

Collecta Distributor Update: September 2020:

Coronavirus Research Products (COVID-19 related)

- SARS Spike-protein Pseudotyped Lentivirus and ACE2 Cells

CRISPR Update: Inducible Cas9 Construct

New Digital Spatial Profiling (DSP) Service (Nanostring GeoMx Platform)



CELLECTA

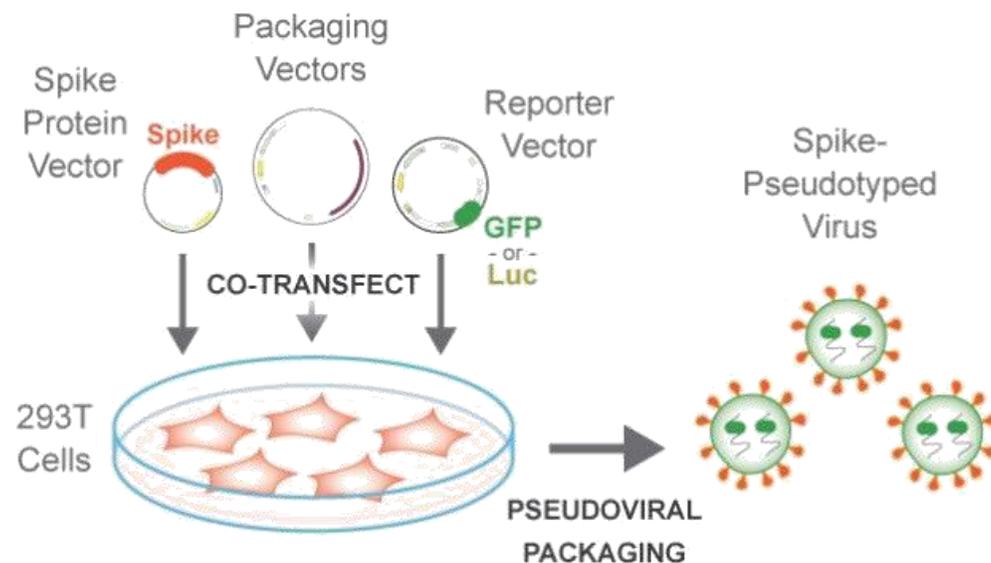
Coronavirus Research Products

COVID-19, MERS, and SARS, are caused by types of coronaviruses that are too pathogenic to study in standard biosafety level (BSL-2) research laboratories. For obvious reasons, though, there is a lot of interest to understand how these viruses function. To help address this need, we now offer a range of products based on **pseudotyping** lentiviruses with coronavirus coat proteins.

Pseudotyping: Lentiviral vectors can be packaged as lentiviral particles with coat proteins from coronaviruses. Since we are using *generation 3 replication incompetent* lentiviruses, the pseudotyped lentivirus reagents are suitable to use in standard BSL-2 research laboratories.

Benefit: Coronaviruses enter cells using the spike glycoprotein (aka S-protein) present on the surface of the coronavirus. Spike-protein pseudotyped lentiviral particles with reporter genes can be used in BSL-2 labs to study spike-protein binding to cells.

Utility: As a result, it is possible for most researchers to conveniently investigate how compounds, factors, and other parameters might inhibit or otherwise affect coronavirus-cell interactions (often called **neutralization assays**).



Studies comparing neutralization assays with spike-protein pseudotyped lentivirus with actual coronaviruses

Chen, X., et al., medRxiv 2020,
doi:<https://doi.org/10.1101/2020.04.06.20055475>

Ju, B., et al., bioRxiv 2020, doi:10.1101/2020.03.21.990770

Pinto, D., et al., bioRxiv 2020, doi:10.1101/2020.04.07.023903

Xiong, H., et al., bioRxiv 2020, 2020.04.08.026948,
doi:10.1101/2020.04.08.026948).

See Tech Page on website for more information:

<https://collecta.com/collections/coronavirus-research-and-neutralization-assays>

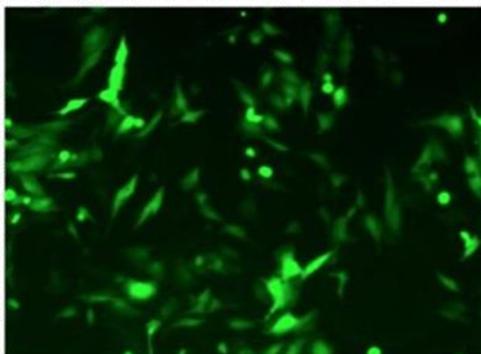
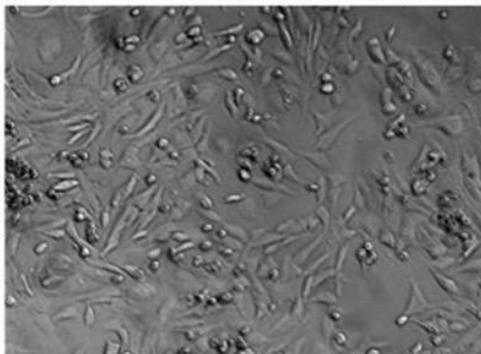
Coronavirus Research Products

Our new portfolio for coronavirus research includes....

