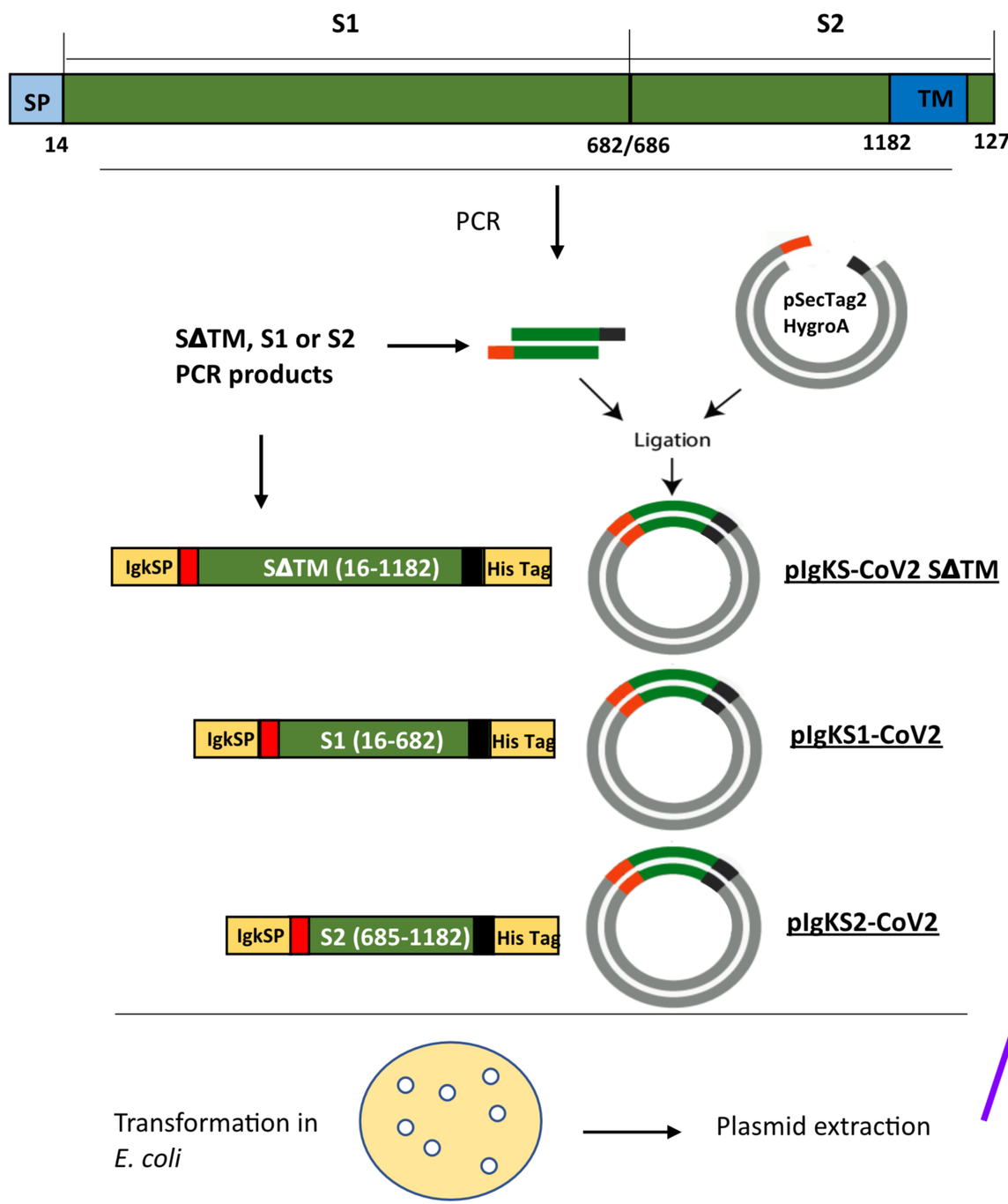
Supplementary figure S1: Schematic representation of the cloning, expression and purification of SARS-CoV-2 recombinant proteins in the HEK293T cel line

- Codon optimized cDNA encoding the Spike protein was used as template for PCR amplification of three different domains of S protein

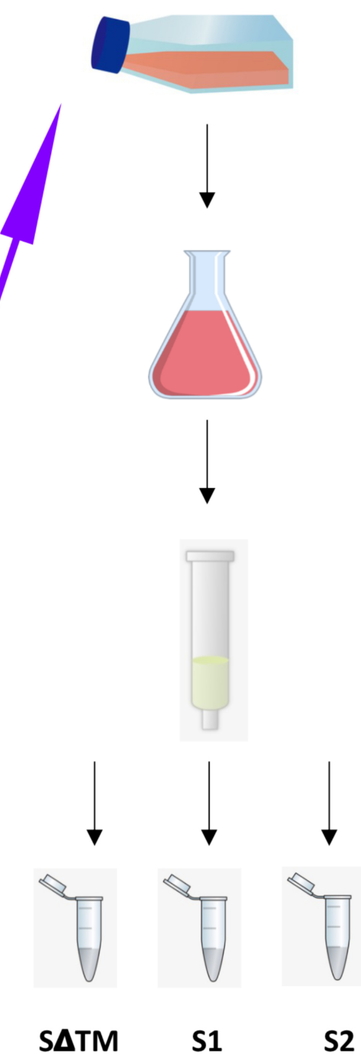
- The coding sequences of the three domains were amplified with the opportune primer pairs including an HindIII restriction site in the 5' end (red) in frame with the Igk signal peptide and an XhoI restriction site in the 3' end (black) in frame with the His Tag of the plasmid backbone

- The obtained amplicons were inserted in pSecTag2HygroA expression vector and transformed in *E. coli.*

- The clones were analyzed and the correct plasmids purificated for transfection



- Expression and purification of the three different recombinant proteins



- Plasmid transfection in HEK293T cell line using polyethylenimine (PEI) transfection reagent

- Supernatant combined with Ni-NTA agarose and gently agitated overnight at 4°C

- Recombinant proteins elution in 0.1 M sodium phosphate, 300 mM NaCl, 250 mM imidazole and 0.05% Tween20, pH 8.0.