

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

Juhye M. Lee<sup>1,4,5,†</sup>      John Huddleston<sup>2,6,†</sup>      Michael B. Doud<sup>1,4,5</sup>  
Kathryn A. Hooper<sup>1,6</sup>      Trevor Bedford,<sup>2,3</sup>  
Jesse D. Bloom<sup>1,3,4\*</sup>

<sup>1</sup>Basic Sciences Division, <sup>2</sup>Vaccine and Infectious Diseases Division, and <sup>3</sup>Computational Biology Program,  
Fred Hutchinson Cancer Research Center, Seattle, WA, USA

<sup>4</sup>Department of Genome Sciences, <sup>5</sup>Medical Scientist Training Program, and <sup>6</sup>Molecular and Cellular Biology Program,  
University of Washington, Seattle, WA, USA

<sup>†</sup>These authors contributed equally

\*Correspondence: [jbloom@fredhutch.org](mailto:jbloom@fredhutch.org)

## Abstract

Abstract text.

## INTRODUCTION

## RESULTS

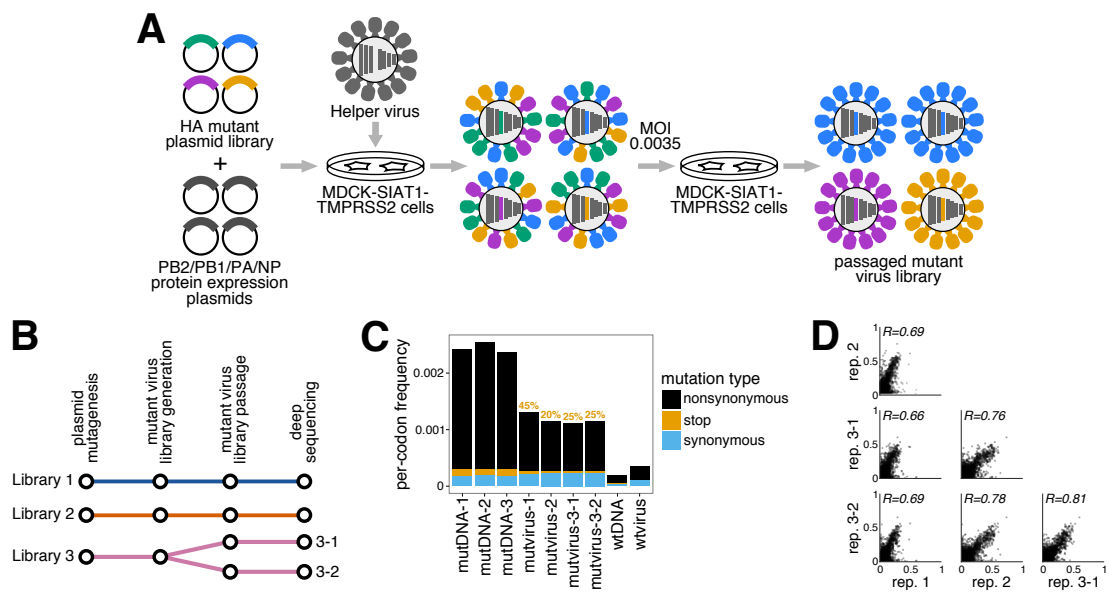
Strategy for deep mutational scanning of an H3 hemagglutinin

