

A Proposal to Construct a BAC Library from *Hydra magnipapillata*

Submitted by Robert E. Steele and Hans R. Bode
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The importance of the organism to biomedical or biological research.

Hydra has been the subject of experimental studies for over 200 years (Trembley, 1744). Its attractive features include (1) its ease of culture in the laboratory, (2) its simple structure, (3) a small number of cell types, (4) three cell lineages, each of which is a stem cell lineage, and (5) an extensive capacity for regeneration. Because of the tissue dynamics of an adult *Hydra*, the developmental processes governing pattern formation, morphogenesis, cell division, and cell differentiation are continuously active. Given its considerable capacity for regeneration as well as its being amenable to a variety of manipulations at the tissue and cell levels, most of the work up to the mid-1980s was focused on aspects of the developmental biology of *Hydra*. Primarily, these efforts involved gaining an understanding of formation and patterning of the single axis of the animal (Bode and Bode, 1984) as well as understanding the nature and control of cell division and differentiation of the stem cell lineages (Bode, 1996). With these aspects fairly well established, the emphasis shifted in the late 1980s to gaining an understanding of the molecular mechanisms underlying the elucidated developmental processes. Efforts were focused on looking for orthologues of genes affecting patterning and stem cell processes in bilaterians, which led to the isolation and characterization of >100 such genes. The variety of cell and tissue manipulations that had been developed proved, and are proving, valuable in sorting out the developmental roles of these genes with some precision. In addition, RNAi has been successfully applied as a tool in these efforts (Lohmann et al., 1999).

As studies of the evolution of developmental mechanisms, or "evo-devo", began to gain prominence, it rapidly became apparent that *Hydra* occupied a strategic place in these efforts. *Hydra* is a member of Cnidaria, an early-diverging metazoan phylum. Its phylogenetic location, coupled with the available experimental manipulations and the extensive understanding of the developmental biology of the animal indicate that *Hydra* plays and will continue to play an important role in defining the evolution of developmental mechanisms.

Uses to which the BAC library would be put, in addition to genomic sequencing.

The most pressing need for the library is to generate end-sequences to aid in assembly of the *Hydra magnipapillata* genome sequence. Other important uses would include the generation of reagents for mapping of DNA-protein interactions (Endl et al., 1999), and the isolation of promoter regions to support the continuing development of transgenic methods in *Hydra* (Bosch et al., 2002; Bottger et al., 2002; Miljkovic et al., 2002). (Thomsen et al., 2004). Also of interest will be experimental studies of regulatory element conservation as has been done with genes from pufferfish and mouse (Brenner et al., 2002; Kimura-Yoshida et al., 2004; Zhang et al., 2003) and *Drosophila* and vertebrates (Haerry and Gehring, 1996; Haerry and Gehring, 1997; Keegan et al., 1997; Malicki et al., 1992; McGinnis et al., 1990). To date such studies have not included comparisons between bilaterians and diploblasts. Such studies could include comparisons of the sea anemone *Nematostella* and *Hydra*. The BACs will also provide valuable reagents for studies of trans-splicing (Stover and Steele, 2001) and possible operon organization in *Hydra*.

The size of the research community that could potentially use the BAC library and the community's interest in and support for having a BAC library.

Hydra is of interest to three different research communities. First, there are about 30 labs worldwide that study *Hydra* exclusively or nearly exclusively. *Hydra* is also of interest to the larger cnidarian research community since it and *Nematostella* are the cnidarians for which genomics tools are the most advanced. Finally, *Hydra* is of growing interest to labs investigating the evolution of metazoans. End-sequencing of BACs to improve assembly of the *Hydra* genome and the availability of clones containing large segments of genomic DNA would be useful to all three of these communities. We have received a number of inquiries from researchers in other areas who are interested in studying *Hydra* genes. These include electrophysiologists interested in the evolution of ion channels and neurotransmitter receptors (e.g. the acetylcholine receptor), molecular biologists interested in the evolution of transcription factors (e.g. myc), pathologists interested in antimicrobial peptides, and developmental biologists interested in the evolution of signal transduction pathways (e.g. the notch pathway).

