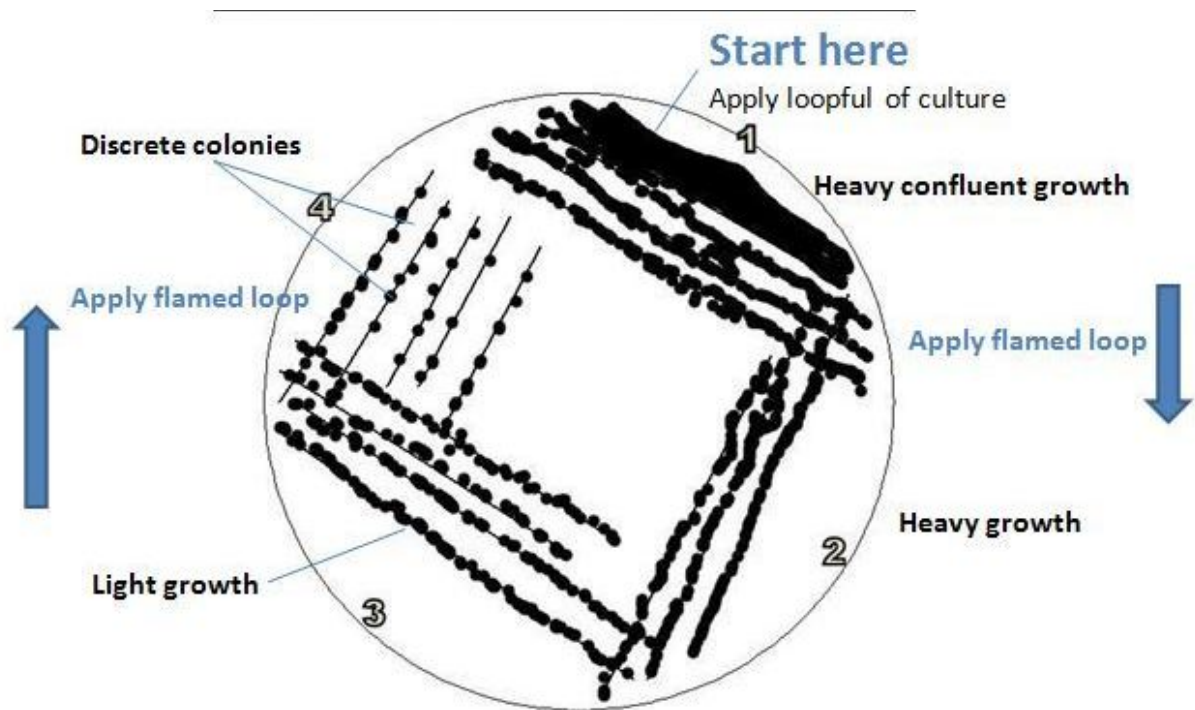


When you receive a plasmid from Addgene...

Work under sterile conditions! (that means under the flame and using pipets that have been sprayed with 70% ethanol)

1. The plasmids come in bacteria (typically E. coli DH5alpha, I don't know if that can vary) in an agar stab.
2. Have growth medium ready: 5mL of sterile LB broth + 5μL of carbenicillin (if your plasmid has the ampicillin resistance gene) or Kanamycin (or any other suitable antibiotic, check the data sheet to confirm the resistance gene of your particular plasmid).
3. Carefully insert your pipet tip through the hole left by the stab and move around (the bacteria are mostly there but they should have grown to the nearby areas too). I always think I did not take anything, but (almost) always you do take bacteria! (all you need is one ☺)
4. Transfer pipet to the LB broth and pipet up and down a few times. You can also leave the pipet tip in the broth.
5. Grow at 37°C, shaking at ~200rpm for a few hours (2-6h). The solution will turn cloudy.
6. Have your agar plates ready at RT (again, check compatibility of antibiotics).
7. Drop 20μL of culture on the side of the agar plate and using a looped glass pipet streak like this (or ask Evelyn if you need assistance):



8. Culture overnight at 37°C (no shaking)
9. Pick one single colony and transfer it to the desired volume of LB broth-antibiotic. The amount will vary depending on whether you want to proceed with a MiniPrep (2-5mL) or MaxiPrep (100-200mL).