Introduction

This protocol outlines how to shear up to 192 samples of genomic DNA for subsequent library preparation using the 2010 Geno/Grinder® Automated Tissue Homogenizer and Cell Lyser as well as the FastPrep-96™ High Throughput Bead Beating Grinder and Lysis System. We have used both devices to fragment 20 ng/µl of genomic DNA (extracted from GM24149 cells) and generated sequencing read lengths distributions up to 10 kb using the Ligation Sequencing Kit.

We recommend **10 minutes at 1200 RPM without beads** as the optimal parameters for shearing conditions to generate reproducible N50s of 7.4-10 kb fragments for a range of extracted gDNA, including human, mouse, roundworm and yeast. Further optimisation may be required depending on the DNA sample and required fragment size. For details of our results, please see the results section below.

Materials

For the **2010 Geno/Grinder® Automated Tissue Homogenizer and Cell Lyser**:

- Adhesive PCR Plate Seals (ThermoFisher, AB0558)
- Hard-Shell® 96-Well PCR Plates, low profile, thin walled, skirted, white/clear (BioRad, HSP9601)
- Nesting Tray Set (SPEX Sample Prep, 2189T)
- 2010 Geno/Grinder® - Automated Tissue Homogenizer and Cell Lyser (SPEX Sample Prep)
- DNA QC equipment, e.g. Agilent Bioanalyzer 2100, or Agilent Femto Pulse

For the **FastPrep-96™ High-Throughput Bead Beating Grinder and Lysis System**:

- Adhesive PCR Plate Seals (ThermoFisher, AB0558)
- Hamilton PCR ComfortLid (Hamilton, 814300)
- Hard-Shell® 96-Well PCR Plates, low profile, thin walled, skirted, white/clear (BioRad, HSP9601)
- FastPrep-96™ High-Throughput Bead Beating Grinder and Lysis System (MP Biomedicals, 116010500)
- DNA QC equipment, e.g. Agilent Bioanalyzer 2100, or Agilent Femto Pulse

Method

Note: This protocol has been validated for inputs of 20 ng/µl of gDNA for up to two 96-well plates.

1. Adjust the volume of genomic DNA sample(s) to 50 µl with nuclease-free water and transfer to your 96-well plate(s).
2. Thoroughly seal the 96-well plate(s) with an Adhesive PCR Plate Seal.
   Note: We found ineffective sealing with AriaMx Adhesive Plate Seals (Agilent, 401492) and do not recommend for this shearing method.
3. Load your plate(s) into your chosen device:

   2010 Geno/Grinder®: Place the nesting tray into the device and load up to two 96-well plates of samples side-by-side. If there is only one plate of samples, use an empty plate to balance. We recommend adding water to the empty plate to reflect the sample plate.

   FastPrep-96™: This system must be loaded with a total of six 96-well plates, equally distributed on both sides of the plate holder to ensure a tight fit. We recommend loading four empty plates and up to two plates of samples on top with ComfortLids. If there is only one plate of samples, use another empty plate to balance. We recommend adding water to the empty plates to reflect the sample plate(s).
4. Clamp the plates in securely by turning the wheel until a tight fit is obtained.

5. Run your chosen system for 10 minutes at 1200 RPM to fragment gDNA to N50s of 7.4-10 kb.

   Note: Shearing conditions can be altered to fit user needs, as further illustrated in the results section.

6. Analyse 1 µl of the fragmented DNA on an Agilent Bioanalyzer 2100 or Agilent Femto Pulse, to assess fragment size. This gDNA can subsequently be prepared for sequencing using the Ligation Sequencing Kit.

Results

We found using inputs of 20 ng/µl in 50 µl sheared for 10 minutes at 1200 RPM without beads were the optimal parameters to generate reproducible N50s of 7.4-10 kb with offline sizes of 11-16 kb. Further optimisations can be made for your specific input.

Below are figures and tables of the experiments performed to identify the optimal conditions, including a summary table of all the specific conditions performed. In all conditions, the fragmented DNA was analysed using the Agilent Femto Pulse.

Speed:

Table 1. A summary of the average peak size following shearing at different speeds. Samples were sheared for 5 minutes at a concentration of 55 ng/µl.

