

Advanced Tools For COVID-19 Coronavirus Research From GeneCopoeia

U.S. Centers for Disease Control and Prevention **Presenter:** 

Ed Davis, Ph.D. Senior Application Scientist GeneCopoeia, Inc.

September 9, 2020

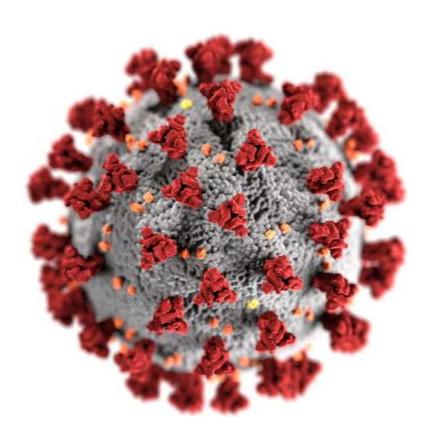
- Spike protein-pseudotyped lentivirus
- Coronavirus protein antigen microarrays
- SARS-CoV-2 RT-qPCR-based testing kits
- COVID-19-related plasmids, including open reading frame (ORF) clones expressing viral proteins and human host factors, CRISPR sgRNA, and shRNA.
- COVID-19-related lentivirus and adenoassociated virus (AAV)







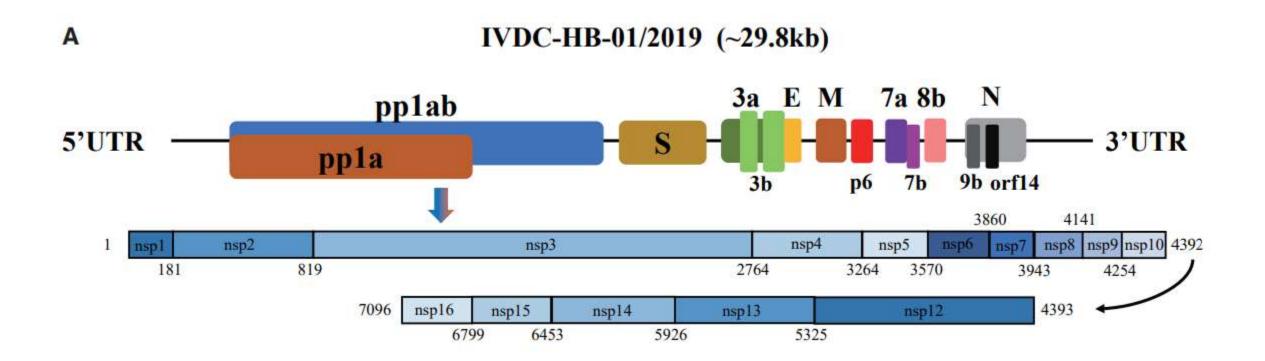
### Background: SARS-CoV-2 virus



- SARS-CoV-2: Member of genus Betacoronavirus
- Highly similar to SARS-CoV-1, which caused the 2002-2004
   Severe Acute Respiratory Syndrome (SARS) outbreak
- Less similar to MERS-CoV, which caused the ongoing <u>Middle Eastern Respiratory Syndrome (MERS)</u> outbreak from 2012
- Surrounded by surface glycoprotein "Spike", which is essential for virus entry
- ✤ ~30 kb genome, positive strand RNA



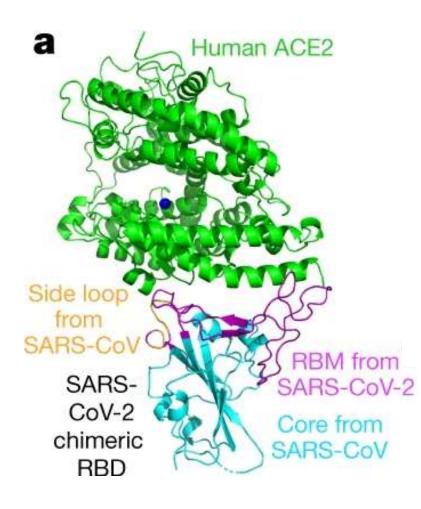
#### Background: SARS-CoV-2 virus



From Wu, A., et al. (2020). Cell Host and Microbe 27, 325



### Background: SARS-CoV-2 interaction with host

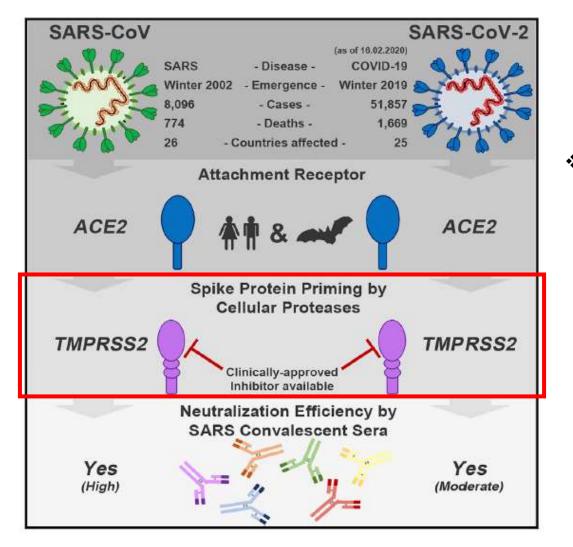


- SARS-CoV-2 Spike protein binds to Angiotensin Converting Enzyme 2 (ACE2)
- Same receptor as SARS-CoV-1. MERS-CoV uses DPP4.
- Entry into host occurs via lung airway epithelial cells
- ACE2 is also highly expressed in other tissues, including vascular epithelial cells and kidney, which could explain some of the secondary pathology of COVID-19 (blood clots, kidney failure)



From Shang, J., et al. (2020). Nature 581, 221

### Background: SARS-CoV-2 interaction with host



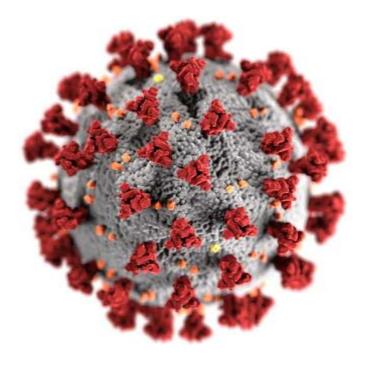
 Serine protease TMPRSS2 is required for Spike binding to ACE2

> GeneCopoeia Expressway to Discovery

From Hoffmann, M., et al. (2020). Cell 181, 271

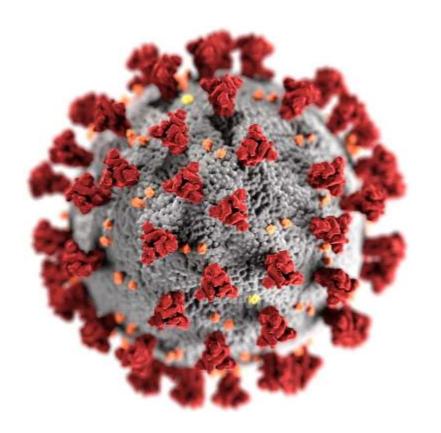
What is it?

