Quarterly Progress Report for the Period 1/1/00 to 3/30/00

Project Title: Multiple-Locus VNTR Analysis (MLVA) for Bacterial Strain Identification

DOE Project Number: DE-FG03-00NN20102 Northern Arizona University

B&R CODE: **Date:** April 12, 2000

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Progress During the Quarter:

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

We have identified > 48 potential VNTR markers from the genomic sequence. This represents the first step towards development of a MLVA system.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

Analysis of 12 core samples from USAMRIID has been completed with the full 48 VNTR markers.

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections.

The MLVA analysis of 96 California samples has been completed. No progress has been made on New Mexico or Arizona samples.

Goal 4. A 25 marker MLVA system for *B. anthracis*

We have a robust 8 marker MLVA system developed for *B. anthracis*. We have identified an additional ~30 potential VNTR regions.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

We have identified a set of 89 unique B. anthracis strains by analysis with the 8 marker MLVA system.

DISCLAIMER

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

We have identified four potential VNTR regions in the *F. tularensis* genome.

Comments

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

We will be developing primers, optimizing reactions and testing for primer compatibility in multiplex reactions in the next few months. A 25 marker MLVA system will be developed by the end of the contract period.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

The core set of *Y. pestis* samples from USAMRIID represent a very diverse collection and its analysis is intended as a broad assay of diversity. A full analysis of 40 samples may not be necessary in order to accomplish this goal and we have selected a representative core set of 12, instead.

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections

MLVA analysis of the California samples will be accomplished with this contract period. MLVA will be accomplished on the New Mexico samples and Arizona samples if they are obtained in the next 3-6 months. Negotiations with the state health labs is still in progress.

Goal 4. A 25 marker MLVA system for *B. anthracis*

With identification of the additional ~30 potential VNTR regions, the prospects for a 25 marker MLVA system appear very good. We will be screening these regions for variation over the next few months. In addition, we will be attempting to combine these markers into multiplex reaction cocktails.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

The new markers identified as a part of goal number 4 will be used to analyze the unique set of 89 strains. This will be accomplished over the next few months.

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

We have screened all available *F. tularensis* genomic sequence. We have contacted the consortium of labs performing the sequence determination, but they have refused to release additional information. Identification of 21 additional potential VNTR regions is dependant upon the availability of these data.

Funding Status	**Operations**	**Capital**
Uncosted from previous FY.	0	0
Current FY	$ 270,000	$ 30,000
Total Funding Available	$ 270,000	$ 30,000
$ Spent this quarter:	$ 31,229	$ 0
$ Spent year-to-date	$ 31,229	$ 0
$ remaining for this FY	$ 238,770	$ 30,000
Anticipated uncosted current FY funds	0	0

Technical Reports/Presentation

- Talk: The 50th Annual Southwest Conference on Diseases in Nature Transmissible to Man. San Antonio, Texas, March 28-30, 2000.

- Talk: Santa Fe NN20 BioFoundations progress report meeting March 2000.

- Journal Article: Keim, P., L.B. Price, A.M. Klevytska, K.L. Smith, J.M. Schupp, R. Okinaka, P. Jackson, and M.E. Hugh-Jones. 2000. Multiple-Locus VNTR analysis (MLVA) reveals genetic relationships within *Bacillus anthracis. J. Bacteriology.* Accepted for publication – Not yet published.

- Adair, DM, PL Worsham, KK Hill, AM Klevytska, PJ Jackson, AM Friedlander, and P. Keim. 2000. Diversity in a Variable Numbers of Tandem Repeat (VNTR) from *Yersinia pestis. J. Clinical Microbiology.* 38:1516-1519.