Protein Engineering

20.109 Module 2 Day 3
Tuesday Oct 14th, 2008

What's my job?

Let's start with some DNA sequence…

GCATGTATAATCTACAGTAGTCCGAATTGTAAATG
TGGCCATTCTTGCAACCAGTTAACAAGGAAGAA

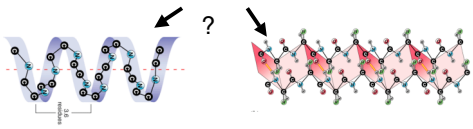
http://www.expasy.ch/tools/dna.html

What other DNA features are relevant?

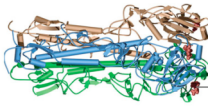
TTGACA ---- 17±1 ---- TATAAT

GCATGTATAATCTACAGTAGTCCGAATTGTAAATG
TGGCCATTCTTGCAACCAGTTAACAAGGAAGAA

What's my job?



and even if you knew this…



Figures from Molecular Cell Biology, 6th edition

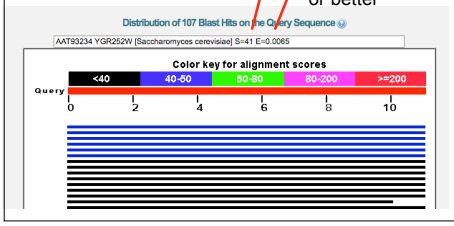
What's my job?

S = 41 where S is sum of letter-to-letter and letter-to-null position in the alignment.

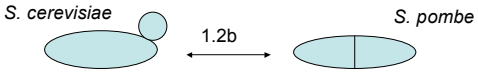
E = 0.0065 where E is expected number of chance alignments with a score of S or better

One approach: profiling

NCBI "BLAST"



The reason "profiling" works



evolution conserves critical aspects of proteins

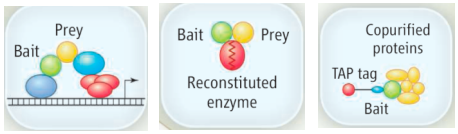
Profiling enables wiser expt'l choices

To identify the localization of functional Fus2p, we attempted to epitope tag both the N and C termini of the protein. In both cases, the resulting proteins were only partially functional (7 and 29% of wild-type levels of mating, respectively). Immunofluorescent microscopy showed two different patterns of localization in shmoos; the N-terminally tagged protein localized to the shmoo-tip and the C-terminally tagged protein localized to the nucleus (unpublished data). However, both proteins localized to the ZCF in prezygotes. Both termini are strongly conserved in other yeasts, which suggests that these regions are required for normal function. To identify functional sites to tag Fus2p, we compared the *FUS2* sequence of closely related yeasts (Cliften et al., 2003; Kellis et al., 2003) to identify regions that were especially nonconserved and therefore likely to reside in nonessential surface loops. Two regions were chosen for internal epitope tagging (residues 104–109 and 410–419). In both cases, the FLAG-tagged proteins were fully functional (100% of wild-type mating efficiency) and localized to the shmoo tip and ZCF (unpublished data).

J Cell Biol. 2008 May 19;181(4):697-709

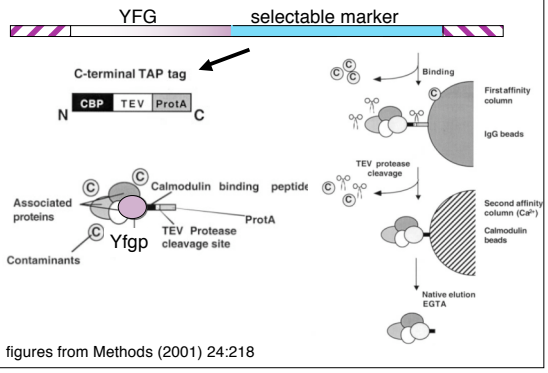
What's my job?

Another approach: associated protein partners



http://www.sciencemag.org/cgi/content/full/322/5898/56

TAP tag to purify complexes

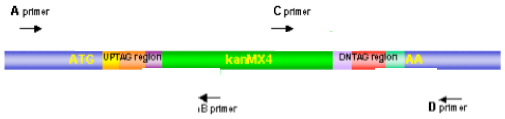


figures from Methods (2001) 24:218

Verification of deletion by colony PCR

Saccharomyces Genome Deletion Project

Successful Deletion , kanMX4 module replaces ORF

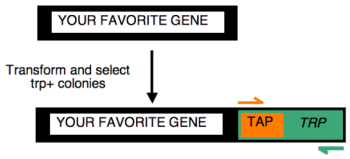


PCR with primers A + B or C + D

Product IFF KanMX replacing ORF

http://www-sequence.stanford.edu/group/yeast_deletion_project/project_desc.html

Verification of TAP TRP tag by colony PCR



Summary

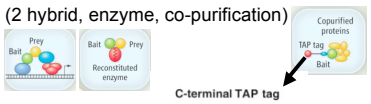
Conjunction Junction: what's my function…

Guilt by profiling (DNA promoters, BLASTP)

GCATGTATAATCTACAGTAGTCCGAATTGTAAATG
TGGCCATTCTTGCAACCAGTTAACAAGGAAGAA

Protein: W P F L Q P V N K E E

Guilt by association (2 hybrid, enzyme, co-purification)



C-terminal TAP tag

N CBP TEV ProtA C

PCR verification of inserts