I. SIGNIFICANCE

Just as penicillin was a life-saving drug to combat infections at the turn of the century, food fortification was critical in preventing life-threatening disease and fetal deformities. Despite these discoveries, we are now plagued with both antibiotic-resistant 'superbugs' [1,2], and our low-fiber Western diet is further increasing mortality and disease risk [3,4]. **We hypothesize that precision dietary prebiotic fibers may prevent or reduce deadly infections and improve cancer outcomes.** Dietary fiber is critical to support the health of the bacteria in the gut, the microbiome, especially microbes which possess the ability to evict pathogens [5]. Deaths from pathogen infections accounts for approximately 700,000 death each year globally, with 23,000 U.S. deaths attributed to antibiotic resistant bacteria [1]. Among the major culprits are *C. difficile,* which is related to approximately 15,000 deaths per year in the U.S., and enterotoxic *B. fragilis* (ETBF), which is a major cause of hospital-related infections. The standard treatment for these infections is a combination of antibiotics, however, as we run out of antibiotics to combat these 'superbugs', we must look for alternatives and preventative strategies, which has ignited a new effort to develop pre- and probiotics to address this critical situation. Currently, we have very little understanding of *which types* of dietary fibers support those microbes that convey protection from infection nor the mechanisms by which specific dietary fibers are protective [6-8]. To address this issue, we have designed a multi-phase strategy to develop precision medicine prebiotics by **utilizing a new tool the Mini-Bioreactor Array system (MBRA)[9].** This new system will allow us to test dietary fibers in a controlled gut microbiome community setting. The purpose of this research is to identify the prebiotics and microbial factors conveying resistance to infection while accounting for inter-individual microbiome variability. Indeed, a recent study showed a 5-fold improved response to cancer immunotherapy with a high fiber diet, which supports our focus on cancer treatment [10].The results from our initial study (Phase I) will allow us to pursue a multi-phase research strategy (**Fig. 1**) designed to address an outstanding need in our field to identify critical microbes and metabolites that are altered as a result of exposure to dietary fibers and pathogens. *Ultimately, this evidence will expand our understanding of the fiber-microbiome relationship, and allow us to develop preventative interventions using prebiotics*. (**Initiative=Health/Data Science)**.

INNOVATION: **1)** Application of the *MBRA system* to study diet-microbiome interaction, as well as, drug-microbe, microbe-microbe, or nanomaterial-microbe interaction in a controlled environment **(Initiative=Health)**. **2)** Innovative use of microbial populations to study dietary fibers through *metabolic modeling* **(Initiative=Data Science/Health)** and their effects over time on resilience, stability, and resistance to pathogens. **3)** Development of a precision medicine prebiotic; a) **Phase I,** will set the stage for several more powerful studies **(Initiative=Health)**: b) **Phase II -** *in vitro* studies to investigate the differential response to dietary fibers between stool communities from healthy individuals and those with colon cancer, and c) **Phase III -** *in vivo* studies to determine the response to fiber-treated microbiota in healthy and cancer models.

IMPACT: The NIH released its draft Strategic Plan for Nutrition Research; this research will position Baylor to take advantage of these funding opportunities. Specifically, our research addresses 8 priorities (**see Appendix).** *Results from this pilot data will be used to develop a large (R01) multi-phase strategy (**Fig. 1**) that includes multiple study inputs and outputs poised to address questions regarding host-microbe and fiber-microbe interactions.* Thus, this study, in combination with future studies, has the potential to lead to development of precision medicine prebiotics for infection resistance and improvement of cancer treatment.

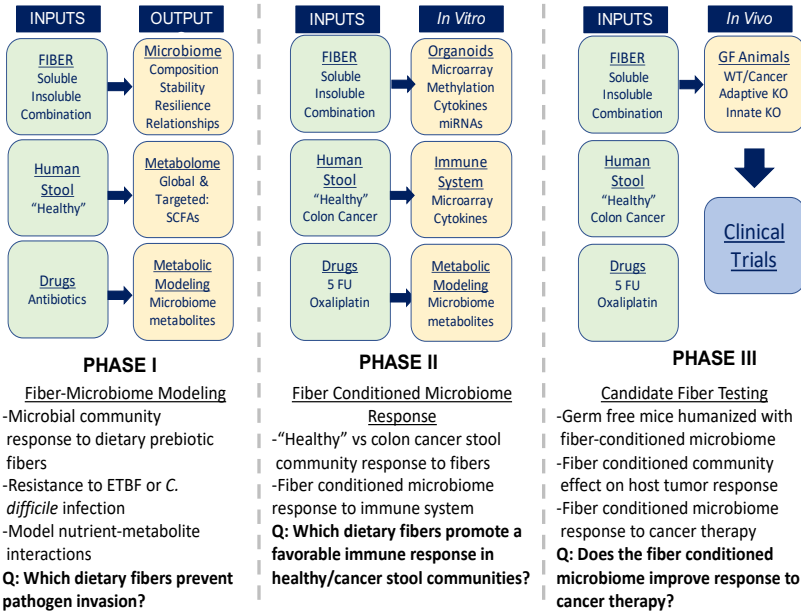


Fig. 1. Three Phase Strategy for Developing Precision Medicine Prebiotics. Phase I is described in this proposal.

II. RESEARCH QUESTIONS ADDRESSED

This proposal seeks to illuminate the dietary fiber-microbiome relationship using a **powerful new tool - fecal mini-bioreactor arrays (MBRAs)**[9]. First used to study *Clostridium difficile* infection, the MBRA allows for continuous cultivation of complex fecal microbial communities to study infection [9]. Using the MBRA system, developed by **Co-PI Dr. Robert Britton**, we seek to develop a prebiotic that is capable of preventing or reducing infection, focusing initially on the effects of two types of fermentable fiber, inulin and resistant starch in Phase I of this research strategy (**Fig. 2**).

Studies indicate that fermentable dietary fibers (prebiotics) are paramount in maintaining a healthy microbiome that is resistant to disease, including infection and cancer [8,11]. Little is known; however, about *which types* of dietary fiber are responsible for these protective effects nor their mechanisms [12]. With the global prebiotics market size anticipated to reach $7.91 billion by 2025, we are facing a critical need to move beyond our current 'one size fits all' application of dietary fiber to that of a tool for precision medicine [13]. The bacteria in the gut, collectively termed the microbiome, is significantly affected by our diet [14]. Our understanding of how this dietary fiber-microbiome relationship impacts response to infection however remains rudimentary, in particular with respect to the effect on the microbiome. Dietary fiber is one of the main sources of carbohydrates the microbiome uses for fuel in the production of short chain fatty acids (SCFAs), which supports gut barrier function [8,15]. Without fiber, the gut bacteria feed off the mucus lining the gut, leaving the host susceptible to infection and disease, including cancer [11,13,15] .

Our long-term goal is to develop prebiotics for precision medicine using the MBRA system in our multi-phase strategy (**Fig. 1**). The goal of this proposal (**Fig. 2**) is to operationalize the MBRA system by testing two types of dietary fibers in healthy stool communities, and their ability to resist pathogen colonization**.** We are choosing to focus on two of the most common dietary fibers, insulin and resistant starch, because they have consistently demonstrated the ability to favorably modify the gut microbiome to produce SCFAs [7,16]. Additionally, by establishing this new tool, **we will be able to train graduate and undergraduate students in using the MBRA** system (**see Appendix)**, which will give them critical skills to enhance learning and the ability to be competitive for fellowships or jobs in the STEM fields. To meet these goals, we have developed the following specific aims:

A.1. Specific Aim 1: Quantify changes in model stool community composition and fatty acid metabolism after exposure to inulin or resistant starch. The gut microbiome is reliant upon dietary fiber for nutrients, which in turn supplies energy for the intestinal epithelial cells. Data show that lack of dietary fiber results in dysbiosis, changes in metabolites (e.g. SCFAs), and increased disease risk [15,17]. However, human dietary fiber intervention studies with inulin or resistant starch each show different effects on the microbiome and SCFA production [18-21]. *Thus, we hypothesize that the gut microbiome composition and SCFA production will be differentially altered in response to inulin or resistant starch, which will be dependent upon the donor microbial stool community supplied.*

A.2. Specific Aim 2: Measure the ability of fiber-conditioned stool communities to resist pathogen colonization. The ability of microbial communities to resist pathogen colonization involves mechanisms that are, in part, reliant upon fermentation of prebiotic fibers to produce SCFAs. Specifically, commensal *Bacteroides spp.* confer colonization resistance again *Salmonella* infection through the production of the SCFA propionate [22]. Further, the combinations of prebiotic fibers with the probiotic *Lactobacillus reuteri* 1063 prevents colonization of the pathogenic strain of *E. coli* by limiting mucus adhesion [23]. *Thus, we hypothesize that inulin and resistant starch will confer differential colonization resistance to the pathogens, enterotoxic B. fragilis (ETBF), and C. difficile.*

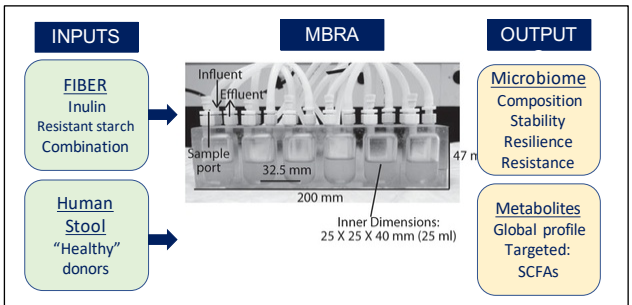


Fig. 2. Study Design Schema for Phase I.

