Bauer Core Standard Protocol		
Title: Hydroshear Protocol		
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1. Purpose

This protocol describes how to use the GeneMachines (Genomic Solutions) Hydroshear to reproducibly fragment DNA. The protocol is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

2. Materials

2.1. DNA sample(s) (see preparation instructions in section 4.1).
2.2. Wash Buffers (see preparation instructions in section 4.2).
2.3. Tubes to hold sheared DNA.
2.4. Shearing assembly.
  2.4.1. The Bauer Center provides standard shearing assemblies which can be used to shear DNA to sizes between 1 and 9KB. Users who would like to use either small (650B to 4KB) or large (4KB to 40KB) assemblies must provide their own.

3. Instrumentation

GeneMachines Hydroshear (now made by GenomicSolutions)

4. Reagent preparation

4.1. DNA sample(s)
  4.1.1. Resuspend the DNA sample in your buffer of choice (ddH_2O or TE, pH 8.0).
    4.1.1.1. The concentration should be from 0.02ug/ul to 0.25 ug/ul.
    4.1.1.2. The volume should be from 40ul to 500ul.
      Note that for sample volumes >300ul, a larger output tube must be used.
    4.1.1.2. The DNA **must** be completely in solution or it will clog the assembly.
      Follow the instructions below to resuspend the DNA.
  4.1.2. Incubate for 30 minutes at 37°C, vortexing every 10 minutes.
  4.1.3. Immediately before shearing, centrifuge at 14,000 rpm for 15-25 minutes.
  4.1.4. If a pellet forms after centrifugation repeat steps 4.1.2 and 4.1.3.
  4.1.5. If a pellet still forms after repeated incubations, add more resuspension buffer.
  4.1.6. Whether or not you see a pellet, transfer the DNA solution to a clean tube, leaving behind the pellet if there is one.

4.2. Wash Buffers

4.2.1. Buffer 1: 0.2M HCl, 0.2 micron filtered.

4.2.2. Buffer 2: 0.2M NaOH, 0.2 micron filtered.

4.2.3. Buffer 3: ddH_2O or TE, pH 8.0, 0.2 micron filtered.

5. Procedure

5.1 Setup.

5.1.1. Open the Hydroshear software.

5.1.2. Set shearing parameters.

5.1.2.1. Volume: sample volume (40ul to 500ul).

5.1.2.2. Number of Cycles: 20 cycles is standard.

5.1.2.3. Speed Code: The standard assembly can shear at speeds from 3 to 40. Small speed codes indicate faster speeds which produce smaller fragments.

5.1.3. Click "Edit Wash Scheme" and choose how many times to wash in each buffer. A typical wash consists of 4 cycles in each buffer.

5.2. Shearing.

5.2.1. Hit "Start".

5.2.2. Click "OK" to perform a wash unless the device has just been cleaned.

5.2.2.1. Follow the prompts on the screen.

5.2.3. Follow the sample loading prompts until "turn the valve to output and click OK".

5.2.3.1. **Only turn the valve halfway so the handle is vertical**

5.2.3.2. Click "OK" and watch the bubble rise as air is pushed out of the syringe.

5.3.2.3. When the bubble rises to the top of the sample, turn the valve to output.

5.2.4. Continue following the prompts for shearing, sample ejection, and washing.

5.2.4.1. Always perform a wash after each sample.

5.3. Shut down.

5.3.1. Always finish with a water rinse

5.3.2. Shut down the software.

5.3.3. Clean the area and remove any buffers or tubes that were used.

5.4 Tips and Tricks

5.4.1. To maximize sample recovery after shearing, aspirate and expel air instead of immediately performing a wash. The last bit of sample will be expelled.

5.4.2. When using the "standard" assembly, use a speed code of 3 or higher. When using a "small" assembly, determine the fastest possible speed (lowest number) by starting at 15 and trying faster and faster speeds. Using a speed that is too fast can cause leaks or over pressure failures.

5.4.3. When using a "small" assembly, use the manual settings to perform washes to allow for slower speed codes.

5.4.4. There are two ways to unclog the assembly:

5.4.4.1 Perform the sonicating washes described in the manual.

5.4.4.2. Turn the assembly around and run "backwards".