



Glycerol Stocks

Last Revision: August 4, 2017

Version: 1.0

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Validated by / Date:

Glycerol stocks allow for long-term storage of plasmids. This protocol assumes you are starting from bacterial colonies on an agar plate.

1. Create sterile conditions.
 - a. We use a benchtop flame to create sterile conditions. Approximately 6 inches around the flame can be considered sterile.
 - b. Always wear gloves and a lab coat.
2. Prepare a bacterial culture tube (CAT #352059) with 5 mL of LB and 5 μ L 1000X of appropriate antibiotic (see buffers and media protocol).
3. Pick a single colony using a sterile inoculating loop, and then submerge the loop into the culture tube LB.
 - a. One can substitute a 10 μ L micropipette tip for an inoculating loop, but care must be taken to ensure sterility of the gloves holding the tip.
4. Grow culture overnight at 37°C in shaking incubator at 200 rpm (16-18 hours).
 - a. If the plasmid of interest is lentiviral or is an empty gateway vector, grow at 30°C (it may take up to 24 hours).
5. The next day, under sterile conditions, take 250 μ L of overnight culture and add to 250 μ L sterile 50% glycerol (CAT #G33-1) in a sterile 2 mL cryotube (CAT #377267).
6. Label tube with plasmid name, bacterial strain, resistance and date, and store at -80°C.
7. Record the information in LabGuru.