Tech Notes

Efficient Fractionation of Periplasmic, Cytoplasmic, and Membrane Proteins From Escherichia coli Using the Epicentre PeriPreps™ Periplasting Kit.

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Introduction
The separation of bacterial cells into periplasmic, cytoplasmic, and membrane fractions is essential to accurately determine the localization of specific proteins, and to enrich for proteins found in these subcellular compartments prior to further purification. The Epicentre PeriPreps™ Periplasting Kit is a commercially available kit for isolating either the periplasmic or spheroplast fraction (the spheroplast fraction consisting of the cytoplasmic components and membranes) by utilizing a combination of lysozyme digestion and osmotic shock procedures.1 We show here that the PeriPreps Periplasting Kit can be effectively used to separate the spheroplast fraction into its individual cytoplasmic and membrane fractions. The PeriPreps Kit therefore provides an efficient means to isolate periplasmic, cytoplasmic, and membrane proteins.

Methods
Bacterial strains
To facilitate testing the PeriPreps Periplasting Kit for the fractionation of E. coli, we first constructed a strain that expressed proteins whose cellular localization could easily be detected. The strain BF202 was constructed by first integrating the plasmid pCD11PKS (R. Platt, C. Drescher, S.-K. Park, and G.J. Phillips, manuscript submitted) into the bacteriophage attachment site (attB) of MC4100 (F-, araD199, D[ara-leu]7696, rpsL150, relA1, flbB301, deoC1, ptsF25, rbsR). pCD11PKS expressed chloramphenicol transacetylase that served as a marker for cytoplasmic localization. This strain was then transformed with pMtlA-PhoA-156 yielding BF202. This latter plasmid expressed a mannitol permease-alkaline phosphatase hybrid protein that is localized to the membrane, as well as the periplasmic protein β-lactamase. Table 1 summarizes the relevant features of BF202, including the localization (cytoplasmic, periplasmic, or membrane) of the proteins monitored in this study.

Periplasting of E. coli
Periplasting was performed essentially as described in the protocol provided with the PeriPreps Kit. This included growing cells at 30°C to an optical density (OD_{600}) of 1.0. A 10 ml volume of cells was pelleted by centrifugation at 12,000 g and the supernatant was discarded. The pellet was resuspended in 0.5 ml of Periplasting Buffer (20% sucrose, 1 mM EDTA, 30,000 U/ml Ready-Lyse™ Lysozyme). The sample was incubated on ice for 5 minutes. Osmotic shock was accomplished by quickly adding 0.5 ml of ice-cold, purified water and gently mixing by slow pipetting. The sample was then incubated on ice for an additional 5 minutes. The sample was centrifuged at 12,000 x g for 2 minutes to remove the spheroplasts and intact cells from the supernatant. The supernatant was recovered as the periplasmic fraction. The resulting pellet contained the spheroplasts and intact cells.

Lysis of spheroplasts
According to the PeriPreps Periplasting Kit protocol, spheroplasts are lysed using a detergent lysis buffer (10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, and 0.1% deoxycholate). We substituted purified water containing 400 U/ml OmniCleave™ Endonuclease (Epicentre) to prevent loss of any membrane proteins that may be solubilized in the detergent. The sample was allowed to incubate at room temperature for 5 minutes to permit spheroplast swelling and lysis. The sample was then sonicated with a micro-tip at approximately 30-40% full power in 2 second bursts to help lyse spheroplasts and dissociate proteins that may be loosely associated with the membranes. Prolonged sonication was avoided to prevent foaming of the sample and overheating. Non-spheroplasted cells were removed from the purified water by low-speed centrifugation at 12,000 x g. The supernatant was removed and again centrifuged as above to ensure complete removal of unlysed cells. The supernatant was reserved as the spheroplast fraction.

Isolation of the membrane fraction
The spheroplasmic fraction was further fractionated by ultra-centrifugation to separate the membranes from the cytoplasmic fraction. The spheroplasmic fraction was centrifuged at 138,000 x g for 1 hour. The supernatant was removed and reserved as the cytoplasmic fraction. The membrane pellet was then dissolved in membrane solubilization detergent (0.5% sarkosyl, 10 mM Tris-HCl (pH 8.0), 5 mM EDTA) and kept as the membrane fraction (containing both inner and outer membranes).3

SDS-PAGE and Western blotting
SDS-PAGE and Western blotting were performed according to standard protocols.4 In brief, samples were separated by electrophoresis on 12.5% SDS-polyacrylamide gels.

Table 1. Proteins Used as Reporters of Subcellular Localization

<table>
<thead>
<tr>
<th>Protein Assayed</th>
<th>Protein Location</th>
<th>Reporter Gene (location of gene in BF202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactamase bla (plasmid)*</td>
<td>Periplasm</td>
<td>bla (plasmid)*</td>
</tr>
<tr>
<td>Chloramphenicol transacetylase (CAT)</td>
<td>Cytoplasm</td>
<td>cat (chromosome)</td>
</tr>
<tr>
<td>Adenosine triphosphatase b subunit of F₀ (ATPase)</td>
<td>Membrane</td>
<td>atp (chromosome)</td>
</tr>
<tr>
<td>Mannitol permease-alkaline phosphatase hybrid protein (MtlA-AP)</td>
<td>Membrane</td>
<td>mtlA-phoA (plasmid)*</td>
</tr>
</tbody>
</table>

*pBR322 derived plasmid encoding a fusion between the mannitol permease (mtlA) at the alanine 156 position of MtlA and alkaline phosphatase.2
and transferred to nitrocellulose by electrophotting. Immunological detection was performed with rabbit polyclonal antibody specific to the proteins listed in Table 1 and anti-rabbit secondary antibody conjugated to alkaline phosphatase.

**Results**

Figure 1 shows the results of a Western blot performed on each individual fraction obtained using the PeriPreps protocol. Inspection of this figure reveals that each of the proteins chosen as a reporter for localization was predominately found in the correct subcellular fraction. CAT remained localized in the cytoplasm, β-lactamase was exported to the periplasmic space, and both the ATPase b subunit of F₀ and the MtlA-PhoA fusion remained associated with the membrane fraction.

**Conclusion**

The PeriPreps Periplusting Kit, with minor modifications, provides an efficient, reproducible means for fractionation of bacterial cells into periplasmic, cytoplasmic, and membrane components. This protocol should be useful for the characterization and purification of protein localized to specific subcellular compartments.

**References**