Rapid identification of yeast artificial chromosome clones by matrix pooling and crude lysate PCR

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Yeast artificial chromosome libraries are extremely valuable for isolating large fragments of genomic DNA (1). One such library, which has been used extensively for screening purposes (2), has been made available to our laboratory and several others to maximize its use. This library, representing a fivefold coverage of the human genome, is currently being screened by a combination of PCR and hybridization protocols (3). Yeast colonies are grown on single filters in 24×16 gridded arrays. DNAs from master pools of five filters are screened, followed by screening of five single filter pools for each positive master pool. Finally, individual positives are identified by colony hybridization (3). This final step of colony hybridization is often difficult and time-consuming. We have devised a simple and rapid method for screening single filters by PCR of matrix pools, circumventing the need for colony hybridizations.

Three copies of a single filter array known to contain a target YAC were grown as described earlier (3). The first filter was cut into 12 columns, each containing two lanes of colonies. The second filter was cut into 8 rows, each containing two lanes of colonies. Strips were cut with a scalpel blade and placed into 1.5 ml microfuge tubes using sterile forceps. To each tube was added 0.5 ml AHC culture medium [40 mM ammonium sulfate, 110 μM adenine hemisulfate, 0.17% (w/v) yeast nitrogen base without amino acids, 1% casamino acids (w/v), pH 5.8]. Tubes were vortexed for several seconds and 10 μl was removed to a fresh tube; the remainder was frozen in glycerol for future screenings. To this was added 60 μl lysis solution [1 U/μl Lyticase (Sigma), 0.45% (v/v) Tween 20, 0.45% (v/v) NP-40, 50 mM KCl, 10 mM Tris-Cl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin]. Samples were vortexed briefly, incubated at 37°C for 60 min, then at 100°C for 10 min. Following this, samples were centrifuged 2 min in a microcentrifuge, and 2 μl of the supernatant was removed for PCR. PCR reactions were performed in 25 μl volumes containing 50 mM KCl, 10 mM Tris-Cl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 1 μM each oligonucleotide primer, 250 μM each dNTP, and 1 unit Taq polymerase. Amplification consisted of one denaturation step at 94°C for 4 min followed by 35 cycles, each 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. After amplification, 24 μl of the PCR product was analyzed by agarose gel electrophoresis.

As an example, the data in figure 1A indicate that a positive colony lies at the intersection of column 5 and row 4. The four colonies at this position were picked from the third filter with a pipette tip (approximate volume 1 μl) and each washed into 60 μl lysis solution and screened as described above. Figure 1B demonstrates the single positive identified among these four colonies.

The PCR process has been performed successfully on pooled lysates of rows and columns from 4 filters by conceptually grouping the four filters in a single rectangle. The rectangle of four filters then includes 24 columns down and 16 rows across with two lanes of colonies in each column or row. Thus all four filters can be screened using 40 (24 + 16) PCR reactions. Screening a single filter requires 20 (12 + 8) PCR reactions.

The method described above circumvents the need for colony hybridizations in screening YAC libraries, and introduces a method for PCR of crude yeast lysates that avoids tedious and time-consuming DNA extractions. This method for complete identification of individual clones by PCR may also be more amenable to automation.

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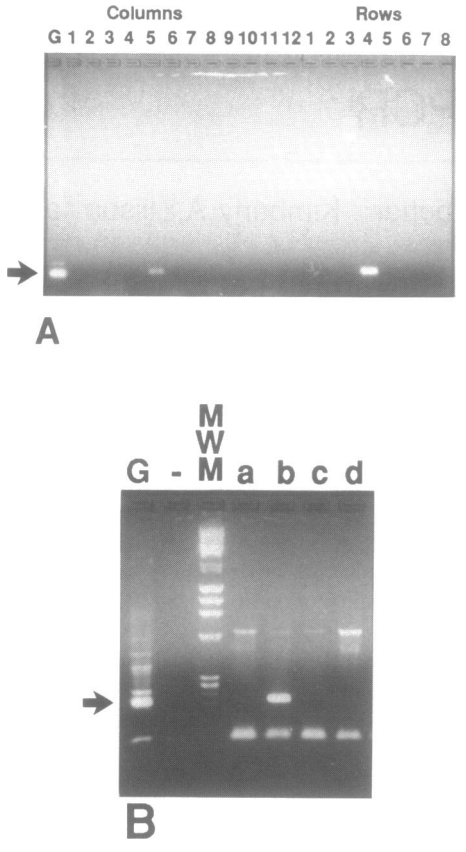

Figure 1. Agarose gel electrophoresis of PCR products. A = products of columns and rows of yeast colonies from a single filter. B = products of the four individual yeast colonies (a–d) at the intersection of column 5 and row 4. 'G' = genomic DNA (40 ng) '–' = no DNA. Arrows indicate the expected size of PCR product.