Fiscal Year:	FY 2019	**Task Last Updated:**	FY 02/27/2019
PI Name:	Cornforth, Michael Ph.D.		
Project Title:	Molecular Characterization of Transmissible Chromosome Aberrations Produced By Ions of Intermediate and High Atomic Number		
Division Name:	Human Research		
Program/Discipline:			
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:	(1) **Cancer**:Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	77555-5302	**Congressional District:**	14
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2013-14 HERO NNJ13ZSA002N-RADIATION
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No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: Extended to 3/10/2020 per NSSC information (Ed., 3/12/19)		
Key Personnel Changes/Previous PI:	January 2016: No changes.		
COI Name (Institution):	Loucas, Bradford Ph.D. (University of Texas Medical Branch, Galveston)		
Grant/Contract No.:	NNX15AG74G		
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Performance Goal Text:			

Task Description:	During deep space exploration, personnel will be exposed to charged particles of intermediate and high atomic number, often collectively referred to as densely ionizing radiations. For a given dose these are almost certainly more likely to cause cancer than the sparsely ionizing types of radiation typically encountered on Earth, such as x- and gamma rays. Since it is not possible to determine directly the carcinogenic potential of such radiations, it becomes necessary to rely on surrogate experimental systems to provide this information. For a number of reasons, the formation of nonlethal (transmissible) chromosome aberrations, mainly reciprocal translocations and inversions, is considered by many to represent the best surrogate endpoint. And yet, only recently have we begun to really understand the molecular processes governing their formation, including possible differences that probably exist in the way that aberrations produced by sparsely- versus densely-ionizing radiations are formed. We propose using advanced molecular methods, including genome sequencing, to characterize structural changes to the DNA of human cells that accompany the formation of transmissible chromosome aberrations caused by exposure to various types of radiation likely to be encountered in deep space.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Radiation-induced reciprocal chromosome translocations and inversions are particularly important in that regard as they relate to crewed space activities. In addition to causing cancer, their appearance also accompanies ongoing genome instability processes associated with their progression. The fact that these particular chromosome aberrations are transmissible (non-lethal) also makes them ideal candidate biomarkers of accumulated radiation exposure. We argue that molecular analysis of breakpoint junctions formed as the result of translocations and inversions is vital to understanding the process of exchange aberration formation, since it is here where underlying repair/misrepair pathways leave their “molecular fingerprints.” Regarding relevance to NASA’s concerns, the study of chromosome aberrations stands to tell us much about mechanisms underlying the cancer process itself. The relationship between particle energy/track structure and radiogenic changes to the genome represents an important first step in understanding 1) basic dose-response relationships at low fluences and 2) fundamental carcinogenic processes that may ultimately form the basis for subsequent mitigation strategies.
Task Progress:	As previously mentioned, there were originally three objectives in this proposal. Following subsequent negotiations with NASA management, two objectives remain. Objective 1 of this proposal involves the Isolation and cytogenetic characterization of cell clones to be used in further molecular analysis of chromosomal inversions and translocations. We have collected and cryopreserved several human cell clones that represent the survival and clonal expansion of single cells exposed to gamma rays, 56Fe and 7Li ions. These cell clones harbor a range of nonlethal chromosome translocations and inversions. Over 20 human cell clones have been collected and cryopreserved that represent the survival of single cells exposed to various ionizing radiations. A compilation of data relating to clones in various stages of analysis has been updated with additional data to include mate-pair analysis of clones surviving exposure to gamma rays and 56Fe ions. As before, the 7Li clones still require further analysis by mFISH and G-banding before they are ready for sequencing and analysis using the newer 3-color/3-chromosome dGH probe sets for inversion analysis. For reasons related to issues with Objective 2, this was temporarily postponed. Objective 2 of this proposal involves the molecular characterization of these clones through the use of Next-Generation Sequencing (NGS), in order to determine the nature of the illegitimate junctions formed at the DNA level. Our workflow had been predicated on the following sequence of tasks. Aberration-bearing clones are isolated and characterized on the