

## **EXPRESSION OF THE HUMAN $\mu$ -OPIOID RECEPTOR IN A STABLE Sf9 CELL LINE FOR AFM AND NSOM STUDIES**

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The opioid receptors belong to a family of G-protein coupled receptors which share a seven transmembrane domain topology. Their key role in pain perception, affective behaviour, and neuroendocrine physiology has been the major impetus for their study. Towards this end we have sought to streamline the process of receptor production. Stably transfected cell lines, as compared to transiently transfected cells, offer the advantage of circumventing the infection step, thus reducing the overall protocol time. The cDNA that encodes the human  $\mu$ -opioid receptor (hMOR) was cloned and expressed in *Spodoptera frugiperda* (Sf9) cells using a non-lytic vector system. The coding sequence was fused to the glycoprotein gp64 signal peptide, and a C-terminal His-tag was used to enable fast, large-scale production of pure protein. Immunofluorescent images showed that more than 75% of the cells expressed hMOR at the plasma membrane. Despite successful expression results, the level of stable expression was almost 400 times lower than transient expression using the baculovirus system. Therefore, hMOR was subsequently produced using transiently transfected Sf9 cells. A protocol has been developed for the deposition of bilayers from insect cell membrane patches that contain the temperature-sensitive hMOR. Preliminary AFM and NSOM studies of the hMOR-containing bilayers, made from either pure lipid or Sf9 cell membrane patches, demonstrate the feasibility of studying the opioid receptor system in whole cells.