# A GENOME-WIDE CRISPR SCREEN TO GENERATE HIGH-YIELD CELL LINES FOR PANDEMIC INFLUENZA VACCINE PRODUCTION

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All influenza vaccines currently sold in Canada require one fertilized chicken egg to produce roughly one dose of vaccine. During pandemic influenza outbreaks, the limited availability of eggs stresses the ability of this method to deliver vaccine in a timely manner (1). Unlike eggs, cell lines grow exponentially, resulting in virtually limitless substrate for cultivating influenza vaccines. This ability to rapidly scale production during periods of increased demand is ideal for effectively responding to pandemic influenza outbreaks. While promising, cell-based influenza vaccine production suffers from low volumetric yield (~10-fold lower) compared to egg-based methods (2).

In this study, a genome-wide screen was used to identify gene knockouts that increase influenza yield in the HEK-293SF cell line. Viral replication is dependent upon a myriad of cellular factors, for example an estimated 9.5% of human protein-coding genes affect HIV-1 replication (3). Many of these cellular factors are components of the innate immune system and actively inhibit virus replication. Identifying these factors will allow the generation of cell lines where they have been knocked out, enhancing influenza yield.

To carry out the screen, knockouts were induced with a lentivirus-vectored, pooled CRISPR/Cas9 library. Cells were then infected with Green Fluorescence Protein (GFP)-tagged influenza. Cells with a favorable environment for influenza replication expressed high amounts of GFP, allowing them to be collected using Fluorescence Assisted Cell Sorting. Next Generation Sequencing was then used to determine which knockouts enhanced influenza replication.

The results of the screen will inform the generation of high-yield vaccine production cell lines based on the HEK-293SF parent line, advancing efforts towards cell-based vaccine production methods that are able to effectively address pandemic outbreaks. The results also offer insights into the host determinants of influenza infection within the unique environment of bioreactor culture.

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