

Pennsylvania Department of Health Final Performance Summary Report Formula Grants

Overview of the Health Research Project Performance Review Process and Criteria

An applicant that receives a health research grant under Tobacco Settlement Act / Act 77 of 2001, Chapter 9, is subject to a performance review by the Department of Health upon completion of the research project. The performance review is based on requirements specified by Act 77 and criteria developed by the Department in consultation with the Health Research Advisory Committee.

As part of the performance review process, each research project contained in a grant is reviewed by at least three experts who are physicians, scientists or researchers. Reviewers are from the same or similar discipline as the research grant/project under review and are not from Pennsylvania. Reviewers use the applicant's proposed research plan (strategic plan), the annual progress report and final progress reports to conduct the review. A grant that receives an unfavorable performance review by the Department may be subject to a reduction in funding or become ineligible for health research funding in the future. The overall grant evaluation rating is based on the ratings for the individual research projects contained in the grant.

This performance review report contains the outcome of the review for the grant as a whole (outstanding, favorable, or unfavorable), strengths and weaknesses of each research project, as well as recommendations for future improvement.

The following criteria were applied to information submitted by research grant recipients:

- **Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?**
 - Did the project meet the stated objectives?
 - Were the research design and methods adequate in light of the project objectives?
 - Consider these questions about data and empirical results: Were the data developed sufficiently to answer the research questions posed? Were the data developed in line with the original research protocol?
 - If changes were made to the research protocol, was an explanation given, and, if so, is it reasonable?
 - Consider (only for clinical research projects) the extent of laboratory and clinical activities initiated and completed and the number of subjects relative to the target goal.
 - Were sufficient data and information provided to indicate or support the fact that the project met its objectives or made acceptable progress?
 - Were the data and information provided applicable to the project objectives listed in the strategic research plan?

- **Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?**
 - What is the significance of this project for improving health?
 - Consider the value of the research completed towards eventual improvement in health outcomes.
 - Consider any changes in risk factors, services provided, incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of impact and effectiveness of the research being conducted.
 - Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment, which are attributable to the completed research project.
 - What are the future plans for this research project?

- **Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?**
 - If leveraging of funds were expected, did these materialize?
 - Are the researchers planning to apply for additional funding in the future to continue or expand the research?

- **Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted/filed?**
 - If any of the above listed were expected, did these materialize?
 - Are the researchers planning to submit articles to peer-reviewed publications, file for any licenses, or patents or begin any commercial development opportunities in the future?
 - Consider the number/quality of each.

- **Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?**
 - Were there improvements made to infrastructure?
 - Were any new investigators added or were any researchers brought into the institution to help carry out this research?
 - Were funds used to pay for research performed by pre- or post-doctoral students?

- **Criterion 6 - Did the project lead to collaboration with research partners outside the institution, or new involvement with the community?**
 - Are the researchers planning to begin any collaborations as a result of the research?
 - For clinical research only: consider the number of hospitals and health care professionals involved and the extent of penetration of the studies throughout the region or the Commonwealth.

Overall Evaluation Rating

An overall evaluation rating is assigned to each research project. The rating reflects the overall progress the project attained in meeting the stated goals and objectives. The rating is based on a scale of 1–3, with 1 being the highest. An average rating is obtained from all the reviews (minimum of 3) of each project and is the basis for the determination of the final overall rating for each project as follows:

1.00 – 1.33 = *Outstanding*

1.34 – 2.66 = *Favorable*

2.67 – 3.00 = *Unfavorable*

The grant level rating is an average rating from all projects as above. The numerical rating appears in parentheses for the grant and each project in the ***Overall Grant Performance Review Rating*** section of the report.

Overall Grant Performance Review Rating

Grant Rating: Favorable (1.67)

Project Rating:

Project	Title	Average Score
0988701	16S FISH-based FACS Purification of Unculturable Bacteria for Whole Genome Amplification and Sequencing	Favorable (1.67)

Project Number: 0988701
Project Title: 16S FISH-based FACS Purification of Unculturable Bacteria for
Whole Genome Amplification and Sequencing
Investigator: Ehrlich, Garth

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The overall goal of this project is to develop a unique methodology for the purification of unculturable bacteria of a single species as part of a comprehensive strategy to identify, characterize, and manipulate important natural product biosynthetic pathways for drug discovery and development. The objectives are to identify complex medical and environmental microbiomes using 16S FISH technique, FACS sort a specific bacterial population, purify its DNA, amplify and sequence its whole genome. As a proof-of-concept, the investigators used *Staphylococcus aureus* as a test organism and demonstrated the utility of 16S rRNA FISH-based FACS sorting to isolate and identify a certain population of bacteria. Subsequently, they used this technique to FISH-SORT unknown bacteria that live symbiotically with the tunicate species *Ecteinascidia turbinata*. The symbiotic microorganism(s) have been postulated to produce the anticancer drug Trabectedin (ET-743). The investigators were able to FISH 60,000 specific bacteria from the tunicate tissue and, through significant optimization work, were able to amplify bacterial genomic DNA from as low as 100 cfu.