Our research plan is supported by a highly-experienced team of scientists at Baylor University, Baylor College of Medicine, and Mayo Clinic, with expertise in microbiology, metabolic modeling, and metabolite analysis [24-28]. Completion of this research will provide a tool (MBRA) to **enhance the current space available** and allow recruitment of students and faculty of high research caliber. This research will also provide the foundation for an externally funded research program dedicated to elucidating the dietary fiber-microbiome relationship. *These findings will impact our field by not only answering outstanding questions that remain with regards to microbiome resilience to infection, but also lead to novel prebiotic interventions to prevent or reduce infection.* ***Our research will benefit Baylor by providing cutting-edge tools, collaboration, student training, and a sustainable research focus that can be used to make Baylor a leader in prebiotics and infectious disease research.***

A.3. Research Approach

Approach: To initiate our study, we (**Dr. Greathouse** and a doctoral student) will begin by training with **Dr. Britton** to setup and run the MBRA system at Baylor College of Medicine. This training will allow us to fully operationalize the MBRA system in the **laboratory of Dr. Greathouse** and begin experiments **(Fig. 2)**. Further, this doctoral student will be co-mentored by both Dr. Greathouse and Dr. Britton through the Biomedical Science (BMS) program at Baylor University. (**see Appendix – Training Plan)**

To address **Aim 1,** we will collect donor stool from three healthy individuals according the methods previously described [9]. Three replicate reactors will be used for each of the three donor stool samples (n=9 reactors). Reactors will be inoculated with 25% fecal supernatants by adding them to pre-reduced growth medium for 16hrs, and then fresh medium will be continuously flowed in through the MBRA system. To determine changes from baseline in community composition we will extract 1mL from each reactor on day 0 (inoculation), and daily thereafter for 20 days and store at -80 degrees. To compare the effects of inulin vs. resistant starch, 2.0 g/L of inulin or resistant starch (dose derived from previous studies) will be added to each donor sample reactor with fresh medium flowed in; provided for 20 days [9]. Samples will be collected in the same manner as at baseline. DNA will be extracted from each sample using bead beating and the Qiagen DNeasy Tissue Kit, and will be quantified with QuantIT. Samples (n=594) will be sequenced on the Illumina MiSeq (**Dr. Petrosino, BCM**) using 16S rRNA sequencing (V4 region) and bioinformatically processed as previously described [9,29,30]. For analysis of metabolites, SCFAs, a separate 1mL sample will be collected at all time points previously indicated. Samples from each donor will be pooled together by time point and sent to **Dr. Lavado (ENV)** for SCFA metabolite analysis (pooled, n=36). Data from the microbial and metabolite profiling will integrated by summing microbe-microbe metabolic interactions to construct an interaction landscape (**Dr. Chia**) from response to dietary prebiotics.

To address **Aim 2,** we will perform invasion studies using *C. difficile* (CD2015 from **Dr. Britton**) or ETBF (**Dr. Greathouse**), both of which are responsible for life-threaten infectious diarrhea, chronic disease, or colon cancer. Prior to inoculation, we will test samples for *C. difficile* or ETBF presence. Using an experimental design similar to the one described previously [9], we will inoculate three reactors with either CD2015 or ETBF at a dilution of 1:100 from a culture grown to an OD_{600} of 1.5 to measure CFU/mL in pure culture by plating and by qPCR. We will test the ability of the pooled communities to resist pathogen invasion by pretreating with i) antibiotics, ii) inulin + antibiotics, iii) resistant starch + antibiotics, or iv) control media. To measure resistance to invasion, we will take 1 mL samples at 15 min and 3 hours post-inoculation, and once daily for 15 days, and measure CFU/mL by plating and by qPCR. To determine community structure changes, we will also collect 1mL samples for 16S rRNA sequencing (n=405). Using these data, microbial and metabolic, we will use the interaction landscape to identify which prebiotic fibers can support the microbes capable of evicting or preventing *C. difficile* or ETBF engraftment (**Dr. Chia**).

Specific Aim	Year 1 Benchmark	Year 2 Benchmark	Year 3 Benchmark
Aim 1	Trained/Initialize MBRA Model communities growing	Samples collected from inulin/starch exposure	Community/metabolite analysis finalized
Aim 2	CD2015/ETBF growing in MBRA reactors	Samples collected from pathogen exposure	Community/invasion analysis finalized
Manuscripts/Grants		Grant funding identified/manuscript outlined	Grant/manuscript submitted

I. CLEAR LINK TO INITIATIVES AND PILLARS

Pillar #1. Commitment to an unambiguously Christian educational environment. This proposal will support the first pillar through two mechanisms, a) opportunity for students to practice communicating their science with a Christian worldview to children and young scientists through mechanisms such as “Present your PhD”, b) demonstration of scientists working through their Christian faith to make important scientific breakthroughs, and c) Christian mentorship of students from Baylor research faculty during this study.

Pillar #3. Excellence in Research & Scholarship. To achieve the University’s goal of R1 status, establishing this MBRA system and research area will be paramount in allowing us to compete for external funding with R1 Universities and Medical Centers. This area of research is highly critical both scientifically and monetarily.

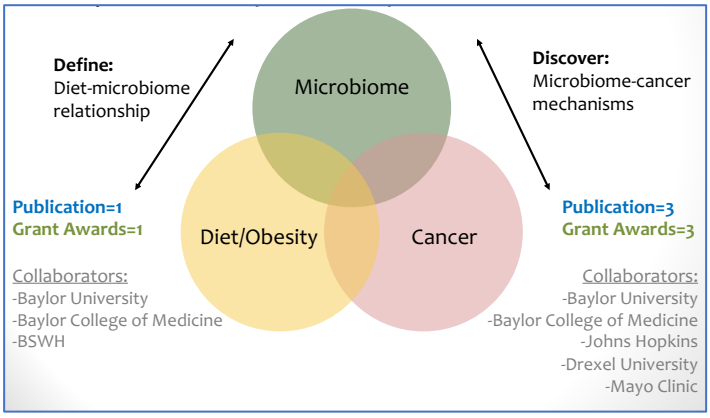
Several funding agencies, private, state, and federal, have issued multiple requests for research proposals focused on the elucidating the diet-microbiome relationship; indicating this is a highly sought after and fundable project. Also, multiple high-impact research articles regularly report a lack of understanding of the mechanisms governing the resistance to infection and disease conferred by the diet-microbiome relationship, which is addressed this proposal.

Collaboration between faculty/schools/Universities

Collaboration between faculty and schools will be a key aspect of this research: 1) **Dr. Greathouse + Dr. Lavado** (Nutrition/ENV) – will work together to identify microbial metabolites using state-of-the-art equipment in the Lavado Laboratory and provide training for undergraduate and graduate students; 2) **Dr. Kearney** (Biology) – will be able to use MBRA system to develop and test his antimicrobial peptide engineered bacteria [31], 3) **Dr. Bruce** (ENV) – will be able to test multiple drug combinations using the MBRA. This research will also take advantage of established and new collaborations between hospitals and Universities by integrating the expertise of multiple investigators: 1) **Dr. Robert Britton** (Baylor College of Medicine) – will provide training and expertise in MBRA system setup and establishment at BU, study design, and microbiome analysis.; 2) **Dr. Nick Chia** (Mayo Clinic) – with whom I have an established publication record [26], will provide metabolic modeling and network analysis [32,33] from data generated from this study, and future studies using this system **(Initiative – Data Science/Health)**, and will be actively engaged in postdoctoral training (**see Appendix)**.

II. PROJECT SITUATED WITHIN A BROADER RESEARCH AGENDA

The main focus of my research is to understand and identify biomarkers and molecular mechanisms that can be used to define the relationship between obesity, the microbiome and diet. Using big data and machine learning techniques, we seek to elucidate the relationship between diet, the microbiome and cancer. Our goal is to **1)** delineate the dietary factors that modify the microbiome and its function, **2)** develop microbial predictors that improve stratification of patients for obesity or cancer treatment (**2 publications/1 grant awarded/2 grants pending funding**), and **3)** identify key functional pathways and mechanisms of the microbiota-host communication (**2 grants awarded/2 grants pending)**. Currently, my lab is working together with several collaborators inside and outside of Baylor University. One of our projects involves the study of the prebiotic Prebiotin as part of an RCT in medical residents to prevent excess weight gain. We are analyzing microbial (stool), stress, activity, and dietary data collected from physicians taking Prebiotin or placebo X12 weeks (**1 URC grant awarded)**. We are using both microbiome data and serum to identify key features that we can use to understand how prebiotics alter the microbiome and mediate weight gain and stress. A separate project involves assessing the microbiome-host relationship by asking whether sRNA, contained in outer membrane vesicles (OMVs) shed by bacteria, can activate the Toll-like receptors (TLRs) on epithelial or immune cells, and thus trigger inflammation. This project is profiling the sRNAs (RNA-seq) contained in colon-cancer associated bacteria (*B. fragilis*) and those associated with the TLRs to identify mechanisms of immune evasion and activation (**3 abstracts published/2 grants awarded/2 grants pending funding).** This research proposal is linked to my broader agenda through my aforementioned goals. These goals will be achieved by testing the effects of two fermentable dietary fibers in a controlled polymicrobial community, which will allow us to identify the dietary and microbial factors that are key in protecting from pathogen invasion and infection, as well as, discovering novel mechanisms through which the microbiome may mediate the response to cancer therapy. Further, the proposed work augments ongoing studies in which we are investigating the mechanisms of host-microbiome communication between known colon cancer promoting pathogens (e.g. *B. fragilis*) and colon epithelial cells. In the future, we are planning to study how different dietary fibers differentially impact the microbiome in individuals with and without colon cancer, which will address an outstanding need in colon cancer prevention and treatment.



