Proposal for Sequencing the Genome of the Tick, *Ixodes scapularis*

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I. Justification for an *Ixodes scapularis* Genome Project

This proposal represents the cooperative efforts of the international tick research community to develop the first large scale genomic analysis of a medically significant tick, namely *Ixodes scapularis.* Ticks transmit the greatest variety of human and animal pathogens of any arthropod vector and are second only to mosquitoes as vectors of human disease (Fivaz *et al.,* 1992; Sonenshine and Mather, 1994). Diseases transmitted by blood feeding ixodid ticks (subphylum Chelicerata; class Arachnida; subclass Acari; family Ixodidae) are global medical and veterinary health problems (Sonenshine, 1993) and include a wide variety of bacterial, rickettsial, viral and protozoan diseases. Other forms of pathogenesis attributed to ticks include anemia, dermatosis, toxemia and paralysis (Gothe, 1999; Roberts and Janovy, 1996; Sonenshine, 1991; Sonenshine, 1993). Important tick-borne diseases include Lyme disease (LD), tick-borne relapsing fever, babesiosis, anaplasmosis, Rocky Mountain spotted fever, Boutonneuse fever, Queensland tick typhus, Q fever, and numerous arboviruses (Sonenshine, 1993). The resurgence of LD and the emergence of other tick-borne diseases such as human granulocytic anaplasmosis (HGA) (Childs and Paddock, 2003; Gratz, 1999; Paddock and Childs, 2003) pose increasing public health concerns. Since the discovery of the causative agent of LD, *Borrelia burgdorferi*, fifteen previously unrecognized tick-borne bacterial pathogens have been described (Parola and Raoult, 2001). Furthermore, due to their efficiency as vectors of a wide variety of pathogens, broad vertebrate host range and worldwide distribution (Sonenshine, 1991), ixodid ticks are recognized as potential vectors of a number of pathogens considered to be possible bioterrorism agents for use against humans and livestock including Crimean-Congo hemorrhagic fever virus, *Rickettsia rickettsii* (Rocky Mountain Spotted Fever), the tick-borne encephalitis complex of flaviviruses (Central European tick-borne encephalitis, Far Eastern tick-borne encephalitis, Siberian tick-borne encephalitis, Kyasanur forest disease and Omsk hemorrhagic fever), *Coxiella burnetii* (Q Fever) and *Francisella tularensis* (tularemia) (Centers for Disease Control and Prevention Select Biological Agents and Toxins, 2004; http://www.cdc.gov/od/sap/docs/salist.pdf).

In the United States, *I. scapularis* is the most important tick species from a human health perspective. *Ixodes scapularis* transmits LD in the northeastern and north-central US, HGA and babesiosis and possibly the flaviviral agent of Powassan encephalitis (POW) which is related to West Nile virus. Recent studies by Anderson *et al*., (2003) also suggest that West Nile virus can be transmitted trans-stadially by *I. scapularis* although vector competence has yet to be established. LD is arguably one of the most important vector borne diseases in the US, Europe and Asia. Over 17, 000 positive LD cases were reported to the US in 2000 (Centers for Disease Control and Prevention, 2002). LD and other tick-borne diseases have important long term health consequences. Of further concern is the fact that the incidence and geographic spread of LD and other tick-borne disease are increasing and many cases are suspected to be vastly under-reported or misdiagnosed (Walker, 1988). *Ixodes scapularis* is a member of the Prostriata, an evolutionarily primitive phyletic line of the Acari that includes a number of medically significant tick species. *Ixodes scapularis* genome data will be widely applicable to studies of other prostriates including *I. pacificus*, the vector of LD on the US Pacific Coast, *I. ricinus* and *I. persulcatus,* the Eurasian *Ixodes spp*. vectors of LD and tick borne encephalitis and *I. holocyclus*, an Australian ixodid responsible for transmission of *Rickettsia* and *Borrelia* and human cases of tick paralysis. These factors, particularly the wide range of human diseases that it transmits, make *I. scapularis* the best overall candidate for a genome project that seeks to have an ultimate impact on human welfare through development of novel vector suppression measures, therapeutics and vaccines.

Control of arthropod-borne pathogens is complicated by lack of vaccines (Walker, 1998) and the development of drug resistant pathogens (Molyneux, 1998) and acaricide resistant ticks (Mitchell, 1996). Current methods for tick control rely primarily on avoidance of tick bites and the use of approved repellants. Despite the medical significance of *I. scapularis* and other ixodid ticks, there are currently no