The strength of this project resides in the investigators' success in using FISH-SORT technique to isolate symbiotic bacteria from the tunicate tissue, which may lead to new technical capabilities in exploiting biosynthetic machineries from unculturable symbiotic microorganisms. Although the idea itself is not new, the success in using this technique to isolate symbiotic bacteria from the tunicate tissue is a significant accomplishment.

The weaknesses include the lack of description or discussion in the report about the whole genome sequencing (WGS) results. Also, during the initial metagenomic sequencing, the investigators identified a number of contigs that may contain putative non-ribosomal peptide synthase domains expected to be involved in the biosynthesis of ET-743. However, it is not clear how, or if, this information will be used to help identify the producing strain or streamline the whole genome amplification (WGA)/WGS part of the project.

Overall, the research design and methods were adequate in light of project objectives; the data were developed sufficiently to answer most of the research questions and in line with the original research protocols. Sufficient data and information were provided to support the fact that the project made significant progress or in most part met its stated objectives.

Reviewer 2:

The project met all three of the stated aims in the original proposal to identify from a metagenomic study the probable bacterial producer and biosynthetic pathway to the anticancer agent ET-743 from a marine invertebrate. The data were indeed developed appropriately and should positively impact future goals to produce this clinical agent in a recombinant microbial system for human use.

Reviewer 3:

At the outset the project had three objectives: 1) to identify unculturable bacteria for whole genome shotgun sequencing (WGS); 2) to develop a method for preparing single cell suspensions from invertebrate symbiote; and, 3) develop a FACS-based purification method for high molecular weight (HMW) DNA. The final report suggests the investigators have made some progress toward achieving these stated objectives although there are significant changes from the original plans.

In answering Aim 1, the authors provided 16S DNA sequencing and WGS using 454 from a tunicate DNA sample. The results were useful: 60% of the sample was bacterial, and several novel pathways were identified idiosyncratically. The group had originally planned to use the Nimblegen microarray platform to construct a metagenomic sequence pull-down system. PCR based gap closure was also mentioned. Wisely, these plans appear to have been dropped as they would have yielded little extra information for the money spent. There appear to have been difficulties in following up on the next specific aims, particularly aim 3. A number of FISH probes for tunicate bacteria were designed based on the earlier study (does not mention how many in the report), and 60,000 cells were isolated by FACS. However, lysing these cells for WGS appears to have been challenging. Instead, *H. influenzae* DNA spiked into the samples was successfully isolated as a surrogate.

Specifically, to answer aims 2-3 they developed a proof of concept study that showed that they could identify *S. aureus* from a clinical sample using IBIS/16S sequencing, design a specific FISH probe and pull *S. aureus* cells out of the suspension of clinical cells using FACS. This proof of concept study was not mentioned in the original proposal and was probably added because of slow progress with the work on the tunicate microbiome.

In summary, there has obviously been considerable effort towards technically difficult goals and refinement of methods. Direct pull down and amplification of individual bacteria from the tunicate sample seems to remain elusive.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The likely beneficial impact of this project is that it provides a new means to access genomic and metabolic potential of unculturable microorganisms. This, in turn, may lead to the discovery of new natural products, which can be developed as new drugs to combat diseases such as cancers,

infectious diseases, and others. A significant percentage of clinically used drugs are of natural product origin or are based on pharmacophores first identified in natural products. Therefore, this project may play an important role in improving health outcomes in the future. However, in the final report, the future plans for this research project are not clearly stated. It is only mentioned that the researchers plan to apply for external funding.

Reviewer 2:

This study strongly shows the potential for 16S FISH-based FACS purification of unculturable bacteria for whole genome amplification and sequencing that should impact not only the ET-743 story but also provide a new method to interrogate other complex environmental systems that may support microbes that produce new drugs and other chemicals that could benefit mankind. There are many marine systems whereby this methodology could have significant success, and thus the future of this method is quite bright.

Reviewer 3:

Strengths: The benefits to health lie in the potential discovery of new compounds through DNA after analysis of metagenomic sequence data (i.e., novel metabolic pathways). As an example, the investigators cite discovery of non-ribosomal peptide synthases similar (level of identity not stated) to that of drug candidate biosynthetic pathway. The development of methods to isolate specific bacteria may also have direct clinical applications as well as potentially enhancing novel compound discovery.

Weaknesses: The downside is that any impact of discovery is many years away from translation to the bedside, if anything of interest is actually found. This type of bioprospecting is a very high-risk endeavor; many if not all putative leads are likely to fail to generate useful targets.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not leverage additional funds, and the investigators have not submitted grant applications as a result of this project. However, the investigators plan to apply for external funding to support future work. But, no specifics were given as to where the grant application(s) will be submitted.

Reviewer 2:

No leveraged additional funds were awarded, although a patent was filed, thereby suggesting a strong potential for future funding success. However, the PI did not articulate a future funding direction, which I found quite puzzling, since this project might generate strong enthusiasm at the NIH or the American Cancer Society.

