E10

Synthesis of double-layered particles using IRES vector system in stably transformed insect cells

Jong-Min Lee[1], Bong-Hee Sohn[1], Pil-Don Kang[1], Sang-Uk Lee[1] and In-Sik Chung[2]

[1]Department of Agricultural Biology, NIAST, RDA Suwon 441-100, Korea and [2]Department of Genetic Engineering, Kyung-Hee University, Suwon, 449-701, Korea.

Stably transformed *Drosophila melanogaster* S2 cells was established using cotransfection of pMT/BiP-Wa-VP2/IRES/BiP-Wa-VP6 and pCoHygro. The full-length Wa-VP2, VP6, and NSP4 genes were amplified by reverse transcription-PCR (RT-PCR) from viral RNA extracted from human Wa-rotavirus. Stably transformed S2 cells coexpressed recombinant VP2 and VP6 with molecular weights of approximately 95 kDa and 46 kDa, respectively. Recombinant VP2 and VP6 were only detected in the extracellular fractions of transformed S2 cells. Stably transformed S2 cell expressing NSP4 showed two major bands corresponding to monomers and dimers of NSP4, with molecular weights of 25 – 30 and 51 - 62 kDa. To examine the Wa-rotavirus 2/6-DLPs synthesis in stably transformed S2 cells, DLPs were purified with 35% sucrose cushion and analyzed by transmission electron microscopy. In this TEM, the DLP appeared as wheel-like particles with short spikes with diameters around 50 to 55 nm. Therefore, rotavirus VLP can be synthesized from stably transformed *Drosophila* S2 cells as well as baculovirus/insect cell system. Also, our results prove that the new dual-expression vector system using the internal ribosomal entry site (IRES) derived from *Encephalomyocarditis* virus (EMCV) can effectively function in *Drosophila* S2 cells.