Facilitated Chromate Reduction by *Deinococcus radiodurans* Engineered for Fuel Hydrocarbon Catabolism, and the Development of a Genetic System of Transformation for *Deinococcus geothermalis*

Hassan Brim,[1] James K. Fredrickson,[2] Lawrence P. Wackett,[3] and Michael J. Daly[1] (PI)

[1]Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland; [2]Pacific Northwest National Laboratory, Richland, Washington; [3]Department of Biochemistry, BioTechnology Institute and Center for Biodegradation Research and Informatics, Gortner Laboratory, University of Minnesota, St. Paul, Minnesota

The end of the Cold War in the early 1990s ushered in a shift in emphasis from nuclear weapons production to environmental cleanup. This enormous remediation challenge has driven the development of microbiological approaches to detoxify the immense volumes of ground that have been, or have the potential to be, contaminated by leaking radioactive storage tanks. However, the lethal effects of radiation have constrained consideration of microbial treatment of environments adjacent to highly radioactive tanks, where intervention could begin before contaminants become widely dispersed. Among the classes of toxic organic compounds identified in stored radioactive wastes, the fuel hydrocarbon toluene is one of the most prevalent, frequently occurring together with Cr(VI) and other toxic metals. We have engineered the extremely radiation-resistant bacterium *Deinococcus radiodurans* (DEIRA) to derive energy from complete toluene mineralization to support Cr(VI) detoxification and immobilization in natural sediment and groundwater analogues of U.S. Department of Energy (DOE)-contaminated environments. In addition, the engineered DEIRA utilizes carbon/energy from toluene oxidation for cellular biosynthesis and survival. During early operation, short-lived radionuclide heating of tanks raised their temperatures as high as 150°C, and in many cases contributed to their rupture and ongoing release of contaminants into the environment. Temperatures in some contaminated environments remain as high as 70°C because of long-lived radionuclide decay, and there is a need to develop radiation-resistant bioremediating organisms that function at elevated temperatures. *Deinococcus geothermalis* (DEIGEO) is thermophilic and closely related to the mesophilic DEIRA. We have shown that DEIGEO is naturally transformable with autonomously replicating and integration vectors derived from DEIRA, yielding a Hg(II)-resistant DEIGEO strain capable of reducing Hg(II) at elevated temperatures and in the presence of 60 Gy/hour. Additionally, like DEIRA, DEIGEO is capable of reducing Fe(III)–NTA, U(VI), and Cr(VI). These characteristics support the prospective development of engineered DEIGEO for bioremediation of radioactive mixed waste environments with temperatures as high as 55°C.