

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 (2.00)

Project Rating:

Project	Title	Average Score
0862601	Analysis of Epigenetic Modifications at Imprinted Loci in Mouse	Favorable (2.00)

Project Number: 0862601
Project Title: Analysis of Epigenetic Modifications at Imprinted Loci in Mouse
Investigator: Davis, Tamara

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 project met the stated goals only partially. The investigator was not able to complete all of the analyses intended. However, this appears largely to be due to the technical difficulties encountered in implementing the chromatin immunoprecipitation (ChIP) procedure that was used as the main analytical approach. Many labs encounter difficulties in getting these analyses running. The data included shows that the principal investigator made progress in optimizing the procedure to work on animal tissues. This was a significant challenge since most published procedures utilize suspensions of dispersed cells (i.e., yeast or cultured cells).

Reviewers without specific experience in this area would have deemed the originally proposed research design to be adequate. To her credit, the principal investigator modified the experimental approach and utilized enzymatic fragmentation of chromatin when they found out that sonication alone was insufficient for certain tissues.

Despite the difficulties encountered, analyses were completed to compare the association of acH3 and H3K9me3 with the paternal and maternal alleles of the *Rasgrf1* gene in hybrid mice. The results obtained were not those expected based on the principal investigator's original hypothesis. This suggests that the situation is more complicated than originally expected and could spur further investigation with the potential for novel findings.

Reviewer 2:

Genomic imprinting occurs when only one of the two parental alleles is expressed, a process which may involve differential methylation of the two alleles. This project sought to determine how histone modifications work in concert with DNA methylation during the process of genomic imprinting. The basis for the project was the observation that the *Rasgrf1* is imprinted and expressed monoallelically in some tissues but biallelically in others, despite sharing the same pattern of methylation. The hypothesis to be tested was that these differences reside in the differential acetylation/methylation of the two alleles.

The overall approach was to use ChIP in different tissues derived from a murine model to compare histone modification patterns and then relate this to allele-specific transcription. The principal investigator identified an appropriate mouse model system, validated allele-specific

PCR to differentiate parental and maternal alleles, and clearly described the approaches, potential problems and alternate methods.

Overall, this was a well-written and designed project which was able to achieve all of the stated goals. The principal investigator was able to conclude that the *Rasgrfl* expression status did not correlate with the histone modifications measured.

Research design and methods: The initial progress report detailed efforts to create chromatin from mouse liver and brain tissue which could then be used for ChIP experiments. Optimization of sonication and ChIP was achieved for the liver samples, and control PCR amplification of the H19 locus was demonstrated. The second progress report indicated that DNA fragmentation was optimized for liver and kidney tissue but that it was not possible to create chromatin fragments suitable for ChIP from brain tissue. Since the liver and kidney represented monoallelic and biallelic tissues, these samples were therefore suitable to test the proposed hypothesis.

Next, the principal investigator was able to optimize and carry out ChIP for H3K9Ac and H3K9me3 on liver and kidney tissue and to use allele specific analysis using the *AvaII* restriction enzyme. This analysis indicated that both alleles in both tissues contained these histone marks, indicating that allele-specific expression was not linked to these histone modifications within the regions tested.

To further improve the reproducibility of the chromatin fragmentation, the principal investigator switched from sonication to the use of micrococcal nuclease, using both liver and kidney tissue. This proved effective, and the principal investigator then examined H3K9Ac and H3K9me3 on the alleles in the two tissues. Again, the results indicated that all four alleles had similar levels of these marks. Further analysis of the promoter regions again failed to identify any differences between the alleles in the two tissues. Importantly, the principal investigator included a nice control indicating that imprinting at the H19 locus does reveal differential patterns of histone modifications in kidney tissue, validating the sensitivity of the approach.

Overall, the methods and approach were adequate to address the questions, and the principal investigator made several changes to the research protocol to address technical issues that arose. These changes were entirely justified and were essential to allow the principal investigator to complete the project. The data were well-developed and described and strongly supported the overall conclusions of the project.

Summary: Strengths of the proposal include a well-written experimental plan and a clear hypothesis. The overall approach was well-described, and the protocols were altered to quickly address technical issues that arose during the project. No weaknesses were noted.