large-scale genome efforts dedicated to a tick species of public health importance. This is a major impediment to vector biology and vector-borne disease research and ultimately to the development of new tick and tick-borne disease control strategies. Among the most important outcomes of this genome project will be opportunities to identify new acaricide, drug and anti-tick vaccine targets. *Ixodes* genome sequence offers an opportunity to investigate aspects of tick biology that can be exploited for tick control. Ticks are thought to have developed unique strategies for obligate hematophagy and parasitism. Tick saliva contains a potent cocktail of pharmacologically active peptides and other molecules that modulate or suppress the haemostatic, inflammatory and immune responses of the host (Valenzuela *et al.,* 2002). These and other molecules "mined" from genome sequence offer an exciting possibility to identify new vaccine targets, potential bio-pharmaceuticals, anti-microbial peptides and other novel human therapeutics. Furthermore, tick saliva and modulation of host defenses is increasingly being linked to pathogen transmission (Nuttall *et al*., 2000; Schoeler and Wikel, 2001; Wikel *et al.,* 1994; Wikel 1996). Sequence data may lead to the identification of molecules associated with the acquisition, development and transmission of infectious agents in the tick. In combination with the completed *Homo sapiens* (Lander *et al.,* 2001; Venter *et al*., 2001) and *Borrelia burgdorferi* (Fraser *et al.,* 1997) genome projects and the anticipated tick-borne pathogen genomes (*Babesia bovis, Anaplasma marginale, Theileria parva and T. annulata*), it will be possible to apply the *Ixodes* genome sequence to unravel the complicated molecular and genetic basis of tick-pathogen-host relationships and tick borne disease transmission.

Ticks likely appeared during the Paleozoic or early Mesozoic era, approximately 225 million years ago (Klompen *et al*., 1996) and are expected to have diverged significantly from the subphylum Mandibulata and the class Insecta. The *Ixodes* project will provide the first genomic overview of the taxonomically diverse subphylum Chelicerata and will significantly expand the scope of comparative and evolutionary eukaryotic analyses. The *I. scapularis* genome is predicted to be highly unique in comparison to the other sequenced invertebrate genomes such as *Drosophila melanogaster, D. pseudoobscura,* the malaria mosquito *Anopheles gambiae* and the honeybee *Apis mellifera,* all of which are phylogenetically restricted to the subphylum Mandibulata and the class Insecta. The subdivision of Arthropoda and Mandibulata extends back to approximately 750 million years ago and is therefore one of the most ancient among metazoan animals. This suggests that the predictive power of Insecta sequences for interpreting Chelicerata ESTs may be limited. In fact many ixodid tick ESTs have no matches to the current entries in sequence databases (Hill and Gutierrez, 2000; Nene *et al.* 2002; Valenzuela *et al.,* 2002). In addition to the *Drosophila* and *A. gambiae* genomes, the *Ixodes* genome project will complement and add value to other vector genome projects including the ongoing *Aedes aegypti* (yellow fever mosquito) project and the proposed *Culex pipiens quinquefasciatus* (Southern house mosquito) and Tsetse fly genomes. Genetic information will also facilitate studies of tick phylogenetics, population biology, ecology and behavior; areas of tick research that have been hindered by lack of sequence data. Genome data will improve gene prediction capabilities and enable the identification of conserved arthropod specific genes, divergent orthologs and differentially expanded paralogous gene families and metabolic pathways amongst the arthropoda. As the first large scale genome analysis of an acarine species, it is expected that the *Ixodes* genome will also act as a powerful catalyst for molecular, genomic and comparative studies between other acarine species and a range of prostriate and metastriate ticks.

II. Status of the *Ixodes scapularis* Genome Effort

Efforts to develop the critical preliminary molecular tools to support an *I. scapularis* genome project are presently funded in a number of labs and are well advanced (refer to sections III, V and VI). There are no efforts underway or currently proposed by other agencies or organizations to determine the genome sequence of *I. scapularis*.

III. Interest of the Scientific Community in the *Ixodes scapularis* Genome and Relationship to Other Research

There is international interest in the *I. scapularis* genome project from the biomedical and entomological research communities. The tick community has conducted many decades of research on various aspects of ixodid tick biology, physiology, genetics, population biology, ecology and pathogen transmission and the control of ticks and tick-borne disease. Many of these studies have focused on *I.*

scapularis because of its medical significance. The tick community has met several times to mobilize for a tick genome project and on each occasion, the *I. scapularis* genome has been acknowledged as a high priority for sequencing due to the importance of *I. scapularis* as a vector of human disease. Several internationally attended meetings dedicated to tick-borne disease and tick genomics demonstrate the global interest in the *I. scapularis* genome project. These meetings include the "*Working Group on Tick Genomics"* Meeting convened by the Institute for Genome Research (TIGR) and the USDA and held in Rockville, MD in February 2003 and the *4th International Conference on Ticks and Tick-Borne Pathogens* (TTP-4) held in Banff, Canada in July 2002 and previous meetings held in 1992, 1995 and 1999. The "*Tick-Borne Diseases: Genomics and Proteomics Approaches"* meeting planned for May 2004 (Dr. A. Azad, Dr. J. Ribeiro and Dr. S. Wikel organizers) will provide a forum for the community to organize for the genome project. We have received correspondence from researchers in the United Kingdom, Europe, Australia, Africa, Canada and the United States who have expressed their support for this effort (refer to appended letters).