III. REQUESTED FUNDING LEVEL

PERSONNEL

K. Leigh Greathouse, PhD, MPH, MS, RD, *Principal Investigator (9.6 calendar months for years 1-3).*

As PI, Dr. Greathouse will be responsible for maintaining the overall scientific and procedural integrity of the project and executing the scientific agenda described in this proposal. She will supervise and/or directly perform IRB applications, training, data collection, and data analysis. She will coordinate meetings and communications amongst her collaborators and students regarding study progress and will have ultimate responsibility for monitoring of research progress and safety. She will be responsible for budgeting resources and for communications regarding the progress of the study with the Baylor University IRB. Dr. Greathouse will request class release in lieu of salary support.

Robert A. Britton, PhD, *Co-PI (1.2 calendar months for years 1-2).* Dr. Britton, Professor at Baylor College of Medicine, will oversee training on the MBRA system, but will not require salary coverage, only $5048 to cover supplies and services for one week of bioreactor runs along with training.

Ramon Lavado, PhD, *Co-PI (2 calendar months for years 2-3).* Dr. Lavado will conduct SCFA analysis in his laboratory and oversee student training in SCFA measurement and analysis. He will not require any salary coverage for this project.

Nicholas Chia, PhD, *Co-PI (1.2 calendar months for years 2-3).* Dr. Chia, Mayo Clinic Co-Director of Microbiome Research, will oversee postdoctoral training and conduct the metabolic modeling of data obtained from the MBRAs. He will require 0.1FTE at $13,852.80/year for this work for years 2-3.

RESEARCH BUDGET

We are requesting a total of $237,560 in Other Personnel, Materials and Supplies, Equipment in years 1-3 as follows:

OTHER PERSONNEL

Postdoctoral assistant. We are requesting 50% coverage for postdoctoral assistant (0.5 FTE) $28,312.50/year for years 2-3 to assist with metabolic modeling to share with Dr. Chia (other 50% covered by Dr. Chia).

Graduate research assistant. We are requesting as part of the research budget, funding to cover one graduate student, who will be working under the guidance of Dr. Greathouse and Dr. Lavado to assist with collecting, storing, and processing biospecimens for metabolite analysis for the duration of the study ($24,000/year).

MATERIALS AND SUPPLIES

Research supplies. We are requesting a total of $ 41,098 to cover consumables, labware, bacterial reagents, DNA extraction, and qPCR reagents (**Dr. Greathouse**). For metabolite SCFA analysis, we are requesting $20,000 (**Dr. Lavado**).

Contract services. For the 16S rRNA sequencing of the microbiome we are requesting: $25,000 for Year 2 and $25,000 for Year 3. (BCM, **Dr. Petrosino**)

EQUIPMENT

To initiate the setup and installation of the Mini-bioreactor array (MBRA) system we are requesting $89,208. This cost includes an anaerobic chamber and necessary components (oxygen sensors, heaters, H2S scrubbers, and pumps). The cost is based on the established system developed by Dr. Robert Britton (**see Appendix**). If this proposal is funded, it has been agreed (Drs. Meohnke and Chambliss) that space within the Molecular Bioscience Center in the Baylor Sciences Building would be utilized making this available to a larger user base.

TRAVEL AND PUBLICATION FEES

To support dissemination of findings from this study we are requesting $4,500 in Year 3 for travel to conferences ($1,500) and fees associated with publication ($3000).

Budget Items	YEAR 1	YEAR 2	YEAR 3	Total
TOTAL BUDGET	**$146,855**	**$87,312**	**$91,814**	**$366,184**

MEASURES OF SUCCESS

I. Expected impact and project deliverables

Impact on:

Field of Research - The results of *this research* will address outstanding questions in our field by **1)** identifying prebiotics key in conveying resistance to infection while accounting for inter-individual microbiome variability, **2)** establishing a model system capable of simulating microbial response to dietary nutrients and pathogens, and **3)** uncovering mechanisms that can be used to develop precision prebiotics to prevent infection, reduce colon cancer risk, and improve cancer therapy response. *Conducting this study is expected to identify those dietary prebiotic fibers capable of preventing infection.* Through metabolic modeling of prebiotics in healthy and diseased stool communities, dieticians and clinicians would be able to optimize dietary recommendations for high-risk patients, and personalize treatment strategies. Ultimately, this evidence is expected to result in reduced infections, improved therapeutic response and, subsequently, reduced deaths from infections.

Baylor University achieving R1 – By establishing this microbial modeling system (MBRA) and area of research, we will become a major stakeholder and **leader in precision prebiotic therapy and infectious disease research**, which will ultimately lead to the following outcomes: **1**) Established leader in microbial modeling of dietary nutrients and prebiotic trials, combining the expertise from Baylor University (Greathouse/Lavado), Baylor College of Medicine (Britton), and Mayo Clinic (Chia), **2)** Competitive position for multiple government and non-governmental grants, especially large center and program grants, **3)** Potential to become a nationally recognized leader in prebiotic research, leveraging the Baylor CPRIT Synthesis and Drug-Lead Discovery Laboratory to synergize these efforts to have major impact on drug design/delivery, **4)** Increased drug to market potential through Baylor's new Technology Transfer and Industry Engagement Program.

Project deliverables:

- Establishment of the first microbial bioreactor system (MBRA) at Baylor University
- MBRA system capable of stable culture of up to 28 independent fecal communities
- Microbial simulation model of response to dietary prebiotic fibers and pathogen resistance
- Preliminary data for larger R01-level grant and scientific leads for future hypothesis testing or intervention/clinical trials
- Graduate and undergraduate students trained in using fecal bioreactor systems, metabolic modeling, data science, and drug discovery (**see Appendix – Training Plan**)

II. Proposed timeframe within which to report back to the ISC

6 Month Report – November 1, 2019; 1 Year Report – June 1, 2020; 2 Year Report – June 1, 2021; 3 Year Report – June 1, 2022

III. Research impact on pillar of pursuing transformational education

Impact on Spiritual Growth – As part of the unambiguously Christian mission of Baylor University, this study will provide the students with the ability to practice science with Christian mentors, and experience the faith journey faced in conducting challenging scientific pursuits. Walking through these challenges with Christian mentors will encourage a deeper reflection on the impact their research has on others.

Impact on Critical Thinking – The complexity of this type of research will provide the students an opportunity to think critically through challenging scientific problems and test their ability to solve complex issues. This experience will build a solid foundation in which to apply these skills in future careers and become transformational leaders themselves.

Impact on Cutting-Edge Skills Development – The ability to combine multiple skill sets from bench to bedside or market is what makes for an exceptional scientist or industry leader. Students involved with this study will have the opportunity to learn from top scientists performing cutting-edge research, and be involved in developing clinical tools or discover novel drugs for use in preventing infections. Ultimately, this will provide greater advantages to our students in the job market, and open doors for multiple STEM career choices.

Impact on Baylor Faculty – Baylor faculty will benefit tremendously from having the MBRA system as a scientific resource (Adair, Bruce, Kearny, Lavado, Sayes, Greathouse), which will expand the scope and capability of their research. Students will also benefit from this collaborative effort through the skills learned by faculty participating in this collaborative study, as well as, exposure to guest lectures from faculty involved in studies from other participating organizations. **This resource will also make students and faculty more competitive for funding and enhance publication opportunities in high-impact journals.**

IMPACT ON R1 METRICS

I. Compelling case on funding source possibilities with citation of external funding sources' RFPs.

I.A. The National Institutes of Health will be announcing in March 2019 a new nutrition research initiative. The Strategic Plan for NIH Nutrition Research will focus efforts in "advancing the scientific understanding of interactions between diet, nutritional status, biological processes, and the environment." Specifically, it will focus on the effect of nutrition across the lifespan with an emphasis on maternal and child health, *which is a focus of my current research.* Multiple initiatives listed in this plan are directly related to this research, as well as, my overarching research focus. The following scientific and *training* priorities listed in their strategic plan directly align with this research:

1-1. Advance Nutritional Biochemistry and the Bioinformatics of Nutrition-Related Pathways
1-3. Identify and Leverage Interrelationships between Diet, Host, and the Gut Microbiome to Promote Health
2-5. Determine Mechanisms by which Dietary Patterns Affect Health Status and Chronic Disease Susceptibility
3-1. Elucidate the Biological Factors Underlying Individual Variation in Response to Dietary Interventions
4-1. Identify and Leverage Interactions Among Nutrition, Disease States, and Treatments
4-3. Identify Triggers and Endpoints for Nutritional Support in Clinical Settings
6-4. Develop and Improve Tools Using Big Data for Systems Science Approaches to Nutrition Research
6-5. Develop Sensors for Continuous Monitoring of Nutrients and Metabolites for Personalized Nutrition Research
7-1. Facilitate Training in Host, Gut Microbiome Metabolism, and Diet Interrelationships
7-2. Enhance Training in the Application of Big Data Approaches to Nutrition Research