Lentivirus that expresses the SARS-CoV-2 Spike (S) glycoprotein in its envelope. Limits the tropism of viral infection to cells that express ACE2.





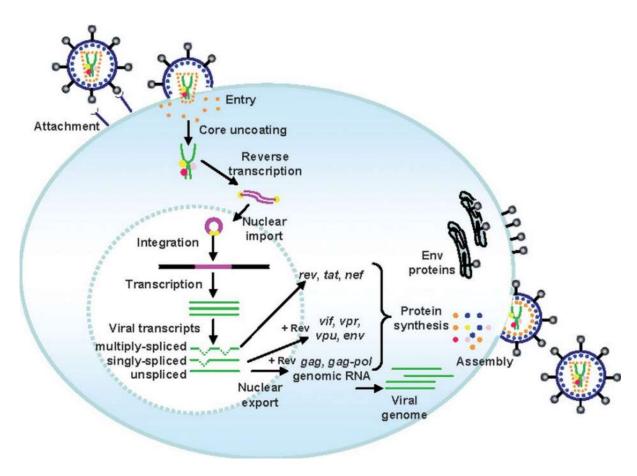
### Spike Protein-pseudotyped lentivirus Useful for several applications in a <u>SAFE</u> environment



- Vaccine development for prevention of infection by SARS-CoV-2 virus
- Studying the efficacy and mechanism of neutralizing antibodies against SARS-CoV-2 virus
- Development of antiviral therapeutic agents
- Studying the mechanism of virusreceptor interaction



#### Lentivirus technology



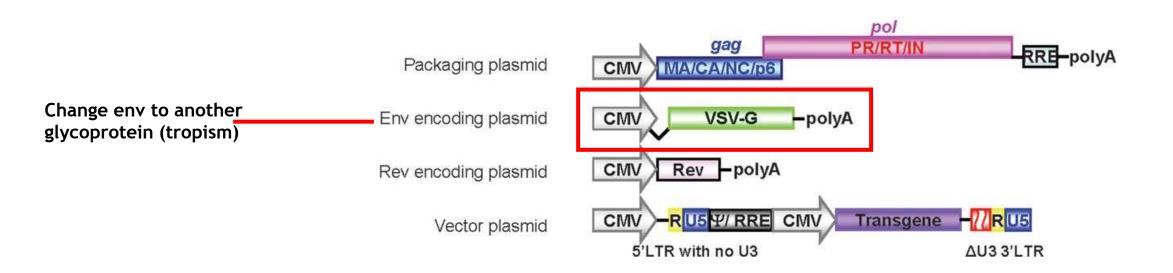
Sakuma, et al. (2012). Biochem. J. 443, 603.

- Class of retroviruses that includes human immunodeficiency virus (HIV)
- Single stranded RNA genome of ~9.7 kb
- Integrates into genomic DNA
- Infect dividing & nondividing cells

