Yeast 2-Hybrid Homework

Advanced Genetics, Biol 5491

Due March 5th, 2024 (please email Doris and Ena)

1. Describe/draw a typical yeast-two hybrid experiment.

2. What are the 5 key components of a typical yeast-two hybrid experiment?

3. List at least 2 ways for assay readout.

Dreze et al. 2009:

1. Describe the overall logic (not method details) behind the approach that the authors took to investigate CED-9 Bcl2 function within the network of protein-protein interactions identified. How did the authors isolate edgetic separation-of-function alleles using a Yeast Two-Hybrid-based scheme?

2. Previous studies demonstrated that EGL-1 and CED-4 each independently bind to CED-9.
   a. Based on the information provided at the beginning on the Results (also in Ref 14), indicate the wild type function of egl-1, ced-9, ced-3, and ced-4.
   b. Using epistatic analysis, determine whether the CED-9 pathway is a developmental/synthetic or regulatory/binary switch.
   c. Draw out the pathway by which these four genes function to control whether a cell does or does not undergo apoptosis.

3. What did Dreze et al. (2009) find, in terms of effects on structure and protein stability, for edgetic and non-edgetic CED-9 alleles?

4. How do the authors deduce the function of a ced-9 edgetic allele in vivo?
5. For *spd-5*, what is the RNAi phenotype and the wild-type function with respect to apoptosis?

6. Based on the effect of the CED-9(W214R) transgene on apoptosis, what is the function of the interaction between CED-9 and SPD-5?

7. List two lines of evidence suggesting that edgetic and nonedgetic alleles result from distinct molecular defects.

8. In the transgene assay, edgetic alleles in the *ced-9* null background have the following phenotypes:

1. CED-9(G169R), which is defective in interaction with EGL-1, has no apoptosis
2. CED-9(K207E), defective in interaction with CED-4, has increased apoptosis
3. CED-9(W214R), defective in interaction with SPD-5, has increased apoptosis

Indicate for each CED-9 mutant whether it is loss-of-function or gain-of-function and why.