Thus, buying into critical cutting-edge technologies, like the MBRAs, will be paramount in positioning Baylor University to take full advantage of this coming opportunity, and to coordinate research and student training efforts around Baylor to build capacity for this new strategic plan at the NIH. (**see Appendix – NIH Strategic Plan for Nutrition Research and Training Plans).**

The results of this research have far-reaching impacts on:

1. Broad basic mechanisms governing host diet-microbe relationships (NSF)
2. Development of prebiotic therapy for infection and cancer prevention (NIH/Foundations/Industry)
3. Novel precision prebiotics for improvement of cancer therapy and treatment outcomes (NIH/Foundations)

I.B Several organizations, both governmental and non-governmental, are key targets for funding of this research. Specifically, one of the National Cancer Institute's (NCI) *Provocative Questions in Cancer* is "**PQ11: Through what mechanisms do diet and nutritional interventions affect the response to cancer treatment?",** which is addressed by this research. **The following funding mechanisms or RFPs are listed below:**

NCI Provocative Questions (RFAs):

- R01 RFA-CA-18-019
- R21 RFA-CA-18-020

Other RFAs or Funding Mechanisms Specifically Aligned with this Research:

- Source: NIH - R01 Advancing Mechanistic Probiotic/Prebiotic and Human Microbiome Research
- Source: NIH - Nutrigenetics and Nutrigenomics Approaches for Nutrition Research (R01 Clinical Trial Optional)
- Source: NIH - Early-Stage Preclinical Validation of Therapeutic Leads for Diseases of Interest to the NIDDK (R01)
- Source: NIH - Food Specific Molecular Profiles and Biomarkers of Food and Nutrient Intake, and Dietary Exposure (R01 Clinical Trial Optional)

- Source: NIH/NCI - R01 (NIH/NCI) Age-related Microbiota Changes and their Implications in Chronic Disease Prevention, Treatment and Progression
- Source: Dannon Institute - Dannon Gut Microbiome, Yogurt and Probiotics Fellowship Grant Program
- Source: NIH - R21 (NIH) Mechanisms of Cancer and Treatment-related Symptoms and Toxicities (PA-16-258)
- Source: Cancer Prevention Research Institute of Texas - Multiple RFAs and general funding awards
- Source: NASA - Translation Research in Space Health (TRISH)

I.C. Plans to communicate with external funding sources that will increase the likelihood of your proposal to the them being accepted

Once this proposal is funded, myself and our team will begin to immediately reach out to program officers from the organizations listed, as well as, those organizations with which our colleagues have favorable connections or previous funding success. Most of the scientists on this team have previous funding from DoD, NIH, CPRIT and Industry. Thus, we have established success in communicating with and obtaining external funding sources. Specifically, I have two contacts at the NCI that are Program Officers, and they are highly interested in this work; Phil Daschle and Roberto Flores. In addition, Dr. Britton has already been successful in obtaining grant funding using this technology, as well as, producing high impact factor publications in *Nature* using this technology. This support and prior success in funding and publications indicates a clear desire from multiple funding agencies for conducting this research once preliminary data is obtained showing feasibility.

II. Return on Investment to Baylor University for this project

A. Increased national recognition in research scholarship – This research is highly regarded by those in the nutrition, cancer, and microbiome fields as a top priority as indicated by the NIH Strategic Plan for Nutrition Research. In consideration of the dramatic increase in spending on pre- and probiotics (>$1 billion/year) there is a critical need for prebiotic research, which could be met in part through this research initiative. Unfortunately, very few investigators have the skills, team, and resources with which to conduct this type of research. Setting up the MBRA system will be an important first step to launching a much larger multi-phase research project, which will involve multiple investigators/teams and stool donors to test potential prebiotic therapies. Having this type **cutting-edge scientific tool** (e.g. MBRAs) is the key to **a)** discovering new mechanisms and potentially new treatment modalities for infection and cancer prevention, **b)** unlocking federal funding opportunities, and **c)** impacting consumers and patients' lives to promote wellness. **This research will significantly impact nutrition, infectious disease, and microbial research on multiple fronts, from basic mechanisms to treatment, which will lead to high-impact publications and subsequent funding opportunities**. Further, this tool and new research area will allow Baylor to attract highly-research active and successful scientists as faculty. All of these factors together will propel Baylor University to R1 status.

B. Improved quality of education and research training – Access to high-quality research and training is key to preparing students for success in the STEM field, as well as, for career success beyond STEM fields. This research will provide **1)** access to experts in the fields of microbial ecology and pre/probiotics (Dr. Britton/Dr. Greathouse), **2)** training in use of the MBRA system (Dr. Britton), 3) skills in **Data Science** (Dr. Greathouse/Dr. Chia), **4)** project management and leadership skills (Dr. Greathouse), and **5)** exposure to **Artificial Intelligence methodology** during development of metabolic modeling (e.g. machine learning) (Dr. Chia/Dr. Greathouse). Further, with the NIH's Strategic Plan on Nutrition Research focused on methods to *Facilitate Training in Host, Gut Microbiome Metabolism, and Diet Interrelationships,* the research outlined in this proposal will dramatically increase our ability to compete for training funding opportunities that align with this objective. In combination, these experiences will dramatically enhance the educational and research training, which will also increase the competitiveness for advanced training opportunities at other R1 institutes, as well as, training fellowships or postdoctoral awards.

C. Production and recruitment of high-quality graduate and postdoctoral trainees – Exposure to the research outlined in this proposal will lead to retainment of high-quality postdoctoral fellows and

production of highly-trained and competitive doctoral students. Among the fields of infectious disease, microbial ecology, and cancer research, all continue to garner significant interest from potential graduate and postdoctoral trainees. Currently, I have 2 doctoral students (Biology/Statistics) and 5 undergraduate research students in my laboratory working on various aspects of microbe-host interactions. To conduct future research, however, I would also require additional top-quality postdoctoral fellows. With my previously established connections at the NCI and other research institutions in the cancer and microbiome fields, I have no doubt I or my team would be able to recruit multiple highly successful and productive individuals for this position. Further, the production of highly trained graduate students is a significant need in our field currently, and would likely garner **support for training grant** funding from the NIH for both undergraduate and graduate students.

D. Establishment of long-term collaborations between multiple academic and health care institutes – One of the keys to research success and recognition is establishment of collaborative scientific teams that span multiple disciplines and institutions. Rarely are highly impactful scientific discoveries made by single investigators, but more often take the effort of several independent investigators working together to solve an outstanding problem. For this research to be successful, multiple disciplines are required, including medicine, mathematics, next-generation sequencing, bioinformatics, epidemiology, chemistry, and computer engineering. Bringing together three entities, **Mayo Clinic, Baylor College of Medicine**, and **Baylor University,** to conduct this research has the potential to lead to long-term powerful collaborations with leverage to obtain multi-site and/or multi-PI program and project grants, including P01 and U01 awards (outlined above). Obtaining these types of awards will continue to propel Baylor to R1 status.

E. Recognition by Baylor Alumni and Businesses for Targeted Investments to the University – With research success and high-profile discoveries, especially those that impact the lives of loved ones (e.g. infectious disease and cancer), Baylor would be well-positioned to attract significant funding and investments from both Baylor alumni and business or industry partners. The potential impact of this research has the ability to **lead to novel prebiotics that could be patented or developed for clinical use by industry partners.**

F. Enhance research space and resources for Baylor and Baylor faculty – As we transition to R1, we will initially be confined to current available space. Investment in equipment, like the MBRAs, that can **enhance space already available at Baylor University** will add to the return on investments by: 1) providing a multi-user resource to Baylor Faculty to increase grant funding/publishing, 2) providing opportunity to recruit and train doctoral students, and undergraduates, 3) incentivize new faculty recruits to commit to Baylor, 4) encourage outside collaboration and/or move the MBRAs to a fee-for-service system that would generate income.

III. Previous and future proposals planned to advance the larger research agenda

A. Previous proposals addressing this research agenda:

Grants Funded

2019 *Baylor University (URSA)*
Project title: Characterization of Outer Membrane Vesicle RNA During the Phases of Growth of *B. fragilis*
Investigators: Leigh Greathouse
Role: PI

2018 *Baylor University (URC – mid-range)*
Project title: A fiber intervention to prevent weight gain and reduce stress levels for physicians in training.
Investigators: LesLee Funderburk (PI), Leigh Greathouse (Co-PI), Pete Grandjean (Co-PI)
Role: Co-PI

2017 *Summer Research Sabbatical*
Project title: COLON-MD (Pilot): COlon cancer LONgitudinal study of the Microbial metabolites and Dietary factors that influence response to treatment
Investigators: K. Leigh Greathouse
Role: PI
***preliminary background research from this sabbatical on conducting the pilot study lead to the publication of book chapter describing how to conduct a cancer microbiome study**

Baylor University (URC – mid-range)
Project title: Mediation of Host-Pathogen Interaction by Bacterial Outer Membrane Vesicle Small RNAs in Colon Cancer
Investigators: K. Leigh Greathouse (PI), Joseph Taube (Co-PI)
Role: PI

Grants Submitted, Not Funded

2018 *Career Development Award (PI: Greathouse)* ****received 1.7/Excellent (funded at 1.5 or higher)***
Project period: 05/01/2019-03/31/2022
Source: DoD
Funding: $485,000
Project title: Identification of the dietary and microbial factors that predict chemotherapy-induced diarrhea in colon cancer. The goal of this study is to identify the dietary and microbiome factors among colon cancer patients undergoing chemotherapy that predict chemotherapy induced diarrhea.
Role: PI

