Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** West Chester University of Pennsylvania

2. **Reporting Period (start and end date of grant award period):** January 1, 2010 - June 30, 2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Erin E. Gestl, Ph.D.

4. **Grant Contact Person's Telephone Number:** 610-436-2760

5. **Grant SAP Number:** 4100050915

6. **Project Number and Title of Research Project: 1** DNA Polymerase Expression of Human Colon Cell Lines following Chemotherapeutic Treatment

7. **Start and End Date of Research Project:** January 1, 2010 - June 30, 2012

8. **Name of Principal Investigator for the Research Project:** Erin E. Gestl, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $3558.46

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
None			

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Gestl	Principal Investigator	5%
Barchi	Research Assistant, Student	10% Yr 1
Rowlands	Research Assistant, Student	25% Yr 2
Kriza	Research Assistant, Student	20%, Yr 2-3

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None, just supplies		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____XX_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___XX_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No___XX_____

If yes, please describe your plans:

12. **Future of Research Project.** What are the future plans for this research project?
Samples in the form of DNA, RNA, and protein have been isolated and further analysis could be completed including but not limited to examining the expression levels of other genes at the RNA and protein levels.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___XX_____No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	2			
Female	1			
Unknown				
Total	**3**	**0**	**0**	**0**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	3			
Unknown				
Total	**3**	**0**	**0**	**0**

	Undergraduate	Masters	Pre-doc	Post-doc
White	3			
Black				
Asian				
Other				
Unknown				
Total	**3**	**0**	**0**	**0**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____XX_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____XX_____No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.
It provided a source of funding to investigate the possible changes in expression of several different DNA polymerase genes. By conducting this study, three undergraduates were also trained in performing biomedical research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____XX_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____XX_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___XX_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goals

1. To determine if there is a difference in DNA polymerase levels between human normal and carcinoma colon cells.

2. To determine if there is a difference in DNA polymerase levels between human colon cells (normal and carcinoma) following treatment with cyclophosphamide, doxorubicin, and methyl-nitrosourea.

Cell Culture and Initial Sample Isolation

The first funds were received in April of 2010. From April until the end of June, five colorectal cell lines HCT-116 40-16, HCT-116 386, HCT-116 379.2, HCT-8, and HCT-15 have been cultured. In relation to the first goal, a normal colon cell line was not analyzed. In the proposal LS123 was listed as normal where in fact it is not and no other "normal" colon cell lines were commercially available. Protein samples have wree isolated using Thermo Scientific NE-

PER protein extraction kit. The start of western blot analysis for the normal levels of DNA polymerases is beginning. In addition, RNA samples have also been isolated using TRIZOL Reagent for the quantification of the level of gene expression for the same DNA polymerase genes by quantitative, reverse-transcriptase polymerase chain reaction. As described in our proposal's time line, during the period of June-August of 2010 our goal was to "Maintain cell lines and isolate untreated samples," which was achieved.

Two West Chester University Biology students, Elizabeth Barchi and William Rowlands have been performing the cell culture and isolations. Elizabeth Barchi graduated from the university in May is will be attending Penn State College of Medicine. William is a senior and will be completing the project.

Cell Maintenance and Cell Growth Curves

The cells were grown in 100 mm dishes with sufficient media and grown at 37C in a 5% CO_2 environment. When they grew to confluence, the cells were removed with trypsin, pelleted, resuspended, and a fraction of the suspension was added to a new plate with new media. Before treatments a cell dose-response curve was determined by adding known concentrations of cells with media to a 96 well plate and determining the effect of the chemotherapeutic treatment after a few days. The cells were added to each well and allowed to grow for 1 day. Then they were treated for 1 hour, after which the treatment was removed, the cells were washed, and new media was added. The experiment was set up according to the Figure 1. Cell growth/death was assayed from days 1-4 using Dojindo's cell counting kit 8. An example of the results is shown in Figure 2. Using this data, concentrations of 1mM as a threshold dose, 2.5mM as a 50% effective dose, and 0mM as a control were selected for use in larger scale treatments.