Reviewer 3:

Specific Aim 1 was to characterize the allelic pattern of chromatin modification at the *Rasgrfl* locus in mouse neonatal brain. The project did not meet this objective. The principal investigator cited technical difficulties in isolating DNA from mouse brain and instead resorted to the use of liver, which they reported to have the same distribution. Data were not provided ensuring this supposition. Unfortunately, the data from the liver are questionable, since there

were no negative controls to ensure that the experimental technique was adequately separating unbound from bound fractions. There are examples of this in the progress reports which are explained away as technical issues; (e.g., Figure 3 of the first progress report indicates a positive in the mock lanes). These should be corrected and gels provided that do not show these issues. This also brings up the question of just how many times the experiments were repeated (which was never stated) if the best gel is one that shows these defects.

Specific Aim 2 was to examine the allele-specific chromatin modification patterns in tissues exhibiting biallelic expression of *Rasgrfl*. The data provided here are also inconclusive for many of the same reasons as above. Figure 4 of the final report indicates no reaction in the liver for the H3ac and reaction of both alleles (paternal and maternal) in the H3K9me3 lane, where it should indicate a 300 bp band in the H3ac and a maternal allele of 400 bp in the H3K9me3 lane. In addition, the supposition that increases and decreases in band intensity on these types of PCR indicate anything about template concentration is a glaring weakness in the analysis. Further, the idea of using mouse crosses with the paternal and maternal alleles coming from different sources is a strength of the experimental design that was never actually used in the analysis.

The overall weaknesses are as follows: 1) the experimental design uses a single technique; 2) the technique was found to have several technical difficulties; 3) there was a lack of follow-through on the experimental plan; 4) data were presented in a manner that made it difficult to interpret or, at times, were presented incorrectly; and, 5) the analysis of the data showed several flaws.

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:

In the long-term, this project could benefit the treatment of diseases caused by the inheritance of imprinted genes bearing mutations. The work completed demonstrated the feasibility of future work in this area and represents a good investment given the small expenditure involved. As indicated in the report, the data obtained have been incorporated into an application to NIH to sponsor further work on the project.

Reviewer 2:

The project has only limited impact for improving current health. However, given the limited resources available, the outcome may provide information that could inform other studies on the link between genetic disease and imprinting.

Future plans: The principal investigator plans to continue to examine differences in histone modifications between alleles using other histone modifications and to improve the chromatin preparation technique to allow use of brain tissue. These experiments are logical and will build on the work funded in the current proposal. No weaknesses were noted in this section.

Reviewer 3:

The likely benefit of the project is expected to be small considering the meager funds allocated to it. The amount of funds provided was on the small side even for a pilot project. This amount is what would be expected in order to provide preliminary data for submission of grant applications to funding agencies or enhance the teaching environment of the laboratory. The dissemination of the data in meetings or publications indicates little impact on teaching. There was one poster presentation, but the lead author was the principal investigator, not one of the students. The lack of student poster presentations at local or national meetings indicates insufficient performance in regard to enhancement of teaching.

The overall weaknesses are as follows: 1) the benefit to advancement of the field is negligible considering the results in Criterion 1; 2) the benefit to teaching has no measurable outcomes; and, 3) it is unlikely the data generated can be used to leverage additional funding.

A strength is that the faculty member has likely developed some expertise in this area of research, which should help with future work in the field.

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:

An NIH R01 proposal has been submitted to apply for additional funding.

Reviewer 2:

The project provided data which were used for submission of an NIH R01 grant (“Analysis of epigenetic modifications at imprinted loci in mouse”). This grant is still pending. It is also stated that the principal investigator plans to apply for additional funding in the future.

Reviewer 3:

It is a weakness that no funds were leveraged, nor are any expected.

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 report indicates that some of the preliminary results obtained have been communicated via a poster at a scientific meeting. More data need to be collected before it would be worthwhile to submit a paper for publication, but this should be feasible in the future now that the principal investigator has the ChIP procedure working well enough.

Reviewer 2:

No publications were produced during the project, although a poster detailing the results was presented at the annual meeting of the Society for the Study of Reproduction (2009). The principal investigator states that publications are planned, but no details are supplied.

Because the results of the project were essentially negative, the data derived is unlikely to be publishable unless supplemented with additional, positive studies. However, a poster was presented, so that the data was made publicly available. Overall, although publication in a peer-reviewed journal would have been preferred, given the essentially negative result obtained, dissemination of the data through a poster presentation is an appropriate method for making the data public.

Reviewer 3:

It is a weakness that these funds did not generate any measurable indicators of success.

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

STRENGTHS AND WEAKNESSES

Reviewer 1:

Improvements to infrastructure were not proposed originally, and none were documented in the report. The funds were largely used to purchase the reagents necessary to perform the work. In turn, this enabled undergraduate students to acquire hands-on experience with contemporary techniques in molecular and cell biology.

