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Database-driven protein solubilization

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is a protocol for using the extraction database found at www.polymerscreen.yale.edu for optimal extraction of membrane proteins into native nanodiscs.

1 Choose your protein of interest.

2 Visit www.polymerscreen.yale.edu to search for your protein in the extraction database. This will provide you with the polymer to use for optimal protein extraction.

3 As an example, below are representative organellar markers and the optimal polymer for extraction:
TMEM192 --> AASTY650
TGN46 --> ChloroSMA60
OMP25 --> SMA200
VAPA --> AASTY650
KRas --> ChloroSMA80

4 Once the optimal polymer has been determined, express the protein of interest, or harvest cells for endogenous solubilization.

5 Resuspend cellular membranes in the chosen polymer and incubate at 4 °C with rotation for 02:00:00 hours. 2h

6 Ultracentrifuge samples ay 200,000xg for 01:00:00 hours to remove insoluble material. 1h