PCR-based screening of YAC clones without DNA extraction

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PCR-based screenings of DNA or spheroplasts from individual clones or pools have proven to be efficient for identification of gene-specific clones within YAC contigs (Ref. 1). While colony-PCR-based methods have been shown to be simple, rapid and efficient approaches for screening individual recombinant bacterial colonies, conflicting data concerning the application of colony-PCR-based screenings of YAC clones have been published (Ref. 2, 3, 4). Two papers showed that PCR can be performed on yeast cells without extensive DNA extractions, using whole yeast cells or spheroplasts as templates for PCR (Ref. 2, 3), while a third report states that colony-PCR procedures are ineffective for yeast cells (Ref. 4). Data presented here demonstrate that inoculates of whole cells, from solid plates and from liquid cultures, are satisfactory templates in PCR reactions, at least when amplified fragments are relatively short

Materials and methods

YAC clones and cell culture

YAC clones, spanning and flanking the *CUB* locus (Ref. 5) were obtained (Fondation Jean Dausset—C.E.P.H., Paris France). The *CUB* gene encoding the human intrinsic factor-vitamin B_{12} receptor *Cubilin*, located on chromosome 10p is a strong candidate gene for autosomal recessive megaloblastic anemia (*MGA1*).

Direct PCR and gel electrophoresis

All PCR reactions were performed in 15 μl reaction volumes. PCR mastermix was composed of:

0.17 mM of each dNTP (Pharmacia Biotech)
0.5 U AmpliTaq (Perkin Elmer)
in 1×reaction buffer (50 mM KC1, 10 mM Tris-HC1, pH 8.3, 1.5 mM $MgC1_2$, 0.001% gelatin)

using 10 pmol of each primer
CUB5FOR: CTACAGAATCAACAGGGACC
CUB5REV: CCACAGTATCTTCCAAGGGA

amplifying a 210 bp fragment of the *CUB* gene. Gel-loading buffer, at final concentrations of 3 mM cresol red (Aldrich) and 12% sucrose (USB), was included in the PCR mastermix (Ref. 6). 15 μl PCR mastermix was pipetted into individual wells of a 96-well PCR thermoplate. Minute amounts of yeast cells were then inoculated from a master microplate with a 10 or 200 μl disposable pipette tip and swirled vigorously in individual wells containing PCR mastermix. PCR on cell suspensions was performed by adding 1 μl aliquots of well-mixed cell suspensions into individual wells of a PCR thermoplate containing 14 μl PCR mastermix. PCR thermoplates were heat sealed using Thermoseal foil (Advanced Biotechnologies), briefly vortexed, centrifuged and placed in a PTC-225 DNA Engine Tetrad thermocycler (MJ Research). Samples were pre-denatured for 10 min at 95°C, followed by 37 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 30 s. A final extension for 5 min at 72°C concluded the thermocycling program. PCR products were electrophoresed in 2% agarose gels, 1×TBE and photographed.

Results and discussion

Inoculum from microplates were used as templates in PCR reactions and several YAC clones within the contig produced PCR products of the correct length when compared with positive controls obtained from human genomic DNA templates (Figure 1). These data verified that PCR performed directly on inoculum from microplates is a reliable and rapid tool for screening YAC clones. Data were further authenticated by using DNA extracted from liquid cultures of individual clones as templates in the PCR reaction (Figure 1, gel C).

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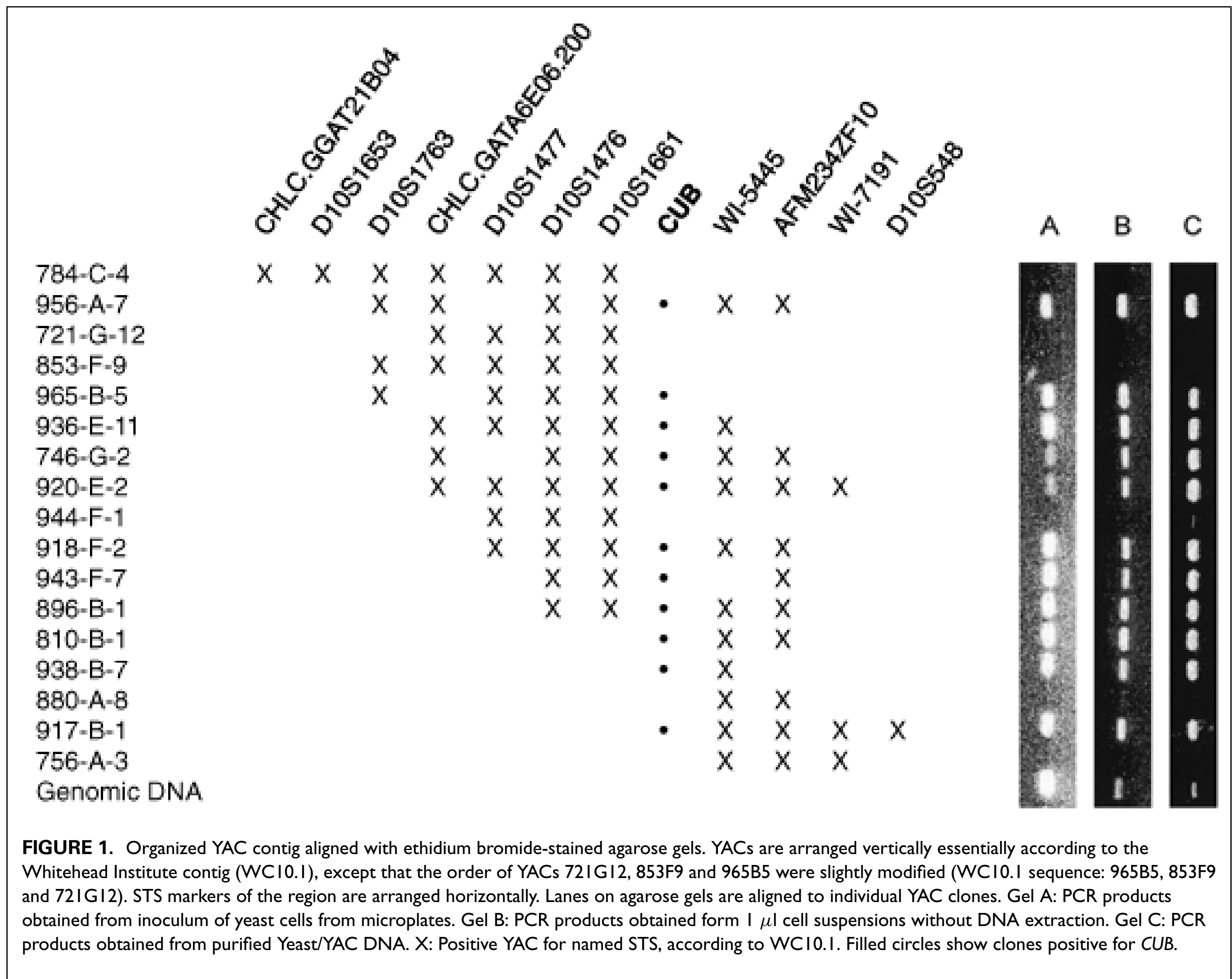

