HW3, finding eQTLs of immunoglobulin genes
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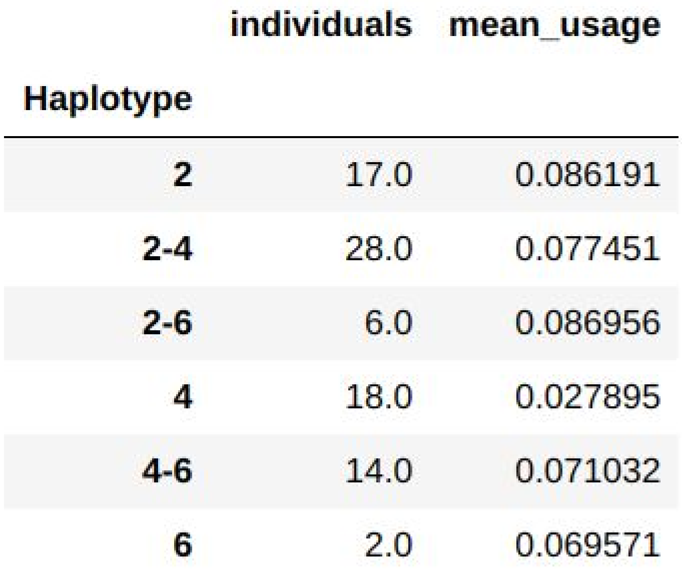
The goal of this homework assignment is to learn techniques for finding eQTLs of antibody repertoires. To complete this assignment, perform the following steps:

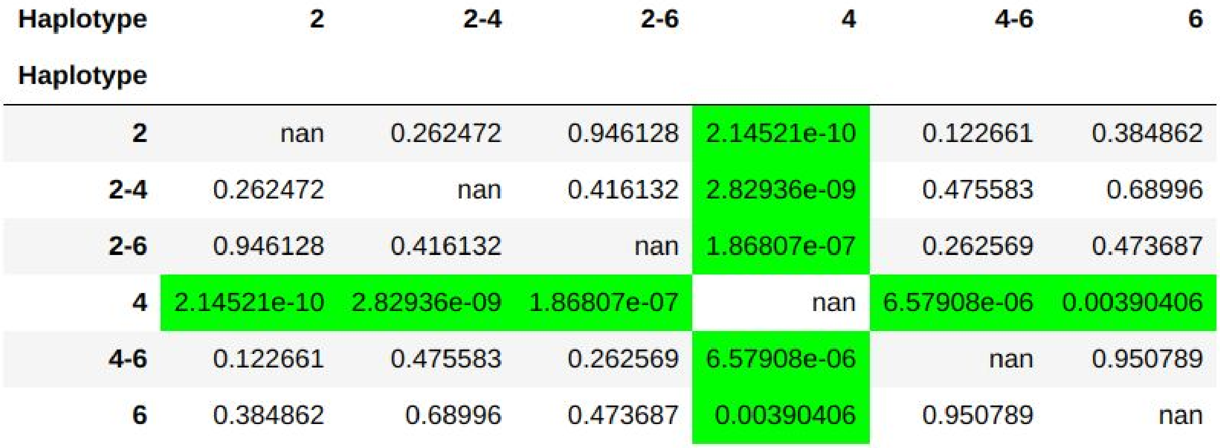
1. Download a dataframe containing usage values of gene IGHV1-2 collected across 85 healthy individuals. Usage values are provided in the "Usage" column. For each individual, haplotypes of IGHV1-2 were also computed and written to the "Haplotype" column. Haplotypes are described by IDs of alleles of 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):



	individuals	mean_usage
Haplotype		
2	17.0	0.086191
2-4	28.0	0.077451
2-6	6.0	0.086956
4	18.0	0.027895
4-6	14.0	0.071032
6	2.0	0.069571

