Title:	Protocol for investigating the biogenesis of SARS-CoV-2 S pseudoviruses in HEK293T cells transduced to express the virus-specific intrabodies
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Abstract:	This protocol provides detailed methodology used in the published article; Robust anti-SARS-CoV-2 single domain antibodies cross neutralize multiple viruses.1 The protocol describes a strategy for the intracellular expression of sdAbs targeting the polybasic cleavage site (anti-CSP sdAb) of the viral entry mediator, SARS-CoV-2 S protein. We also analyze the intracellular fate of the protein in the anti-CSP intrabody (IB)-expressing cells. For generating an anti-CSP IB construct, the sequence of the selected sdAb specific to peptide (NSPRRAR/SVAS) which encompasses the polybasic cleavage site (CSP) of SARS-CoV-2 S protein was cloned into a pLenti-GFP vector (a lentivirus-based mammalian expression vector) downstream to CMV promoter.2 The cloning scheme used is: 50 LTR-CMV promoter-VHH - c-Myc-tag - e-GFP -30 - LTR. The anti-CSP sdAb sequence was amplified using the forward primer, VHH-FR1-pLenti (containing XbaI restriction site), and reverse primer, FR4-c-Myc pLenti, that encoded c-Myc tag at 30 end of VHH with a BamHI site) (Figure 1). To generate the lentivirus-based pseudoviruses expressing surface SARS-CoV-2 S protein, HEK293T cells were transfected with plasmids such as a reporter construct, pCMVR8.74, SARS-CoV-2 S construct, tat and rev as well as pMD2.G.3 The plasmids required for producing LV(CoV-2 S) pseudoviruses were prepared and the purity was confirmed before beginning with the experiments.
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