basis of translocations via mFISH (and inversions via dGH). Next, these clones are subjected to conventional G-band analysis, in order to more succinctly localize the rearrangement breakpoints. Following G-banding, the next step in the workflow was paired-end sequencing, followed by PCR, and eventual Sanger sequencing at the base-pair level from the amplified fragment, an approach that eventually we successfully implemented for one of the clones, and which we recently published. We discovered a 4 bp microhomology at the t(3;4) translocation junction of clone K1-400C4, which substantiated our working hypothesis that such rearrangements are characteristic of mmNHEJ misrepair pathways. It was also discovered that both junctions of the reciprocal translocation occurred in repetitive DNA. The breakpoint on chromosome 3 mapped to an LTR sequence, while that on chromosome 4 mapped within a LINE element. To our knowledge, this is the very first report involving the sequencing and validation of a known radiation-induced translocation in human cells using modern massively parallel sequencing (Cornforth, et al., Radiat Res, 2018. 190(1): p. 88-97). The analysis of paired-end libraries was deemed far too slow to meet the timely objectives of the grant proposal, and the sequencing of a single translocation is hardly proof of a consensus mechanism, so more work remained. This included analyses of clones that originated from cells exposed to high LET (linear energy transfer) radiation, which we fully recognize is of particular interest to NASA. Consequently paired-end sequencing was scrapped in favor the newer technique of mate-pair analysis, which allows for the assembly of longer-read fragments. Despite encouraging preliminary results using mate-pair described in last year’s progress report, we discovered some glaring inconsistencies between what the cytogenetic data was telling us about certain aberration-bearing clones, compared to the sequencing data derived by mate-paired analysis. Given the preponderance of repeat DNA elements in the human genome, we began to realize the chromosome exchange breakpoints occurring with repetitive DNA is likely to be the rule, rather than the exception. Further, that this fact could explain the aforementioned inconsistencies between mate-pair and cytogenetic endpoints, and the preponderance false-positive calls to the reference genome that plagued our early attempts. We now believe that strategies making use of longer insert libraries should circumvent repetitive DNA problems related to short reads, as is the case for paired-end and, to a lesser extent, mate-pair approaches. For that reason, we have re-assessed our sequencing strategy to embrace newer long-read technologies. This includes SMRT (single-molecule real-time) sequencing that can generate very long sequencing reads (~20 kb) using a Sequel System (PacBio) that was acquired and implemented in the McDermott NGS core at UTSW (University of Texas Southwestern). While this approach was, until recently, too expensive to be applied for our studies, these costs have dropped substantially, making SMRT sequencing now affordable for our project. We also plan to employ a newer less-expensive approach known as Linked-Read sequencing that utilizes molecular barcodes to tag reads that come from the same long (~50 kb) DNA fragment, thereby providing the long range information missing from standard approaches for a more complete characterization of SVs (structural variations) in the genome. For that approach we will use the

	Chromium platform from 10x Genomics that was recently acquired and implemented in the McDermott NGS core at UTSW. Linked-read libraries maintain haplotype and other long-range information and are compatible with standard short-read whole genome Illumina sequencing. It is expected that these two approaches, either by themselves, or likely in combination with short read technologies, will allow for a more rapid and accurate characterization of breakpoint junctions of radiation-induced large-scale SVs, such as the translocations and inversions we have already identified by mFISH and dGH.
Bibliography Type:	Description: (Last Updated: 06/11/2025)
Abstracts for Journals and Proceedings	Cornforth, MN, Loucas BD, Kittler R, Kollipara R, Williams ES, Ray FA, Robinson E, Bedford JS, Peto M, Anur P, Wang N, Spellman P, Gray JW, Bailey SM. "Molecular Cytogenetics Guides Massively Parallel Sequencing of a Radiation-Induced Chromosome Translocation In Human Cells." Poster presentation. 64th Annual Meeting of the Radiation Research Society, Chicago, IL, September 23-26, 2018. 64th Annual Meeting of the Radiation Research Society, Chicago, IL, September 23-26, 2018. , Sep-2018
Abstracts for Journals and Proceedings	Cornforth, MN, Loucas BD, Kittler R. "Molecular Characterization of Transmissible Chromosome Aberrations Produced By Ions of Intermediate and High Atomic Number." Poster presentation. 2019 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 22-25, 2019. 2019 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 22-25, 2019. , Jan-2019
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