Genome-wide Purification of Extrachromosomal Circular DNA from Eukaryotic Cells

Extrachromosomal circular DNAs (eccDNAs) are common genetic elements in Saccharomyces cerevisiae and are reported in other eukaryotes as well. EccDNAs contribute to genetic variation among somatic cells in multicellular organisms and to evolution of unicellular eukaryotes. Sensitive methods for detecting eccDNA are needed to clarify how these elements affect genome stability and how environmental and biological factors induce their formation in eukaryotic cells. This video presents a sensitive eccDNA-purification method called Circle-Seq. The method encompasses column purification of circular DNA, removal of remaining linear chromosomal DNA, rolling-circle amplification of eccDNA, deep sequencing, and mapping. Extensive exonuclease treatment was required for sufficient linear chromosomal DNA degradation. The rolling-circle amplification step by φ29 polymerase enriched for circular DNA over linear DNA. Validation of the Circle-Seq method on three S. cerevisiae CEN.PK populations of 10(10) cells detected hundreds of eccDNA profiles in sizes larger than 1 kilobase. Repeated findings of ASP3-1, COS111, CUP1, RSC30, HXT6, HXT7 genes on circular DNA in both S288c and CEN.PK suggests that DNA circularization is conserved between strains at these loci. In sum, the Circle-Seq method has broad applicability for genome-scale screening for eccDNA in eukaryotes as well as for detecting specific eccDNA types.

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Contributors: Møller, H. D., Bojsen, R. K., Tachibana, C., Parsons, L., Botstein, D., Regenberg, B.
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