2018 *DNA Genotek Innovation Award*
RECRUITMENT, LONGITUDINAL RETENTION AND REPRODUCEABLE METHODS FOR COLON CANCER MICROBIOME STUDIES
Role: PI
Funding: $30K

Collaborative Faculty Research Investment Program ****not funded because BSWH withdrew from the CFRIP award mechanism***
Project title: "COLON-MD (Pilot): COlon cancer LONgitudinal study of the Microbial and Dietary factors that influence response to treatment"
Source of Funding: Baylor University

2017 *Cancer Prevention, Control, Behavioral Sciences, and Population Sciences Career Development Award (K07)*
Project title: "COLON-MD (Pilot): COlon cancer LONgitudinal study of the Microbial metabolites and Dietary factors that influence response to treatment"
Investigators: **K. Leigh Greathouse (PI)**
Source of Funding: National Cancer Institute ($444K, 3 years)

MRC2 Pilot/Feasibility Grants
Project title: "COLON-MD (Pilot): COlon cancer LONgitudinal study of the Microbial metabolites and Dietary factors that influence response to treatment"
Source of Funding: University of Michigan

2016 *Collaborative Faculty Research Investment Program*
Project title: "COLON-MD (Pilot): COlon cancer LONgitudinal study of the Microbial and Dietary factors that influence response to treatment"
Source of Funding: Baylor University

Department of Defense (Lung Cancer Concept Award)
Project title: "Investigating the relationship of commensal microbiota and DNA methylation in early stage Lung Cancer"
Investigators: As Co-PI, together with Curt Harris (PI)
Source of Funding: Department of Defense

B. Future Proposals Planned:

Due Date	Funding Mechanism	Title	Collaborators
June 19, 2019	R15 AREA (NIH)	Microbial Response to Specific Dietary Fibers Using Model Gut Communities	Rob Britton/Nick Chia/Jun Chen/Ramon Lavado
October 3, 2019	R35 MIRA (NIH)	Using MBRAs to Explore Diet-Microbiome Relationships	Rob Britton/Nick Chia/Jun Chen/Ramon Lavado

C. Future Users of the MBRA System:

Tamara Adair (BIO) – **The MBRA will enrich multiple undergraduate courses** by allowing students to design creative and relevant experiments and to analyze large genomic data sets. The following courses would be enhanced by having the MBRA system: BIO 1406 - Analysis of soil microbial communities for types and concentrations of bacteriophage or specific host organisms related to various locations or conditions; BIO 1106 - Analysis of soil microbial communities and experimental design using additions of different types of ciliates, microbes, or pollutant; Independent undergraduate research projects - This technology will provide the opportunity to have large data sets generated in several research labs and generate a rich repository that could be used for genomics and bioinformatics training and analysis. (**see Appendix – Training Plan**)

Erica Bruce (ENV) – Mini bioreactor arrays are an innovative tool that allow for a complex system of microbes and other constituents to be evaluated over multiple endpoints. Specifically, Dr. Bruce could utilize this system to evaluate metabolites of novel drugs, evaluating changes in the microbiome following single dose and multiple dose drug trials, evaluating changes in efficacy of drugs following incubation with microbes, evaluating relationships between co-cultured cells from the GI tract, and evaluating the resulting effects from transformation products. This proposal will add this valuable system to resources available at Baylor University that can be utilized by several scientists, it stimulates collaborative, interdisciplinary project development and represents a meaningful tool to sustain long term funding.

Chris Kearney (BIO) – will be able to use MBRA system to develop and test his antimicrobial peptide engineered bacteria for growth and functionality in a polymicrobial stool model community

Christie Sayes (ENV) – will use the model to test the metabolic effects after cellulose nanocrystal exposure to the microbiome. Metabolism of engineered materials is a largely under-studied area and needs data to help establish toxicokinetic profiles of nano-enabled food and drug products.

REFERENCES

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8 Makki, K., Deehan, E. C., Walter, J. & Backhed, F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe* **23**, 705-715, doi:10.1016/j.chom.2018.05.012 (2018).

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10 Christine N. Spencer, V. G., Jennifer McQuade, Miles C. Andrews, Beth Helmink, M.A. Wadud Khan, Elizabeth Sirmans, Lauren Haydu, Alexandria Cogdill, Elizabeth Burton, Rodabe Amaria, Sapna Patel, Isabella Glitza, MIchael Davies, Eliza Posada, Wen-Jen Hwu, Adi Diab, Kelly Nelson, Hussein Tawbi, Michael Wong, Robert R. Jenq, Lorenzo Cohen, Carrie Daniel-MacDougall, Jennifer A. Wargo. The gut microbiome (GM) and immunotherapy response are influenced by host lifestyle factors. in *AACR Annual Meeting 2019 Online Proceedings.*

11 O'Keefe, S. J. *et al.* Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun* **6**, 6342, doi:10.1038/ncomms7342 (2015).

12 Martens, E. C., Kelly, A. G., Tauzin, A. S. & Brumer, H. The devil lies in the details: how variations in polysaccharide fine-structure impact the physiology and evolution of gut microbes. *J Mol Biol* **426**, 3851-3865, doi:10.1016/j.jmb.2014.06.022 (2014).

13 Zeevi, D. *et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell* **163**, 1079-1094, doi:10.1016/j.cell.2015.11.001 (2015).

14 Zmora, N., Suez, J. & Elinav, E. You are what you eat: diet, health and the gut microbiota. *Nat Rev Gastroenterol Hepatol* **16**, 35-56, doi:10.1038/s41575-018-0061-2 (2019).

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27 Hale, V. L. *et al.* Distinct microbes, metabolites, and ecologies define the microbiome in deficient and proficient mismatch repair colorectal cancers. *Genome Med* **10**, 78, doi:10.1186/s13073-018-0586-6 (2018).
28 Battaglioli, E. J. *et al.* Clostridioides difficile uses amino acids associated with gut microbial dysbiosis in a subset of patients with diarrhea. *Sci Transl Med* **10**, doi:10.1126/scitranslmed.aam7019 (2018).
29 Morales, E., Chen, J. & Greathouse, K. L. Compositional Analysis of the Human Microbiome in Cancer Research. *Methods Mol Biol* **1928**, 299-335, doi:10.1007/978-1-4939-9027-6_16 (2019).
30 Greathouse, K. L. *et al.* Interaction between the microbiome and TP53 in human lung cancer. *Genome Biol* **19**, 123, doi:10.1186/s13059-018-1501-6 (2018).
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33 Sung, J., Hale, V., Merkel, A. C., Kim, P. J. & Chia, N. Metabolic modeling with Big Data and the gut microbiome. *Appl Transl Genom* **10**, 10-15, doi:10.1016/j.atg.2016.02.001 (2016).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: K. Leigh Greathouse

eRA COMMONS USER NAME (credential, e.g., agency login): Leigh_Greathouse

POSITION TITLE: Assistant Professor of Nutrition Science

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Stephen F. Austin State University, Nacogdoches, TX	B.S.	05/1997	Nutrition and Food Sci.
Texas Woman's University, Denton, TX	M.S.	08/2001	Sports Nutrition
University of Texas Houston Health Science Center and M.D. Anderson Cancer Center, TX	Ph.D.	05/2010	Molecular Carcinogenesis
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD	M.P.H.	05/2011	Epidemiology and Biostatistics
National Cancer Institute, Cancer Prevention Fellowship Program, Bethesda, MD	Post-doc	06/2014	Molecular Epidemiology of Lung Cancer

A. Personal Statement

My role in this project is that of PI. As a dietician and cancer biologist the main focus of my research group is to identify biomarkers and elucidate molecular mechanisms that can be used to define the relationship between diet, the microbiome and colon cancer pathogenesis. Using big data and machine learning techniques, we seek to define the relationship between diet, the microbiome and cancer. Our goal is to 1) delineate the dietary factors that modify the microbiome and its function, 2) develop microbial predictors that improve stratification of patients for obesity treatment, and 3) identify key functional pathways and mechanisms of the microbiota-host communication. Ultimately, our goal is to discover microbial and metabolic targets for the development of clinical tools to improve the treatment of and reduce mortality from colon cancer. As Co-PI of a dietary fiber intervention (RCT) investigating the effects on the microbiome, this research is poised to compliment these efforts *in vitro.* Currently, my lab is working together with several collaborators inside and outside of Baylor University, and have recently published research in *Genome Biology* and *BMJ Open Gastroenterology* that shows my ability to conduct this research.

