1. PURPOSE

1.1. This SOP describes the lysis of expression cells by sonication to harvest whole cell lysate and supernatant to assess recombinant protein expression and solubility.

2. SCOPE

2.1. These methods have been developed by the Center for Structural Genomics of Infectious Diseases (CSGID) group. It is the responsibility of all research associates in this group to read, understand, and adhere to this document.

3. REQUIREMENTS

3.1. Material Requirements

3.1.1. Lysonase™ Bioprocessing Reagent-1mL (Novagen: Cat# 71230-4)
3.1.2. 1M DTT (kept in -20ºC, see Appendix)
3.1.3. High Salt Buffer (pH 7.3, for components see Appendix)
3.1.4. Cold water

3.2. Equipment Requirements

3.2.1. 12-channel Rainin pipettor
3.2.2. Misonix Sonicator* 3000 Homogenizers
3.2.3. Misonix* Sonicator* 3000 Microplate Horn
3.2.4. NanoDrop™ ND-1000 Spectrophotometer
3.2.5. Hardshell PCR 96-well plate, clear-well (BioRad; Cat# HSP-96)
3.2.6. Plate Centrifuge
3.2.7. Hearing Protectors
3.2.8. Ice and Ice bucket
3.2.9. Timer

3.3. Environmental Requirements (storage conditions)

3.3.1. High Salt Buffer (store at 4ºC. Expires in 4 months after initial prep).

4. PROCEDURE

Note: Please be extremely meticulous when handling plasmids, culture, and/or isolated proteins to avoid well-to-well cross-contamination. Cross-contamination can happen at any step and will lead to protein mixtures inevitably complicating downstream expression and solubility screening.

4.1. Pellet Resuspension

4.1.1. Remove one of the Hardshell PCR 96-well plates containing overexpression pellets from the -80^{0}C (see SOP #: CSG-004) and thaw on ice.

4.1.2. Prepare High Salt **Lysis** buffer fresh before each sonication:

Component	Volume (ml)	Final Concentration
High Salt Buffer (pH 7.2)	10	-
Lysonase	0.0057	1:1754
DTT (1 M)	0.01	1 mM

4.1.3. Using the Biomek method, add 50 µl High Salt Lysis Buffer to each well. The method is under the directory \\Netvenus\pfgrc\Biomek_Central\Biomek_Multi96\Multi96_Methods\CSGID-HSB To OE Pellet Plate.bmf

4.1.4. Check the absorbance, $OD_{600,}$ for a couple of wells before and after sonication using Nanodrop. Choose the "Cell Cultures Module" when prompted.

Note: A ten-fold decrease in OD_{600} value after sonication is a good indication the cell lysis is performed successfully.

4.1.5. Seal the plate with clear tape pad seal and remove the white edge. Keep the plate on ice at all times.

4.2. Sonication

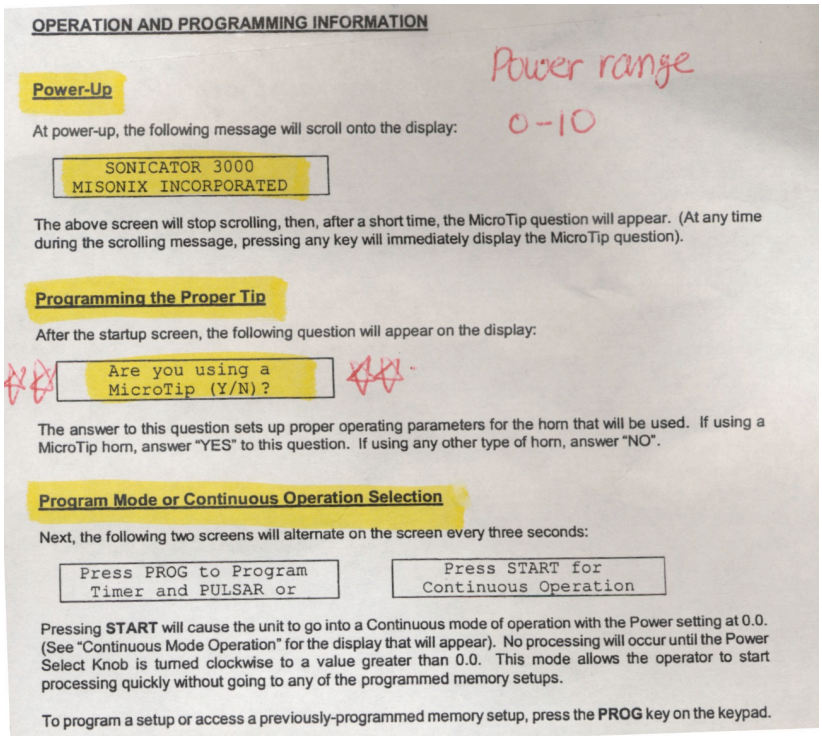
4.2.1. Attach the power cable to the reservoir in the Microplate Horn.

4.2.2. Fill the reservoir with water so that the plate can float when placed in the reservoir. Add ice to cool the water down.

4.2.3. Remove the ice. Place an empty beaker under the drainage tube that is connected to the Microplate Horn. Drain the extra water by opening the valve. Leave enough water in the reservoir to let the plate float.

Note: After removing enough water, don't forget to close the valve.

4.2.4. Place the plate in the reservoir. Put down the lid of the Microplate Horn. Follow the programming information below to start the sonication.



OPERATION AND PROGRAMMING INFORMATION

Power range 0-10

Power-Up

At power-up, the following message will scroll onto the display:

```
SONICATOR 3000
MISONIX INCORPORATED
```

The above screen will stop scrolling, then, after a short time, the MicroTip question will appear. (At any time during the scrolling message, pressing any key will immediately display the MicroTip question).

