INTERNATIONAL BIOLOGY UNIVERSIADE

Do not open the tasks until the signal

4 hours

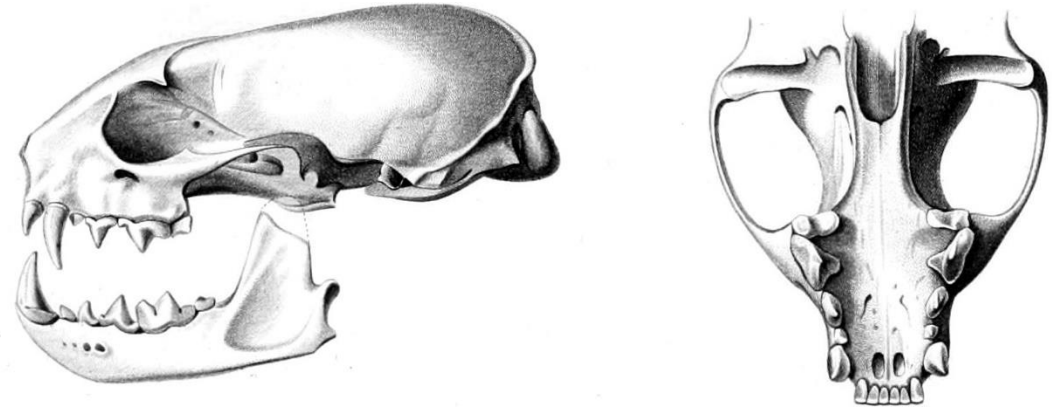
100 points

Lomonosov Moscow State University
November 21-24, 2019

Part I (25 points)

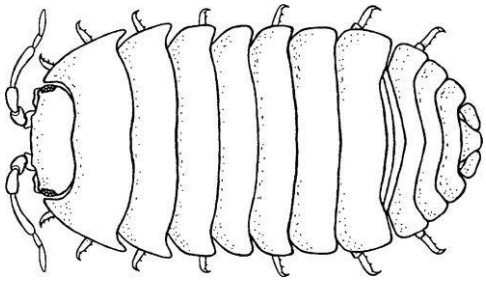
Multiple choice tasks (from 1 to 5 correct answers in each task)

1. Select the correct statements about the dental formula of this animal:



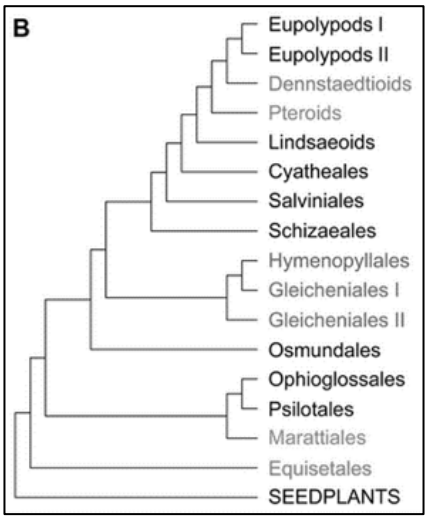
A) There are 3 premolar teeth on each side of the upper jaw;
B) There is one molar on each side of the lower jaw;
C) The total number of teeth on the upper jaw is 16;
D) We should expect the presence of exactly 4 incisors on the lower jaw;
E) The number of teeth on both jaws does not match.

2. The animal depicted belongs to the group(s):



A) Chelicerata;
B) Myriapoda;
C) Crustacea;
D) Arachnida;
E) Mandibulata.