Chemotherapeutic Treatments and Sample Isolation

Treatments of five different colorectal cell lines (HCT-8, HCT-15, HCT-116 40-16, HCT-116 386, HCT-116 379.2) were treated with Methyl-nitrosourea (MNU) at concentrations of 1 mM as a threshold dose, 2.5 mM as a 50% effective dose, and 0 mM as a control were completed, as well as Doxorubicin at concentrations of 12.5, 5, 2.5, 1.25 and 0 uM. DNA, RNA, and proteins were purified and collected using Quigen multiprep DNA, RNA and extraction kit. This kit allowed us to extract the DNA, RNA and protein from the same cell pellet which will allow us to determine better cause and effect relationships. The DNA and RNA concentrations after the extractions were determined using a nanodrop spectrometer (Table 1), and the protein concentrations were determined by the D_C Protein Assay kit.

Sample Analysis

Western blot analysis has begun to determine polymerase expression at the protein level, while primers for SYBR Green quantitative reverse-transcriptase polymerase chain reaction (Q-RT-PCR) has been tested and confirmed for analysis of gene expression at the transcript level. The genes being analyzed include polymerase delta 1, polymerase delta 2, polymerase beta, polymerase eta, polymerase iota, polymerase kappa, and PCNA. We have focused on determine the expression levels of the indicated genes at the RNA level and have utilized quantitative-reverse-transcriptase-polymerase chain reaction (qRT-PCR) as the procedure of choice. This was in part a practical issue due to the longer western blot assay requiring several consecutive days.

During the spring semester (Jan-May 2012), Chase Kriza, a junior at West Chester University, began analyzing the RNA samples using the method of quantitative reverse transcriptase polymerase chain reaction (q-RT-PCR). The genes being analyzed include polymerase delta 1, polymerase delta 2, polymerase beta, polymerase eta, polymerase iota, polymerase kappa, and PCNA. Some of the results are shown in Figure 3. Analysis of the fold change caused by varying MNU doses shows several trends, within individual genes and cell lines. In general, the HCT 116 386 cell line was affected most by the treatments of MNU, causing overall gene expression to increase. This includes a 12 and 20 fold increase in pol eta and PCNA expression at 2.5 mM MNU. Possible reasoning for the increase in pol eta is the fact that it is a translesion polymerase, which will be used to work around the damage caused by MNU. As the damage increases with the dose, more pol eta will be synthesized. The pol kappa gene showed interesting results, having changed nearly 80 fold in gene expression in the HCT-8 cell line, while the other cell lines changed minimally. Pol beta, most likely because it is specialized for repair, shows slight increased in expression. It can be concluded that the MNU has some effect on these gens, causing gene expression to be turned on as a response. Pol delta 2 is involved in the replication of DNA, which is not effected by the MNU, explaining why gene expression is minimally effected. Any of the remaining samples from the treated cell lines have been saved and can be used in future studies if the need arises.

Figure 1. Diagram of Chemotheraeutic Dose-Response Curve with Colorectal Cell Lines.

