The APPEAL project
Antivirus Pandemic Preparedness EuropeAn pLatform:

Identification of host-directed, broad-spectrum antiviral molecules

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INTRODUCTION

The development of novel antivirals, particularly against emerging and re-emerging viruses prioritized by the World Health Organization (WHO)[1], is a **major public health priority.**

Viruses rely on **host dependency factors (HDFs)** for infection, while **host-restriction factors (HRFs)** limit viral replication. HDFs and HRFs could be targeted or induced, respectively, for the development of **host-directed antiviral therapies,** which represent a promising strategy for the development of **broad-spectrum antivirals**, notably because they reduce the risk of emergence of viral resistant mutants.

However, identifying relevant host targets remains **challenging** due to the **variability and limited reproducibility** of genome-wide high-throughput screens, as observed in studies on SARS-CoV-2[2] and influenza A virus.

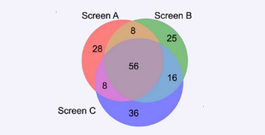
OBJECTIVES

The APPEAL project proposes an approach integrating multidisciplinary and interconnected expertise aimed at :

- Developing a **bioinformatics** and **experimental pipeline** to identify and prioritize host genes involved in the replication of the pathogenic viruses of concern such as coronaviruses, influenza viruses, nipah virus, lassa virus, zika virus.
- Validating these candidate genes in **physiologically relevant cellular models from healthy donors** in 3 virology labs including ours.
- **Identifying** and/or **repurposing** molecules that target these host factors or their associated pathways, to support the development of **novel antiviral strategies.**

MACHINE LEARNING

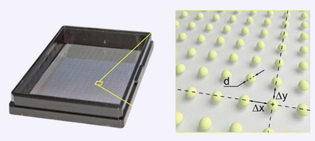
Identification of **consistent patterns** across heterogeneous screening data by integrating diverse omics information from virus-infected cells

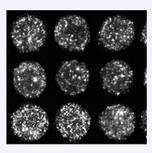


Generation of a comprehensive list of ~1600 HDF and ~ 800 HRF candidates

CRISPR SCREENS USING HIGH-DENSITY CELL ARRAYS (HD-CAs)

High-throughput screening platform using **microscopy-based phenotypic analysis** assays[2]

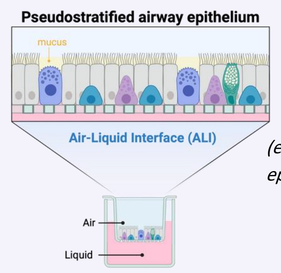




CRISPR screens on HD-CAs and with pooled libraries for the selection of promising HDF/HRF candidates

3D PRIMARY CELLULAR MODELS

Physiologically relevant primary cellular models for the characterization of host-pathogen interactions (through CRISPR studies) and **better prediction** of compounds efficiency and toxicity



(e.g., primary human airway epithelia cultured at the Air-liquid interface (ALI))

Selected HDF/HRF and drug candidates validation on relevant models, bypassing misleading results often obtained from cancer cell lines

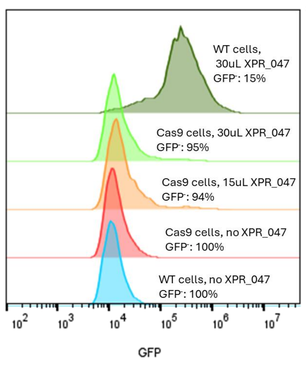
FINAL COMPOUND ASSESMENT

Candidate compound is selected for **preclinical** and **clinical** evaluation as a **repurposed drug** in APPEAL partner laboratories

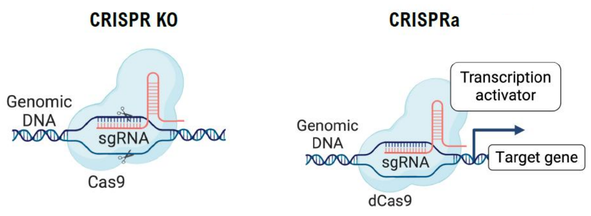
Better preparedness for future viral pandemics, reinforcing the world's capacity to respond to health crises

ANALYSIS OF A BROAD SPRECTRUM OF VIRUSES AND HOST CELLS

Establishing the screening platform

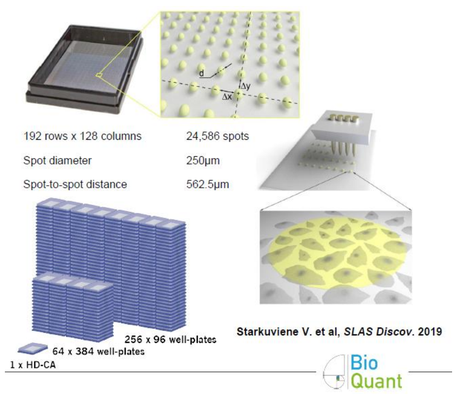


- Establishment of cellular models (A549, Calu-3, Caco-2, NHBE) for CRISPR knock-out (CRISPR KO; Cas9-expressing lines) or CRISPR activation (CRISPRa; dCas9-VP64-expressing lines)



Cas9 activity test in NHBE-Cas9. *WT or Cas9-expressing NHBE cells (MATTEK) are transduced with a lentivector coding for a destabilized GFP and a GFP targeting sgRNA (XPR_047). A strong Cas9 activity results in little residual GFP expression.*