Members of the tick research community listed below represent an international consortium of scientists that will participate in the *I. scapularis* genome effort. One major goal of the consortium is to expand and strengthen ties with members of the community who work on all aspects of tick-borne disease and tick control. The collaboration and consultation between scientists involved in the *Ixodes* genome project is summarized below.

a) Dr. C. Hill has funding from Purdue University and Dr. F. Collins is supported by the Indiana Center for Insect Genomics to develop *I. scapularis* BAC libraries for genome sequencing. Dr. Hill will initiate nuclear staining techniques as a method to rapidly estimate the size of the genome.
b) Dr. S. Wikel will supply the reference *I. scapularis* strain for sequencing. Dr. D. Sonenshine and Dr. M. Roe will establish satellite *I. scapularis* colonies for maintenance and distribution of the reference strain to the community.
c) A. Ullmann and Dr. W. Black IV will complete a C_ot analysis to determine the size of the *I. scapularis* genome in April 2004.
d) The consortium will consult with Dr. J. Oliver, Dr. T. Kurtti and Dr. U. Munderloh to develop *I. scapularis* physical mapping for genome assembly. Independent funding will be pursued by the community for this effort.
e) Dr. F. Collins, Dr. D. Severson and Dr. V. Nene will provide expertise on the sequencing, assembly and annotation of the *Ixodes* genome. These researchers have extensive experience in sequencing of the *A. gambiae*, *A. aegypti* and *T. parva* genomes respectively.
f) Dr. S. Wikel has funding from the U.S. Army Medical Research and Materiel Command, an NIH RO1 and the CDC and Drs. J. Ribeiro and J. Valenzuela are supported by intramural NIH funding to sequence and analyze *I. scapularis* ESTs. These ESTs will act as a community resource and genome annotation tool.
g) Dr. D. Sonenshine, Dr. T. Mather, Dr. T. Schwan and Dr. S. Wikel will provide *I. scapularis* tissue (including ticks infected with various pathogens) and Dr. A. Azad will provide *I. scapularis* cell lines for production of cDNA libraries.
h) Dr. R. Rozenberg, Dr. J. George and Dr. F. Guerrero are supported by USDA to develop and sequence ESTs and BACs for the Southern cattle tick, *Boophilus microplus*. They will collaborate within the consortium to facilitate comparative genomics studies between pro- and metastriate ticks.
i) Dr. F. Collins is the principal investigator of an NIAID contract proposal to develop ***VectorBase***, a relational bioinformatics resource for invertebrate vectors of human disease. ***VectorBase*** will provide access to *I. scapularis* sequence and functional data and will act as a bioinformatics tool to investigate the *Ixodes* genome.
j) Other members of the international tick community (Dr. P. Willadsen, Dr. S. Barker, Dr. P. Nuttall and Dr. R. Bishop) will collaborate within the consortium to utilize the genome resource, facilitate comparative studies between ixodid tick species and contribute to third party genome annotations. The International Livestock Research Institute (ILRI) bioinformatics unit has established strong collaborative linkages and in-house capacity in prediction of protein structures from sequence data and would be interested in applying these approaches to add value to annotation of the *Ixodes* genome.

k) Dr. A. Azad and members of the Indiana Center for Insect Genomics plan to develop an *I. scapularis* micro-array for the tick research community using cDNA clones from the genome project.

IV. Management of the *Ixodes scapularis* Genome Project

We have assembled the **International *Ixodes scapularis* Sequencing Committee (IISSC)** to coordinate and manage the *Ixodes* genome project. The **IISSC** will have ultimate responsibility for the *I. scapularis* genome sequence. The constitution of the **IISSC** reflects the interest of the wider tick research community in the proposed genome project and includes internationally recognized experts on the biology, genetics, genomics and bioinformatics of ixodid ticks. Current members of this committee are: Dr. A. Azad, Dr. F. Collins, Dr. C. Hill, Dr. V. Nene, Dr. J. Oliver, Dr. J. Piesman, Dr. J. Ribeiro, Dr. R. Rozenberg, Dr. D. Severson, Dr. J. Valenzuela, Dr. S. Wikel and Dr. N. Zeidner. Dr. Hill (10% time) and Dr. Wikel (5% time) will chair the committee and will coordinate the involvement of other community personnel as needed. Dr. Hill will assume primary responsibility for interaction with the Microbial Sequencing Center (MSC). Dr. Wikel will provide the reference strain for sequencing. The committee will elect a representative of the MSC following selection of the MSC by NIAID. In addition, expert advice will be provided by Dr. Collins and Dr. Severson who have extensive experience in the development and coordination of the *A. gambiae* and *A. aegypti* genome projects, respectively. **IISSC** membership will be modified and expanded as needed.

V. Utility of the *Ixodes scapularis* Genome Sequence

The work outlined in this proposal will build on and complement *Ixodes* research being undertaken by many labs throughout the world. The primary intended beneficiary communities of the *Ixodes* genome include labs that study various aspects of tick biology, genomics, genetics, tick-borne disease, arthropod evolutionary biology and the broader vector biology community in general. The tick community has conducted many decades of research on various aspects of ixodid tick biology and there are dozens of tick research laboratories worldwide. The maturity of the tick research community is demonstrated by organizations such as the Systematic and Applied Acarology Society, the International Arachnology Society, the Acarological Society of America and the International Consortium on Ticks and Tick-Borne Diseases (ICTTD). It is anticipated that many labs and researchers will be drawn to molecular analysis of the tick genome. As detailed below, the wealth of data and ongoing research and expertise within the scientific community will enable investigators to maximally exploit the *Ixodes* genome sequence.