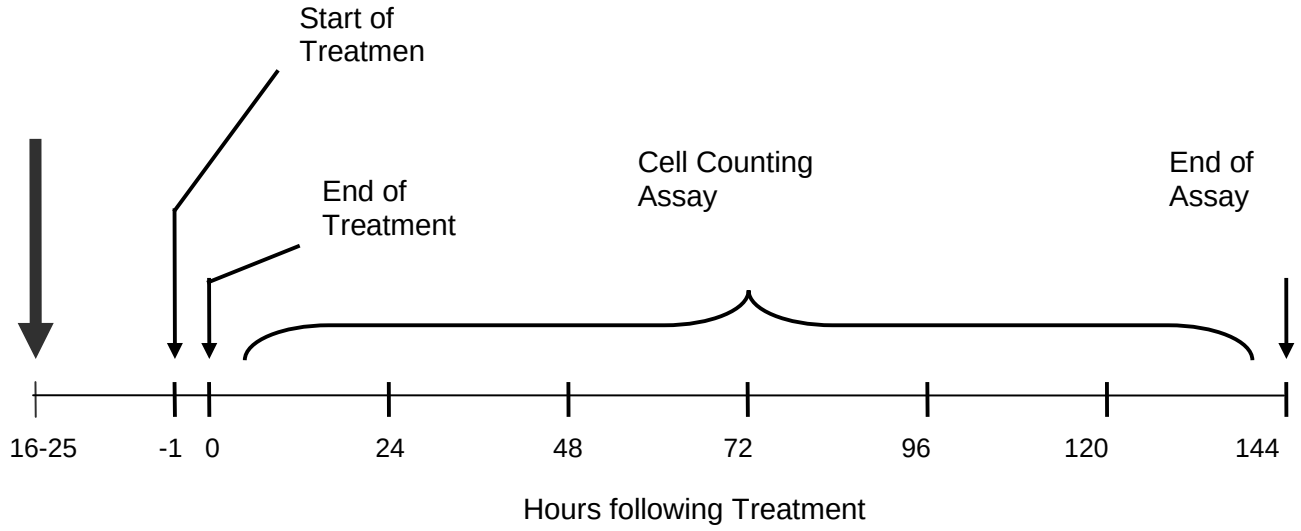


Figure 2. Example of Dose-Response curve using the HCT-8 cells.

