PTAT/PTAT-HA PLASMIDS AND PROTEIN PURFICATION PROTOCOL (VECTORS ARE ON DEPOSIT WITH ADDGENE)

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T-000717

Technology Description:

Researchers at Washington University have developed a method that allows the transduction of full-length proteins by utilizing the pTAT or pTAT-HA protein expression plasmids. A protein of choice can be fused to a HIV-TAT transduction domain under the expression system. Bacterial expression, followed by solubilization of protein aggregates with a denaturing agent, results in high yields of transducible fusion protein. Once added to the culture medium, the fusion protein can cross the cell membrane and then be degraded or refolded by the cellular machinery. Correct targeting and function of the fusion protein can be easily examined by fluorescent microscopy or immunohistochemistry. This strategy was established and improved to its current state by the purification and transduction of a variety of fusion proteins, including sequestering proteins, proenzymes, viral proteins, enzymes, GTPases, and transcriptional regulators. No special equipment is necessary to generate or transduce fusion proteins, although the use of fast protein liquid chromatography is recommended (but not required) to reproducibly bind and elute denatured fusion proteins. The pTAT/pTAT-HA vectors are now available from Addgene.

Publications: Nat Med. 1998 Dec;4(12):1449-52., Methods. 2001 Jul;24(3):247-56., Curr Protoc Cell Biol. 2003 May;Chapter 20:Unit 20.2.