FINAL REPORT

PROJECT TITLE: Contribution to Sequencing of the *Deinococcus radiodurans* Genome

NAME AND ADDRESS OF PRINCIPAL INVESTIGATOR:

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PROJECT AND BUDGET PERIOD: 9/1/96 - 8/31/97

RECIPIENT ORGANIZATION:

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DOE AWARD NO.: DE-FG02-96ER62231

TOTAL BUDGET (direct and indirect): $44,406

AMOUNT OF UNEXPENDED FUNDS: $917

DISCLAIMER

TECHNICAL REPORT:

The stated goal of this project was to supply The Institute for Genomic Research (TIGR) with pure DNA from the bacterium *Deinococcus radiodurans* R1 for purposes of complete genomic sequencing by TIGR. We subsequently decided to expand this project to include a second goal; this second goal was the development of a *Not*I chromosomal map of *D. radiodurans* R1 using Pulsed Field Gel Electrophoresis (PFGE).

With respect to the first goal: Total genomic DNA of *D. radiodurans* R1, purified by CsCl-EtBr isopycnic centrifugation, was delivered to TIGR for their initial sequencing efforts early in the grant period. In these early sequencing efforts they found that this DNA preparation showed over representation of the *D. radiodurans* R1 natural plasmid pS16. In response, we generated a series of ultrapure minipreparations of high molecular weight *D. radiodurans* R1 DNA and forwarded these samples to TIGR. They found that these latter preparations did not over represent plasmid DNA, but instead contained largely chromosomal DNA, and were used by TIGR for the subsequent successful *D. radiodurans* genomic sequencing project.

With respect to the second goal: We provided TIGR with the results of high resolution studies on determining the size of the *D. radiodurans* chromosome (3.1 Mbp) and its plasmid pS16 (46 kbp). Determining the size of the chromosome was achieved by generating a *Not*I restriction map by PFGE; we found that the chromosome contains 12 *Not*I sites and pS16 contains a single *Not*I site. As a result of genetic disruption studies we were able to resolve all PFGE bands clearly by eliminating doublets, thereby correcting a previously published erroneous chromosome size estimate based on 11 PFGE chromosomal fragments - there is a 420 kbp chromosomal *Not*I doublet. Finally, by cloning out the *Not*I sites plus flanking sequences, and using them as probes, we were able to align the *Not*I fragments into contigs using PFGE Southern blot analysis. These studies have provided partial *Not*I maps of the *D. radiodurans* R1 chromosome.