



High throughput genomic DNA shearing

FOR RESEARCH USE ONLY

Contents

[Introduction](#)

[Materials](#)

[Method](#)

[Results](#)

1. Overview
2. Concentration
3. Volume
4. Speed
5. Time
6. Fragment length optimisation
7. Buffer Types
8. Spatial trends
9. Cross-contamination
10. Precision
11. Tolerance

[Discussion](#)

[Change log](#)

Introduction

This protocol outlines how to shear up to 192 samples of DNA for library preparation using the 2010 Geno/Grinder® Automated Tissue Homogenizer and Cell Lyser (GenoGrinder) as well as the FastPrep-96™ High Throughput Bead Beating Grinder and Lysis System (FastPrep). We have used both devices to optimise fragmentation of DNA. Sequencing read length N50 distributions of 10, 12.5, 15 and 20 kb have been generated with this method. In no cases are these instruments using beads to process samples.

To shear gDNA to 15 kb we recommend the following settings of **1600 SPM** for **5 minutes** with gDNA samples of **30 ng/μl** in **120 μl**. Further optimisation may be required depending on the DNA sample and required fragment size. The quality of gDNA being sheared will also affect outcomes. For further details on results, please see the sections 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)
- [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: FastPrep speed units are in rotations per minute (RPM), GenoGrinder units are in strokes per minute (SPM). They are used interchangeably here. This protocol has been validated for gDNA in 96-well plates. To shear samples to 15 kb please follow the method outlined here using 3.6 μg per sample.

1. Adjust the volume of genomic DNA sample(s) to 120 µl with nuclease-free water and transfer to your 96-well plate(s).
Note: We found sub-optimal shearing performances when not using the recommended 96-well plate.
2. Thoroughly seal the 96-well plate(s) with an Adhesive PCR Plate Seal. Run a seal applicator through each column and row for effective sealing.
Note: We found ineffective sealing with AriaMx Adhesive Plate Seals (Agilent, 401492) and do not recommend it for this shearing method.
3. Load your plate(s) into your chosen device:
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 in wells that 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 sealed samples on top. If there is only one plate of samples, use another empty plate to balance. We recommend adding water to the empty plate in wells that reflect the sample plate.
4. Clamp the plates in securely by turning the ratchet nut/turning knob until a tight fit is obtained.
5. Run your chosen system for 5 minutes at 1600 SPM to fragment gDNA to N50 of 15 kb.
Note: Instrument settings 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 Femto Pulse, to assess fragment size. This gDNA can subsequently be prepared for sequencing using the Ligation Sequencing Kit.

Results

Overview

Four variables were found that affect shearing outcomes: shearing time and speed, and sample concentration and volume (summarised in Figure 1). A desired fragment length can be obtained by optimising these on your instrument of choice. How fragment lengths are affected when changing these is shown in result sections 1–5. Fragmentation in these sections was carried out with the FastPrep, and the results are as follows:

- Increasing sample concentration or volume increases fragment size and distribution •
- Increasing shearing time or speed, decreases fragment size and distribution.

Further results are shown in sections 6–11 including results on sequencing fragment length optimisation, sample buffer types, plate spatial trends, cross-contamination, shearing precision and tolerance. Fragmentation in these sections was carried out with the GenoGrinder.

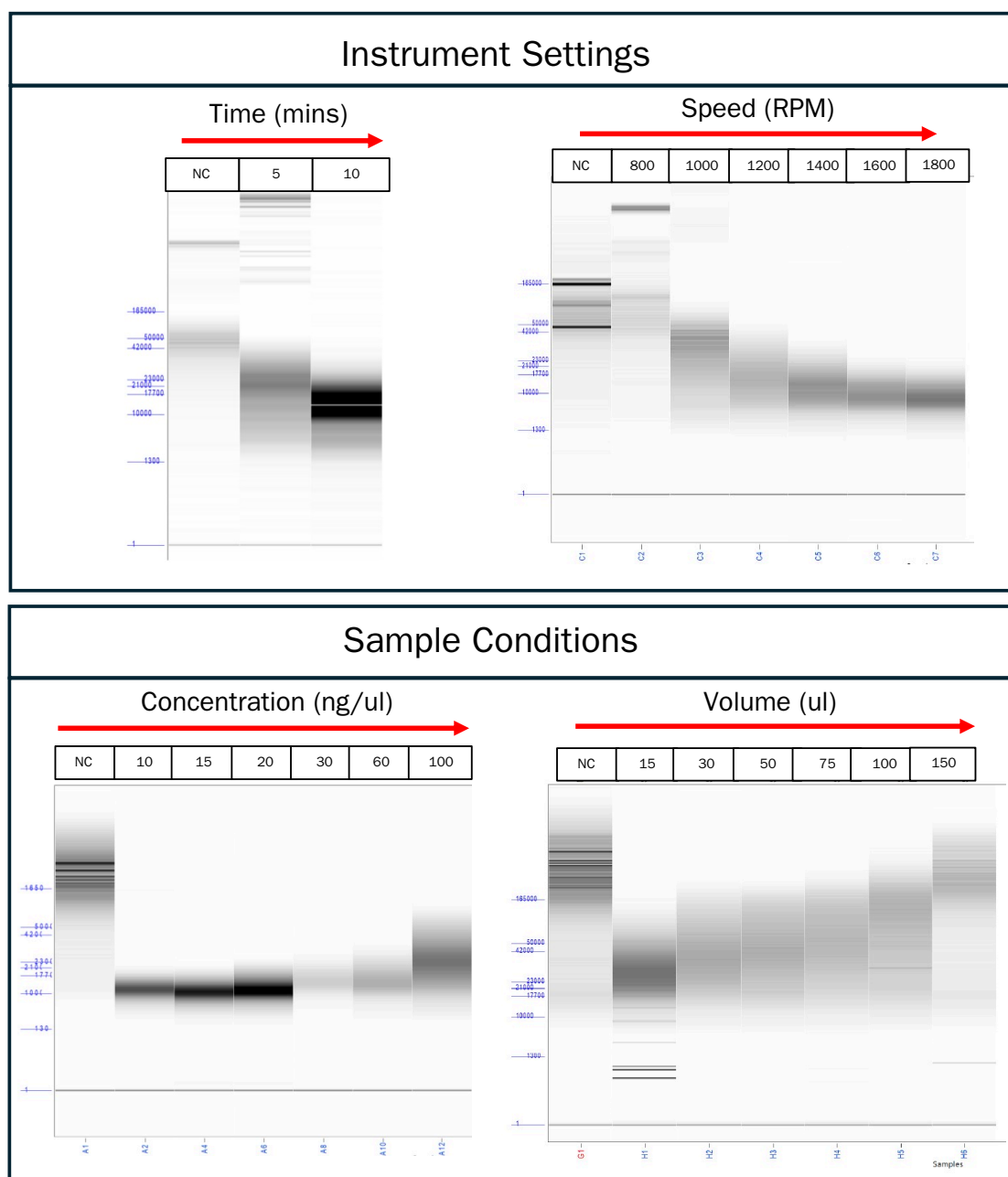


Figure 1. The effect of instrument and sample conditions on shearing outcomes. Human gDNA was sheared in duplicate, changing one variable at a time and analysed on the Femto Pulse. The results of one representative replicate are shown on digital gel images. The red arrow indicates an increasing value for the respective parameter.

