# Deep mutational scanning of an H3 hemagglutinin can inform evolutionary forecasting of human H3N2 influenza virus

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#### **Abstract**

Abstract text.

#### INTRODUCTION

#### **RESULTS**

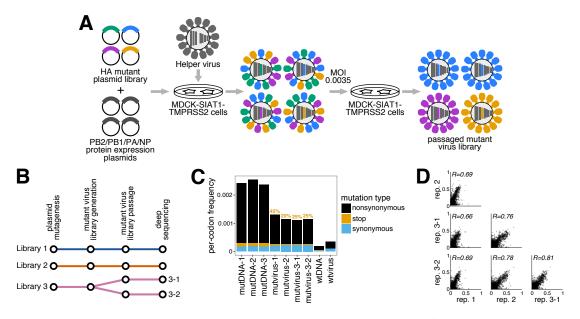
Strategy for deep mutational scanning of an H3 hemagglutinin

H3 site-specific amino-acid preferences

Estimating mutational effects from an H3N2 phylogeny

Comparing H1 and H3 preferences

#### **DISCUSSION**



**Figure 1: Overview of deep mutational scanning experiments of H3 hemagglutinin** Figure caption text

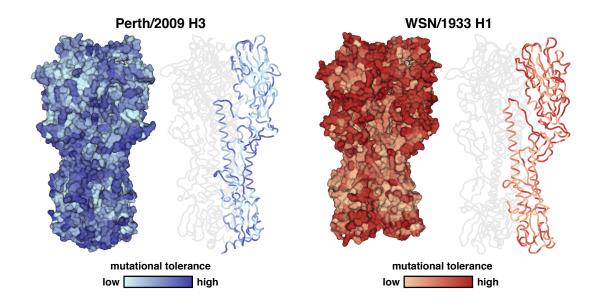


Figure 2: The mutational tolerance of HA Figure caption text

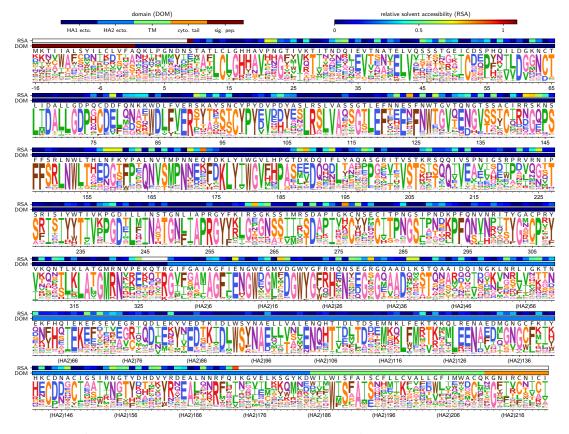


Figure 3: The site-specific amino-acid preferences of H3 hemagglutinin Figure caption text

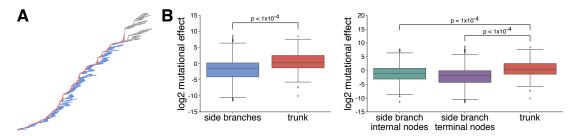


Figure 4: The trunk of a human H3N2 phylogeny has higher mutational effects than those of side branches Figure caption text

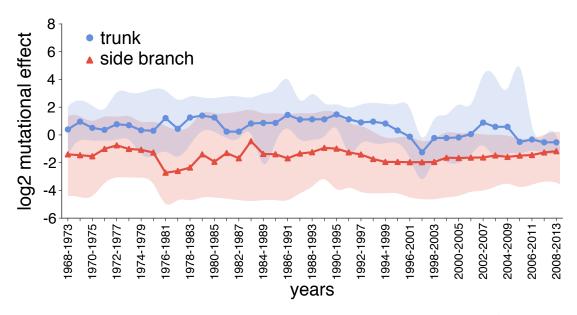
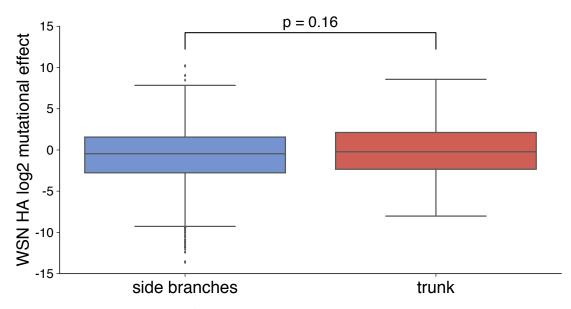


Figure 5: Sliding window analysis of mutational effects of trunk vs side branches. Figure caption text



**Figure 6: Trunk vs side branch mutational effects calculated using WSN preferences** Figure caption text

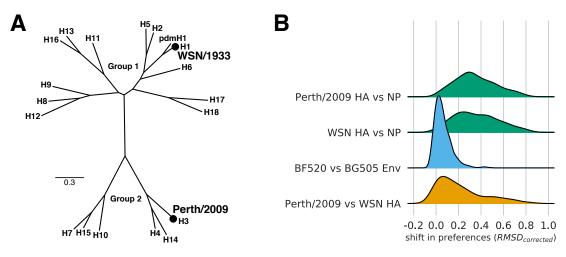


Figure 7: Distribution of preference shifts for protein homologs Figure caption text

## **METHODS**

### HA numbering

Generation of HA codon mutant plasmid libraries

Generation and passaging of mutant viruses

Barcoded subamplicon sequencing

Analysis of deep sequencing data

Quantification of mutational effects and sequence preferences from an H3N2 phylogeny

Data availability and source code

Deep sequencing data are available from the Sequence Read Archive under BioSample accession [add accession].

#### **ACKNOWLEDGMENTS**

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