- Spike-protein-pseudotyped lentiviral particles with fluorescent protein or Luciferase reporters.
- Cell lines engineered to express the Spike protein receptors for binding assays (i.e., Ace2 for SARS-CoV and SARS-CoV2 and Dpp4 for MERS-CoV).
- Packaged lentivirus expressing the receptor protein binding target to make your own response cell lines for the binding assay.
- Antibodies to Spike and related coronavirus proteins.

For details on specific products...

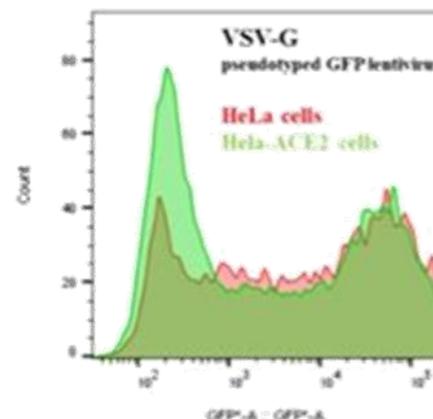
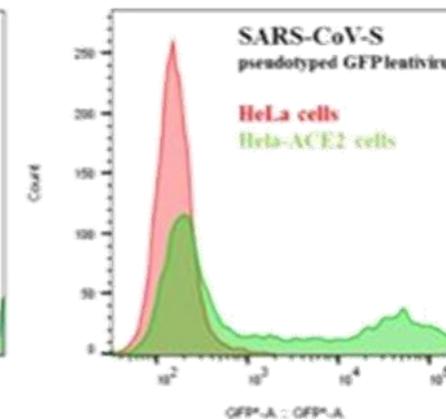
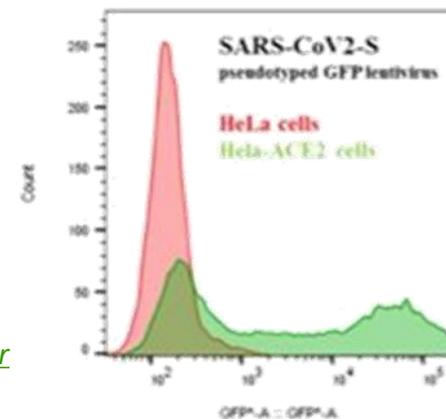
- See [Coronavirus Products Category on Price List](#)
- Product information on website: [https://collecta.com/collections/all?_pf&pf_pt_type=Products&pf_t_category=Coronavirus%20\(COVID19\)](https://collecta.com/collections/all?_pf&pf_pt_type=Products&pf_t_category=Coronavirus%20(COVID19))



ACE2-expressing HeLa cells transduced with SARS-CoV2-S pseudotyped lentiviral particles.

In addition to the off-shelf items, we have added 2 related coronavirus services:

RCSCL-VR-X	Virus Receptor Cell Line Engineering Service (e.g., ACE2/DPP4 expressing cells)
RCSVPT-X	Lentivirus Pseudotyping Service (e.g., Lentivirus with RFP reporter pseudotyped with coronavirus spike protein)

