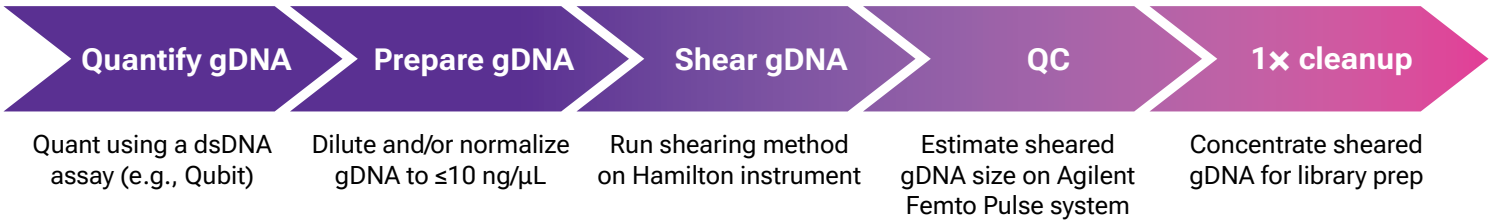
Technical note

HIGH-THROUGHPUT DNA SHEARING USING HAMILTON MICROLAB PREP FOR PACBIO® LONG-READ WHOLE GENOME SEQUENCING

Overview



Application: Human, plant, and animal WGS

Target DNA fragment size ranges: 15–22 kb

DNA input per well: ≤3 µg

Introduction

The whole genome sequencing (WGS) application requires an up-front mechanical DNA shearing step to transform genomic DNA into fragment sizes appropriate for HiFi sequencing. With the Hamilton Microlab Prep system, this step can be performed in as little as 22 minutes for 24 samples at the cost of a pipette tip per sample. Here we describe the required equipment and consumables, best practices, and procedure for shearing DNA on the Microlab Prep system.

Lab equipment, consumables, and materials

Common laboratory equipment such as microcentrifuge tubes, single- and multi-channel pipettes, and filtered pipette tips are assumed to be available and not listed below.

Equipment

- Hamilton Microlab Prep (PacBio 103-283-600)
- Agilent Femto Pulse system (Agilent M5330AA or PacBio 103-283-300)
- Qubit Flex fluorometer (Thermo Fisher Scientific Q33327)

Consumables

- Femto Pulse gDNA 165 kb analysis kit (Agilent FP-1002-0275 or PacBio 103-391-800)
- Qubit 1x dsDNA HS (High Sensitivity) assay kit (Thermo Fisher Scientific Q33231)
- Qubit Flex assay tube strips (Thermo Fisher Scientific Q33252)
- 300 µL CO-RE II tips (filtered, black, non-sterile), (Hamilton 235903)
- Hamilton 5 × 60 mL reagent reservoir rack (Hamilton 6600700-01)
- Hamilton 60 mL trough (Hamilton 56694-03)
- Abgene 96 well 0.8mL polypropylene DeepWell storage plate (Thermo Fisher Scientific AB0859)
- SMRTbell® cleanup bead kit (PacBio 103-306-300)
- Bio-Rad 96 PCR full skirt 200 µL plate (Bio-Rad HSP9601)
- 1000 µL CO-RE II tips (filtered, black, non-sterile) (Hamilton 235905)
- Alpaqua Magnum FLX magnet (Alpaqua ALPQ-0008)

Material

- ≤3 µg of genomic DNA at 10 ng/µL in low TE buffer (pH 8.0 or 9.0) per well

Parameter	Setting
DNA concentration	10 ng/µL or less
DNA volume	300 µL in low TE buffer
Number of mixes	300 cycles
Pipette mixing speed	500 µL/sec
Range of mean fragment size	15–22 kb

Table 1. Overview of the settings for the high-throughput shearing method on Hamilton NGS liquid handler systems