3. The illustration shows the phylogenetic tree of ferns (Polypodiophyta) (Shen et al., 2018). Based on this tree, it can be concluded that:



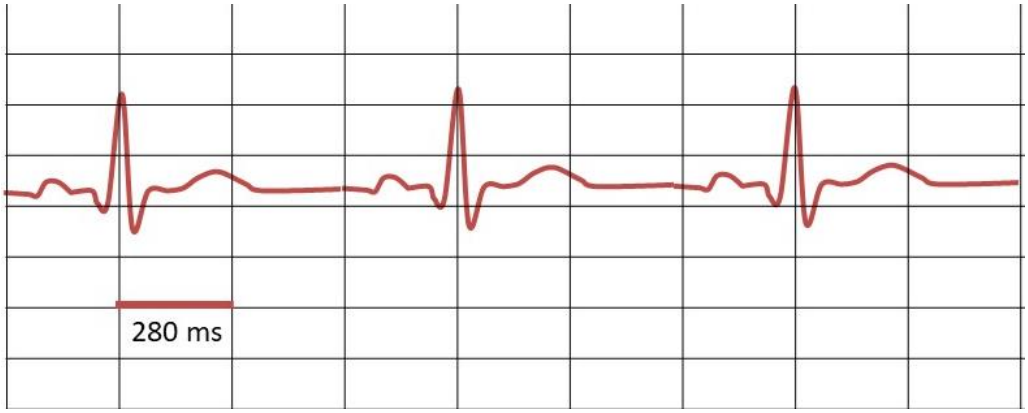
A) The sister group of Cyatheales is Salvinales;
B) The last common ancestor of Ophioglossales and Psilotales lived at the same time as the last common ancestor of the groups Eupolypods and Dennstaedtioids;
C) At least two classical orders of ferns are paraphyletic;
D) Representatives of Osmundales can be selected as an outgroup for studying the evolutionary relationships in Maratiales;
E) Eupolypods is a polyphyletic group.

4. All copies of the alleles of a particular gene in an existing population can be retrospectively traced to one ancestral copy in the past. The time elapsed since the life of the ancestor which possessed this copy is called the coalescence time. Choose the right statements:

A) Selection against certain gene alleles increases the average coalescence time calculated from the sample;
B) The passage of a population through a bottleneck reduces the average coalescence time calculated from the sample;

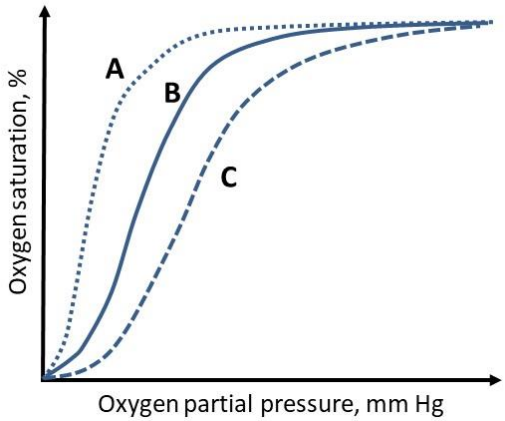
C) With sample of individuals growth, the average coalescence time increases in proportion to the cubic root of the sample size;
D) In mammals, the coalescence time of mitochondrial DNA is less than the coalescence time of most autosomal genes;
E) For all genes of one biological species, the average coalescence time is the same.

5. Figure depicts a human ECG record in lead I. Analyze the figure and select the correct statements:



A) The heart rate is approximately 85 beats / min;
B) There are signs of His bundle blockade on the record;
C) The heart rate is approximately 71 beats / min;
D) the RR interval is approximately 840 ms;
E) the RR interval is approximately 280 ms.

6. Figure displays oxygen affinity curve of an adult hemoglobin, fetal hemoglobin and myoglobin. Analyze the graph and select the correct statements.



A) Curve A corresponds to the hemoglobin of an adult, B to the fetal hemoglobin, C to myoglobin;
B) Curve B corresponds to the hemoglobin of an adult, A to the fetal hemoglobin, C to myoglobin;
C) Curve C corresponds to the hemoglobin of an adult, B to the fetal hemoglobin, A to myoglobin;
D) Curve A corresponds to a lower affinity for O_2 than curve C;
E) Curve C corresponds to a higher affinity for O_2 than curve A.

