and species, limiting accurate identification. Additionally, there is a pressing need for a universal sample preparation method that can be applied to various plants and insects susceptible to BCTV.

To address the challenges mentioned, our project aims to develop a portable microfluidic device specifically designed for the rapid detection of BCTV in agricultural settings. Building upon our expertise in molecular diagnostics, we will optimize the existing microfluidic device developed for molecular diagnostics to overcome the limitations of current methods. Our device will incorporate innovative technologies that eliminate the need for specialized equipment and trained personnel, enabling on-site testing and providing immediate results.

Alveo has already developed and commercialized a microfluidic system for pathogen detection, which consists of 3 main components: (1) the be.well Analyzer; (2) the be.well cartridge; (3) the be.well Mobile App that connects via Bluetooth (Figure 1). A key technical innovation of our project is the development of a portable microfluidic. device tailored specifically for BCTV detection. This will be accomplished by designing Loop-Mediated Isothermal Amplification (LAMP) primers specific to the BCTV virus. The device will integrate multiple cutting-edge technologies, including sample pretreatment on the cartridge, isothermal amplification of the pathogen's nucleic acid target, and signal detection using our patented Alveo electrical impedance detection technology.

Additionally, we will integrate a universal sample preparation method suitable for various plants and insects into the device. This innovative approach involves an integrated, user-friendly disposable cartridge system that simplifies the testing process. The cartridge will contain all the necessary chemistry, fluidics, and patented technologies for one-step sample preparation and real-time detection of multiple pathogens, including BCTV. By utilizing low-cost, disposable test cartridges, we ensure convenience and affordability for agricultural professionals.

The cartridge offers multiplexed testing capabilities, allowing for the detection of a broader range of plant pathogens while maintaining high selectivity. Results will be delivered in less than

20 minutes, facilitating prompt decision-making and intervention measures. To enhance usability and data management, the device will connect to a mobile phone application via Bluetooth. This connection will enable data analysis, remote access to experts, and streamlined data management, tracking, and monitoring.

The success of the project will be evaluated based on several criteria. These include the successful development and optimization of a sensitive assay capable of detecting various BCTV strains and species, the development and implementation of a streamlined sample preparation method, and the establishment of a systemic approach for detection, tracking, and monitoring the virus's spread. By achieving these objectives, the device will enable farmers and agricultural professionals to promptly identify and control BCTV outbreaks, minimizing crop losses and economic damage.

The primary beneficiaries of this project will be agricultural professionals, including farmers and field workers, who will benefit from the portable microfluidic device's rapid and accurate BCTV detection. By providing a portable and user-friendly solution for BCTV detection, our device will enable early intervention, accurate diagnosis, and improved disease management. It will empower them to make informed decisions and take timely intervention measures, safeguarding crop yield and minimizing economic losses.

In summary, our project aims to develop a portable microfluidic device tailored specifically for the rapid detection of BCTV in agricultural environments. By leveraging innovative technologies and integrating a universal sample preparation method, we strive to provide a rapid, user-friendly and affordable solution that revolutionizes BCTV detection. By empowering agricultural professionals with this innovative tool, we aim to enhance disease management strategies, promote sustainable agriculture, and ensure the resilience of our food systems.

C. Justification.

BCTVCP Mission and Research Priorities:

This research project aligns directly with the BCTVCP Research Priorities and contributes to the mission of advancing the environmentally safe and agronomically sound use of insecticide or pesticide materials. By developing a portable microfluidic device for the rapid detection of BCTV in the context of agricultural environments, the project addresses the critical need for improved disease management strategies. The technology will enable early and accurate BCTV detection and disease diagnosis, allowing farmers to implement targeted control measures and reduce usage on insecticide materials. By providing a user-friendly and on-site testing solution, the project supports the mission of the BCTVCP to promote sustainable agricultural practices.

The project acknowledges the potential health effects associated with pesticide use, particularly with regards to long-term exposure. Certain pesticides, including Pyrethroids, neonicotinoids, and organophosphates, have been found to have diverse impacts on human health. Prolonged exposure to these pesticides can affect the nervous system, cause skin or eye irritation, disrupt the hormone or endocrine system, and even have carcinogenic properties (6). Therefore, it is of utmost importance to take measures to mitigate the risks associated with pesticide exposure. The project also aims to address research priorities by focusing on early diagnosis of the disease and implementing measures to minimize economic losses.

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The proposed project directly contributes to addressing the unknown long-term health effects associated with pesticide exposure. By developing a reliable and rapid detection method for BCTV, the project aims to reduce unnecessary pesticide use. Accurate and early diagnosis will prevent misdiagnosis and subsequent ineffective pesticide applications, saving time, expenses, and reducing the risk of continued plant decline. This approach aligns with the goal of promoting environmentally safe and agronomically sound practices by minimizing pesticide usage and its potential adverse impacts.