Procedure

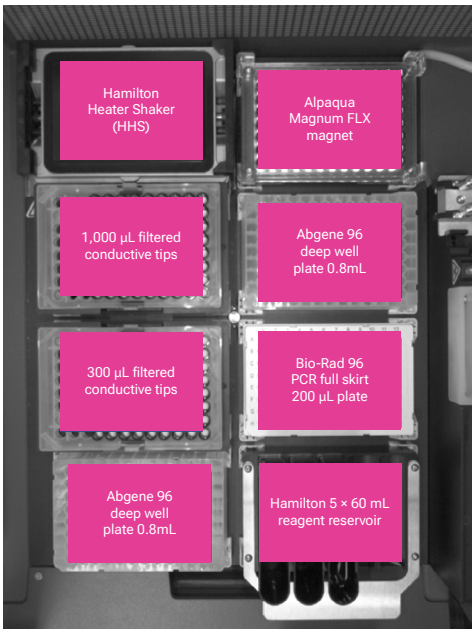
Shearing module

1. Prior to beginning, equilibrate the following reagents to room temperature:
   - Low TE Buffer (provided in SMRTbell® cleanup bead kit or SMRTbell prep kit 3.0)
   - Quant-IT 1× dsDNA HS kit or Qubit 1× dsDNA HS kit (follow manufacturer's instructions)
   - Femto Pulse gDNA 165 kb analysis kit (follow manufacturer's instructions)
2. Accurately quantify the genomic DNA samples using either Quant-IT or Qubit assays for dsDNA.
3. Calculate the volume of genomic DNA needed for a ≤**10 ng/µL** concentration in a **300 µL** volume.
4. Transfer the appropriate volume of DNA calculated in prior step to the appropriate plate well. Start at position A1 and fill the plate by moving down the column. Continue adding any additional samples to each adjacent column.
5. Bring the final volume of each sample up to **300 µL** using PacBio low TE buffer. Add 300 µL of low TE buffer to any empty wells in a column containing samples.

6. Seal and briefly centrifuge the plate to collect liquid at the bottom of the well and ensure no air bubbles are present.
7. Load the sample plate into position 8 and the appropriate number of Hamilton 300 µL CO-RE II conductive filtered tips on to the Microlab prep into position 4 as shown in picture.
8. Begin the automated shearing program.
9. It is recommended to confirm shearing size distribution using an Agilent Femto Pulse system. Note that instruments built for short-read NGS workflows (e.g., TapeStation) may be able to provide a qualitative answer but will not provide accurate size measurements for long-read sequencing applications. Seal and store the sheared DNA in a refrigerator set to 4°C while waiting on QC results.
10. Proceed to the post-shear cleanup method using 1× SMRTbell cleanup beads. It is *not* recommended to store diluted DNA at 4°C for long periods of time.

Post-shear cleanup module

1. Remove the base plate for the 1,000 µL tip position (#2). Then place the tip rack adapter in position #2 for the 1,000 µL tips.
2. Calculate the volume of reagents you will need to add to the 60 mL reagent troughs based on the number of samples you will be running. Add 2 mL to account for dead volume of the troughs. Remove the middle insert of the trough before adding reagent.
3. Gather and load the following on the deck as shown in the picture:
   - 96-well, deep well plate containing the sheared samples
   - Bio-Rad 96 PCR full skirt 200 µL plate
   - Hamilton 300 µL conductive filtered tips
   - Hamilton 1,000 µL conductive filtered tips
   - Alpaqua Magnum FLX magnet
   - Hamilton 5 × 60 mL reagent reservoir rack
   - Hamilton 60 mL trough



4. Select the post-shearing cleanup process. Enter the number of samples and follow the loading dialog prompts to place all consumables in the appropriate positions.

Number of samples	8	16	24
SMRTbell bead volume (µL)	4,400	6,800	9,200
80% ethanol volume (µL)	3,600	5,200	6,800
Elution buffer volume (µL)	2.400	2,800	3,200

5. Once complete, the samples are ready for library prep. Store DNA at 4°C until ready to proceed.

Expected performance for DNA shearing on the Microlab Prep system

Shearing DNA on the Microlab Prep systems produces similar DNA fragment size profiles to that of the Diagenode Megaruptor 3 system when following PacBio recommendations (figure 1). The profiles shown in figure 1 are optimal for HiFi sequencing on the Sequel® II and Revio™ systems.