Speed (RPM)	Average peak size (kb)
800	73.9
1000	26.6
1200	16.4
1400	12.8
1600	10.4
1800	9.4

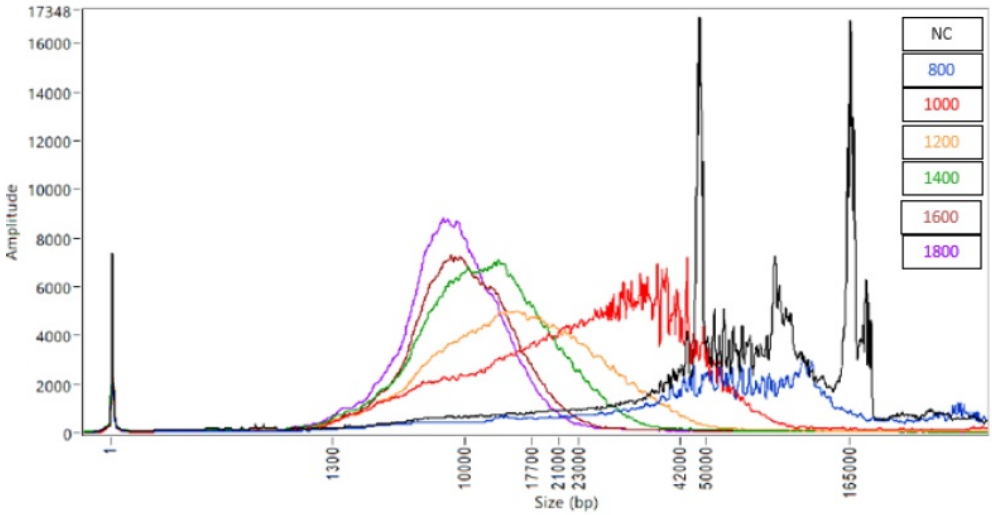

Figure 1. DNA fragment lengths following fragmentation at different speeds for 5 minutes. Analyses of fragment lengths showed increasing speed reduced fragment length and distribution.

Concentration:

Table 2. A summary of the average peak size following shearing at different sample concentrations. Samples were sheared for 5 minutes at 1,800 RPM.

Sample concentration (ng/µl)	Average peak size (kb)
10	11.82
15	11.08
20	12.05
30	15.50
60	15.78
95	26.37

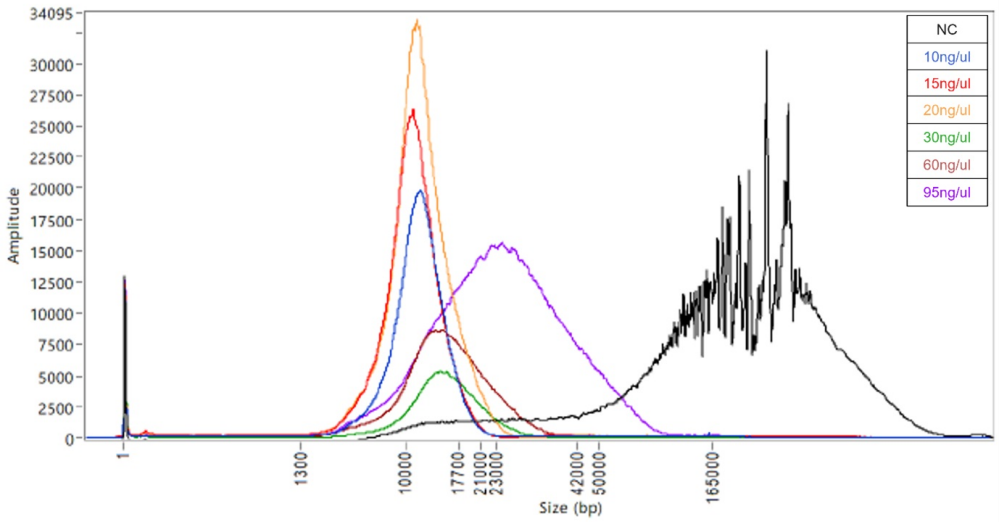

Figure 2. DNA fragmentation lengths following shearing at 1,800 RPM for 5 minutes using different input concentrations. Analysis showed increased shearing for samples at lower concentrations.

Volume:

Table 3. A summary of the average peak size following shearing of samples at different volumes. Samples were at a concentration of 55 ng/µl and sheared for 5 minutes at 1,400 RPM.

