Background

Emerging viruses are a global threat to human health and economic stability, as exemplified by the current COVID-19 pandemic caused by novel coronavirus SARS-CoV-2, which is efficiently transmitted from human to human. COVID-19 has spread rapidly, creating a global emergency situation. The scientific community reacted swiftly and massively world-wide, supplying diagnostic tests, generating new therapies, and designing much needed vaccines.

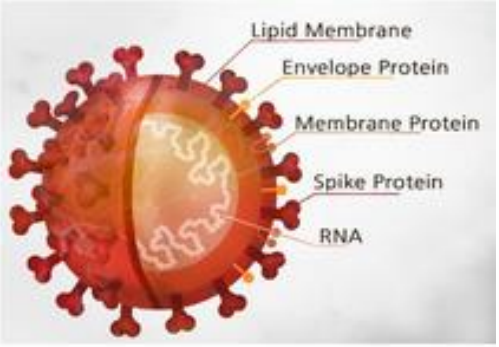

Figure 1: SARS-CoV-2

Scientists at BPS Bioscience have been actively working on developing COVID-19 research tools to support the scientific community in these efforts. These research tools include an entirely new line of lentiviruses to study the first step of SARS-CoV-2 infection.

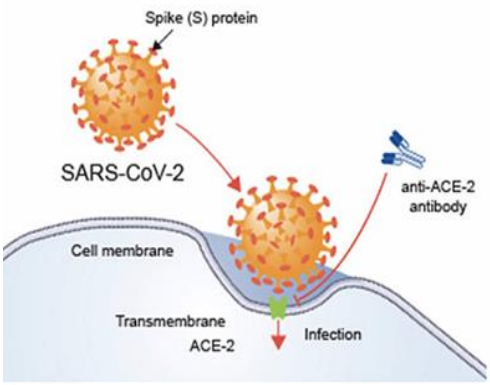

Figure 2: SARS-CoV-2 virus entry into host cells

The Spike protein consists of two subunits (S1 and S2) organized in a homo-trimer structure. SARS-CoV-2 infects human cells when the RBD (Receptor Binding Domain) region of the Spike S1 subunit binds to ACE2 (angiotensin converting enzyme 2) on the surface of cells in the respiratory system including the lungs, in the arteries, heart, kidney, and intestines. Based on this observation, drugs or neutralizing antibodies that target the interaction between the Spike protein of SARS-CoV-2 and human ACE2 may offer protection against viral infection. Spike then needs to be primed by extracellular proteases Furin and TMPRSS2 (human transmembrane serine protease 2), primarily expressed by endothelial cells across the respiratory and digestive tracts. These proteases cleave Spike at the S1/S2 site, exposing a peptide in the S2 subunit that promotes virus fusion with the cellular membrane, thereby allowing the virion to enter the cell. Since TMPRSS2 is involved in viral entry, it has been proposed that blocking TMPRSS2 activity could also be an effective therapeutic strategy.

The emergence of SARS-CoV-2 mutants is a serious threat, particularly when new mutations result in higher transmissibility and infectivity. Thousands of variants have now been described, with notable variants of concern: Variant (B.1.1.7) identified in the United Kingdom, Variant (B.1.351) identified in South Africa, or Variant (P.1) identified in Brazil. Fast-spreading Variant B.1.1.7, for example, contains several mutations including a N501Y mutation that is thought to make it easier for the virus to attach to human ACE2. Research on these variants will be critical to update vaccines and therapeutics.

Lentivirus-based Tools

To better understand the mechanism of SARS-CoV-2 entry into cells, BPS Bioscience has developed new, off-the-shelf lentivirus-based virus particles that can infect most types of mammalian cells, **including primary and non-dividing cells**. These viruses are ready for infection and do not require other components. Infection rates as high as 90% and efficient gene transduction lead to robust expression of proteins of interest or reporter proteins. Importantly, none of the HIV genes (gag, pol, rev) are expressed in the infected cells, therefore our lentiviruses

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are replication-incompetent and **can be used in a Biosafety Level 2 facility**, which makes them accessible to many research laboratories.

Our first family of lentiviruses for COVID-19 research are replication incompetent, pseudotyped lentiviral particles that use protein VSV-G (Vesicular stomatitis virus G protein) as an envelope protein to mediate cellular entry via the LDL receptor (low-density lipoprotein receptor), allowing infection of various cell types. These viruses are used to drive transient or stable expression of proteins of interest ACE2 (BPS Bioscience #79944), TMPRSS2 (BPS Bioscience #78011), and Spike (BPS Bioscience #78010).

