New method for developing Pichia pastoris yeast strains with high productivity of useful proteins

July 8 2022

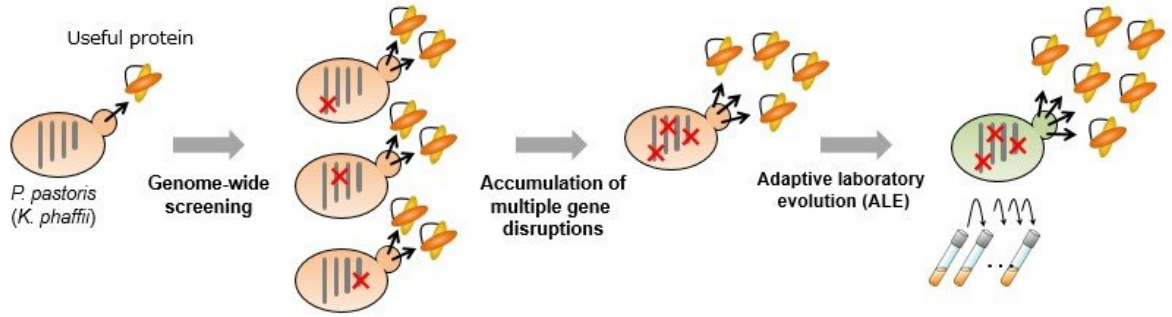

Figure 1. Strategy for developing a Pichia pastoris strain for the production of useful proteins. Credit: Modified version of Ito et al, "A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii," *Communications Biology*, 2022.

A collaboration including researchers from Kobe University, University of Tokyo and Tohoku University has successfully identified and disrupted genes in the yeast Pichia pastoris in order to increase its secretory production of useful proteins. Through a series of processes that involved combining gene disruptions and then serially cultivating the resulting multiple disruption strains, they developed P. pastoris strains that can produce high yields of useful proteins. It is hoped that this discovery will lead to the development of techniques to improve protein

production for biomedical antibodies and industrial enzymes, among other applications.

From Kobe University, the research collaboration included Project Associate Professor Ito Yoichiro and Associate Professor Ishii Jun (both of the Engineering Biology Research Center), and Professor Kondo Akihiko (Graduate School of Science, Technology and Innovation).

These results were published in *Communications Biology* on June 8, 2022.

Main points

- The researchers used a multi-well formatted high-throughput assay to identify P. pastoris gene disruptions that could increase the secretory production of useful proteins.
- Secretory production of useful proteins could be increased when multiple disruptions to identified genes were combined.
- These genes with multiple disruptions were found to increase secretory production, even when the useful protein or its expression were different.
- By cultivating these multiple-gene disruption strains (i.e. useful protein production strains), the researchers successfully increased secretory production of useful proteins.

Research background

In recent years, attempts have been made worldwide to produce proteins for enzymes (used in industry) and antibodies (used in biomedicine) via secretory production by the yeast P. pastoris (syn. Komagataella phaffii). However, various difficulties hamper this process, for example, P. pastoris has a low production rate for some target proteins. In light of

this, the researchers came up with the following approach (Figure 1) aiming towards high productivity of difficult-to-secrete proteins (or proteins with low secretory production) in P. pastoris:

1. Identify effective gene disruptions by screening a library of random genome-disruption strains.
2. Add multiple disruptions to the identified genes.
3. Improve secretory production through serial cultivation.