7. CRISPR/Cas9 genome editing is based on introducing a break in the DNA. Changes are introduced into target locus during repair of this break. Double-stranded break produced by Cas9 nuclease can be repaired by non-homologous end joining (NHEJ) or by homologous recombination. In mammalian cells, repair is more likely to occur by NHEJ. It often leads to the formation of small deletions or insertions at break site, which is used to obtain knockouts. In order to make a particular change to the targeted locus, repair is necessary to occur by the second path using specific donor DNA for recombination. Which of the following approaches will lead to an increase in the efficiency of homologous recombination over the NHEJ?

A) nucleofection of Cas9-ribonucleoprotein complexes and donor DNA into a synchronized cell culture in G2 phase of the cell cycle;

B) nucleofection of Cas9-ribonucleoprotein complexes and donor DNA into a synchronized cell culture in G1 phase of the cell cycle;

C) treatment of cells with nocodazole, an inhibitor of microtubule polymerization, followed by nucleofection of Cas9-ribonucleoprotein complexes and donor DNA;

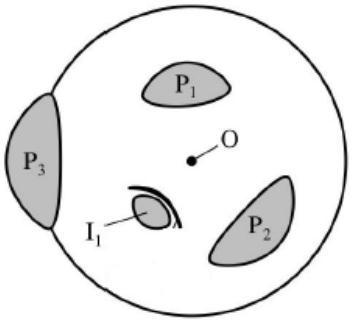
D) treatment of cells with a NU7026 substance which inhibits the non-homologous end joining followed by nucleofection of Cas9-ribonucleoprotein complexes and donor DNA;

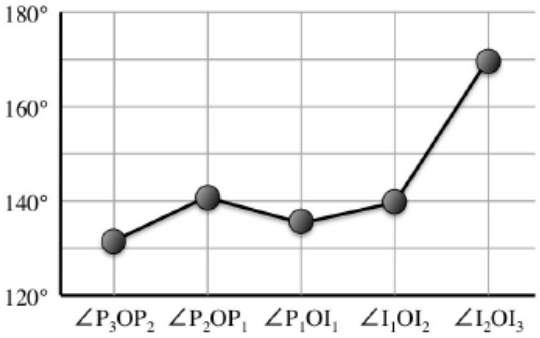
E) knockdown of the *RAD51* and *RAD52* genes encoding homologous recombination proteins followed by nucleofection of Cas9-ribonucleoprotein complexes and donor DNA.

8. It is known that bumblebees can fly even at low ambient temperatures. To fly, they need to warm up the flying muscles located in the thoracic region. It is suggested that they can use a futile cycle (a cycle in which work is not performed, and energy is dissipated in the form of heat), consisting of phosphofructokinase and fructose-1,6-bisphosphate phosphatase reactions. The activity of these enzymes in several species of bumblebees is shown in the table. Choose species of bumblebees that are potentially capable of implementing the discussed futile cycle:

	Species	Phosphofructokinase activity, arbitrary units	Fructose-1,6-bisphosphate phosphatase, arbitrary units
A)	*Bombus terrestris*	75	40
B)	*Bombus affinis*	50	5
C)	*Bombus impatience*	95	2
D)	*Bombus rufocinctus*	100	40
E)	*Bombus citrinus*	55	1

9. There is a regular arrangement of leaves that is characteristic of each plant. The angle between consecutive leaves (centered on the stem) is constant and is about 135-140°, so that the leaves form a regular spiral. This pattern is due to the action of the leaf, which precedes its position, when a new leaf develops on the periphery of the shoot apical meristem. The figure shows the shoot apex O, leaf primordium I1, which just had a cut on the shoot, partially separating the primordia from the rest of the apex, two new primordia I_2 - I_3 (they are not visible at the time of the cut) and the three previous sheets P_1 - P_3.