Reviewer 3:

Weaknesses: There was no co-funding of the work, and the investigators were unable to use the work to get new funding. This is a weakness. However, in mitigation, with the current funding

climate, it may take several years to find funding. The group states that they continue to seek funding.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not result in any peer-reviewed publications but did have a patent application filed on April 15, 2010. The investigators indicated a plan to prepare a methods paper describing the protocols that were developed in this project.

Reviewer 2:

The project resulted in a highly visible publication last year in *ACS Chemical Biology* as well as a patent application. The quality of both is very high and appropriate.

Reviewer 3:

Weaknesses: There has not been any commercial development based on this work and no publications in press. Overall, this is a weakness, although publications are in the works.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did enhance the quality and capacity for research at the grantee's institution, since the technology developed in the project may be used as tools for future research projects. It is mentioned in the report that the CGS had acquired a BD Influx cell sorter, but it is not clear whether or not this acquisition was leveraged by the health research funds. On the other hand, funding from the health research grant was able to support a fraction of the PI's salary and seven other members of the PI's institution, including two pre-doctoral students.

Reviewer 2:

The project does indeed appear to have contributed to improving the infrastructure of the PI's institution by involving a large number of co-workers who were able to collaborate with the Sherman group at Michigan on this multi-PI/multi-institution project.

Reviewer 3:

Strengths: There are certainly strengths that have been realized through this funded project. A research team, including two pre-doctoral investigators, was trained in these specialized techniques. Additionally, links within the institution (e.g., use of the core FACS machine) have been established. The work yielded invaluable preliminary data, which is a form of infrastructure. Finally, the investigators have a repository of negative results that could only be

gained from attempting to develop these tools. The value of this experience is often overlooked, but these data will mean that more rapid progress can be made in future research in this field.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

In the final report, the researchers indicate that the health research funds did not lead to collaboration with research partners outside of the institution, and there is no mention of plans to begin any collaboration as a result of the research. However, the invention does include David Sherman of the University of Michigan as one of the inventors.

Reviewer 2:

The project was set up as a collaboration with David Sherman's laboratory at the University of Michigan. No new collaborations appear to have resulted from the outcome of the research project described.

Reviewer 3:

Weakness: This work does not seem to have led to any new partnerships outside the institution.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. There is no description or discussion in the report about the whole genome sequencing (WGS) results. Whereas this part of the project may take a longer time to accomplish, a description of its current status may be helpful.
2. During the initial metagenomic sequencing, the investigators identified a number of contigs that may contain putative non-ribosomal peptide synthase domains expected to be involved in the biosynthesis of ET-743. However, it is not clear how, or if, this information will be used to help identify the ET-743 producing strain or streamline the WGA/WGS part of the project.
3. The future plans for this research project are not clearly stated. It is only mentioned that the researchers plan to apply for external funding. More detailed descriptions of the future plans related to the research project are recommended. Also, more information related to infrastructure and outside collaborations is needed.

Reviewer 2:

The next step forward is to convert the promise of ET-743 biosynthesis as nicely shown in this progress report to a genetically engineered ET-743 produced in economic titers. The PI has not

articulated how they propose or whether they have plans to move their research in this applied direction.

Reviewer 3:

Weakness: It is unclear if this is strategically the best approach to move forward in the future, given the many developments in the field over the last two to three years.

Recommendations: At this point, I would recommend that the group perform an in-depth survey of the current state of the art and re-formulate their research plans. I would consider these questions:

- Is isolation of individual bacteria worth the extra time and money over WGS of the community? Factors in this equation are the speed and cost of WGS (and the likely path of these factors over the next five years). Also, how many species are present in the communities being investigated and how metabolically interesting are the rare community members?
- What is the best sequencing platform for this application? 454 is much less cost efficient but has longer reads. PacBio and Illumina are viable alternative approaches. One way to investigate this is to create synthetic model microbial community data and model the yield.
- Is FISH still the best approach to single cell amplification? Even though significant resources have been spent on developing the FISH based amplification, it may not be the best approach to solve the problem. Several groups are working on this problem, and they may have developed more cost-efficient methods (e.g., single cell dilution).

ADDITIONAL COMMENTS

Reviewer 2:

This project resulted in solving a long-standing question in the field of marine natural products concerning the anticancer agent ET-743 which recently was clinically approved in Europe and is pending approval in the U.S. through the FDA. The PI and his colleagues used a dizzying array of modern omics approaches to show that the ET-743 chemical isolated from a marine ascidian invertebrate is produced by one of the dominant bacteria associated with the animal. Since they were unable to culture the bacterium, they resorted to the sequence analysis of the ascidian metagenome and the assembly of the major bacterial genomes, whereupon they were able to identify the putative biosynthetic genes that their Michigan collaborators were able to show participated in ET-743 biosynthesis. This work nicely opens the door for the production of this clinical agent in an engineered microbe, since its current production for the clinic relies on an expensive semi-synthesis. So, while much work remains to be seen whether this promise can be turned into reality, the PI provided important first information on the molecular basis of ET-743 biosynthesis to clearly show the way forward.