Volume (µl)	Average peak size (kb)
15	34.26
30	52.46
50	58.56
75	69.20
100	89.18
150	158.61

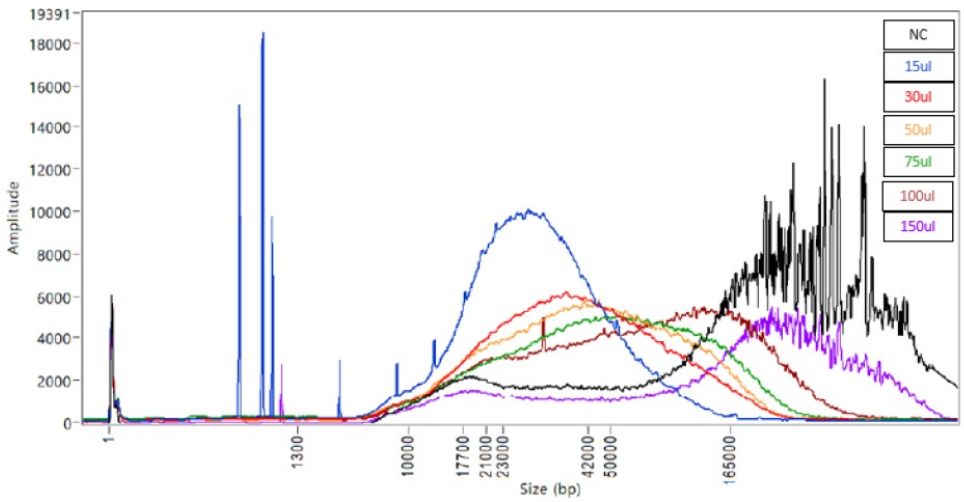

Figure 3. DNA fragment lengths of samples at different volumes following shearing at 1,400 RPM for 5 minutes. Analysis showed increasing sample volume increased fragment length but with less effect on fragment size distributions.

Time:

Table 4. A summary of the average peak size following fragmentation for 5 and 10 minutes. Samples were at a concentration of 120 ng/µl and sheared at 1,800 RPM.

Time (min)	Average peak size (kb)
5	18.20
10	13.28

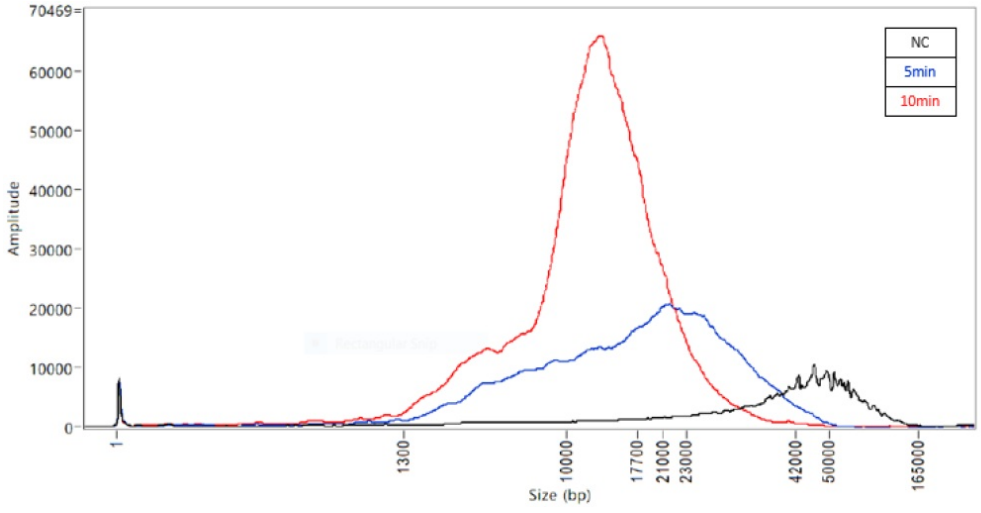

Figure 4. DNA fragment lengths following fragmentation at 1,800 RPM for 0, 5 and 10 minutes. Analysis showed increased shearing duration decreased fragment size distribution.

Comparing shearing mechanisms:

Table 5. A summary of the average peak size and shearing parameters used for comparing shearing mechanisms. Samples were at a

concentration of 20 ng/µl.

Mechanism	Time (min)	Speed	Average peak size (kb)
FastPrep-96	10	1200 RPM	11.67
MegaRuptor	-	25 30 35	34.11 15.67 10.76
g-TUBE	2	700 RPM 1000 RPM 1300 RPM	17.82 13.06 12.15