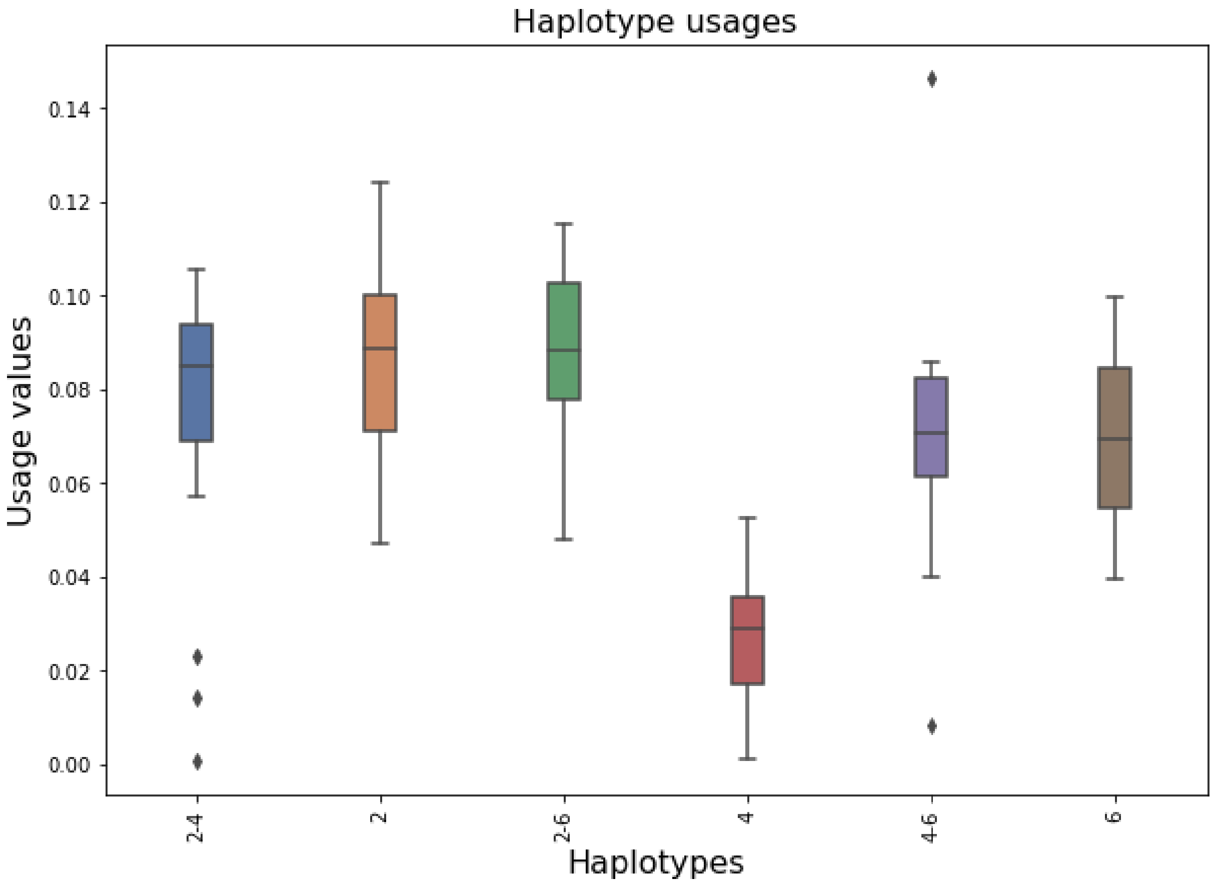
Table 1.

3. 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.



