2-35

Generation of gene disruption cassettes with long flanking regions via fusion PCR.
Andreas Karoly Gombert (1), Björn Johansson (2), Nelson Lima (3), José Teixeira (3), Lucília Domingues (3)
(1) Chemical Engineering, University of Sao Paulo, C.P. 61548, Sao Paulo, SP, 05424-970, Brazil; (2) University of Minho, Department of Biology, Braga, Portugal; (3) University of Minho, Department of Biological Engineering, Braga, Portugal

In studies on yeast physiology and functional genomics, gene disruption is an essential tool to investigate the function of specific genes. Protocols for gene deletion in laboratory strains of *Saccharomyces cerevisiae*, which are based on the generation of disruption cassettes directly by PCR with chimeric primers, are well established. However, industrial strains of *S. cerevisiae*, as well as many non-conventional yeasts, due to their low capacity of promoting homologous recombination, require the use of disruption cassettes with long flanking regions (consisting of some hundreds of base pairs up- and downstream of the marker gene), when targeted gene disruption is aimed. The generation of such cassettes is not possible via direct PCR amplification with chimeric primers and requires enzyme digestions, ligation and cloning steps. Alternatively, such disruption cassettes may also be generated by fusion PCR, a technique which is in theory straightforward, but still not widespread, probably due to the lack of reliable and reproducible protocols. We have investigated several experimental conditions, aiming at the generation of a disruption cassette with long flanking regions with homology to the pyruvate decarboxylase gene of *Kluyveromyces marxianus* CBS 6556. Negative and positive results, the latter confirmed by DNA sequencing, will be reported and discussed, aiming at the establishment of a successful protocol for generation of such disruption cassettes.