Figure 3 illustrates the structure of the ACE2 vector used to transduce the gene encoding ACE2, while Figure 4 shows ACE2 expression following infection of HEK293 cells with ACE2 Lentivirus (BPS Bioscience #79944) measured by flow cytometry.

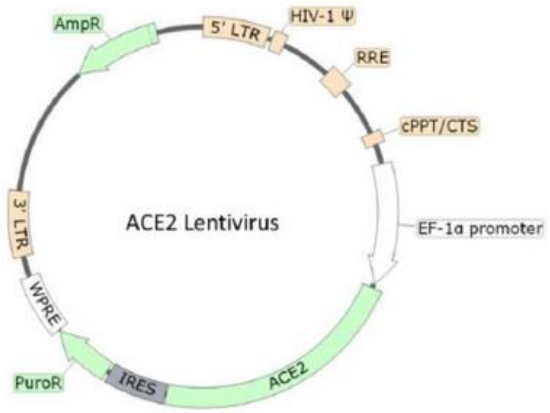

Figure 3: ACE2 lentivirus construct. Under the control of EF1a promoter, the virion transduces ACE2 (NM_021804.3) allowing its transient expression in target cells or the generation of a stable cell line expressing ACE2 (following puromycin selection).

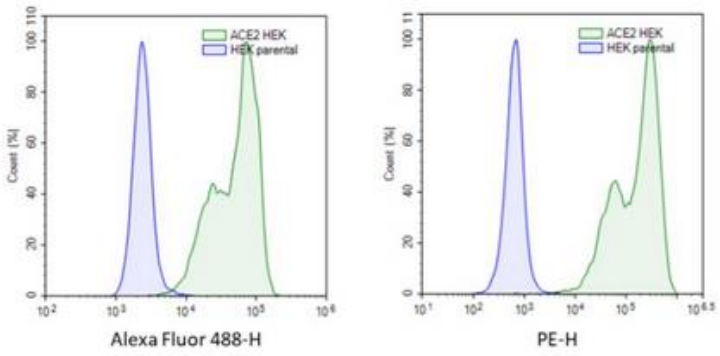

Figure 4: Flow cytometry analysis of ACE2 expression in HEK293 cells transduced with ACE2 Lentivirus (BPS Bioscience #79944). Blue: HEK293 parental cells; Green: HEK293 cells transduced with ACE2 lentivirus (left panel: Alexa Fluor 488; right panel: PE)

Pseudotyped SARS-CoV-2 Lentiviruses

Our second generation **off-the-shelf pseudotyped lentiviruses** are designed to study the interaction between the SARS-CoV-2 Spike protein and human ACE2 in a physiologically relevant context. These viruses use SARS-CoV-2 Spike protein (GenBank #QHD43416.1) as the envelope protein to infect COVID-19-relevant target cells. They drive the expression of Firefly luciferase for precise and robust quantification of infection, enhanced GFP (eGFP) for fluorescence-based experiments, or both. For example, Spike SARS-CoV-2 Pseudotyped Lentivirus Luciferase reporter (BPS Bioscience #79942) contains the vector shown on the left in Figure 5. Spike SARS-CoV-2 Pseudotyped Lentivirus eGFP corresponds to BPS Bioscience #79981 (not shown), and pike SARS-CoV-2 Pseudotyped Lentivirus dual reporter (BPS Bioscience #79982) contains the vector shown on the right in Figure 5.

