Baker's yeast breeding and engineering to improve biologics manufacturing

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Abstract

The regulatory-friendly baker's yeast Saccharomyces cerevisiae has a bright future for cost reduction and improved access to medicines, especially for lower and middle-income countries. It has been used safely for biopharmaceutical manufacturing since 1987, although this work only used a limited number of closely related strains. These were improved by chemical mutagenesis and rational genome engineering for first-generation platforms, producing biologics that currently worth over $40 billion/year in market value.

Phenotypeca has developed a next-generation biologics platform using genetically diverse baker's yeast from different geographical and ecological niches. Four parent strains with around 100,000 segregating functional SNPs were engineered for protease reduction, chaperone overexpression and other modifications known to improve product yield and quality during cGMP manufacturing of recombinant products. Advanced multi-generational breeding produced libraries of approximately a billion genetically distinct progeny for the intracellular production or secretion of a range of recombinant proteins. These populations contain industrially stable, exchangeable, high copy number expression plasmids and consist of only yeast DNA plus a codon-optimised synthetic gene of interest. Results for mCherry fluorescence showed that phenotypes improved from the diverse progeny generated.

Results

Intracellular Products:

Multi-Generational Breeding

Figure 2: Four diverse parental yeast strains are bred to generate diverse populations for phenotyping screening.

Figure 3: Phenosort feature study and Phenodex™ strain optimisation workflows.

Expression Plasmids:

High-copy number episomal expression plasmids based on the whole 2-micron plasmid were designed for stable maintenance during industrial scale production. Plasmid copy number is enhanced by complementary genome engineering in the parental strains to elevate the transcript levels for the recombinant product. Promoter, leader and terminator sequences are selected from libraries for each recombinant protein of interest. Recombinant protein production is repressed during breeding and activated afterwards for selection of strains with improved phenotypes from the diverse progeny generated. Fluorescent protein tags, e.g. mCherry, were used to facilitate selection during flow cytometry. Plasmids expressing mCherry-tagged proteins can be exchanged for the final plasmids without the mCherry-tag by growth in non-selective media and re-transformation with the desired final expression plasmid. Similarly, strains improved for one recombinant product can easily be tested for new products.

Conclusion

• Yeast breeding generated progeny phenotypes better than the best parent and worse than the worst parent.
• Combining yeast breeding with rational genome engineering offers a powerful strategy for improving recombinant protein production from S. cerevisiae.
• Phenotypic improvement of production strains promises significant cost reductions for biologics production.

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Figure 5: Comparison of S. cerevisiae parental and progeny strains for intracellular mCherry production.

Secreted Products: A range of SARS-CoV-2 spike protein fragments were secreted with C-terminal mCherry tags using the invertase leader sequence. Figure 6 shows flow cytometry results for cell sorting of 4th generation progeny secreting a SARS-CoV-2 Spike protein fragment (RBD 344-504) fusion. Microtiter plate cultures for individuals selected for high mCherry signal by flow cytometry were visibly red compared to individuals with low mCherry signal.

Figure 6: Single cells were sorted for mCherry fluorescence using a Beckman Coulter Aria II E cell sorter for antigenic analyses.