Separation of the Bacterial Periplasm by Osmotic Shock (or "How I learned to stop worrying and love the bug")

Background This protocol is slightly adapted from the 1965 paper by Neu and Heppel [1], with the main difference being the deletion of the wash steps before resuspending the cells in the sucrose solution. Several manufacturers provide osmotic shock purification protocol with their kits that deviate more from the paper (e.g. Qiagen), but I've found sticking closer to the original works better.

Osmotic Shock

1. Pellet the cells. 2000-4000 g for 15' at 4 °C usually works. Spin longer if supernatant is not clear.
2. Resuspend cells in 20% Sucrose, 1 mM EDTA, 30 mM Tris-HCl, pH8 (at room temperature). Use 80 ml per 1 g wet weight of pellet. If you don't feel like weighing your pellet, guess half of your culture volume.
3. Shake gently 10 min at room temperature.
4. Spin 13,000 g for 10' at 4 °C.
5. Drain pellet well, then rapidly resuspend with ice cold pure water. Use the same volume as above. You will get poor yields if the water isn't chilled.
6. Shake gently 10 min at 4 °C.
7. Spin 13,000 g for 10' at 4 °C.
8. The supernatant will contain the periplasmic fraction. If you expect to do chromatography, 0.2 μm filtering of the supernatant is advisable to remove any remaining debris. It's also advisable to add protease inhibitors at this point. I generally use 1 mM PMSF.

Notes

- Do not freeze the cell pellet before processing, as this will cause the cells to lyse.

References

[1] H C Neu and L A Heppel. The release of enzymes from escherichia coli by osmotic shock and during the formation of spheroplasts. 240:3685–3692, 1965.