Concentration

DNA size and distribution increases as concentration increases. Human gDNA was sheared at 1800 RPM for 5 mins in 25 μ l nuclease-free water with a changing concentration and analysed on

the Femto Pulse (see Figure 2). Samples more dilute than 20 ng/μl did not observably change in size or distribution (see Figure 3).

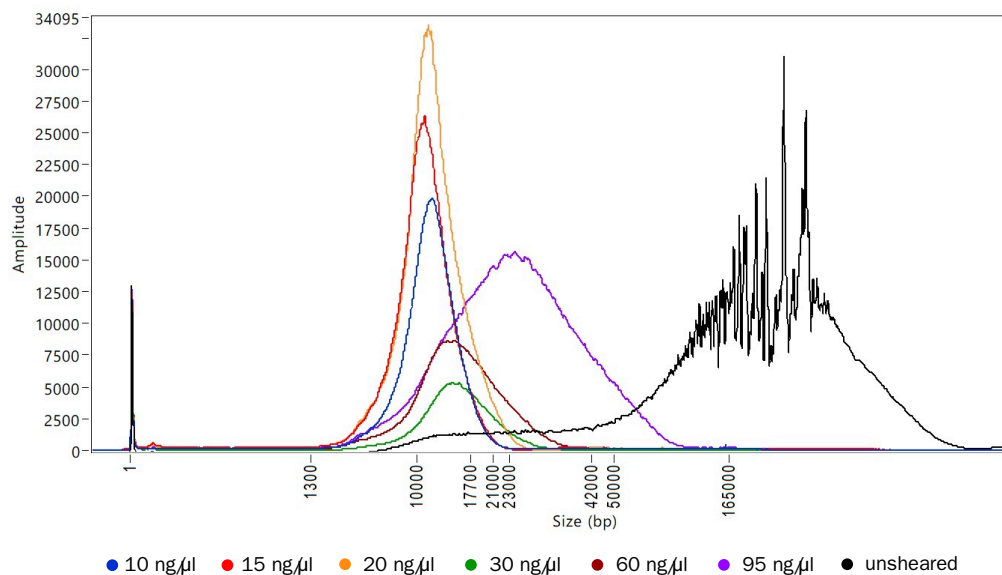


Figure 2. The effect of changing sample concentration on DNA length. Human gDNA was sheared with changing concentrations. Samples were sheared in duplicate. The results of one representative replicate are shown.

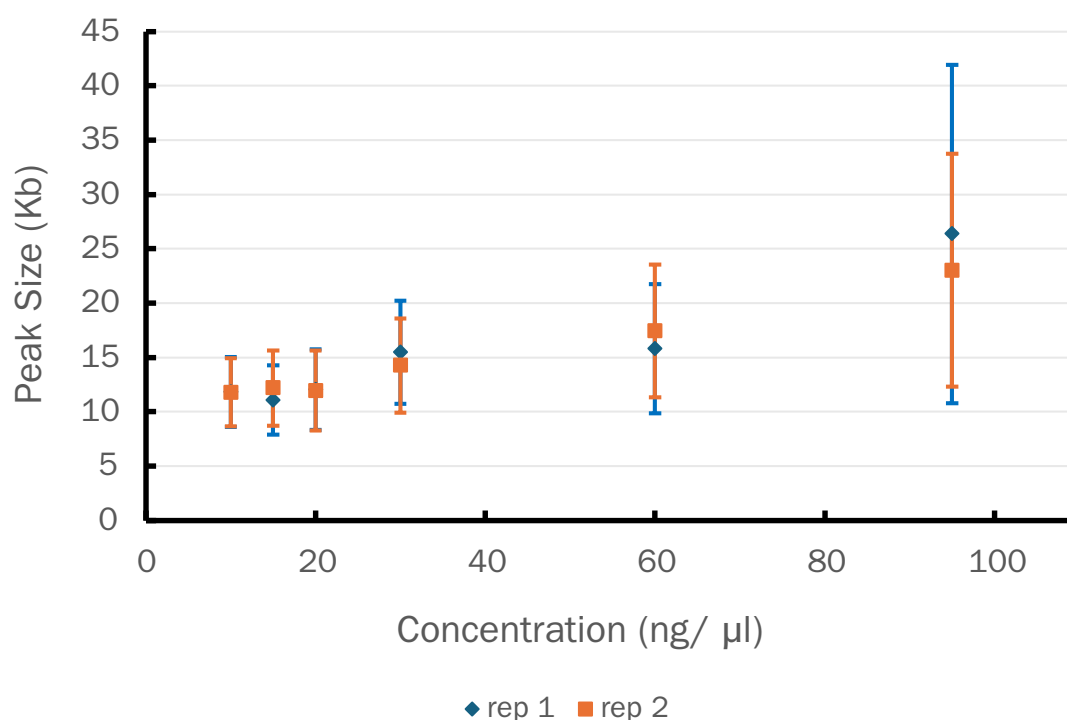


Figure 3. Peak size and distribution are shown of samples sheared at different concentrations. The average peak size is marked with standard deviation error bars using smear analysis in ProSize. Data for both replicates are shown.

Volume

Increasing sample volume increases DNA size but with less effect on the distribution shape (see Table 1 and Figure 4). Human gDNA was sheared at 1400 RPM for 5 minutes at 55 ng/µl in a changing volume of nuclease-free water and analysed on the Femto Pulse. Volumes at 150 µl were not sheared effectively.

Table 1. Average peak sizes following shearing of samples at different volumes. Samples were sheared in duplicate. The results of one representative replicate are shown. Sizes were determined using smear analysis in ProSize.

