# SARS2 Entry Helix Bundle HTS Campaign


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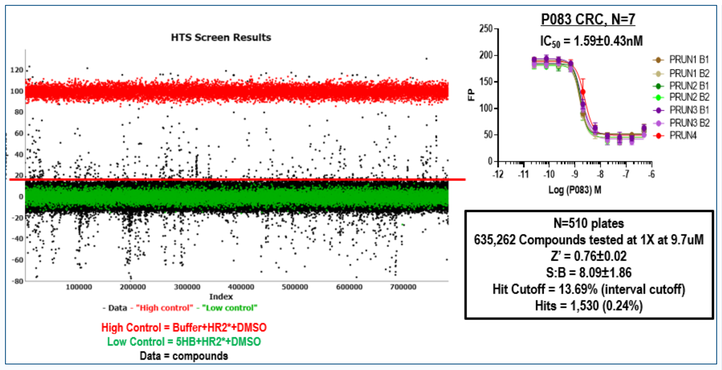
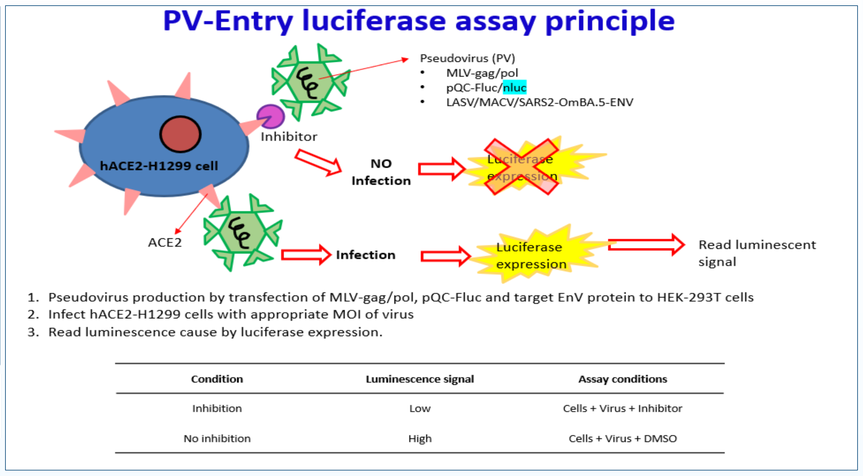
## Abstract


SARS-CoV-2 and other related viruses enter host cells via receptor recognition and membrane fusion. The 5-Helix bundle (5HB) pentamer assay was constructed at the University of Minnesota for the purpose of identifying potential inhibitors SARS-CoV-2 virus entry. 5HB is capable of binding to the viral spike heptad repeats (HR2), which is a critical component in the virus entry pathway and makes 5HB a potential inhibitor of virus entry. Following miniaturization and optimization into a 1536 well format, we completed a pilot HTS vs 5HB and were able to find small molecule inhibitors that appear to compete with the 5HB binding to HR2. We continued to complete the full HTS campaign by screening 635,262 compounds. Following the completion of the HTS around the 5HB pentamer, we tested a monomer version of the 5HB against a pilot screen which would help confirm on target activity. Here in, we illustrate the implementation of the ultra high throughput assay (uHTS) and the comparison of the pentamer vs the monomer assay outcomes. At completion, we screened 130 compounds in dose response format against the 5HB assay. We also screened the same compounds in a secondary cell-based assay that looked for inhibitors of either Machupo entry or SARS2 entry in a dual luciferase transient transfection system (assay optimized and implemented by Yuka Osuka at UF-Scripps). At the conclusion of the screens, as well as a cytotoxicity screen, 41 compounds were found to be selective inhibitors of the 5HB pentamer assay. From these assays, 31 compounds and analogs were selected. The 5HB monomer assay was tested against the Maybridge pilot of 14,000+ compounds. After comparison with the 5HB pentamer assay outcomes 52 compounds, including the 31 compounds and analogs from the 5HB pentamer screen were tested in both assays. 5 compounds showed good potency and are currently being tested in pseudovirus and live virus assays.


## 5HB Pentamer Primary Screen



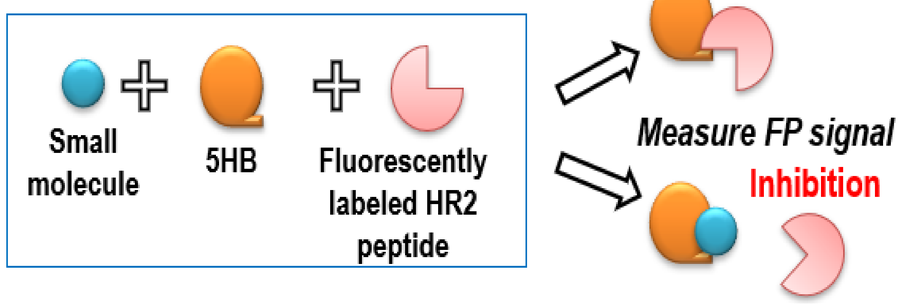

## Machupo and SARS2 DLR Assay




1. Pseudovirus production by transfection of MLV-gag/pol, pQC-Fluc and target EnV protein to HEK-293T cells
2. Infect hACE2-H1299 cells with appropriate MOI of virus
3. Read luminescence cause by luciferase expression.