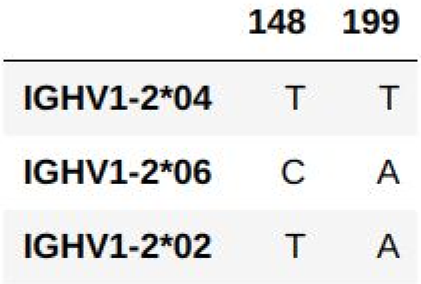
Haplotype	2	2-4	2-6	4	4-6	6
Haplotype						
2	nan	0.262472	0.946128	2.14521e-10	0.122661	0.384862
2-4	0.262472	nan	0.416132	2.82936e-09	0.475583	0.68996
2-6	0.946128	0.416132	nan	1.86807e-07	0.262569	0.473687
4	2.14521e-10	2.82936e-09	1.86807e-07	nan	6.57908e-06	0.00390406
4-6	0.122661	0.475583	0.262569	6.57908e-06	nan	0.950789
6	0.384862	0.68996	0.473687	0.00390406	0.950789	nan

Table 2.



Ну да, тут видно, что 4-й очень сильно выбивается и на боксплоте, и pval у него ххороший по сравнению со ВСЕМИ другими.

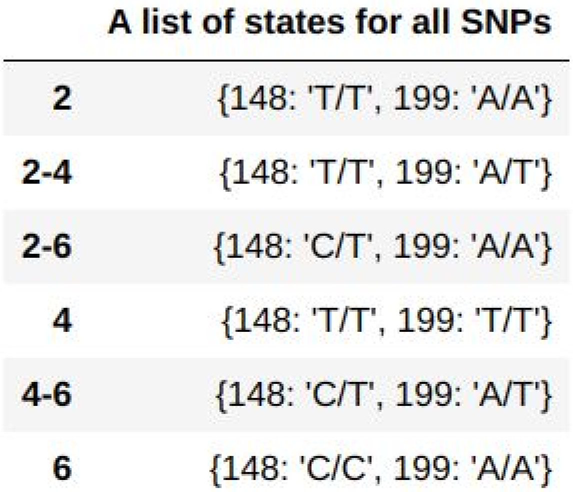
4. Extract sequences of alleles forming haplotypes in Table 1 from IGHV.fa and compute their multiple alignment. Identify SNPs (=differences) between alleles and, for each allele, describe them as pairs (N, P), where N is the nucleotide at position P in the multiple alignment. Fill Table 3 (add rows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



	148	199
IGHV1-2*04	T	T
IGHV1-2*06	C	A
IGHV1-2*02	T	A

Table 3.