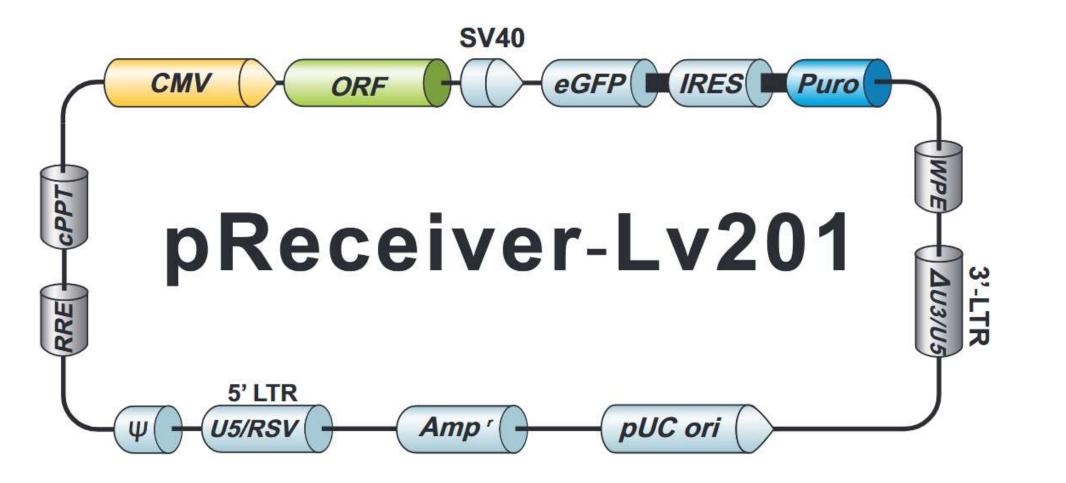


#### Lentivirus technology

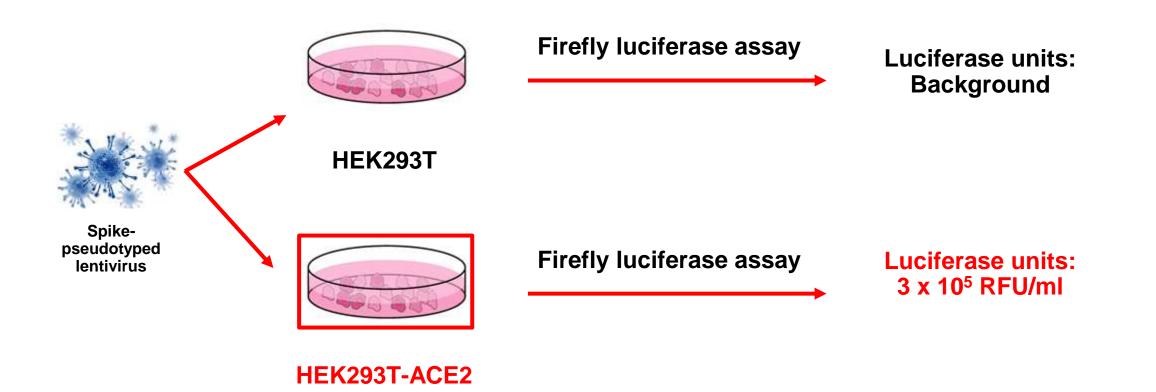
#### 3rd generation lentivirus



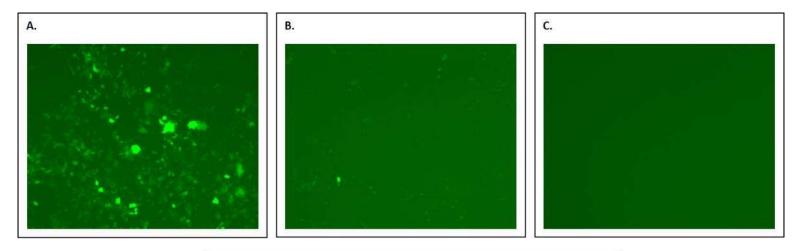


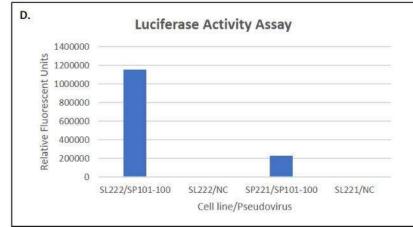






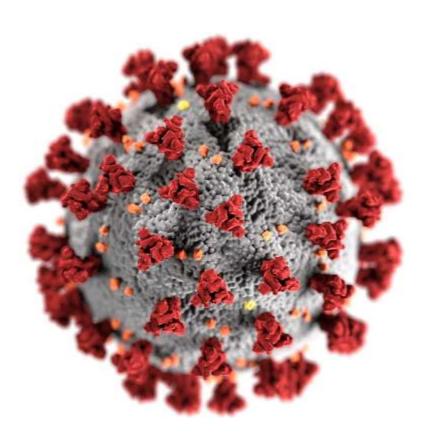








### Lentifect<sup>™</sup> Spike Protein-pseudotyped lentivirus



#### Advantages

- <u>Accurate functional titers</u>. Measured by FACS sorting of ACE2-expressing HEK293T cells infected with GFPexpressing particles.
- <u>Complete system</u>. Can be used with GeneCopoeia's ACE2-expressing HEK293T cells. Kits for do-it-yourself packaging are also available.
- ✤ <u>Application versatility</u>.
  - Can be packaged for either *in vitro* grade or ultrapurified *in vivo* grade.
  - Can express either commonly-used markers such as GFP and luciferase, or most human, mouse, and rat ORFs in GeneCopoeia's >80 lentiviral transfer vector types.



Product cat. #	Length of Spike	Spike variant
SP101-100	Full	D614
SP001-100	Truncated	D614
SP103-100	Full	G614
SP003-100	Truncated	G614



### **COVID-19 Antigen microarray**

#### What are antigen microarrays?

- \* Antigen arrays are a type of protein microarray. Proteins of interest are spotted onto membranes.
- ✤ In this case, the proteins are known to be associated with specific diseases.
- Antigen arrays are used for detection of antibodies in patient body fluids. Can also be used to validate specific antibodies.
- Usually, the antigens are proteins that are known autoantigens in autoimmune diseases, but can be other proteins associated with other disorders, such as cancers or allergies.



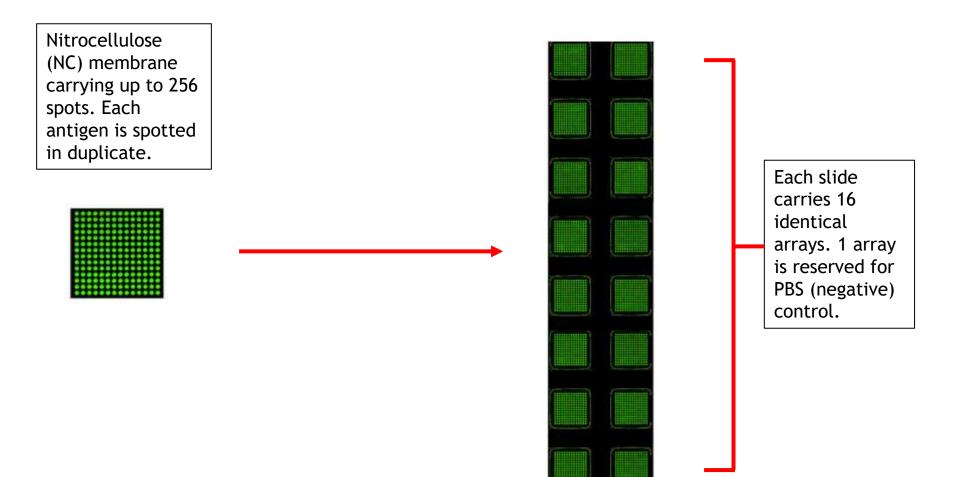
### OmicsArray<sup>™</sup> SARS-CoV-2 Coronavirus Antigen Microarray

Antigen	Associated disease
SARS-CoV-2 Spike protein S1	COVID-19
SARS-CoV-2 Spike protein S1 RBD	COVID-19
SARS-CoV-2 Spike S2	COVID-19
SARS-CoV-2 Spike S2 ECD	COVID-19
SARS-CoV-2 Spike S1 + S2	COVID-19
SARS-CoV-2 Nucleocapsid protein (NCP)	COVID-19
SARS-CoV-1 Spike protein	SARS (2002-2004)
SARS-CoV-1 NCP	SARS (2002-2004)
MERS-CoV Spike Protein	MERS (2012-present)
HCoV-229E Spike protein	Common cold (seasonal)
HCoV-HKU1 Spike protein	Common cold (seasonal)
HCoV-NL63 Spike protein	Common cold (seasonal)
HCoV-OC43 Spike protein	Common cold (seasonal)
Influenza A antigen	Seasonal influenza
Influenza B antigen	Seasonal influenza
RSV Glycoprotein G	Common cold-like respiratory disease