It is true that:

A) Developing leaves have an environmental impact to prevent the emergence of new leaves;

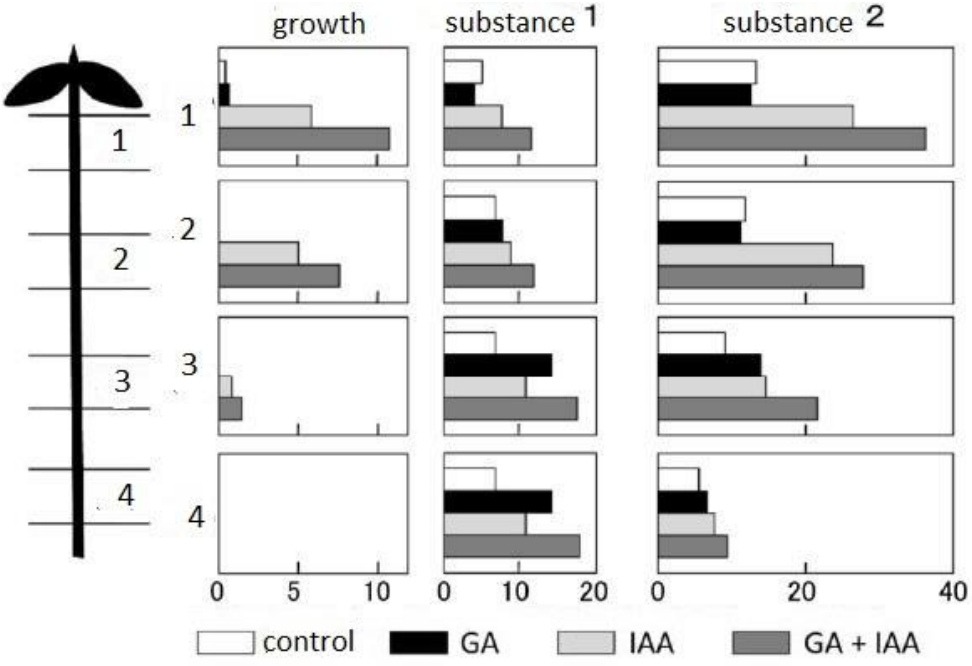
C) Only one of the developing leaves is involved in controlling the location of next new leaves;

C) When the leave begins to develop, the position of the following leave has already been determined;

D) The spiral arrangement of leaves allows them to shade less each other;

E) Primordium determines the concentration of phytohormones, probably auxin.

10. An experiment to determine the sensitivity of plant parts of different ages to phytohormones was performed using bean hypocotyls and the hormones of auxin (IAA) and gibberellin (GA). After 20 hours of bean incubation at 25° C with phytohormones, the elongation of four sections of the hypocotyl, as well as the% content of two polysaccharides of the cell wall (substance 1 and substance 2) were measured. The results are shown in the figure below.



It is true that:

A) The older tissue grows more in response to auxin;

C) Substance 2 is probably cellulose;

C) The greater elongation leads to the more active synthesis of cell wall polysaccharides in young tissues;

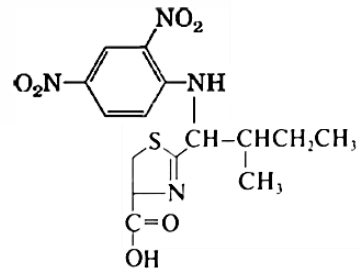
D) Synthesis of cell wall polysaccharides practically does not occur in old tissues;

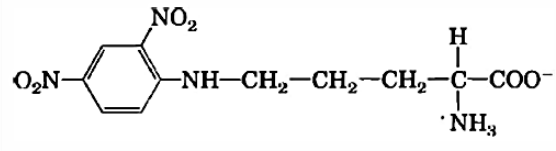
E) The synthesis of certain cell wall polysaccharides controlled by plant hormones does not necessarily correlate with elongation.