Furthermore, the project builds upon related research conducted by Alveo in the field of molecular diagnostics. The success and validation of the be.well™ COVID-19 Test, a rapid molecular test utilizing electrical impedance sensing (EIS) and isothermal nucleic acid amplification technology (8), provide a strong foundation for the proposed BCTV assay. Leveraging this expertise, the project aims to adapt the existing platform for BCTV detection, ensuring robustness for viral target detection. The project will contribute to expanding our scientific knowledge about the point-of-site molecular diagnostics for plant diseases, specifically early detection methods for BCTV and their impact on reducing pesticide overuse and misuse from farmers, therefore reducing agroecological and environmental damage, and economic losses.

By addressing BCTVCP Research Priorities, mitigating the risks associated with pesticide exposure, and utilizing prior research successes, the project presents a comprehensive and innovative approach. It aligns with the mission of the BCTVCP to advance the environmentally safe and agronomically sound use of insecticide materials, while also promoting sustainable agricultural practices. Ultimately, the project's outcomes will help improve disease management practices, reduce the need for excessive insecticide use, and enhance overall agricultural sustainability.

Impact:

The development of an easy-to-use handheld device that can be used in the field for sensitive and early identification of BCTV will have significant agronomic, economic, environmental, and societal implications on a local, regional, and statewide basis.

Agronomic impact:

1. Rapid and accurate diagnosis: The handheld device will enable farmers to quickly and accurately identify BCTV-infected plants in the field. This early detection capability will facilitate timely interventions and targeted control measures, improving disease management strategies and minimizing the spread of BCTV within and between fields.
2. Enhanced crop health: By providing farmers with a user-friendly tool for early BCTV detection and identification, the handheld device will contribute to maintaining the overall health of the crops. Prompt actions based on accurate diagnosis will prevent extensive damage, reducing yield losses and ensuring higher crop quality.

Economic impact:

1. Yield protection: The sensitive and early identification of BCTV through the handheld device will help farmers protect their crop yields. By detecting infections at their early stages, farmers can implement appropriate measures to control the spread of BCTV and

minimize yield losses. This will contribute to maintaining their productivity and economic return.

2. Cost savings: The handheld device's ability to provide accurate and rapid BCTV diagnosis will reduce misdiagnosis and unnecessary pesticide use. By optimizing the use of insecticide materials, farmers can save on input costs, improving their economic efficiency. Additionally, early detection and intervention can prevent extensive crop damage and potential crop failure, further safeguarding farmers' investments.

Environmental impact:

1. Reduced pesticide usage: The handheld device's sensitive identification of BCTV will help reduce the need for broad-spectrum insecticides. By accurately pinpointing BCTV-infected plants, farmers can implement targeted control measures, minimizing the reliance on chemical pesticides. This reduction in pesticide usage will contribute to the preservation of beneficial insects, biodiversity, and ecological balance in agricultural ecosystems.
2. Soil and water conservation: The decreased application of insecticides associated with the use of the handheld device will reduce the risk of soil and water contamination. By minimizing pesticide usage, the device promotes soil health, water quality, and overall environmental sustainability.

Societal impact:

1. Easy-to-Use solution: The handheld device's user-friendly design and simplicity will enable farmers of all skill levels to effectively detect BCTV in their crops. This accessibility will empower a wider range of agricultural practitioners to benefit from early disease identification and implement appropriate measures, promoting more inclusive and resilient farming practices.
2. Enhanced food safety: By facilitating early and accurate BCTV identification, the handheld device will help prevent infected plants from entering the food supply chain. This will enhance food safety, reduce the risk of consumers being exposed to BCTV-infected produce, and contribute to public health.

Overall, the development of an easy-to-use handheld device for sensitive and early identification of BCTV will revolutionize disease management strategies in agriculture. It will provide farmers with a practical tool for rapid BCTV detection, enabling timely interventions and targeted control measures. The widespread adoption of this device will lead to improved crop health, increased economic efficiency, reduced pesticide usage, environmental preservation, and enhanced food safety. The local, regional, and statewide agricultural communities, as well as consumers, will benefit from this technological advancement, fostering sustainable and resilient agricultural practices.

Long-Term Solutions:

This project has strong potential for measurable progress toward long-term solutions for BCTV detection and management. By developing a portable microfluidic device that addresses the limitations of current methods, we provide a practical solution for on-site testing, enabling timely

and accurate detection of BCTV. The integration of innovative technologies, such as Loop-Mediated Isothermal Amplification (LAMP) primers and a universal sample preparation method, and innovative electrical impedance detection of amplified signal will enhance the sensitivity and efficiency of BCTV detection. These advancements lay the foundation for future improvements in disease management strategies and contribute to the long-term goal of minimizing the impact of BCTV on crop productivity.

