Response from NEB (received 11/02/2010)

Why certification of K. lactis as a host-vector system and use of wild type strains

In 2000, New England Biolabs took an interest in heterologous protein expression in yeast. At the time, the two yeast systems that were widely available to the research and biotech communities were *Saccharomyces cerevisiae* and *Pichia pastoris*. There was an additional very well known yeast system, *Kluyveromyces lactis*, but it was being exclusively used in food industry manufacturing and it was not generally accessible to the research and biotech communities. We undertook a comparison of the various systems. We found *S. cerevisiae* to be a wonderful genetic model, but its expression power was significantly less than the other two yeasts. *P. pastoris* was an excellent system except that it required the use of methanol for induction of gene expression which posed safety and logistical issues for New England Biolabs. In addition, *P. pastoris*' genetics was not as well characterized. *K. lactis* had attractive characteristics of both *S. cerevisiae* and *P. pastoris*: it was a great genetic system, it did not require methanol, and it produced proteins as well as *P. pastoris* (in several head-to-head tests). Finally, Gist-Brocades (the Dutch food company that owned the rights to *K. lactis*) presented New England Biolabs un-fettered access to the technology for internal use at New England Biolabs and to develop a reagent kit for the research community.

About a year ago, a customer in Hawaii alerted us that despite wanting to use *K. lactis*, he was unable to because the NIH did not list it as an exempt host-vector system. Additionally, we have received similar comments from a couple fermentation service providers who were familiar with *K. lactis* and its food industry history, but were surprised to find it absent from the NIH exempt host-vector system list. For these reasons we have elected to petition the NIH for inclusion of *K. lactis* on the list of approved host vector systems.

In regards to the question on why we are requesting to certify two wild type *K. lactis* strains, New England Biolabs is interested in *K. lactis* for the purpose of heterologous protein expression. Over the years, we have found that *K. lactis* strains having auxotrophic markers commonly used in yeast genetics (e.g. *ura3*, *his3* etc) are severely hampered in their ability to efficiently produce foreign proteins in comparison to *wt* cells. In the case of *ura3*, this reduced performance occurred even when the *URA3* gene was expressed *in trans* in Δ*ura3* cells (restoring the cells ability to grow in the absence of exogenous uracil, but not restoring the cells exceptional protein expression ability). We believe that certain *wt K. lactis* backgrounds are finely tuned to be efficient protein producers and that altering nucleotide or amino acid biosynthetic pathways (the markers generally used in yeast genetic experiments) severely alter the cell's metabolic balance. In heterologous protein production strategies, this can result in orders of magnitude less target protein being produced. Therefore, for performance reasons, we have adopted the use of primarily "untouched" *wt* backgrounds into which we introduce expression vectors that integrate site-specifically on the chromosome. Dominant selection strategies (e.g. antibiotic resistance or acetamide selection – a nitrogen source selection) are then used to identify and maintain the integrated vectors. For these reasons, certain wild-type strains are quite important to this process.