

Supplemental Files

1: Non-hotspot analysis (Sup. Table 1)	2
2: ML estimation of omega, kappa, and tree length	3
3: Effects of empirical equilibrium frequencies	5
4: Ancestral state reconstructions	6
5: Fully context dependent simulations	10

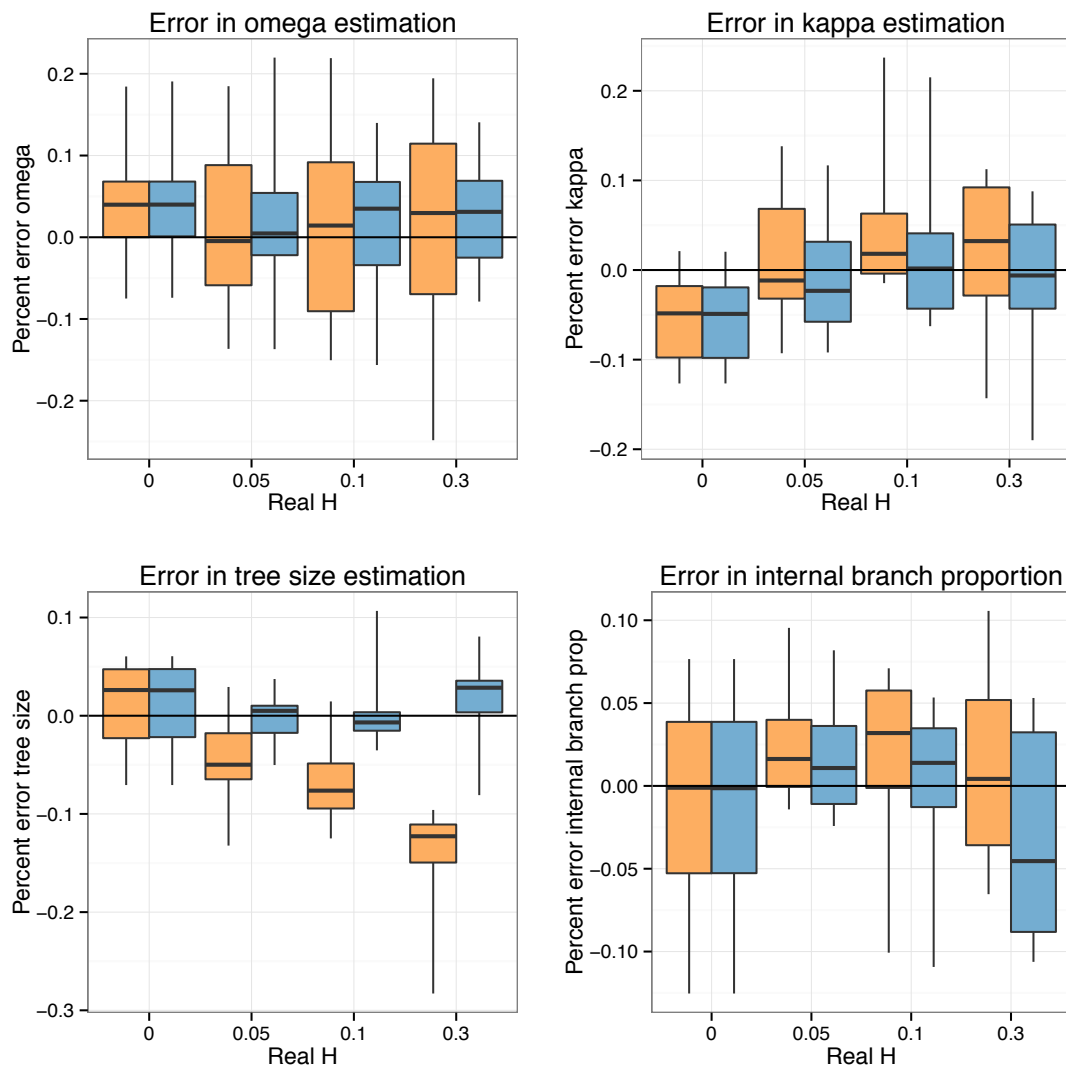
a)

Lineage	Trimer motifs		Dimer motifs	
	Mean non-hotspot correlation	Non-hotspot correlation > hotspot correlation	Mean non-hotspot correlation	Non-hotspot correlation > hotspot correlation
CH103	0.05	0.00	0.03	0.52
CAP256	0.07	0.00	-0.05	0.91
VRC01	0.06	0.00	-0.04	0.79

