DNA Sonication using Sonic Dismembrator 550

I. Purpose

To sonicate DNA and cDNA for Illumina library construction or other application

II. Scope

All procedures are applicable to the BCGSC FG-Library Construction Core.

III. Policy

All production procedures shall be documented and controlled by approved systems.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Production Coordinator to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
N/A	N/A

VI. Related Documents

SOP Title	SOP Number
N/A	N/A

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information. Note that Optime 105 – Over-the-head Earmuff must be worn to ensure ear protection during the sonication (step 4.1). This device has an NRR (noise reduction rating) of 29dB and is certified for ear protection from sonication.

Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue	
Fisherbrand Textured Nitrile gloves	Fisher	270-058-53		✓
Ice bucket – Green	Fisher	11-676-36		✓
wet ice	In house	N/A	N/A	N/A
1.5mL Eppendorf tube	Ambion	12400		✓
Premium 2.0mL Microtubes (RNase/DNase/Pyrogen Free)	Diamed	PRE2000-N		✓
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Neptune barrier tips 10µL	CLP	Bt10XL		✓
Neptune barrier tips 20µL	CLP	Bt20		✓
Neptune barrier tips 200µL	CLP	Bt200		✓
Neptune barrier tips 1000µL	CLP	Bt1000		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100		✓
Large Kimwipes	Fisher	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Benchcote (Bench Protection Paper)	Fisher	12-007-186		✓
Small Autoclave waste bags 10”X15”	Fisher	01-826-4		✓
TE (pH8.0)	Ambion	9849		✓
Sonic Dismembrator 550 (cup horn)	Fisher	Discontinued	N/A	N/A
Foam tube holder	Ambion Sample	N/A	N/A	N/A
Optime 105 – Over-the-head Earmuff	McMaster-Carr	9206T2		✓

IX. Procedure

NOTE: PERFORM ALL PROCEDURES IN THE SONICATOR WORKSTATION:

1. Retrieval of reagents and equipment preparation

1.1. Put on a clean pair of gloves and lab coat.

1.2. Wipe down the workbench, small equipment, and ice bucket with DNAway.

1.3. Lay down new benchcoat.

1.4. Change gloves.

1.5. Retrieve fresh ice and all required reagents.

2. Preparing sample for sonication (00:00 – 0:30 hr)

2.1. Retrieve DNA sample. The minimum amount of material varies depending on protocols, discuss with APC to see how much starting material to use. Once the amount of sample is determined, aliquot the DNA sample into the labeled 2.0ml **conical** microcentrifuge tube.

2.2. Sonication volume can vary from a minimum of 30µl to a maximum of 500µl. Please discuss with APC if unsure of how much volume the sample should be in. Make up sample to the final volume with TE.

2.3. Vortex the sample briefly to mix the sample.

2.4. Spin down sample briefly with the Galaxy mini-centrifuge.

3. Setting up Sonicator

3.1 Connect either end of coaxial High Frequency Cable to SHV connector on rear panel of generator, and connect other end of cable to SHV connector on the convector. Push the connectors on and turn the chrome rings ¼ turn to secure the connectors.

3.2 Mount the convertor and the cup horn onto a table mounted holder. Ensure the converter is secure by turning the lever attached to the mounting apparatus. Do not over tighten

3.3 Put the two black screws into the cup horn.

3.4 Fill the cup with water and a little bit of ice to the black mark on the cup horn.

3.5 Turn generator on by pressing "I" on Power Switch, switch will illuminate when power is on.

3.6 Press CLEAR to select Programmed Mode.

3.7 Press PROG?DATA to select programmed mode; display will show program screen and memory location.

3.8 Press PROG key a second time to begin programming the selected memory location.

3.9 Enter the Process Time. If unsure of the process time, discuss with PC/APC.

3.10 Press ENTER to save your entry in memory; display will switch to Pulse On data screen.

3.11 Enter pulse on time 30 sec and press Enter.

3.12 Enter pulse off time 30 sec and press Enter.

4. Sonication (0:30hr – 1:30hr)

4.1. Post the sign "sonication in progress, do not enter" on the door of the sonication room. Wear the Over-the-head Earmuff to ensure proper ear protection.

4.2. Secure sample (2.0mL tube) in the cup horn by using the tube holder. Sit the sample right on top of the hole on the radiating surface of the cup horn.

4.3. Press start to begin processing sample as programmed. Adjust amplitude setting to "7".

4.4. Press PAUSE if you want to interrupt sample processing, then press PAUSE again to continue.

4.5. Once the program finishes, it will stop on its own. The program timer will automatically reset itself.

4.6. Return amplitude setting to "0".

4.7. Press "O" on the Power Switch to turn generator off.

4.8. Disconnect cable from SHV connector on the convector.

4.9. Dispose all waste (including pipette tips waste bag, benchcoat), ice, and partially used reagents aliquots.

4.10. Wipe dry the inside of the cup and lay it down on the workbench.