PIXUL™ gDNA Shearing Kit Manual

Catalog No. 53131

(version A1)

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Overview

Highly consistent shearing of genomic DNA (gDNA) to an appropriate size for conversion into functional next-generation sequencing (NGS) libraries is integral to high quality and comparable next-generation sequencing data. Manual shearing with a probe sonicator or shearing with an inconsistent high-throughput sonicator can lead to sample-to-sample variability.

The PIXUL gDNA Shearing Kit delivers highly consistent average fragment lengths and low standard deviations across entire plates time after time. PIXUL sonication is compatible with a wide range of purified gDNA (500 ng - 20 µg) or cells (100,000 - 1,000,000) with no changes in sonication efficiency or processing time.

PIXUL Advantages

- Process 1 - 96 samples in parallel
- Extremely consistent sonication
- Up to 12 different sonication conditions per run

product	format	catalog no.
PIXUL™ gDNA Shearing Kit	1 X 96 reactions	53131

Kit Components and Storage

The kit contains sufficient reagents to sonicate 96 wells in one PIXUL run. The reagents in this kit have multiple storage temperatures. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Cell Shearing Buffer	1 mL	RT
RNase A	2 X 45 µL	-20°C
Proteinase K	600 µL	-20°C
Low EDTA TE	5.6 mL	RT
SPRI Beads	13 mL	4°C
PIXUL™ 96-well plate with sealer	1 unit	RT

Additional Materials Required

- 100% Ethanol
- Nuclease-free water
- 1X PBS
- PIXUL™ Multi-Sample Sonicator (Cat. No. 53130)
- Microcentrifuge tubes
- PCR tube strips (300 µL well volume capacity)
- Centrifuge to spin 96-well plate
- Thermal cycler or other 37°C incubator
- 96-well plate magnet or bar magnet
- Single channel and multi-channel pipettors and tips
- Hemocytometer and light microscope

PIXUL gDNA Shearing Kit Protocol

IMPORTANT: PIXUL sonication requires using PIXUL 96-well round bottom plates. These plates are available as Active Motif Catalog No. 53139, and these are the same plate as Corning Catalog No. 3799. Using any other plates will result in inefficient sonication, may damage the instrument, and will void the instrument warranty.

Sample Preparation

- If sonicating purified gDNA in water, add concentrated Tris-HCl pH 8.0 to a final concentration of 10 mM Tris-HCl.
- If sonicating cells growing directly in the PIXUL 96-well plate, aspirate media, wash with 1X PBS, and add 100 µL Cell Shearing Buffer per well.
- If sonicating cells grown in a different plate or dish, resuspend the desired number of cells in 100 µL Cell Shearing Buffer per well and add to wells in the PIXUL 96-well plate.

PIXUL Sonication of gDNA

1. Add 100 µL of purified genomic DNA or cells in Cell Shearing Buffer to wells in the PIXUL 96-well plate and seal the plate with a PIXUL plate seal.

   Note: All wells lacking sample in the columns being sonicated MUST be filled with 100 µL liquid (water, coupling buffer, etc.) prior to starting the run.

2. Turn on and run the instrument as specified in the PIXUL Multi-Sample Sonicator User Manual or Quick Guide.
3. We recommend using the following parameter specifications as a starting point, and adjusting Process Time to optimize for your specific sample and application:

Sonication Parameter	Setting
Pulse [N]	50
PRF [kHz]	1.00
Process Time [min]	36:00
Burst Rate [Hz]	20.00

Note: The instrument software will not allow settings that could damage the transducers. The upper limits are a maximum Pulse of 50 or a maximum PRF of 1.00. We recommend a minimum Burst Rate of 20.00. Varying the Process Time is typically the only parameter that needs adjusting.

DNA Purification using SPRI Beads

Take SPRI Beads out of 4°C and warm to room temperature by simply placing the vial of SPRI beads on the benchtop. Prepare fresh 80% ethanol (4.8 mL 100% ethanol + 1.2 mL nuclease-free water).

1. Add 0.5 µL RNase A to each well and incubate for 30 minutes at 37°C.
2. Add 5 µL Proteinase K to each well and incubate for 30 minutes at 55°C.
3. Add 120 µL (1.2 volumes) of SPRI Beads to each well. Pipette the bead-lysate mixture 10 times to mix and incubate for 5 minutes at room temperature.
4. Place plate on a magnet for 5 minutes to collect beads, and then aspirate supernatant.
5. Keeping the plate on the magnet, add 180 µL of 80% ethanol and incubate for 30 seconds at room temperature.
6. Aspirate the supernatant and perform a second wash with 180 µL of 80% ethanol.
7. Completely aspirate the supernatant and air dry the beads until they are no longer shiny (2-5 minutes).
8. Remove the plate from the magnet and elute DNA by adding 50 µL of Low EDTA TE.
9. Pipette the beads 10 times and incubate for 3 minutes at room temperature.
10. Place tube or plate back on the magnet for 5 minutes to collect beads.
11. Carefully remove eluted DNA in the supernatant and transfer to a new tube or plate.
12. Analyze gDNA yield using a spectrophotometric (Nanodrop) or fluorometric (Qubit) method of your choice and analyze fragmentation efficiency using either agarose gel electrophoresis, Agilent TapeStation, or Agilent Fragment Analyzer. For most applications, we recommend shearing DNA to 200 - 600 bp. This gDNA is suitable and ready for library preparation.

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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