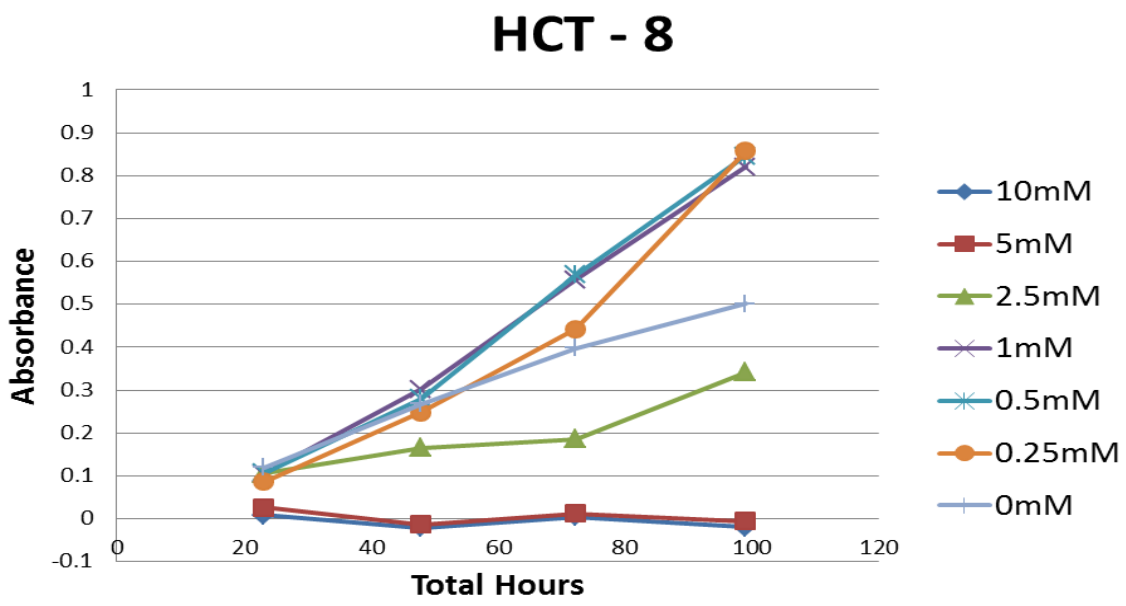
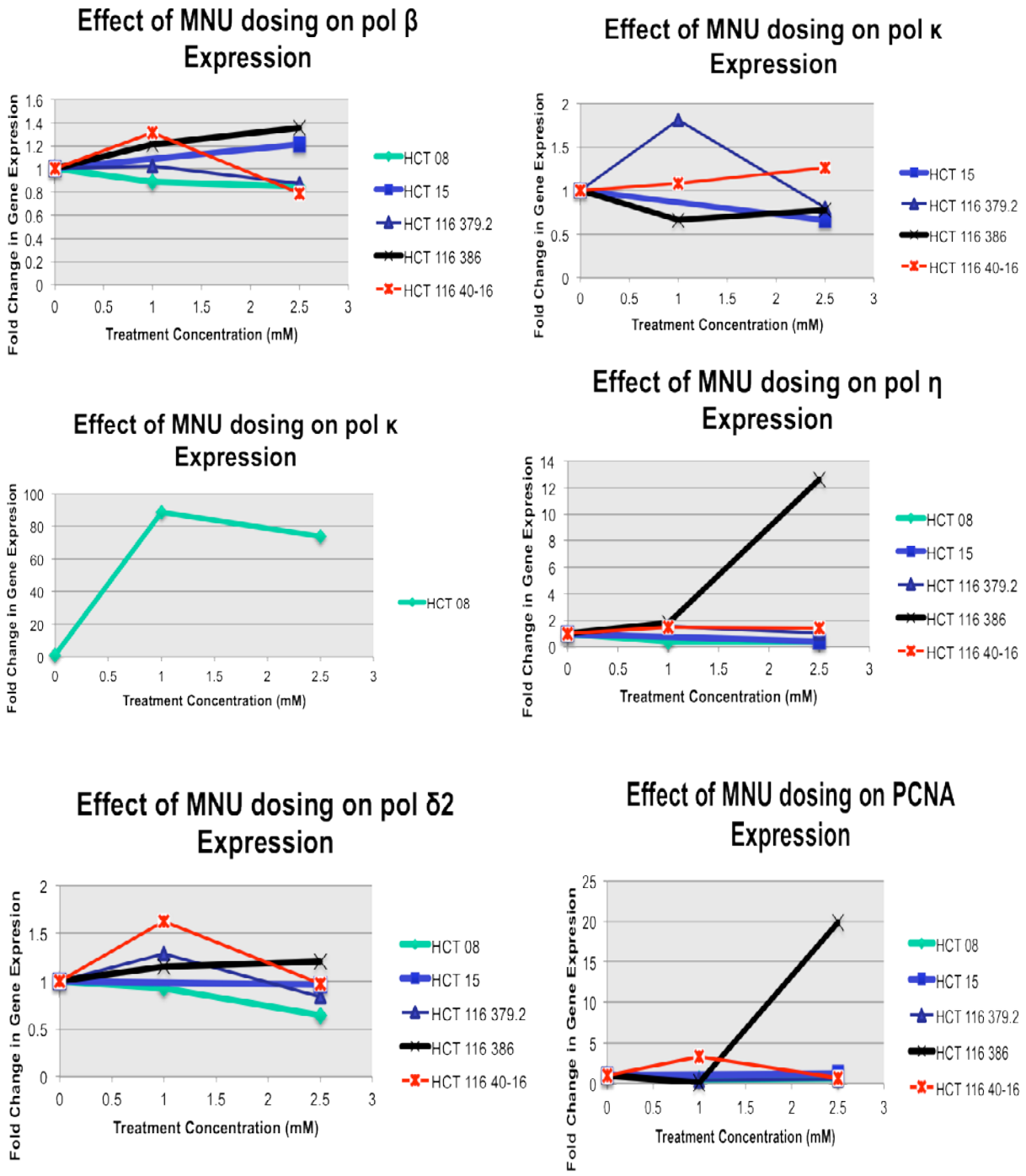


Table 1. DNA and RNA concentrations from Colorectal cells following Treatment

MNU Treatment:				Doxorubicin Treatments		
	DNA Concentration (ng/ug)	RNA Concentration (ng/ug)			DNA Concentration (ng/ug)	RNA Concentration (ng/ug)
HCT-116 379.2				HCT-116 379.2		
0mM	189.6	376.6		0µM	232.1	2766.1
1mM	301.4	821.3		0.234µM	659.3	2114.6
2.5mM	393.9	752.6		0.468µM	117.9	1002.9
				0.938µM	368	1590.9
HCT-116 386				2.34µM	715.3	1210.7
0mM	306.1	384.3				
1mM	421.8	1814.6		HCT-116 386		
2.5mM	264.7	2407.7		0µM	229.7	1636.9
				0.234µM	312.3	3212.7
HCT-116 40-16				0.468µM	703.2	1704.7
0mM	536.3	1031.2		0.938µM	369.9	1249.6
1mM	314.7	1214.3		2.34µM	704.7	1428.4
2.5mM	327.5	1297.9				
				HCT-116 40-16		
HCT-8				0µM	292	2994.5
0mM	242.9	1158		0.234µM	313.9	1666.1
1mM	248.7	1164.8		0.468µM	332.2	1251.2
2.5mM	222	2697.4		0.938µM	417.5	3145.1
				2.34µM	230.9	3230.5
HCT-15						
0mM	163.7	717.1				
1mM	118.6	661.5				
2.5mM	240.8	1826.2				