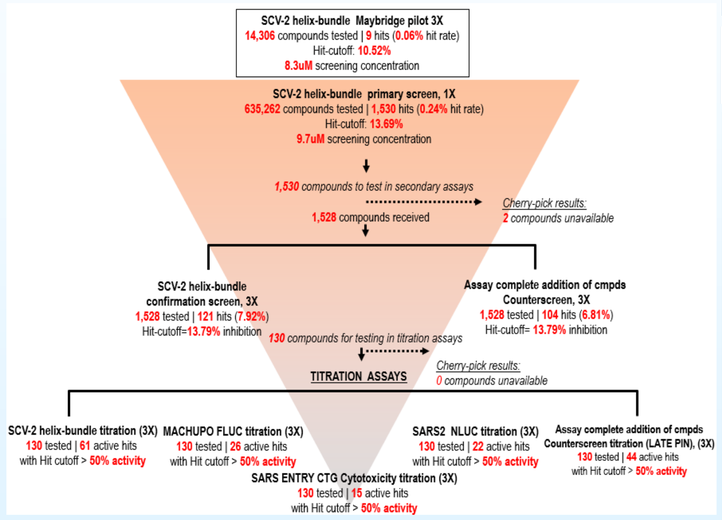
| Condition | Luminescence signal | Assay conditions |
|---|---|---|
| Inhibition | Low | Cells + Virus + Inhibitor |
| No inhibition | High | Cells + Virus + DMSO |

## Competition FP binding Assay Principle

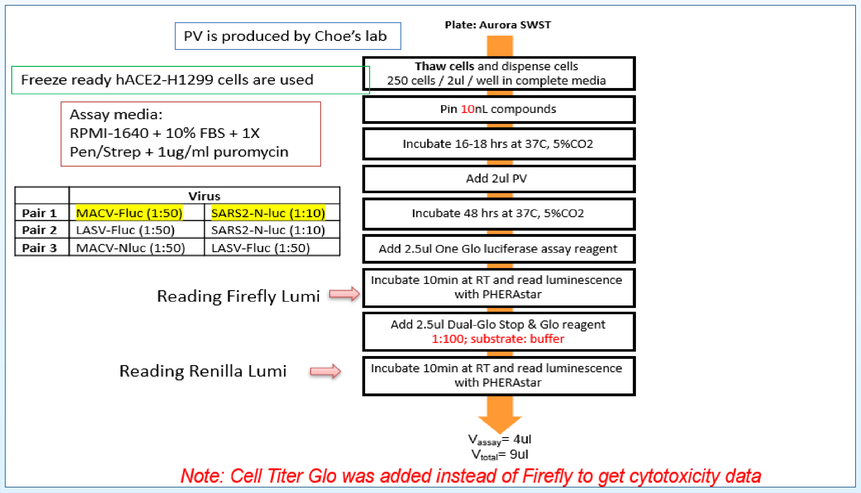



| Condition | FP Signal | Assay Conditions |
|---|---|---|
| No Inhibition | High | 5HB+ AF546-HR2 + DMSO |
| Inhibition | Low | Buffer + AF546-HR2 + DMSO |
| Inhibitor | Low | 5HB+ AF546-HR2 + Inhibitor |

