Additional BAC Coverage of Male C57BL/6J Mouse: A Request for BAC Library Construction Through the BAC Resource Network

Submitted to the NHGRI, summer 2002

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Overview. This is a request for an additional male C57BL/6J mouse library providing 10X autosomal coverage. Combined with the existing RPCI-24 library, the requested library will provide sufficient coverage of the mouse Y chromosome so that BACs for sequencing can be selected to avoid unnecessarily large overlaps and so that the Y-chromosome BAC map will have few gaps. Large overlaps are costly to sequence because even though the overlap does not have to be finished in two clones, full depth of subclone shotgun reads must be generated for both overlapping clones.

In the remainder of this request we address the issues enumerated in the NHGRI's "Instructions for Proposing Organisms From Which to Make New BAC Libraries."

1. The importance of the organism to biomedical or biological research. The importance of the laboratory mouse for biomedical research is well established, and the NHGRI's goal is to produce a finished sequence for the C57BL/6J mouse strain in 2005.

2. Uses to which the BAC library would be put, in addition to genomic sequencing. In addition to genomic sequencing, the clones in this library (accompanied with high-density hybridization filters) would provide a source of mouse clones for knockout and transgenic experiments.

3. 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. The BAC library would be of use to hundreds of mammalian genetics laboratories, which could use clones from the library in their genetic experiments. In addition, by enabling cost-effective clone selection and gap closure for sequencing the mouse Y chromosome, this library will ultimately be of great value to those studying genes on the mouse Y chromosome. The additional male C57BL/6J BAC coverage will support a R01 competing renewal that we are submitting. This proposal aims to provide a sequence ready map of the mouse Y chromosome, and with clones sequenced at the Washington University Genome sequencing Center in St. Louis ("WashU"), the finished sequence of the mouse Y chromosome. We have attached letters from nine researchers who are interested in the mouse Y chromosome and who have agreed to act as consultants on the proposed project.

4. 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. Finished sequence of the C57BL/6J mouse by 2005 is a goal of the NHGRI. The requested library will enable cost-effective clone selection and gap closure for sequencing the 3% of the haploid mouse genome represented by the Y chromosome.

5. Other genomic resources that are available that will complement this resource. The value of the requested library will be increased by the existing fingerprint maps and BAC end sequences available from other C56BL/6J libraries, since they will permit the design of overgos and STSs to be used in identifying and characterizing clones in the requested library.

6. The strain of the organism proposed and rationale for its selection. The mouse strain requested is C56BL/6J. This is the strain selected for the reference sequence of the laboratory mouse, and the requested library will complement the existing C57BL/6J libraries RPCI-24 (male) and RPCI-24 (female).

7. The size of the genome. ~3 Gb.

8. The availability of a source of DNA for construction of the BAC library. C57BL/6J mice from which DNA can be extracted are readily available. Ideally DNA that was used to construct the RPCI-24 library could be used for this purpose. Otherwise a mouse that is closely related patrilineally to the RPCI-24 animal should be used.

9. Specifications for the library (e.g., library depth, BAC insert size) and supporting scientific rationale for these specifications. 10X autosomal coverage in the requested library would provide an additional 5X BAC coverage of the mouse Y chromosome. Taken together, the existing RPCI-24 library and the requested library would provide a total of 10X coverage of the mouse Y chromosome. Insert size of 150 to 160 kb, as for the RPCI-24 library, will be suitable. Availability of high-density hybridization filters will be essential to identify BACs in the library. To provide better probability of filling clone gaps in the RPCI-24 library, an enzyme other than *Mbo*I (the one used in constructing the RPCI-24 library) or a non-enzymatic fragmentation technique should be used.

10. The time frame in which the library is needed. April 2003, the activation date for the proposed project to map and sequence the mouse Y chromosome.

11. 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 for construction of an additional BAC library representing the genome of a male C57BL/6J mouse.

12. The need for an additional BAC library if one or more already exists. The existing RPCI-24 library represents the mouse C57BL/6J Y chromosome at 5.4X coverage, and no other libraries from male C57BL/6J are available. Our experience mapping and (with WashU) sequencing the human Y chromosome indicates that at this low level of coverage there will be an unacceptably high number of gaps (~100) in the BAC map of the 95-Mb mouse Y chromosome. We anticipate that many of these gaps will be spanned by BACs in the requested library.

In addition, at only 5.4X coverage we would be forced to select for sequencing many clones with large overlaps, simply because there will be few BAC choices in many areas. This would increase the cost of sequencing the mouse Y chromosome. Even though such redundant overlaps will not have to be finished in both overlapping BACs, in many cases both BACs will have to be fully shotgun sequenced at substantial expense.

13. Other relevant information. None.