Strain of the organism proposed and the rationale for its selection.

The library would be made from *Hydra magnipapillata* strain 105. This is the strain that is being used for the *Hydra* EST Project and the *Hydra* Genome Project. This is also the strain that was used for the *Hydra* Peptide Project (Bosch and Fujisawa, 2001). This strain has been in continuous culture for about 30 years and is one of the most widely used *Hydra* strains.

Whether the organism will be, or has been, proposed to NHGRI or another publicly funded agency for BAC-based genomic sequencing and the status of that request.

This organism has not been proposed to NHGRI or other publicly funded agencies for BAC-based genomic sequencing. The NHGRI-funded sequencing project will use the whole genome shotgun approach (see below).

Other genomic resources that are available that will complement this resource.

The NSF-funded *Hydra* EST Project (www.hydrabase.org) has to date generated 97,230 sequences. Another 19,611 sequences have been generated by a smaller EST project carried out at the National Institute of Genetics in Japan. When the *Hydra* EST Project is completed, we expect to have about 300,000 sequences. The sequencing is being done by the Genome Sequencing Center at Washington University, and the sequences are rapidly deposited in the public databases. When the Project is complete we will generate a unigene clone set. We are in the process of planning with Pieter de Jong for the deposition of this clone set at CHORI. Individual clones or replicas of the entire clone set will be readily available to interested researchers at cost. NCBI has generated a UniGene dataset for *Hydra magnipapillata*, which is now at Build #6. By the end of 2005, a draft sequence of the *Hydra magnipapillata* genome should be completed. The sequence will be available in the public databases. Within the next year we plan to submit a proposal to NSF and/or NIH for funding to support the construction and maintenance of a *Hydra* genome database.

The size of the genome.

The genome size for *H. magnipapillata* strain 105 has recently been determined by Feulgen staining and microdensitometry in the laboratory of Thomas Bosch at the University of Kiel (T.

Bosch, personal communication). The haploid genome size is 1.296×10^9 base pairs. All species of *Hydra* have 30 chromosomes in the diploid set (Zacharias et al., 2004).

The availability of a source of DNA for construction of the BAC library (evidence of its quality for this purpose).

Hydra DNA is available in unlimited amounts from one “individual” since the animal reproduces asexually by budding. The *Hydra* polyp is naked, consists of two epithelial layers, and is cultured in a simple medium (essentially synthetic pond water). We foresee no problems in obtaining sufficient quantities of high molecular weight DNA. There were no problems getting DNA of sufficient quality from the *Nematostella* polyp, and we don't expect the *Hydra* polyp to behave differently. Another indication that DNA of the appropriate size can be isolated from *Hydra* is the work showing that pulsed-field gels can be done successfully with *Hydra* polyps (Gauchat et al., 2000). *Hydra* is not cultured axenically, so there are bacteria associated with the polyp. The level of bacteria can be reduced considerably by treating the cultures with antibiotics for several days prior to DNA isolation.

Specifications for the library (e.g., library depth, BAC insert size) and supporting scientific rationale for these specifications. (Note: any request for an unusual vector for a particular application must be thoroughly discussed.)

We anticipate making a library with 10x genomic coverage and an average insert size of 150 kb. For *Hydra magnipapillata* this will require the generation of 86,400 clones. The library will be made using standard methods (Osoegawa and de Jong, 2004). These include digestion with EcoRI in the presence of EcoRI methylase and cloning into the pTARBAC2.1 vector. The mitochondrial genome of *Hydra* consists of two 8 kb linear molecules (Warrior, 1987). Thus, incorporation of mitochondrial DNA into the library will not be an issue. Of some concern is the base composition of the *Hydra* genome, which is 71% A+T. BACs produced from A+T-rich genomes have shown stability problems (Pieter de Jong, personal communication). The two cases that have been most extensively tested are *Dictyostelium discoideum* (77% A+T) and *Plasmodium falciparum* (82% A+T). Given that the Bosch lab has had what appears to be success in making a small BAC library from *Hydra magnipapillata* (see below), it appears that *Hydra* DNA is not sufficiently A+T-rich to cause BAC instability problems.