H3 site-specific amino-acid preferences

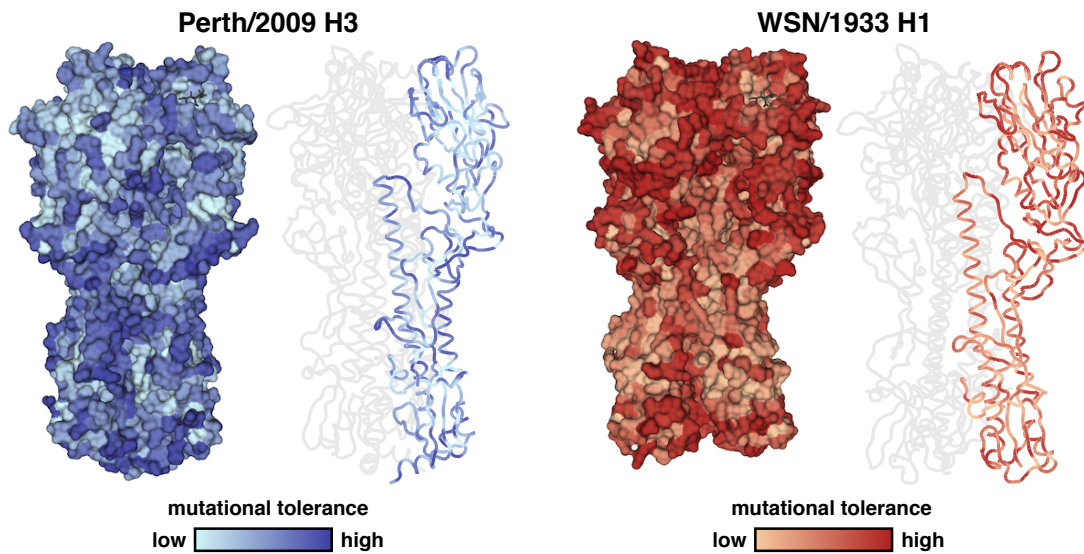
Estimating mutational effects from an H3N2 phylogeny

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

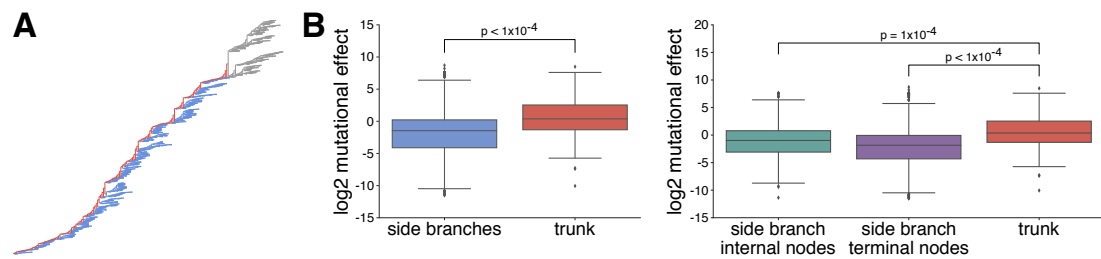
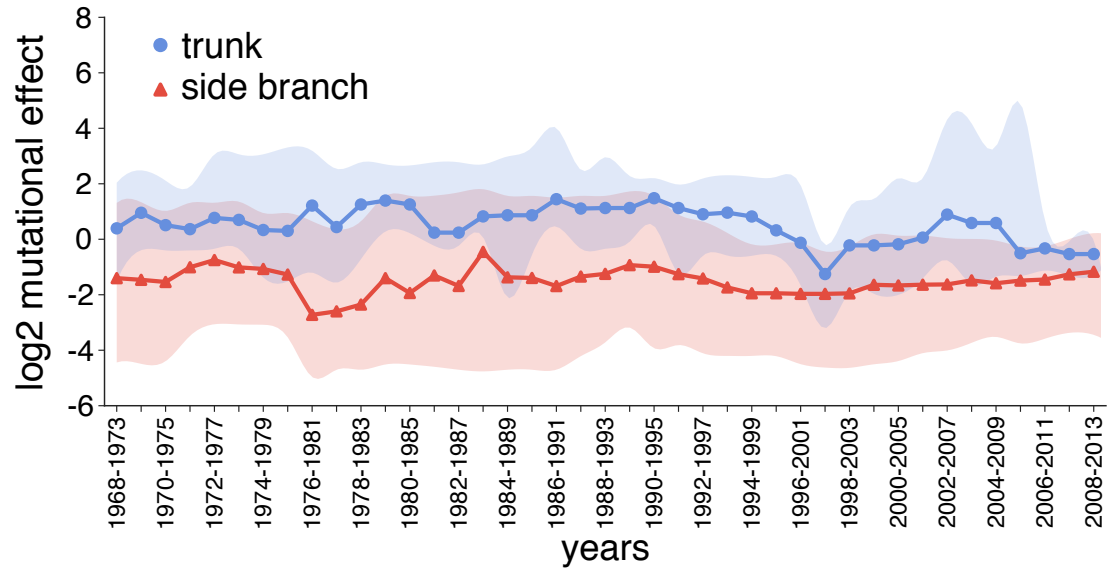
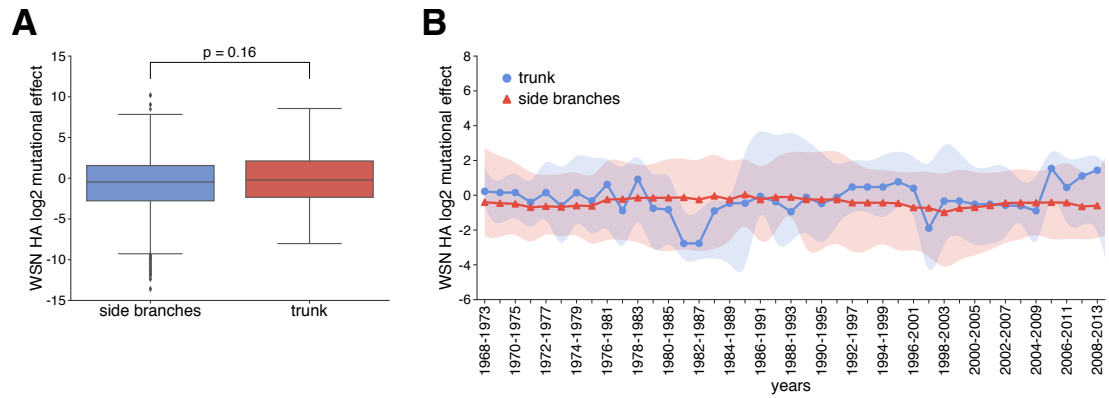


