Producing Chemically Competent Cells (Modified *Campylobacter jejuni* Protocol (1)):

Materials Needed:

Two TSA plates (Bacterial Lawn Grown)
One empty TSA plate
Wash Buffer (264mM Sucrose, 15% Glycerol)
Centrifuge
1.5mL Eppendorf Tubes
TSB
Pipets

Protocol (estimated time 1 hour):

1. Remove the bacteria from plates by pipetting 2mL TSB onto the plates, followed by scraping the cells off with an inoculation loop.
2. 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 (contains the cells)
    a. The solution is then dispensed into the 1.5mL eppendorf tubes.
3. Repeat steps 1 and 2 with the other plate.
4. Pellet the cells by centrifugation at >10000g for 5 minutes.
5. Remove the supernatant without disturbing the bacterial pellet.
6. Resuspend the bacterial pellet in 1mL ice cold wash buffer.
    a. The pellet can be resuspended by gently pipetting up and down close to the bacterial pellet.
7. Repeat steps four through six, three times.
    a. each time, resuspending the cells will get easier and easier.
8. For the last time, resuspend the bacterial pellet in 400µL of ice cold wash buffer.
9. The cells can immediately be used or can be frozen at -80°C in 100µL aliquots.
10. Streak out the competent cells to ensure they survived the washes and no contamination.

Producing Electrically Competent Cells (Modified *Salmonella* Protocol (2)):

Materials Needed:

Two TSA plates (Bacterial Lawn Grown)
Centrifuge
1.5mL Eppendorf Tubes
50ml Sterile Falcon Tubes
TSB
Pipets
1mM HEPES Buffer (pH:7)
10% Glycerol Solution
Ice Bucket
One Empty TSA Plate

Protocol (estimated time 2 hours):

1. Remove the bacteria from the plates by pipetting 4mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
2. 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 (contains the cells)
    a. The solution is then dispensed into a 50mL falcon tube.
3. Repeat steps one and two.
    a. However, place the cells into the same 50mL falcon tube as used in step one and two.
4. Chill the cells on ice for 15 minutes
    a. during this time, set a centrifuge to cool down to 4°C.
5. Pellet the cells by centrifugation at 2300g for 10 minutes (4°C).
    a. Remove the supernatant by pouring it out the top.
        i. Be sure to quickly flame the lid of the falcon tube.
6. Resuspend the cells by gently shaking in 40mL chilled 1mM HEPES buffer.
7. Pellet the cells by centrifugation at 2300g for 10min and remove the supernatant.
8. Repeat steps five and six with 20mL chilled 1mM HEPES buffer, followed by 20mL chilled 10% glycerol, and lastly 3mL chilled 10% glycerol.
    a. Cells can immediately be used or can be frozen at -80°C in 100µL aliquots.
9. Streak out the competent cells to ensure they survived the washes and no contamination.

Producing Electrically Competent Cells (Modified *Pseudomonas* Protocol (2)):

Materials Needed:

Two TSA plates (Bacterial Lawn Grown)
Centrifuge
1.5mL Eppendorf Tubes
50ml Sterile Falcon Tubes
TSB
Pipets
Ice Bucket
One Empty TSA Plate
Magnesium Electroporation Buffer (1mM $MgCl_2$, 1mM HEPES, pH:7)

Protocol (estimated time 2 hours):

1. Remove the bacteria from the plates by pipetting 4mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
2. 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 (contains the cells)
    a. The solution is then dispensed into a 50mL falcon tube.

3. Repeat steps one and two.
    a. However, place the cells into the same 50mL falcon tube as used in step one and two.
4. Pellet the cells by centrifugation at 2300g for 10 minutes (4°C).
    a. Remove the supernatant by pouring it out the top.
5. Resuspend the bacterial pellet in 20mL ice cold MEB
6. Repeat steps four and five and four again.
7. Resuspend the bacterial pellet in 1mL ice cold MEB (with glycerol or sucrose added)
    a. Cells can immediately be used or can be frozen at -80°C in 100µL aliquots.
8. Streak out the competent cells to ensure they survived the washes and no contamination.

S. enteritidis **Electroporation Protocol**

Materials

dH2O
50% glycerol
1.5 mL Eppendorf Tubes
liquid N2
100 mL TSB culture of *G. apicola*

Protocol

1. Grow culture of *G. apicola* for O/N
2. Spin down cells at 5000g for 20 min. Decant supernatant.
3. Resuspend pellet with 25mL of pre-chilled dH2O
4. Spin down cells at 5000g for 10 min. Decant supernatant.
5. Resuspend pellet with 12.5mL of pre-chilled dH2O
6. Spin down cells at 5000g for 10 min. Decant supernatant.
7. Resuspend pellet with 6.25mL of pre-chilled dH2O
8. Spin down cells at 5000g for 10 min. Decant supernatant.
9. Resuspend pellet with 1.8mL of dH20 and 0.2mL of 50% glycerol
10. Aliquot 50uL of cells into epitube and freeze snap with liquid N2

References

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

2. Nickoloff, J. A. (Ed.). (1995). *Electroporation Protocol for Microorganisms.* Totowa, NJ: Humana Press Inc.