Inoculation into liquid culture

To transfer bacterial colonies from a LB agar plate to liquid culture (LB broth).

We picked 8 colonies from each plate for colony PCR and subsequent sequencing. The number of colonies will be variable according to the level of confidence we have on the Gibson assembly reaction and transformation.

A typical cloning procedure is:
PCR -> Gel running-> PCR purification -> Gibson Assembly or digestion and ligation-> Transformation -> **inoculation**-> Colony PCR (this process can be skipped according to our project schedule) -> miniprep to obtain the plasmid

Materials:

› Liquid Growth Media (LB broth)
› Use LB (high growth media) for colony PCR and miniprep
› LB agar plates with bacterial colonies
› Antibiotics (ampicillin for most of the time)
› Falcon tubes (15 mL or 50 mL)
› Shaking Incubator

Procedure:

1. In the hood, pour in an appropriate volume of medium in a 10 or 15ml falcon tube. Precision is not very important here.
2. In the hood, add antibiotics according to the ratio of 1: 1000 in volume for the antibiotics and media.
3. In the hood, invert the falcon tube a few times to mix the antibiotic with the media.
4. Identify the target colonies.
5. The colony should be relatively small, a seemingly big colony can sometimes come from merging of several neighboring colonies
6. The colony should be spatially isolated, so that you can easily pick the colony without accidentally touching any other colonies on the plate, ensuring that we have a clonal population of cells in each culture tube.
7. If you can rely on an expected visual phenotype (GFP or RFP under strong expression is visible with naked eyes), make sure your colony satisfy this criteria.
8. Using a new pipette tip, gently scrape off the cells from your colony from the surface of the agar.
9. You don't have to press strongly against the agar-- just a gentle brushing of the surface is sufficient to get the cells off the agar and onto your tip

10. Swirl the pipette tip around in the liquid media in a culture tube, or you can just leave the tip in the liquid but make sure the tip is absolute uncontaminated.
11. You will have to tilt the tube so the tip can reach the liquid.
12. Set the tube aside and proceed to the next colony.
13. Put the culture tubes in the shaking incubator
14. Make sure the incubator is set to the right settings (for minipreps we use 250 rpm at 37°C). If you are doing an experiment or using another strain, it may be different.
15. Make sure that, once in the incubator, the caps on the tubes are loosened so that air can flow easily into the tubes.
16. It takes around 14 hours for the cultures to be ready for miniprep.
17. If you use the culture too soon then the yield of the plasmid DNA will be lower. If you isolate the plasmid too late the culture may be too old and will have lower yield