Speed (μl)	Average Peak Size (kb)
800	73.90
1000	26.60
1200	16.40
1400	12.80
1600	10.40
1800	9.40

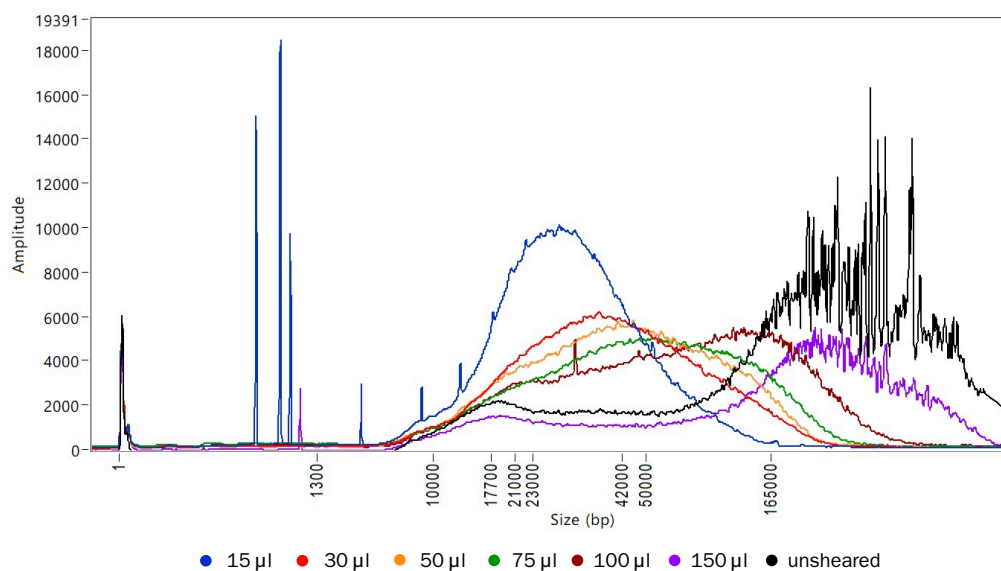


Figure 4. The effect of changing sample volume on DNA length. Human gDNA was sheared in changing volumes. Samples were sheared in duplicate. The results of one representative replicate are shown.

Speed

Increasing shearing speed decreases DNA size and distribution (see Table 2 and Figure 5). Human gDNA was sheared for 5 minutes at 55 ng/μl in 25 μl nuclease-free water at a changing speed and analysed on the Femto Pulse. At 800 RPM samples were not sheared effectively.

Table 2. Average peak sizes following shearing at different speeds. Samples were sheared in duplicate. The results of one representative replicate are shown. Sizes were determined using smear analysis in ProSize.

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

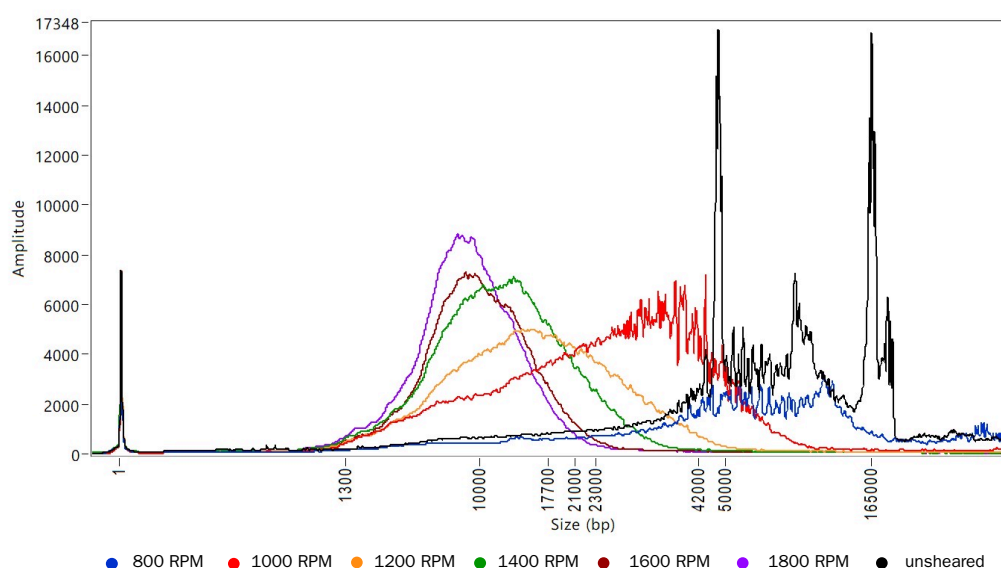


Figure 5. The effect of changing shearing speed on DNA length. Human gDNA was sheared at changing speeds. Samples were sheared in duplicate. The results of one representative replicate are shown.

Time

Increasing shearing time decreases DNA size and distribution (see Table 3 and Figure 6). Human gDNA was sheared at 1800 RPM at 120 ng/μl in 25 μl nuclease-free water for either five or ten minutes and analysed on the Femto Pulse.

Table 3. Average peak sizes following shearing at five and ten minutes. Samples were sheared in triplicate. The results of one representative replicate are shown. Sizes were determined using smear analysis in ProSize.

Time (μl)	Average Peak Size (kb)
5	18.20
10	13.28

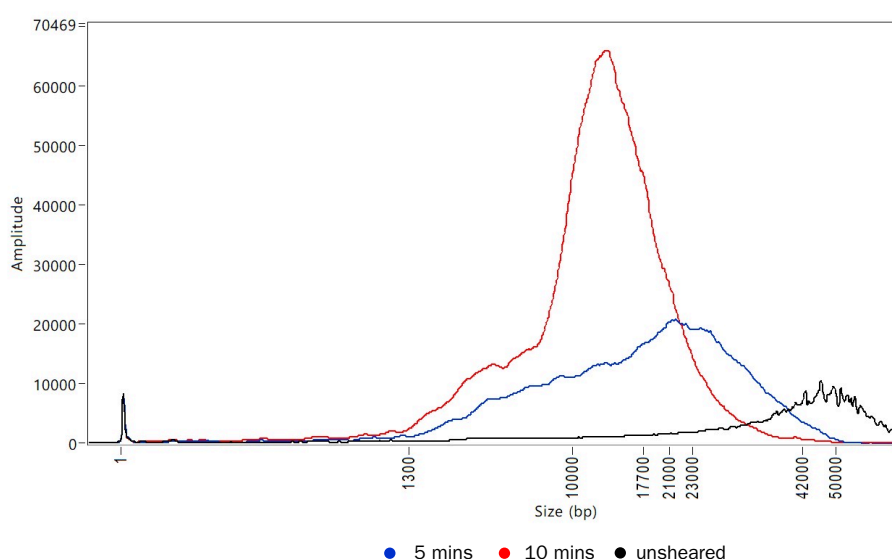


Figure 6. The effect of changing shearing time on DNA length. Human gDNA was sheared for either five or ten minutes. Samples were sheared in triplicate. The results of one representative replicate are shown.

