BIO-411 Week 6 Exercises Functional genomic screens 28 OCT 24

Questions 1-5 pertain to Liu et al., questions 6 and 7 stem from the lecture.

1) The authors work strictly with lethal lines (9'106 of them, see Fig. 1a). Would you have done the same? Justify your answer.

2) Explain why it is important that flies are cultured at 18°C until eclosion of the pupae (see Fig. 1a)?

3) What impact do you expect on male fertility of *tum*^{RNAi} and *Nedd8*^{RNAi} (Fig. 2c and 2d)? How about on female fertility?

4) The 218 genes whose RNAi-mediated knockdown results in GSC loss include a nucleoporin (page 7). Formulate a plausible hypothesis to explain how such a protein could be required for germline stem cell maintenance.

5) In what way would the design of the screen have been different if you used CRISPR/Cas9-mediated gene inactivation (targeting the same 13019 genes to begin with)?

6) You want to identify *S. cerevisiae* genes that are dispensable for growth at 32°C but necessary at 37°C. How could you design an efficient screen to identify such genes?

7) The schematic below represents a pooled sgRNA CRISPR/Cas9-based screening strategy in human cells, aimed at identifying genes that exhibit synthetic lethality in acute myeloid leukemia (AML) cell lines.
a) Which multiplicity of infection (MOI) would you use for such a screen?
b) What types of changes in the genome do you expect to recover primarily?
c) What experiment would you conduct to verify that a candidate gene identified in this manner is truly responsible for the synthetic lethality, as opposed to this being due to an off-target effect?
d) What would you change to the experimental strategy -if anything- to identify genes that would represent promising therapeutic targets for AML?