Average insert size will be determined by pulsed-field gel electrophoresis on a minimum of 100 clones. The results from this analysis will allow reporting of the number of clones that have no inserts (empty clones). The number of empty wells will be determined by visual inspection of the plates produced by arraying of the library. The library will be considered acceptable if the number of empty clones and empty wells together is <5% of the total. Depth of coverage will be determined in two ways. First, coverage will be calculated from the average insert size and the number of clones produced. Second, filters from the arrayed library will be generated and hybridized with 30 overgo probes generated from *Hydra* EST Project sequences. Except for cases in which probes overlap an intron/exon boundary, we would expect all probes to hybridize to multiple clones. The overgo probes will be designed from genes which the *Hydra* EST Project has sequenced a sufficient number of times that the consensus sequence will correct for sequencing and reverse transcription errors.

The time frame in which the library is needed.

One of the important uses of the library is to generate end-sequences for aiding the assembly

of the genome sequence. Thus it would be ideal to have the library available before the end of 2005. For the construction of the *Nematostella* BAC library, the first test plugs of DNA were shipped to the de Jong lab in mid-March of 2003. The library was listed as available on the CHORI web site in December, 2003. If no unforeseen problems arise, production of the *Hydra* BAC library should take a similar amount of time.

Other support that is available or has been requested for the construction of the desired library.

No other support is available or has been requested.

The need for an additional BAC library if one or more already exists.

The Bosch lab has produced a small BAC library from *Hydra magnipapillata*. This library has only 2x coverage, extensive quality control analyses have not yet been done on this library, and there are currently no plans in place for archiving and distribution. We believe that it is preferable for the research community to have a library made by a lab which has extensive experience in BAC library construction, quality control testing of the libraries, and the resources for archiving and distributing the libraries.

Any other relevant information.

Attached to the proposal are letters of support for the project from prominent members of the cnidarian research community, from Chris Amemiya, PI of one of the BAC Library Production Centers, and from Karin Remington, the PI for the *Hydra* Genome Project.

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J. Craig Venter

I N S T I T U T E

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September 20, 2004

Re: Proposal to construct a BAC library from *Hydra magnipapillata*

Dear Dr. Steele,

As principal investigator for the NHGRI's *Hydra magnipapillata* sequencing project, I am extremely enthusiastic about your proposal to construct a BAC library for this organism. As you are well aware, and note in your proposal, high-quality BAC-end sequences are a crucial input for large-scale whole-genome shotgun assembly projects. This is especially the case where there is no closely related reference sequence nor significant density of reliable mapping information, as is the situation for the Hydra project. While our planned strategy of short and medium insert libraries and a 50kb deletion library will provide the bulk of the sequence data for the target 8x draft genome, it is the clone coverage provided by a well-constructed BAC library that assures long-range contiguity of the assembled fragments. With end sequences from such a BAC library, it is my expectation that scaffolds comprising nearly complete chromosome arms will be possible. Given the AT-richness of this organism, such long range information may help span long heterochromatic stretches and provide order and orientation where otherwise more labor intensive strategies would be required for resolution. Even after the sequencing project is complete, such a BAC library, if maintained, provides a key community resource, greatly facilitating regional finishing in areas of particular interest where the draft sequence does not provide sufficient resolution.

The Venter Institute has genetic material for the *Hydra magnipapillata* presently in house, and will begin random shotgun library construction and sequencing this fall. Our budget for this project is split over the coming two years, and we anticipate completing the random sequencing toward the end of calendar 2005. At that time, availability of a high-quality BAC library for end-sequencing will be of extremely high value to the project. This timing appears to be a very good fit with your proposal. I greatly look forward to participating actively with your group, and am confident this will be a very productive and exciting collaboration.

Sincerely,



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30 August 2004

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Dear Rob and Hans,

I am pleased that the NHGRI has decided to sequence the genome of *Hydra magnipapillata*. The sequence will be a big boon to investigators interested in many aspects of its biology. These include stem cell biology, regeneration, developmental genetics, and the evolutionary origins of bilateria, all areas of interest to a broad swath of biologists and with obvious ties to human health. Moreover, having the *Hydra* sequence will allow direct comparisons with that of the anthozoan, *Nematostella vectensis*, which is being sequenced by the JGI. These comparisons are likely to reveal exciting findings of general biological interest.