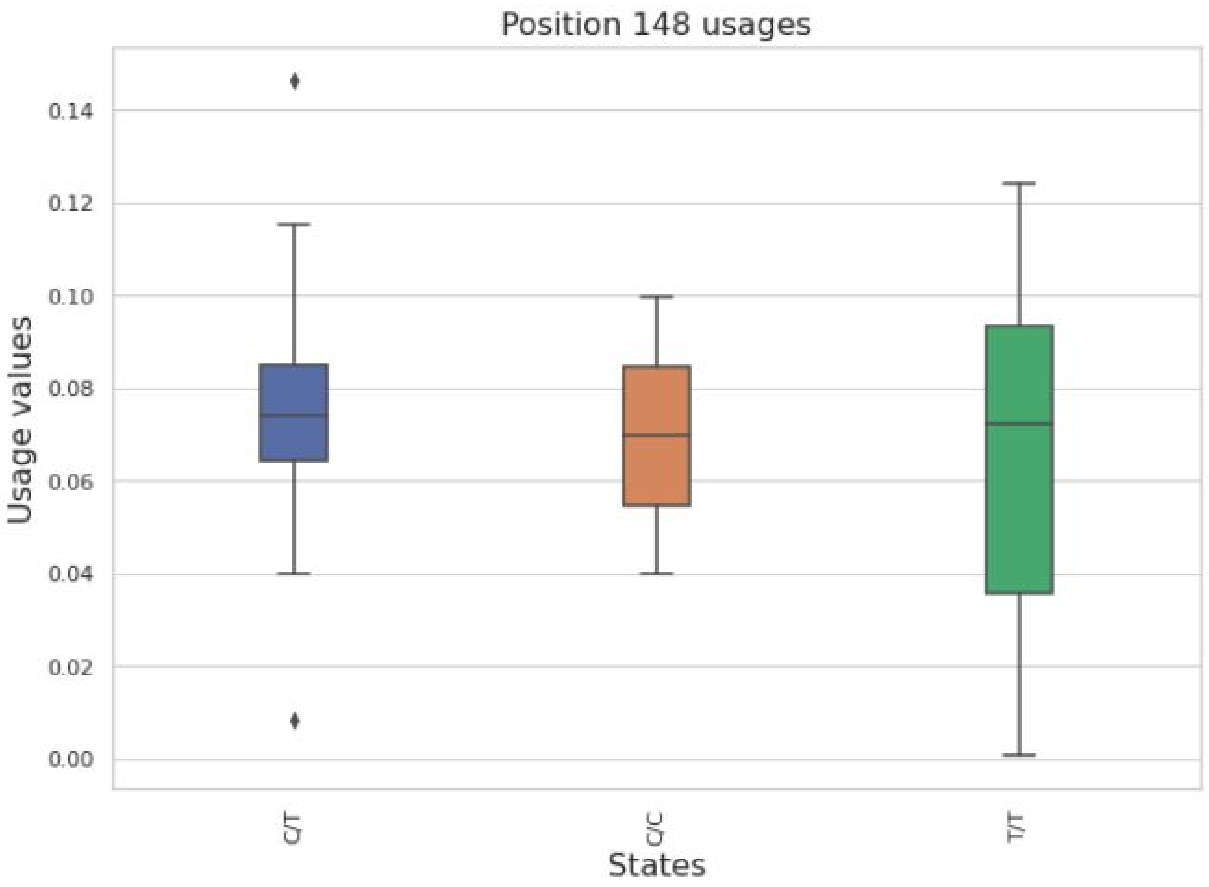
5. For each haplotype, compute a state for each SNP as a list of allele nucleotides. If a haplotype is homozygous, then its state N. If a haplotype is heterozygous, then its state is either N (if two alleles have the same nucleotide N), or N1/N2 (if two alleles have different nucleotides N1 and N2).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

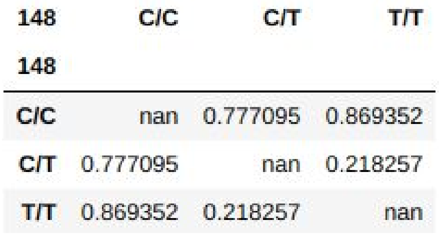


	A list of states for all SNPs
2	{148: 'T/T', 199: 'A/A'}
2-4	{148: 'T/T', 199: 'A/T'}
2-6	{148: 'C/T', 199: 'A/A'}
4	{148: 'T/T', 199: 'T/T'}
4-6	{148: 'C/T', 199: 'A/T'}
6	{148: 'C/C', 199: 'A/A'}