As the platform continues to evolve and develop, there is great potential for long-term solutions. The expandability of the platform allows for the incorporation of additional assays for different plant pathogens. This multiplex format holds immense promise for disease prevention, not only in California but also in other states and even globally. By incorporating assays for various plant pathogens, the platform can address a wide range of plant diseases and contribute to comprehensive disease management strategies. This versatility will enable farmers and researchers to detect and monitor multiple pathogens simultaneously, facilitating early detection and prompt action. This broader reach will benefit agricultural communities globally by providing them with a valuable tool for disease prevention and control.

Related Research:

Previous research efforts at Alveo have been dedicated to the development and successful commercialization of a handheld device specifically designed for the rapid and user-friendly detection of SARS-CoV-2, the virus responsible for the COVID-19 pandemic. This device comprises four distinct components: a swab for convenient sample collection, a sample buffer for efficient sample elution and lysis, a microfluidic cartridge containing reagents for target amplification, and an analyzer that runs the test, delivering accurate and reliable results (Figure 1). The successful implementation of this handheld device in various countries has revolutionized the field of diagnostics, enabling widespread and accessible testing for the virus.

In addition to its application in COVID-19 testing, this handheld device has also been explored for its potential use in agriculture-related applications. Collaborative research endeavors in partnership with an agriculture company have focused on utilizing the device to detect fungal infections in wheat crops. As a result, specific assays have been developed and implemented within the device, demonstrating its capability to detect early-stage fungal infections with impressive speed (as early as 8 minutes) while maintaining sensitivity comparable to that of established PCR technology. These findings have significant implications for early detection and prompt intervention, leading to enhanced crop management and improved agricultural productivity.

Continuing in the realm of agricultural diagnostics, ongoing research initiatives are underway to develop a point-of-need test for avian influenza, with a specific focus on H5 and H7 subtypes. This collaborative effort, conducted in partnership with a European company, aims to design a diagnostic solution suitable for farm settings. By leveraging the capabilities of the handheld device, this research seeks to enable quick and accurate detection of avian influenza facilitating timely interventions to prevent disease spread and minimize economic losses within the poultry industry.

Furthermore, the handheld device's potential is being further expanded through ongoing research and development efforts. The focus is on advancing and refining the current platform to enhance

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simplicity, broaden the range of target detection, and enable multiplexing capabilities. The aim is to streamline the testing process, making it more user-friendly and accessible to a wider range of end-users. Notably, research is currently being conducted on sample preparation techniques, specifically exploring bacterial direct lysis strategies for both gram-positive (e.g., Group A streptococcus) and gram-negative bacteria (e.g., Chlamydia trachomatis and Neisseria gonorrhoeae). These innovative sample preparation methods will be integrated into the handheld device's cartridge, paving the way for future target detection applications.

The collective efforts in developing and expanding the capabilities of this handheld device exemplify the commitment to advancing diagnostic technologies for both human and agricultural health. Through these research endeavors, we aim to improve disease management strategies, promote early detection, and facilitate effective interventions, ultimately enhancing the well-being of individuals and the sustainability of our agricultural systems.

Figure 1: The portable pathogen detection system.

The figure showcases the components of the be.well COVID-19 test, a portable pathogen detection system. It includes a swab for sample collection, a buffer for sample elution, a microfluidic cartridge for target amplification, an Analyzer device for isothermal analysis, and a user-friendly app for test management. The be.well COVID-19 Test, developed by Alveo, utilizes isothermal nucleic acid amplification technology and EIS for qualitative detection of SARS-CoV-2 RNA. The test is performed in a single-use cartridge, requiring the be.well Analyzer, a mobile device (e.g., iPhone) with an integrated camera and Bluetooth capability, and a HIPAA-compliant Software Application. The test workflow involves four steps: sample collection with a swab, sample elution with a buffer, loading the eluted sample into the microfluidic cartridge, and detection of SARS-CoV-2 using target amplification and EIS. Test results are reported on the be.well mobile app and stored securely in a HIPAA-compliant cloud database.

Contribution to Knowledge Base:

This project contributes to the current knowledge base by developing a portable and point-of-site test for agriculture usage. It would advance plant diagnostics methodologies by introducing a user-friendly testing approach for on-site agricultural applications. Early disease diagnostics and prevention would be facilitated, enabling farmers to implement timely disease management strategies. The incorporation of a cloud system for disease spread monitoring would provide valuable insights into disease epidemiology, aiding in proactive measures. The user-friendly on-site testing capability would revolutionize disease monitoring and reduce dependency on external diagnostic services. Additionally, the project's outcomes would provide insights into the

adaptability of the testing methodology to other plant diseases. The focus on cost effectiveness would make the test affordable in low-and-middle income countries, ensuring accessibility. Furthermore, the project's integration of technology and connectivity would contribute to advancements in agricultural machinery and foster farmer connectivity. Overall, these contributions would enhance disease management practices, improve agricultural productivity, and support sustainable agriculture.