I write in strong support of the white paper that you are submitting for the generation of a communal BAC library for *Hydra magnipapillata*. This library will be useful for assembling of the genomic shotgun sequences and, equally important, for providing reagents for isolating, characterizing and empirically testing regulatory elements and as templates for transgenic analysis.

My laboratory previously was able to generate 100+ kb BAC clones from a related hydra, *Hydractinia symbiolongicarpus*, however, we were not successful in generating an entire library due to low cloning efficiency. *Hydractinia* apparently also has a high A-T content like *Hydra*, however, this high A-T content may or may not have a bearing on the efficacy of BAC cloning. I am not convinced our previous attempts weren't flawed because of the poor quality of high molecular weight DNA that we were given. With the greatly improved methods for both preparation of high molecular weight DNA and BAC cloning, I think the chances of success are good. The small pilot BAC library that you discussed in the proposal certainly is supportive of this.

Yours sincerely,



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October 7, 2004

Dear Rob and Hans:

I have read with great enthusiasm a draft of your white paper requesting support for a *Hydra magipapillata* BAC library. Such a resource would be a tremendous boon to researchers in the fields of gene regulation, animal regeneration, evolutionary developmental biology and evolutionary genomics. In recent years, there has been a veritable explosion of papers using molecular and genomic approaches to study cnidarian development, and these papers are providing important insights into the ancestral mechanisms of animal development. A wider and wider community has come to realize the merits of studying these "basal animals" to illuminate fundamental properties of animals. Our own work on the sea anemone *Nematostella* falls into this category, and we would benefit if more comparative genomic data were made available for *Hydra*. In the coming years, I expect that comparative genomics work on *Hydra* and *Nematostella* will move forward hand-in-hand, progressing much more rapidly because of the natural complementarity of these two important model systems.

Best wishes in getting this important proposal funded,

John R. Finnerty

Department of Biology
Program in Bioinformatics

Dr. Rob Steele

Sept. 24, 2004

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Dear Dr. Steele,

It is a great news that you and Dr. Bode are preparing a white paper to submit to NIH for construction of a BAC library from *Hydra magnipapillata*.

We have found that some of the subpopulations of *Hydra* nerve cells form compartments along the body axis. We are trying to identify what kind of transcription factors would be involved in regulating region specific expression of these neuronal genes. For that purpose we need 5' upstream regions of these genes. Sequence comparison of these regions would narrow the possible motifs that the factors recognize. Having the sequence information of 5' upstream regions in the form of BAC library, it would be a great help for us. Although the neuronal genes are immediate targets, we also would like to see the promoter regions or even enhancers of other genes that we are working or will be working. I strongly hope and endorse that the above-mentioned proposal would be granted so that we can enjoy the rich information of Hydra genomic sequences.

Best wishes

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Geneva, September 25th 2004

OBJECTIF: construction of a BAC library from *Hydra magnipapillata*

As a scientist investigating the regulation of developmental genes in the hydra model system for the last 15 years, I consider that making available a sequenced BAC library from hydra would be a definitive asset for our field and more precisely to support the strategies elaborated in my laboratory. Indeed during the last years, we learnt that genes encoding developmental proteins are incredibly conserved from cnidarians (the phylum to which hydra belongs to) to vertebrates. However very little is known concerning the evolutionary conservation of the regulation of these genes during evolution: what sequences are absolutely required to give a stem cell fate? what sequences are important to give a neuronal cell fate to a stem cell? What are the trans-acting factors involved in these regulations? All these questions and many others imply that we have a precise knowledge of the genomic sequences surrounding coding sequences.

For this reason I consider the project of Dr. Robert Steele and Hans Bode as extremely important not only for researchers using cnidarian model systems but obviously also for those active in the field of developmental biology, cell biology, evolution and regenerative medicine. I strongly support this proposal and look forward to using in a close future the resulting data.