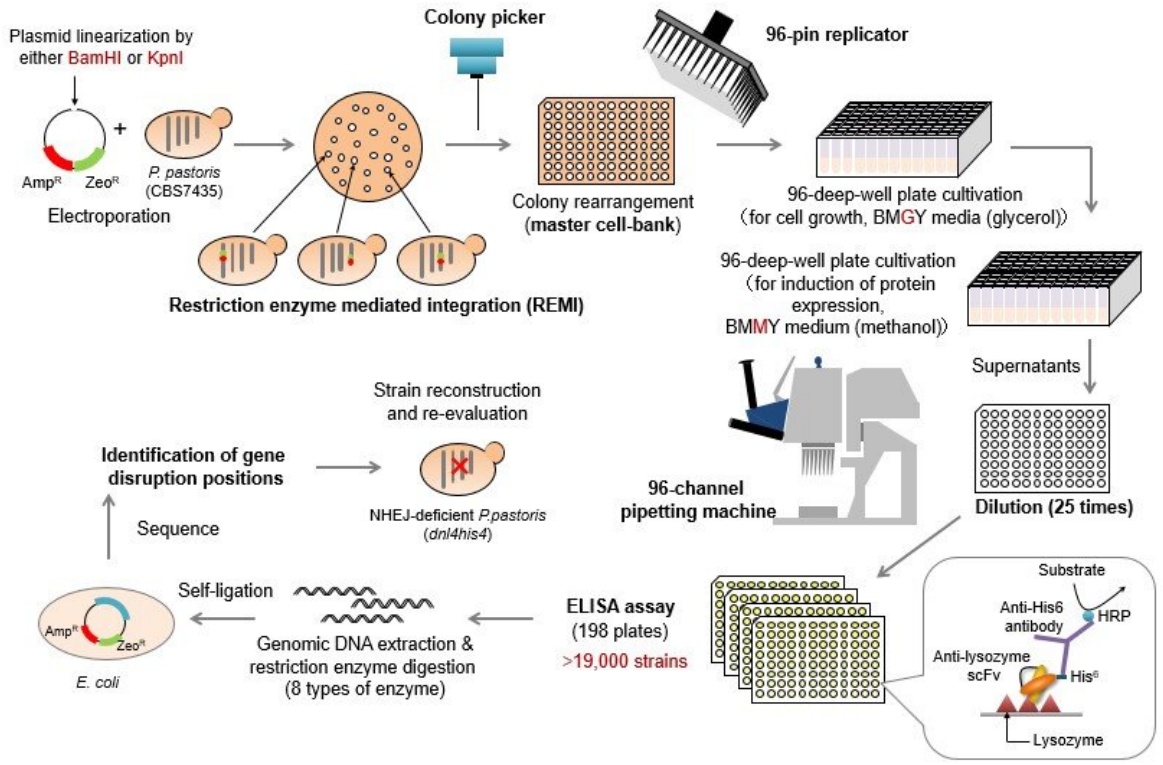

Figure 2. High-throughput screening system. Credit: Modified version of Ito et al, "A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii," *Communications Biology*, 2022.

Creation of a random genome-disruption library and screening:

In order to find effective gene disruptions in a random genome-disruption library, the researchers first of all developed a simple method to evaluate how well various genetically modified strains could produce the target proteins (Figure 2). Anti-lysozyme single-chain variable fragment (scFv) antibody is difficult to secrete in P. pastoris. Using scFv as a model protein, the researchers set up a high-throughput multi-well screening system so that they could easily evaluate the secretion of scFv by a large number of mutant strains. They created a random genome-disruption library from P. pastoris strains that can produce scFv, and using the method explained above, they were able to evaluate the scFv productivity of over 19,000 different strains. From this, they succeeded in identifying six genes in which gene disruption could increase scFv production (five of the six types of gene disruption were new) (Figure 3).

Coincidentally during this screening, they also discovered a mutation in a secretory signal sequence (that greatly increases scFv secretion (79G10 in Figure 3a). This is a 1 amino acid mutation (V50A) in the Saccharomyces cerevisiae-derived MFα signal peptide.

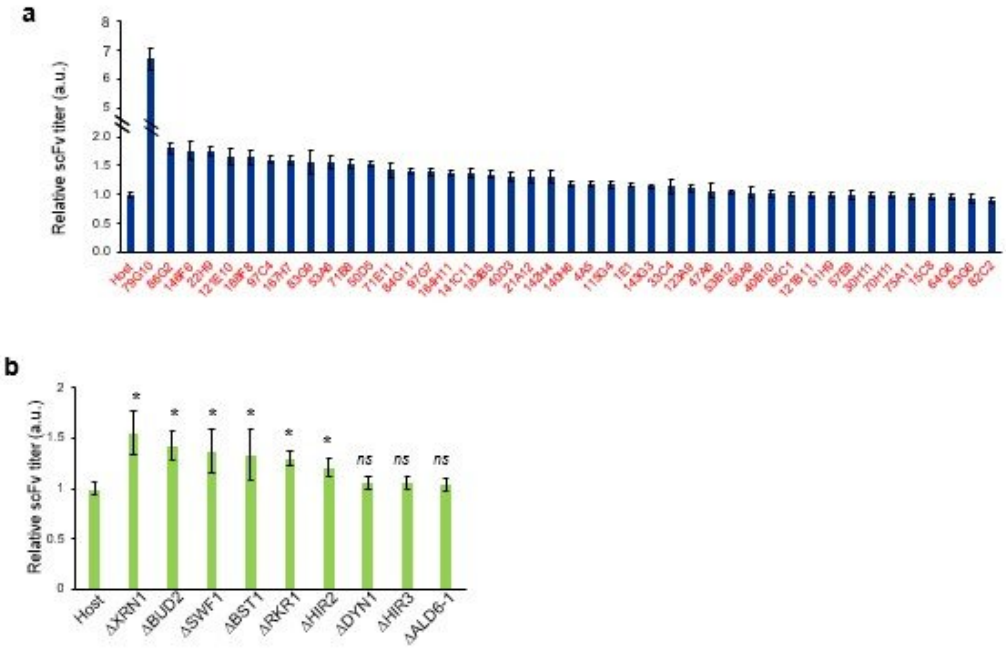

Figure 3. Screening of the random genome-disruption library and the useful gene candidates obtained. Credit: Modified version of Ito et al, "A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii," *Communications Biology*, 2022.