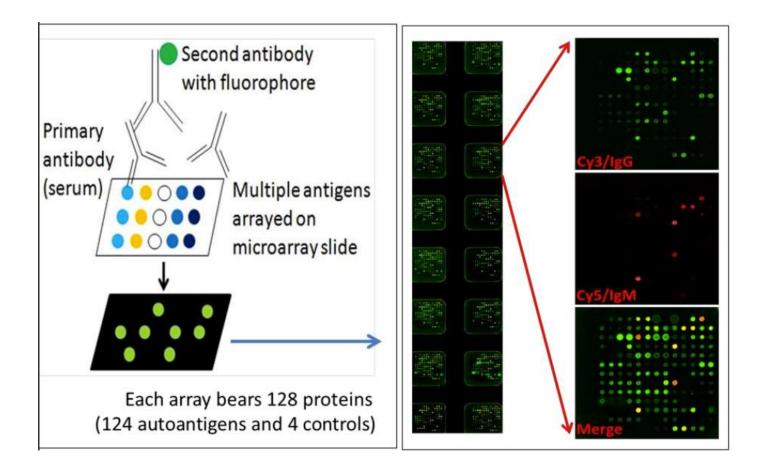
### What are antigen microarrays?

#### Proteins in <u>native conformation</u> spotted onto membranes





#### Autoantigen microarray workflow



From Zhu, H., et al. (2015). Genomics, Proteomics, & Bioinformatics <u>13</u>, 210



### Applications for antigen microarrays

- Autoimmune disease profiling and diagnostics (e.g. Systemic lupus erythematosus, Rheumatoid arthritis, Crohn's disease, Type 1 diabetes, Sjögren's Syndrome, and ~100 others.
- Cancer biomarker profiling.
- Infectious disease/pathogen marker profiling
- Allergen profiling.
- Transplantation evaluation.
- Evaluation of the efficacy, toxicity, and other side effects from clinical applications of drugs and therapies.
- Evaluation of human body responses to pollutants and impacts on health.
- Vaccine efficacy evaluation



### Custom antigen microarray services

- <u>Custom printing and spotting</u>. GeneCopoeia will create custom antigen microarrays built to customer's specifications.
- <u>Sample processing</u>. Customer can send blood, plasma, tissue, or other biological sample and we will prepare it for processing and incubation with any of our predesigned antigen microarrays or custombuilt antigen microarrays
- <u>Data analysis</u>. Once samples are processed and incubated with an antigen microarray, we will analyze the raw data. The standard analysis service includes: 1) An Excel file of the Net Signal Intensity (NSI) for each antigen on the array, normalized to internal controls; and 2) a heat map
- Additional analysis services, including proteomic analysis, pathway analysis, and more, are also available.



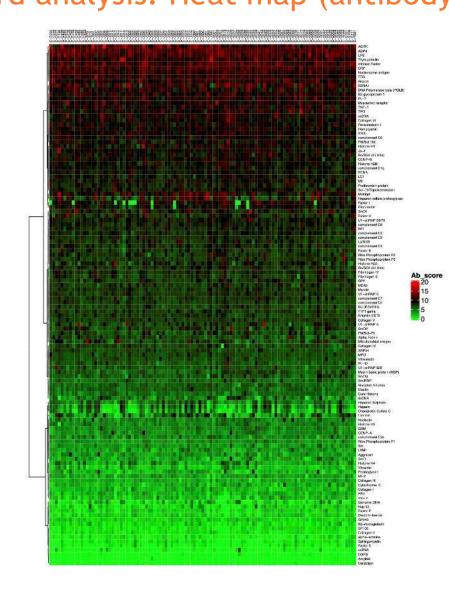
#### Custom antigen microarray services

Standard analysis: Normalized data (Net signal intensities, or NSI)

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	38.384423		85.884423		101.88442		36.884423		30.884423		109.38442		28.884923		81.88442		6.384423		3.384423		85.384423	
	12.115192		13.615192		22.615192		15.115192		82.615192		52.615192		44.615192		36.61519		25.61519		54.61519		36.615192	
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omplem	142.80769	13.246	204.30769	14.865	235.30769	12.017	217.80769	11.888	468.30769	19.9355	412.30769	7.175	383,30769	9 6.9815	266.3076	9 13.0315	163.8076		405.8076		462.80769	
omplem	37.153846	4.5055	79.653846	1.923	49.653846		34.653846	2.0895	148.15384		168.65384		45.653846	50.873	50.65384		137.1538		77.15384		191.65384	
omplem	193	9.95	439	9.3935	261.5	7.5635	551	9.444	324.5	8.323	2860	8.372	997.5	7.4765	\$67.5	9.854	841.5	10.8875	604.5	10.068	358	4.726