B. Positions and Honors

Positions and Employment:

2010- 2014 **Postdoctoral Fellow***,* Cancer Prevention Fellowship Program, NCI, Bethesda, MD
2014- 2015 **Research Fellow**, National Cancer Institute, NIH, Bethesda, MD
2015-present **Assistant Professor** of Nutrition Sciences, Baylor University, Waco, TX; Adjunct Professor of Biology, Baylor University, Waco, TX

Other Experience and Professional Memberships:

2015-present Active Member of the American Association for Cancer Research
2015-present Editorial Board Member – *Carcinogenesis*
2018-present Editorial Board Member – *Genetic Testing and Molecular Biomarkers*

Honors:

2008	R.W. Butcher Award, Graduate School of Biomedical Science, University of Texas M.D. Anderson Cancer Center, Houston, TX
2008	Schissler Foundation Fellowship in Human Genetics of Disease, Graduate School of Biomedical Science, University of Texas, M.D. Anderson Cancer Center, Houston, TX
2010	Cancer Prevention Fellowship, National Cancer Institute
2012	National Institutes of Health Merit Award
2013	Aspen Cancer Conference Fellow
2016	Rising Star Young Investigator, Baylor University
2017	Fellow of the Texas Hunger Institute, Waco, TX
2019	URSA Leadership Award, Baylor University

C. Contributions to Science

***Selected Publications**

K. Leigh Greathouse, James R White, R. Noah Padgett, Brittany G Perrotta, Gregory D Jenkins, Nicholas Chia, Jun Chen. Gut microbiome meta-analysis reveals dysbiosis is independent of body mass index in predicting risk of obesity-associated CRC. bioRxiv 367466; doi: https://doi.org/10.1101/367466. *BMJ Open Gastroenterology.* 2019

K. Leigh Greathouse, J. White, V. Bliskovsky, A. Vargas, E. Polley, E. Bowman, M. Khan, A. Robles, B. Ryan, A. Dzutsev, G. Trinchieri, M. Pineda, S. Bilke, P. Meltzer, C. Deming, S. Conlan, J. Oh, J.A. Segre, C.C. Harris. Interaction between the microbiome and TP53 in human lung cancer. 2018 *Genome Biol, 19*(1), 123. doi:10.1186/s13059-018-1501-6

Daquigan N, Seekatz AM, **Greathouse KL**, Young VB, White JR. High-resolution profiling of the gut microbiome reveals the extent of *Clostridium difficile* burden. *NPJ Biofilms Microbiomes*. 2017 Dec 5;3:35. doi: 10.1038/s41522-017-0043-0. eCollection 2017. PubMed PMID: 29214047; PubMed Central PMCID: PMC5717231. 4 citations

Complete List of Published Works in MyBibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1RGAUfG1s69Q8/bibliography/53509358/public/?sort=date&direction=ascending.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

University Research Committee (URC) Funding: $7318 (2018-2019)
Project title: *A fiber intervention to prevent weight gain and reduce stress levels for physicians in training.*
Investigators: LesLee Funderburk, PI, Leigh Greathouse, Co-PI.

My role in this project is as Co-PI. I conceived of this idea, designed the study and experiments and co-wrote the grant. Our hypothesis is that increased dietary fiber will prevent weight gain, increased adiposity and reduce perceived stress levels in residents at the Family Health Clinic as the result of changes in distal gut microbiota composition and function.

Undergraduate Research Student Award (URSA) Funding: $4946 (2019-2020)
Project title: *Characterization of Outer Membrane Vesicle RNA During the Phases of Growth of B. fragilis.* The goal of this study is to characterize the size and concentration outer membrane vesicles secreted at each phase of growth, as well as, sequence their RNAs to analyze the differences in gene expression.
Role: PI

BIOGRAPHICAL SKETCH

NAME: Ramon Lavado

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Barcelona, Barcelona, Spain	Bachelor	07/1999	Biological Sciences
University of Barcelona, Barcelona, Spain	Ph.D.	12/2005	Animal Physiology
University of California Riverside, Riverside, CA	Postdoctoral	06/2011	Toxicology

A. Positions and Honors

Positions and Employment

2016- Assistant Professor, Department of Environmental Science, Baylor University, Waco, TX.
2014-2016 Research Associate in Virology, Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, IA.
2012-2013 Scientist / Technical Support Officer, Institute for Environment and Sustainability, European Commission Joint Research Centre, Ispra, Italy.
2011-2012 Assistant Specialist, Department of Environmental Sciences University of California Riverside, Riverside, CA.
2006-2011 Postdoctoral Research Associate, Department of Environmental Sciences University of California Riverside, Riverside, CA.

Other Experience and Professional Memberships

2018- Grant Proposal Reviewer, California Sea Grant, California Department of fish and Wildlife.
2018- Grant Proposal Reviewer, Polish National Science Centre, Poland.
2018- Board of Advisors, Society of Environmental Toxicology and Chemistry (SETAC) South Central Regional Chapter.
2017- Faculty Advisor of Baylor University Student SETAC Chapter.
2017- Editorial Board Member of Journal of Environmental and Toxicological Studies.
2016- Selected as Baylor University “Rising Star” through the OVPR.
2012- Editorial Board Member of Bulletin of Environmental Contamination and Toxicology (BECT).
2007- Member of the Society of Environmental Toxicology and Chemistry (SETAC) North America.
2006- Member of International Society for the Study of Xenobiotics (ISSX).
2006- Member of American Chemical Society (ACS).
2001- Member Society of Environmental Toxicology and Chemistry (SETAC) Europe.

Honors

2016- Selected as Baylor University “Rising Star” Research Program.
2012-2013 Marie Curie Actions – International Incoming Fellowship (IIF) – European Commission Postdoctoral Fellowship.

B. Recent Contributions to Science (Last 5 years)

1. Ishmaeel, A., **Lavado, R.**, Smith, R. S., Eidson, J. L., Sawicki, I., Kirk, J. S., Bohannon, W. T. and Koutakis, P. Effects of limb revascularization procedures on oxidative stress. Journal of Surgical Research 232 (2018), 503-509.

2. Franco, M. E., Sutherland, G. E. and **Lavado, R.** (2018). Xenobiotic metabolism in the fish hepatic cell lines Hepa-E1 and RTH-149, and the gill cell lines RTgill-W1 and G1B: Biomarkers of CYP450 activity and oxidative stress. Comparative Biochemistry and Physiology. Part C: Toxicology & Pharmacology 206-207 (2018), 32-40.
3. Oziolor, E.M., Howard, W., **Lavado, R.** and Matson, C.W. Induced pesticide tolerance results from detoxification pathway priming. Environmental Pollution 224 (2017), 615-621 (2017).
4. Maldonado, A., **Lavado, R.**, Knutson, S., Slattery, M., Goldstone, J.V., Watanabe, K., Hoh, E., Gadepalli, R.S., Rimoldi, J.M., Ostrander, G.K. and Schlenk, D. Biochemical mechanisms for geographical adaptations to novel toxin exposures in butterflyfish. PLOS One (2016), doi:10.1371/journal.pone.0154208.
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6. Crago, J., Tran, K., Budicin, A., Schreiber, B., **Lavado, R.** and Schlenk, D. Exploring the impacts of two separate mixtures of pesticide and surfactants on estrogenic activity in male fathead minnows and rainbow trout. Archives of Environmental Contamination and Toxicology 68 (2015), 362-370.
7. Maryoung, L.A., **Lavado R.** and Schlenk, D. Impacts of hypersaline acclimation of the acute toxicity of the organophosphate chlorpyrifos to salmonids. Aquatic Toxicology 152 (2014), 284-290.
8. Lyons, K., **Lavado, R.**, Schlenk, D. and Lowe, C. Bioaccumulation of organochlorine contaminants and EROD activity in southern California round stingrays (Urobatis halleri) exposed to planar aromatic compounds. Environmental Toxicology and Chemistry 33 (2014), 1380-1390.
9. Forsgren, K.L., Qu, S., **Lavado, R.**, Cwiertny, D. and Schlenk, D. Trenbolone acetate metabolites promote ovarian growth and development in adult Japanese medaka (*Oryzias latipes*). General and Comparative Endocrinology 202 (2014), 1-7.
10. **Lavado, R.**, Li, J., Rimoldi, J.M. and Schlenk D. Evaluation of the stereoselective biotransformation of permethrin in human liver microsomes: contributions of cytochrome P450 monooxygenases to the formation of estrogenic metabolites. Toxicology Letters 226 (2014), 192-197.

Complete List of Published Works (36 publications total):
https://www.ncbi.nlm.nih.gov/sites/myncbi/1jl8k0F2aX7kl/bibliography/42763479/public/?sort=date&direction=ascending

C. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Title: "Spatially-explicit profiles of endocrine disruption activity during low flows in East Canyon Creek, Utah"
Dates: 07/01/2018-10/30/2019
Grantor: Carollo Engineers, Inc. **Role:** PI **Funding:** $199,976.00

Title: "Evaluation of biomass health in the wastewater treatment basins"
Dates: 07/01/2017-06/30/2019
Grantor: Dow Chemical Company **Role:** PI **Funding:** $108,861.00

Title: "Use of a novel cell-based approach for assessing potential toxicity of seafood"
Dates: 06/01/2018-05/30/2019
Grantor: Baylor University (YIDP2019 Program) **Role:** PI **Funding:** $25,000.00

Completed Research Support

Title: "MutEndocrintool – Rationally mutated estrogen and androgen receptors: a novel approach to improve the detection of endocrine disruptor chemicals in the environment"
Dates: 12/01/2012-11/30/2013
Grantor: European Commission (Marie Curie Program) **Role:** PI **Funding:** $260,000.00

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Robert Allen Britton

eRA COMMONS USER NAME (credential, e.g., agency login): MSUBRITTONR

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Nebraska	B.S.	1985-1989	Biology
Baylor College of Medicine	Ph.D.	1990-1996	Cell and Molecular Biology
Massachusetts Institute of Technology	Postdoc	1996-2002	Bacterial genetics/genomics

A. Personal Statement. The role of bacteria in human and animal health has undergone a renaissance in the past decade. The overall focus of my laboratory is therapeutic microbiology, in which we aim to develop both traditional probiotic bacterial strains for the prevention and treatment of disease as well as engineer bacterial communities to express therapeutic proteins. During my PhD work under James Lupski at Baylor College of Medicine and my postdoctoral training under Alan Grossman at MIT I received excellent training in microbial genetics, physiology and genomics. I trained extensively in both Gram-negative and Gram-positive model systems and it is this expertise that I now bring to the current work in the areas of probiotic bacteria and the intestinal microbiota. My laboratory has been investigating microbial community structure and function using next generation sequencing technology to address how microbial ecology impacts health. We also have developed powerful genetic tools for the exploration of mechanistic insights into the benefits of probiotic lactic acid bacteria. Recently, we have invented human fecal Mini-Bioreactor Arrays (MBRAs) to investigate how human intestinal microbiota interacts with *C. difficile* as well as a humanized microbiota (Hmb) mouse model of *C. difficile* disease. The MBRAs completely reproduce the *C. difficile* invasion dynamics that are observed in humans and animal models as well as other aspects of human intestinal communities. We also use MBRAs to study microbiome:diet interactions, drug metabolism by the microbiota and other infectious disease interactions with microbial communities. Thus, I am well-positioned to act at Co-PI on this proposal.