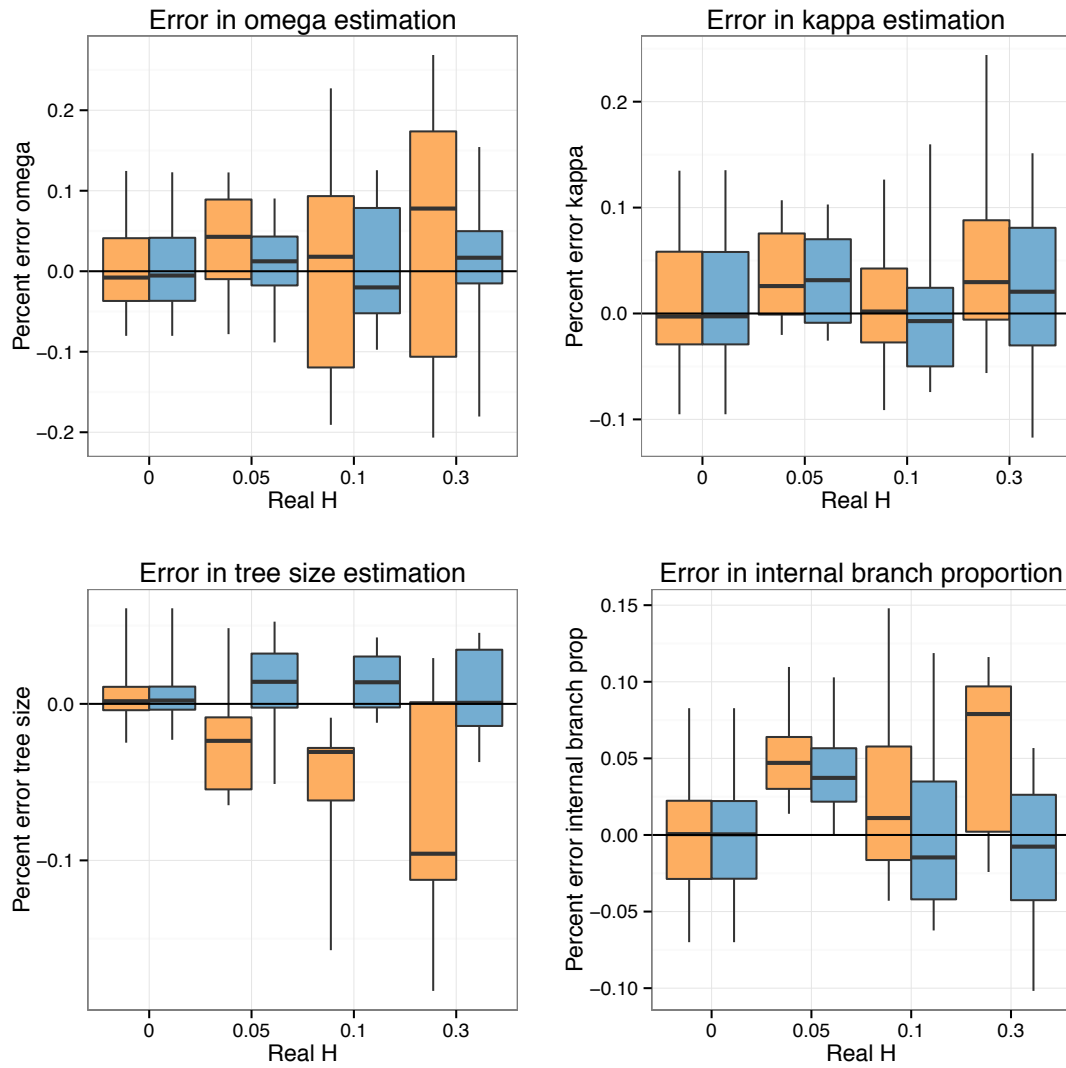
b)

Lineage	Trimer motifs: WRC/GYW			Dimer motifs: WA/TW		
	Mean p value	P < 0.05	P < hotspot p	Mean p value	P < 0.05	P < hotspot p
CH103	0.54	0.04	0.02	0.58	0.04	0.36
CAP256	0.60	0.02	0.01	0.49	0.08	0.61
VRC01	0.58	0.05	0.05	0.44	0.06	0.63

Supplemental File 1: Null tests for hotspot decay. **a)** Shows the results of repeating observed correlation tests in the three bNAb lineages using non-hotspot motifs (see Methods). **b)** In these analyses, both the correlation significance test was repeated using 100 non-hotspot motifs assigned as hotspots (see Methods). Here, the mean p value across the 100 different non-hotspot values is shown in the first column, followed by the proportion of the 100 tests that showed a p value < 0.05, and finally by the proportion of tests that non-hotspot p values showed a p value at least as low as that observed for real p values.



