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Construction of PLC cells stably overexpressing Csseverin

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- 2 The PEZ-LV203 vector and Csseverin gene fragments were digested with EcoRI and Apa I, respectively, and subsequently ligated using T4 DNA ligase.
- 3 The recombinant plasmid pEZ-LV203-Csseverin was identified by enzyme digestion and sequencing.
- 4 To generate the lentivirus, the pEZ-LV203-Csseverin plasmid or PEZ-LV203 control plasmid was cotransfected into 293T cells.
- 5 Supernatant containing the recombinant lentiviral particles was collected at 48 h post-transfection, filtered by a Millipore filter and subjected to ultracentrifugation.
- 6 The lentiviral particles were re-suspended in cold phosphate-buffered saline (PBS) and used to infect PLC cells.
- 7 After 48 h, the cells were incubated in selection medium containing puromycin (3 mg/ml) for 7 days to select stably cells.