Part II (75 points)

Task 1 (10 points)

A specific peptide with antimicrobial activity has a molecular mass of about 1.4 kDa. Complete acid hydrolysis of the peptide followed by analysis of the products demonstrated the presence of the amino acid L-Ile, as well as equimolar amounts of D-Phe, D-Asp, D-Glu, L-Asn, L-Lys, L-His, D-Orn and non-proteinogenic amino acid X. The indicated peptide was not hydrolyzed by treatment with carboxypeptidase. When treated with 1-fluoro-2,4-dinitrobenzene (a substance that reacts with free amino groups) followed by complete hydrolysis, only free amino acids and derivatives of the following structure were obtained (in the case of the second compound, a 2,4-dinitrophenyl group is attached to the amino group of the ornithine side chain):





Partial hydrolysis of the peptide resulted in the formation of oligopeptides of the following sequences (from N- to C-terminus):

A: X-Leu-Glu-Ile,

B: Orn-Ile-Phe,

C: Ile-Lys-Orn,

D: Asn-Lys,

E: Phe-His.

Write the amino acid sequence of the peptide. Describe in detail the process of finding the peptide formula using all these tasks.

Task 2 (15 points)

Parvoviruses (family *Parvoviridae*) are viruses with a small single-stranded linear DNA genome. Parvoviruses include the adeno-associated virus (AAV) which requires a concomitant infection of the cell with an adenovirus, or hepresvirus for a productive infection. Despite their small size, parvoviruses have complicated replication mechanism. The ends of the parvovirus genome are equipped with T-shaped hairpins. In the case of an adeno-associated virus, the right and left hairpins have similar sequences (A-C, see figure 1).

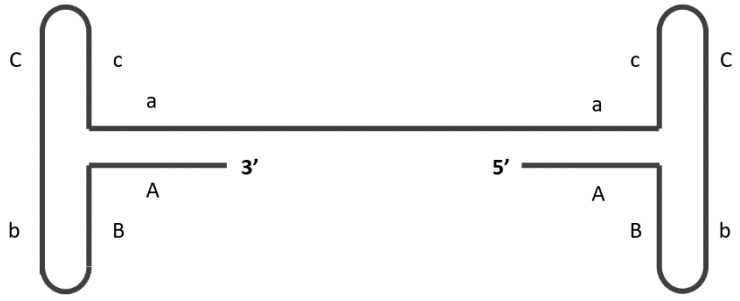


Fig. 1. The structure of the adeno-associated virus genome. The same letters indicate the same sequences in the terminal repeats. Uppercase and lowercase letters indicate complementary sequences.

Parvovirus genome is replicated by the strand displacement mechanism, as shown in the figure 2. Viral Rep protein is crucial for all stages of AAV genome replication. Rep protein has nickase (nick is a single-strand DNA gap) and helicase activities.

