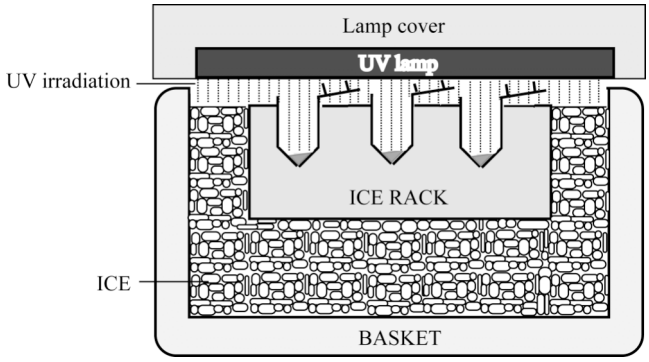
Vanadate-Induced Nucleotide Trapping for Detection of ABC Proteins

1. Membrane proteins (20 µg) were incubated in a buffer mixture composed of 10 µM 8-azido-[α-^{32}P]ATP (specific activity 740 GBq mmol^{-1}, ICN Biochemicals, Irvine, California), 2 mM ouabain, 0.1 mM EGTA, 3 mM $MgSO_4$, and 40 mM Tris-HCl (pH 7.5), 200 µM orthovanadate in a total volume of 8 µl for 10 min at 37°C.
2. The reactions were stopped by adding 400 µl of ice-cold TGM buffer (40 mM Tris-HCl [pH 7.5], 0.1 mM EGTA, 1 mM $MgSO_4$).
3. The membrane proteins were sedimented by centrifugation (20,000x*g*, 10 min, 4°C) to separate unbound ATP.
4. After being washed with Tris-EGTA buffer, the pellet was irradiated with UV light at 254 nm (5.5 mW cm^{-2}) in 8 µl of TGM buffer for 5 min on ice.
5. Samples were electrophoresed on a 7% SDS-polyacrylamide gel, and autoradiographed.
6. The radioactivity trapped by ABC proteins was analyzed with a radioimaging analyzer BAS 2000 (Fuji Film Co., Tokyo, Japan).



Reference

Kazuyoshi Terasaka, Nobukazu Shitan, Fumihiko Sato, Fumio Maniwa, Kazumitsu Ueda and Kazufumi Yazaki (2003)

Application of Vanadate-Induced Nucleotide Trapping to Plant Cells for Detection of ABC Proteins

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