Engineering the ribosomal DNA in a megabase synthetic chromosome

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Abstract

We designed and synthesized a 976,067–base pair linear chromosome, synXII, based on native chromosome XII in Saccharomyces cerevisiae. SynXII was assembled using a two-step method, specified by successive megachunk integration and meiotic recombination mediated assembly, producing a functional chromosome in S. cerevisiae. Minor growth defect “bugs” detected in synXII, caused by deletion of tRNA genes, were rescued by introducing an ectopic copy of a single tRNA gene. The ribosomal gene cluster (rDNA) on synXII was left intact during the assembly process and subsequently replaced by a modified rDNA unit used to regenerate rDNA at three distinct chromosomal locations. The signature sequences within rDNA, which can be used to determine species identity, were swapped to generate a Saccharomyces synXII strain that would be identified as Saccharomyces bayanus by standard DNA barcoding procedures.

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