Figure 4: The trunk of a human H3N2 phylogeny has higher mutational effects than those of side branches Figure caption text



**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

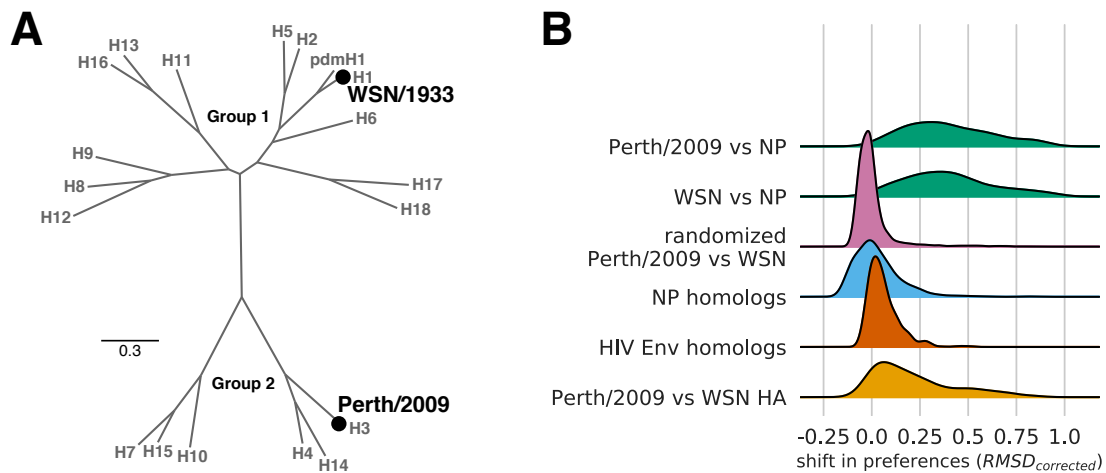


Figure 7: Distribution of preference shifts for protein homologs Figure caption text

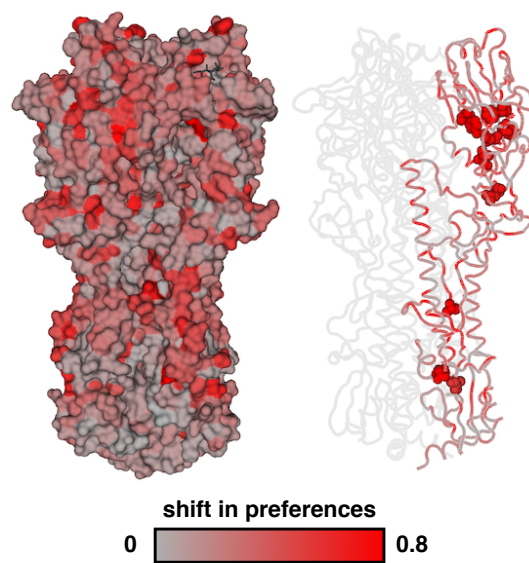


Figure 8: Shifts in preferences mapped onto the structure of HA Figure caption text

## **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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