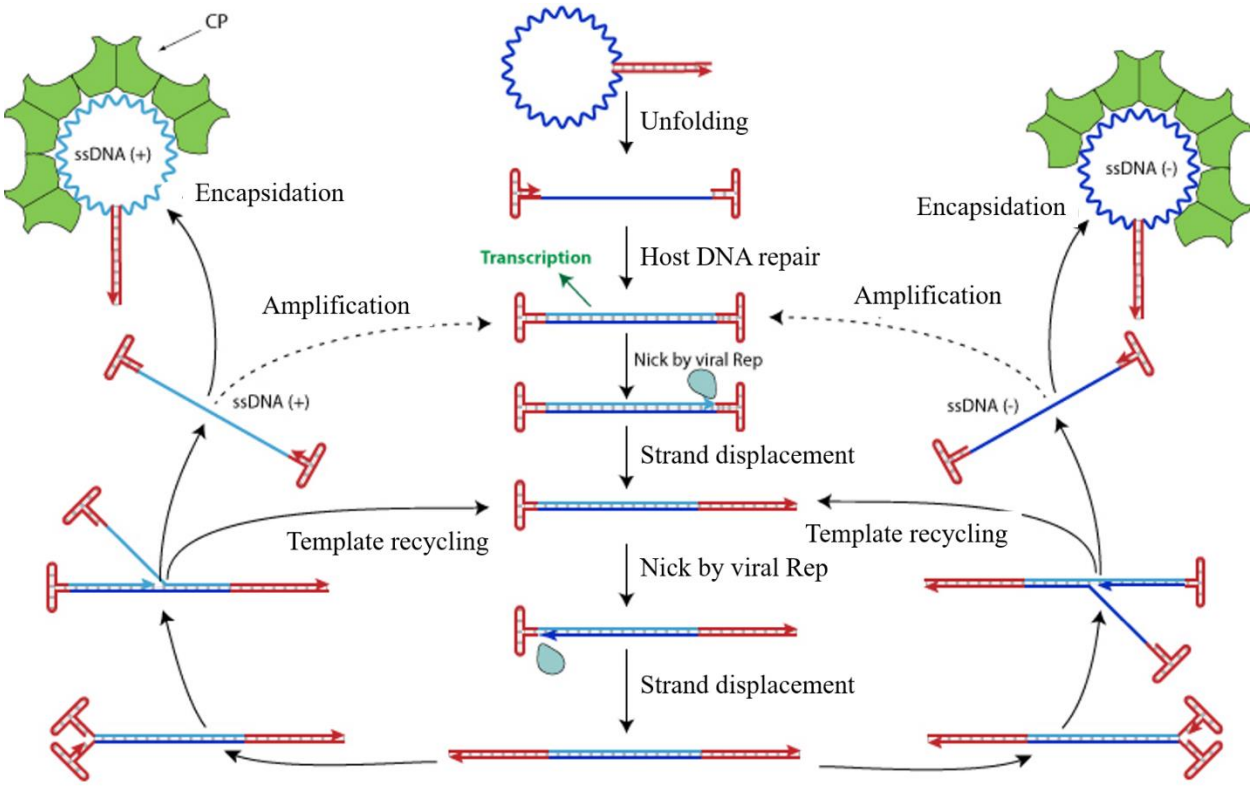


Fig. 2. AAV replication mechanism. Note that the Rep protein cuts only one of the chains in each terminal repeat. The arrow marks the 3' end of the DNA molecules.

1. Both the "+" strand (coinciding in sequence with mRNA) and the "-" strand of the genome can be packaged into AAV viral particles. Why both types of viral particles are infectious (cause a productive infection)?

2. Based on the above scheme of replication, how many total single strand genome sequence variants are possible for AAV (take into account different combinations of terminal repeats)? Explain your answer.

According to the current point of view, the adeno-associated virus replication occurs through the formation of concatemers – double-stranded DNA molecules containing repeating copies of the viral genome (see figure 3) – which are then cut into individual genomic DNA molecules. Helicase activity of Rep protein is necessary for the formation of concatemers.

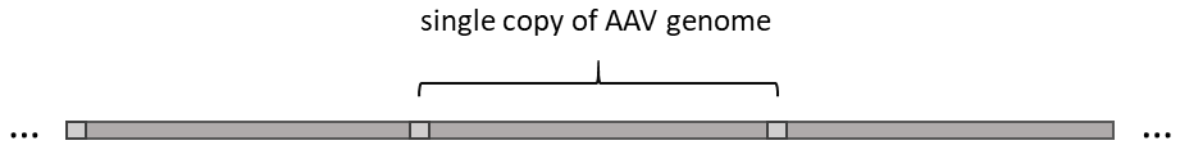


Fig. 3. Concatemer of AAV genomes.