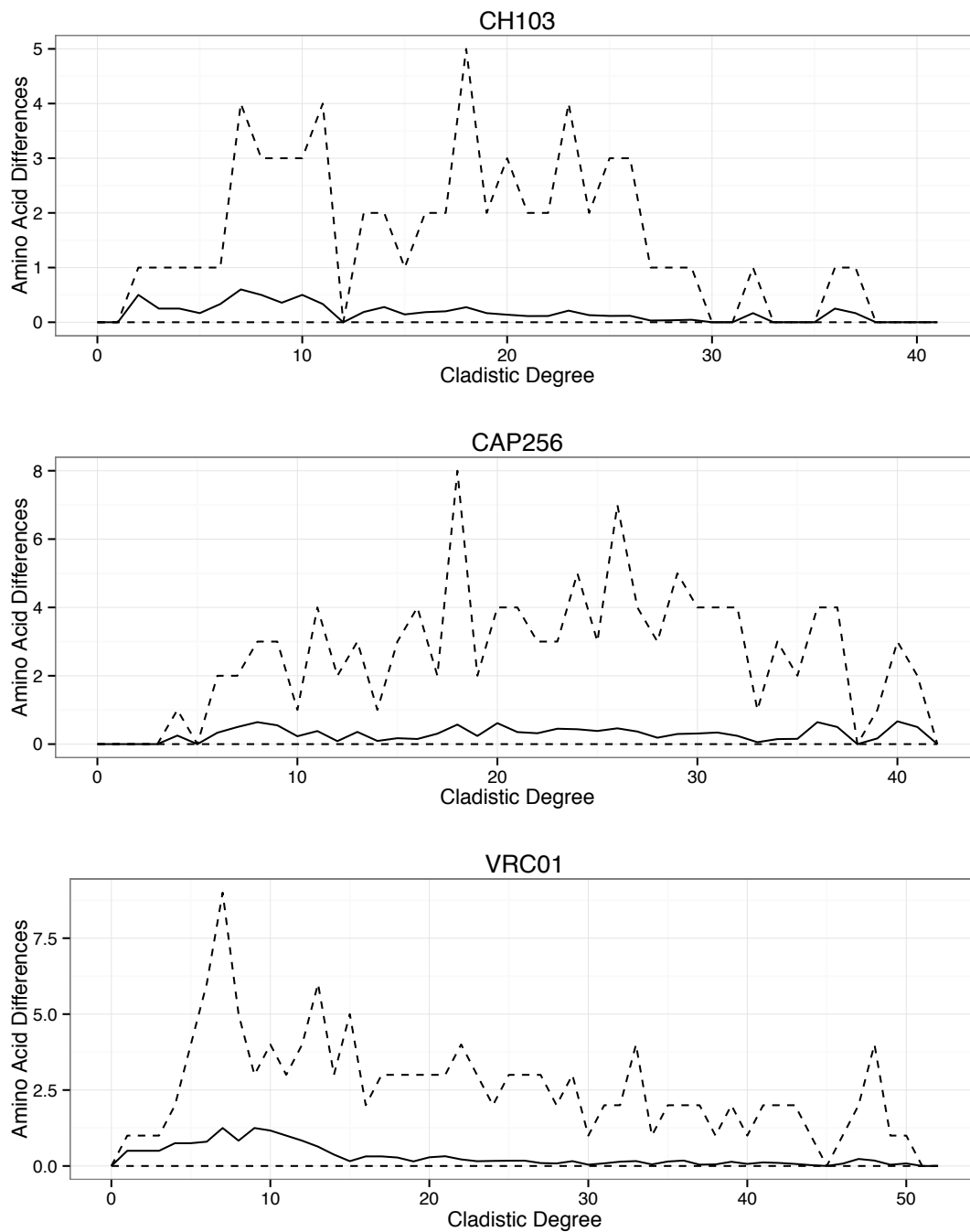
Supplemental File 2a: Percent error of parameter estimation compared to true values for **CH103** fit with GY94 (blue – h_0) and the hotspot model (orange – h_{MLE}). Edges and center of boxplots show 1st, 2nd, and 3rd quartiles, while whiskers show range.