Tick/Host/Pathogen Relationships

The *Ixodes* genome project offers an opportunity to study tick/host/pathogen relationships, to identify novel biologically active molecules and to develop novel vaccine targets against *I. scapularis* and other tick species (Valenzuela *et al*., 2002). Various laboratories are currently focused on the genomic and proteomic analysis of ticks and in particular, tick salivary gland proteins (the sialome) as a source of targets for vaccine development (de la Fuente and Kocan, 2003; Ribeiro and Francischetti, 2003; Trimnell *et al.,* 2002; Valenzuela, 2002; Wikel *et al*., 2003). Ticks remain attached to the host for prolonged periods and have evolved unique blood feeding and osmo-regulation strategies and mechanisms to modulate and avoid host immune responses. Tick salivary glands play a critical role in survival and maintenance of water balance and supply a sophisticated pharmacopoeia of compounds that promote blood feeding and pathogen transmission. Patents have already been issued for application of ixodid cement as a surgical adhesive and ixodid histamine binding proteins and a mast cell tryptase inhibitor as novel anti-inflammatory drugs, with the former already at the stage of phase IIb human clinical trials for treatment of allergic conjunctivitis (P. Nuttall, pers. comm.). The TickGARD vaccine commercialized for control of *B. microplus* in Australia is based on a tick gut glycoprotein and is one of the few successful recombinant vaccines to be developed for tick control (reviewed by Willadsen *et al*., 1995). The safe and efficacious recombinant vaccine against *B. burgdorferi* based on a tick-expressed antigen (Fikrig *et al*., 1990; Sigal *et al*., 1998) for protection against Lyme disease in endemic areas of the United States was recently withdrawn from the market. Numerous studies have investigated the molecular cross-talk of the tick-host-pathogen interface (Anguita *et al.,* 2002, Leboulle *et al*., 2002) and many laboratories have established the necessary animal and pathogen models for these studies (T.

Mather, pers. comm.). There is strong evidence that components of ixodid saliva can promote virus transmission (reviewed by Nuttall, 1998; Schoeler and Wikel, 2001) but the specific components of saliva responsible for this have not been identified. Ultimately, genome sequence will be used to understand the role of ticks in the transmission of emerging and re-emerging infectious disease.

Genetic Basis for Disease Transmission and Vector Competence

The competence of ixodid ticks to vector many diseases has been the focus of detailed studies (Edlow, 2002). Many current studies are directed toward understanding the ability of ticks to vector newly identified spirochete *spp*. (A. Barbour and T. Schwan, pers. comm.) and potential agents of bioterrorism (Azad and Radulovic, 2003). Vector competence for many tick-borne diseases is presumed to be under genetic control (Ochanda *et al.,* 1998; Young *et al.,* 1995). Despite the fact that genes determining vector competence are obvious targets for control strategies, the genetic basis of vector competence in ticks has received limited research attention. The *I. scapularis* genome sequence will help to unravel the complicated molecular mechanisms that underpin pathogen acquisition and transmission of a wide range of emerging and resurging tick-borne diseases. The genome project will provide a major boost to current efforts to develop added markers for phylogenetic analysis to help solve outstanding questions regarding the evolution of vectoring capabilities in ticks (H. Klompen, pers. comm.).

Comparative Genomics Studies

The tick genome represents the first chelicerate genome and would make an invaluable contribution to comparative and evolutionary eukaryotic genomics (Rubin *et al.,* 2000). There is considerable interest from the veterinary entomology community to develop genome sequence for a tick species of veterinary significance. Dr. J. George and Dr. F. Guerrero have initiated an EST project for the Southern cattle tick, *Boophilus microplus* and have future plans for production of BAC libraries. *Boophilus microplus* is a one-host tick that causes significant losses in animal production systems in the southern hemisphere. A BAC library has also been generated from *R. appendiculatus,* an important vector of East Coast fever to cattle, as part of a collaboration between ILRI and USDA. Both *B. microplus* and *R. appendiculatus* are members of the Metastriata, a tick lineage comprising 17 genera of ticks that vector many pathogens of veterinary significance (Ahmed and Mehlhorn, 1999; Kocan *et al*., 2002; McQuiston *et al*., 2003; Wagner *et al.,* 2002). *Ixodes scapularis* data will complement these proposed genomics efforts and permit valuable comparative studies between highly divergent ixodid tick species. The *Ixodes* project will be used in conjunction with molecular studies in other tick species (refer to Table 1) to significantly extend comparative and phylogenetic studies of the Ixodidae that will have an important bearing on disease transmission and epidemiology.