Reviewer 2:

The project had a significant impact in that it allowed the principal investigator to purchase the reagents which were used by three undergraduate research assistants who worked on the project. This research experience provided a significant opportunity for these students to develop their technical and analytical skills. A major strength of this project was, therefore, the ability to provide a novel research experience to undergraduate workers.

Reviewer 3:

It is a weakness that these funds did not generate any improvements to infrastructure, any new investigators to the institution, or pay for research for pre- or post-doctoral students. In all fairness to the principal investigator, these funds were limited; and, they did potentially improve the principal investigator's skills in this experimental area and pay for research for undergraduate students. This appears to be the only strength of the project.

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:

The report does not note any plans for new collaborations as a result of the work.

Reviewer 2:

No outside collaborations or community involvement were noted.

Reviewer 3:

It is a weakness that there were no collaborations in this project. The principal investigator would have benefited from a mentor in the field to assist with technical difficulties.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The principal investigator should consider comparing a range of antisera for the initial fractionation of chromatin into active and inactive groups. The source and catalog number of the acH3 antisera employed was not given, but I suspect the antisera used may have been one raised against multiple sites. If this is indeed the case, it may precipitate chromatin based on the cell cycle dynamics of H3 acetylation in addition to transcriptional activity.

Moreover, the principal investigator should explore whether sequential ChIP with two different histone modification antisera can provide more stringent fractionations into active vs. active fractions. Alternatively, they could ChIP for the elongating form of RNAPII using antisera specific for CTD phosphorylations.

2. I am concerned that the methods used to dissociate tissues (mincing and pushing the suspension through a 20-gauge needle) may not have been adequate. One could mince, add HCHO to the suspension and then dounce with a loose fitting pestle.

Reviewer 2:

None.

Reviewer 3:

1. The benefit to advancement of the field is negligible considering the results. The specific aims of a grant should be commensurate with the level of funding provided. Given the level of funding provided, the investigator should have concentrated on providing a first-rate learning experience for the undergraduates or producing preliminary data for submission of a grant application for further funding.
2. The benefit to teaching has no measurable outcomes. It seems the applicant was concentrating on increasing undergraduate exposure to research. If this is true, it should have included dissemination of research results at local or state levels, perhaps with the dedication of part of the funding to sponsoring a local meeting.
3. It is unlikely the data generated can be used to leverage additional funding. Given that the data were inconclusive, it is unlikely to be compelling for a grant review by other funding agencies. The applicant should have used several experimental techniques rather than one to

illustrate the points. This would provide reviewers with the impression that the applicant is examining the experimental question from multiple angles. For example, there are no quantitative RT-PCR results showing differential expression of the paternal and maternal alleles.

4. It is a weakness that no collaboration existed in this project. The principal investigator would have benefited from a mentor in the field to assist with technical difficulties and experimental design.
5. The funds allocated to the proposal were too modest. I would advise allocating at least \$20,000 per year for a project. The modest level of funding provided made the planned experiments infeasible. The applicant was using a mouse-based experimental approach which is cost-intensive. Molecular biology reagents also tend to be expensive. These cost variables greatly contributed to the feasibility of this project.

Generic Recommendations for Bryn Mawr College

Reviewer 1:

This is work that is likely to provide novel information in the long term on an important scientific problem and should be supported.

Reviewer 2:

The project did extremely well given the small amount of money allocated to it. I would recommend increasing the budget to allow more detailed experimental exploration of the hypothesis (e.g., using additional histone modification antibodies) and to allow the principal investigator to recruit additional undergraduate assistants.

ADDITIONAL COMMENTS

Reviewer 2:

Given the small size of the grant, the project more than met its objectives and had several major strengths, including a well-written hypothesis and plan, a flexible experimental approach which allowed the principal investigator to draw strong conclusions, presentation of the completed work at a meeting, and the involvement of three undergraduate research assistants in the project. No weaknesses were noted.

Reviewer 3:

The grant failed to achieve either specific aim of the grant. Furthermore, no additional grant applications were submitted, no publications were produced, and the grant did not foster any collaborations. This reviewer would like to qualify this by stating that the funds allocated were fairly modest. The benefits seen from the grant were the increase in expertise of the principal investigator and the exposure of four undergraduate students to scientific research. These benefits alone would be sufficient given the level of investment if there were some measurable outcome of these results, such as poster presentations by the students or publication of results by the principal investigator (there was a poster presentation).