Fiscal Year:	FY 2008	**Task Last Updated:**	FY 07/11/2008
PI Name:	Bacher, Jeff Ph.D.		
Project Title:	A Novel Biodosimetry Method		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	jeff.bacher@promega.com	**Fax:**	FY 608-273-6989
PI Organization Type:	INDUSTRY	**Phone:**	608-277-2608
Organization Name:	Promega Corporation		
PI Address 1:	Genetic Analysis		
PI Address 2:	2800 Woods Hollow Road		
PI Web Page:			
City:	Madison	**State:**	WI
Zip Code:	53711-5399	**Congressional District:**	2
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2007 Space Radiation NNJ07ZSA001N
Start Date:	09/01/2007	**End Date:**	08/31/2010
No. of Post Docs:	2	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
Contact Monitor:	Cucinott1a, Francis	**Contact Phone:**	281-483-0968
Contact Email:	noaccess@nasa.gov		
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Bailey, Susan (Colorado State University) Halberg, Richard (University of Wisconsin)		
Grant/Contract No.:	NNX07AQ02G		
Performance Goal No.:			
Performance Goal Text:			
	Exposure of astronauts to space radiation during extended space missions may cause serious health problems. Accurate methods for measuring the biological effects of radiation exposure are, therefore, critical for estimating an individual¿s health risks. Biodosimetry measurements reflect variation in radiation sensitivity and consequently result in highly individualized estimates of dose and risk. Our novel biodosimetry approach is based on the hypothesis that non-coding repetitive DNA sequences are sensitive to radiation-induced mutations and that these mutations are not harmful to a cell. Therefore, mutations in non-coding repetitive DNA sequences can accumulate and provide a stable molecular record of genetic damage that can be used to determine cumulative radiation exposure and health risk. In our previous NASA grant, we demonstrated the feasibility of using radiation-induced mutations in non-coding repetitive DNA sequences to estimate radiation dose. Our initial data indicate that radiation-induced mutations in non-coding repetitive DNA markers		

Task Description:	are stable over time and additive over multiple exposures. In this successor proposal, we plan to extend our previous work by developing optimized multiplex marker panels for human and mouse biodosimetry, validate our approach by comparing our assay to current gold standard cytological methods and then utilize the novel system to assess risks from space radiation and improve our understanding of how these risks are affected by variations in dose rate, dose fractionation and genome stability. The main contribution of the proposed research to manned space exploration is the validation of a novel biodosimetry method for estimating dose and risks from exposure to space radiation. Completion of this research should provide new insights into the effects of space radiation on DNA mutagenesis and establishes panels of human and mouse biomarkers with broad utility for future studies in radiation biology, toxicology and cancer research.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Mutational load profiling, through analysis of mutations in tandem DNA repeat sequences, is a simple, non-invasive and generalized approach for monitoring an individual's cumulative record of mutations that may be useful for determining health risks and effectiveness of countermeasures for astronauts or other individuals exposed to ionizing radiation or chemical mutagens. Biomarkers identified in this study are also sensitive to free radical DNA damage and therefore may be useful markers for detection of cancer and other degenerative diseases in which oxidative stress is involved. Completion of this research should provide new insights into the effects of space radiation on DNA mutagenesis and establishes panels of human and mouse biomarkers with broad utility for future studies in radiation biology, toxicology and cancer research.
