HW3, finding eQTLs of immunoglobulin genes

The goal of this homework assignment is to learn techniques for finding eQTLs of antibody repertoires. To complete this assignment, perform the following steps:

1. Download a dataframe containing usage values of gene IGHV1-2 collected across 85 healthy individuals. Usage values are provided in the “Usage” column. For each individual, haplotypes of IGHV1-2 were also computed and written to the “Haplotype” column. Haplotypes are described by IDs of alleles of IGHV1-2. For example, while a homozygous haplotype of individual 2 is described by allele IGHV1-2*04, a heterozygous haplotype of individual 1 is described by two alleles: IGHV1-2*02 and IGHV1-2*06.
2. For each unique haplotype, compute the number of individuals representing it and the mean usage of IGHV1-2. Fill Table 1 (add rows if needed):

Haplotype	mean	count
2	0.086191	17
2-4	0.077451	28
2-6	0.086956	6
4	0.027895	18
4-6	0.071032	14
6	0.069571	2

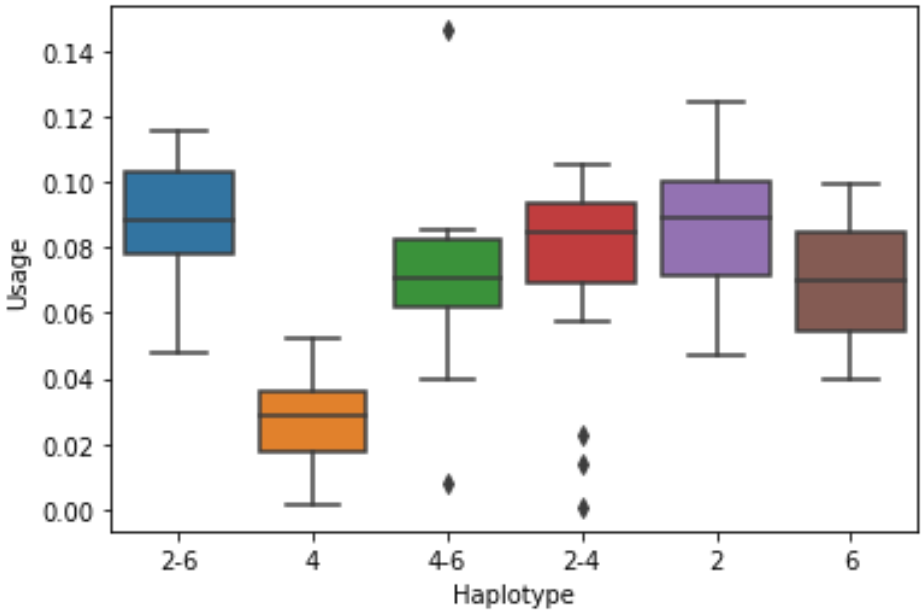
3.

Table 1.

4. For each pair of haplotypes (H1, H2), compare their usages (U1 and U2) and compute a p-value showing the probability that U1 and U2 have the same means. For computing p-value, use the one-way ANOVA test. Fill Table 2 (add rows and columns if needed) and mark statistically significant pairs with * (e.g., H2-H3). Visualize usages across all haplotypes as a boxplot and add it below.

	2	2-4	2-6	4	4-6	6
2	1	0.2625	0.9461	0.0000	0.1227	0.3849
2-4		1	0.4161	0.0000	0.4756	0.6900
2-6			1	0.0000	0.2626	0.4737
4				1	0.0000	0.0039
4-6					1	0.9508
6						1

Table 2.



5. Extract sequences of alleles forming haplotypes in Table 1 from IGHV.fa and compute their multiple alignment. Identify SNPs (=differences) between alleles and, for each allele, describe them as pairs (N, P), where N is the nucleotide at position P in the multiple alignment. Fill Table 3 (add rows if needed). Clustal Omega:

Allele 1	A list of pairs (N, P) for all positions of SNPs
IGHV1-2*02	-
IGHV1-2*04	(T,199)
IGHV1-2*06	(C,148)

Table 3.

