

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.

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?

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?

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

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?