High resolution restriction mapping of YACs using chromosome fragmentation
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The large insert size of yeast artificial chromosomes (YACs; 1) has made them ideal for the analysis of complex genomes. YACs can be restriction mapped with rare cutting enzymes but this does not provide the resolution required to investigate gene structure (such as locating exons and introns). Detailed mapping normally requires sub-cloning into cosmid or phage vectors. Here we describe a method which generates high resolution restriction maps of YACs without the need for sub-cloning. Chromosome fragmentation (2) was used to produce a set of nested deletion mutants of a 200 kb YAC. Partial digestion with EcoRI and indirect end-labelling was used to map the end of each deletion mutant. The overlapping restriction maps were then aligned to produce a high resolution map of the YAC.

A 200 kb telomeric YAC clone (yIgH6; 3) containing a YAC vector arm at one end (pTYAC1; 4) and a human telomere repeat sequence at the other was isolated by complementation of telomere function in yeast strain AB1380 (MATa ade2-1 can1-100 lys2-1 trp1 ura3-52 his5). Deletion mutants were created using the Alu fragmentation vectors, BP108 and BP109 (2). Use of these vectors requires the YAC to be in a his3 background. yIgH6 in strain AB1380 was therefore mated with YPH857 (MATα ade2-101 lys2-801 trp1-D63 ura3-52 his3-Δ200 leu2-Δ1 cyh2 R; ref. 2). Diploids were identified by histidine auxotrophy, sporulated and random spores were recovered and germinated on rich medium (5). Clones were analysed by replica plating on omission media and by test crosses to determine the genotype. From ~60 germinated spores, one was found to be of the desired genotype.

This clone was transformed with 1 µg of either SalI digested BP108 or SalI digested BP109 using a lithium acetate transformation procedure (6), and transformants were identified by histidine auxotrophy. With conventional YAC clones (made in pYAC4; 1) recombination with BP108 or BP109 results in replacement of the URA3 containing vector arm by the HIS3 arm of the fragmentation vector. Replica plating of HIS3 transformants onto medium lacking uracil can therefore be used to identify clones in which a deletion has occurred. However, the YAC clone yIgH6 (constructed using pTYAC1) has a vector arm at one end and a human telomere repeat sequence at the other. Transformation with BP108 and BP109 results

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in deletion of the human telomere repeat end without loss of a selectable marker and hence deletion mutants could not be identified by replica plating. Instead, 36 HIS<sup>+</sup> transformants were analysed directly by pulsed-field gel electrophoresis and Southern hybridisation resulting in the isolation of three different length deletion mutants (yd75, yd95 and yd165; Fig. 1). To isolate additional deletion mutants, 250 transformants were combined into 18 pools (12–15 clones/pool) and these pools were analysed by pulsed-field gel electrophoresis and Southern hybridisation (data not shown). Clones from three of these pools were then analysed individually resulting in the isolation of six additional deletion mutants (Fig. 1). One of these clones contains YACs of both 28 and 200 kb; hybridisation with the HIS3 probe specific for the fragmentation vector demonstrates that the 28 kb YAC arose by fragmentation but that the 200 kb YAC was wild-type ylgH6 (Fig. 1B). This clone probably results from the transformation of a cell containing two copies of ylgH6, only one of which was fragmented.

A map of ylgH6 was produced by restriction mapping the individual deletion mutants using partial digestion and indirect end-labelling with a fragmentation vector-specific probe. DNA from each deletion mutant (5–10 µg) was partially digested with 0.1 U EcoRI for 10 min at 37°C and digestion stopped by heat inactivation. Samples were electrophoresed on an Autobase field inversion gel electrophoresis apparatus (Q-life systems Inc., Ontario, Canada) using a preprogrammed setting for separation in the range 8–80 kb. Southern blotting and hybridisation with the HIS3 probe (Fig. 2) reveals a ladder of bands corresponding to the EcoRI sites in the deletion mutants (the clone containing both yd28 and ylgH6 was useful in this experiment since only yd28 has the HIS3 gene). Restriction mapping the 30–50 kb adjacent to the fragmentation vector arm of each deletion mutant produced a set of overlapping restriction maps. These were aligned to produce a restriction map of the 200 kb YAC (Fig. 3). In this example, 10–15 kb at the human telomeric end of the clone could not be mapped due to the absence of a suitable probe in this region. However, probes can be derived from both vector arms of conventional YACs allowing restriction mapping of the entire insert.

The method described here enables the construction of restriction maps at a resolution similar to that obtained using cosmid subclones of the YAC. The method is not limited by the length of the YAC, only by the frequency of the deletions and the resolution of the electrophoresis system used. Furthermore, two or more markers which fall into a single deletion interval (as produced by fragmentation) can be ordered according to the size of their respective restriction fragments (5). The most laborious part of the technique described here is the transfer of ylgH6 from a his5 strain to a his3 strain. However, strains have now been developed which remove the requirement for sporulation enabling YACs to be transferred to a his3 strain in a fraction of the time (7). The availability of fragmentation vectors for mouse YACs (8), together with new yeast strains and vectors which remove the need for sporulation (7,9) make this method widely applicable.

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REFERENCES