Combining multiple disruptions in the identified useful genes

The researchers proceeded to disrupt the identified genes (obtained through the step above) in a scFv production strain of P. pastoris, revealing that the accumulation of gene disruptions led to increased protein productivity. The strain with multiple disruptions to four of the identified genes demonstrated a five-fold improvement in productivity compared to the parental strain (Figure 4). In addition, the researchers showed that (multiple) disruptions to these genes had the same effect,

not only in the scFv production strain used in the screening, but also in a β-glucosidase production strain and an scFv strain with a different promoter to the one used in the screening (Figure 4).

Serial cultivation of strains with (multiple) gene disruption(s)

In the strains with (multiple) disruptions in each gene, they found that the speed of P. pastoris cell multiplication decreased as the gene disruptions accumulated (Figure 4). To restore the multiplication speed in these strains, they repeated the cultivation cycle over 50 times, allowing the yeast to grow in a fresh culture medium each time. This is called Adaptive Laboratory Evolution (ALE). Using this technique, the researchers succeeded in restoring cell multiplication in the majority of the disrupted strains, in addition to boosting their scFv productivity (Figure 5).

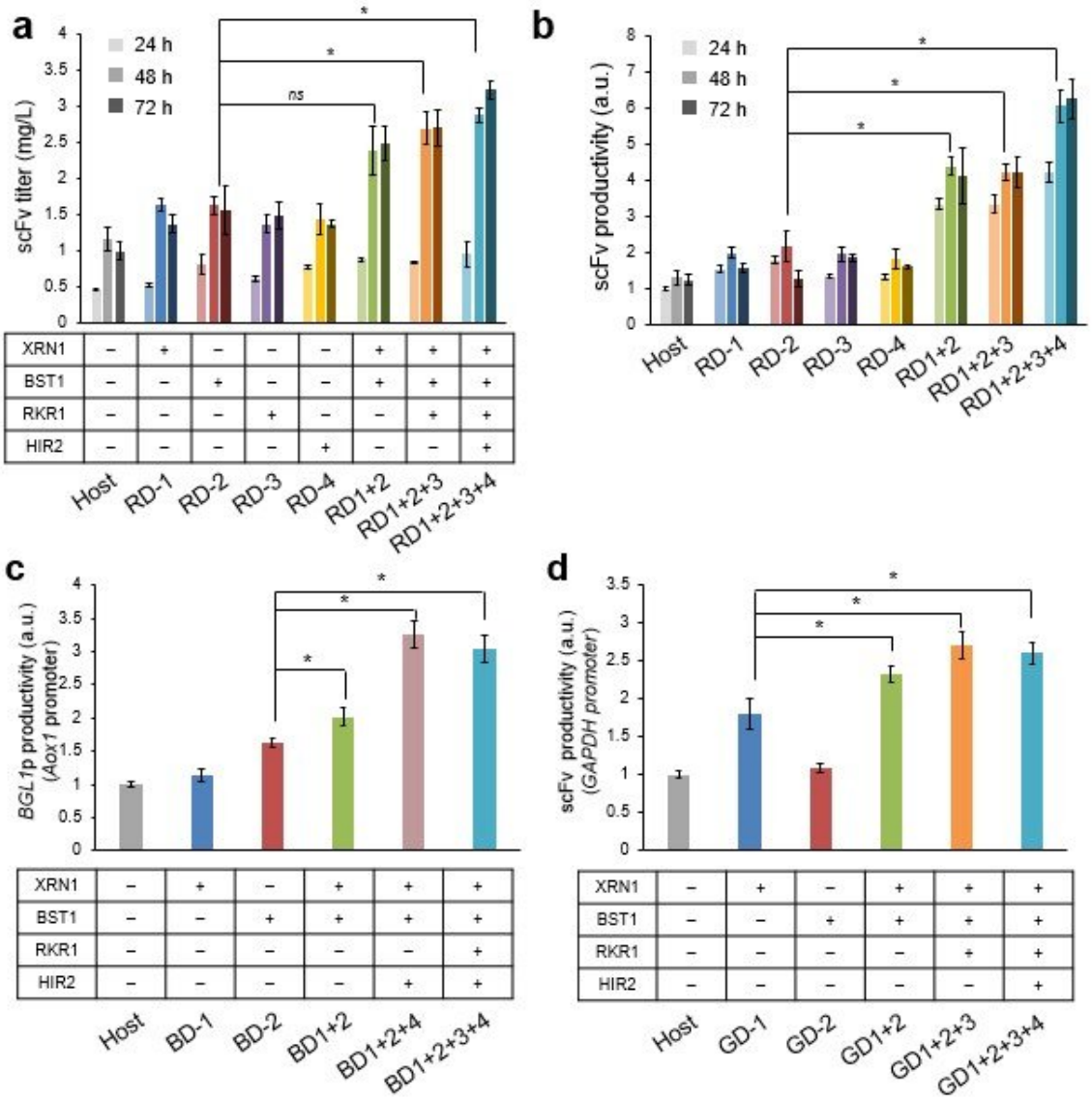

Figure 4. Evaluating the production of target proteins from multiple disruptions in the useful genes. Credit: Ito et al, "A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii," *Communications Biology*, 2022.

Through the three steps described above (Creation of a random genome-disruption library and screening; combining multiple disruptions in the

identified useful genes; cultivation of strains with (multiple gene disruption(s)), the researchers successfully increased the production of target proteins. This can serve as a method for developing new host strains to increase the production of useful proteins in P. pastoris.

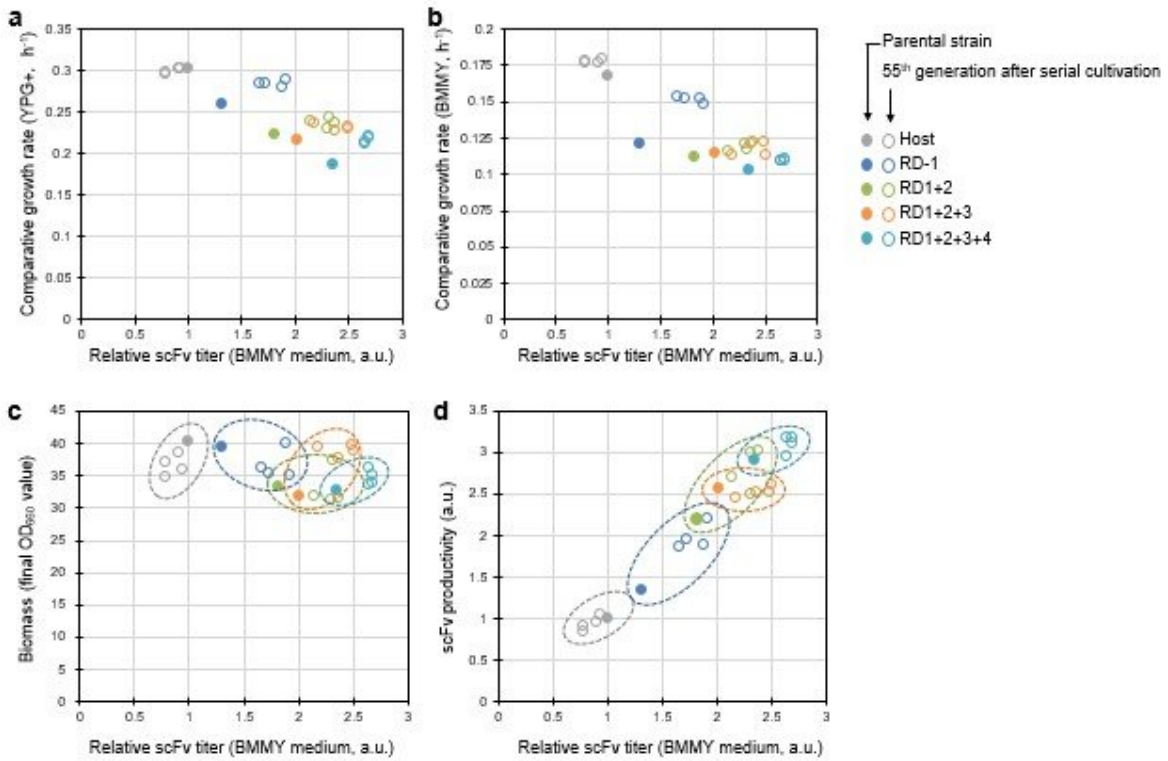

Figure 5. Evaluating the long-term cultivation of strains with (multiple) useful gene disruption(s). Credit: Ito et al, "A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii," *Communications Biology*, 2022.

Further research

Using the insight obtained from this study, the researchers plan to

develop P. pastoris stains with even higher productivity of difficult-to-express target proteins.

More information: Yoichiro Ito et al, A streamlined strain engineering workflow with genome-wide screening detects enhanced protein secretion in Komagataella phaffii, *Communications Biology* (2022). DOI: 10.1038/s42003-022-03475-w

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