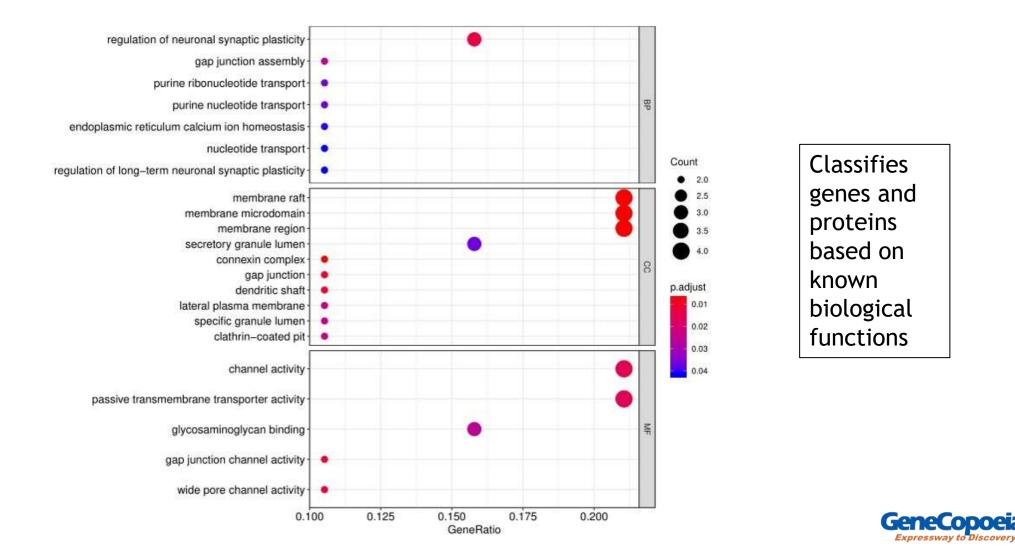


### Custom antigen microarray services Standard analysis: Heat map (antibody score)

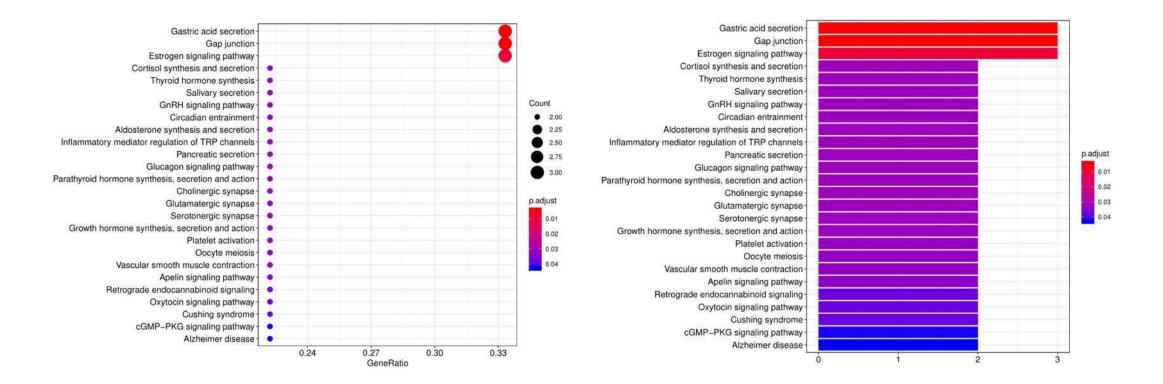




### Custom antigen microarray services Additional analysis: Gene Ontology (GO) analysis



### Custom antigen microarray services Additional analysis: Pathway analysis



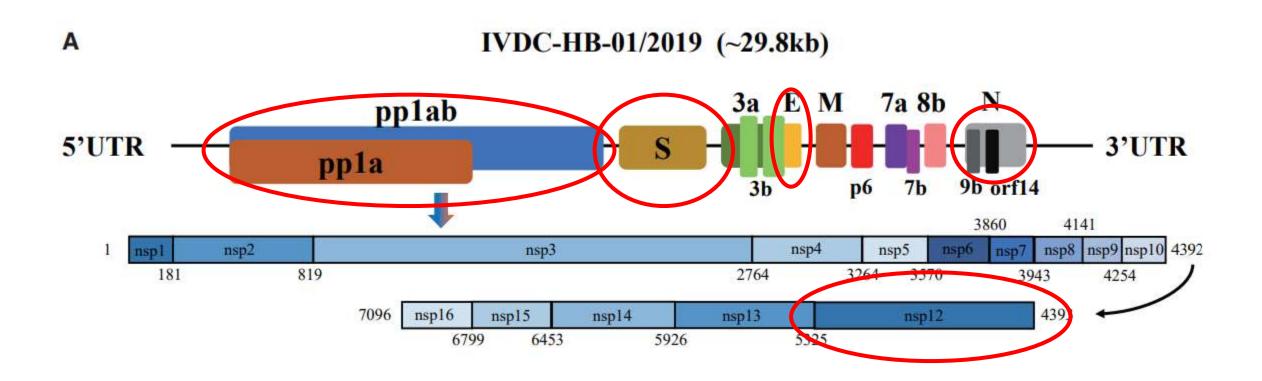
Groups positive antibody-antigen interactions based on defined biological pathways



#### <u>Application: For detection of SARS-CoV-2 viral RNA via RT-</u> <u>qPCR</u>

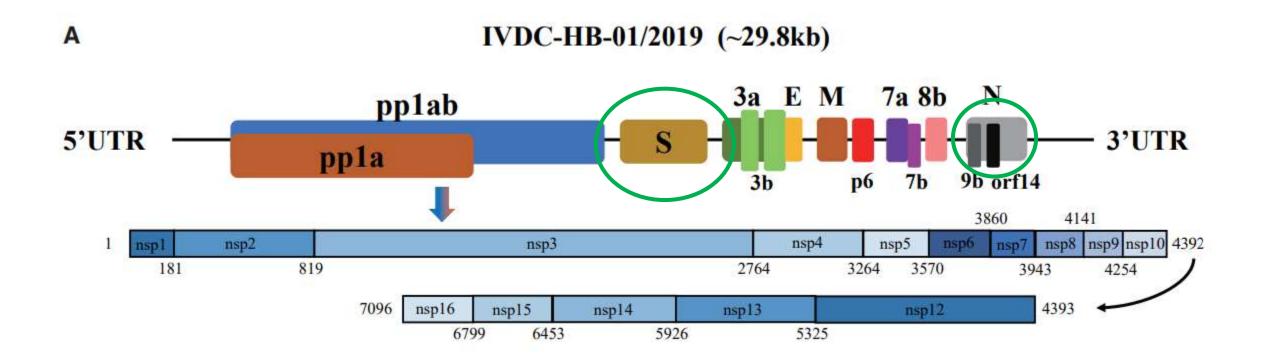


#### SARS-CoV-2 detection via RT-qPCR



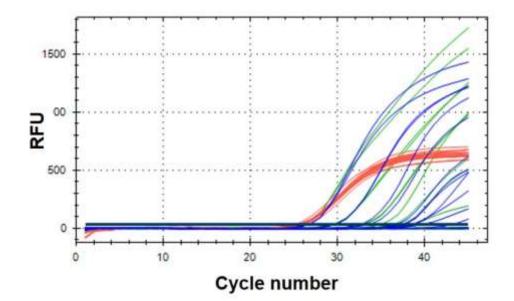
From Wu, A., et al. (2020). Cell Host and Microbe 27, 325





From Wu, A., et al. (2020). Cell Host and Microbe 27, 325





Copies/Rxn	Fan	n (S)	Нех	(N)	Cy5 (GAPDH)		
10,000	26.09	26.12	26.53	26.46	25.33	25.58	
1,000	29.67	29.66	29.54	29.55	25.82	26.61	
100	33.2	33.1	33.4	32.39	25.98	26.13	
10	36.54	35.13	37.2	39.54	25,98	25.95	
5	37.48	N/A	38.52	38.27	25.89	26.14	
2.5	N/A	38.97	37.37	N/A	25.87	26.28	
1.25	N/A	N/A	N/A	43.01	26.23	26.16	
0.625	N/A	N/A	N/A	N/A	26.19	25.94	
NTC	N/A	N/A	N/A	N/A	N/A	N/A	

#### **Features**

- High sensitivity. Can detect as few as 10 copies of the viral target sequences.
- <u>Broad-spectrum detection</u>. Proprietary primer/probe designs enable detection of most SARS-CoV-2 strains.
- <u>Specificity</u>. Inclusion of UDG and dUTP in the reaction reduces carryover contamination, resulting in fewer false positives.
- Instrument compatibility. Optional ROX dye ensures compatibility with virutally all qPCR instruments in use.



