Sample: Biology - Principles of Genetics

Part A: Questions from the *Drosophila melanogaster* cross practical

1). In the first cross wild-type (wt) female flies and male flies with vestigial wings (vg) were crossed. Our class results for the F2 generation of the Drosophila melanogaster cross are the follows:

F_2	Males	Females
Normal wings	592	691
Vestigial wings	162	120

In the beginning I checked the possibility of vestigial or long wings alleles location on the X chromosome. In this case we can write down crosses results in the next view.

P: ♀ $X^{wt} X^{wt}$ x ♂ $X^{vg}Y$

F1: ♀ $X^{wt} X^{vg}$ x ♂ $X^{wt}Y$

F2:

♀ ♂	X^{wt}	Y
X^{wt}	$X^{wt} X^{wt}$ normal winged female	X^{wt} Y normal winged male
X^{vg}	$X^{wt} X^{vg}$ normal winged female or vestigial winged female	X^{vg} Y vestigial winged male

If vestigial wings were X-linked recessive trait, we would have only normal winged female in F2 generation. If vestigial wings were X-linked dominant trait, we would have equal number of flies of four phenotypic classes in F2 generation. But in accordance with our class results, females have normal and vestigial wings. There are smaller number of vestigial winged flies than normal winged flies. Thus, *vg* and long wings alleles are not X-linked and are located on autosomes.

2) To investigate if the trait is X-linked, reciprocal cross might be carried out. In the case of X-linked nature of vestigial wings gene, this trait would no behave equally in reciprocal crosses:

Reciprocal cross

P: ♀ $X^{wt} X^{wt}$ x ♂ $X^{vg}Y$ P: ♀ $X^{vg} X^{vg}$ x ♂ $X^{wt}Y$

F1: ♀ $X^{wt} X^{vg}$, ♂ $X^{wt}Y$ F1: ♀ $X^{wt} X^{vg}$, ♂ $X^{vg}Y$

normal winged flies normal vestigial

Male flies with vestigial wings will appear in reciprocal cross. But if the vestigial wings were coded by autosomal gene, the results of reciprocal crosses would equal and all flies would have normal wings

Reciprocal cross

P: ♀ wt wt x ♂ vgvg P: ♀ vg vg x ♂ wt wt

F1: wt vg F1: wt vg

normal winged flies normal winged flies

3) We defined the autosomal location of our gene in previous step

P: ♀ wt wt x ♂ vg vg

F1: ♀ wt vg x ♂ wt vg

F2:

♀ ♂	wt	vg
wt	wt wt normal winged flies	wt vg normal winged flies
vg	wt vg normal winged flies	vg vg vestigial winged flies

We can observe two phenotypic classes in the F_2 progeny: flies with normal and rudimentary (vestigial) wings. Thus, two alleles of one gene are involved in the cross. Due to smaller quantity of flies with vestigial wings we can conclude that vestigial wings is a recessive trait and normal wings is a dominant trait. In this case segregation ratio 3:1 should be observed in F2 progeny, with 3 normal winged flies **:** 1 vestigial winged flies

4) Our **null hypothesis** is: in the F2 progeny we will observe 3:1 phenotypic distribution (3 normal winged flies **:** 1 vestigial winged flies)

Alternative hypothesis: In the F2 progeny we will not observe 3:1 phenotypic distribution (3 normal winged flies **:** 1 vestigial winged flies)

Chi square test will help us to accept or reject the null hypothesis.

To calculate the chi squared value we will use the next formula $(O-E)^2/E$, where O is the observed number of flies in each category, and E is the expected number of flies.

	Observed	Expected	$(O-E)^2/E$
Normal winged flies	592 + 691 = 1283	1565/4*3 = 1173,75	10.17
Vestigial winged flies	162 + 120 = 282	1565/4 = 391,25	30.51
Total	1565	1565	40.68

We have two phenotypic classes, giving us 1 degree of freedom. The total chi squared value of the cross is too large.

I found significance level for chi squared value of normal winged flies (10.17). Entering the Chi square distribution table with 1 degree of freedom and reading along the row I found our value of x^2 (10.17) lies between 6.635 and 10.827. The corresponding probability is $0.01<P<0.001$. Significance level is smaller than 1% (0.01), so there is strong evidence against the null hypothesis in favor of the alternative. Thus, we can reject our null hypothesis and accept that in the F2 progeny we don’t observe 3:1 phenotypic distribution.

Indeed our distribution is 4:1. Thus, vestigial winged flies demonstrate high mortality levels. I think, that vestigial wings allele has sub vital nature, and causes poor survival rate of vestigial winged flies.

Part B: Questions from the polytene chromosomes practical

1) The width of a polytene chromosome on my slide is 10 μm

2) Polytene chromosomes (giant chromosomes) composed of hundreds of DNA strands aligned side by side. They do not change their shapes throughout the mitotic cycle and reach the lengths of up to 600 μm and a thickness – 25 μm. They are encountered, for example, in the salivary glands of the Diptera (flies, mosquitoes), in macronucleus ciliates and ovary tissues beans. Most often, they are seen in the haploid number, because polytene chromosomes represent paired homologs. Cells with such chromosomes grow to an unusually large size.

Polytene chromosomes arise due to multiple repetitive DNA replication process, but without strand separation or cytoplasmic division. Interphase polytene chromosomes due to their huge size are clearly visible in the light microscope. Staining of these chromosomes visualizes alternating transverse bands and interbands.

3) Polytene chromosomes are found in salivary, midgut, rectal, and malpighian tubules in the larvae of some representatives of Diptera, in several species of protozoans and plants.

