Saccharomyces cerevisiae is far from being the only yeast of potential scientific and economic importance. Many of the 2000 other known yeast species have highly unusual metabolic, biosynthetic, and physiological capacities. However, significant hurdles lay in front due to the missing radical technologies to effectively engineer nonconventional microorganisms. Our research is geared towards illustrating how to design systematic rules and enable generalizable technologies that can be effectively applied from one species to another.

This presentation will start with an example introducing how we integrated an *in silico* chromosome scanning with a high throughput screening to efficiently identify centromeric DNA that significantly stabilizes episomal plasmid expression. A common technology hurdle for engineering nonconventional species is that majority of them preferentially employ non-homologous end joining (NHEJ) to repair DNA double-strand breaks (DSB), which prohibits precise CRISPR/Cas9 genome editing. Most recently, we identified the shortcomings of the conventional genome editing strategies and developed a new CRISPR platform, named Lowered Indel Nuclease system Enabling Accurate Repair (LINEAR), which enabled precision editing without NHEJ disruption with efficiencies of 67-100% in four industrially relevant yeasts. With NHEJ preserved, we demonstrated its ability to survey genomic landscapes, identifying loci whose spatiotemporal genomic architectures yielded favorable expression dynamics for heterologous pathways. A case study will be presented to demonstrate how to leverage an antagonizing pair of DNA DSB repair mechanisms to rapidly engineer a microbial factory to produce (S)-norcoclaurine, an entrance molecule of the large group of benzylisoquinoline alkaloids with medicinal applications. I will conclude my presentation by illustrating the potential of developing yeast models to benefit disease studies. Taken together, the development of radical technologies pushes the frontier of synthetic biology to explore novel species and accelerates strain engineering for generating interesting biochemical or biomedical products.