TRANSFORMATION OF COMPETENT E. COLI

If transforming with supercoil plasmid DNA use as little as possible, the transformation efficiency of most frozen competent cells that we use is in the order of 106 colonies/ug supercoil DNA. (Nicked circular DNA and ssDNA are 50% as efficient, linear DNA is 0.1% as efficient).

When transforming after a ligation reaction there may be some inhibition of DNA uptake because of the pH of the ligation reagents. You can transform up to 2.5 ul of a ligation reaction. If you don't get enough colonies you will have to lower the pH by adding MES buffer or ethanol precipitate the DNA before transforming. Use MES buffer at 0.1M final, and transform the entire ligation reaction.

1. Place 2.5 ul of ligation rxn (or 0.5 ul of supercoiled DNA) into a sterile microfuge tube.
2. Add 50 ul ready-to-use competent E. coli. (Thaw frozen stocks on ice. Do not heat). Place on ice 30 min.
3. Heat shock tubes at 42C 45 sec, add 500uL (up to 1mL) warm (room temperature) LB and incubate at 37C for 45 min. This allows the bacteria to express the antibiotic resistance gene in your plasmid (eg. make some lactamase and become AMP resistant). If you leave the bugs at 37C longer than 45 minutes they will begin dividing (once every 20 min) and all your colonies will be the same.
4. Spin down bacteria in the microfuge, 15 sec, remove the supernatant resuspend the pellet in 50 - 100 ul antibiotic broth. Plate out the bug suspension on the appropriate agar plate. If using intact supercoil plasmid DNA there is no need to spin down the bugs, just add antibiotic and plate out 50-100 ul after incubation.
5. Incubate the plates at 37C 12-16 h.

NOTE 1: Most protocols do not suggest the final addition of antibiotic that we use. That is because standard agar plates are much thicker than we use. Our thin plates are much cheaper (agar is very expensive) but since they have less antibiotic surrounding the colony you tend to get satelites unless you add antibiotic when plating out the cells.

NOTE 2: Kanomyacin resistant plasmids do not transform well into DH10B or DH5a cells; use HB101 or TOPP2 strains instead.