Programming the Proper Tip

After the startup screen, the following question will appear on the display:

```
Are you using a
MicroTip (Y/N)?
```

The answer to this question sets up proper operating parameters for the horn that will be used. If using a MicroTip horn, answer "YES" to this question. If using any other type of horn, answer "NO".

Program Mode or Continuous Operation Selection

Next, the following two screens will alternate on the screen every three seconds:

```
Press PROG to Program
Timer and PULSAR or
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Press START for
Continuous Operation
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Pressing **START** will cause the unit to go into a Continuous mode of operation with the Power setting at 0.0. (See "Continuous Mode Operation" for the display that will appear). No processing will occur until the Power Select Knob is turned clockwise to a value greater than 0.0. This mode allows the operator to start processing quickly without going to any of the programmed memory setups.

To program a setup or access a previously-programmed memory setup, press the **PROG** key on the keypad.

Note: You can put on the Hearing Protectors.

4.2.5. The answer to "***Are you using a microtip***" is **N**.

4.2.6. Set a timer and sonicate for **2 minutes** by pressing the start button and turning the Power Select Knob to **5.5.**

4.2.7. After 2 minutes, stop sonication by turning the Power Select Knob counterclockwise bringing it to **0.0.**

4.2.8. Remove the plate from the reservoir and put it on a bucket of ice. While the plate is cooling down, sonicate another plate.

Note: It is possible to work on four plates in a sonication/chilling cycle at a time. Do a total of seven sonications per plate (it takes ~45 min). After sonicating two plates, add ice to cool down the water in the reservoir. Remove the ice by hand and the extra water by opening the drainage valve. After removing enough water, don't forget to close the valve.

4.2.9. Take OD_{600} reading of the same wells that were checked before the sonication started.

4.2.10. If the lysis is complete, prepare the plate for expression and solubility screening.

4.3. Expression and Solubility screening

4.3.1. Prepare a whole cell Lysate plate for Caliper analysis

Note: Compare the absorbance, $OD_{600,}$ values before and after IPTG induction (see SOP #: CSG-004, 4.3.4 and 4.5.1). If there is no significant change in the values, when preparing the plates for Caliper dilute the samples in less than 35 µl water and adjust the program accordingly. But if the OD_{600} values have changed significantly, dilute the samples in 35 µl water as stated in the table below.

Final OD_{600}	Sample (µl)	HT protein Express Buffer (µl)	Water (µl)	Total (µl)
If > 1.5	2	7	35	44
If < 1.5	8	7	29	44

4.3.1.1. Using the Biomek method transfer and mix 2 µl (or 8 µl) of whole cell lysate into 7 µl of HT Protein Express Sample Buffer in a 96 clear-well PCR plate. The Biomek method is found under the directory:

\\Netvenus\pfgrc\Biomek_Central\Biomek_Multi96\Multi96_Methods \CSGID-Lys or Sup to Caliper Buffer Plate.bmf

4.3.1.2. Centrifuge the remaining cell lysates at 2000 RCF for 30 min

4.3.2. Prepare a Supernatant plate for Caliper analysis

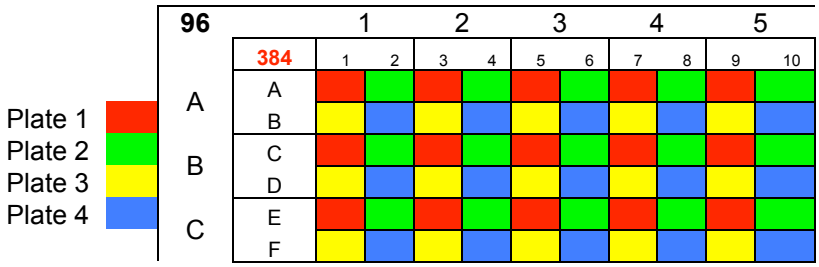
4.3.2.1. Without mixing or disturbing pellet, carefully transfer approximately 20 µl of the supernatant to a new Hardshell PCR 96-well plate.

4.3.2.2. Using the Biomek (see 4.3.1.1) transfer and mix 2 µl (or 8 µl) of supernatant into 7 µl of HT Protein Express Sample Buffer in a 96 clear-well PCR plate.

4.3.3. Rearraying the plates

4.3.3.1. Boil the Lysate and Supernatant plates from 4.3.1.1 and 4.3.2.2, using the thermocycler, at 100ºC for 5 min and quick spin. Use the Biomek method under the directory \\Netvenus\pfgrc\Biomek_Central\Biomek_Multi96\Multi96_Methods \CSGID- Caliper MJR96 to Corning384 Plate.bmf to add 35 µl (or 29 µl) H2O and rearray the plates as necessary so the lysate and supernatant are next to each other (see diagram below).

Note: Two lysate and two supernatant 96-well plates can fit in one 384-well plate.



4.3.3.2. If not running caliper after, plates can be stored at -20^{0}C. To run caliper, follow the SOP #: PEP-009 which is posted here: https://intranet.jcvi.org/cms/node/2466

5. APPENDIX

5.1. High Salt Lysis Buffer [pH 7.3 at RT, pH 7.8 at 4^0C]

Reagent	Formula weight	Mass of Reagent Or Volume	Concentration (Final)
Tris (Trizma)	121.14	6.056 g	50 mM
NaCl	58.44	17.53 g	100 mM
Imidazole	68.08	0.68 g	10 mM
0.5M EDTA		1.0 ml	0.1 mM

Bring up to 1 L with Milli-Q water. Adjust the pH with HCl to 7.3.

5.2. DTT

Reagent	Formula weight	Mass of Reagent (g)	Solvent	mL	Concentration
DTT	154.25	0.154	Milli-Q water	1.0	1 M