B. Positions and Honors

Positions and Employment

1988-1990	Research Assistant with Robert Klucas, Ph.D., in the Dept. of Biochemistry, Univ. of Nebraska, Lincoln, NE.
1989-1990	Research Assistant with Anne Vidaver, Ph.D., in the Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE.
1995	Guest Researcher in the laboratory of Dr. Donald Court, Laboratory of Chromosome Biology and Gene Expression, NCI/FCRDC, Frederick, MD.
1991-1996	Completed Ph.D. Thesis Research with James R. Lupski, M.D./Ph.D., Dissertation Title: Suppressor Analysis of *E. coli dnaG* Mutations. Baylor College of Medicine, Houston, TX.

1996-2002	Postdoctoral fellow in the laboratory of Dr. Alan Grossman, Department of Biology, MIT, Cambridge, MA.
2003-2008	Assistant Professor, Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.
2008-2014	Associate Professor, Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.
2014-2014	Professor, Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.
2014-present	Professor, Department of Molecular Virology and Microbiology; Member, Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX.

Professional Memberships

1999-present	American Society for Microbiology

Honors and Service

2016	International Ocular Surface Society Award Lecture, Seattle, WA.
2010	Teacher-Scholar Award, College of Natural Science, Michigan State University
2009	NSF Fall Genetics Panel, Reviewer
2008-present	Participant in the Joint Genomes Institute Undergraduate Annotation Research Initiative.
2005-2010	Editor, Gene – Functional Genomics
2006-2009	Delegate, International Society of Probiotics and Prebiotics
2005-2007	Scientific Foundation of Ireland, Reviewer, Genetic Panel and Equipment grants
2000-2002	Co-director of the MIT Microarray Club.
1994	O.B. Williams Award Winner, Best Oral Presentation, Texas Branch American Society for Microbiology.

C. Contributions to science.

3. Investigating the interaction between the intestinal microbiota and *Clostridium difficile*. We are interested in understanding how the intestinal microbiota provides a barrier to incoming pathogens and how perturbations of the microbiota result in an established infection.

1. Collins J, Robinson C, Danhof H, Knetsch CW, van Leeuwen HC, Lawley TD, Auchtung JM, **Britton RA.** (2018) Dietary trehalose enhances virulence of epidemic *Clostridium difficile*. Nature. 2018 Jan 18;553(7688):291-294. doi: 10.1038/nature25178. Epub 2018 Jan 3. PMID: 29310122

2. Robinson CD, Auchtung JM, Collins J, **Britton RA**. (2014). Epidemic *Clostridium difficile* Strains Demonstrate Increased Competitive Fitness Compared to Nonepidemic Isolates. Infect Immun. 2014 Jul;82(7):2815-25. doi: 10.1128/IAI.01524-14. Epub 2014 Apr 14

3. Collins J, Auchtung JM, Schaefer L, Eaton KA, **Britton RA**. (2015). Humanized microbiota mice as a model of recurrent *Clostridium difficile* disease. Microbiome. 2015 Aug 20;3:35. doi: 10.1186/s40168-015-0097-2

4. Auchtung JM, Robinson CD, **Britton RA**. (2015). Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRA). Microbiome. 2015 Sep 30;3:42. doi: 10.1186/s40168-015-0106-5. PMID: 26419531.

Full publication record available at:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1TKIVdmmlv5/bibliography/41856232/public/?sort=date&direction=descending

D. Research Support (selected)

Ongoing research support:

U19AI116482 (Britton - collaborator) 3/1/15 – 2/29/20
NIH/NIAID
Engineered human intestinal organoids: a modular system to model enteric disease
The goal of this project is to interface human intestinal organoids with intestinal microbes.

U01 AI124290-01 9/1/16-8/31/21
NIH/NIAID (Savidge, Britton, Sorg, Garey, Iliopoulus multi-PI)
Decoding antibiotic-induced susceptibility to *Clostridium difficile* infection
The goal of this project is to study *C. difficile* pathogenesis using a systems biology approach.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Chia, Nicholas Lee-Ping

eRA COMMONS USER NAME (credential, e.g., agency login): NICHOLAS_CHIA

POSITION TITLE: Associate Professor of Surgery

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Georgetown University, Washington DC	B.S.	06/2001	Physics
The Ohio State University, Columbus, OH	Ph.D.	06/2006	Physics
Institute for Genomic Biology, University of Illinois, Urbana-Champaign, IL	Post-Doctoral Fellowship	06/2011	Biophysics

A. Personal Statement

My role in the proposed project is that of Co-PI. I have a broad background in biophysics and theoretical biology, with specific training in the systems and computational biology approaches needed to make this project successful. I have always believed that the most interesting subject a physicist can study is life. From my undergraduate days in microscopy, when I developed a glass-only total internal reflection microscope from spare parts, to my present focus on understanding how we can model the microbial-host interface to create better tools for diagnosis and prevention, biology has been a constant. As PI of an R01 on the role of the microbiome in causing colon cancer, I have demonstrated my ability to manage a large, interdisciplinary project involving metabolic modeling, metagenomic sequencing, and human samples. In addition, in my position as Co-Director of the Microbiome Program at Mayo Clinic, I am ideally placed to ensure that this project receives the clinical, bioinformatics, and statistical support required. This work builds logically on my prior experience and interests, and the direction of Principal Investigator Dr. Leigh Greathouse will provide complementary expertise in using MBRA models to study the effects of dietary prebiotics on the microbiome; we have published and presented work on the microbiome together in the past. Below, I list publications that exemplify my work in building and strengthening computational pipelines for clinical and basic research at Mayo Clinic; many recent publications from Mayo Clinic in the areas of Microbiology and Microbiome research have made use of these pipelines to generate novel findings. Of course, this proposal also requires expertise in microbiome bioinformatics, and representative publications in this area can be found in section C.

B. Positions and Honors

Positions and Employment

2006-2008 Postdoctoral Researcher under Nigel Goldenfeld and Carl Woese, University of Illinois at Urbana-Champaign, Champaign, IL
2011-2012 Senior Research Scientist, Institute for Systems Biology, Seattle, WA
2012-2014 Associate Consultant, Department of Surgery, Joint appointment in Health Sciences Research, Mayo Clinic, MN
2012-2018 Associate Director, Microbiome Program, Center for Individualized Medicine, Mayo Clinic, MN
2015-Present Senior Associate Consultant, Department of Surgery, Mayo Clinic, MN
2015-2018 Assistant Professor, Department of Surgery, Mayo Clinic, MN
2018-Present Associate Professor, Department of Surgery, Mayo Clinic, MN

Honors

2001 Awarded Fowler Fellowship by the Ohio State University Department of Physics
2006 Awarded the Alexander von Humboldt Fellowship for work in the area of Bioinformatics

C. Contribution to Science

My research career contains three key ingredients that relate to the success of this proposal, namely (1) creating new bioinformatics tools; (2) improving the accuracy of metabolic modeling, especially as regards the microbiome; and (3) designing theoretical models that predict biological reality. *Selected publications:

