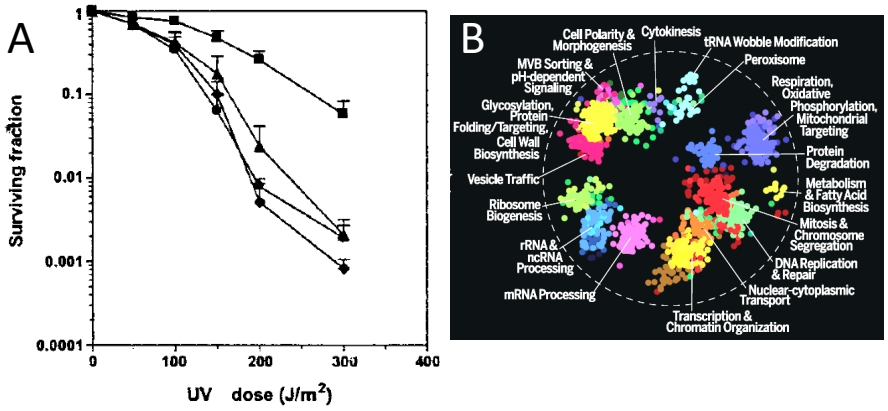
BIO-411 **Group project #2** **November 2024**

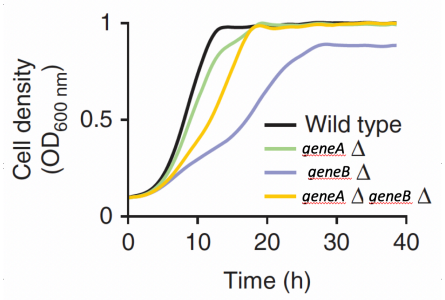
Using your knowledge of the approaches covered in class, as well as your scientific culture and sharp reasoning, respond to the five questions below in approximately half a page each, always justifying your answers. Drawings are of course allowed. Several answers are possible in some cases, but only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

1) Let's imagine that Bud411 is a gene that belongs to the group of previously "unknown proteins" discovered by Ni and Snyder as having a role in diploid budding pattern in *S. cerevisiae* (p. 34 of week 6 lecture). What experiments would you conduct to better understand how Bud411 contributes to this process (mention four possibilities, stemming from the contents of week 5, 6, 7 and 8, respectively) (1 point each)?

2) See image below. Panel A: square -wild-type *S. cerevisiae*; other symbols: three distinct deletion mutant strains. Panel B: outcome of a comprehensive screen in *S. cerevisiae*.
a) Would you expect the three mutant strains in panel A to be alive in the Lavaux?
b) Explain what type of data lead to the clustering in functional groups in panel B?
c) Where do you think the three mutant strains might map in panel B?
d) You subject the three mutants to low doses of 1) rotenone; 2) cisplatin. What impact could each of these treatments have on the mutant strains compared to wild-type cells?



3) The image below represents the outcome of an experiment conducted in *S. cerevisiae*. (Δ = gene deletion). Address the following four questions, briefly explaining your reasoning in each case.
a) What type of experiment does this represent?
b) What impact on fitness do you expect from a drug blocking activity of protein A (encoded by *geneA*) in cells lacking *geneB*?
c) Do you expect the proteins A and B to belong to the same cellular compartment?
d) Let's assume that protein B has a human homologue that is overexpressed in breast cancer. How would you design an experiment to identify a drug that specifically prevents proliferation of cells overexpressing B?



4) How would you generate a reversible conditional mutant allele of *rosetless* (this will be introduced in week 8 -patience if you read this before…). Using knowledge harnessed in class, provide two methods and explain how you would proceed in each case (~half a page in total). (2 points each)

5) Below are the results of a clonogenic assay in HeLa cells targeted with two shRNAs (KD1, KD2) against the cell cycle checkpoint protein RAD17, or a negative control shRNA (SCR). Cells were also treated with increasing concentrations of the casein kinase inhibitor D4476. We have asked the four following questions from ChatGPT, and have obtained the responses hereafter (abbreviated for the sake of space).
For each question, explain whether you agree with ChatGPT or not (and of course justify your answer in each case).
a) How effective was the RNAi-mediated depletion of RAD17 in your opinion?
ChatGPT: From the clonogenic assay, if RNAi-mediated depletion of RAD17 via KD1 and KD2 resulted in a significant reduction in colony formation compared to the SCR control, then the knockdown was likely effective.
b) And how would the data look if you combined RNAi-mediated depletion of RAD17 with CRISPR/Cas9-mediated targeting of casein kinase?
ChatGPT: If RNAi depletion of RAD17 were combined with CRISPR/Cas9 targeting of casein kinase, you might expect an additive or synthetic lethal interaction.
c) How does this synthetic interaction compare to that of cells lacking BRAC2 and treated with PARP inhibitors?
ChatGPT: The scenario you're exploring here is conceptually similar to synthetic lethality seen in BRCA2-deficient cells treated with PARP inhibitors.
d) Do you think that D4476 is a promising candidate to target cancer cells with hyperactive RAD17?
ChatGPT: The casein kinase inhibitor D4476 may hold promise if the assay data show that it selectively reduces clonogenic survival in RAD17-depleted cells more than in control cells.