Use of polymerase chain reaction for rapid detection of gene insertions in whole yeast cells

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The polymerase chain reaction (PCR) (1) can be performed to generate sequence-specific DNA molecules using complex biological samples, such as whole cells or tissues, as a substrate. This method has been exploited for the rapid analysis of recombinant DNA constructions carried in whole bacterial cells or viruses (2, 3). We have extended these techniques for use in analyzing the genomic structure of the yeast *Saccharomyces cerevisiae*. Gene disruption/replacement technology (so called 'reverse genetics') has become routine in *S.cerevisiae* (4). The usual method for identifying yeast cells containing chromosomal insertions and/or deletions is to prepare genomic DNA from individual yeast transformants, digest the DNA with restriction enzymes, and analyze the restricted DNA by Southern blotting. This procedure takes ~2–3 days. We demonstrate here that it is possible to directly screen whole yeast cell transformants by PCR for those carrying the desired genomic construction. In the example shown below, cells selected to contain a *URA3* (5) gene disruption of the *RBP1* gene (6) were easily distinguished from wild-type cells following a PCR reaction, using freshly-grown cells as the substrate, and *RBP1*-specific oligonucleotide primers that flank the *URA3* insertion. Identification of a chromosomal alteration by this method takes less than 3 h and eliminates the need to prepare genomic DNA, restrict it and probe it with a radiolabeled DNA fragment. The amplified product can be used for subcloning, restriction digest analysis and DNA sequencing. While the majority of cells appeared intact following the PCR reaction as visualized by phase-contrast microscopy, apparently some cells disrupted during the denaturation step (94°C), liberating a small quantity of genomic DNA. Preboiling of cells was not essential and did not affect the yield of the PCR product.

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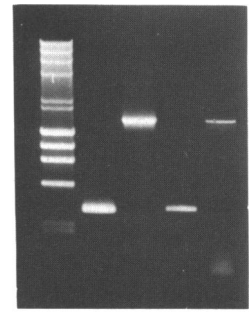

Figure 1. Cells of *S.cerevisiae* strain GL45 (*MATα trp1Δ1 leu2–3,2–112 ura3–52*) were transformed with a fragment of the *RBP1* gene (6) which was engineered to contain the *URA3* gene (1.1 kb *Hin*dIII fragment, Klenow-filled) (5) inserted into the unique *Nde*I (Klenow-filled) site. Ura^+ transformants were selected on synthetic complete agar medium lacking uracil, and cells were directly added to a 100 μl PCR reaction containing 10 μl 10×reaction buffer (500 mM KCl, 100 mM Tris HCl pH 8.3, 15 mM $MgCl_2$, 0.1% gelatin), 16 μl dNTP (1.25 mM each), 1 μl each of two primers (1 μg/μl), 71 μl H_2O, 5 units of Taq polymerase (Boehringer Mannheim; 5 units/μl). Each reaction contained approximately $1.0–2.5\times10^7$ cells. Primers were 5'-CCGGCTCGAGATGT-CTGAAGTAATTGAAGGTAACGTC-3'and 5'-CCGGGGATCCAAGCAG-AAAGGCGGCTCAATTGATAG-3'(6). Samples were subjected to 35 rounds of temperature cycling: 94°C 1 min, 52°C 1 min, 72°C 3 min, with a final 7 min 52°C step, using a Perkin Elmer thermocycler. A portion of each reaction mixture was analyzed on a 1.0% agarose gel with 1 μg/ml EtBr. Lane 1: λ *Hin*dIII and øX174 *Hae*III markers (New England Biolabs). Lanes 2–4: samples of individual PCR reactions using either purified plasmid DNA or whole yeast cell substrates (as indicated). Lane 2: pGEM–7Zf(+)RBP1 (5 μl). Lane 3: PGEM–7Zf(+)RBP1::URA3 (5 μl). Lane 4: GL45 cells (10 μl). Lane 5: MM3 cells (*MATα trp1Δ1 leu2–3,2–112 ura3–52 rbp1::URA3*) (15 μl). pGEM–7Zf(+) was obtained from Promega Corp.

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