Grower Use:

This device offers practical applications and incentives for growers to adopt sustainable agriculture practices. With on-site and rapid diagnostics, farmers can make timely decisions to manage diseases, leading to improved crop health, reduced losses, and increased productivity. The device is convenient and easy to use, eliminating the need for specialized equipment and external services. This promotes self-sufficiency and empowers farmers to take immediate action. Its cost-effectiveness makes it attractive, especially in low-and-middle income farmers, offering affordable disease monitoring and control measures. By using this device, farmers can protect their crops, reduce the use of harmful chemicals, and improve their profits. It also helps the environment by promoting sustainable farming practices. Incentives for growers include improved yields, reduced pesticide reliance, environmental stewardship, and long-term economic benefits. Policymakers can encourage farmers to use this device by offering incentives like better crop yields, lower costs, and long-term benefits. This project will make farming more efficient and sustainable, leading to a healthier food system for everyone.

D. Objectives

The goal of the proposed project is to develop a portable and field-deployable pathogen detection technology using electrical impedance detection and loop-mediated isothermal amplification (LAMP) technologies for the rapid detection of plant pathogens. The device will be designed for agricultural end-users in both laboratories and remote environments, such as farm fields and, where traditional laboratory analysis is not feasible. Listed below are the specific objectives of this project:

1. Develop a rapid and sensitive nucleic acid isothermal amplification assay (s) for the early detection of BCTV in agricultural crops, with a reduced test time of 15-20 minutes, ensuring accurate and reliable results.
2. Implement sample treatment and virus lysis efficient improvement strategies to enhance the performance and reliability of the diagnostic test for BCTV detection.
3. Modify the software system and cloud-based data storage system for user-friendly testing protocols, enabling easy implementation of the diagnostic test by farmers and agricultural practitioners in the field.
4. Validate the test using diverse BCTV-infected plant samples under various environmental and field conditions, collaborating with agricultural extension services and industry partners to facilitate adoption into disease management strategies.
5. Disseminate research findings through scientific publications, conferences, and workshops to contribute to the knowledge base on BCTV detection and promote wider adoption of the developed diagnostic test.

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6. Monitor and evaluate the implementation of the diagnostic test in real-world agricultural settings, gathering user feedback to further refine and improve its usability and effectiveness.

These objectives focus on the development, validation, adoption, and evaluation of a rapid and sensitive diagnostic test for BCTV detection. The objectives also include educational outreach efforts, economic and environmental assessments, and dissemination of research findings to ensure the successful integration of the diagnostic test into BCTV management practices.

E. Work Plans and Methods

Work Plan: we propose to complete the project in 8 months to 1 year. The early phase of the project will focus on assay development feasibility and development studies and the late phase will focus on verification and validation studies using field samples.

Plan:

Task 1.1: Feasibility and Development of the diagnostic test for BCTV detection

Activities:

1. LAMP assay design and selection

a. Bioinformatics analysis and target region identification: A reference sequence (GenBank accession AF379637.1) will be used to align with all other BCTV sequences and sequences for other plant and plant pathogen sequences obtained from the NCBI public database. Conserved and unique sequence regions will be identified for LAMP primer design.

b. Design and optimize the loop-mediated isothermal amplification (LAMP) assay for BCTV detection. Alveo proprietary LAMP primer design method will be used to design primers. Multiple primer sets (2-5) recognizing different distinct regions in the vial target regions will be designed and evaluated. A best performed primer set will be selected based on the performance of the primers. A LAMP primer set will be also designed against a known plant housekeeping gene to be used as a positive control.

2. Assay optimization and integration into the microfluidic cartridge

a. LAMP primer composition and enzymatic reaction condition will be further optimized by design of experiment (DOE)

b. Drying condition of LAMP Primers and reaction reagents such as dNTP and enzyme in the microfluidic cartridge will be optimized and storage stability of the cartridge will be determined.

3. Assay performance optimization in cartridge:

a. Studies will be performed to determine the assay sensitivity of the cartridges (100-1000 copies/mL).

b. Studies will be performed to determine the test cut off time (10-20 minutes)

c. Assay specificity evaluation against common plant pathogens.

d. Assay amplification precision and robustness studies

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Interim task product: Prototype LAMP assays for BCTV detection

Completion date/milestone: 6 months (July-December)

Task 1.2: Sample treatment and virus lysis improvement strategies

Activities:

1. Research and develop efficient sample treatment and virus lysis methods: Mechanical, enzymatic, and chemical methods will be evaluated for plant leaf tissue, beet leafhopper (*Circulifer tenellus*) and virus lysis efficiency.

2. Development of methodology to removal inhibitory plant materials such as Polysaccharides including cellulose, starch, dextran sulfate and pectin and Polyphenols including flavanol, gallic acid, resveratrol, and secoisolariciresinol. Different approaches such as gel filtration or membrane filtration will be evaluated.

3.Integration of sample treatment, inhibitor removal and cartridge loading: A device with sample pretreatment containing sample lysis, inhibitor removal and sample loading functions will be integrated into and evaluated for virus nucleic acid recovery and amplification efficiency.