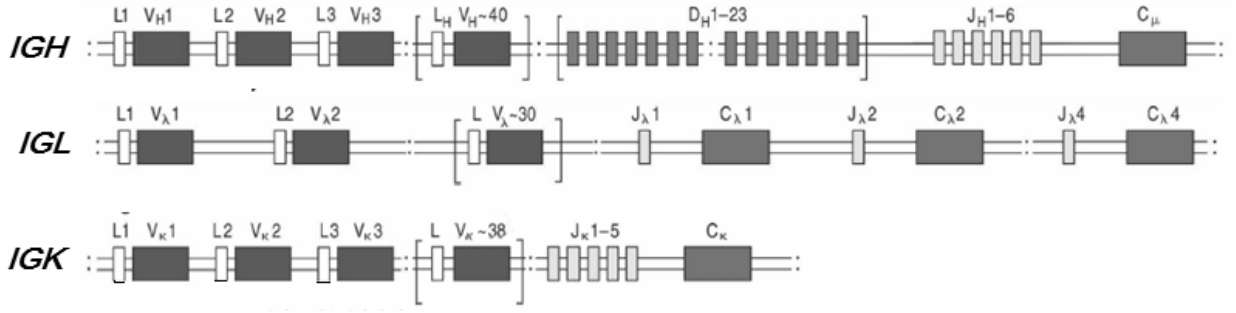
3. Modify figure 2 (replication mechanism) by a diagram illustrating the formation of a concatemer consisting of two copies of the AAV genome (you should not redraw the entire figure 2).

Task 3 (15 points)

B and T lymphocytes build their variable receptor genes from individual segments during a process called somatic rearrangement. Maturating B-cell (pro-B-cell) first recombines one of the D-segments with one of the J-segments (DJ_H recombination) at the antibody heavy chain locus (*IGH*), and then selects one of the V segments (VDJ_H recombination). Thus, an exon coding the variable domain of heavy chain is formed by two recombinations; with the help of alternative splicing it is combined with exons that encode constant domains (μ chains or δ chains).

A cell that has assembled a full-lenght heavy chain gene (pre-B cell) displays it on the surface together with a surrogate light chain, forming a pre-B receptor, which is necessary for the survival of B cell. DJ_H recombination occurs on two homologous chromosomes, and VDJ_H usually occurs on one, and only if recombination fails, the other homologous is recruited. The recombination might failure due to the fact that additional nucleotides appear between two joined segments, and the sequences of the D and J segments appear in a random reading frame, which may not coincide with the frame of the V segment. If the second attempt also fails, the pre-B cell dies.

1. Calculate the probability with which the B-cell will correctly rearrange first VDJ_H if a) the D-segments do not have stop codes in any frame, b) if 80% of the D-segments have stop-codons in the third frame (real situation for *H. sapiens*).
2. Calculate the probability of VDJ_H assembly in two attempts if a) D segments do not have stop codes, b) 80% of D segments with stop codons in box 3.
3. Evaluate the combinatorial diversity of human Ig heavy chains.



Next, the assembly of light chains takes place, usually it starts with the *IGK* (κ-chain locus), in which VJ_K recombination occurs. If it fails, it can be repeated with other V- and J-segments, usually the cell makes no more than three attempts with a kappa locus and then proceeds to the *IGL* lambda locus. *IGL* can only be rearranged once per one homologous chromosome.
4. What is the maximum number of times *IGK* loci can try to rearrange?
5. Calculate the likelihood of successful assembly of the VJ_K and VJ_L lymphocytes respectively.

Task 4 (20 points)

Introduction

The coevolution of parasite fungus and host plant is often carried out according to the "gene-for-gene" principle: in response to the parasite overcoming basic incompatibility (aggressive phenotype ***V***), plant varieties that were initially sensitive to the parasite (phenotype ***r***) develop resistance mechanisms (variety with phenotype ***R***), to which the fungus can respond by the appearance of non-aggressive strains (phenotype ***v***). Plant pathogens ***v*** cause less damage to hosts ***R*** and ***r***, but at the same time bypass the resistance mechanisms developed by the variety ***R***. Moreover, strains ***v*** can use the mechanisms of plant resistance to their own advantage.

