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Next-generation sequencing library preparation using the Bioruptor.

Shearing of both gDNA and dscDNA

Author: Dr. Brian D. Gregory
Damon Runyon Postdoctoral Fellow, Ecker Laboratory, Plant Biology Laboratory
The Salk Institute for Biological Studies, 10010 North Torrey Pines Road LaJolla, CA 92037

Shearing of DNA molecules is often required to obtain properly sized substrates for production of genome-wide libraries for sequencing by next-generation high-throughput deep-sequencing technologies. Using the Bioruptor, we have demonstrated that appropriately sized DNA molecules can be obtained in more than sufficient quantities for production of a number of different libraries for sequencing by next-generation sequencing technologies.

The recent advances in sequencing technologies have ushered in an era where analysis of a whole genome's worth of sequence can routinely be obtained in a single experiment. These sequencing technologies can be employed for a myriad of purposes including empirical annotation of the transcriptome, chromatin immunoprecipitation-sequencing (ChIP-seq) studies, analysis of alternative RNA splicing, characterization of the methylation state of cytosine bases throughout a genome (methylome), and DNA polymorphism discovery. However, in order to make the library of DNA molecules that will be sequenced using one of the next-generation sequencing technologies the large genomic DNA (gDNA) or double-stranded complementary DNA (dscDNA) molecules must be sheared to obtain DNA molecules which are much smaller in size (~200-600 nucleotides (nts) on average). Here, we describe a protocol that employs the Diagenode Bioruptor for shearing of larger DNA molecules from Arabidopsis thaliana into properly sized molecules that can then be used directly to make libraries for sequencing on the researchers next-generation sequencing technology of choice. In fact, this protocol (as outlined below) can be used for shearing both gDNA and dscDNA into properly sized molecules that are then directly incorporated into library production protocols for sequencing by next-generation sequencing technologies. Furthermore, upon completion of genomic DNA shearing by the Bioruptor, the now properly sized DNA molecules are not too damaged to introduce bias into the corresponding sequencing libraries which they are used to create (1, 2). Overall, this Bioruptor specific protocol, with minor modulation, can be adapted to shear gDNA and dscDNA from any organism into properly sized DNA molecules for next-generation sequencing library construction.

Bioruptor Cycle for shearing Arabidopsis gDNA or dscDNA (as seen for library construction in 1 and 2):

1) Sample (gDNA or dscDNA) is placed into Bioruptor making sure that all of the DNA-containing liquid is below surface of water in Bioruptor resevoir.
2) 30 seconds on, 2 minutes off for 5 treatments consecutively (10 minutes 30 seconds total).
3) Upon completion of cycle sample is centrifuged to collect all liquid to bottom of the sample tube, and then replaced into Bioruptor making sure that all of the DNA-containing liquid is below surface of water in Bioruptor resevoir.
4) This procedure is repeated 2 more times for a total of 3 cycles.

Bioruptor UCD-200

This cycle can be modulated to obtain properly sized DNA molecules for next-generation sequencing library preparation using DNA from all organisms as starting material.

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Workflow for next-generation sequencing library preparation following preparation of proper-sized DNA molecules using the Bioruptor:

1) Shear gDNA or dscDNA into appropriately sized DNA molecules for next-generation sequencing library preparation (~200-600 nts)
2) Use population of sheared DNA molecules obtained from Bioruptor protocol in library preparation protocol as per manufacturer's instructions
3) Sequence library on next-generation sequencing instrument to depth as desired.

References

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Dr. Brian D. Gregory
Damon Runyon Postdoctoral Fellow
Ecker Laboratory
Plant Biology Laboratory
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