## 5HB Pentamer HTS Campaign Funnel



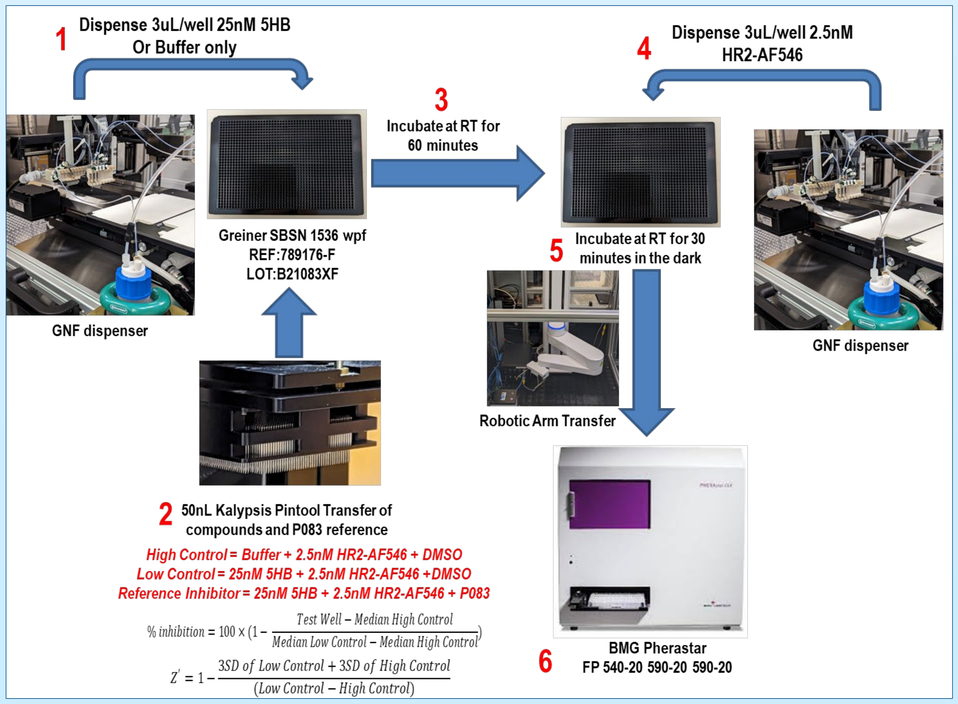

## Machupo and SARS2 DLR Protocol



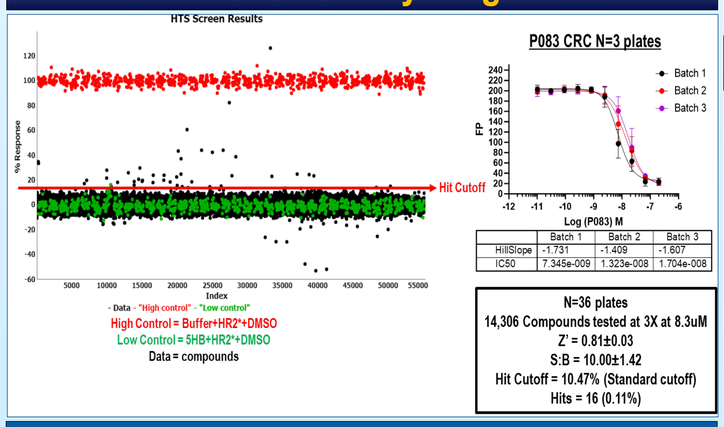

| | Virus | |
|---|---|---|
| Pair 1 | MACV-Fluc (1:50) | SARS2-N-luc (1:10) |
| Pair 2 | LASV-Fluc (1:50) | SARS2-N-luc (1:10) |
| Pair 3 | MACV-Nluc (1:50) | LASV-Fluc (1:50) |

*Note: Cell Titer Glo was added instead of Firefly to get cytotoxicity data*

## HTS Protocol

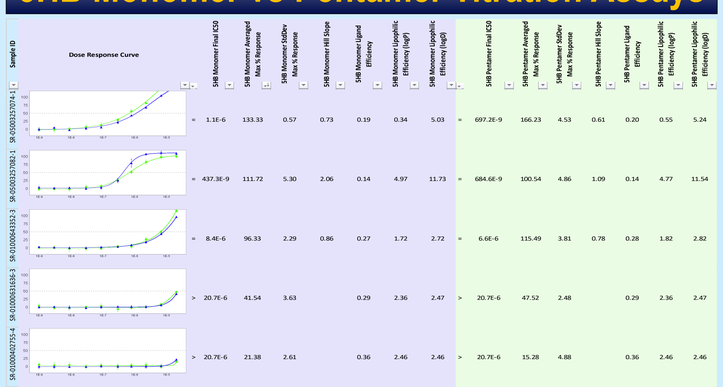



## 5HB Monomer Maybridge Pilot




| | Batch 1 | Batch 2 | Batch 3 |
|---|---|---|---|
| HillSlope | -1.731 | -1.409 | -1.607 |
| IC50 | 7.345e-009 | 1.323e-008 | 1.704e-008 |

## 5HB Monomer vs Pentamer Titration Assays




## Summary And Next Steps

- 5HB, HR2-FI, and HR2-AF546 were provided by the Fang Li lab at the University of Minnesota
- HR2-FI and HR2-AF546 labeled peptides responded the same and AF546 was used to minimize the number of fluorescent artifacts in the assay
- The HTS campaign for the 5HB Pentamer assay was run on the entire UF-Scripps 640K library
- Machupo and SARS2 PV-Entry assays from Hyeryun Choe's Lab (Harvard) were used to further characterize the titration assay compounds from the 5HB pentamer HTS campaign
- 41 compounds selectively inhibit 5B pentamer, but others that overlap with the other assays may be useful too.
- Medicinal chemistry isolated 31 compounds that were tested along with the 21 active Maybridge pilot hits from the 5HB monomer assay in the 5HB monomer and 5HB pentamer assays.
- 5 compounds showed activity in both the monomer and pentamer assays
- The 5 compounds will be tested in pseudovirus or live virus assays

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