

Using your knowledge of the approaches covered in class, as well as your scientific culture and sharp reasoning, respond to the five questions below in approximately half a page each, always justifying your answers. Drawings are of course allowed. Several answers are possible in some cases, but only one is required. Each question is worth 4 points (i.e. 20 points in total).

As mentioned in class, only one member of each group (the person listed first) should submit your answers. Please write the names of all group members, as well as the group number, on the top of the first page, and number pages of your document. Then submit a single PDF by 11:59PM, Sunday November 9th 2025.

Note that questions 1a), 1b), 2, 3a), 4 stem from weeks 5 and 6, the rest from week 7.

Questions

1) Let's imagine that you conduct a functional genomic screen in *Drosophila* to identify genes essential for cell proliferation, using the genome-wide UAS-shRNA collection crossed to a driver expressing GAL4 in the eye. You discover that targeting the gene *pro* in this manner results in a minuscule eye phenotype, indicative of severely defective cell proliferation.

a) Would you expect the same phenotype in flies homozygous for an EMS-induced mutant allele of *pro* (referred to as *pro*^{EMS})? (1 point)

b) Let's assume that *pro* is required for progression through a specific stage of the cell cycle. Propose an experiment that could reveal which one. (1 point)

c) Let's imagine that the above experiment establishes that *pro* acts during mitosis. Given this, do you expect taxol to enhance the phenotype of shRNA-mediated depletion of *pro*? And what about cisplatin? (1 point each, 2 points in total)

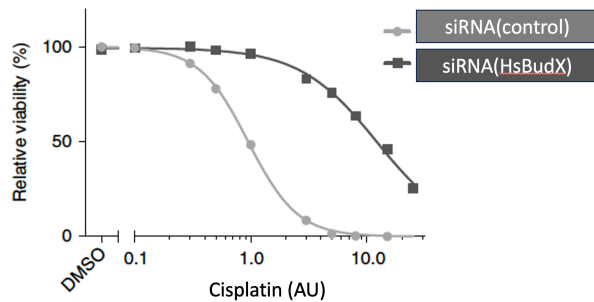
2) In your attempts to make a new type of beer, you would like to identify *S. cerevisiae* genes that are dispensable for growth at 32 °C but necessary for growth at 37 °C. Using approaches covered in class, how could you design an efficient screen to identify such genes? (4 points)

3) Let's imagine that BudX is a gene that belongs to the group of previously "unknown proteins" discovered by Ni and Snyder as having a role in diploid budding in *S. cerevisiae* (see p. 34 of week 6 lecture). BudX encodes a kinase present on the mitotic spindle. This makes you wonder whether, in addition to having a role in diploid budding, BudX may also function during mitosis. Therefore, you decide to test whether BudX is synthetic lethal with a mutant of a Cdc gene acting during mitosis (see p. 56 of week 5 lecture).

a) Which Cdc mutant would you choose for this analysis? (1 point)

b) Describe in detail how you would proceed to conduct this experiment. (3 points)

4) You now turn to human cells, conducting experiments with the BudX human homologue HsBudX. You deplete HsBudX using siRNAs, and compare the viability of such cells to that of cells treated with negative control siRNAs, when subjected to increasing concentrations of the chemotherapeutic agent cisplatin (expressed here in arbitrary units, AU).



- How efficient was siRNAi-mediated depletion of HsBudX in your opinion? (1 point)
- Would you expect CRISPR/Cas9-mediated targeting of HsBudX to yield the same outcome as siRNAi-mediated depletion? (1 point)
- What impact on cell viability do you expect from a drug blocking HsBudX activity in the presence of 10.0 AU of Cisplatin? (1 point)
- Let's assume that HsBudX is overexpressed in breast cancer. How would you design an experiment to identify a drug that specifically prevents proliferation of cells overexpressing HsBudX? (1 point)

5) The image below represents the outcome of an experiment conducted in *S. cerevisiae*.

- What type of experiment does this represent? Explain how such experiments are conducted in general. (1 point)
- In this schematic representation, how were the regions with different colors defined (e.g. the purple region within which the point 1 is located)? And how were the points positioned in a specific location within a given region (i.e. why is point 1 located specifically in that position within region 1)? (1 point)
- Which specific commercially available chemical reagent (e.g. see <https://www.sigmaaldrich.com>) could the points labelled 1 and 2 correspond to (other than reagents from the corresponding paper)? (1 point each, 2 points in total)