Fragment length optimisation

Human gDNA was sheared to a range of N50 distributions (see Figure 7). Sample concentrations and volumes were 30 ng/μl and 120 μl respectively. The following instrument conditions achieved the following approximate lengths:

- 10 kb (1750 RPM, 10 mins) • 12.5 kb (1750 RPM, 5 mins) • 15 kb (1600 RPM, 5 mins) • 20 kb (1400 RPM, 5 mins)

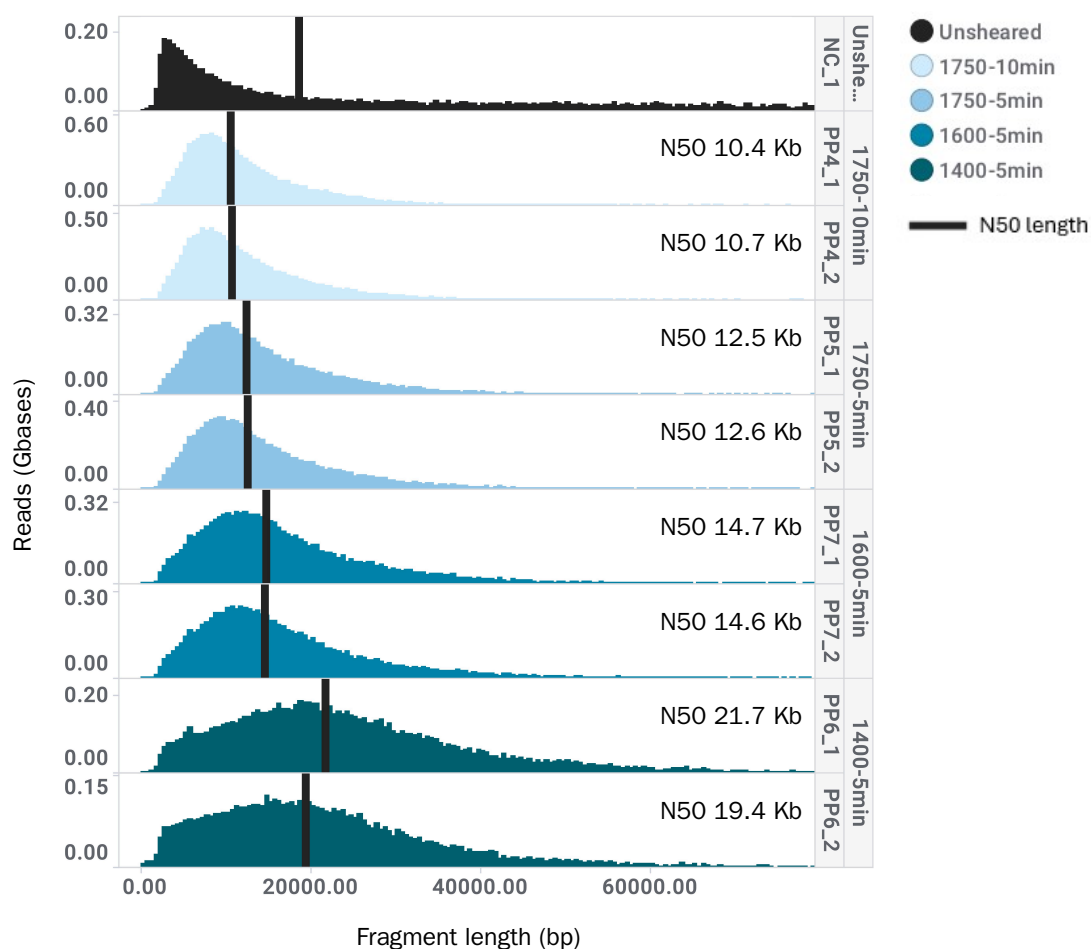


Figure 7. Shearing gDNA to a range of N50 lengths. Fragments were sheared to sizes between approximately 10 and 20 kb in duplicates by changing the instrument settings for time and speed. Samples were sequenced for 24 hours with MinION/GridION Flow Cells on a GridION.

