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Construction of a CRISPR-based plasmid library for targeted gene deletion in yeast

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Understanding the relationship between genotype and phenotype is a fundamental goal in biology. In model organisms, mutant collections of loss-of-function genetic variants present powerful means to link genes to precise molecular functions. In the model yeast Saccharomyces cerevisiae, we dispose of a collection of more than 6,000 loss-of-function mutants that covers almost the entire yeast genome. Taking advantage of this resource, multiple large-scale forward genetic screens have been conducted, revealing key pathways involved in important cellular functions. Most notably, using an automated strategy called Synthetic Genetic Array (SGA), nearly every combination of double mutants have been systematically studied across the yeast genome, leading to an unparalleled understanding of the cellular functional diagram compared to any eukaryotic models so far. While extremely useful, the construction of such mutant collections is often tedious, even in lab-friendly organisms like yeast. The first deletion collection in the reference strain S288c, using a PCR-based homologous recombination strategy, required international collaborations of several labs in a span of several years to be completed. As a result, studies using the yeast deletion collection are almost exclusively conducted in this single laboratory genetic background, leading to potential biases and an incomplete view of the genetic and phenotypic diversity within the species. To further exploit the power of yeast genetics in an efficient and unbiased manner, we are currently constructing a plasmid library that allows for highly efficient targeting of each individual yeast gene across the genome based on the CRISPR-Cas9 system. This plasmid collection, upon completion, will allow one-stop PCR-free generation of a complete genome-wide barcoded deletion collection for any background of interest in the S. cerevisiae species in one month. This powerful resource will greatly benefit the yeast community in studying background-specific mutation effects, higher-order genetic interactions and gene-environment interactions.

Biography

He is a Senior Research Associate in the University of Toronto in the Boone Lab and his research is focused on genetic interaction; molecular biology technique, especially at yeast mutant construction.

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