

## **Cloning and Sequencing with Trace Amount of DNA on Roche/454 and Illumina Platforms**

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As a user facility, The US Department of Energy's Joint Genome Institute, in collaboration with scientists around the world, are able to generate DNA sequences from a diversity of organisms and environmental communities. Often times, the amount of genomic DNA provided for library construction is very limited. It is imperative to develop a protocol to minimize the amount of genomic DNA required for library construction for the second-generation of sequencing platforms. We have begun constructing Roche/454 Shotgun and Illumina Paired End libraries with less than 1ug of genomic DNA by altering two key components from the standard operating protocol for library construction. The two key components that help minimize loss of genomic DNA are: shearing DNA via Covaris Adaptive Focused Acoustics™ (AFA) process instead of nebulization and utilizing Agencourt® AMPure® purification system to purify and select the size range of DNA fragments from contaminants and enzymes, with minimal loss of sample. This approach enables us to create Roche/454 libraries with as little as 300ng of genomic DNA and Illumina libraries with only 1ng of genomic DNA as the starting material.