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		<u>Note</u> : The BlazeTaq™ Probe One-Step SARS-CoV-2 Detection Kit is for research purposes intended for diagnostic or treatment usage in humans.								
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	Ordering information	Ordering information In addition to the standard size kits listed in the table below, custom size kits are also available. For more information, please contact								
	inquiry@genecopoeia.com.   Buy Catalog# Designation	scription	Price							
	□ QP201 BlazeTaq <sup>™</sup> Probe One-Step SARS-CoV-2 D	etection Kit with ROX dye (20 ul x 200rxn)	\$795							
	□ QP202 BlazeTaq <sup>™</sup> Probe One-Step SARS-CoV-2 D	etection Kit with ROX dye (20 ul x 1,000rxn)	\$2795							
		etection Kit without ROX dye (20 ul x 200rxn)	\$795							
	□ QP204 BlazeTaq <sup>™</sup> Probe One-Step SARS-CoV-2 D	etection Kit without ROX dye (20 ul x 1,000rxn)	) \$2795							
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Note: The BlazeTaq<sup>™</sup> Probe One-Step SARS-CoV-2 Detection Kit is for research purposes only. It is not intended for diagnostic or treatment usage in humans.



#### Plasmids

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Gene delivery Cancer research Drug/target screening	For more information or to re	quest a quote, p	lease contact inquiry@	genecopoeia.com.		
Molecular & cell biology	Test kits & RT-qPCR	Plasmids	Lentivirus/AAV	CRISPR/shRN	A Cell lines	Related products
COVID-19 coronavirus research tools						
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Promotions	down menus below. We also have plasmid clone	es for the related	I virus SARS-CoV, whic	h caused the 2002-	2004 SARS pander	nic.
	+ COVID-19 ORF	cDNA plas	mid clones-viral	proteins		
	+ COVID-19 ORF	cDNA plas	mid clones-ACE	2		
	+ COVID-19 ORF	<sup>c</sup> cDNA plas	mid clones-TMP	RSS2		
	+ COVID-19 ORF	cDNA plas	mid clones-Cycl	ophilin A		
	+ COVID-19 ORF	cDNA plas	mid clones-FKB	P1A		
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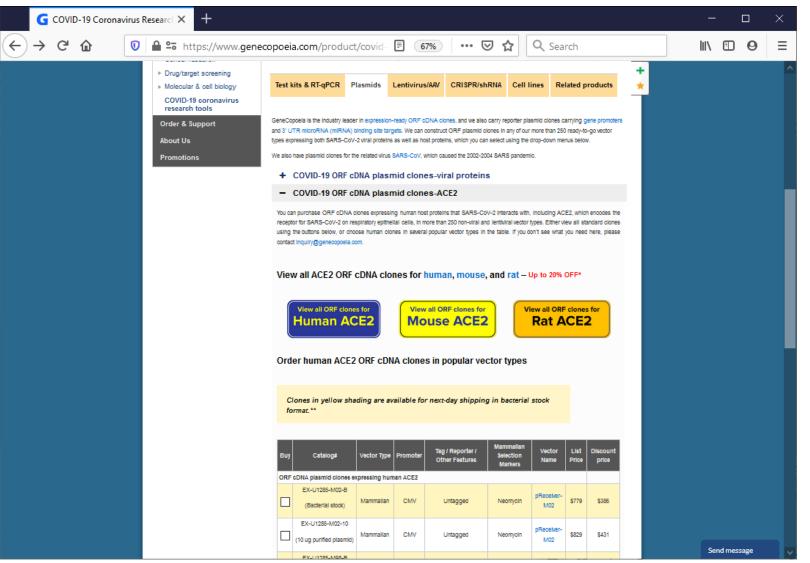


#### **ORF** clones

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		Product ID	Protein accession	Symbol	Alias	Description			
		CoV201 (please inquire)	YP_009724389.1	orf1ab	GU280_gp01	orf1ab polyprotein [Severe acute respiratory syndro coronavirus 2}, full-length gene	ome		
		CoV202	YP_009725297.1	orf1ab	GU280_gp01	leader protein [Severe acute respiratory syndrome mature peptide*	coronavirus 2],		
		CoV203	YP_009725298.1	orf1ab	GU280_gp01	nsp2n [Severe acute respiratory syndrome coronal peptide*	virus 2], mature		
		CoV204	YP_009725299.1	orf1ab	GU280_gp01	nsp3n [Severe acute respiratory syndrome coronal peptide*	virus 2], mature		
		CoV205	YP_009725300.1	orf1ab	GU280_gp01	nsp4n [Severe acute respiratory syndrome coronal peptide*	virus 2], mature		
		CoV206	YP_009725301.1	orf1ab	GU280_gp01	3C-like proteinase [Severe acute respiratory syndro coronavirus 2], mature peptide*	ome		
		CoV207	YP_009725302.1	orf1ab	GU280_gp01	nsp6n [Severe acute respiratory syndrome coronav peptide*	virus 2], mature		
		CoV208	YP_009725303.1	orf1ab	GU280_gp01	nsp7n [Severe acute respiratory syndrome coronal peptide*	virus 2], mature		
		CoV209	YP_009725304.1	orf1ab	GU280_gp01	nsp8n [Severe acute respiratory syndrome coronav peptide*	virus 2], mature		
		CoV210	YP_009725305.1	orf1ab	GU280_gp01	nsp9n [Severe acute respiratory syndrome coronav peptide*	virus 2], mature		
		CoV211	YP_009725306.1	orf1ab	GU280_gp01	nsp10n [Severe acute respiratory syndrome cord mature peptide*	Send message		

