Imaging of transformed P.chlororaphis

We have transformed P.chlororaphis with a plasmid and either stored the plates at 4°C or set up a frozen glycerol stock at -80°C.

1. Make 3-5mL ON liquid culture of P.chlororaphis with plasmid, in LB or SOC with antibiotics (for example Streptomycin 400), incubate ON at 28°C shaking at 180rpm. Note that P.chlororaphis generally takes longer to grow than E.coli, so you may want to set up the liquid culture in the morning the day before rather than in the evening.
2. Next morning, dilute to OD600 of 0.2 (in 3mL LB or SOC with antibiotics), let grow to OD0.4-0.6 (approximately 2-4h) at 28°C shaking at 180rpm.
3. Induce with IPTG (10mM -> 2.5µl of a 100mM stock in 25µl) -> let grow at 28°C shaking 180rpm for 2-4h total. Depending on the protein, shorter or longer induction is better (ON induction is also possible, but didn't work for us. We had the best results with 2h). Different IPTG concentrations can also be used, literature recommends 0.1-1mM but for us, 10mM yielded better results.
   Note that we induce in 25µl so as not to use more IPTG than necessary as we only need 5µl in the end. You may consider doing 2.5mL if you additionally want to run an SDS-page with the induced bacteria.
4. If the cells are to be infected: Infect 25µl bacteria with MOI 20 (Phage Lysate). Infect for min 1h. (So if we do a 2h induction, we infect after 1h)
5. 30mins before imaging, add DAPI (2.5µl in 25µl from a 10X stock)
6. Prepare slides: microwave 1% agarose in dH2O until liquid, pipette 10µl onto a clean slide, press second slide onto it to flatten (left/right: coverslip with tape for hight), leave to dry for 1.5mins, carefully slide off top slide.
7. Add 5µl of bacteria onto agarose pad on slide, let dry for 3mins, cover with coverslip (clean).
8. Ready to use on the microscope and have some fun!