

Development of new two hybrid system suitable for proteomic study in periplasm of *Escherichia coli* based on anchored periplasmic expression (APEX) system.

**Ki Jun Jeong, Barrett R. Harvey, Brent L. Iverson, and George Georgiou
Ki Jun Jeong^{1,2}, Barrett R. Harvey², Brent L. Iverson^{2,3}, and George
Georgiou^{1,2,4}**

¹Department of Chemical Engineering, ²Institute for Cellular and Molecular Biology, ³Department of Chemistry and Biochemistry, ⁴Department of Biomedical Engineering, University of Texas, Austin, TX 78712, USA

Anchored Periplasmic Expression (APEX) system is the new technology developed for the isolation of ligand-binding proteins from combinatorial libraries anchored on the periplasmic face of the inner membrane of *Escherichia coli*. Based on APEX system, we have developed new two hybrid system (APEX 2-Hybrid) suitable for high throughput antibody selection in proteomics applications, epitope mapping or when searching genomes for interacting pairs of proteins. In this system, one protein is expressed in the periplasm and tethered to the inner membrane of *E. coli* via lipidation of a small *N*-terminal 6 amino acids, and the other protein is exported into the periplasm and becomes associated with the cell via its interaction with the membrane-tethered protein. Following permeabilization of the outer membrane, the unbound protein can be removed and the bound protein can be detected by flow cytometry followed labeling with 2nd antibody-fluorescent dye conjugate. The efficient screening of antibodies with markedly improved ligand affinities from random mutagenized libraries by using APEX 2-Hybrid will be discussed.