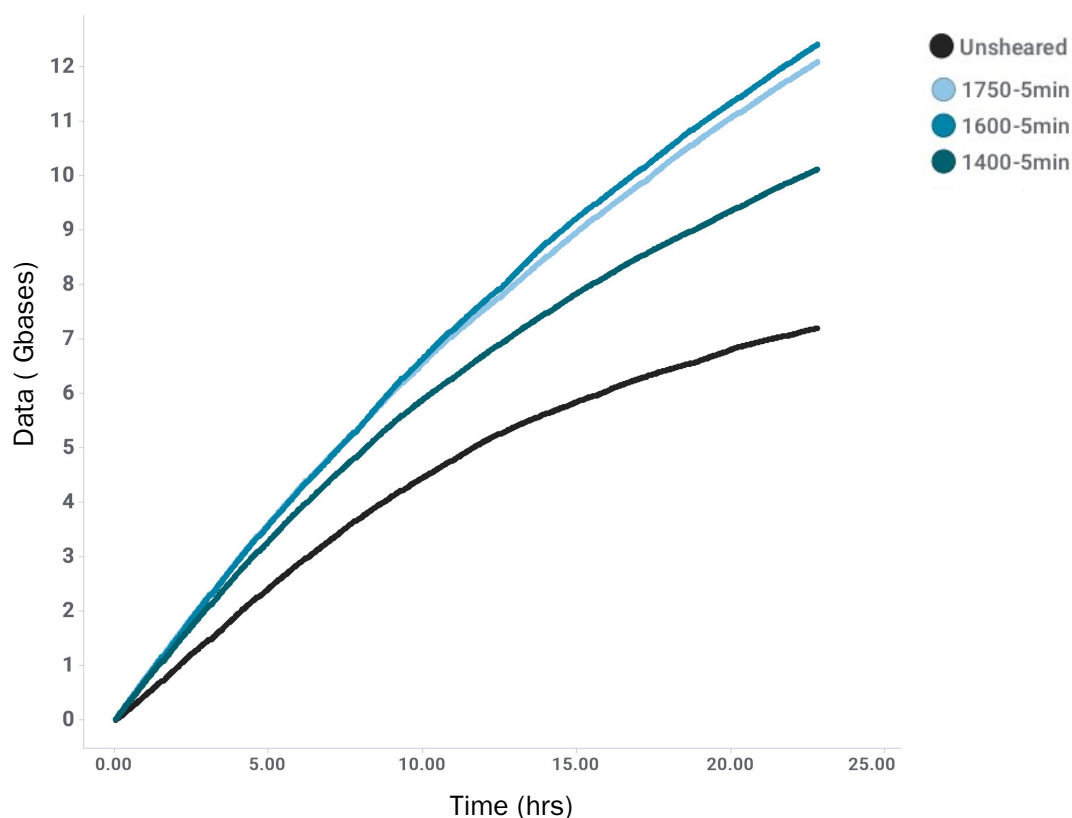


Figure 8. Sequencing data outputs of sheared gDNA. Outputs of sheared samples shown as average cumulative sum line of two samples. Samples were sequenced for 24 hours with MinION/GridION Flow Cells on a GridION.

Buffer Types

No effect on fragment lengths was seen if gDNA was sheared in either nuclease-free water, Tris or low-TE buffer (see Figure 9). Samples had a concentration and volume of 60 ng/μl and 50 μl respectively and were sheared at 1800 RPM for 5 minutes. All samples sheared to an N50 of 17 kb.

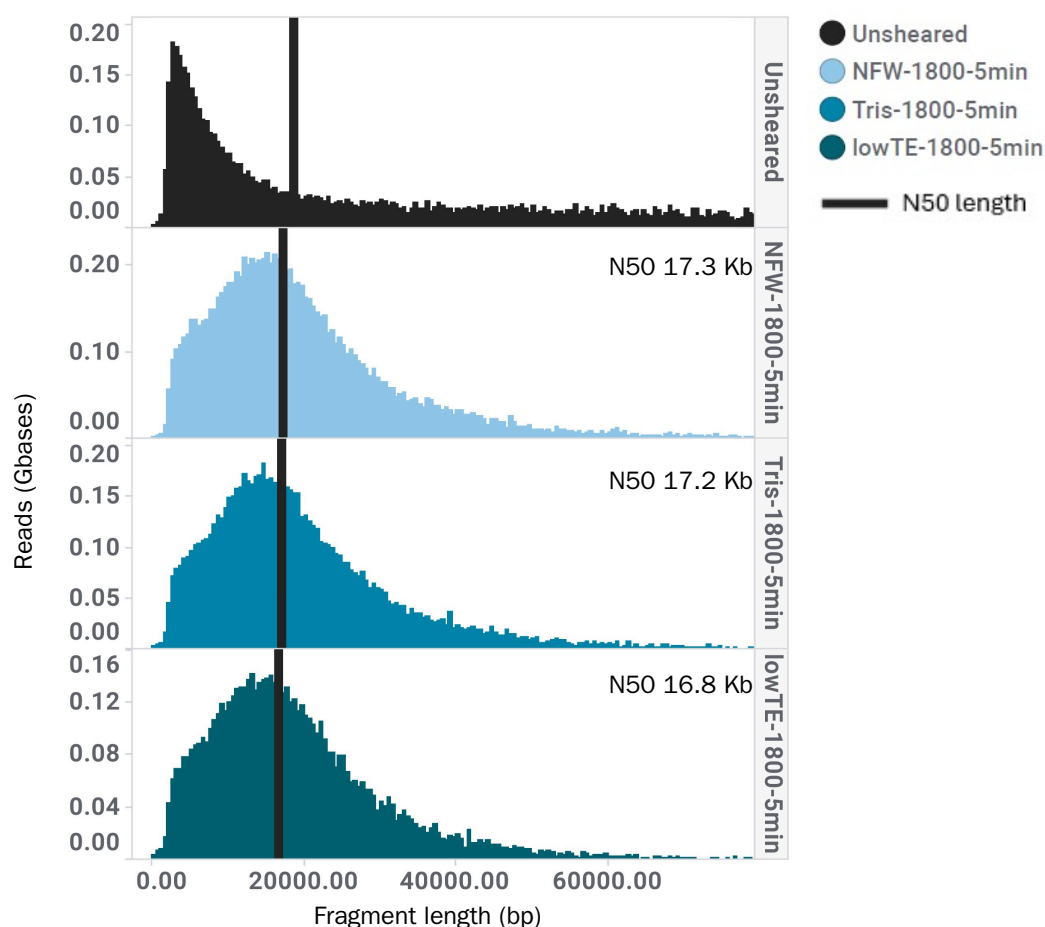


Figure 9. Shearing gDNA in different sample buffers had no effect on size distribution. Samples were sheared in either nuclease-free water, Tris or low TE buffer and sequenced for 24 hours with MinION /GridION Flow Cells on a GridION.

Spatial trends

Shearing samples on 96-well plates is independent of their position on the plate. NEB 48 kb Lambda DNA was used to line the edges of a plate and chequerboard across the middle. Samples had a concentration and volume of 60 ng/μl in 50 μl respectively and were sheared at 1800 RPM for 5 minutes. Eight samples from across the plate were sequenced for 24 hours. The N50s ranged from 12.43–13.58 kb with no observable correlation between plate position and fragment length (see Figure 10).

