Rapid generation of improved lipid-producing yeast strains using PicoShell screening

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Summary

Saku identified and validated two high-yielding lipid-producing yeast variants using PicoShell screening technology. Saku isolated a novel *Rhodotorula toruloides* variant (E2) that increased lipid yields from 38.6 grams per liter to 50.4 grams per liter (31% increase) and identified a *Yarrowia lipolytica* variant (E6) that increased lipid yields from 7.4 grams per liter to 10.8 grams per liter (47% increase). At commercial scales, increases of this magnitude can materially reduce cost of goods by improving volumetric productivity and reducing fermentation time or reactor utilization. From parental inoculation to internally finalized analyses, both campaigns from bioassay development, screening, re-culture, and validation were completed in 5 – 6 weeks.

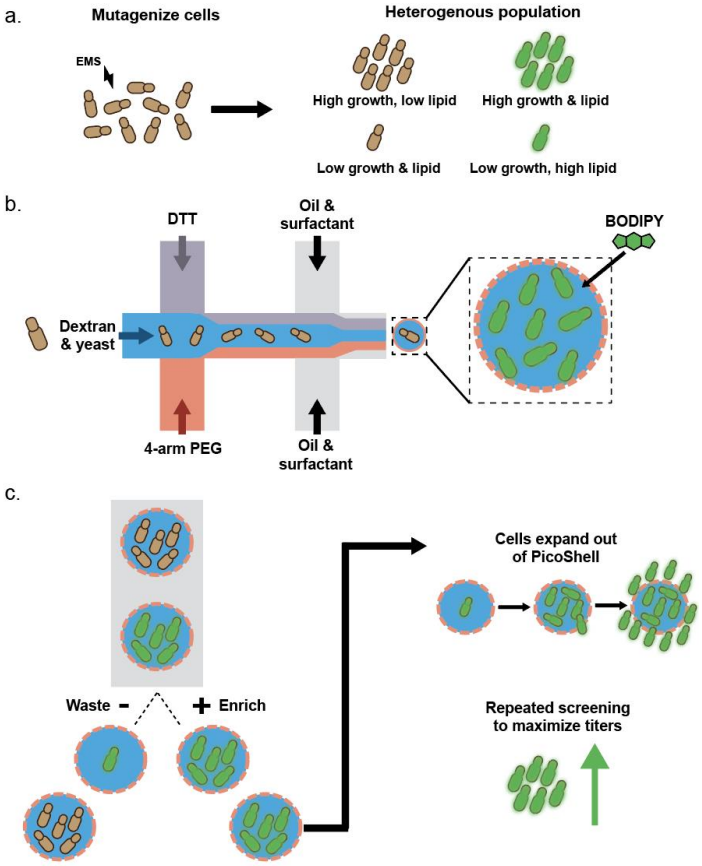

Fig 1: Overview of PicoShell screening process. a) Mutagenizing yeast strains yields diversity in growth and lipid production rates. b) Millions of individual yeast clones can be rapidly encapsulated in PicoShell particles and stained for lipid accumulation. c) Flow cytometry is used to enrich the top growers and producers, which can be iteratively improved upon through multi-generational screening.

Background: Why the Lipids and Fatty Acid Markets Are Bottlenecked

Fermented lipids and fatty acids support a growing range of applications spanning cosmetics, food ingredients, and other specialty chemicals.[1] Despite strong demand, many lipid fermentation processes remain economically challenged, with production costs often limiting commercial viability. A primary driver of these costs is the performance of the host strain or chassis used in fermentation, which directly determines achievable titers, yields, and overall process efficiency.[2]

Traditional strain improvement approaches based on rational design frequently extend development timelines due to the inherent complexity of lipid metabolism and its tight coupling to growth and cellular fitness. In many cases, rational design strategies also rely on targeted genetic modifications that trigger regulatory classification as genetically modified organisms (GMO), introducing additional development, approval, and market adoption hurdles in certain applications.[3]

While high-throughput screening has the potential to accelerate strain engineering without direct genetic engineering, commonly used tools such as well plates and conventional microfluidic systems suffer from fundamental limitations. First, microbial growth is often impaired by inadequate oxygen transfer and rapid nutrient depletion within confined compartments. Second, screening is typically performed under conditions that differ substantially from end-state fermentation environments, leading to poor translation of screening results to scaled processes

Saku's PicoShell technology addresses these limitations by enabling high-throughput encapsulation, screening, and sorting of large strain libraries directly in shake flasks and bioreactors.[4] PicoShell-based workflows are compatible with natural heterogeneity as well as established regulatorily accepted library generation methods such as chemical and UV mutagenesis, allowing strain performance to be improved without introducing targeted genetic modifications. By allowing microbes to grow and produce under fermentation-relevant conditions with appropriate mass transfer, PicoShell-based screening preserves realistic growth and production phenotypes and improves the likelihood of successful scale-up. This white paper summarizes two campaigns demonstrating improved lipid accumulation in two widely used yeasts for lipid production: *Rhodotorula toruloides* and *Yarrowia lipolytica* (Figure 1).

