

Introduction:

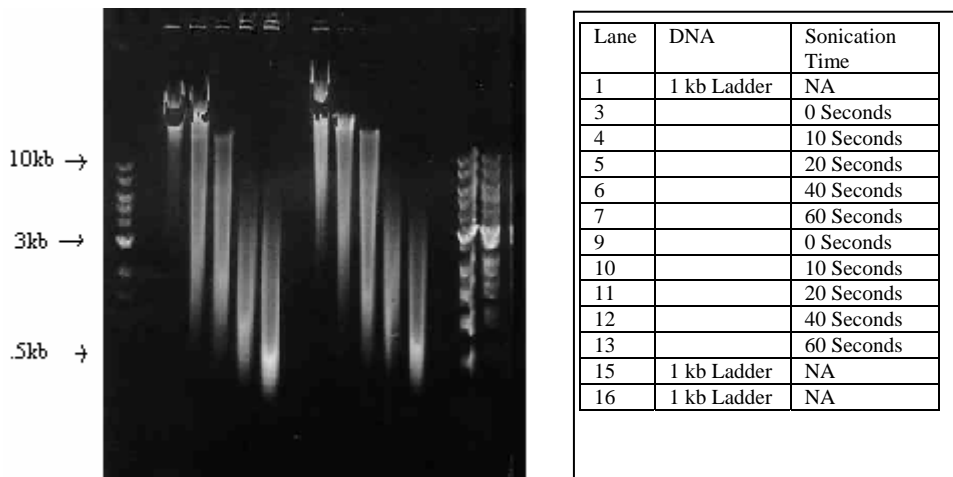
Small-insert random DNA fragments are necessary to create efficient libraries for genomic sequencing projects. The success and efficiency of sequencing a large genome is dependent on the randomness of the fragments generated by the shearing of target DNA. Physical shearing methods (i.e., sonication, nebulization, and hydrodynamic shearing^{1,2,3}) are preferentially chosen over enzymatic digestion due to the randomness and size of the fragments produced resulting in a suitable overlapping collection of fragments for sub-cloning. The SonicManTM offers these properties in a high-throughput sonication format allowing for a straightforward, user-friendly, customizable method to generate random DNA fragments.

Benefits of the SonicManTM

- Fragment size is correlated with sonication settings (power/time) and is controllable by the user. Fragments centered around a desired length can be repeatedly generated in seconds.
- Samples may be sonicated in volumes ranging from 25 μ L to 1.5mL per well and is scalable to 96 well format and 384 well formats.
- Unlike enzyme based digestion, the random fragments generated by sonication are suitable for the sequencing of large genomes and sonication procedures allow for an uncomplicated, quick process as opposed to techniques like hydrodynamic shearing.
- No specialized reagents are needed minimizing solution variation and allowing for easy carryover to downstream applications.

Data:

Figure 1A



Bacterial DNA was thawed and a 70 μ L aliquot was transferred to a well in a 384 plate (MGB101-1-1, MatriCal, Inc. Spokane, Wa). The plate was sonicated in a SonicMan using a 384 disposable pinlid (SLO-384 p11, MatriCal, Inc. Spokane, Wa) at 100% Power for times of 10, 20, 40, and 60 seconds.

After each sonication a 5 μ L aliquot was loaded into a Gel. The .7 agarose gels ran at 160 V for 70 min. Lane 1, 11, and 12 are 1kb DNA markers (NEB, Ipswich, MA),

Results:

As shown in figure one, genomic DNA may be sheared to fragments with the distribution of sizes centered around the users preferred size. DNA may be sheared to generate fragments with a distribution of sizes centered around 10kb (lanes 4) or sheared to smaller fragments (4kb: lane 10, 3kb: lane 4, 2kb: lane 5). Fragments can be repeatedly produced with minimal production of unwanted 'small fragments' (<300bp), as in lanes 4 & 10 of figure 1A, which can lower transformation efficiency. A simple configuration step is all that is required by a new user or with new DNA to correlate ideal settings to ideal fragment length.

References:

- 1.) Deininger PL 1983. Anal. Biochem. 129: 216-223.
- 2.) Bodenteich AS, Chisoe S, Wang Y-F, and Roe BA 1994. In Automated DNA sequencing and analysis techniques (ed. MD Adams, C Fields, and C Venter), pp.42-50. Academic Press, London, UK.
- 3.) Thorstenson YR, Hunicke-Smith SP, Oefner PJ, Davis RW 1998. Genome Res. 8:848-55.