Dr. Brigitte Galliot
Principal Investigator



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29-Sep-04

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Supporting letter for a proposal to construct a BAC library from *Hydra magnipapillata*

This is a letter to strongly support the efforts associated with the *Hydra* genome project. There are a number of reasons, why sequencing the *Hydra* genome is indispensable for biological and biomedical research.

- (i) *Hydra* is famous for its regeneration capacity. It is a simple animal with an unlimited regeneration capacity. Unraveling the mechanisms of regeneration in *Hydra* will have an impact in our understanding of regeneration and why regeneration may have been lost in higher metazoans. There are no other organisms having a comparable regeneration capacity.
- (ii) There is an absolute need for the library to generate reagents for mapping DNA-protein interactions, for the isolation of promoter regions, and to study the conservation of regulatory elements cnidarians and vertebrates. These studies will give us important clues why the regeneration capacity may have been lost during evolution.
- (iii) Choosing *Hydra magnipapillata* strain 105 is an ideal choice, since this strain has been used for many major projects, i.e. the peptide project and the EST project, and many molecular and genetic tools, as well mutants are for this strain available, worldwide.

Our lab has been working on *Hydra* for a number of years. On the genomic level, the Darmstadt-lab (now in Heidelberg) contributed to the NSF-funded *Hydra* EST project (www.hydrabase.org) by providing the normalized regeneration-specific cDNA library, roughly 1/4 of the >80,000 sequences are from this library. Our lab also performed an EST project on *Nematostella* embryos, and we provided the genomic DNA for the BAC library, which has a 10X genomic coverage and an average insert size of ~150 kB; for the ongoing *Nematostella* genome project.

Thus, the planned *Hydra* BAC library is indispensable for our future work, and I heartily hope that it is granted.

Sincerely,

A handwritten signature in black ink, appearing to read 'Thomas W. Holstein'.

(Thomas W. Holstein)

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September 18, 2004

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Dear Rob and Hans,

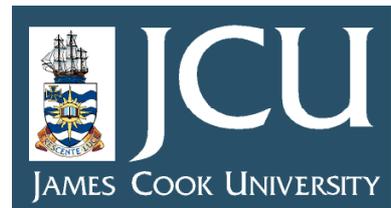
Thank you for showing me your “white paper” proposal to NIH to prepare a BAC library to *Hydra magnipapillata*. I am writing in complete support of this important project and hope that you will include this letter in your final submission. As you clearly outline in your proposal, there is over two hundred years of experimental knowledge about the biology of this model cnidarian organism. It is by far the most heavily studied cnidarian around the world to this day and represents an important model system for regeneration and stem cell research in basal metazoans. The pivotal position of cnidarians possessing common ancestry with both deuterostome (including vertebrates) and protostome animals provides a great deal of insight in to the molecular evolution of patterning systems in virtually all other animals on Earth.

The fact that both NIH and NSF have made substantial investments in this model system, most recently by funding total genome sequencing and EST screens, respectively, indicates the importance of this BAC library project. This BAC library is the last piece of the puzzle. Not only will it facilitate the assembly of the entire sequenced genome, but it will also jumpstart the field of comparative genomics. I know that my lab will use this information to compare synteny and regulatory regions for developmental genes to the cnidarian that we work on, the anthozoan *Nematostella vectensis*.

The combined experience of your two labs make you the most appropriate place to generate this important reagent for the scientific community. Not only is this obviously important for cnidarian biologists, but every molecular biologist interested in understanding the evolutionary history of genes and gene families will utilize this resource. Good luck on this endeavor, and please let me know if there is anything else I can do to facilitate this project. I know I will be anxious to search the database when the information becomes publicly available.

Sincerely,

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8th October 2004

Dear Sir

I am writing to indicate my strong support for the proposal from Rob Steele and Hans Bode for construction of a BAC library from *Hydra magnipapillata*.