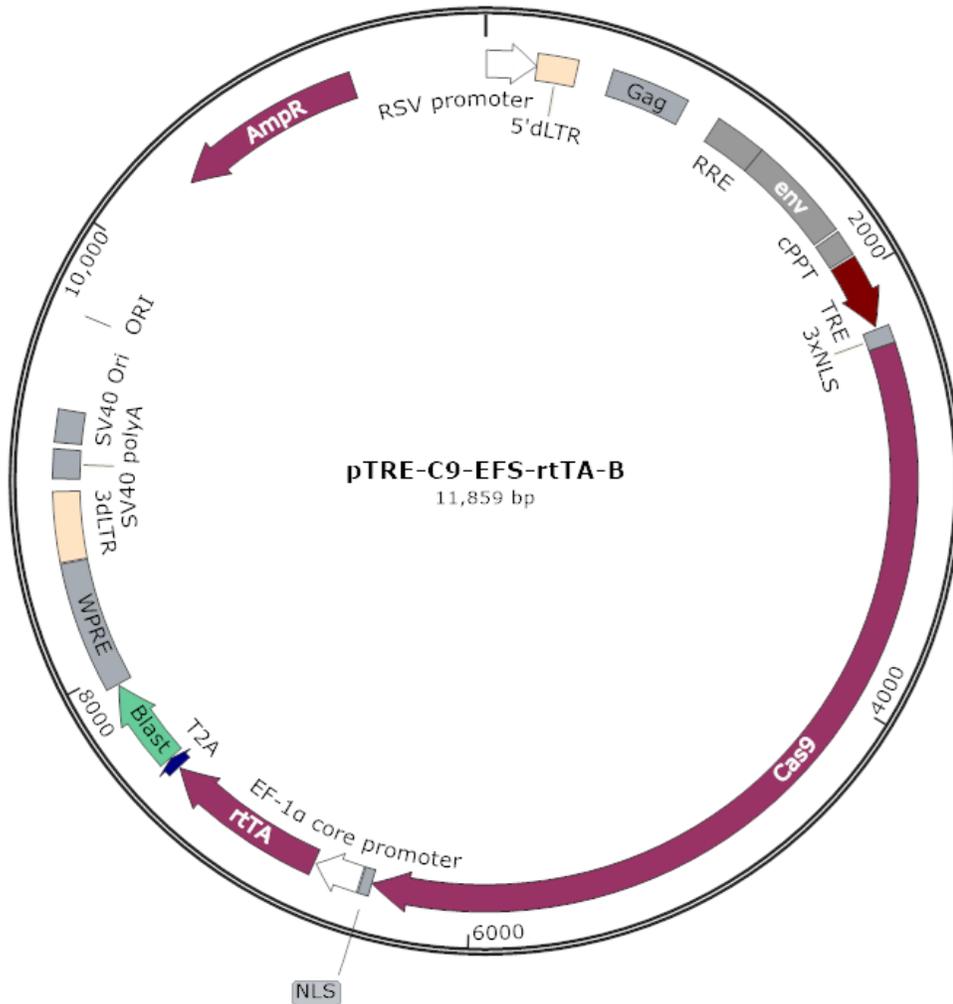


Upper two panels: Fluorescent ACE2-expressing HeLa cells resulting from transduction of SARS coronavirus Spike protein pseudotyped lentiviral particles.

Lower panel: Same cells transduced with VSV-G pseudotyped lentivirus containing the same fluorescent GFP vector



New CRISPR Product: Inducible Cas9 Vector



- Self-contained tet-inducible Cas9 gene expression
- Expresses spCas9 under the Tet-Responsive Promoter (P_{TRE}) and the rtTA tetracycline regulator (i.e., Tet-On)
- Packaging is similar to the standard Cas9 vectors (i.e., low efficiency).

Webpage for product:

<https://collecta.com/products/tet-inducible-crispr-cas9-lentiviral-vector?variant=35036608528550>

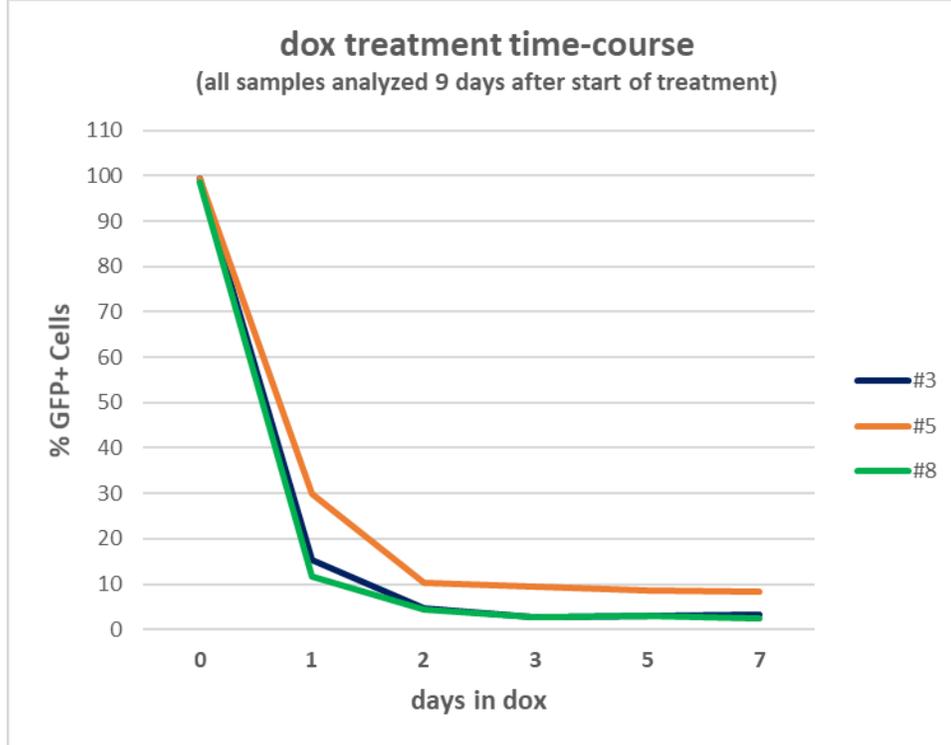
Also note that we have increased the price for our other constitutive Cas9 Vectors.