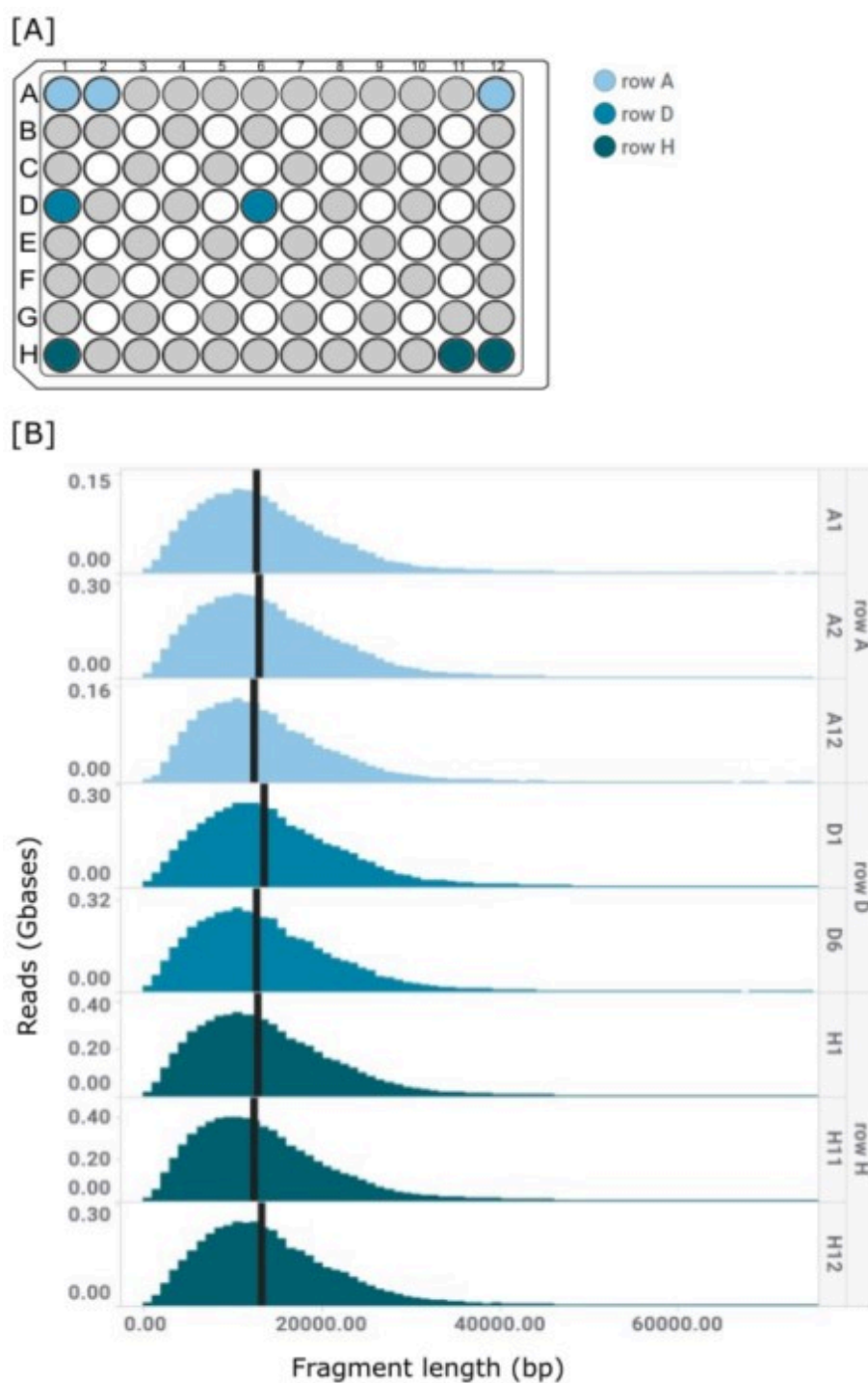


Figure 10. Sheared sample sequence distributions are independent of spatial 96-well plate positioning. [A] Sample positioning on the plate during shearing shown here. The row each analysed sample was taken from is indicated in the legend and plate. [B] The library fragment distributions of the analysed fragments shown here. Black line shows the N50.

Cross-contamination

Processing a 96-well plate with an adhesive seal did not result in cross-contamination between wells. NEB lambda and blank nuclease-free water were loaded onto a plate, sheared and sequenced. All sequenced data of blank wells was unaligned showing no cross contamination took place (see Figure 11).

Additional tests were done using chequerboarded plates of food dye and nuclease-free water blanks. Of 67 plates tested, 65 plates showed no well-to-well leakage had taken place. The two plates that did exhibit leakage had undergone an incubation with a heated lid before shearing. This heating compromised the seal adhesive and so is not recommended for this method (data not shown).