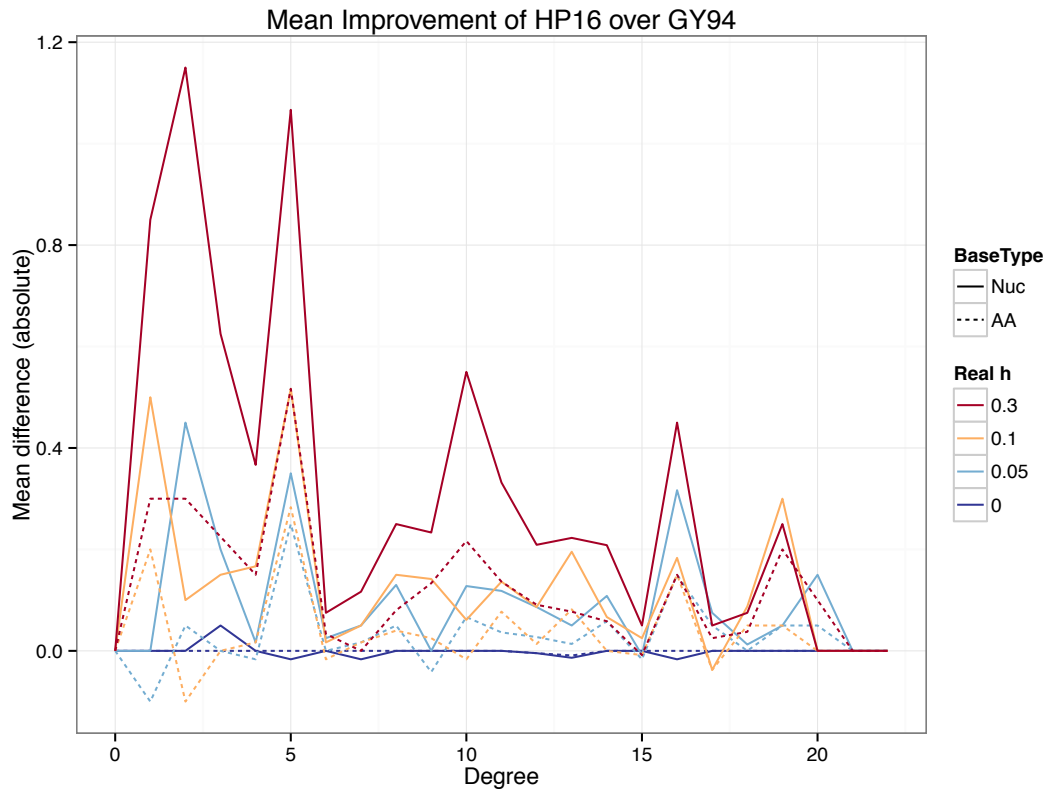
Supplemental File 2b: Percent error of parameter estimation compared to true values for CAP256 fit with GY94 (blue – h_0) and the hotspot model (orange – h_{MLE}). See caption for Supplemental File 2a for further details.

Set	h	Mean \hat{h}	Bias	Variability	Type 1 error	Type 2 error
CH103	0.30	0.281	-1.9E-02	7.6E-04	0.00	0.10
	0.10	0.092	-8.4E-03	1.2E-04	0.00	0.20
	0.05	0.050	-2.4E-05	7.0E-05	0.00	0.10
	0.00	0.002	2.5E-03	7.2E-06	-	0.30
CAP256	0.30	0.280	-2.0E-02	4.8E-04	0.00	0.20
	0.10	0.099	-1.1E-03	1.4E-04	0.00	0.20
	0.05	0.051	8.2E-04	1.1E-04	0.00	0.30
	0.00	0.003	2.6E-03	7.6E-06	-	0.30
VRC01	0.30	0.231	-6.9E-02	1.3E-03	0.00	0.90
	0.10	0.081	-1.9E-02	3.4E-05	0.00	0.70
	0.05	0.042	-8.0E-03	2.0E-05	0.00	0.70
	0.00	0.001	9.0E-04	2.4E-06	-	0.10

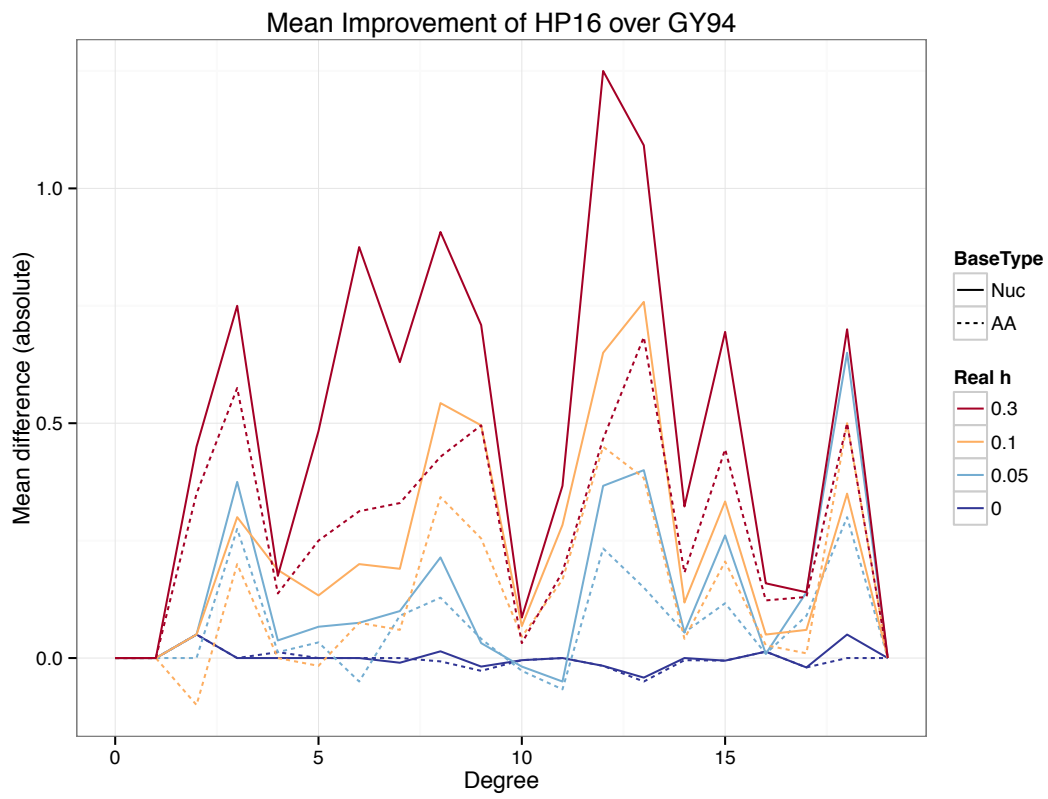
Supplemental File 3: Effects of empirical equilibrium frequencies on h estimation. Type 1 error rate shows the proportion of data sets that incorrectly failed to reject the null hypothesis of $h = 0$. Type 2 error rate shows the proportion of data sets that rejected the true value of h shown in the first column. Both of these hypothesis tests used an alpha value of 0.05. See Methods for explanation of how summary statistics were calculated.



