

Designing Yeast Assays for Human Disease

A. Determining severity of disease phenotype

We are again working with the human gene CBS and its yeast analog *cys4*. Recall that these genes each encode the protein cystathionine β -synthase that is responsible for converting homocysteine into cystathionine in the cellular pathway of creating cysteine.

Human disease homocysteinuria results from CBS enzyme deficiency. Human cells deficient in CBS enzyme accumulate homocysteine—a product of the previous step in the pathway of making cysteine. This accumulation leads to a host of symptoms in humans, but not in yeast.

In Section 22 we discussed why we expect wild type human CBS protein to complement CBS protein deficiency in yeast.

1. Briefly describe the procedure for expressing human CBS protein in *cys4* yeast.

To express the human protein in the yeast cell, we can

- 1) *design PCR primers to selectively amplify the mature mRNA for CBS protein (avoid introns and human promoter);*
- 2) *clone that human gene into a yeast vector under a yeast promoter;*
- 3) *transform a *cys4* strain of yeast with the library of vectors*
- 4) *select for the cells that picked up a vector (special tricks can be applied to make sure only the vectors with inserts are picked for analysis);*
- 5) *induce the yeast promoter to make the cell produce human protein.*

And, as a bonus, we can now

- 6) *check to see whether the deficiency is complemented.*

We will now consider various disease alleles of homocysteinuria in the context of the same assay.

Aside from scientific curiosity, there are clinical reasons for us to consider analyzing various disease alleles.

2. We do not expect all the disease alleles to behave similarly in this assay. Why not?

Disease phenotype is caused by a number of different alleles of the CBS gene. We know that mutants with the same or similar phenotype may be a result of mutations in different parts of the CBS gene. Each of the mutant alleles will produce a protein that has different shape, and may behave differently depending on conditions. Because yeast system where the protein will be expressed is somewhat different from the human cells, we can not predict the behavior of each individual allele.

When the wild type and various human disease alleles are put through this test, only WT human and V320A alleles gave significant growth. A114V allele showed small but significant number of slow-growing colonies. A few colonies were seen with G307S, and none with I278T.

3. Can you formulate a hypothesis about the severity of the phenotypes associated with the human CBS alleles we used today?

We could hypothesize that V320A is approximately equal in phenotype to WT human allele, followed by A114V, and the remaining two alleles—I278T and G307S, the order of which could not be determined from our data. Notice that V320A and A114V both convert one hydrophobic amino acid into another, with the only difference being the size. Thus, these alleles are likely to cause less of a problem than I278T and G307S alleles, both of which substitute polar amino acids for hydrophobic amino acids and result in a new amino acid of a very different size than the amino acid present in the wild type protein.

4. How can the assay we used today be used to diagnose the severity of a new patient's disease phenotype?

For any new patient, one would need to isolate his/her CBS mRNA, reverse transcribe it into cDNA, and clone resulting DNA product into a vector we used in this experiment. Next, the yeast strain we used in this experiment needs to be transformed with the resulting vector, and the yeast need to be grown out as in the original experiment to determine the results.

The only available treatment for the disease is supplementation with the CBS co-factor—vitamin B₆. It turns out that this assay also diagnoses the severity of the phenotype with respect to the likelihood of success of the B₆ treatment. The alleles that respond well to that treatment, like V320A and A114V show various degrees of growth in the experiment.

5. Why do you suppose that supplementation works for some alleles? How is the severity of phenotype connected with the protein structure?

The reason supplementation works for some alleles is that it drives up the effective concentration of the cofactor, allowing some forms of defective enzyme to achieve a certain level of function.

It is reasonable to suppose that alleles that are susceptible to treatment are defective in the regions of the protein involved in the co-factor binding. We can also suggest that the alleles that do not respond to treatment are defective in the areas of protein that perform functions other than co-factor binding.

B. Developing a novel screening assay

Recall that when we discussed homocysteinurea, and its primary symptom of mental retardation, we also talked about additional phenotypes of early strokes and heart disease.

1. What type of inheritance does the mental retardation phenotype show? Explain. (Feel free to ask your TA for additional information if you think you need it.)

The phenotype is autosomal recessive, since parents of the affected individuals were generally not observed to be affected, and the rates of male: female affected individuals is about 1:1.

2. Propose a hypothesis for what kind of inheritance is shown by the secondary phenotypes. Justify your answer.

What is the biochemical basis for your proposed inheritance pattern?

(Feel free to ask your TA for additional information if you think you need it.)

We know that these phenotypes are not dominant, because if they were, we would see both parents of the kids with homocysteinurea drop with early strokes and heart disease at the same rate as their children.

This does not happen. A reasonable alternative hypothesis might be that these phenotypes are co-dominant, i.e. that heterozygotes (e.g. parents) would have a higher risk of heart disease and strokes than homozygous wild type individuals.

3. What are the public health implications of your inheritance hypothesis?

If heterozygotes have a higher risk of heart attacks and strokes, the questions of when and who is it prudent to test for heterozygosity become very important.

4. Design an experimental protocol to test your working hypothesis.

You need to describe your model, predictions, and control.

Be very clear about how you would determine an individual's genotype from your experimental data. *To test this hypothesis, a study might be conducted to determine whether there is a higher percentage of heterozygotes for cbs^- alleles among people who suffered early heart disease or strokes. A design of such a study would involve getting an experimental group from among those who have suffered early heart disease or strokes and a control group from among those who have not. Both groups would then be typed by the assay we designed above to determine how many functional copies of the CBS gene each subject of the study has.*

Notice that if a person is a heterozygote for the CBS gene the assay would result in two different types of transformants. If one of the alleles a person is carrying is a cbs^- allele that shows little or no growth in the assay, then the assay would result in about half the number of transformants compared to the number produced if the assay is performed on a homozygous wild type subject.

The study would then compare the number of heterozygotes in the experimental and control groups, and analyze the data to determine whether any differences found are statistically significant.

If the hypothesis is confirmed, the questions on what to do about the finding would arise. The questions would include medical considerations, such as whether it is medically indicated to test all people of a given age or only those who have family history of early heart disease or strokes; and ethical considerations, such as whether it is in a patient's best interest for his/her insurance company to have access to the data from the test.

We have now gone from investigating cysteine auxotrophy in yeast all the way to clinical considerations in humans. The story of Cys4/CBS illustrates how basic science can lead to important discoveries in medicine. Rarely does research in model organisms start with express goal of achieving a medical breakthrough, but it is always a building block that leads to such breakthroughs.

The Genetic Code

	U	C	A	G	
U	UUU phe (F) UUC phe UUA leu (L) UUG leu	UCU ser (S) UCC ser UCA ser UCG ser	UAU tyr (Y) UAC tyr UAA STOP UAG STOP	UGU cys (C) UGC cys UGA STOP UGG trp (W)	U C A G
C	CUU leu (L) CUC leu CUA leu CUG leu	CCU pro (P) CCC pro CCA pro CCG pro	CAU his (H) CAC his CAA gln (Q) CAG gln	CGU arg (R) CGC arg CGA arg CGG arg	U C A G
A	AUU ile (I) AUC ile AUA ile AUG met (M)	ACU thr (T) ACC thr ACA thr ACG thr	AAU asn (N) AAC asn AAA lys (K) AAG lys	AGU ser (S) AGC ser AGA arg (R) AGG arg	U C A G
G	GUU val (V) GUC val GUA val GUG val	GCU ala (A) GCC ala GCA ala GCG ala	GAU asp (D) GAC asp GAA glu (E) GAG glu	GGU gly (G) GGC gly GGA gly GGG gly	U C A G