***Ixodes scapularis* Genome Project Bioinformatics**

Ixodes scapularis is one of five key invertebrate vectors proposed for the development of "***VectorBase***: A Bioinformatics Resource Center for Invertebrate Vectors of Human Pathogens". This is a contract proposal to the NIAID coordinated by Dr. F. Collins to develop a centralized relational bioinformatics database for invertebrate vectors of human disease. This proposal is considered to be within the competitive range for funding and is the subject of contract negotiations with NIAID. ***Ixobase***, a component of ***VectorBase***, will integrate *I. scapularis* sequence data and associated annotations and analyses. Dr. C. Hill and Dr. S. Wikel will act as the main contacts between the scientific community and ***IxoBase***. ***VectorBase*** and other public databases will allow the scientific community to use *Ixodes* sequence data rapidly and effectively.

US National Tick Collection

The US National Tick Collection maintained by the Institute of Arthropodology and Parasitology (IAP) at Georgia Southern University is the largest and most representative tick collection in the world. Duplicate specimens stored in ethyl alcohol may be available to the tick genome project for destructive sampling and analyses. These specimens together with the genome sequence, will promote and complement studies of tick morphology, molecular systematics, phylogeny and population biology (Klompen *et al*., 2000; Norris *et al.,* 1996).

Ixodid Tick Genomics

The extensive number of ESTs from various *I. scapularis* stages and tissues and from other ixodid tick species (refer to Table 1 for a summary) demonstrates the readiness of researchers to exploit genome data. These sequences provide invaluable functional data on ixodid ticks and are useful tools to annotate the *I. scapularis* genome. ESTs are also a valuable gene mining tool to identify novel biopharmaceuticals and antimicrobial peptides, targets for acaricide and vaccine development, acaricide resistance and vector competence genes.

Table 1. Type and Number of Ixodid Tick ESTs

Tick Species	Stage/Tissue	No. ESTs	Reference
Ixodes scapularis[1]	female SG blood fed 18-24 hr	2000	S. Wikel,
"	SG 3-4 day	2000	J. Ribeiro &
"	SG 16-24 hr	600	J. Valenzuela
"	adult unfed	900	"
"	nymph SG blood fed	900	"
"	nymph SG unfed	900	"
"	as above *Borrelia* infected	1000	"
"	cell lines	*	A. Azad
"	IDE8 cell lines	*	J. de la Fuente
Ixodes pacificus[2]	3-4 days SG	500	"
Ixodes ricinus[3]	various	112	multiple
Dermacentor andersoni[4]	female SG blood fed 18-24 hr	1,157	S. Wikel
Dermacentor variabilis[5]	SG	1000*	"
"	MG	1000‡	"
Amblyomma americanum[6]	adult	1,462	C. Hill
"	larva	480	"
"	normalized, fed, unfed	*	A. Barbour
"	adult female SG blood fed 50-300 mg	NA	A. Bior†
"	as above, normalized	NA	
"	adult female SG unfed, feeding, replete	NA	"
"	adult SG blood fed 3 day	NA	"
Amblyomma variegatum[7]	adult female SG blood fed 4 day	3, 499	V. Nene
Boophilus microplus[8]	larvae	234	A. Crampton†
"	normalized cDNA, mixed stages	20, 419	V. Nene
Rhipicephalus appendiculatus[9]	various	79	multiple
"	adult female SG blood fed 4 day	18, 422	V. Nene

1, Lyme disease/black-legged tick; 2, western black-legged tick; 3, sheep tick; 4, Rocky Mountain wood tick; 5, American dog tick; 6, lone star tick; 7, tropical bont tick; 8, southern cattle tick; 9, brown ear tick; * in progress; ‡ planned; MG, midgut; SG, salivary gland; ND, not determined; † Refer to reference section for citation.

VI. Suitability of *Ixodes scapularis* for a Genome Project

Ixodes scapularis is arguably the most important tick disease vector in the US and is one of the most extensively characterized tick species at the genetic level. These factors together with other considerations presented below, distinguish *I. scapularis* as the most suitable tick species for a genome project.

Life History Traits and Experimental Suitability of *Ixodes scapularis*

Numerous *I. scapularis* colonies have been established from wild collected material and are maintained by various labs throughout the US. *Ixodes scapularis* is a three-host tick; the larva, nymph and adult stages each feed on separate hosts. It is possible to maintain *I. scapularis* colonies by feeding the developmental stages on laboratory animals. Although the life-cycle of *I. scapularis* is relatively long in the wild, one generation can be produced in the lab within 9 to 12 months and colonies can be rapidly expanded to produce large numbers of larvae, nymphs and adults for experimental purposes and sequencing.