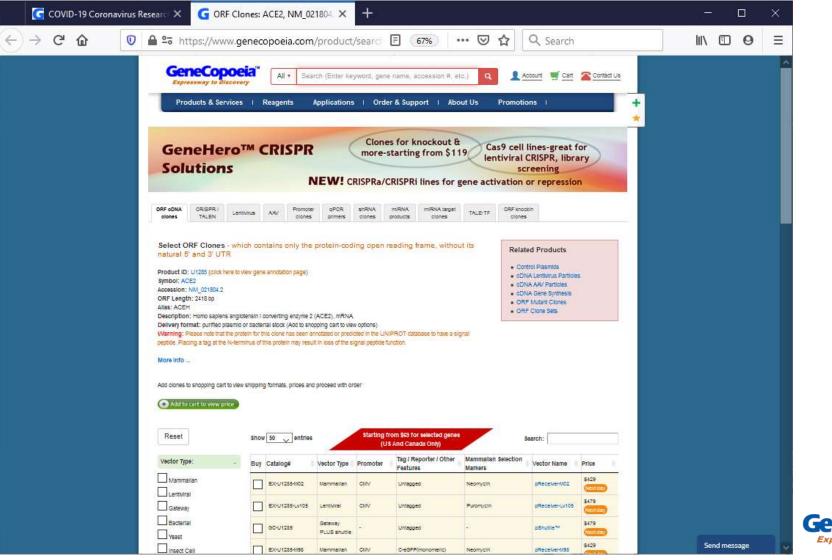


#### **ORF** clones

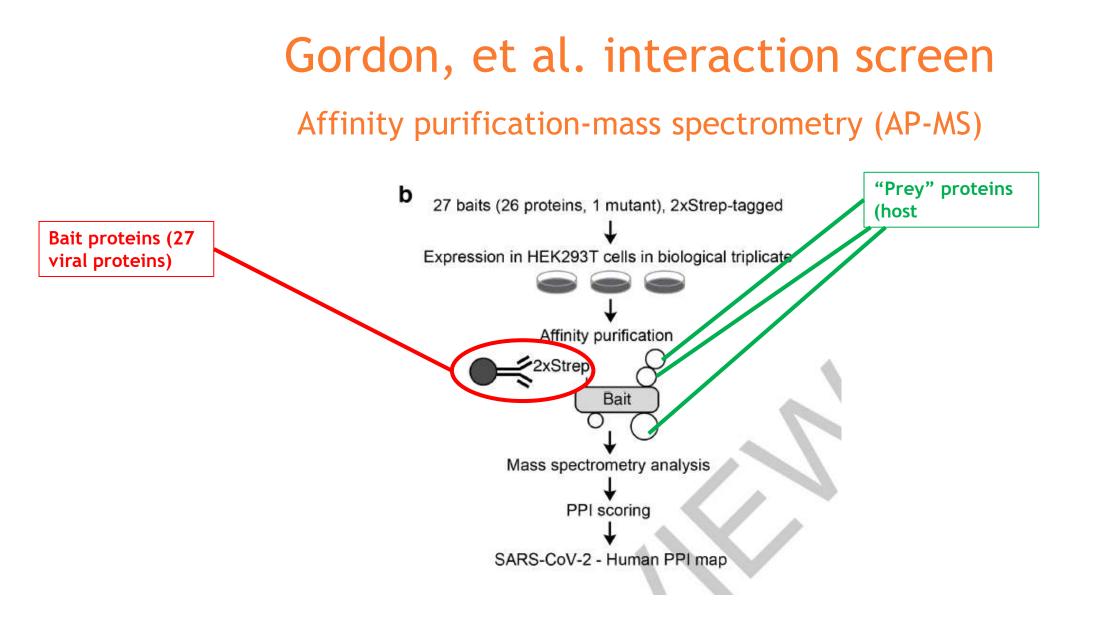




#### **ORF** clones



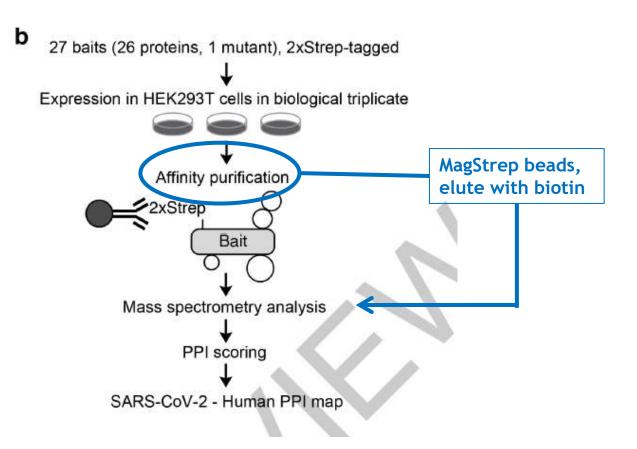






# Gordon, et al. interaction screen

Affinity purification-mass spectrometry (AP-MS)





# 332 interacting proteins identified

#### Caveats

- Possible false positives. Because this is an artificial system, it's possible that some, most, or even all of the reported interactions are not biologically relevant
- Possible false negatives. 2 host proteins of note, ACE2 and TMPRSS2, were not identified in the screen. TMPRSS2 is an enzyme, so it might not physically interact despite its essential role in viral pathogenesis.



# Repurposing of drugs for antiviral use

Search 2 pharmacological sources for ligands of host proteins

✤ IUPHAR/BPS Guide to Pharmacology (2020-3-12) and ChEMBL25 databases

- 16 drugs previously approved for other uses.
- 3 "investigational new drugs" ("A substance that has been tested in the laboratory and has been approved by the U.S. Food and Drug Administration (FDA) for testing in people"-NCI)
- ✤ 18 "pre-clinical candidates".



# Repurposing of drugs for antiviral use

Search 2 pharmacological sources for ligands of host proteins

#### ✤ Literature searches

- ✤ 13 drugs previously approved for other uses.
- ✤ 9 "investigational new drugs"
- ✤ 10 "pre-clinical candidates".
  - ✤ 69 total drugs targeting 66 human proteins.



## Implications

- Repurposing of existing or development of new antiviral drugs that act on host proteins instead of viral proteins. Drug resistance is less likely to be caused by genetic drift for host-directed drugs than for virus-directed drugs.
- Basic research to learn more about virus life cycle and biology.