Interim task product: An easy to use and efficient sample treatment and virus lysis device.

Completion date/milestone: 5 months (July 2023-Dec 2023)

Task 1.3: Software system and testing protocol modification

Activities:

1. Modify the existing software system and develop a user-friendly testing protocol by software development and interface design

2. New call algorithms will be created to make a call for the reaction results, based on the assay cut off time developed and assays for target detection and control reactions.

3. Software team will establish the new cloud database for data storage and determine the portal access.

Interim task product: Modified software system, cloud database, and user-friendly testing algorithms and protocol

Completion date/milestone: 5 months (Nov 2023-March 2024)

Task 1.4: Verification and Validation of the BCTV test

Activities:

1. Perform system integration studies.

2. Verify assay analytical performance such as LoD and assay specificity using manufacturing lot of tests

3. Conduct verification studies by detecting the pathogen detection test using diverse BCTV-infected plant samples under various environmental conditions.

Interim task product: Validated diagnostic test results.

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Completion date/milestone: 5 months (Jan 2024 -March 2024)

Task 1.5: Field Testing and Assessment of environmental implications and Monitoring and evaluation of diagnostic test implementation

Activities:

1. Test the BCTV tests in at least three (3) remote locations (farms) and test at least 200 samples, with different stages of infections in parallel with another detection method to determine the assay field sample specificity and sensitivity.

2. Implement the test in the farm and evaluate the reduction in insecticide use and environmental contamination due to the diagnostic test implementation

3. Surveys, interviews, data analysis

4. Analyzing the test results, assessing environmental implications, and evaluating user feedback.

5. Field sampling: Collecting diverse BCTV-infected plant samples for diagnostic test validation.

6. Laboratory testing: Conducting the diagnostic tests using the developed assay on collected samples.

7. Data analysis: Analyzing the test results, assessing environmental implications, and evaluating user feedback.

Methods: Data collection on insecticide use, environmental monitoring, statistical analysis

Interim task product: Environmental impact assessment report

Completion date/milestone: 12 months (March 2024-Oct 2024)

Task 1.6: Dissemination of research findings

Activities:

1. Publish research findings in scientific journals, present at conferences, and conduct workshops.
2. Methods: Manuscript writing, conference presentations, workshop organization
3. Interim task product: Published articles, conference presentations, workshop materials
4. Data analysis: Analyzing the test results, assessing environmental implications, and evaluating user feedback.
5. Statistical analysis: Analyzing data related to insecticide use and environmental monitoring.

   Manuscript writing: Writing research articles for publication in scientific journals.

   Conference presentations: Presenting research findings at relevant conferences and workshops.

   Surveys and interviews: Gathering user feedback on the diagnostic test.

   Completion date/milestone: 6 months (Oct 2024-Dec 2024)

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Alveo Technologies is organized into different divisions, including Technology, Quality, Program management, Business (product management, marketing, and sales), regulatory, clinical affairs, and manufacturing. The project team will consist of highly qualified and experienced individuals who are experienced in their respective fields. The team is organized into core team led by the program manager. Here is an overview of the key roles and responsibilities:

F. Project Management, Evaluation, and Outreach

1. Management: The role of the project leader for this project is to ensure the successful implementation of the project if it is funded. His key responsibilities include various critical aspects, starting with creating a detailed project plan that aligns with the grant objectives, budget, and timeline. Collaborating closely with the program manager, the project leader will build and manage a project team, including expertise from all required fields, such as R&D, engineering, software, etc. He will delegate tasks based on the plan and oversee project execution to achieve the project's goals and deliverables. He will communicate with project stakeholders, including the BCTVCP and collaborating partners in agriculture field studies, providing regular updates on project progress and addressing challenges as they arise. Additionally, the project leader is responsible for ensuring strict compliance with the grant's terms and conditions, including reporting requirements, financial accountability, and adherence to ethical guidelines.

2.. Evaluation: The project will be managed using our company's proven design control process and quality management system, which has already been successfully employed in the development of other products. To ensure the best outcome, a specific plan will be followed for the evaluation:

1) Establish product requirements with specific, measurable, and time-bound objectives and performance metrics that align with objectives; The objectives will align with the aim of this project, i.e. to develop a sensitive, specific, cheap, fast, easy to use point of site test for BTCV.
2) To achieve our objectives, we will implement a rigorous design control process and a comprehensive quality management system. This system will be carefully adhered to throughout the different stages of research and product development, ensuring that we maintain high standards at every step.
3) The project will progress through distinct phases, starting with feasibility and development, followed by verification and validation. Each phase will have specific goals and checkpoints to ensure that the product is on track and meets the required criteria.
4) During the feasibility and development phase, we will explore various options and assess their viability, considering factors such as technical feasibility, resource availability, and market demand. Once a viable concept is established, we will move on to the verification phase.