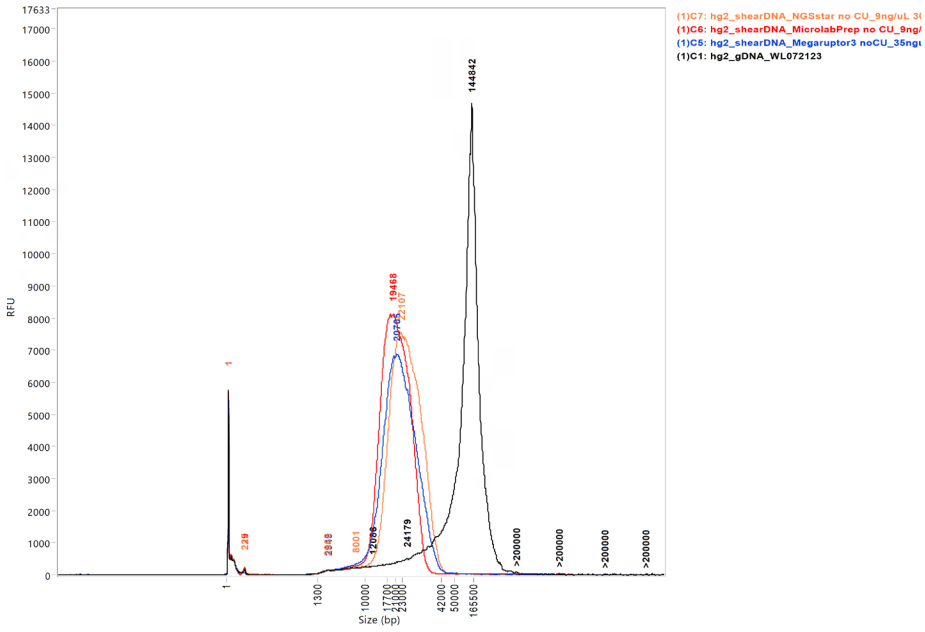


Figure 1. Agilent Femto Pulse electropherogram showing the size profile of HG002 genomic DNA unsheared (black), sheared on the Hamilton NGS STAR MOA system at 9 ng/µL (orange), sheared on the Hamilton Microlab Prep system at 9 ng/µL (red), and sheared on the Megaruptor 3 system at 35 ng/µL (blue).

The fragment size distributions, and resulting HiFi read length distributions, will shift from a lower mean size to higher mean sizes as the DNA concentration is increased (table 2). However, we do not recommend exceeding 10 ng/µL because the distributions will begin to broaden out with a greater fraction of the fragments remaining outside of the HiFi sequencing range as the concentration is increased. This will result in lower overall SMRT® Cell loading and sequencing yield. Samples with concentrations exceeding 15 ng/µL may fail to shear altogether.

The Hamilton Microlab Prep system cannot shear samples below a mean size of 14 kb except in circumstances where the DNA is highly damaged or degraded to start.

DNA concentration (ng/µL)	Mean HiFi read length (Sequel II)
15	21,290 bp
12.5	20,300 bp
10	**18,735 bp**
7.5	16,590 bp
5	16,864 bp
2.5	15,512 bp
1	15,335 bp
0.5	15,047 bp
Megaruptor 3 @ 30 ng/mL	17,806 bp

Table 2. Mean HiFi read lengths of human whole-blood derived DNA at varying concentrations sheared on the Hamilton NGS STAR MOA system with the 96 MPH. The sheared DNA was prepared using SMRTbell prep kit 3.0, pooled, and sequenced on a single Sequel II SMRT Cell. The demultiplexed results for each concentration are shown in the table.

Sequencing performance on the Revio system

HiFi sequencing of HG002 DNA sheared on the Hamilton NGS STAR system.

Metric	Value
HiFi reads	6.1 M
HiFi yield	101.22 Gb
HiFi read length N50	18,016 bp
HiFi read quality (median)	Q34
Base quality ≥ Q30 (%)	92.78%

Table 3. Sequencing performance on a Revio system. Sequencing metrics for HG002 cell-line derived DNA sheared and prepared on the Hamilton NGS STAR system. Degraded genomic DNA was depleted using the Short-Read Eliminator kit and the SMRTbell library was size selected using 3.1× concentration of a 35% v/v mix of AMPure® PB beads.

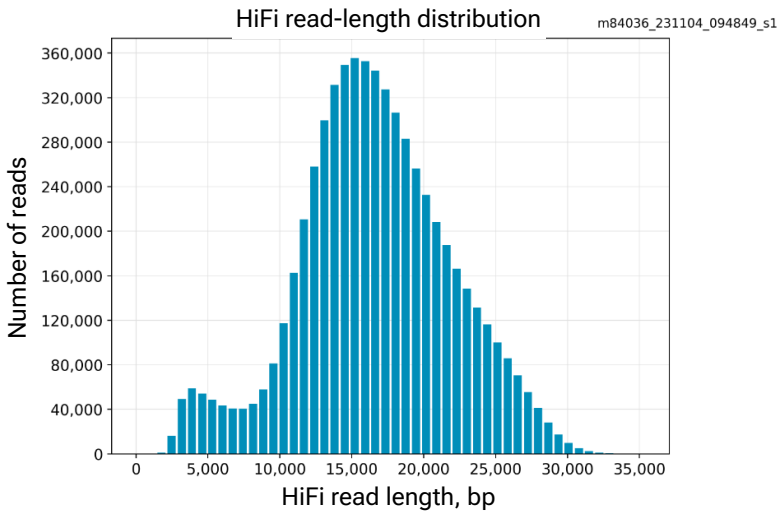


Figure 4. HiFi read length distribution of HG002 cell-line derived DNA sheared on the Hamilton NGS STAR system and sequenced on the Revio system.

Questions? Visit **pacb.com/contact** or contact your local customer service representative