SVRTC9E2B-PS	CRISPR rtTA (Dox-On) Cas9 pRTCE2Bla-TRE-Cas9-EFS-rtTA-2A-Blast (plasmid)	25 ug	\$700
SVRTC9E2B-VS	CRISPR rtTA (Dox-On) Cas9 pRTCE2Bla-TRE-Cas9-EFS-rtTA-2A-Blast (virus)	1 x 10 ⁶ TU	\$900



COLLECTA

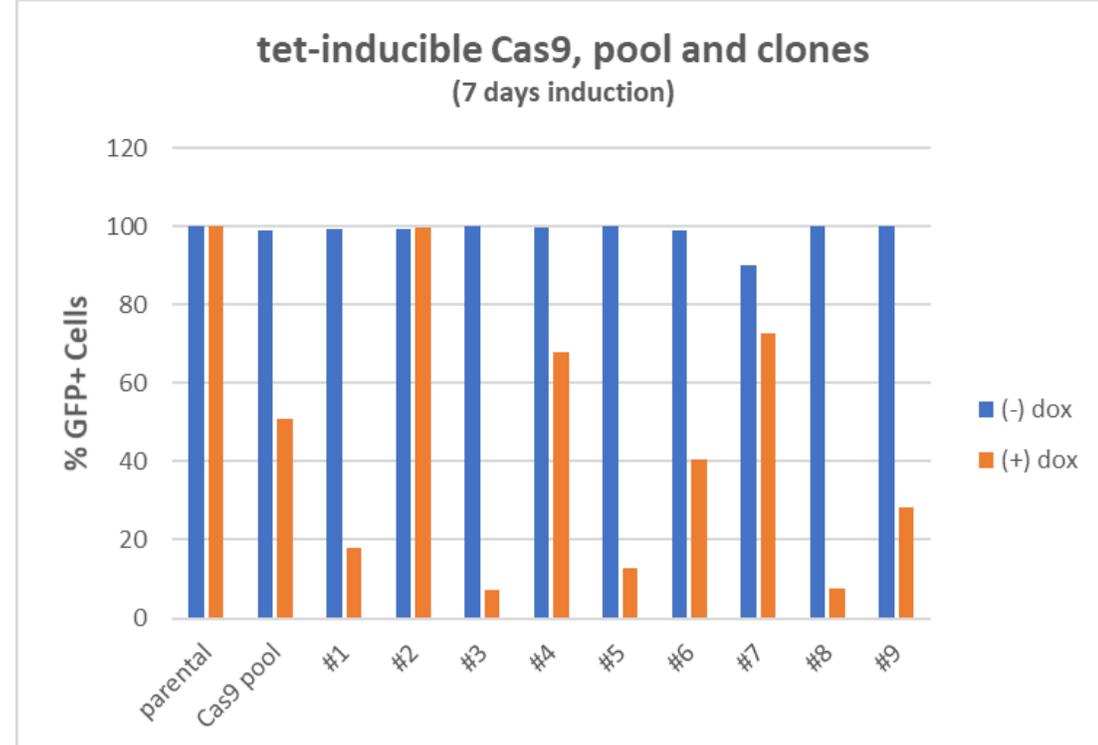
Inducible Cas9 Vector



Dox-Treatment Time Course

Percentage of GFP measured 9 days after the start of treatment, with different treatment lengths in cells transduced with inducible Cas9 and a construct expressing GFP-targeting sgRNA. Treatment for all cultures started at same and continued over the indicated number of days (X axis). All cultures were harvested together on the 9th day.

The results show that only 3 days of dox treatment are needed for maximum knockout KO after 9 days of culture.



Pools vs. Clones: Number of Responding Cells.

Cells were transduced with GFP-targeting sgRNA and the tet-responsive Cas9 construct, then incubated 7 days with 1ug/ml dox. After this induction, individual cells from were isolated and expanded to make clonal cultures.