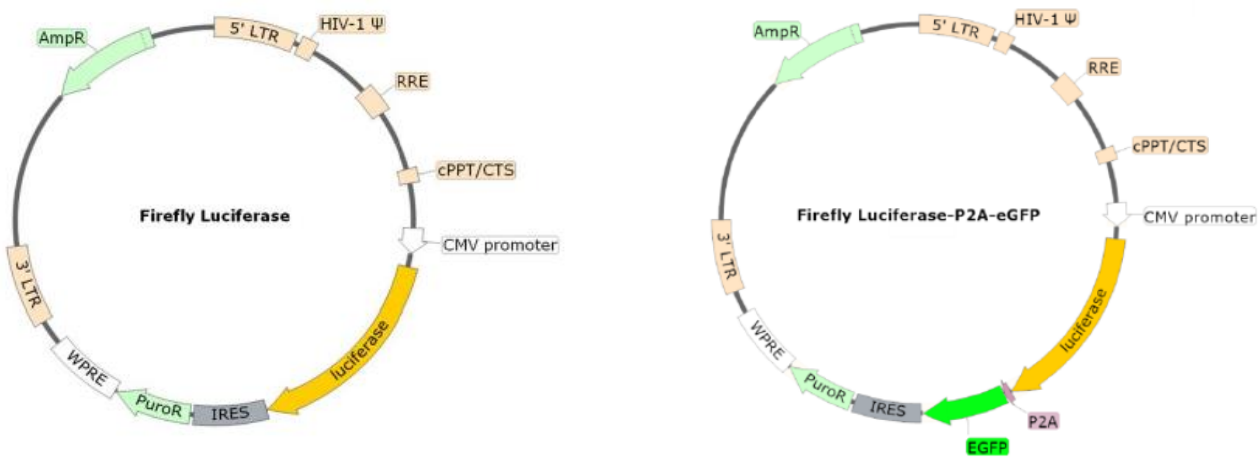

Figure 5: Spike SARS-CoV-2 Pseudotyped Lentivirus Luc Reporter (BPS Bioscience #79942) and Dual Reporter (BPS Bioscience #79982).

These pseudotyped lentiviruses are particularly useful to measure the effect of neutralizing antibodies on Spike-mediated infectivity and gene transduction, as shown in Figure 6.

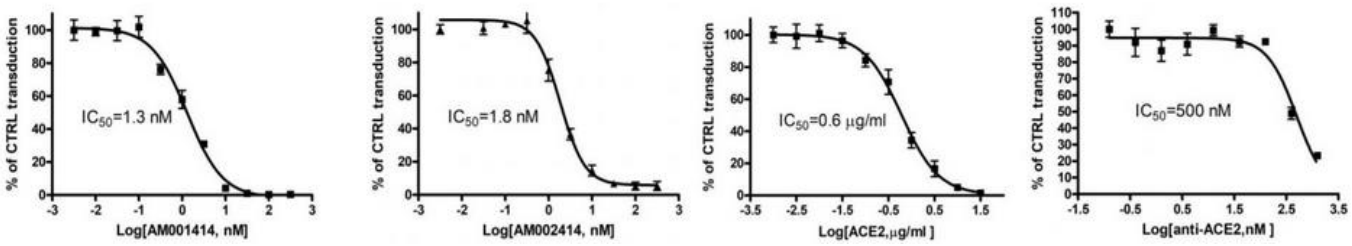

Figure 6: Neutralization assays performed with two distinct anti-SARS-CoV-2 Spike antibodies, a recombinant ACE2 protein, and an anti-ACE2 antibody (from left to right). HEK293 cells expressing ACE2 were transduced with a mix containing the Spike (SARS-CoV-2) pseudotyped lentivirus and different concentrations of the corresponding neutralizing compounds. Control transduction (100%) corresponds to the luciferase activity measured in presence of the virus without neutralizing compounds. Three independent experiments showed dose-dependent decreases in the transduction efficiency of the Spike (SARS-CoV-2) Pseudotyped Lentivirus due to neutralizing effects of the tested compounds.

To serve as negative controls for this family of reporter pseudoviruses, bald lentiviruses were designed without envelope glycoprotein (neither VSV-G nor SARS-CoV-2 Spike) so they cannot infect cells, but contain the firefly luciferase gene and/or eGFP: Bald Lentiviral Pseudovirion Luciferase Reporter (BPS Bioscience #79943), eGFP Reporter (BPS Bioscience #79987), and Luc-eGFP Dual Reporter (BPS Bioscience #79988).

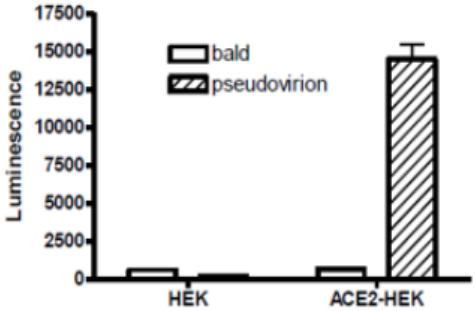

Figure 7: Infection of ACE2-HEK293 cells monitored by luciferase activity. HEK293 cells were infected with ACE2 Lentivirus or with Bald Lentivirus Luc-eGFP Dual Reporter (BPS Bioscience #79988).

