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Whole Genome Shotgun Library Approach For Microbial Sequencing Projects at the JGI

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The US Department of Energy's Joint Genome Institute is a high-throughput sequencing center and user facility that has sequenced a large number of microbial genomes. The strategy for most projects calls for construction of whole genome shotgun libraries from high-molecular weight DNA isolated from an axenic culture. In general, the JGI produces 3 insert size-selected libraries for all whole genome shotgun projects. We generate a 3kb high-copy pUC18 library, an 8kb low-copy pMCL200 library, and a 40kb pCC1FOS fosmid library. The DNA is randomly sheared, fragments are end-repaired for blunt-end cloning, and then size selected on an agarose gel, extracted and purified. 3 & 8kb inserts are cloned into the appropriate vector and transformed into *E. coli*. 40kb inserts are cloned, packaged and infected by phage into *E. coli*. PCR using primers flanking the inserts are used to determine the percentage of clones with inserts for both the 3 and 8kb libraries, before proceeding to production sequencing. Clones (10-384-well plates) from each of the 3 & 8kb libraries are initially sequenced and library quality is assessed at this stage before full sequencing is completed. Both 3 & 8kb libraries are sequenced to 4x sequencing coverage and the 40kb library is sequenced to 30x clone coverage. The 3 library approach generally results in more complete genome coverage at the draft stage, and pairing information allows for contig order and orientation and repeat resolution in the sequence. Finishing using standard methods is also facilitated by this approach.

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