Estimate of *Ixodes scapularis* Genome Size, Complexity and Composition

The only analysis of genome size and complexity for an ixodid tick was performed in the lone star tick, *Amblyomma americanum* (Palmer *et al.*, 1994). Based on re-association kinetics, the *A. americanum* genome is estimated to be approximately 1.08 x 10^9 bp, comprising 36% unique DNA, 4% fold back sequences, 18% highly repetitive and 42% moderately repetitive sequences. A. Ullmann, W. Black, IV and colleagues are conducting C_ot analysis to determine the size and complexity of the *I. scapularis* genome. In addition, the tick community also has plans to estimate the size of the *I. scapularis* genome using nuclear staining techniques prior to sequencing of the genome through collaboration with Spencer Johnston at Texas A & M University. Preliminary studies indicate that the *I. scapularis* genome exhibits a long period interspersion typical of anopheline mosquitoes and *Drosophila*. Ongoing investigations suggest that the *I. scapularis* genome may be smaller than that of *A. americanum* (A. Ullmann and W. Black, IV, pers. comm.). If so, the *I. scapularis* genome may be comparable in size to that of *A. aegypti*.

***Ixodes scapularis* Cytogenetics and Genetic Mapping**

Cytogenetic studies have been undertaken for 103 of the approximately 830 known tick species (Oliver, 1977). *Ixodes scapularis* has 28 chromosomes (2n=28) with 26 autosomes and XX (female) and (XY) male sex chromosomes (Oliver *et al*., 1993; Chen *et al*.,1994). Karyotypes for various *I. scapularis* cell lines (IDE8, IDE12 and ISE18) have also been determined and shown to be similar (Chen *et al*., 1994). Microsatellite loci are of low abundance in *I. scapularis* (Fagerberg *et al*., 2001). Ullmann *et al.* (2002) produced a preliminary linkage map for *I. scapularis* based on segregation amongst 127 loci, including RAPD, STAR, cDNA and microsatellite markers. The researchers generated a linkage map of 616cM across 14 linkage groups, presumably corresponding to the 14 chromosomes, with one marker every 10.8cM. The relationship of physical to genetic distance was calculated as ~300kb/cM. This map provides a mechanism for mapped-based positional cloning of candidate genes for traits such as vector competence, host preference and acaricide resistance**.** Additional markers, including SNPs will identified using the genome sequence and will be used to expand mapping capabilities and as a basis for phylogenetic studies of ticks. The DNA from this mapping family has been amplified using Multiple Displacement Amplification (Gorrochotegui-Escalante and Black 2003) and is a permanent genetic resource available to the tick community for linkage mapping of new and/or problematic markers in a genome project.

Development of *Ixodes scapularis* Physical Mapping

I. scapularis does not yield usable polytene chromosomes. However, mitotic chromosome spreads have been produced from *I. scapularis* cell lines and male and female nymph immature gonad tissue (Chen *et al*.,1994; U. Munderloh, pers. comm.). Tick chromosomes range in size from 2-8µm and the sex chromosomes are often significantly larger than the autosomes. Chen *et al*., (1994) observed approximately 100 G-bands per haploid set in chromosomes from cells in metaphase with three to 18 G-bands in each chromosome arm. Fluorescent *in situ* hybridization (FISH) techniques already available for genetic studies in mosquitoes are applicable to *I. scapularis* chromosomes as a rapid method to generate physical maps (Brown and Knudson, 1997; Brown *et al*., 2001). FISH mapping offers a method to correlate genetic and physical maps and to map clones, genetic markers and BACs to chromosomes.

***Ixodes scapularis* Genetic Manipulation**

Several researchers have identified transposons (C. Hill, unpublished) and retro-transposons (P. Nuttall, pers. comm.) from tick EST sequences suggesting that genome sequence may enable the identification and development of tools for the genetic manipulation of ticks. Aljamali *et al*. (2002) and Narasimhan *et al.,* (2004) have demonstrated silencing of tick salivary gland transcripts using RNA interference (RNAi). RNAi offers a powerful functional genomics tool to investigate the many genes that will be identified by the genome project. These studies highlight opportunities to investigate the function of proteins associated with pathogen transmission or host avoidance and to silence undesirable tick genes for tick-borne disease control. Dr. T. Kurtti and Dr. U. Munderloh have developed a number of tick cell lines from *I. scapularis* embryonic tissue (Munderloh *et al*., 1994), several of which are available

through the ATCC. These cell lines represent a resource for high through-put functional studies of *Ixodes* genes.

VII. Goals, Strategy and Rationale for the *Ixodes scapularis* Genome Project

The long term goal of the tick genome effort is to generate a draft of the *I. scapularis* genome. We have explored sequencing strategies with representatives from TIGR and have consulted with Dr. F. Collins (*A. gambiae* genome project) and Dr. D. Severson (*A. aegypti* genome project) to develop the current *Ixodes* genome proposal. In an attempt to achieve a balance between fiscal considerations and the generation of sequence information that will be immediately useful to the scientific community, we propose a two-step genome project. Due to the paucity of tick cDNA and genome data, we propose a partial genome project based on extensive sequencing of normalized tick cDNAs, complete sequencing of selected large BACs and BAC-end sequencing (BES) in Phase I. Phase I will provide sequence data which the community can begin to use and will allow a preliminary analysis of the genome to guide Phase II in which we propose whole genome random shotgun sequencing (WGS) to draft coverage of possibly 4 to 6X. The draft genome is a desired end point when sequencing a new species as the physical organization and structure of the genome and genetic elements are revealed making it easier to study the genome and to carry out comparative genome studies. The direct products from this project include an index of expressed genes, a BAC based physical map of the tick genome, and several thousand annotated contigs describing genome sequence organization and coding capacity. Other benefits which will accrue from this sequence database have been described (Kirkness *et al*., 2003).