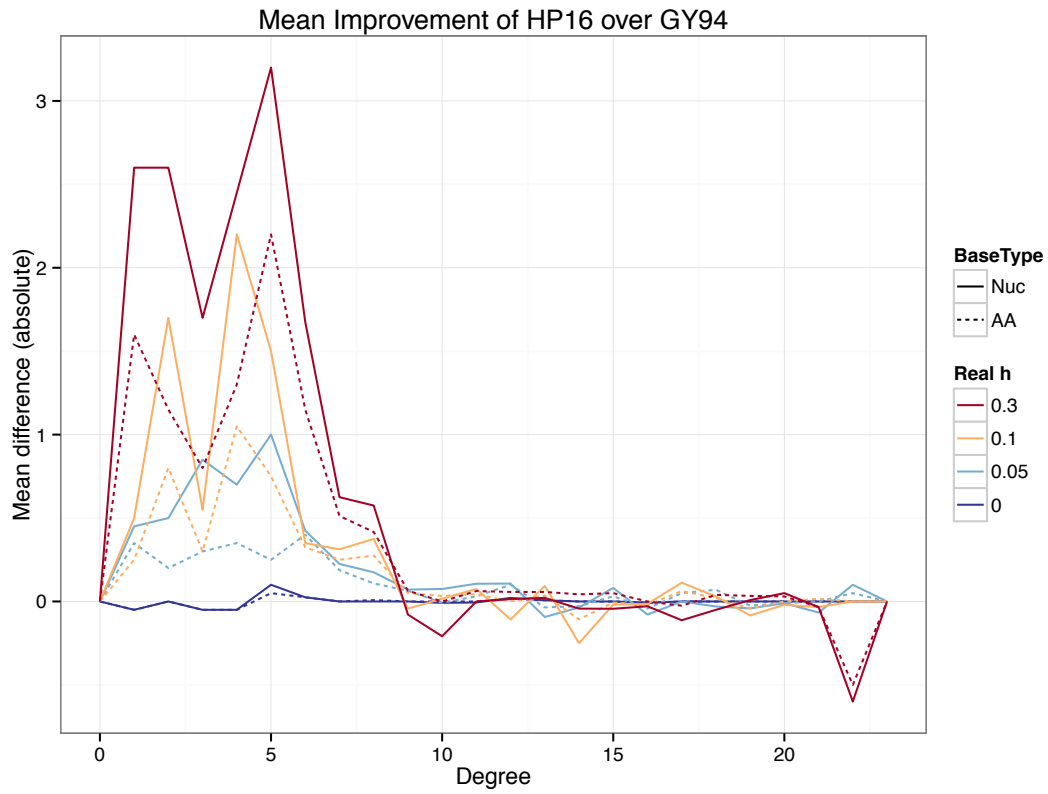
Supplemental File 4a: Differences between true and predicted ancestors for three bNAb lineages by cladistics degree (nodes from root sequence). Solid line shows the mean difference for each node, dashed lines show the minimum and maximum values for each degree.



Supplemental File 4b: Improvement in accuracy of ancestral state reconstruction of h_{MLE} over h_0 (GY94) as cladistic degree (number of nodes from root) increases in simulations of **CH103** bNAb lineage. Values to the left are closer to the root, while values to the right are closer to the tips. Accuracy is measured as number of differences (solid line: nucleotides, dotted line: amino acids) from the true ancestral sequence at a given node. Values shown are the means across all nodes of a particular degree across 20 simulated data sets for each value of h (the same as in **Table 3**). Lines are colored by the value of h used in simulations. As expected, larger values of h in simulations lead to greater error when h is not modelled in h_0 .



