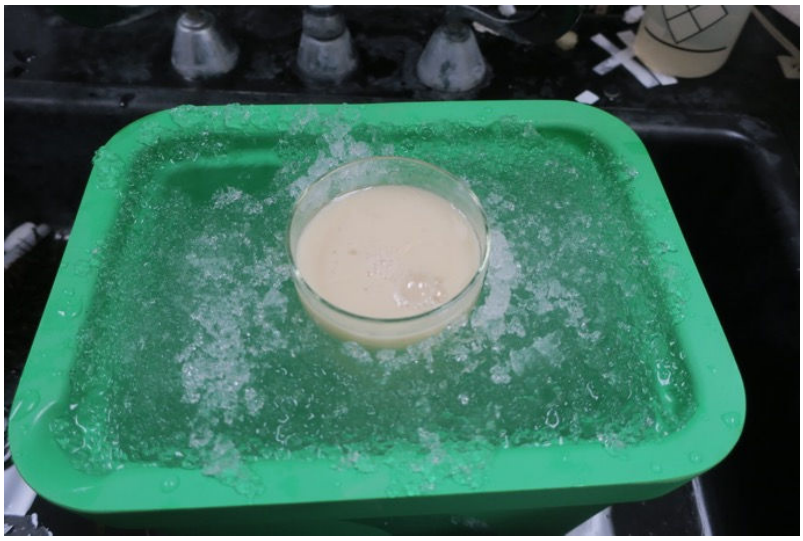
Sonicator Protocol

Sonicating large quantities of *E. coli* cell paste for structural biology.
Written and photographed by Nat Clark, 5/21/2018

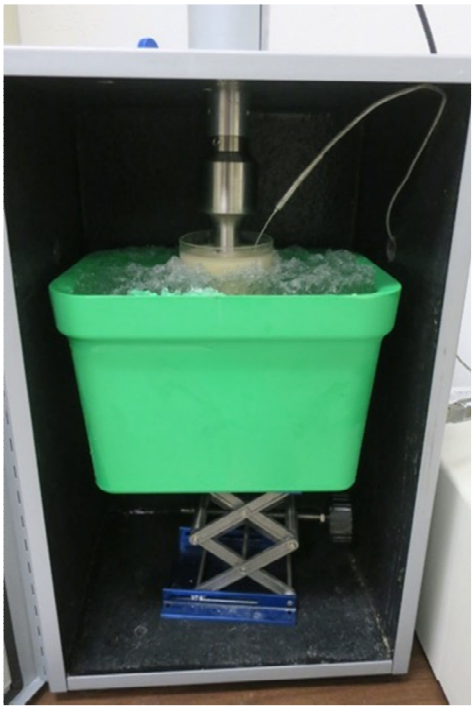
1. Re-suspend frozen E. coli pellet at ratio of 5 ml lysis buffer/ 1 gram cell paste by stirring. Here, 200 grams of E. coli pellet are suspended in 1 L of lysis buffer with protease inhibitors. The 200 grams of cell paste was obtained from 9 liters of BL21 cells grown cultured in terrific-broth.

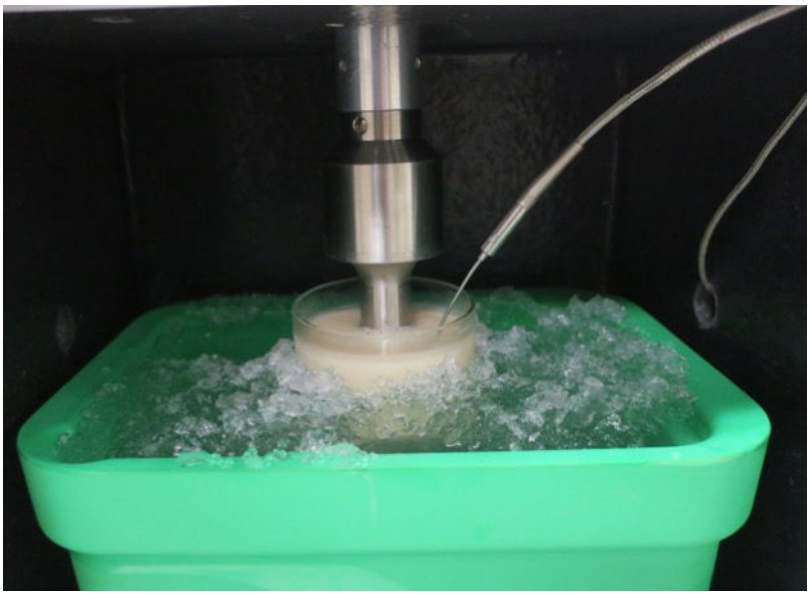
2. Pour 500ml into a Rosette Cooling cell.

3. Place Rosette cell into an ice water bath. The water will significantly improve cooling compared to packing the cell in solid ice only.



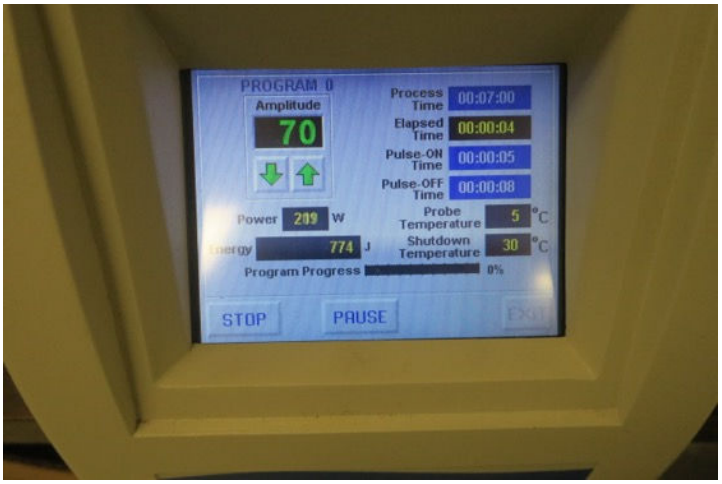
4. Sonicate using the Q700 system with 1" high-intensity sonicator horn (#4311). Insert thermocouple into channel of Rosette cell. Adjust intensity if needed to control overheating.





*Note newer sound reducing enclosures allow the horn to move up/down so a jack stand is no longer required to hold the vessel.

Sonication settings: 70% amplitude; Pulse 5 sec ON/8 sec OFF; 7 min total sonication time.



5. After sonication the color will be darker and viscosity will be decreased.

Pre-sonication Post sonication

6. Clarify by centrifugation then proceed with downstream purification.