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5) In the verification phase, we will thoroughly test and analyze the product to confirm that it meets the predetermined requirements and specifications. This stage involves conducting various tests and experiments to ensure that the product performs as intended and meets all necessary standards.
6) The final stage, validation, is critical to ensure the product's effectiveness and suitability for its intended use. During validation, the product will undergo extensive real-world testing to ensure it performs well in the target environment and delivers the desired outcomes. To validate the test's performance and usability, it will undergo testing in real farming environments. For this purpose, we plan to conduct a minimum of 3 field studies.
7) Throughout this entire process, careful documentation and review will be conducted to maintain transparency and accountability. The study results will be carefully evaluated, and comprehensive reports will be prepared for each study. These reports will then be shared and reviewed by all stakeholders involved. The design control process and quality management system will be continuously monitored and improved, ensuring that we deliver a top-quality product that fulfills its purpose effectively. By following this structured approach, we are confident in achieving our objectives and meeting the expectations of our customers and stakeholders.

G. Budget Narrative

a. Personnel Expenses:

Salary: We will have 8 individuals to work on the project:

Project Leader and Project Manager: 15% of full-time salary - $45,000 per year

Research and Development Scientists: 40% of full-time salary - $120,000 per year

Electrical and Mechanical Engineers: 20% of full-time salary - $52,000 per year

Software Engineers: 15% of full-time salary - $39,000 per year

Benefits: We will allocate 30% of the total personnel expenses to cover benefits, which amounts to $76,800 per year (30% of $256,000).

All personnel will work on the project for the entire project duration, which is one year starting from July 1.

b. Operating Expenses:

Supplies: We estimate the total cost of supplies to be $108,000 for the project duration. These supplies will include raw materials (enzymes, chemicals, buffer, pathogen DNA, leaf samples), laboratory equipment, and other necessary items for research and development.

Equipment: As per our project requirements, we need build Analyzers for this project (>30) and equipment for sample preparation studies. We estimate the cost will be about $18,000. This equipment will be used for field studies and the title to the equipment will vest with the State as per the terms.

Travel: We anticipate conducting multiple trips within California to various farming locations for field studies and outreach activities. Estimated travel expenses, including mileage, accommodation, and per diem, are $8,000.

Professional/Consultant Services: We do not plan to sub-grant any work or services for this project; all project activities will be carried out by the in-house team, consulting the agricultural professionals for some field study activities ($9200).

Other Expenses: We have allocated $26,000 to cover any additional expenses not included in the above categories. These may include unforeseen costs or minor expenses arising during the project's implementation.

c. Other Funding Sources:

We are not receiving funding from any other organizations for this project. The entire project budget of $500,000 will be required, expecting 30% funded by this grant and the rest by the funding Alveo raised from other sources.

Note: The above budget breakdown adheres to the provided budget template and is consistent with the information and justifications provided in Section G: Budget Narrative. The budget meets the requirement of a project start date of July 1 and limits indirect costs to 10%.

H. References

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I. Appendices

1. Project lead: Slava Elagin, PhD, MBA

Education:

MBA | University of Wisconsin: Madison | Madison, WI | 2009
Ph.D | Molecular Genetics | Engelhard Institute of Molecular Biology | Moscow | 1992
M.S. | Genetics | Vavilov Institute of General Genetics | Moscow | 1990
B.S. | Applied Physics & Mathematics Moscow Institute of Physics and Technology Moscow| 1988

Work Experience:

Chief Technology Officer| Alveo Technologies. Alameda, California | 2022-present

Chief Operating Officer and President |MiraVista Diagnostics, Indianapolis, Indiana | *2018 – 2021*

- Led company and departmental goals setting and prioritization.
- Responsible for P&L management of $20MM+ revenue with $3MM EBIDA and operating budgets.
- Provided overall leadership for day-to-day operations.
- Oversaw Manufacturing, Sales and Marketing, Quality Assurance, Regulatory, IT, Clinical Operations, Project Management, and R&D departments.
- Established operating standards to ensure compliance with ISO, CLIA, CAP, NYSDOH, and FDA regulatory requirements.
- Collaborated with the leadership team to create and execute business plans, identify new business opportunities, develop growth initiatives, and explore product licensing and technology acquisitions.

Executive Vice President and Chief Scientific Officer| Meridian Bioscience, Inc., Cincinnati, Ohio | 2009 - 2018

- Part of the Executive Leadership Team with corporate-wide P&L responsibilities for $300MM+ revenue, strategic investments, acquisitions, due diligence, FTO assessments, etc.
- Oversaw global R&D and Project Management organizations with a team of over 90 individuals.
- Led the design, development, and commercialization of the illumigene molecular platform, one of the first POC-comparable molecular platforms on the market.
- Developed and cleared 10 molecular and 5 immunological diagnostic products by FDA and/or CE marking.
- Managed company Clinical Trials for FDA and other regulatory agency submissions.
- Provided strategic guidance for product development from concept to commercialization, including oversight of Project Management Team.
- Ensured compliance with ISO, GLP, CE, and FDA standards for product development.
- Directed patent development and protection of intellectual property.