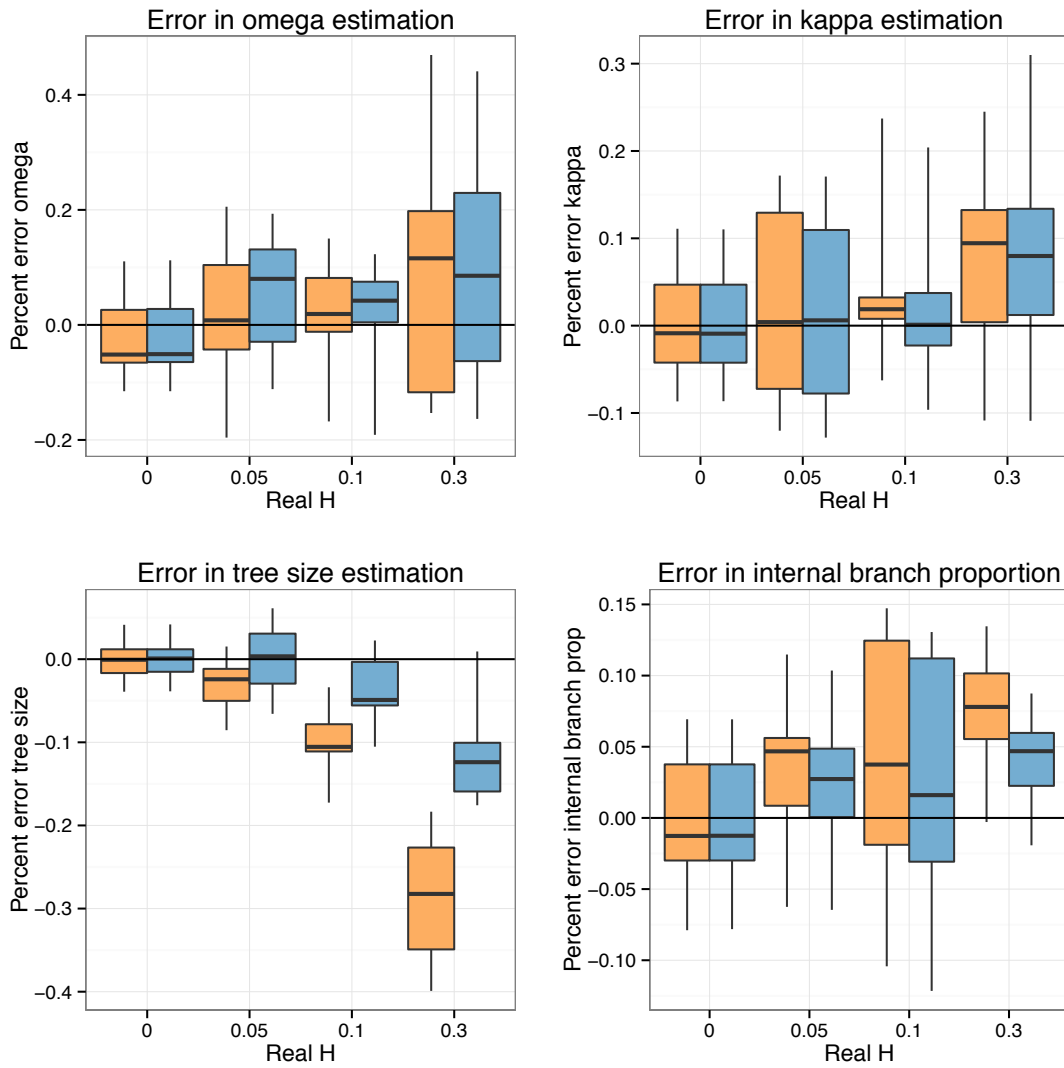
Supplemental File 4c: Improvement in accuracy of ancestral state reconstruction of h_{MLE} over h_0 (GY94) as cladistic degree (number of nodes from root) increases in simulations of CAP256 bNAb lineage. See caption for Supplemental File 4b for details.