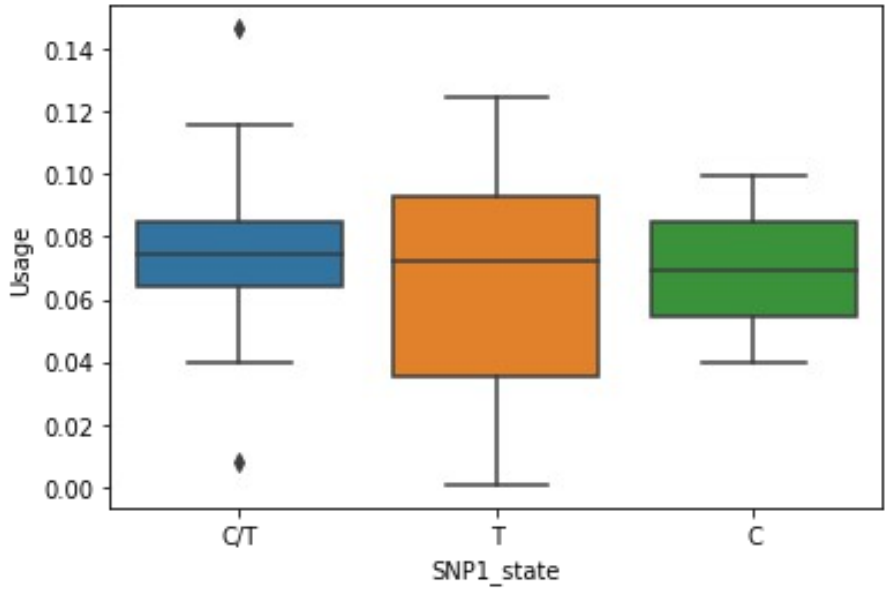
5. For each haplotype, compute a state for each SNP as a list of allele nucleotides. If a haplotype is homozygous, then its state N. If a haplotype is heterozygous, then its state is either N (if two alleles have the same nucleotide N), or N1/N2 (if two alleles have different nucleotides N1 and N2). Note that N1/N2 = N2/N1. Fill Table 4 (add rows if needed).

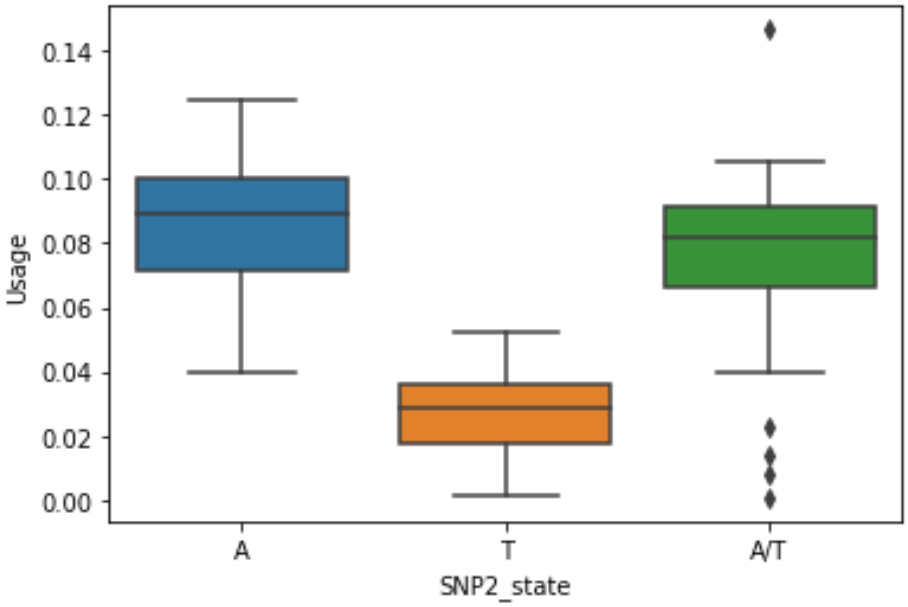
Haplotype 1	A list of states for all SNPs
2	T A
2-4	T A/T
2-6	C/T A

4	T T
4-6	C/T A/T
6	C A

Table 4.

6. As a result, each SNP is described by a set of states (e.g., A, A/C, C) across all haplotypes. For each SNP, add a boxplot showing the distribution of usages across its states. Compute a p-value showing association between SNP states and usages using the one-way ANOVA test. Comment on statistical significance of such association.





SNP1_state	C	C/T	T
C	1	0.7771	0.8694
C/T		1	0.2183
T			1

SNP2_state	A	A/T	T
A	1	0.1433	0.0000
A/T		1	0.0000
T			1

It seems that antibodies with SNP in IGHV1-2*04 allele (nucleotide T on 199 position, starting from 1) are presented less often than antibodies without this SNP. This polimorfism might make antibody less effective, but it still works.
If we have heterozygous haplotype for this SNP, this polimorfism doesn't make any difference.

Deadline: Dec 6 (Sunday), 11:59 pm PST. Please send you reports directly to Nastya Vinogradova (@vinogradovana).

Useful links:
One-way ANOVA in Python:
https://docs.scipy.org/doc/scipy/reference/generated/scipy.stats.f_oneway.html

Visualizing boxplots via seaborn:
https://seaborn.pydata.org/generated/seaborn.boxplot.html