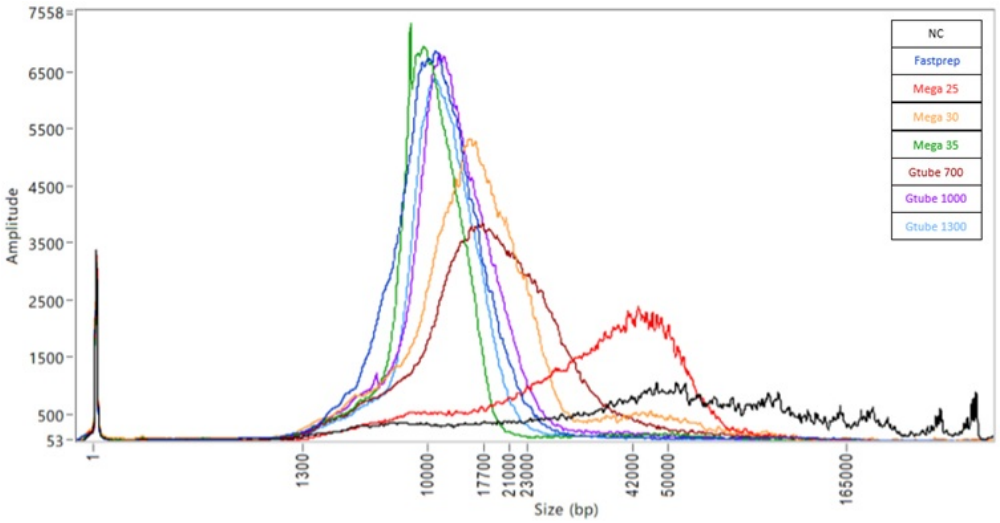

Figure 5. DNA fragment lengths following fragmentation using FastPrep-96, MegaRuptor, and g-TUBE at three different speeds. Analysis showed the FastPrep-96 produced similar fragment lengths to the MegaRuptor and g-TUBE at their fastest speeds.

DNA input from different sources:

Table 6. A summary of the average peak size following shearing of different input sources. Samples were at a concentration of 20 ng/µl and sheared for 10 minutes at 1,200 RPM.

Input	Average peak size (kb)
G-tip Clivome	10.86
ULK Cliveome	10.74
Nanobind Cliveome	13.85
Mouse stem cell	11.50
C. elegans	15.85
S. cerevisae	15.00

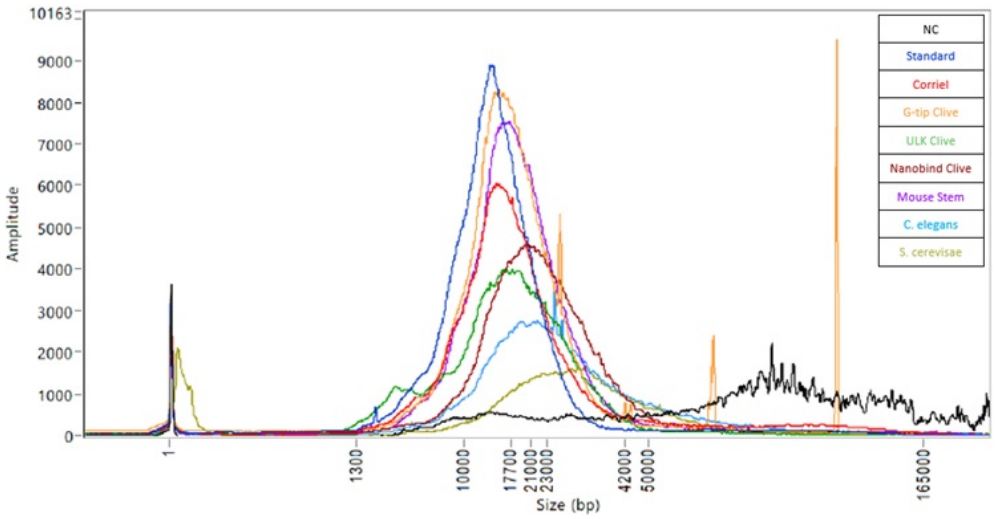

Figure 6. DNA fragment lengths following shearing at 1,200 RPM for 10 minutes. DNA inputs and extraction methods were tested, including human and non-human cell lines, as well as Nanobind and g-tip extraction methods. Analysis showed comparable results for all samples.

Overview of conditions run:

Below we outline the variables of each condition tested used to determine the optimal parameters to generate reproducible results.

Variables	Time conditions Fig. 4	Speed conditions Fig. 1	Concentration conditions Fig. 2	Volume conditions Fig. 3
Time (minutes)	0, 5, 10	5	5	5
Speed (RPM)	1800	800, 1000, 1200, 1400, 1600, 1800	1800	1400
Concentration (ng/µl)	120	55	10, 15, 20, 30, 60, 95	55
Volume (µl)	25	25	25	15, 30, 50, 75, 100, 150

Change log

Version	Change
v4, August 2023	Updated grammatical errors and updated figures 5 and 6. Table 5 has been updated to include g-TUBE units.
v3, June 2023	Reformatted the results section, including table and figure legends, and corrected reproducible N50s read lengths. Updated plate sealing recommendations and plate balancing. Clarified in the shearing parameters to use without beads
v2, May 2023	Updated recommended parameters and included genogrinder recommendations

Version	Change
v1, August 2020	Initial protocol publication