

Title: Legume Genome Initiative at the University of Oklahoma

Principle Investigator: Dr. Bruce A. Roe, Ph. D. George Lynn Cross Research Professor of Chemistry and Biochemistry

Project Summary: Consolidated Appropriations Resolution, 2003 Conference Report for the Department of Energy's Biological and Environmental Research (BER) program provided \$481,000 for the Legume Genome Initiative at the University of Oklahoma. These funds were used to support our research that is aimed at determining the entire sequence of the gene rich regions of the genome of the legume, *Medicago truncatula*, by allowing us to obtain a greater degree of finished BAC sequences from the draft sequences we have already obtained through research funded by the Noble Foundation. During the funding period we increased the number of *Medicago truncatula* BACs with finished (Bermuda standard) sequences from 109 to 359, and the total number of BACs for which we collected sequence data from 584 to 842, 359 of which reached phase 2 (ordered and oriented contigs). We also sequenced a series of pooled BAC clones that cover additional euchromatic (gene rich) genomic regions. This work resulted in 6 refereed publications, see below. Genes whose sequence was determined during this study included multiple members of the plant disease resistance (R-gene) family as well as several genes involved in flavinoid biosynthesis, nitrogen fixation and plant-microbial symbiosis. This work also served as a prelude to obtaining NSF funding for the international collaborative effort to complete the entire sequence of the *Medicago truncatula* genomic euchromatic regions using a BAC based approach.

Relevance: Legumes are one of the world's most important crop families, unique in their ability to fix atmospheric nitrogen. Because of this ability and because *M. truncatula*'s is a forage crop, the life cycle of the plant affects the air, soil, livestock, and, subsequently, our agriculture and economy. By understanding the genome more thoroughly, we will increase the potential to improve the length of the crop's growing season, as well as its durability in a variety of atmospheric conditions.

Among legumes, *M. truncatula* is considered an excellent model for genome research. Unlike most crop legumes, *M. truncatula* has a compact genome, simple genetics, short generation time, relatively high transformation, excellent mutants, and large collections of ecotypes. Because of these desirable features, there is a growing consensus that *M. truncatula* is an ideal candidate to act as a reference species for legume genomics.

Scientific Objectives: Our long term objective is to fully sequence the euchromatic gene rich regions of *Medicago truncatula*, a model plant legume. These DOE funds were used to specifically support our work to:

1. obtain a greater degree of finished BAC-based DNA sequences from the draft DNA sequences we already have obtained through funding by the Noble Foundation, and
2. sequence pooled BACs from the ends of the present ~1200 contigs to provide the additional sequences needed to reduce the present number of contigs to ~300.

This work, which has been accomplished using state-of-the-art DNA sequencing methods that are ongoing in our Genome Center at the University of Oklahoma, provides valuable sequence information and extends our mapped-BAC strategy as a prelude to obtaining the completed uninterrupted sequence of the euchromatic arms of the 8 *M. truncatula* chromosomes to the “Bermuda” standards using the recently awarded NSF funding.

Relevance to the DOE Mission: One of the major missions of the DOE and the Biological and Environmental Research (BER) Program is to provide fundamental science that will result in biological based solutions that address DOE and National needs. *M. truncatula* and related plant species require enormous energy resources to maintain a high crop yield, and a high crop yield is agriculturally important in order to feed livestock. By determining the genomic structure of *M. truncatula*, we will lay the foundation to improve the yield per acre and the disease, heat, and drought resistances. It may also lead to the extension of the growing period and reduce the dependency on the extensive use of energy-dependent high yield fertilizers and irrigation for this important crop. In addition, since legumes are the only plants that allow for nitrogen fixation, a greater understanding of this process will be obtained through these genomic studies that may allow improvements to be made in understanding plant-microbe interactions, resulting in making these processes more wide spread and more efficient.

Benefits to the Public: The public also will benefit from these studies by allowing growers to make more efficient use of the dwindling farmlands that are devoted to forage crops and, subsequently, slow the rise in food costs. This knowledge of the genomic structure and organization of the *M. truncatula* genome will allow plant geneticists to produce new strains of this legume that will be more robust, have a lower dependency on energy resources, and allow increased yields per acre. A greater understanding of plant-microbe interactions will also benefit farmers’ abilities to increase the production and quality of their crops.

Research Publications as an immediate result of this DOE funding:

O. Kulikova, R. Geurts, M. Lamine, D.J. Kim, D.R. Cook, L. Leunissen, H. de Jong, B.A. Roe, T. Bisseling. Satellite repeats in the functional centromere and pericentromeric heterochromatin of *Medicago truncatula*. *Chromosoma*. 113, 276-283 (2004).

H.K. Choi, D. Kim, T. Uhm, E. Limpens, H. Lim, J.H. Mun, P. Kalo, R.V. Penmetsa, A. Seres, O. Kulikova, B.A. Roe, T. Bisseling, G.B. Kiss and D.R. Cook. A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. *Genetics*. 166, 1463-1502 (2004).

B. Roe. Shotgun Library Construction for DNA Sequencing in *Methods in Molecular Biology*, vol 255: Bacterial Artificial Chromosomes, Volume 1: Library Construction, Physical Mapping, and Sequencing. S. Zhao and M. Stodolsky, eds., Human Press Inc, Totowa, New Jersey pp. 171-187 (2004).

B. A. Roe and D. M. Kupfer. Sequencing Gene Rich Regions of *Medicago truncatula*, a Model Legume in Molecular Breeding of Forage and Turf. A. Hopkins, Z.Y. Yang, R. Mian, M. Sledge and R.E. Barker, eds., Kluwer Academic Publishers, The Netherlands, pp. 333-344 (2004).

J.M. Ane, G.B. Kiss, B.K. Riely, R.V. Penmetsa, G.E. Oldroyd, C. Ayax, J. Levy, F. Debelle, J.M. Baek, P. Kalo, C. Rosenberg, B.A. Roe, S.R. Long, J. Denarie, and D.R. Cook. *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. *Science*. 303, 1364-1367 (2004).

H.K. Choi, J.H. Mun, D.J. Kim, H. Zhu, J.M. Baek, J. Mudge, B. Roe, N. Ellis, J. Doyle, G. B. Kiss, N. D. Young, and D. R. Cook. Estimating genome conservation between crop and model legume species. *Proc. Natl. Acad. Sci. USA*. 101, 15289-15294 (2004).