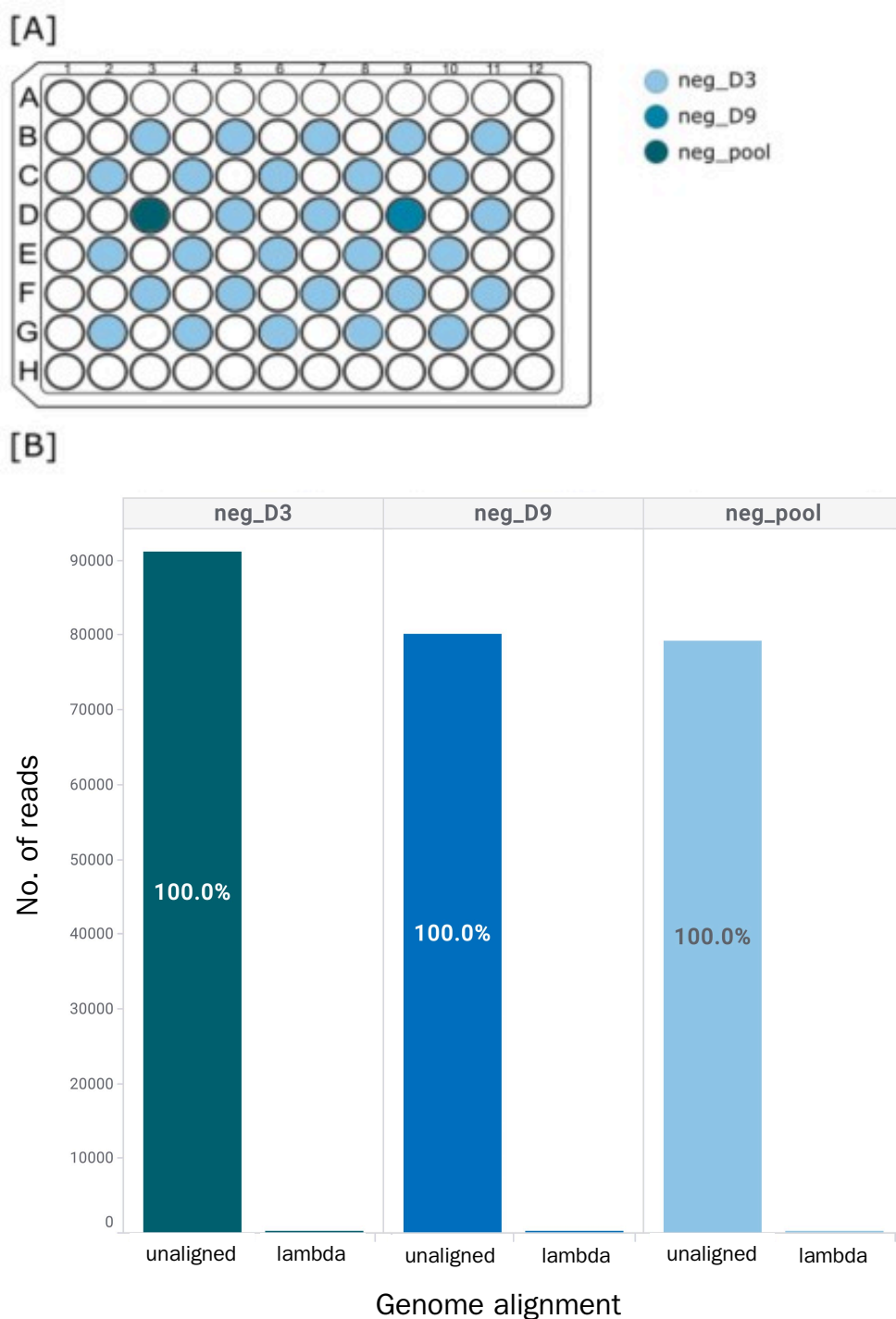


Figure 11. No well-to-well cross-contamination is detected during sequencing. [A] Shown is a layout of the processed plate. The legend indicates the three sample groups collected for analysis, blank well D3 (teal) and D9 (dark blue), as well as a pool of all blank wells (light blue). White wells contained lambda DNA. [B] Unaligned and aligned reads are shown for each sample. Samples were sequenced for 24 hours on MinION/GridION Flow Cells on the GridION.

Precision

Shearing precision was determined by processing nine samples distributed evenly across three plates. Four speeds were tested representing the slowest, fastest and two middle speeds. After sequencing for 24 hours on a MinION/GridION Flow Cell the variability of N50s were compared. An interquartile range of less than 1 kb for samples across each speed was observed (see Figure 12). An inter-plate CV of <10% was observed for samples across plates for every speed (see Figure 13). An intra-plate CV of <10% was also seen for samples on the same plate (not shown).

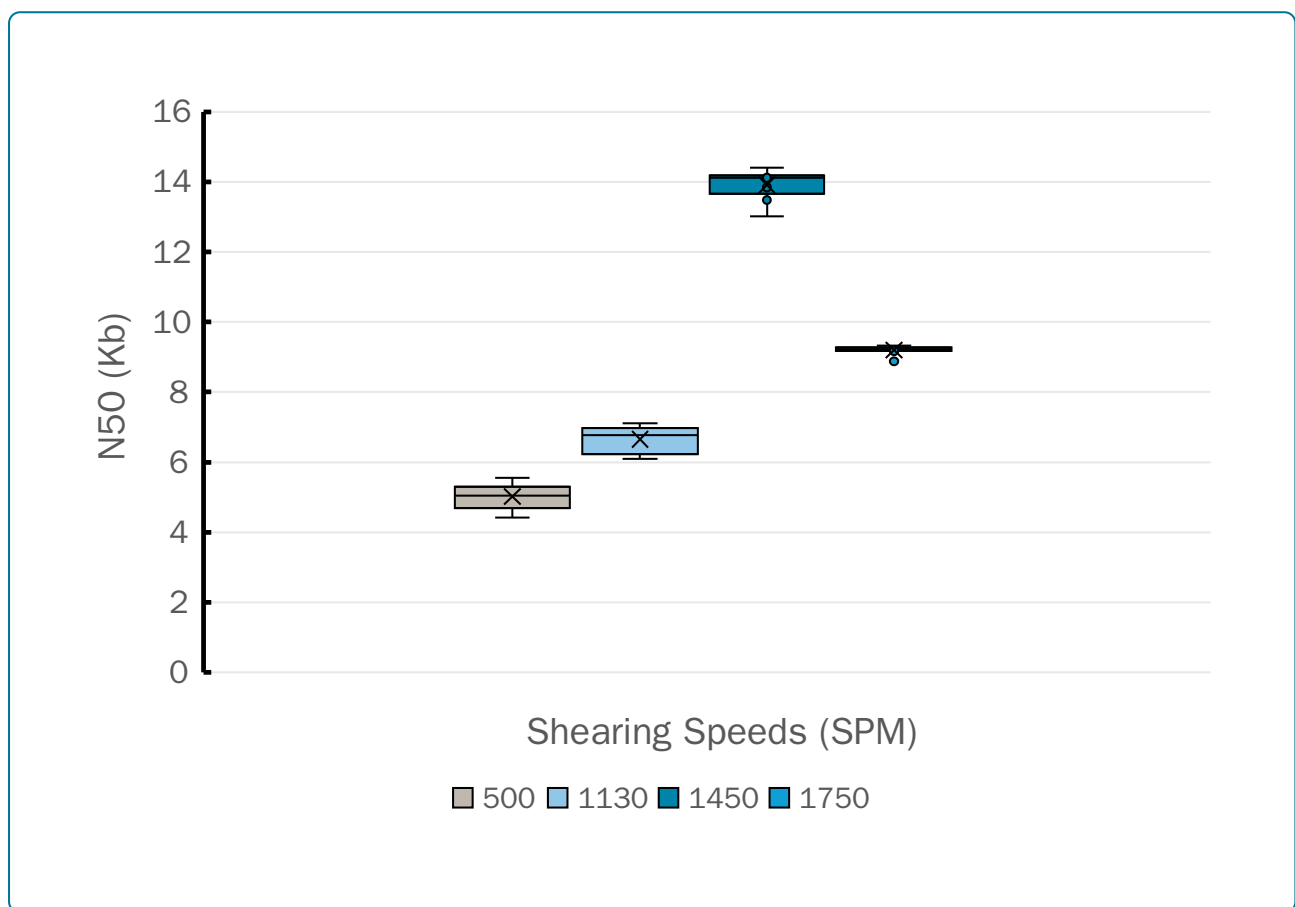


Figure 12. Variance in nine sheared human gDNA samples spread evenly across three plates. Each plate was processed on separate runs, and four speeds were tested.