Task Progress:	We have previously demonstrated that mutations in selected biomarkers exhibit a dose response to radiation exposure in normal AG01522 human fibroblasts cell cultures. A dose-dependent response was observed in vivo in mouse blood, cheek and brain cells in tissue samples collected 10 weeks after exposure. A significant increase in radiation-induced mutations in mononucleotide repeats was detectible in mouse blood and cheek samples up to 26 weeks after radiation exposure and these mutations were additive over multiple exposures. In this successor proposal we plan to build on our previous work and ultimately plan to validate our biodosimetry method for assessing radiation exposure in human lymphoblast cells and in vivo in mouse blood and buccal cells. We will confirm that mutation frequency from fractionated exposures is additive and extend stability studies out to about 2 years post irradiation with gamma, protons and iron ions. The usefulness of our biodosimetry assay as a surrogate biomarker for estimating radiation-induced cancer risk is being investigated by looking for correlation with other known cancer risk factors, such as chromosomal aberrations and mutations in coding repeats. Mismatch repair deficient SupFG1 mutation reporter mice were irradiated and screened for mutations in (C)8 and (G)7 coding repeats of the SupFG1 transgene and compared to mutations observed in tandem DNA repeats. The sensitivity of our PCR-based mutation assay was 10-fold greater than that observed for the SupFG1 transgenic mouse mutation assay. DNA sequencing revealed that almost all (97%) of the SupFG1 mutations were insertions or deletions in the (G)7 or the (C)8 mononucleotide repeats. The susceptibility of short coding repeats to radiation-induced mutations is troubling since there are thousands of similar coding repeats within the human genome that may be vulnerable. Radiation-induced mutations in non-coding repeats were highly correlated with mutations in short coding repeats (which are considered biomarkers for cancer risk) and therefore may be useful as surrogate markers for cancer “risk” as well as “dose”. Correlation with chromosomal aberrations is currently being investigated. We have completed two NSRL runs thus far. During the NSRL-08A run we started experiments designed to test the effects of dose rate on mutation induction in tandem repeats and also experiments to determine the stability of mutations in repeats over time. To do this human AG9389 lymphoblast cells were exposed to 1 Gy of iron ions, protons and gamma rays at various dose rates (0.1, 0.2, 0.5 and 1 Gy/min), allowed to recover for 3 days, then snap frozen for later mutational analysis. We also irradiated C57BL/6 mice with 1 Gy of iron ions, protons or gamma rays and collected blood and tissue samples (cheek swabs, spleen, liver, brain and colon) after 3 days. Additional groups of mice from NSRL-08A run will be tested after 4 months, 1 year and 21 months to determine the stability of mutations in DNA repeat markers and chromosomal aberrations. During the NSRL-08B run we started experiments designed to validate our biodosimetry method by comparing results of obtained using tandem repeat markers to the gold standard, chromosomal aberration analysis. Human AG9389 lymphoblast cells were exposed to doses of 0.1, 0.2, 0.5, 1 and 2 Gy of iron ions, protons or gamma rays. The cells were allowed to recover for 3 days then either snap frozen for mutational analysis or shipped to co-investigator Susan Bailey for chromosomal analysis. The cells will be tested for DNA repeat mutations using small-pool PCR and for chromosomal aberrations using Giemsa staining and whole chromosome painting for translocations. This validation experiment is will be repeated at NSRL-08C so we can compare results from two independent experiments. Other confounding effects that may affect biodosimetry measurements are being investigated. For example, the deleterious effects of radiation exposure may increase with age. To test this, 20-month-old C57BL/6, CBA/Ca and Balb/c mice will be irradiated and results for tandem repeat mutations, mismatch repair gene expression, mismatch repair gene methylation status and telomere stability will be compared to 2-month-old mice. Mice are currently being aged and irradiation experiments are planned in 2009 at NSRL-09C. We are also investigating the effects of dose rate, dose fractionation, dual ions and the effect of radiation on DNA mismatch repair gene expression and how these may alter the frequency of radiation-induced mutations in tandem repeats, and thus the accuracy of biodosimetry measurements.
Bibliography Type:	Description: (Last Updated: 04/16/2019)
Abstracts for Journals and Proceedings	Ensenberger MG, Megid WA, Halberg RB, Steffen LS, Bourdeau-Heller JM, Stanhope SA, Kent-First MG, Prolla TA, Storts DR, Bacher JW. "A Novel Biodosimetry Method." Presented at the NASA Human Research Program Investigators' Workshop, League City, Texas, January 2008. 2008 NASA Human Research Program Investigators' Workshop, January 2008. , Jan-2008

Abstracts for Journals and Proceedings	Bacher JW, Ensenberger MG, Megid WA, Halberg RB, Steffen LS, Bourdeau-Heller, JM, Stanhope SA, Grochowski E, Storts DR. "A Novel Biodosimetry Method." 19th Annual NASA Space Radiation Investigators Workshop, Philadelphia, PA, June 30-July 2, 2008. Proceedings from the 19th Annual NASA Space Radiation Investigators Workshop, July 2008. , Jul-2008