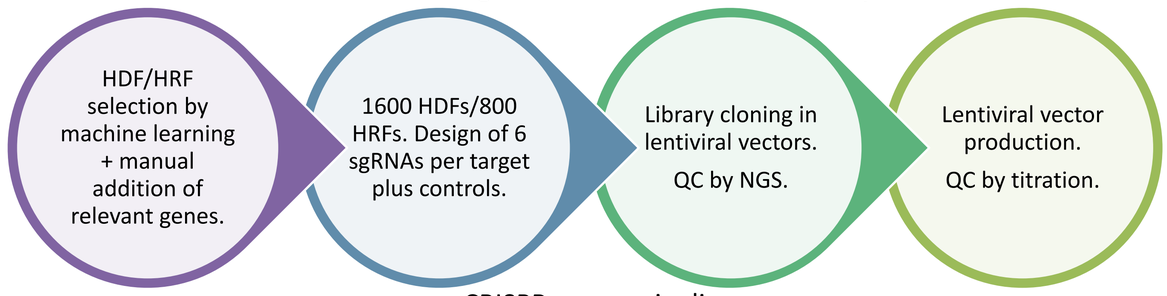
Establishing HD-CAs for CRISPR screens



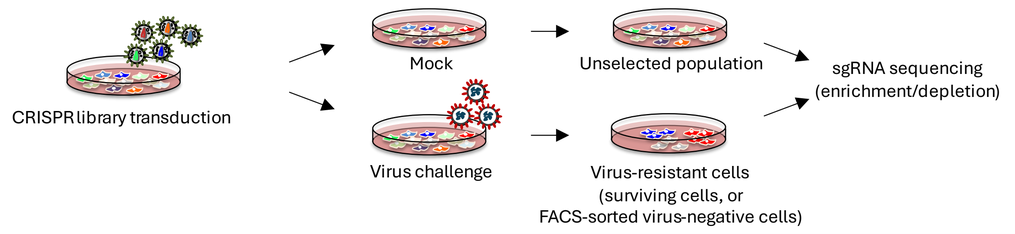
- Ability to "spot" **up to 24,576 sgRNAs per plate** for solid-phase reverse trans-fection of overlaid cells.
- Cost- and time-effective.
- Adapted to CRISPR knock-out (CRISPR KO) or CRISPR activation (CRISPRa).
- To be used with several cell line-virus combinations

Using focused CRISPR libraries

Focused CRISPR libraries: from target selection to lentiviral vector production



CRISPR screen pipeline

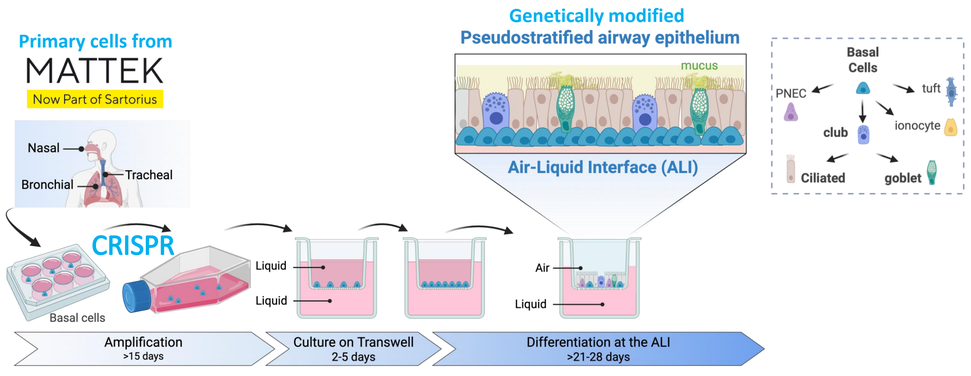


- CRISPR KO (for HDFs) and CRISPRa (for HRFs) screens are currently performed in multiple model cellular systems and with multiple viruses

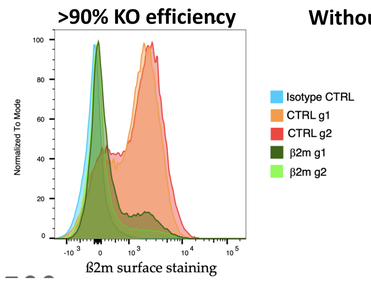
Cellular models used	Virus challenges performed in our lab	Virus challenges performed by other APPEAL labs
• A549-AT Cas9/dCas9-VP64 • Calu-3-Cas9/dCas9-VP64 • Primary NHBE	• IAV H3N2 A/ Victoria/1975 • IAV pH1N1 A/ England/195/2009 • Avian influenza viruses H5N8 and H7N1 • SARS-CoV-2	• Lassa virus • Nipah virus • Ebola virus • MERS-CoV • SARS-CoV-1 • IAV H5N1 • Zika virus

TARGET VALIDATION IN COMPLEX, PRIMARY CELLULAR MODELS IN 96-WELL PLATES

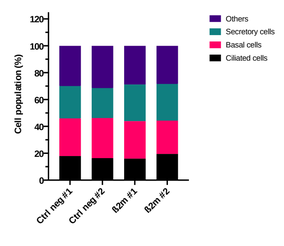
Efficient gene editing in primary airway epithelia for host gene validation



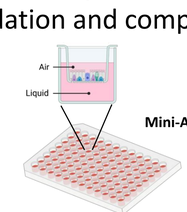
>90% KO efficiency



Without any impact on the epithelium composition



The WT and genetically modified epithelia can be generated in 96-well plates for semi-automated gene validation and compound screens



References:

(1) WHO. Prioritizing Diseases for Research and Development in Emergency Contexts. (2025)

(2) R Rebendenne, A., Roy, P., Bonaventure, B. et al. Bidirectional genome-wide CRISPR screens reveal host factors regulating SARS-CoV-2, MERS-CoV and seasonal HCoVs. *Nat Genet* 54, 1090–1102 (2022).

(3) Starkuviene V, Kallenberger SM, Beil N, et al. High-Density Cell Arrays for Genome-Scale Phenotypic Screening. *SLAS Discov.* 2019;24(3):274-283. doi:10.1177/2472555218818757

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