Vice President, R&D | EraGen Biosciences, Madison, Wisconsin |*2006 - 2008*

- Led Research and Development functions with associated budgets.
- Directed Product Development, Software Development, and Technical Support groups.
- Commercialized molecular diagnostic products for human genetics, oncology, and infectious diseases.

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- Oversaw development and commercialization of the company's product portfolio, resulting in an 800% and 400% revenue increase in 2007 and 2008, respectively.
- Implemented product development cycle, QSR, and Design Control to comply with regulatory requirements, obtaining ISO certification in 2007.
- Formulated strategic product development plans, supported business development, licensing activities, and competitive landscape evaluation.
- Established and maintained industry and academic contacts to achieve business goals and product development strategies.

Vice President, R&D | Third Wave Technologies, Madison, Wisconsin. | *2003 - 2006*

- Managed R&D personnel with an annual budget exceeding $30MM.
- Expanded the total addressable market for the product portfolio from $30MM to $400MM in 3 years.
- Guided research, product support, quality control, and technical operations.
- Led technology assessments, strategic collaborations, licensing, and business development efforts in various diagnostic areas.
- Ensured compliance with ISO, GLP, and FDA standards, including clinical trials and FDA submissions.
- Managed development and clinical trials for the company's first FDA approved diagnostic test.

Senior Scientist/Manager R&D | **Bayer Diagnostics/Visible Genetics, Atlanta, Georgia** | 2000 - 2003

- Managed a group of Scientists in the R&D Department.
- Developed new IVD products: TRUGENE HCV 5'NC and TRUGENE HBV genotyping assays.
- Optimized production efficiency, quality, and manufacturing costs.
- Responsible for Quality Control of raw materials and final products.

Research Assistant Professor| University of Notre Dame, South Bend, Indiana |1996 - 2000

- Cell signal transduction, neurobiology, and neurogenetics.
- Supervised graduate and undergraduate students as well as performed laboratory management duties.
- Five manuscripts published in peer reviewed scientific journals.

Significant Grants Awarded:

- 2006 – 2007 Principal Investigator: "Development of Diagnostics Test for Avian Influenza". Department of Homeland Security and Centers for Disease Control and Prevention, government contract for $1.2 million for two years.
- 1996 – 2000 R01 Grant, Co-Principal Investigator: "Mechanisms of Retinal Degeneration". National Institute of Health, National Eye Institute, grant award of $265,000 annually.
- 1993 - 1996 Principal Investigator: "Innovative Research Award". Howard Hughes Medical Institute

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Selected Publications and Patents

Publications

1. Detection and quantification of hepatitis C virus (HCV) by MultiCode-RTx real-time PCR targeting the HCV 3' untranslated region. Mulligan EK, Germer JJ, Arens MQ, D'Amore KL, Di Bisceglie A, Ledeboer NA, Moser MJ, Newman AC, O'Guin AK, Olivo PD, Podzorski DS, Vaughan KA, Yao JD, Elagin SA, Johnson SC. J Clin Microbiol. 2009 Aug;47(8):2635-8. doi: 10.1128/JCM.02170-08. Epub 2009 Jun 17. PMID: 19535519 Free PMC article.
2. Hepatitis C: sexual or intrafamilial transmission? Epidemiological and phylogenetic analysis of hepatitis C virus in 24 infected couples. Cavalheiro Nde P, De La Rosa A, Elagin S, Tengan FM, Araújo ES, Barone AA. Rev Soc Bras Med Trop. 2009 May-Jun;42(3):239-44. doi: 10.1590/s0037-86822009000300001. PMID: 19684968 Free article.
3. Invader plus method detects herpes simplex virus in cerebrospinal fluid and simultaneously differentiates types 1 and 2. Allawi HT, Li H, Sander T, Aslanukov A, Lyamichev VI, Blackman A, Elagin S, Tang YW. J Clin Microbiol. 2006 Sep;44(9):3443-7. doi: 10.1128/JCM.01175-06. PMID: 16954297 Free PMC article.
4. Evaluation of the TRUGENE HCV 5'NC genotyping kit with the new GeneLibrarian module 3.1.2 for genotyping of hepatitis C virus from clinical specimens. Germer JJ, Majewski DW, Rosser M, Thompson A, Mitchell PS, Smith TF, Elagin S, Yao JD. J Clin Microbiol. 2003 Oct;41(10):4855-7. doi: 10.1128/JCM.41.10.4855-4857.2003. PMID: 14532242 Free PMC article.
5. Hepatitis C virus: molecular and epidemiological evidence of male-to-female transmission. Cavalheiro Nde P, La Rosa Ad, Elagin S, Tengan FM, Barone AA. Braz J Infect Dis. 2010 Sep-Oct;14(5):427-32. doi: 10.1590/s1413-86702010000500001. PMID: 21221468 Free articles.