Phase I – Low Level Sequence Coverage of the *Ixodes scapularis* Genome:

a) *Ixodes scapularis* EST Sequencing

Proposed Research: The first activity we propose is the production and extensive sequencing of normalized *I. scapularis* cDNA libraries. Based on the interests and research priorities identified by the tick community, two normalized libraries composed of pooled tick tissues are proposed:

Library 1 - Pooled Ixodes scapularis Non-infected Library:

Composed of non blood fed and various blood fed and replete whole *I. scapularis*, *I. scapularis* life-cycle stages, adult salivary glands, midgut and ovaries and *I. scapularis* cell lines.

Library 2 – Pooled Ixodes scapularis Infected Library:

Composed of blood-fed *I. scapularis* and *I. scapularis* infected with *Borrelia burgdorferi*, *Babesia microti* and possibly *Anaplasma phagocytophilum*/*Ehrlichia chaffeensis* and Powassan virus/TBE.

Tissue samples will be collected from participating tick research labs and pooled for library production using a standardized protocol. cDNA libraries will be produced at Express Genomics (Frederick, Maryland). To ensure a high rate of gene discovery, Library 1 will be sequenced and analyzed by the MSC in batches of 10-20, 000 ESTs up to a maximum of 80, 000 ESTs. One round of subtraction will be carried out as needed to enrich for rarer cDNAs. Library 2 will be subtracted using abundant ESTs from Library 1 and sequenced up to a maximum of 20, 000 ESTs. The EST data will be used to construct a gene index of expressed *I. scapularis* genes. Dr. A. Azad and researchers associated with the Indiana Center for Insect Genomics plan to develop an *I. scapularis* micro-array for the community to conduct global expression studies.

Justification: Besides their utility in full length gene cloning, genome annotation and the development of micro-arrays, the ESTs will underpin ongoing proteomics studies, inform on the repertoire of expressed genes and gene families and permit evolutionary comparisons between invertebrate genes. ESTs will provide the community with information on the types of genes and gene families that are expressed during tick developmental stages and within tissues and will also enable the identification of genes involved in blood feeding, host finding and pathogen transmission. 3' EST data will be used by Dr. A. Azad and scientists from the Indiana Center for Insect Genomics to develop an *I. scapularis* micro-array as a community resource to determine tissue specific gene expression profiles and to study the vector-pathogen or vector-host interface.

b) *Ixodes scapularis* BAC-End Sequencing (BES) and Complete BAC Sequencing

Proposed Research: We will generate a 10X coverage genomic DNA BAC library (average insert size of approximately 120kb) from *I. scapularis* embryos. Genomic DNA extraction will be undertaken by Dr. C. Hill and BAC libraries will be produced at the Clemson University Genomics Center in collaboration with Dr. J. Tomkins. We propose the complete sequencing of two randomly chosen and two selected BAC clones. The latter will be selected in consultation with the tick research community. These BACs should represent euchromatic regions of the genome and contain genes of defined interest. Complete BAC sequencing and auto-annotation will be undertaken by the MSC with community scientists contributing to manual annotation of the sequence data. We propose extensive BES of approximately 80, 000 clones (160, 000 reads). The proportion of BAC end clones to be sequenced will be determined by the MSC. BAC sequences will be used for FISH mapping to assign scaffolds to chromosomes. *In situ* mitotic chromosome mapping will be carried out within the community. The community will seek funds to map the markers used in generating an *I. scapularis* linkage map to BAC clones and for FISH mapping to assign BAC clones, unique cDNAs and cDNA sequences of interest to mitotic chromosomes. Complete BAC and BES sequencing will be undertaken by the MSC.

Justification: Complete BAC sequences will provide preliminary but valuable information on genome organization, including repetitive sequences, gene structure and density. BACs can also be used to test the feasibility of random shotgun sequencing and genome assembly proposed in Phase II. Complete BACs and BAC-end sequences will provide sequence data for the community to begin analyzing the *Ixodes* genome. Genes identified from annotation of BACs will help train the gene prediction algorithms used in Phase II. In combination with random sequencing, BES will generate important architectural information on the genome and will enable the development of a preliminary physical map for *I. scapularis*. Paired BAC end sequence data are important as sequence-tagged connectors to assist in scaffold assembly and FISH mapping will allow assignment to chromosomes. Directed mapping of several hundred to one thousand clones may be needed to assign scaffolds to chromosomes.