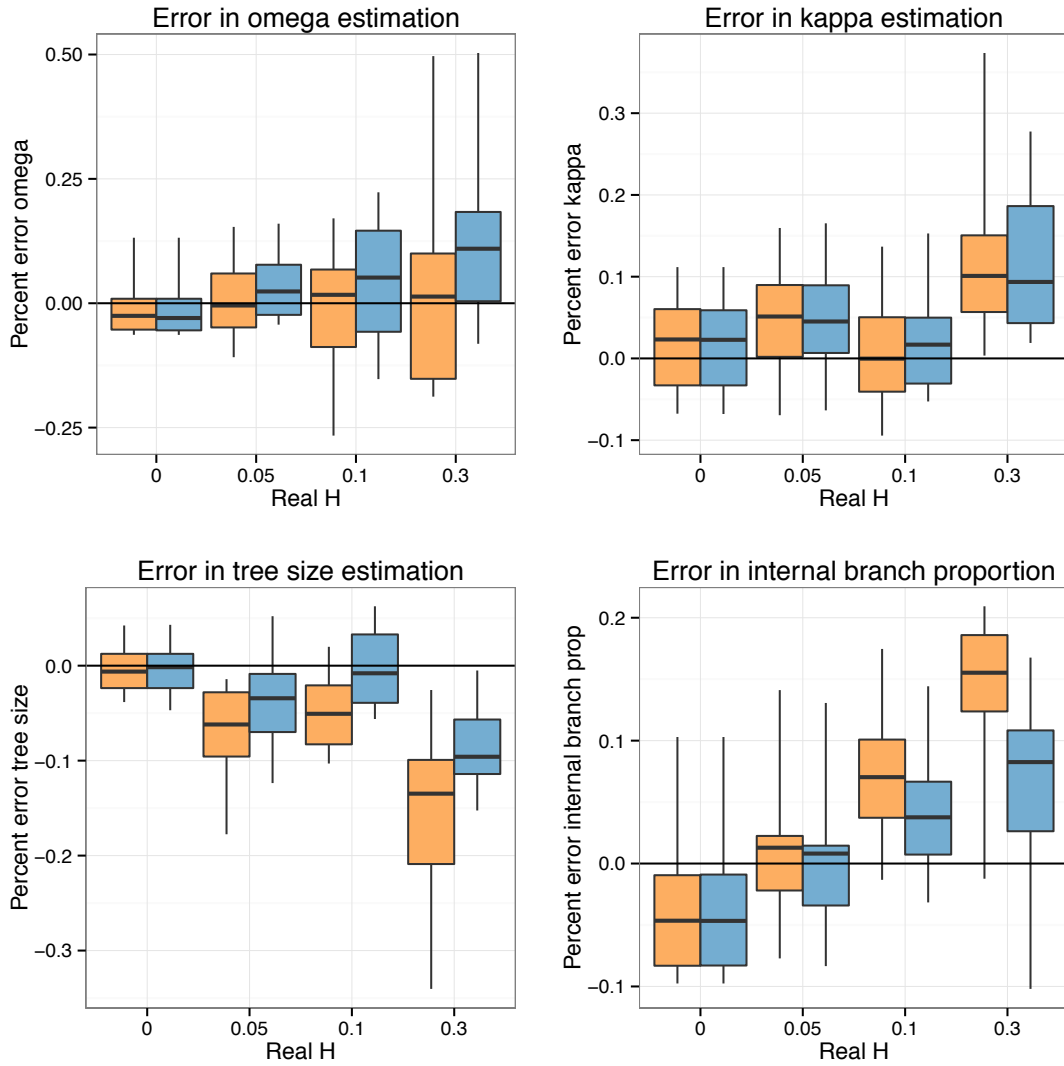
Supplemental File 4d: Improvement in accuracy of ancestral state reconstruction of h_{MLE} over h_0 (GY94) as cladistic degree (number of nodes from root) increases in simulations of VRC01 bNAb lineage. See caption for **Supplemental File 4b** for details.

Set	h	Mean \hat{h}	Bias	Variability	Type 1 error	Type 2 error
CH103	0.30	0.221	-7.9E-02	1.1E-03	0.00	0.60
	0.10	0.085	-1.5E-02	1.3E-04	0.00	0.40
	0.05	0.051	5.0E-04	4.0E-05	0.00	0.00
	0.00	0.001	1.0E-03	1.5E-06	-	0.00
CAP256	0.30	0.242	-5.8E-02	1.3E-03	0.00	0.60
	0.10	0.089	-1.1E-02	1.3E-04	0.00	0.30
	0.05	0.043	-6.6E-03	5.2E-05	0.00	0.40
	0.00	0.002	1.5E-03	5.5E-06	-	0.10
VRC01	0.30	0.130	-1.7E-01	5.6E-04	0.00	1.00
	0.10	0.077	-2.3E-02	7.0E-05	0.00	0.80
	0.05	0.044	-6.2E-03	2.2E-05	0.00	0.10
	0.00	0.001	1.1E-03	1.6E-06	-	0.10

