Cold Osmotic Shock

- Release of periplasmic protein fraction from *E. coli* by cold osmotic shock Modified protocol from Neu & Heppel, 1965:
  - Centrifuge *E. coli* cell suspension for 5 min at 14,000 g (4 °C) to collect the cells
  - Discard the entire supernatant
  - Resuspend the cells in ice-cold Cell Fractioning Buffer 1. The resulting volume should be 1/4 of the former suspension volume
  - Incubate for 20 min on ice. Invert the suspension at regular intervals to counteract sedimentation
  - Centrifuge the cell suspension for 15 min at 14,000 g (4 °C)
  - Discard the entire supernatant
  - Resuspend the cells in ice-cold Cell Fractioning Buffer 2. The resulting volume should be 1/4 of the former suspension volume
  - Incubate for 10 up to 20 min on ice under regular invertion
  - Centrifuge the cell suspension for 15 min at 14,000 g (4 °C)
  - Save the supernatant, which contains the periplasmatic proteins and membrane proteins