Patents

1. US 8,389,245. Detection of HPV
2. US 8,354,232 T-structure Invasive Cleavage Assays, Consistent Nucleic Acid Dispensing, and Low-Level Target Nucleic Acid Detection.
3. US 8,071,750 Determination of Hepatitis C Virus Genotype from Human Patients Using the INVADER Invasive Cleavage Structure Assay.
4. US 7,759,062 T-structure invasive cleavage assays, consistent nucleic acid dispensing, and low-level target nucleic acid detection
5. US 7,527,948. Methods and Oligonucleotides for Detection of HPV Nucleic Acids Using Invasive Cleavage Structure (INVADER) Assay
6. US 7,473,773. Determination of hepatitis C virus genotype
7. US 20090275039. Methods for Detection and Quantification of Small RNA.
8. US 20130149697. Detection of HPV.
9. US 20070207455; WO 2007059348. Invasive Cleavage Detection Assay for Detecting an HCV-1 Subtype.
10. US 2006252032. Methods and Primers for Detection of Human Herpes Viruses Using PCR and INVADER Oligonucleotide Cleavage Assays.

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11. US 20060246475; WO 2006079049. Increased Dynamic Range Detection of Nucleic Acid Molecules Use of Different Levels of Amplification and Probes with Differing Hybridization Properties and Concentrations.
12. US 20060147955; WO 2006050499. Single Step Detection of Nucleic Acids by PCR and Reverse Transcription.

Current Research and Outreach Activities of Dr. Elagin, the Project Leader:

Dr. Elagin is the Chief Technology Officer at Alveo Technologies, leading a range of research, outreach, and product development projects. He is dedicated to leading a collaborative effort that stands at the forefront of infectious disease management. His current research and activities are strategically aligned with the goals of the proposed project, aiming to tackle the challenges posed by infectious agents affecting various domains. Here's a breakdown of his ongoing, planned, pending, and recent work, along with how they impact our grant proposal:

1. Collaborative Pathogen Research:
He is focused on understanding disease-causing agents in humans, animals, and plants. We're crafting tailored tests for each area, including agricultural research.

Time Commitment: Ongoing, about 10% of his time.

Impact on Proposal: Our expertise in impedance-based detection of various pathogens, insights in sequence alignment and assay design are vital for our project. We use this knowledge to design precise assays, like the BCTV assay, which helps contain plant viruses.

2. Advancing Detection Tech:
We are developing an improved pathogen detection platform, including simpler sample handling, patented pathogen measurement system, and proprietary primer design. Our goal is a cost-effective and scalable system.

Time Commitment: About 20% of my time, coordinating multiple departments.

Impact on Proposal: Our innovations in sample prep align with the main project's goals, improving virus and bacterial testing. Our insights also enhance the proposed project's efficiency and impact.

3. Point-of-Site Assay Development:
He is collaborating on creating quick assays for agricultural and animal pathogens. We're focusing on efficient sample prep and streamlined detection.

Time Commitment: About 20%, leading the collaboration.

Impact on Proposal: Our findings help the proposed project, especially for plant samples, expanding its reach and impact.

4. Human Infectious Disease Assay Development:

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He is working on assays for human diseases, aligning with the project's objectives. These assays optimize design and form the project's foundation.

Time Commitment: A significant 30%.

Impact on Proposal: These assays support the project, refining design, and functionality. The platform we're developing is essential for the project's system.

In conclusion, His role involves research, outreach, and projects that advance disease management. These efforts combine to position us well for achieving the goals in our grant proposal.

2. Cooperators.
The project is solely managed by Dr. Elagin/Alveo Technologies, and no external co-operators are involved in its execution.
3. Supporters. As there are no supporters associated with this project, there is no requirement for letters from supporters detailing the rationale for their support. The project is proceeding without external supporters, and thus this section does not apply.

J. CEQA 1. Project lead is responsible for compliance with all applicable State and federal laws and regulations. Importantly, it should be noted that this project, which does not come under the scope of CEQA and NEPA, has been designed in a manner that avoids any adverse impacts on the environment.

Reagents/Supplies	$/per cartridge	$/2000 cartridge+overage	Supplies
Reagents	20	88000	108000
Lab supplies		20000	
Instrument-Analyzer	150	9000	18000
QS-Maintainance		9000	

Function	Personnel	Cost/Year
Project Management/Quality Manager	2	$ 150,000.00
Mechanical Engineer/Software	4	$ 130,000.00
Assay Development and Sample Prep	2	$ 120,000.00

Personel	Roles
Slava Elagin	Project Lead
Helen Belcstro	Project manger
Anand Hindupur	R&D Lead
Claudina Kwok	R&D Scientist
Aman Ullah	Engineer Lead
Joseph Pham	Hardware Engineer
Raji Limaye	Softer Lead
Sunita Pokkunuri	Software engineer