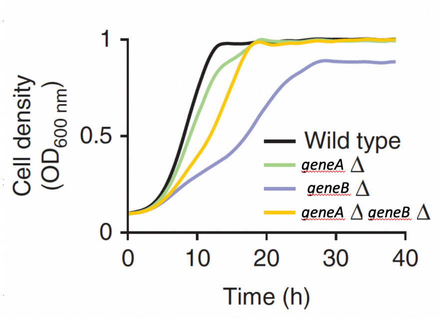
BIO-411 **Group project #2** **November 2023**

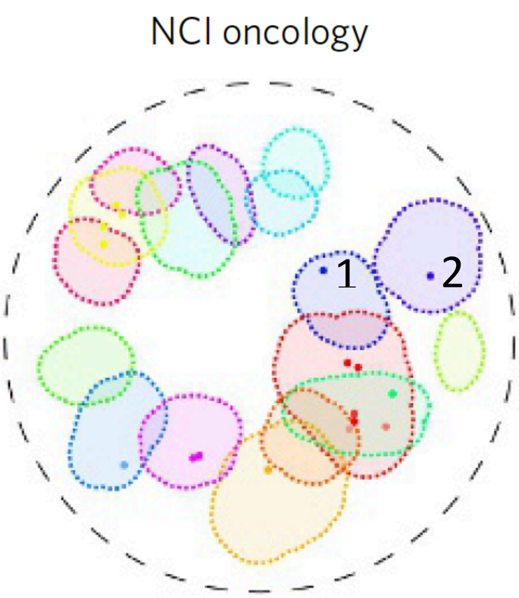
Using your knowledge of the approaches covered in class, as well as your scientific culture and sharp reasoning, respond to the five questions below in approximately half a page each, <u>always justifying your answers</u>. Drawings are of course allowed. Several answers are possible in some cases, but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

1) *YAF9* is one of the previously uncharacterized genes discovered by Birrell et al. as contributing to UV resistance in *S. cerevisiae* (see exercises of week 6). What type of experiments <u>that were covered in class</u> could you envisage to conduct to better understand how Yaf9p (the protein encoded by the *YAF9* gene) contributes to UV resistance (mention at least four, 1 point each)?

2) 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. (1 point each)
a) What type of experiment does this represent?
b) What impact on fitness do you expect from a drug blocking *geneA* function in cells lacking *geneB*?
c) Given this image, do you expect the proteins A and B to belong to the same cellular compartment?
d) Let's now assume that protein B has a human homologue that is overexpressed in liver cancer. How would you design an experiment to identify a drug that specifically prevents proliferation of cells overexpressing B?



3) The image below represents the outcome of another experiment conducted in *S. cerevisiae*.
a) What type of experiment does this represent? Explain how such experiments are conducted in general. (1 point)
b) How are the various dots positioned on this schematic representation (e.g. why is the dot labelled 1 positioned in that blue area, and the specific position within that blue area)? (1 point)
c) Which specific commercially available compounds could the dots labelled 1 and 2 correspond to (provide one name for each)? (2 points)



4) How would you generate a 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) We have asked ChatGPT to explain how to design an experiment aimed at identifying gene pairs, the protein products of which could represent promising for combination drug therapy in CML. Below is the text we got back. We will focus on steps 2-4 here. For each of these steps, explain why what is proposed might be useful, and whether you, as an astute and critical scientist, would do each step in this manner (or not) (1 point each). Moreover, inspired by the material covered in class, suggest another experimental strategy than the one suggested for the identification of relevant gene pairs (step 2 below) (1 point).

ChatGPT's answer

Designing an experiment to identify gene pairs whose protein products could be promising targets for combination drug therapy in Chronic Myeloid Leukemia (CML) involves a systematic approach. Here's a step-by-step guide to designing such an experiment:

Step 1: Understand the Biology of CML

1. **Literature Review:** Conduct an in-depth literature review to understand the molecular pathways and genetic mutations involved in CML. Focus on genes and proteins associated with disease progression, drug resistance, and cell survival.

Step 2: Identify Potential Gene Pairs

1. **Genomic Analysis:** Utilize genomic data from CML patients to identify gene pairs that are frequently co-mutated or co-expressed. Bioinformatics tools and databases can aid in this analysis.
2. **Pathway Analysis:** Identify genes involved in critical signaling pathways related to CML, such as BCR-ABL1, JAK-STAT, and PI3K-AKT. Look for gene pairs that interact within these pathways.

Step 3: Experimental Setup

1. **Cell Lines:** Choose relevant CML cell lines (like K562 or KU812) representing different stages of the disease and drug sensitivity levels.
2. **Gene Silencing or Overexpression:** Use techniques like RNA interference (RNAi) or CRISPR/Cas9 to silence or overexpress the identified gene pairs individually in CML cell lines.

Step 4: Functional Assays

1. **Cell Viability and Proliferation Assays:** Assess the effect of silencing or overexpressing gene pairs on cell viability, proliferation, and apoptosis. Use assays like MTT, CellTiter-Glo, or flow cytometry for this purpose.
2. **Drug Sensitivity Assays:** Treat the modified cells with existing CML drugs (such as Imatinib, Dasatinib, or Nilotinib) individually and in combination. Assess cell viability and apoptosis to determine the synergistic effects of the drugs and gene alterations.

Step 5: Mechanistic Studies

1. **Signaling Pathway Analysis:** Investigate changes in signaling pathways upon gene pair modifications. Use techniques like Western blotting or quantitative PCR to analyze the expression levels of key proteins in relevant pathways.
2. **Functional Interaction Studies:** Perform co-immunoprecipitation or proximity ligation assays to validate the physical interaction between proteins encoded by the identified gene pairs.

Step 6: Data Analysis and Validation

1. **Statistical Analysis:** Analyze the experimental data using appropriate statistical methods to identify significant effects of gene alterations and drug combinations.
2. **Validation:** Validate the findings in primary CML patient samples or patient-derived xenograft (PDX) models to confirm the relevance of the identified gene pairs and drug combinations in a clinical context.

Step 7: Preclinical and Clinical Translation

1. **Animal Models:** Test the promising gene pairs and drug combinations in relevant mouse models of CML to assess their efficacy, toxicity, and potential side effects in vivo.
2. **Clinical Trials:** Collaborate with clinicians and pharmaceutical companies to design early-phase clinical trials targeting the identified gene pairs using combination drug therapies. Monitor patient responses and adjust treatment strategies based on the outcomes.
3. **Biomarker Development:** Identify potential biomarkers associated with treatment response or resistance to guide patient selection and personalize therapy.

Continuous collaboration with experts in CML biology, genetics, and clinical research is essential throughout the experimental design and validation process. Additionally, consider ethical guidelines and regulations when conducting experiments involving human samples or animal models.