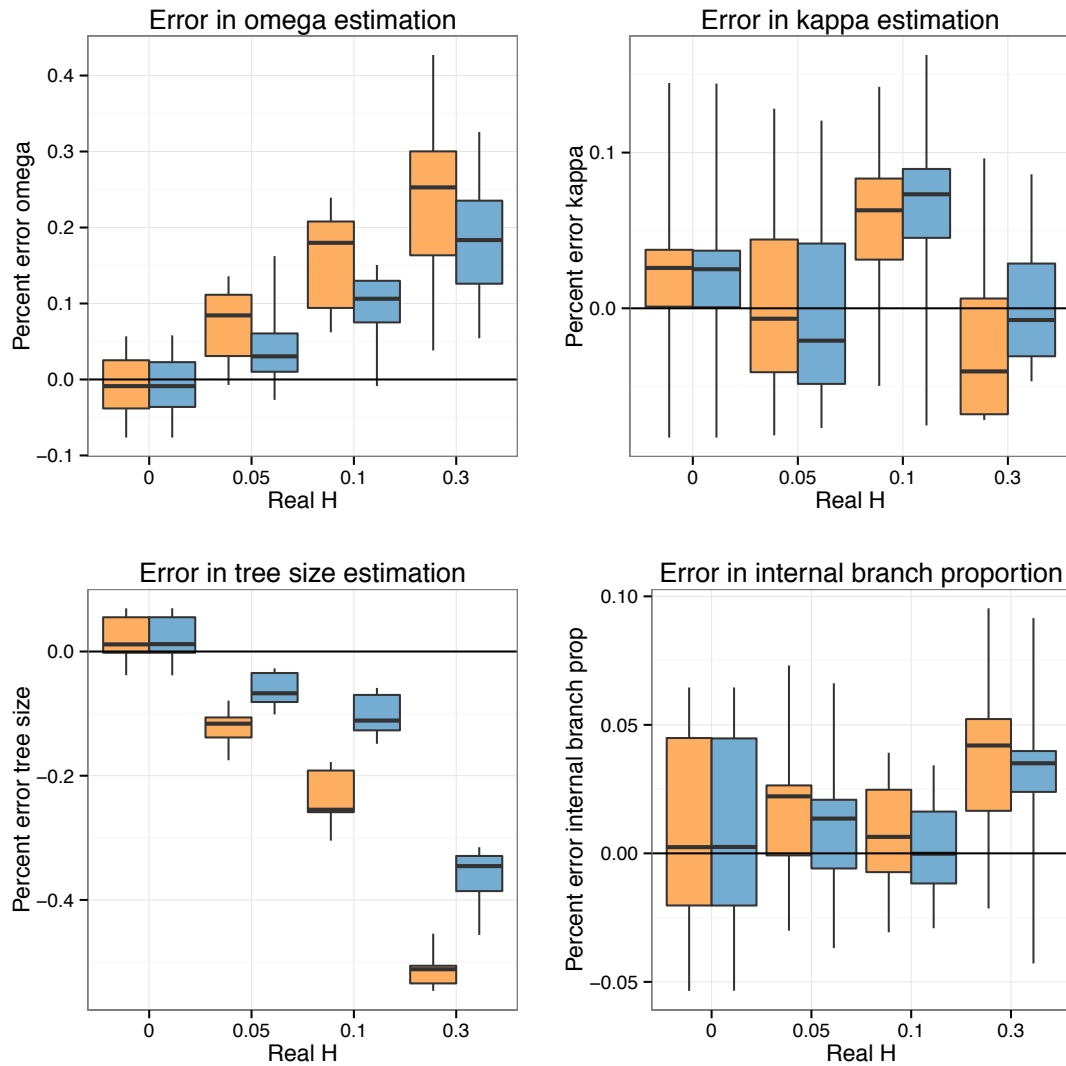
Supplemental File 5a: Inference of \hat{h} from data simulated from a fully context dependent version of the HLP16 model. Type 1 error rate shows the proportion of data sets that incorrectly failed to reject the null hypothesis of $h = 0$. Type 2 error rate shows the proportion of data sets that rejected the true value of h shown in the first column. Both of these hypothesis tests used an alpha value of 0.05. See Methods for explanation of how summary statistics were calculated. Boxplots of other parameters from these simulations are shown in **Supplemental Files 5b-d**.



Supplemental File 5b: Inference of other parameters and tree statistics from sequences simulated from fully context dependent HLP16. Percent error of parameter estimation compared to true values for **CH103** fit with GY94 (blue – h_0) and HLP16 (orange – h_{MLE}). Edges and center of boxplots show 1st, 2nd, and 3rd quartiles, while whiskers show range.



Supplemental File 5c: Inference of other parameters and tree statistics from sequences simulated from fully context dependent HLP16. Percent error of parameter estimation compared to true values for **CAP256** fit with GY94 (blue – h_0) and HLP16 (orange – h_{MLE}). Edges and center of boxplots show 1st, 2nd, and 3rd quartiles, while whiskers show range.



Supplemental File 5d: Inference of other parameters and tree statistics from sequences simulated from fully context dependent HLP16. Percent error of parameter estimation compared to true values for **VRC01** fit with GY94 (blue – h_0) and HLP16 (orange – h_{MLE}). Edges and center of boxplots show 1st, 2nd, and 3rd quartiles, while whiskers show range.