Hydra is the textbook cnidarian and, given the over 200 years of experimental work on this animal, the extensive literature on its cell biology and extent of its representation in the molecular databases, it is certainly the best understood cnidarian at the cellular and molecular levels. The Irvine group has coordinated a massive EST collection, and *H. magnipapillata* will be the second cnidarian whose genome will be completely sequenced, again largely due to the efforts of the Irvine group which Bode and Steele lead. These are (or will be) invaluable resources for the ever-expanding 'evo-devo' community. Their utility would, however, be increased substantially by the availability of a BAC library – this would greatly facilitate the assembly of the genomic sequence, and provide reagents for transgenesis and a wide range of other applications. It is therefore extremely important that appropriate support be available to the Irvine group to enable the generation of BAC libraries.

My sincere best wishes with your proposal, and if I can be of any further support, please do not hesitate to contact me.

Sincerely

Dr David J Miller
Group Leader, Coral Genomics Group
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To whom it may concern

This is a letter to strongly support the proposal of a BAC library for *Hydra magnipapillata*. *Hydra* is by far the best studied model organism among the cnidarians, which represent the crucial outgroup of comparison for the Bilateria. Many labs in the world that work on *Hydra* will benefit directly from such a resource, together with the ongoing EST screens and the planned genome project. However, since researchers of other fields become increasingly interested in the evolutionary roots of developmental mechanisms, gene families, genetic cascades etc. this will provide an important tool and resource for many labs outside the *Hydra* field.

To my knowledge, from other Cnidaria BAC libraries are only available from the anthozoan *Nematostella vectensis*, which is also being sequenced to date. Being involved in this project and also working on *Hydra* for the last 10 years I clearly see the great advantage of having the genomes and the genomic resources such as the BAC library from both organisms available. *Hydra* and *Nematostella* represent two distantly related classes of cnidarians, the anthozoans and the hydrozoans. The comparison of both will allow to infer the ancestral cnidarian gene set and from there that of the common ancestor of both Bilateria and Cnidaria, some 700 myr ago.

Rob Steele and Hans Bode are highly renowned scientists within and beyond the *Hydra* community. They also are guiding successfully the large *Hydra* EST screen as well as the *Hydra* genome project. Rob Steele was also the driving force in the *Nematostella* BAC library proposal which was funded in 2003 by NSF. The BAC library is an indispensable tool for the assembly of the genome sequence as well as for the identification and analysis of specific genes and regulatory regions. It is a must!

Uli Technau
Group leader

X-From_: tbosch@zoologie.uni-kiel.de Tue Sep 7 05:12:46 2004
From: "Thomas C.G. Bosch" <tbosch@zoologie.uni-kiel.de>
To: <resteele@uci.edu>
Cc: "Hans Bode" <hrbode@uci.edu>
Subject: AW: BAC libraries
Date: Tue, 7 Sep 2004 14:09:22 +0200
X-Priority: 3 (Normal)
Importance: Normal
X-Virus-Scanned: by AMaViS 0.3.12 (Uni-Kiel/tms)
X-UCIRVINE-MailScanner: No viruses found
X-NACS_ES-MailScanner: No viruses found

Dear Rob

I learned from Hans that you are preparing a NIH proposal for making a large BAC library from Hydra magnipapillata in Pieter de Jong's lab.

I support that proposal in the strongest possible terms.

The library will represent a new and important resource for physical mapping and sequencing and will help to bridge the gap between higher order genome maps and the need to perform detailed analysis of specific clones. Since using the library will be done in a comparative context, it will contribute to reconstruct the evolutionary history of metazoan genomes.

In my lab one primary research focus is to identify and analyze regulatory sequences involved in spatial and temporal control of gene expression in Hydra. To facilitate isolation of specific genomic sequences and for localizing and characterizing Hydra genes or gene families, we just have produced a BAC library from Hydra magnipapillata which (based on a haploid genome size of Hydra magnipapillata of 1290 Mbp) represents about 5 genome equivalents. This library was used for identification and analysis of the genomic structure of members of the ks1 gene family which previously was shown to play a crucial role in head formation in Hydra. We are just writing up the corresponding paper and I have attached below the Material & Methods part describing the experimental details used for constructing this library. If you need more information, please contact me.

Sincerely yours

Thomas

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