1. We accept the fitness of strain ***V*** parasiting on variety ***r***, as well as the fitness of an uninfected ***r*** plant to be equal to 1. The probability of causing damage to the host is equal to the fitness of the parasite. Consider the following **non-negative** parameters:

- ***s*** – fitness reducing of the plant affected by any fungal strain;
- ***k*** – fitness reducing of the non-aggressive fungal strain on any host variety;
- ***t*** - fitness reducing of the aggressive fungal strain on the resistant host variety;
- ***c*** - fitness reducing of the resistant plant variety due to the costs of resistance;
- ***a*** - a positive effect of plant resistance on fitness of the non-aggressive fungal strain.

Fill in the table with fitness values of phenotypes in each host-parasite combination. Values must be expressed in terms of the aforementioned parameters.

	Host phenotype		
	r	***R***	
Parasite phenotype ***V***			parasite fitness
			host fitness
Parasite phenotype ***v***			parasite fitness
			host fitness

2. For some host-parasite system, the following table of fitness values has been compiled (***k*** = 0.2, ***s*** = 0.5, ***c*** = 0.2, ***a*** = 0.1, ***t*** = 0.2):

	Host phenotype		
	r	***R***	
Parasite phenotype ***V***	1	0,8	parasite fitness
	0,5	0,4	host fitness
Parasite phenotype ***v***	0,8	0,9	parasite fitness
	0,6	0,35	host fitness

In an equilibrium community, the average fitness of phenotype ***V*** is equal to the average fitness of phenotype ***v***, the same is true for phenotypes ***R*** and ***r***. Based on this, calculate the proportions of these phenotypes in the equilibrium community **in ordinary fractions** (e.g. 1/6). What are the proportions of the dominant $\mathbf{H}^{R}$ and $\mathbf{P}^{V}$ alleles in ideal populations of these host plant and fungus (typical species of Ascomycota)? Round the answer to **the nearest thousandth**.

3. Calculate the average fitness of the parasite and the host in equilibrium **in ordinary fractions**. Average fitness of species A is equal to weighted average of species A fitnesses calculated for every host-parasite combination.

4. Suppose that in the spring of 2019, a control agent (mycophilic fungus) invaded the described community and started to suppress the parasite population. The spreading of the control agent leads to decreasing of the parameter ***s*** by 0.04 at the end of each growing season for an aggressive strain and by 0.05 for a non-aggressive one (the remaining variables are unchanged). How will the equilibrium ratio of phenotypes ***V*** and ***v*** change from season to season? In the fall of which year, this ratio will change more than 2 times relative to the spring 2019?

Task 5 (15 points)

There are three proteins A, B and C. The amino acid composition (in molar percent), the molecular weight and the number of disulfide bonds in these proteins are shown in the table:

	Protein A	Protein B	Protein C	pK of residue
Alanine	7.8	5.3	5.8	
Asparagine	5.3	7.6	6.8	
Aspartate	5.8	5.0	5.8	3.7
Arginine	4.0	6.6	6.2	12.5
Valine	7.6	8.2	5.8	
Histidine	1.4	1.1	1.3	6.0
Glycine	8.2	5.3	7.9	
Glutamine	5.2	7.8	4.8	
Glutamate	5.1	6.1	7.6	4.3
Isoleucine	6.3	5.8	5.3	
Leucine	9.1	8.9	9.3	
Lysine	3.7	4.8	5.8	10.5
Methionine	2.3	1.8	2.9	
Proline	5.4	5.7	6.5	
Serine	6.6	6.5	6.2	
Tyrosine	3.2	3.8	1.1	10.1
Threonine	5.9	6.2	6.7	
Tryptophan	1.4	1.1	0.9	
Phenylalanine	3.9	1.0	1.4	
Cysteine	1.8	1.4	1.9	8.1
	0	2	0	
Molecular weight	66240	67200	37440	

Considering the average mass of the amino acid residue in a protein to be 120 daltons, calculate what charges the molecules of these proteins will carry at pH 6.0. For convenience of calculations, round off pK values to integer values.

In what sequence will these proteins be located upon electrophoresis in a 0.7% agarose gel at pH 6.0 and 8.0, if we assume that the resistance of the medium is approximately the same for all proteins? (for example, anode - A - start - B - C - cathode).