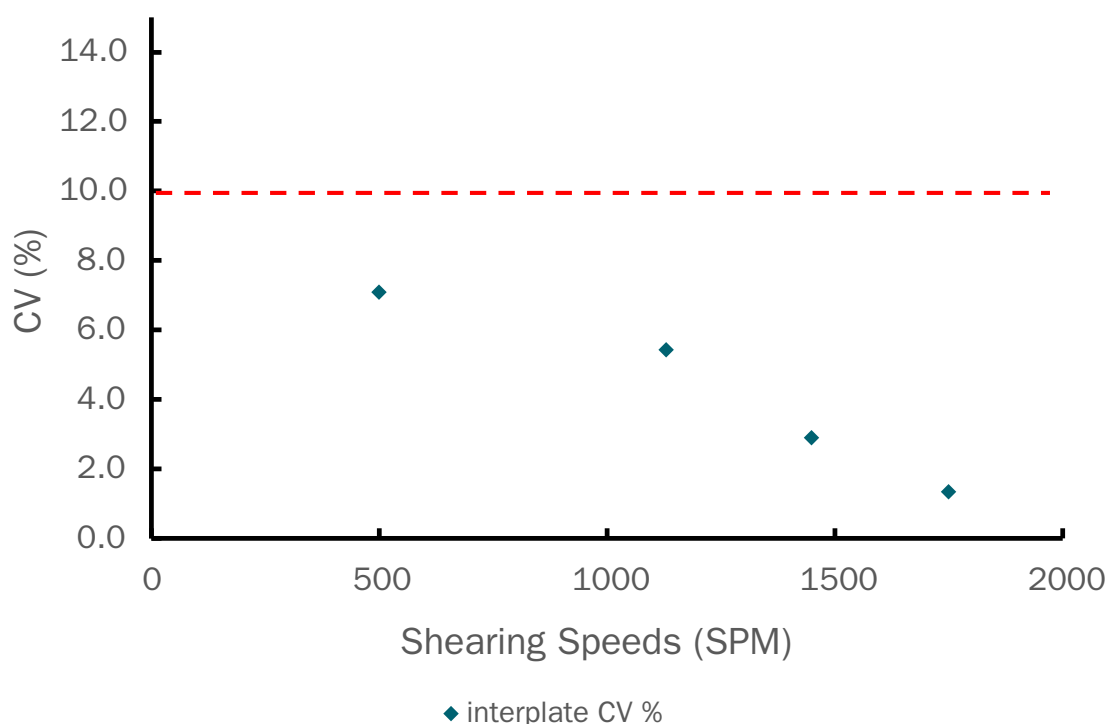


Figure 13. Inter-plate variance of samples sheared at different speeds. Observed CVs of nine sheared human gDNA samples spread evenly across three plates is shown. Four speeds were tested. Red line indicates a CV % of 10%.

Tolerance

Given the shearing condition of 1450 SPM for 5 minutes at 30 ng/μl in 110 μl nuclease-free water, a range of concentrations and volumes deviating from this were tested to approximate outcomes for out of specification samples. Concentrations of 80, 90, 110 and 120% of 30 ng/μl were tested and volumes of 95, 105 and 110% of 110 μl were tested. The effect of concentration deviations on N50 and N10/N90 are shown in figure 13. An N10/N90 equal to one would represent a single fragment length. While the N50 remained unchanged, the N10/N90 shows an increase in distribution broadness when moving from 80 to 120% concentration (see Figure 13). Shearing outcomes were unchanged for the volume range tested.

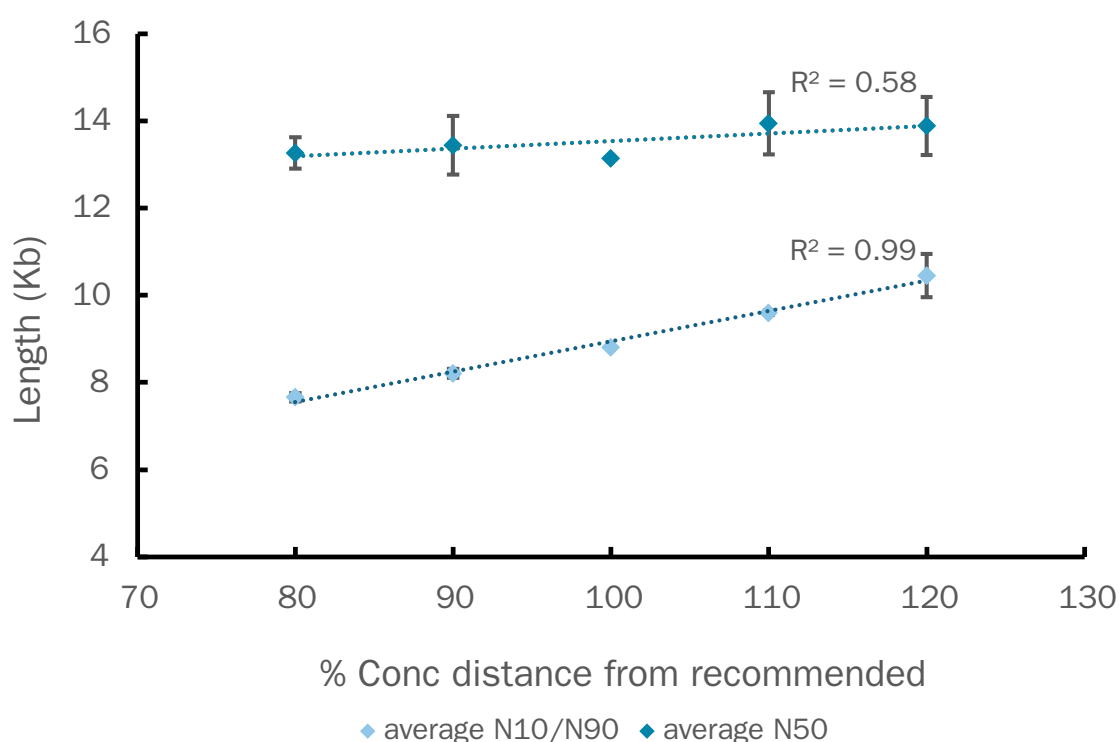


Figure 14. Shown here are fragment distributions of samples sheared with concentrations incrementally changing from a specified condition (which is 30 ng/μl here and is represented as 100%).

Discussion

In this document we show a comprehensive range of tests demonstrating the suitability of the GenoGrinder and FastPrep for DNA shearing applications. The GenoGrinder offers advantages over the Fastprep and is the preferred shearing instrument. For example, speed increments are in 10 SPM on the GenoGrinder as opposed to 100 RPM on the FastPrep which allows for higher precision in fragment size tuning.

Change log

Version	Change
v5, March 2025	Introduction updted Method updated with new recommended instructions and tips: Recommendations for shearing to 15 kb in 96-well plates using 3.6 µg in 120 µl volume per well. Results section overhauled: Added new sections for information and data. This now includes information for: Overview of results, Concentration considerations, Volume, Speed, Time, Fragment length optimisation, Buffer types, Spatial trends, Cross-contamination, Precision, and Tolerance. Added a Discussion section as a summary/conclusion. Moved the Change Log to separate section.
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
v1, August 2020	Initial protocol publication