Electroporation (Modified protocol for cells that cannot grow in liquid media) (1):

Materials Needed:

Electrocompetent Cells
TSA Plates with and without antibiotic
TSB
Glass Spreader
100% Ethanol
Pipets
Electroporation Cuvettes
Kimwipes
Inoculation Loop

Protocol (estimated time 2-3 days):

1. Thaw your electrocompetent cells and plasmid on ice. Additionally, chill two electroporation cuvettes on ice.
    a. Cuvettes are placed on ice to prevent cell shock when transferred to a new environment
2. After about 20 minutes of chilling the electroporation cuvettes, pipet in 1µL of plasmid DNA (50-100µg) and 50µL of cells.
    a. The cells are either allowed to rest with the DNA for 15 - 30 minutes or are taken directly electroporated.
        i. Some protocols recommend resting with DNA, whereas others recommend electroporating right away.
3. Wipe down the sides of the electroporation cuvette with a kimwipe and place it into the electroporation machine.
    a. Wiping down the outside prevents sparks from forming/damaging the machine.
4. Electrocute the cells 1250 kV, 200ohms, 25µF with a time constant less than 4ms.
    a. No arcing should occur.
5. Gently remove the electroporation cuvette from the machine and pipet in 100µL chilled TSB. Mix the cuvette contents by pipetting up and down a couple times.
    a. Pipet the cuvette contents and place onto a TSA plate.
6. Sterilize the spreader by dipping it into 100% ethanol and flaming it
    a. Allow the spreader to cool next to the flame.
    b. Spread the cells around the plate until the plate is dry.
7. Repeat steps 4 through 7 with a no plasmid control.
    a. However instead of plateing all the TSB/cell mixture from the cuvette onto one plate, half is spread directly onto a TSA plate containing antibiotic and the other is spread onto a TSA plate without antibiotic.
8. As soon as visible colonies form, remove the bacteria from the plates by pipetting 1mL TSB onto the plates and scrape the cells off the TSA with an inoculation loop.
9. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up all the TSB and cells

a. The cells that were electroporated and plated on TSA without antibiotic are now being transferred and spread onto an antibiotic plate for selection of the successful transformants.
    i. Antibiotic concentrations used are as follows: TSA ampicillin (100µg/mL) and oxytetracycline, TSA chloramphenicol (12.5µg/mL) and oxytetracycline, TSA kanamycin (25µg/mL) and oxytetracycline, TSA tetracycline (50µg/ml) and oxytetracycline.
b. If there is growth on the negative controls of electroporated cells without plasmid on the antibiotic plates, then the transformation was unsuccessful. This may either be due to contamination or a poor concentration of antibiotic.

References

1. Williams, P., Ketley, J., & Salmond, G. (Eds.). (1998). *Bacterial Pathogenesis*. London, UK: Academic Press.