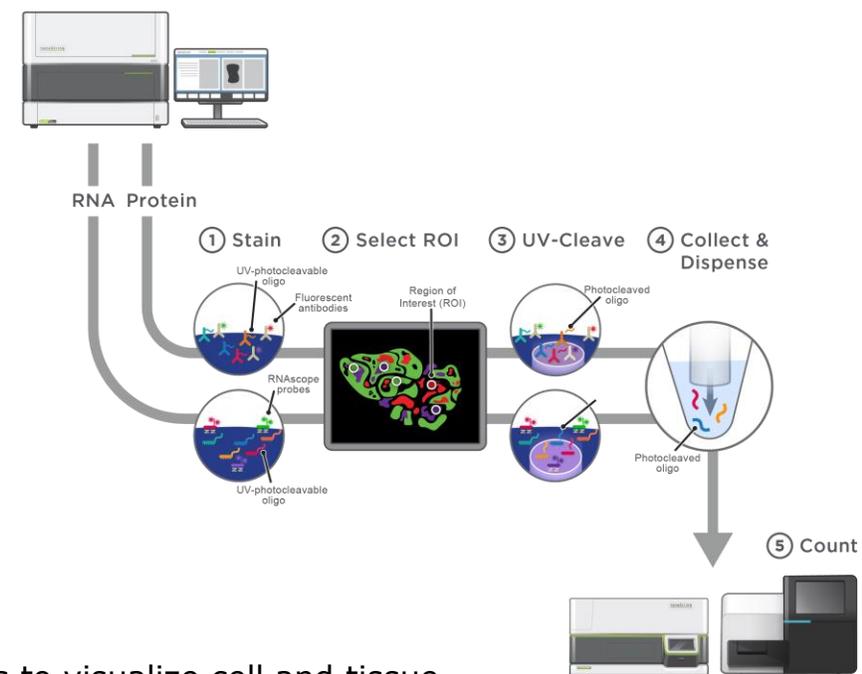
Results show that ~50% of the cell pool show tet-responsive knockout of the GFP gene. However, clones with significantly better responsiveness can be easily isolated.



Digital Spatial Profiling Service

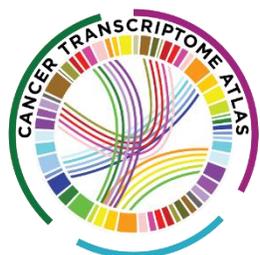
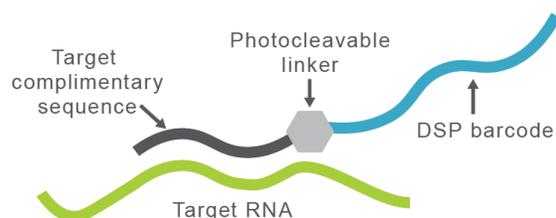
Cellecta is launching a Digital Spatial Profiling (DSP) service with the NanoString GeoMx Instrument

- The GeoMx Digital Spatial Profiler (DSP) is a new platform that enables analysis of RNA and protein present in cells of specific regions of interest on tissue section.
- For example, patterns of gene expression in cells in specific targeted areas of interest on tumor sections (e.g., center of tumor, near tumor/normal tissue interface, immune cell infiltration, etc.) can be assayed and compared to see if there are differences in gene activation.
- There is significant interest from labs interested in understanding tumor biology, and/or looking for markers of treatment prognosis, metastasis, etc.
- Cellecta is focusing specifically on RNA profiling with this platform using the Nanostring Cancer Transcriptome Atlas Probe set (<https://www.nanostring.com/products/geomx-digital-spatial-profiler/geomx-cancer-transcriptome-atlas>). We will also run the genome-wide transcriptome assay when it is released by Nanostring.



RNA reagents

(UV cleavable linker)



How it works

- 1) Tissue sections are stained with fluorescently-labeled markers to visualize cell and tissue morphology (these are similar to H&E and other commonly used cell stains). There are also a number of labeled antibodies that can be used to identify cells with specific protein markers.
- 2) Large panels of pre-mixed biological probes which incorporate UV-cleavable DNA barcodes are added to hybridize to expressed transcripts.
- 3) Regions of interest (ROIs) can then be defined using the fluorescent morphology markers.
- 4) UV light is used to cleave and collect barcodes from hybridized probes in the ROIs.
- 5) Levels of each barcode are then quantified by NGS to measure the relative expression levels of the corresponding probed transcripts for the barcodes.

Cellecta Service webpage in development.

Info on GeoMx platform available at the Nanostring website:

<https://www.nanostring.com/products/geomx-digital-spatial-profiler/geomx-dsp>



CELLECTA