

## Microfluidizer Protocol

*The Rout lab uses a Microfluidizer® (Microfluidics, product # M11 OS) to lyse large quantities of bacteria while keeping them cool. The cells are resuspended in buffer, pushed through a patented chamber at high pressure, and passed through a cooling coil.*

1. Resuspend cell pellet in 4 volumes of the buffer of your choice.
2. Polytron 1 minute at setting 4 to fully resuspend cells.
3. Tighten screws on pressure compartment.
4. Fill tray with ice/water slush (make sure clamp is closed)  
If more than 20mL of cell suspension, pull to drain (<20mL cells push to recycle).  
Use clamp to attach bucket (for <20mL cells bucket is not needed)
5. Open valve to create vacuum.  
The red handle is lowered and raised to start and stop the machine.  
Lower the red handle to start the machine. Let it go 3 chunks to get isopropanol out.
6. Add ~100mL water. Empty until 3 chunks after the top of the base.
7. Repeat water wash.
8. Wash once with ~20mL lysis buffer.
9. Pass cells through microfluidizer  $\geq 5$  times for *E coli* (15 times for yeast). Each time, allow cells to go 3 chunks past the top of the base.
10. After lysis, wash out with buffer until it runs clear. Wipe foam from bucket.
11. Wash well with water (use all of a 500mL bottle).
12. Wash and fill with 70% isopropanol for storage.
13. Turn off vacuum.
14. Open white clamp at the bottom by the bucket to allow liquid to drain from the ice/water tray.  
Cover base with parafilm.