

B18 High-throughput cloning and expression of human ABC transporters in Baculovirus/Insect Cell system customized for X-ray crystallography studies

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Introduction: ATP-binding cassette (ABC) transporters are complex integral membrane proteins that perform key functions in the cell, such as those related to energy transport and other substances moved into and out of the cell and organelles. More than 40 ABC superfamily genes have been identified so far and they are involved in the expression of seven protein subfamilies, which defects were found to be associated to the several diseases such as: Tangier disease, Schizophrenia, pancreatitis, cystic fibrosis and immune deficiencies. It is also known that they are potentially involved in cholesterol homeostasis and translocation of peptides for antigen presentation via MHC class I and based on these fact, they constitute potential targets for drug discovery by structural studies.

Objective: We aimed to clone, express and purify human recombinant ABC transporters by screening different DNA constructs using eukaryotic system in a high throughput-based pipeline developed at the Structural Genomics Consortium/University of Oxford for X-ray crystallography.

Methodology: Clones derived from 9 human ABC transporter targets were obtained by Ligase-Independent-Cloning using different DNA constructs associated to a C-terminal His-Flag expression vector based on FastBacTM (pFBCT10HF-LIC) then transformed into *E.coli* DH10Bac cells. Plasmid containing inserts was transfected into *Spodoptera frugiperda* Sf9 cells and after a small-scale high-throughput test expression, positive proteins were submitted to solubilization test using 12-detergent screening then led to high-scale expression. Membrane proteins were extracted with the chosen detergent then purified by immobilized metal affinity chromatography and analytical gel filtration. SDS-PAGE and LC-MS intact mass analyses were used for quality control.

Results: All the targets analyzed showed at least 67% of the constructs successfully cloned. Proteins were expressed in 8% of the clones in the small-scale test and all of them were successfully scaled up although they exhibited different purification patterns.

Conclusion: High-Throughput LIC using customized vector is an important tool for obtaining successful clones for human ABC transporters. However, additional approaches are required in order to improve yield and stability for crystallography studies.

Keywords: Protein Expression, Baculovirus, Crystallography