Comparative analysis of the structure of polytene chromosomes in seven species of Anopheles mosquitoes in three cell systems - the salivary glands, malpighian tubules (somatic tissue) and ovarian nurse cells (generative tissue) - showed that the polytene chromosomes of salivary gland cells and malpighian tubules are the relative similarity of both the morphology of each chromosome and the number of discs of those or other chromosomes. Some differences are mainly associated with greater (or less) with a thickness of individual sections and reflect, apparently tissue-specific activation (puffing). Nurse cells chromosomes have more significant differences from the chromosomes of somatic tissues due primarily significantly greater seal structure (shortening) at high polytenization. Should also be noted that the polytene chromosomes of nurse cells are subject to strong morphological transformations in the period of its operation, which continues throughout the formation of the oocyte very short time (1.5 - 2 days). However, with sufficient skill and correct timing of cell fixation analysis of these chromosomes (their clear identification), as well as identifying common in nature inversion hetero-and homozygotes presents no special difficulties. In comparison with the chromosomes of the salivary glands and malpighian tubules nurse cells chromosomes look like depleted disks.

4) Puffs is the swelling detected on polytene chromosomes. Puffs are arisen in accordance with a set of active (functioning) genes in the cell at particular stage of differentiation. Puffs represent DNA that was reeled out in order to serve as a template for RNA synthesis during transcription.

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At different stages of differentiation regular changes of location of the puffs on the same chromosomes are occurred. This indicates that some parts of the chromosomes operate relatively independently.

Part C: Questions from the bacterial transformation practical

1) 5µl*2ng/µl = 10 ng (DNA was added to each tube of bacteria)

250pg = 0.25 ng

2ng – 1 µl

0.25 ng – x µl

x = 0.125 µl would have been needed

2) Bacteria from the tube A grew on agar plate in the presence of ampicillin. That is mean, that bacteria from the tube A were transformed with plasmids containing ampicillin resistance gene, which confer host bacteria resistance to ampicillin. And bacteria from the tube B grew on agar plate in the presence of kanamycin – they contain plasmids with kanamycin resistance genes.

3) (Cells from tube A) Number of colonies on plate = 400

Transformation efficiency (transformants/µg) = $\frac{\text{Number of colonies on plate}}{\text{µg of DNA per transformation}}$

µg of DNA per transformation = 5µl*2 ng/µl = 10 ng = 0.01 µg

Number of colonies on plate/µg of DNA per transformation must be multiplied by 10 because we took only ten per cent of the bacteria culture for transformation.

Transformation efficiency = 312 / 0.01 x 10 = 400000 = 4×10^5 (transformants/µg)

(Cells from tube B) Number of colonies on plate = 22

Transformation efficiency = 22 / 0.01 x 10 = 22000 = 2.2×10^4 (transformants/µg)

4) Only competent bacteria cells can be transformed. The events of transformation in natural environment are very rare. In laboratories for research purpose competent cells can be made in different ways (for example, $CaCl_2$/glycerol method). These methods are based on creation of micropores in bacteria' cell walls, thus DNA can enter the cell.

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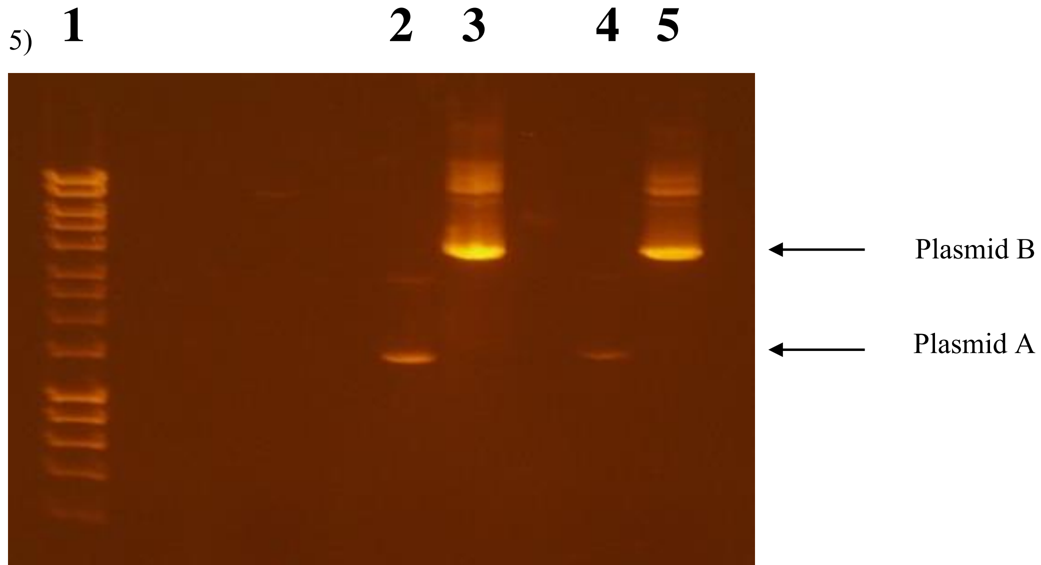
5)



Lane 1. Molecular weight marker

Lane 2. Plasmid A

Lane 3. Plasmid B

Lane 4. Plasmid A

Lane 5. Plasmid B

6) During agarose gel electrophoresis DNA molecules will be separated in accordance with their sizes. Small molecules travel much faster than large fragments. The molecular size of an unknown piece of DNA can be estimated by comparison of the distance that it travels with that of the molecular weight standards. But major part of isolated from the bacteria culture plasmids are in supercoiled form. Supercoiled form of plasmid migrates faster than the linearized plasmid due to its conformation.

The estimated size of plasmid A= 3000 base pair (approximately).

The estimate size of plasmid B = 8000 base pairs (approximately).

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