

2D gel analyses

Cells were grown overnight at 28°C, in 200 ml YPD cultures. When cell concentration reached 10^7 cells/ml, cells were washed, resuspended in fresh YPD medium at a concentration of 0.75×10^7 cells/ml and grown for another 45 minutes at 28°C. Afterwards, 2×10^9 cells were synchronized using 3g/ml alpha-factor, for two hours at 28°C (or two and a half hour for *rad51* cells). G1 arrest was checked by microscope observation. When more than 80% of the cells were arrested, they were centrifugated, washed and then released in 200 ml of fresh YPD medium, at 23°C, to slow down replication in order to increase the chance of catching replication intermediates. Cells were harvested after 30 min, 40 min, 60 min and 90 min, killed by addition of Sodium Azide (0,1% final concentration), cooled down on ice, centrifugated, washed in cold water and frozen at -70°C. The day after, they were incubated in 5 ml spheroplasting buffer (1 M sorbitol, 100 mM EDTA (pH 8.0), 0.1% -mercaptoethanol), with 500 µl of 10 mg/ml Zymolyase 100T (Seikagaku), for 45 min at 30°C. Spheroplasts were centrifugated at 4,000 rpm for 10 min at room temperature, supernatant was carefully removed, and 2 ml water, 200 µl RNase A (10 mg/ml), and 2.5 ml Solution I (2% w/v cetyltrimethyl-ammonium-bromide (CTAB), 1.4 M NaCl, 100 mM Tris HCl (pH 7.6), and 25 mM EDTA (pH 8.0) were sequentially added to the spheroplast pellet, before incubation for 30 min at 50°C. Subsequently, 200 µl Proteinase K (20 mg/ml) were added and tubes were incubated at 50°C for 1.5 hour, then at 30°C overnight. Samples were centrifugated at 4,300 rpm for 10 min, cellular debris pellets were kept for further extraction, while the supernatant was extracted with 2.5 ml chloroform/isoamylalcohol (24/1) and DNA in the upper phase was precipitated by addition of 2 volumes Solution II (1% w/v CTAB, 50 mM Tris-HCl (pH 7.6), and 10 mM EDTA), followed by centrifugation at 8,500 rpm for 10 min. The pellet was resuspended in 2 ml Solution III (1.4 M NaCl, 10 mM Tris HCl (pH 7.6), and 1 mM EDTA). Residual DNA in cellular debris pellets was also extracted by resuspension in 2 ml Solution III and incubation at 50°C for 30 min, followed by extraction with 1 ml chloroform/isoamylalcohol (24/1). Upper phases of both extractions were pooled. Total DNA was then precipitated with 1 volume isopropanol, washed with 70% ethanol, air dried, and finally resuspended in 250 µl TE. Enzymatic restriction was performed on 10 µg total DNA, with ClaI, in NEB 4 buffer (supplemented with BSA) in 200 µl final volume, overnight at 37°C. Digested DNA was precipitated by addition of 225 µl isopropanol and 25 µl 2.5 M potassium acetate pH 6.0, washed with 75% ethanol, air dried, and finally resuspend in 20 µl TE (Lopes et al., 2001).

2D gel electrophoreses were done as originally described by (Brewer and Fangman, 1987). The first migration was performed in 0.35% *seakem* (BMA) agarose, at 1 V/cm during 21h, and the second migration was done in 0.9% *seakem* agarose with 0.3 g/ml ethidium bromide, at 7 V/cm during 4h30. DNA was transferred overnight in 10X SSC on a charged nylon membrane (Sigma). DNA was then UV crosslinked, on a Stratagene Stratalinker. Hybridization was performed with a 750 bp probe covering the 5' end of the *ARG2* gene, labeled by random priming (Feinberg and Vogelstein, 1983).