Construction of PLC cells stably overexpressing Csseverin

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Abstract

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Protocol

Step 1.

Step 2.
The PEZ-LV203 vector and Csseverin gene fragments were digested with EcoRI and Apa I, respectively, and subsequently ligated using T4 DNA ligase.

Step 3.
The recombinant plasmid pEZ-LV203-Csseverin was identified by enzyme digestion and sequencing.

Step 4.
To generate the lentivirus, the pEZ-LV203-Csseverin plasmid or PEZ-LV203 control plasmid was cotransfected into 293T cells.

Step 5.
Supernatant containing the recombinant lentiviral particles was collected at 48 h post-transfection, filtered by a Millipore filter and subjected to ultracentrifugation.

Step 6.
The lentiviral particles were re-suspended in cold phosphate-buffered saline (PBS) and used to infect PLC cells.

Step 7.
After 48 h, the cells were incubated in selection medium containing puromycin (3 mg/ml) for 7 days to select stably cells.