FIGURE 1. Organized YAC contig aligned with ethidium bromide-stained agarose gels. YACs are arranged vertically essentially according to the Whitehead Institute contig (WC10.1), except that the order of YACs 721G12, 853F9 and 965B5 were slightly modified (WC10.1 sequence: 965B5, 853F9 and 721G12). STS markers of the region are arranged horizontally. Lanes on agarose gels are aligned to individual YAC clones. Gel A: PCR products obtained from inoculum of yeast cells from microplates. Gel B: PCR products obtained form 1 μl cell suspensions without DNA extraction. Gel C: PCR products obtained from purified Yeast/YAC DNA. X: Positive YAC for named STS, according to WC10.1. Filled circles show clones positive for *CUB*.

This successful amplification of inoculum, directly from microplates without DNA extraction, suggested that PCR could also be performed directly on small volumes of liquid cultures. Data obtained (Figure 1, Gel B) shows that PCR can be performed successfully on resuspended cells without DNA extraction. Furthermore, cell suspensions do not need to be fresh; data presented in Figure 1 were from cell suspensions that had been left at 4°C for more than two weeks. Before aliquoting out, the tubes were briefly vortexed to resuspend the cells. That PCR can be performed directly on cell suspensions suggests that modifications of this technique are potentially applicable for automation, where handling of liquids in microplates are far less complicated than picking colonies from individual wells. A recent report suggests that growing and storing yeast cultures in Hogness freezing medium allows for repeated freezing and thawing cycles of yeast cultures (Ref. 7). Thus, this could be a valuable modification making the present procedure more applicable for automation.

This technique has been used at length in our laboratory and individual YAC clones encoding, for example, Hoxd13 (Hoxd13FOR: ATCAGCCGGACATGT-GCGTCTAC, Hoxd13REV: GCCGCCGCTTGTCCTTGTTA, 126 bp), *BFSP1* (Fild1UP: CTTTGGCCTATAAGACAGTGG, Fild1down: AAGCATTACCCCTACAGTGG, 208 bp) (Ref. 8) and a hitherto-unknown gene (1472A: ATGCATCCCCCT-GTAAGAG, 1472B: GGTTGGAGGTGGCAGATA, 165 bp) (data not shown) have been identified, suggesting that it works well, at least for short PCR products.

In conclusion, we have presented a simple, inexpensive and apparently robust PCR-based technique to screen individual YAC clones directly from microplates or cell suspension, thus bypassing the time-consuming culturing of yeast, generation of spheroplasts and unnecessary DNA

extractions. Cultures of individual YACs can be established simultaneously during the screening process, and when cultures are adequately dense, DNA can be extracted from positive clones for further characterization or use.

Acknowledgements

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Products Used

dNTP: dNTP from PE Applied Biosystems
dNTP: dNTP from Pharmacia
dNTP: dNTP from Promega Corporation
dNTP: dNTP from Boehringer Mannheim
AmpliTaq DNA polymerase: AmpliTaq DNA polymerase from PE Applied Biosystems
Taq polymerase: Taq polymerase from Boehringer Mannheim
AmpliTaq: AmpliTaq from PE Applied Biosystems
Thermoseal Foil: Thermoseal Foil from Advanced Biotechnologies
PTC-225 DNA Engine Tetrad thermocycler: PTC-225 DNA Engine Tetrad thermocycler from MJ Research Inc