Case study 1: improved *Rhodotorula* strain with 30% increase in lipid yield

A wildtype *Rhodotorula toruloides* strain was provided by collaborators at the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU) at Lawrence Berkeley National Laboratory (LBNL). The parental population was mutagenized using ethyl methanesulfonate (EMS) and individual cells were encapsulated into separate PicoShells to enable clonal growth and screening. These strain-loaded PicoShells were cultured in shake flasks under lipid induction media for 72 hours.

Following culture, the PicoShells containing clonal populations were stained with BODIPY dye to label intracellular lipids (Figure 2a). Samples were sorted using a SONY SH800S cell sorter by

selecting loaded PicoShells within the top 10% of side scatter area (a proxy for biomass accumulation) and the top 10% of BODIPY fluorescence area (lipid content) (Figure 2b).

Following the enrichment process and re-culture, Saku identified a high-performing *R. toruloides* variant (E2) with a 30% increase in total lipid accumulation relative to the parental strain (38.6 g/L to 50.4 g/L), as measured by **Bligh-Dyer extraction**. External validation was performed by the UCLA Lipidomics Core Facility using shotgun lipidomics, which confirmed a 33% increase in total lipid accumulation for clone E2 following an independent culture (Figure 2c).

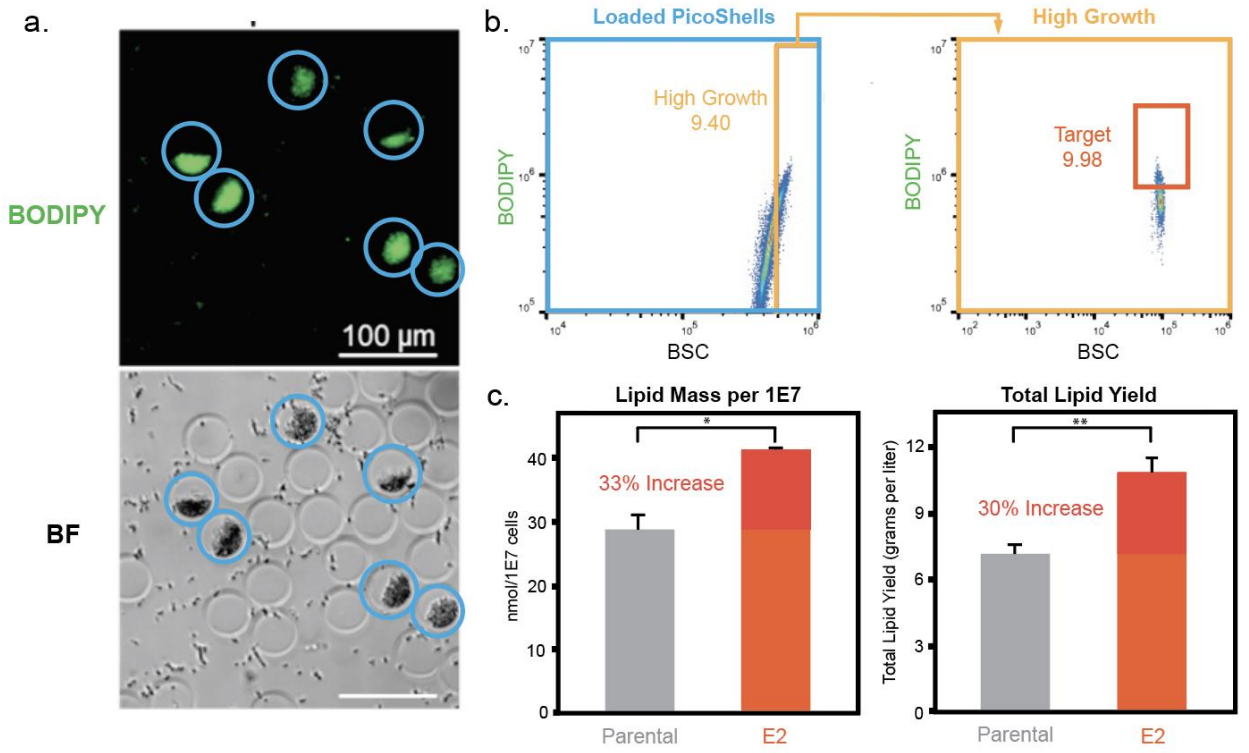

Fig 2: *R. toruloides* lipid yield enhancement. (a) *R. toruloides* colonies within PicoShells that have had accumulated lipids stained with BODPIY. (b) Gating strategy employed to enrich high-growth, high-producing *R. toruloides* strains from PicoShell screening process. (c) Shotgun lipidomics analysis shows increased lipid content per cell (left) and total lipid yield (right) for the E2 variant compared to the parental strain following a 72-hour lipid induction culture. Results confirm improved cellular productivity and higher overall lipid output. Data shown as mean ± standard deviation. Analysis performed by the UCLA Lipidomics Core Facility. Improved per-cell lipid accumulation translated directly into increase total lipid yield at the culture level.

