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Yeast colony size reflects YAC copy number

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ABSTRACT

A novel strategy for separation of co-cloned YACs was developed. For this, yeast cells were grown under non-selective conditions to allow the mitotic loss of multiple YACs. Yeast colonies of different size appear on 'drop-out' selection plates with small clones consistently containing a single-copy YAC. Different auxotrophic marker genes can be used to separate co-cloned YACs or reduce their copy number, which is essential for most YAC-modification procedures.

Multiple yeast artificial chromosomes (YACs) can co-exist in the same yeast cell due to co-cloning or amplification of an originally single-copy YAC. YAC amplification can be explained by inefficient transcription from the promoter of the *TRP*1 gene in pYAC vectors, which in Trp⁻Ura⁻ 'drop-out' medium can lead to selection of cells with two or more YACs (1). However, we have observed amplification of YACs even when selection did not involve omission of tryptophan. Thus, it seems that the copy number of YACs can increase in selective medium regardless of the auxotrophic marker used for selection.

The presence of several YACs, co-cloned or amplified, in one cell can lead to difficulties in YAC manipulation, such as modification, fragmentation, deletion or recombination between two YACs (2,3). Several approaches can be used to reduce the YAC copy number per yeast cell: (i) purification of the YAC DNA and re-transformation into yeast (4); (ii) traditional genetic cross with spore colony analysis after mating and meiosis (5); (iii) retrofitting one of the co-existing YACs to disrupt or replace the *URA*3 gene on the YAC arm with a different auxotrophic marker. Subsequent selection using 5-fluoro-orotic acid (5FOA) results in the loss of the unmodified YAC, but maintains the retrofitted YAC (2); (iv) *Kar*1-mating, where only one chromosome is usually transferred by cytoduction (6). Approach (i) frequently leads to undesired recombinations during re-transformation of the purified YAC DNA. Method (ii) was shown to be associated with YAC instability (2). Whilst methods (iii) and (iv) give reliable results, retrofitting and *kar*1 mating are time-consuming and the latter protocol also involves changing the genotype of the host.

Here we show that the size of yeast colonies growing on selection 'drop-out' plates reflects the YAC copy number, with large colonies containing consistently multiple YACs (Fig. 1). By picking colonies of different size and verifying their YAC content by PFGE and Southern hybridisation we were able to reliably select small colonies with a single-copy YAC. The identification

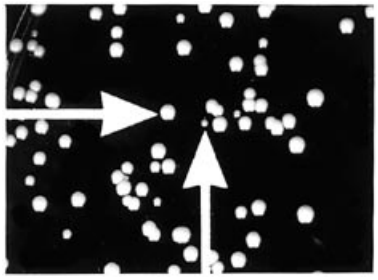

Figure 1. YAC-containing yeast colonies after 4 days growth on an Ade⁻ 'drop-out' medium plate.

was carried out first on a previously described clone, containing a 320 kb YAC, with the core region of the human kappa light chain locus (1). Attempts to introduce defined mutations into this YAC were hampered by the finding that the YAC was present in two or more copies per yeast cell. This YAC was obtained from a library constructed in pYAC4. For our experiments the acentric *URA*3-containing YAC arm was replaced by site-specific integration with an *ADE*2-containing YAC arm and the unmodified YAC was 'discarded' by selection in 5-FOA (7). However, after growth in medium lacking adenine, we found that the cells again contained more than one copy of the *ADE*2-targeted YAC. In order to allow mitotic loss of extra YAC copies, individual clones were picked from selective medium plates (Ade⁻Ura⁺Trp⁺His⁺Lys⁺Ile⁺Thr⁺) and grown in 10 ml YPD rich medium (8) until stationary phase. This procedure was repeated with 50 µl of the resulting culture inoculated into 10 ml YPD. After overnight growth, serial dilutions were made in water and spread on selection Ade⁻ 'drop-out' plates (see above). From the plate shown in Figure 1 five large and five small colonies were picked, grown in 5 ml YPD and their DNA was analysed by PFGE and Southern hybridisation (Fig. 2). The different intensities of the YAC bands are clearly visible on both the ethidium bromide-stained gel (upper panel) and the autoradiogram after hybridisation with a YAC-specific probe (lower panel).