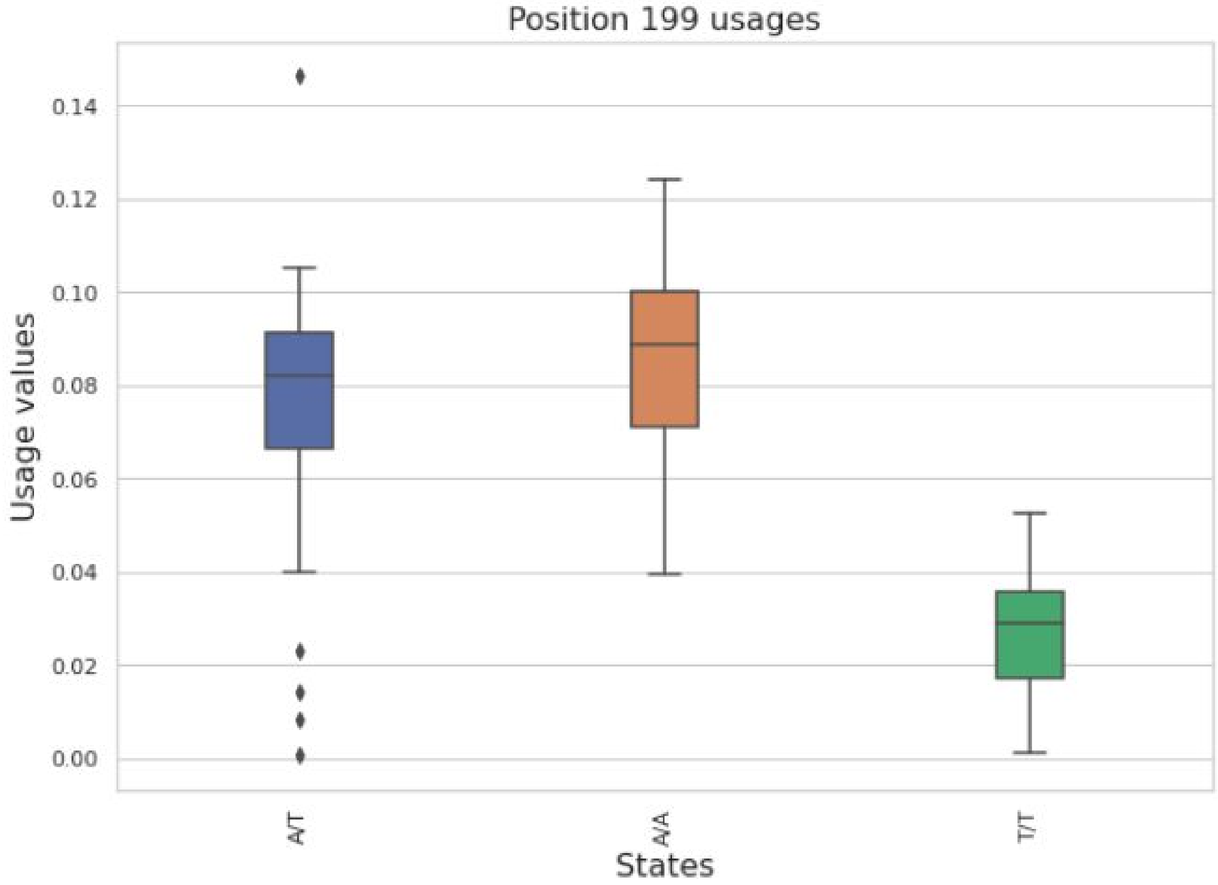
Table 4.

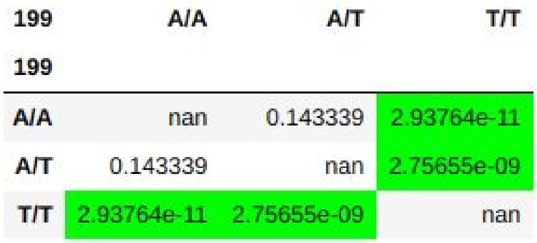
6. As a result, each SNP is described by a set of states (e.g., A, A/C, C) across all haplotypes. For each SNP, add a boxplot showing the distribution of usages across its states. Compute a p-value showing association between SNP states and usages using the one-way ANOVA test. Comment on statistical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





148	C/C	C/T	T/T
148			
C/C	nan	0.777095	0.869352
C/T	0.777095	nan	0.218257
T/T	0.869352	0.218257	nan





199	A/A	A/T	T/T
199			
A/A	nan	0.143339	2.93764e-11
A/T	0.143339	nan	2.75655e-09
T/T	2.93764e-11	2.75655e-09	nan

Можно сделать вывод, что в 148 позиции чего-то статистически значимого нет (не поддерживается отбором), однако в 199 позиции гомозигота по T имеет значительно более маленький usage, чем гомозигота по A и гетерозигота. Этот SNP, по все видимости, и влияет на весь eQTL, меняя его usage.