# Why focus on antiviral drugs?

- The ultimate way to eradicate a disease-causing virus is through a fully-effective vaccine.
- However, there is no guarantee that we will ever have, or soon have, a SARS-CoV-2 vaccine:
  - There are examples of viruses for which a vaccine has never been developed, despite decades of effort (HIV, EBV, RSV). A vaccine for Zika was developed in 2019, but has only been tested on macaques.
  - There has never been a vaccine against any coronaviruses shown to work in humans. 4 coronaviruses cause common colds, so there is less incentive to create vaccines, even though common colds cause workplace disruptions with people getting sick.
  - Vaccines were developed against SARS (2002-2004) and MERS (2012-), which are also caused by coronaviruses. These were tested in mice and shown to produce antibodies, but were never tested in humans because the groups studying them were unable to get funding. SARS is not currently active, MERS still is, although the number of known cases is only about 2,500 globally after 8 years.



# Why focus on antiviral drugs?

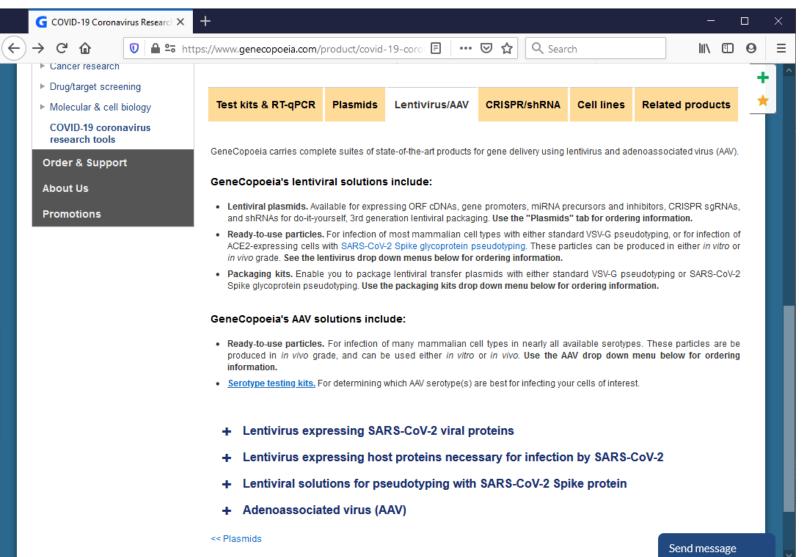
- Therefore:
  - The earliest we will have a mass-produced vaccine will be 2021. So effective antiviral drugs, such as Remdesivir, could fill that gap.

 It is possible that we will never get a fully-effective vaccine, or even any vaccine, so effective antiviral drugs might be one of two treatment options or the only treatment option.



### Other GeneCopoeia SARS-CoV-2 related products

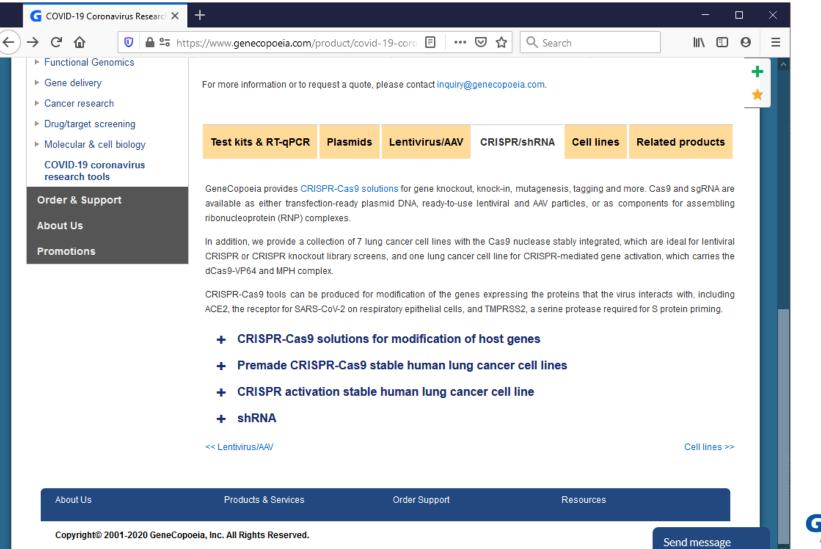
#### Lentivirus & AAV



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### Other GeneCopoeia SARS-CoV-2 related products

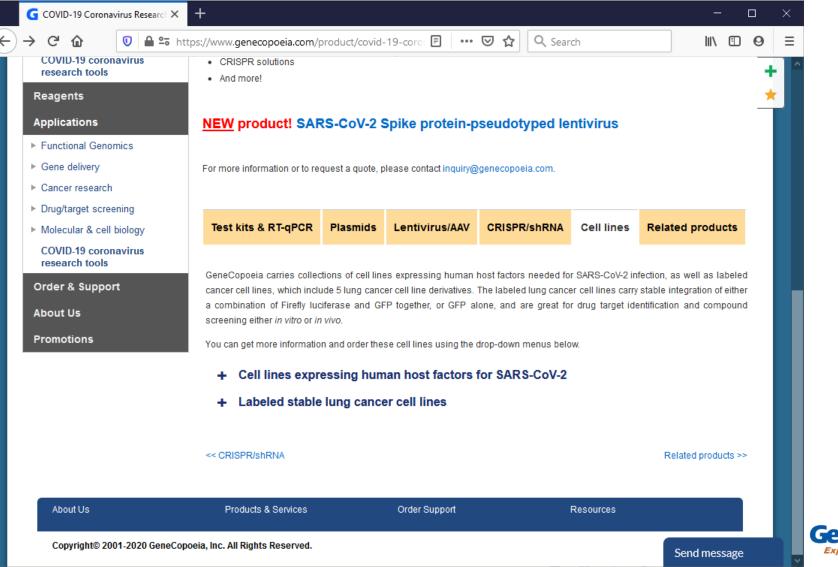
### CRISPR & shRNA





### Other GeneCopoeia SARS-CoV-2 related products

#### Stable cell lines





### Thank You!

If you have any additional questions, please call 1-301-762-0888 x227 Email: edavis@genecopoeia.com Or visit us on the web: www.genecopoeia.com

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