1. Jeraldo P, Kalari K, Chen X, Bhavsar J, Mangalum A, White BA, Nelson H, Kocher JP, **Chia N**. IM-TORNADO: A tool for comparison of 16S reads from paired-end libraries. PLOS One. 2014 Dec 15;9(12):e114804. PMCID: PMC4266640.
2. Sipos M, Jeraldo P, **Chia N**, Qu A, Dhillon AS, Konkel ME, Nelson KE, White BA, Goldenfeld N. Robust computational analysis of rRNA hypervariable tag datasets. PLoS One. 2010 Dec 31; 5(12):e15220. PMCID: PMC3013109.
3. Jeraldo P, **Chia N**, Goldenfeld N. On the suitability of short reads of 16S rRNA for phylogeny-based analyses in environmental surveys. Environ Microbiol. 2011 Nov; 13(11):3000-9.
4. Jeraldo P, Sipos M, **Chia N**, Brulc JM, Dhillon AS, Konkel ME, Larson CL, Nelson KE, Qu A, Schook LB, Yang F, Goldenfeld N, and White BA. Quantifying the role of neutral and niche processes in evolution, Proc Natl Acad Sci U S A.. 109, 9692-9698 (2012) PMCID: PMC3382495.
5. Jeraldo, P., Sipos, M., Chia, N., Brulc, J. M., Dhillon, A. S., Konkel, M. E., ... & Yang, F. (2012). Quantification of the relative roles of niche and neutral processes in structuring gastrointestinal microbiomes. *Proceedings of the National Academy of Sciences*, *109*(25), 9692-9698.
6. Sinha, R., Chen, J., Amir, A., Vogtmann, E., Shi, J., Inman, K. S., ... & Chia, N. (2016). Collecting fecal samples for microbiome analyses in epidemiology studies. *Cancer Epidemiology and Prevention Biomarkers*, *25*(2), 407-416.
7. Sipos M, Jeraldo P, Chia N, Qu A, Dhillon AS, Konkel ME, Nelson KE, White BA, Goldenfeld N. Robust Computational Analysis of rRNA Hypervariable Tag Datasets. PLoS One. 2010 Dec 31; 5(12):e15220. PMCID: PMC3013109
8. Multinu, F., Harrington, S. C., Chen, J., Jeraldo, P. R., Johnson, S., Chia, N., & Walther-Antonio, M. R. (2018). Systematic Bias Introduced by Genomic DNA Template Dilution in 16S rRNA Gene-Targeted Microbiota Profiling in Human Stool Homogenates. *mSphere*, *3*(2), e00560-17.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/nicholas.chia.1/bibliography/47744991/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 CA179243 (PI - N Chia) 06/05/2014 – 05/31/2019
Microbial Metabolic Toxicity Drives Colon Cancer. The aims of this application are to 1) build metabolic models reflective of the microbial production and consumption of potential toxins with special focus on the role of sulfate-reducing bacteria, methanogens, and microbial community structure, and 2) understand the role of microbially-produced metabolites in carcinogenesis in deficient and proficient mismatch repair colorectal cancer.

R01 AR56647 (R Patel) 09/01/14 – 08/31/19
Metagenomic Analysis of Arthroplasty Failure
The goals of this application is to understand the underlying microbial populations in prosthetic joint infections (PJIs) using next-generation sequencing methodologies to directly probe the environment in PJIs.
Role: Co-investigator

Mayo Clinic & University of Minnesota Cooperation Program
(K. Khazaie & R Blekhman) 02/01/18 – 01/31/20
Development of personalized microbiome-based treatment for colorectal cancer
Our objective in this application is to build a systems-level, mechanistic understanding of the functional interactions between tumor neoplastic mutations, standard of care treatments, and microbial factors (taxa, genes, and functions) in colorectal neoplasia.
Role: Co-investigator

BAYLOR
UNIVERSITY

March 21st, 2019

Principal Investigator: Leigh Greathouse, PhD, MPH, MS, RD
Co-PI: Ramon Lavado, PhD
RE: Illuminate Proposal

Dear Review Committee,

As Assistant Professor of Environmental Sciences, I fully endorse the proposed study "Development of precision medicine prebiotics using mini-bioreactor arrays (MBRAs)."

I am happy to be a part of this study and to assist Dr. Greathouse with metabolite analysis of short-chain fatty acids. I am the principal investigator of the Environmental Toxicology and Metabolomics Lab in the Department of Environmental Science at Baylor University. I am excited to have the opportunity to collaborate on this study by leading the lipidomic analysis of the project. This work fits perfectly with research I am currently conducting in my laboratory and with that of my other collaborators. I have extensive experience with LC/MS spectrometry, and I will be responsible for overseeing the data acquisition and analysis of the LC/MS lipidomics identification and quantification. Furthermore, I will interpret the results, and prepare manuscripts for publication. In addition to lab expertise, I am also thrilled to help mentor undergraduate student researchers. We have recently received funding to profile over 360 lipids in cell culture systems and in human muscle, and thus I have shown that I have the expertise and standards necessary to complete this type of analysis.

The results from this study will lay the groundwork for a larger multi-phase study that is well-positioned to address the need for prebiotic formulations and treatments to combat infections. Ultimately, this research will lead to a significant opportunity for other researchers at Baylor University to take advantage of this cutting-edge system, and potentially be used to recruit highly research-active investigators to Baylor.

This project has tremendous promise not only for Dr. Greathouse but also for Baylor University. Further, I believe it will develop into a highly impactful long-term collaboration with exceptional external funding opportunities from multiple agencies, and that will generate innovative research on the diet-microbiome relationship. Overall, I am well positioned to provide excellent support for this study and we look forward to collaboration to facilitate Baylor's mission of R1 Status.

THE DEPARTMENT OF ENVIRONMENTAL SCIENCE
One Bear Place #97266 • Waco, TX 76798-7266 • Office (254) 710-3405 Fax (254) 710-3409

I will totally support her research endeavors, and I will be available at all times to discuss any aspect of the research as well as participate in manuscripts generation and future grant preparation. I am confident that this grant proposal will be submitted as an R01 NIH grant proposal before the end of the project.

Sincerely,

Ramon Lavado, Ph.D.
Assistant Professor
Department of Environmental Science
Baylor University

Baylor College of Medicine

DEPARTMENT OF MOLECULAR VIROLOGY AND MICROBIOLOGY

Robert A. Britton, Ph.D.
One Baylor Plaza, Room 739E
MS: BCM385
HOUSTON, TEXAS 77030-3411
rabritto@bcm.edu

March 5th, 2019

Principal Investigator: Leigh Greathouse, PhD, MPH, MS, RD
Co-PI: Robert Britton, Ph.D.
RE: Illuminate Proposal

Dear Review Committee,

As Professor of Molecular Virology and Microbiology at Baylor College of Medicine, I fully endorse the proposed study **Development of precision medicine prebiotics using mini-bioreactor arrays (MBRAs)**.

I am excited to be a part of this study and to assist Dr. Greathouse and Baylor University in establishing the MBRA system. As original developer of this system, I have expertise in all aspects of set up and experimental design, which makes me well-suited to serve as Co-Principal Investigator on this Illuminate Proposal. Our laboratory is interested in understanding how the intestinal microbiota provides a barrier to incoming pathogens and how perturbations of the microbiota result in an established infection. We have focused most of our attention on the pathogen *Clostridium difficile*, which is the most common cause of antibiotic associated diarrhea. We developed the mini-bioreactors and mice colonized with a human intestinal microbiota to address which members of the community are responsible for inhibiting *C. difficile* invasion. Thus, our research is complimentary and potentially synergistic.

The results from this study will lay the ground work for a larger multi-phase study that is well-positioned to address the need for prebiotic formulations and treatments to combat infections. Given that the NIH will be announcing a large multi-center funding strategy to support nutrition research, this proposal is well timed to ready Baylor to take advantage of this funding opportunity. Ultimately, this research will lead to a deeper understanding of the dietary prebiotic fibers that modulate the gut microbiome, and identify the prebiotics that support microbial communities capable of both resistance to and eviction of pathogens.

I have little doubt that this project will develop into a highly impactful long-term collaboration with exceptional external funding opportunities from multiple agencies, and that will generate innovative research on the diet-microbiome relationship. Overall, I am well positioned to provide excellent support for this study and we look forward to collaboration to facilitate Baylor's mission of R1 Status.

Best regards,

RBritton

Robert Britton, PhD
Therapeutic Microbiology Laboratory
Professor, Department of Molecular Virology and Microbiology

Member, Alkek Center for Metagenomics and Microbiome Research
Baylor College of Medicine, Room 663E
rabritto@bcm.edu

MAYO CLINIC

CENTER FOR INDIVIDUALIZED MEDICINE

Nicholas Chia
Mayo Clinic
200 First St SW
Rochester, MN 55905

March 5th, 2019

Principal Investigator: Leigh Greathouse, PhD, MPH, MS, RD
Co-I: Nicholas Chia, Ph.D.
RE: Illuminate Proposal

Dear Review Committee,

As Co-Director of the Microbiome Program and Associate Professor of Biophysics in the Center for Individualized Medicine, I fully endorse the proposed study **Development of precision medicine prebiotics using mini-bioreactor arrays (MBRAs)**.

Our laboratory is interested in understanding how the gut microbiome influences colon cancer risk and development. Specifically, we are focused on manipulating the gut microbiome using antibiotics, probiotics or prebiotics to determine how to attenuate risk. My research uses metabolic modeling to understand complex microbial interactions to better understand the mechanisms controlling risk. Thus, I am thrilled to have the opportunity to be a part of this study and assist Dr. Greathouse and Baylor University in establishing the MBRA system. As the developer of several metabolic modeling software tools, I have expertise in all aspects of modeling of the data generated from this research, which makes me well-suited to serve as Co-Principal Investigator on this Illuminate Proposal. Overall, our research is complimentary and potentially synergistic with that of Dr. Greathouse.

The results from this study will lay the ground work for a larger multi-phase study that is well-positioned to address the need for prebiotic formulations and treatments to combat infections. Ultimately, this research will lead to a deeper understanding of the dietary prebiotic fibers that modulate the gut microbiome, and identify the prebiotics that support microbial communities capable of both resistance to and eviction of pathogens.

I have little doubt that this project will develop into a highly impactful long-term collaboration with exceptional external funding opportunities from multiple agencies, and that will generate innovative research on the diet-microbiome relationship. Overall, I am well positioned to provide excellent support for this study and we look forward to collaboration to facilitate Baylor's mission of R1 Status.

Sincerely,

Nicholas Chia, Ph.D.
200 First Street SW/Harwick 3