Attn: Chris Detter,
Paul Richardson (925-296-5851)
Joint Genome Institute
2800 Mitchell Drive
Walnut Creek, CA 94598
pmrichardson@lbl.gov

Dec. 1, 2003

Re: *Deinococcus geothermalis* DNA delivery to JGI

Dear Dr. Chris Detter,

The radiation resistant, thermophilic bacterium *Deinococcus geothermalis* was recommended for draft sequencing at the JGI by the DOE prioritization review panel on Sept 8, 2003. We are sending you *D. geothermalis* DNA together with the items and information listed below (A-C). Under separate cover, we are sending TEM images of the *D.geothermalis* cells from which the DNA was isolated. Please feel free to call me or Dr. Elena Gaidamakova.

Sincerely,

Michael Daly

A. Required information for samples:

1. PI: Michael Daly

mdaly@usuhs.mil,
USUHS, Dept of Pathology, Rm. B3153
4301, Jones Bridge Rd
Bethesda, MD20814-4799
Tel: 301-295-3750
Fax: 301-295-1640

2. Persons who isolated DNA:

Elena Gaidamakova, PhD, and Min Zhai, Technical Assistant
egaidamakova@usuhs.mil
USUHS, Dept of Pathology, Rm. B3153
4301, Jones Bridge Rd
Bethesda, MD20814-4799
Tel: 301-295-0063
Fax: 301-295-1640

3. Persons who packed and shipped DNA sample:

Elena Gaidamakova, PhD, and Min Zhai, Technical Assistant
egaidamakova@usuhs.mil
USUHS, Dept of Pathology
4301, Jones Bridge Rd
Bethesda, MD20814
301-295-0063

4. Contents of the shipment:

1) DNA sample in 1.7 ml tube (600 µl with concentration of DNA at 0.17 mg/ml)
2) 1% agarose gel picture for estimation of DNA concentration
3) 0.7% agarose gel electrophoresis of DNA digested with endonucleases: EcoRI, Eco47III, HindIII and XhoI
4) Spectrophotomeric reading (210-350 nm) of sample, diluted 20 times

5) Culture of Deinococcus geothermalis strain DSM 11300 (in 50% glycerol) obtained from the DSMZ collection.

6) Genome size of *D. geothermalis* is unknown, but expected to be similar to *D. radiodurans* (3.3 Mbp). Note, we observed a small band in the agarose gel consistent with a 1-2 kb plasmid.

7) Reprints of our recent paper on *D. geothermalis* (Appl. Environ. Microbiol. 69, 4575-4582).

8) Under separate cover, CD-R disc of images of *D. geothermalis* cells (DSM 11300) from which the DNA was prepared. Alexander Vasilenko, PhD, prepared images. avasilenko@usuhs.mil

B.

· CD-R disc of images of *D. geothermalis* cells (DSM 11300) from which the DNA was prepared.
· Reprints of our recent paper on *D. geothermalis* justifying why it was selected for sequencing (Appl. Environ. Microbiol. 69, 4575-4582).
· Relevant literature refs: http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?CMD=search&DB=PubMed key word: geothermalis
· Names and email of collaborators to be listed on website:

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C. Protocol for genomic DNA isolation:

Deinococcus geothermalis strain DSM 11300 was obtained from the DSMZ collection.

A single bacterial colony was used to inoculate 5 ml of TGY (1% Bacto Tryptone, 0.1% glucose, 0.5% Bacto Yeast Extract). After 24 hours of growth at 50°C, 3 ml of the culture were used to inoculate 300 ml TGY and incubated at 50°C with aeration. Strict precautions were taken to avoid contamination. Contamination was not likely since the only other red-pigmented strain we use routinely is *D. radiodurans*, which does not grow above 39°C. As expected the cell pellets used for DNA isolation were uniformly red. Genomic DNA was purified from 300 ml culture according to the JGI-modified CTAB method (with recommended dilution of cells to OD600 =1). In all cases, we purchased new reagents from vendors recommended by JGI for DNA purification and followed the JGI protocol from step 1 to step 27. After RNase treatment (step 27) DNA was re-precipitated with ethanol, ethanol was removed from the DNA precipitate which was then washed in 70% ethanol, and dissolved in 1.2 ml TE buffer (8.0).

The absorbance of DNA was measured at 260 nm and 280 nm. The ratio of A260 to A280 for the sample is 1.79. A picture of a 1% agarose gel used in determining the DNA concentration is attached. The DNA concentration of the sample is 0.17 mg/ml. Note well, spectrophotometry indicated a higher DNA concentration, which may be due to RNA contamination.

Enclosed: 600 ml DNA sample (~100 mg of DNA). A duplicate sample is maintained at USUHS.

Sincerely,

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