Case study 2: improved *Yarrowia lipolytica* strain with >45% increase in lipid yield

Saku conducted the *Y. lipolytica* campaign using a wildtype parental strain obtained from ATCC. The parental population was mutagenized with EMS and individual cells were encapsulated into PicoShells to enable clonal growth and screening.

Strain-loaded PicoShells were cultured under lipid induction conditions for 72 hours, after which intracellular lipids were stained using BODIPY. Samples were sorted on a SONY SH800S cell sorter by selecting the top 10% of PicoShells by side scatter area and top 10% by BODIPY fluorescence area (Figure 3a).

Following enrichments and re-culture, Saku identified a high-performing *Y. lipolytica* variant (E6) demonstrating a 47% increase in total lipid accumulation relative to the parental strain (7.4 g/L to 10.8 g/L). Triplicate cultures of the E6 variant and parental strain were fermented under lipid induction conditions and total lipid yield was quantified using Bligh-Dyer extraction (Figure 3b).

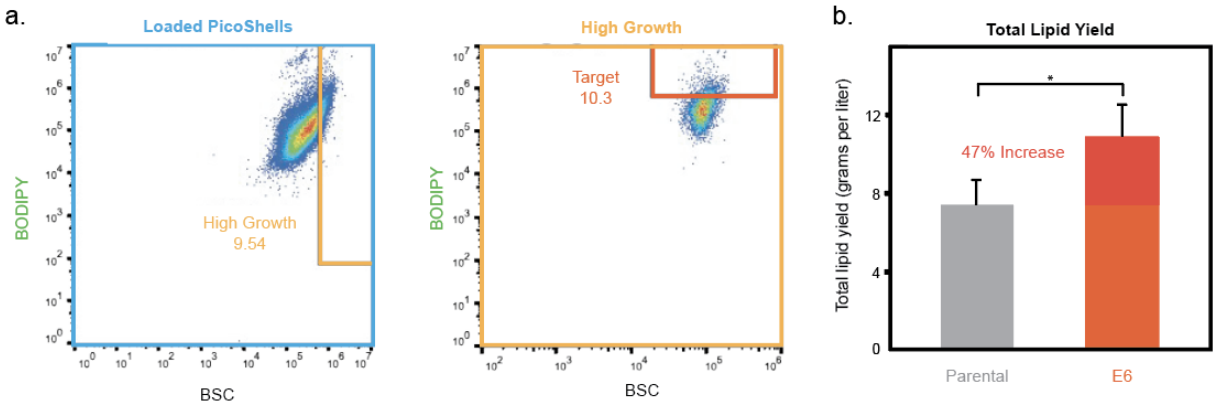

Fig 3: *Y. lipolytica* lipid yield enhancement. (a) Gating strategy employed to enrich high-growth, high-producing *Y. lipolytica* strains from PicoShell screening process. (b) Total lipid yield was measured for the parental *Y. lipolytica* strain and the Saku-enriched E6 variant following lipid induction fermentation. The E6 variant demonstrates a 47.5% increase in total lipid yield relative to the parental strain. Data represent mean ± standard deviation from triplicate cultures. Increased strain productivity directly translated into higher lipid output at the culture level.

Conclusions

These results demonstrate the effectiveness of PicoShell-enabled screening for improving lipid-producing microbial hosts. Across two distinct and industrially relevant yeast species, *Rhodotorula toruloides* and *Yarrowia lipolytica*, Saku identified and validated single-cell-derived variants exhibiting substantial increases in total lipid accumulation relative to their respective parental strains. Notably, these improvements were achieved within a single screening cycle, underscoring the ability of the platform to rapidly identify rare, high-performing producers from diverse mutant populations.

A key outcome of both campaigns is the consistent translation of improved cellular phenotypes to increased culture-level lipid yield. By conducting screening and selection under fermentation-relevant conditions, PicoShell-based workflows preserve the coupling between growth, metabolism, and production, reducing the disconnect that commonly limits the predictive value of conventional screening methods. As a result, gains in per-cell lipid accumulation translated directly into higher total lipid output at the culture level, a critical determinant of fermentation process economics.

Beyond the specific strains described here, these campaigns validate a broader strain enhancement strategy applicable to a wide range of lipid-producing microbes. The ability to rapidly identify improved variants within weeks, rather than months or years, offers a clear path to accelerating strain development timelines, reducing experimental iterations costs, and de-risking subsequent scale-up efforts. Ongoing work will further characterize top-performing variants across expanded cultivation conditions assess phenotypic stability and robustness, and integrate molecular analyses, including sequencing, to better understand the genetic basis of enhanced lipid production and inform subsequent improvement cycles.

More broadly, the same platform capabilities demonstrated in these lipid-focused campaigns including high-throughput single-cell encapsulation, fermentation-relevant screening, and enrichment of rare high-performers, are directly transferable to other biomanufacturing applications. In particular, PicoShell-enabled screening can be adapted to secretion-linked assays for the discovery of ultra-high-producing protein-secreting cell lines in food proteins, biopharma, and other protein-driven industries.[5] In these contexts, the platform offers the potential to compress strain engineering and cell line development timelines while improving the likelihood that early screening results translate into durable production performance at scale.

Acknowledgements

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