Figure 3. Effect of MNU treatment on Polymerase Gene Expression in Colorectal Cell Lines.



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__XX_ No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__XX_ No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics

_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__XX_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_XX__ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include

the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__XX_____ No_____

If yes, please describe your plans:
The plan is to submit an article however, it may be combined with the analysis of some results of additional genes that are being conducted this academic year and therefore would be submitted in the summer of 2013.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be

single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.
None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___XX___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____XX_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Erin E. Gestl, Ph.D.

Education

1988-1992 Pennsylvania State University, University Park, PA
B.S. in Molecular and Cell Biology, Minor in Microbiology

1994-2002 Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA
Ph.D. in Biochemistry and Molecular Biology

Research Interests

Currently, there have been at least 15 DNA polymerases reported in eucaryotic cells, the roles of which have not all been determined. Multiple DNA polymerases within cells allow the evolution of different polymerases for special or unique functions. Although some cross over between polymerases, they can be classified into 3 main groups, Replicative, Repair, and Specialized. The main role of replicative polymerases is to duplicate the organism's genome while another set of polymerases are mainly involved in DNA repair. The final set is hypothesized to have specialized functions such as the bypass of DNA damage or translesion synthesis (TLS). Current models of TLS during cellular DNA replication hypothesize that DNA polymerase switching occurs in order to replace a replicative polymerase that is stalled or inhibited by a DNA lesion with a specialized DNA polymerase or set of polymerases that are more facile at performing TLS. Once the lesion is bypassed, the specialized polymerase is then replaced by the replicative polymerase and normal replication is resumed. This process of switching may result in mutations being incorporated and could be important in the choice of chemotherapy that is given to cancer patients, since may drug therapies cause DNA damage.

I am interested in approaching this area of research by several different angles. The first set of experiments would include *in vitro* (in the test tube) studies to examine the interactions between polymerases and the DNA template which can be examined through the addition of a fluorophore to the polymerase and a dark quencher placed on the 3' end of the DNA template. The combined use of fluorescent markers and quenchers will allow detection of a bound polymerase to the template DNA as a loss of fluorescence due to fluorescence resonance energy transfer (FRET).

The second line of research I would like to continue my current research on regulation of polymerase levels and their affect on DNA replication and stability. This line of research would be include *ex vivo* experiments in human cell lines and would include both normal and tumorigenic cells lines. Our initial results indicate that polymerase levels may be altered following treatment of cell lines with chemotherapeutics. DNA array technology, along with Northern and Western blot analysis would play a role to determine the pathways involved in polymerase regulation.

I would also like to extend my research into a vertebrate model, zebrafish. The zebrafish (*Danio rerio*) animal model was chosen for this research for several reasons. First, the zebrafish is a 3 - 4 cm, hardy, freshwater vertebrate animal and therefore the results obtained from this project are more easily extrapolated to people. A single pair of zebrafish can produce hundreds of eggs biweekly with a generation time of 3 months. The embryos, about 1 mm in diameter, develop ex

utero (outside the body) and are completely transparent allowing visualization of the animal's internal structures during development. The final line of proposed research would include tissue-specific and temporal expression of DNA polymerases in developing zebrafish may help elucidate their function and may easily be completed through RNA and protein analysis. The functions of DNA polymerases can be studied *in vivo* (in the animal) using current techniques such as morpholino technology and microinjection into fertilized eggs.

Professional Commitments

None