Transcriptional activation by hybrid prs in yeast

- His gene acts a reporter gene because it reports to you that system is working

- dealing with mutated yeast strain: can’t make histidine due to physically separated the binding domain from activating domain (so you have a dysfunctional TF) => yeast will not survive if there is no His

- reporter: if they’re grown in media w.out His and they survive -> means that yeast has brought together artificially through 2 hybrids, the 2 domains

- you do NOT make a pr in this process

- **positive selection** -> bait vector contains sequence encoding DNA binding domain of TF and introduce into yeast

 - first yeast cannot make Trp: only way yeast can survive is by incorporating bait vector which allows Trp production -> cells that don’t survive were unable to incorporate vector

 - you’re looking for which pr binds to bait!

 - thousands of fish vectors: isolate all msgs expressed in cell and RT to get cDNA and ligate cDNA invidually into fish vector

 - fish vector = cDNA sequence and activating domain sequence

- how do you know yeast contains fish vector? **Introduce another selection**

 - use serial dilution and statistical approach to ensure yeast that contains bait vector will only take up one specific fish vector -> you do NOT do this individually: statistically make sure each yeast cell only takes in 1 fish vector and grow yeast in masses in media WITHOUT Trp and Leucine (yeast needs both to grow)

- last step: remove His while growing on Trp and Leucine -> test to see what binds to bait

 - on top of plasmids being there, they need to be able to make His (and consequent colony formation)

 - many prs don’t bind to bait, no His -> no colony formation

 - each fish vector only encodes for one pr

 - don’t know what msgs are but you know bait has been attracted by fish

 - if you get colony forming, you know binding domain comes in close proximity to activation domain and His being produced

- how do you know yeast picked up 2 or 3? Very likely if improper dilution (all 3 fish may bind to bait)

- note: you haven’t seen pr but you know yeast is growing and His produced

- isolate and sequence plasmid then see what you caught

- main idea: identifying pr:pr interaction by rescuing a mutation in yeast

Translation

http://www.rockefeller.edu/pubinfo/proteintarget.html