SARS-CoV-2 Variants

Mutant viruses are available to study emerging SARS-CoV-2 variants and to measure the effect of select mutations on the interaction between Spike and ACE2 on virus infectivity. Currently available variant viruses include Spike SARS-CoV-2 Pseudotyped Lentivirus Luc Reporter B.1.1.7 Variant, identified in the UK (BPS Bioscience #78112) and B.1.351 Variant, identified in South Africa (BPS Bioscience #78142), while **Variant P.1** (first identified in Brazil) **is in the pipeline**. Mutant Spike pseudotyped viruses containing only the highly conserved D614G mutation (eGFP reporter BPS Bioscience #78035 or Luc reporter BPS Bioscience #78028) or the 3 mutations K417T, E484K, N501Y present in the RBD (Receptor Binding Domain) of the P.1 variant (Luc reporter BPS Bioscience #78143) have also been released, with more to come. These mutant pseudoviruses can be useful for differentiating the binding site and mechanism of action of neutralizing antibodies. Figure 8 shows an example in which cell entry of Spike mutant (D614G) was visualized using eGFP fluorescence.

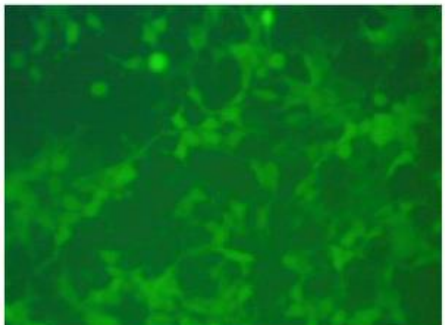

Figure 8: Infection of ACE2-HEK293 cells with SARS-CoV-2 Spike (D614G) pseudotyped lentivirus eGFP reporter (BPS Bioscience #78035).

Finally, our study shown in Figure 9 compared the effect of anti-Spike antibodies on the infectivity of wild-type and Variant B.1.1.7 Spike-pseudotyped viruses in ACE2-HEK293 cells. It was observed that the wild-type virus was neutralized by this antibody in a dose-dependent fashion (left panel), whereas Variant B.1.1.7 was not (right panel).

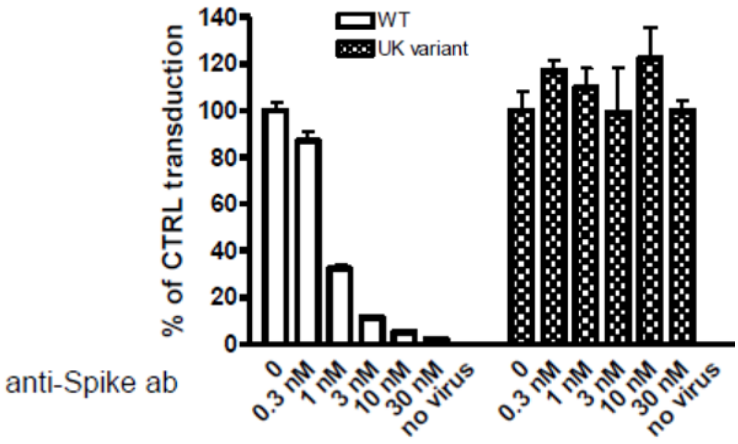

Figure 9: Effect of anti-Spike antibodies on the transduction efficiency of B.1.1.7 Variant (UK) compared to wild-type (WT) Spike. ACE2-HEK293 cells (BPS Bioscience #79951) were infected with Spike Pseudotyped Lentivirus Luciferase Reporter (BPS Bioscience #79942) or with Spike B.1.1.7 Variant Pseudotyped Lentivirus Luciferase Reporter (BPS Bioscience #78112) in the presence of increasing concentrations of an anti-Spike antibody. Control transduction (100%) corresponds to luciferase activity measured in the presence of WT virus without neutralizing antibody.

Conclusion

BPS Bioscience has recognized the urgency and special challenges of conducting coronavirus research during the pandemic. BPS scientists are continually striving to offer novel and innovative products and services to help researchers design new therapeutics and vaccines for COVID-19. We have developed one of the largest portfolios of coronavirus research tools and services. These include cell lines expressing ACE2 and TMPRSS2, unique assays to measure the activity of 3CL, PL^{Pro}, TMPRSS2, Furin, RdRP, and other key SARS-CoV-2 targets, and **a rapidly expanding** portfolio of lentivirus-based virus particles to keep pace with the emergence of new variants of concern and facilitate the study of these new mutations on virus biology, diagnostic test performance (serology or antigen tests) and vaccine efficacy.