96-well Mini-Expression SOP

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Purpose: Do mini-expression and lysis of cells in 96 well plates.

Materials/Reagents/Equipment	**Vendor**
Disposables	
96 sterile deep well 2.2 ml Plate (DWP)	Beckman
96 well PCR plate (PCR)	
Sterile applicator sticks.	
Reagents	
Luria Broth + antibiotic	
M9 minimal media + antibiotic	
10 mM IPTG @ 0.3 mM	
Equipments	
Infors Shaker	
Multichannel pipettor	
Matrix Electrapette pipettor	
Misonic Plate sonicator Model S-3000-001	1-800-645-9846 aalivio@misonix.com (Audrey, X 179)
Lifeline Plate Shaker	

Procedure:

A. Inoculum:

Day 1.

1. Dispense 0.2 ml of LB into snap cap tube. Inoculate with 1 or 2 colonies from a fresh transformation plate using a sterile applicator stick.
2. Shake cells at 37˚C for at least 5 hrs until the cells are dense. Store at 4˚C overnight.

B. Growth:

Day 2 [ ]: temperature at induction

1. Prewarm needed sterile media (with antibiotics) in a 37˚C incubator 30 minutes prior to use. For example, if you are going to need 20 ml, dispense 25 ml into a sterile flask and prewarm. Prewarm two 96 sterile deep well plates (DWP). Prewarm Infors incubator at 37˚C.
2. Dispense 1 ml M9 [**30˚C**] into as many wells as you have clones using the Matrix Electrapette. These will be done on the same 96 DWP [I]. Dispense 1 ml M9 [**18˚C**] into a separate 96 DWP [II]. Tape the aluminum foil over the plate. Tape the plate to an eppendorf rack so that the DWP is at a slant.
3. Take 10 µl from A2 and inoculate each of the wells: 1ml M9[30˚C], 1ml M9[18˚C]. This is a 1:100 dilution. Shake at **37˚C** at 250 rpm. for 3 hr. The cells should reach an OD600 of 0.8.

C. Induction:

It is crucial to not allow the cells to sit for more than 3 minutes during the addition of induction reagents. Cells will rapidly die if not aerated.

1. Take plate I and shake at 30˚C for 15 min. Take plate II and shake at 18˚C for 15 min. Induce all the wells with 30 µl of 10mM IPTG (@ 0.3 mM. Rinse pipet tip up and down. Shake for 4 hr. at 30˚C or overnight at 18˚C.

D. Harvesting/resuspension:

1. Take DWP and spin for 15 min, 4˚C, 4000 rpm (3000 g). Shake upside down 3 X to remove the supernatant, then add 100 µl 100 mM Tris, pH 7.5 to each well. Resuspend the pellet by shaking for 5 minutes at speed 1400 to resuspend the cells.

E. Sonication in Eppendorf tubes:

1. If using a microtip sonicator on a 1 ml culture pellet, transfer each sample to a pre-labelled 1.5 ml eppendorf tube. Place the tubes in an ice bucket. It is of utmost importance to keep the samples cold. Sonicate 3 x 5 sec, chilling the sample for 1 min in between each sonication. Do not allow foaming to occur, as this denatures the protein. Centrifuge for 5 minutes at 12,000 rpm at 4˚C.
2. Carefully pipette the supernatant into a labeled 1.5 ml eppendorf (SN) and add 100 µl 100 mM Tris, pH 7.5 to the pellet, resuspend (P).
3. Remove 10 µl from SN and P tubes, respectively, and mix with 5 µl of 2X sample buffer. Boil for 5 min, run on SDS/PAGE.

Sonication in 96 well PCR plate:

1. Transfer samples (Step D-1 from above) to a 96 well PCR plate using a multichannel pipettor. Chill the Misonic sonicator by placing crushed ice in the horn cup for 10 minutes. Drain the ice and refill the horn cup with ice. Make sure that the bottom of the PCR plate is in direct contact with the water/ice slurry. Sonicate the plate for 1 minute, turning it often. Stop the sonication, drain the water, add more ice. Repeat the process 5 more times (total of 6 minutes) until the cells are lysed. The sonicator can be programmed to do this. Remove 10 µl of each sample to a new PCR plate containing 5 µl of 2X sample buffer/well: **Total protein.**
2. Centrifuge PCR plate for 15 minutes at 3700 rpm (3500xg). Transfer the supernatant to another 96 well PCR plate using a multichannel pipette. Take 10 µl of this supernatant and transfer to a plate containing 5 µl of 2X sample buffer: **Cleared lysate sample.**
3. Heat samples from Steps 1 and 2 above for 3 min at 95˚C in a PCR machine or heat in a water bath. Load SDS/PAGE gel.