

Plating from Frozen Spore Stocks

Important: Be careful not to let any of the stocks thaw.

- 1) Pull the frozen stock of the clones that you want to plate from the coffin -80°C freezer.
- 2) Put them in the -20°C freezer till you are ready for them.
- 3) Start flame on bench.
- 4) Label bottom of plate with the clone name, your name, and the date.
- 5) Label 0.5 mL autoclaved eppendorf tube with name of the clone.
- 5) Flame mouth of test tube containing fresh overnight. Pipette up 200µl of fresh overnight. Flame mouth of test tube and cap.
- 6) Pipette 200µl of fresh overnight into 0.5 mL eppendorf tube
- 7) Get the frozen stock you are plating from the -20°C freezer.
- 8) Use either an autoclaved 1000µL pipette tip (blue) or a flame sterilized loop to scrape up some of the frozen stock.
- 9) Swirl the blue tip or loop into the 200µl of fresh overnight of the appropriate eppendorf tube.
- 10) Vortex the tube for 10 seconds.
- 11) Pipette up the contents of the eppendorf tube and transfer to appropriately labeled SM/5 plate.
- 12) Sterilize spreader with ethanol and flame. Allow spreader to cool (test by touching it to the agar away from the bacteria). Spread the solution evenly over the plate using the sterilized spreader.
- 13) Return the frozen stock to the -20°C freezer.
- 14) Repeat steps 4-10 for other clones you wish to plate out.
- 15) Turn off flame.
- 16) Return the frozen stocks to the -80°C freezer.
- 17) Put the plates in a safe place to develop (agar side down).