Phase II – Draft *Ixodes scapularis* Genome

Research Plan: In phase II we propose whole genome sequencing of *I. scapularis* via random shotgun sequencing of plasmid genomic libraries up to a draft genome coverage of possibly 4-6X, but not full finishing. Random data will be assembled at regular intervals and 2-3X coverage should provide sufficient data to allow construction of a tiling path of the BAC clones from BAC-end sequence data to provide a BAC based map of the genome. The level of draft coverage should be determined as sequence data emerge from staged analysis of the tick sequence and as better estimates are revealed by the *A. aegypti* genome project. Assembly of contigs into scaffolds will be facilitated by BAC-end sequences. Physically and linkage mapped clones and linkage data will be useful in assembly. In this phase we also propose generation of 125 full insert cDNA sequences to provide data for training gene finding programs. We will select cDNA clones likely to be greater than 2 kbp in length as judged by the gene indexing carried out in Phase 1. Phase I sequence data will be used to trouble shoot technical problems in Phase II. The selected MSC will produce genomic libraries and will undertake the automated assembly and preliminary annotation of the draft *Ixodes* genome. The MSC will determine the appropriate ratio of medium (~10kb) and small (~4kb) insert clones to be sequenced. Costs associated with these activities will be governed by the MSC contract guidelines established with NIAID.

Justification: The whole genome shotgun sequencing approach is a robust method for rapidly acquiring large amounts of sequence data that can be combined with targeted sequencing to eventually produce a finished genome sequence. Random sequence will provide an insight into the size, complexity, level of polymorphism and repetitive content of the *I. scapularis* genome. We propose 4-6X sequence coverage as an intermediate stop point in a genome project. Based on the Lander-Waterman calculation, 5X genome coverage should produce contigs with an average length of 24kb (Lander and Waterman, 1988), the threshold for reliable contig annotation (S. Salzberg, pers. comm.). Gap closure may be performed by independently supported research groups with interests in specific regions of the genome. We recommend 125 cDNA sequences in order to produce a minimum of 250 kbp of cDNA sequence for adequate training of gene prediction algorithms.

Public Release of *Ixodes scapularis* Genome Data

Phase I and II sequence data and all subsequent versions of the genome will be approved by the **IISSC** and released to the public domain. We propose that raw, un-annotated genome data be made available to the scientific community through a dedicated MSC website such as the TIGR *Aedes aegypti* website. Trace and analyzed data should be released to an appropriate database at NCBI, to ***VectorBase*** and other public databases such as Ensembl. The tick community is considering the development of a dedicated stock center for the distribution of cDNA and BAC clones to researchers.

VIII. Colony Choice and Availability of DNA for the Project

Following extensive consultation with the tick community, the *I. scapularis* colony maintained by Dr. S. Wikel at the University of Connecticut Health Center has been selected as the most suitable source of material for sequencing. This colony was established in 1996 using field collected material from New York, Oklahoma and a LD endemic area of Connecticut. This colony has been continuously in-bred since establishment and has not been supplemented with field collected material. The colony is commonly used and has been well characterized. The colony is known to be a competent vector of various *Borrelia* and *Babesia* isolates and has been used to produce a variety of cDNA libraries and EST sequences that are the basis of a number of ongoing genomics studies (see Section V). Dr. Wikel's laboratory is able to supply sufficient material for sequencing and to meet community requests for the reference strain long term. We propose the establishment of satellite colonies at Old Dominion University (Dr. D. Sonenshine) and North Carolina State University (Dr. M. Roe) for long term maintenance and preservation of the reference strain. Dr. C. Hill has developed a method to obtain large amounts of high molecular weight genomic DNA for from ixodid ticks (Hill and Gutierrez, 2003). In anticipation of a genome effort, Dr. Hill is currently extracting high quality genomic DNA from *I. scapularis* embryos for production of BAC libraries. We do not propose the production of endosymbiont free *I. scapularis* for sequencing. Ixodid ticks are obligate hematophagous organisms; many studies suggest that *I. scapularis* is associated with endosymbionts that are thought to play a critical role in the lifecycle of this organism (Sonenshine, 1991). Sequencing of these organisms may help to unravel aspects of this dynamic relationship.

IX. Availability of Other Funding Sources

Dr. C. Hill has funding from Purdue University to produce *I. scapularis* BAC libraries and to undertake nuclear staining studies. Dr. F. Collins has funding from the Indiana Center for Insect Genomics to support BAC library production. Dr. S. Wikel has funding from the U.S. Army Medical Research and Materiel Command, NIH and CDC to support colony production and EST sequencing. In consultation with Dr. T. Kurtti and Dr. U. Munderloh, consortium members will explore *I. scapularis* FISH mapping techniques through independent funding sources. Dr. Collins is the lead PI on a contract proposal to develop ***VectorBase***. If funded, ***VectorBase*** will provide a database and bioinformatics resource tool for the *I. scapularis* project. We have initiated discussions with J. Rodgers, Director of Sequencing at the Sanger Institute in the United Kingdom regarding the participation of the Sanger Institute/Wellcome Trust in the *I. scapularis* genome project. Consortium scientists also plan to develop a collaborative proposal for submission to NHGRI and other agencies to obtain additional support for the genome effort. Possible sources of direct or indirect funding for this project are currently being investigated and may include DHS, DOD, DOE, WHO, The Michael Smith Genome Science Centre in Canada (R. Holt) and the Mathers Foundation (provided support for the *B. burgdorferi* genome).

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