



Material needed:

- 1- Growing *E-coli* (mid log phase)
- 2- 80% glycerol stock (in water, autoclaved)

To make the E-coli stock:

- 1- Inoculate 5 ml LB media using a single colony. Allow to grow to mid log phase. Do not allow the solution to saturate.
- 2- In a sterile tube (o-ring tubes), add 800 ul of growing E-coli.
- 3- Add 200 ul of 80% autoclaved glycerol.
- 4- Mix briefly, by vortex, and store at -80 °C

Growing cells from glycerol stock:

With sterile pipet tip, touch the surface of the glycerol stock (DO NOT THAW), and throw the tip in 5 ml LB media.