In order to test the general applicability of this method, yeast clones containing different YACs with various auxotrophic marker genes were used. As an example, clone cosCλ/pRAN4 (3) is shown. This clone contained two YACs: 120 and 130 kb, one with *URA*3 and *TRP*1 marker genes, and the other with *URA*3 gene disrupted with an *ADE*2-containing vector [pRAN4 (9)]. A single colony was picked from Ade⁻Ura⁻Trp⁻ plates, cells were

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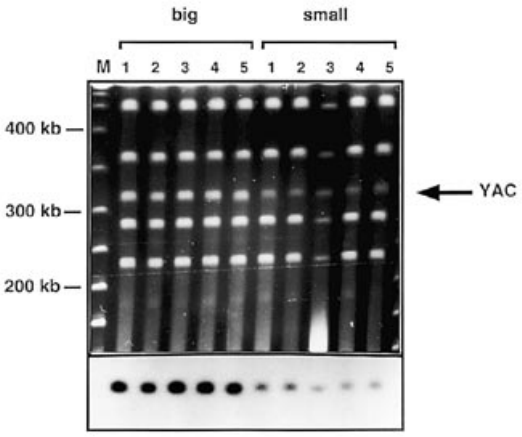

Figure 2. PFGE (upper panel) and Southern blot (lower panel) hybridisation of randomly picked colonies from the Ade⁻ agar plate shown in Figure 1 (left, five big clones; right, five small clones). Yeast cells (1.6×10^7) were used to prepare one 120 µl agarose block and half of the block was loaded onto the gel. The blot was hybridised with a YAC-specific probe [human Ig Cκ probe (1)]. The amount of DNA in lane 3 (small colony) was decreased due to insufficient spheroplasting. Lane M contains λ ladder (New England Biolabs).

passaged in YPD as above and selection of large and small colonies was performed on plates lacking tryptophan ($Trp^-Ura^+Ade^+His^+Lys^+Ile^+Thr^+$). The results, presented in Figure 3 show that not only was the hybridisation signal from individual YACs weaker in cells obtained from small colonies, but also that the separation of different YACs was achieved.

Thus, culturing YAC-containing yeast under non-selective conditions with subsequent screening for cells with different growth rates on 'drop-out' medium plates allows the reliable isolation of clones with reduced YAC copy number. We speculate that under selective conditions, cells containing more than one YAC copy with an essential auxotrophic marker gene, divide faster and subsequently form bigger colonies. In summary, sequential growth and colony size selection make it possible to separate co-cloned YACs which simplifies many yeast manipulation procedures.

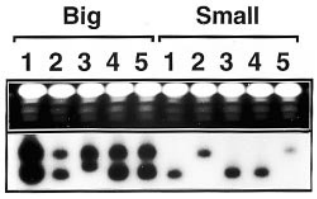

Figure 3. PFGE (upper panel) and Southern hybridisation (lower panel) analysis of DNA from five large and five small colonies after selection on Trp^- 'drop-out' plates. The right, *URA*3-containing arm of this 120 kb YAC was modified by pRAN4 (9), resulting in the appearence of two YACs of different sizes. The left, *TRP*1-containing arm on both YACs remained unchanged. The blot was hybridised with a YAC-specific probe [human Ig Cλ3 (3)].

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REFERENCES

1 Davies,N.P., Rosewell,I. and Brüggemann,M. (1992) *Nucleic Acids Res.*, **20**, 2693–2698.
2 Heikoop,C., Steensma,Y., van Ommen,G.-J.B. and den Dunnen,J.T. (1994) *Trends Genet.*, **10**, 40.
3 Popov,A.V., Bützler,C., Frippiat,J.-P., Lefranc,M.-P. and Brüggemann,M. (1996) *Gene*, **177**, 195–201.
4 Connelly,C., McCormick,M.K., Shero,J. and Hieter,P. (1991) *Genomics*, **10**, 10–16.
5 Green,E.D and Olson,M.V. (1990) *Science*, **250**, 94–98.
6 Spencer,F., Hugerat,Y., Simchen,G., Hurko,O., Connelly,C. and Hieter,P. (1994) *Genomics*, **22**, 118–126.
7 Boeke,J.D., LaCroute,F. and Fink,G.R. (1984) *Mol. Gen. Genet.*, **197**, 345–346.
8 Markie,D. (1996) *Methods Mol. Biol.*, **54**, 359–371.
9 Markie,D., Ragoussis,J., Senger,G. Rowan,A., Sansom,D., Trowsdale,J., Sheer,D. and Bodmer,W.F. (1993) *Somat. Cell Mol. Genet.*, **19**, 161–169.