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# Construction of a human chromosome 4 YAC pool and analysis of artificial chromosome stability

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## ABSTRACT

**In order to construct a human chromosome 4-specific YAC library, we have utilized pYAC4 and a mouse/human hybrid cell line HA(4)A in which the only human chromosome present is chromosome 4. From this cell line, approximately 8Mb of chromosome 4 have been cloned. The library includes 65 human-specific clones that range in size from 30kb to 290kb, the average size being 108kb. In order to optimize the manipulation of YAC libraries, we have begun to investigate the stability of YACs containing human DNA in yeast cells; these studies will also determine if there are intrinsic differences in the properties of chromosomes containing higher eukaryotic DNAs. We are examining two kinds of stability: 1] mitotic stability, the ability of the YAC to replicate and segregate properly during mitosis, and 2] structural stability, the tendency of the YAC to rearrange. We have found that the majority of YACs examined are one to two orders of magnitude less stable than authentic yeast chromosomes. Interestingly, the largest YAC analyzed displayed a loss rate typical for natural yeast chromosomes. Our results also suggest that increasing the length of an artificial chromosome improves its mitotic stability. One YAC that showed a very high frequency of rearrangement by mitotic recombination proved to be a mouse/human chimera. In contrast to studies using total human DNA, the frequency of chimeras (i.e., mouse/human) in the YAC pool appeared to be low.**

## INTRODUCTION

Yeast artificial chromosomes (YACs) enable the cloning of fragments up to 1Mb in size (1). Insert sizes of this scale greatly assist efforts to map the human genome as these cloning vectors are able to bridge the gap between the resolution of genetic linkage and physical mapping technology. Chromosome-specific YAC libraries have special utility for human gene mapping projects as a more specific source of new polymorphic markers, as well as starting material for chromosome walking experiments to bridge gaps between known genetic and physical markers. YACs should meet several requirements to maximize their use in mapping the human genome. First, there should be relatively little bias with respect to the types of sequences present in the YAC. YAC clones should exhibit mitotic stability, replicating and segregating with fidelity during mitosis. Finally, YAC clones must faithfully represent their genomic origin; this requires that they be structurally stable in the yeast host. YACs have already been used successfully for characterizing regions of the human genome corresponding to the gene involved in cystic fibrosis (2), Duchenne muscular dystrophy (3), and the fragile X syndrome (4).

The mitotic stability of YACs containing human chromosome 4 DNA may be compared to authentic yeast chromosomes by analysis of the rate of loss of the chromosome. Natural chromosomes in *Saccharomyces cerevisiae* are replicated and segregated with high fidelity, with loss rates of only $10^{-4}$ to $10^{-5}$ per chromosome per generation (5–7).

The fidelity of chromosome transmission in *S. cerevisiae* is dependent upon at least three DNA components: autonomous replicating sequences (ARS), centromeres (CEN), and telomeres (TEL). Circular plasmids containing only the ARS component are mitotically unstable and present in many copies per cell (8,9). The insertion of one CEN into an ARS plasmid stabilizes the plasmid and maintains its copy number at one per cell (10,11). Circular centromeric plasmids 3–140kb in length are lost at a rate of $10^{-2}$ to $10^{-3}$ per mitotic division (10,12,13). The cloning of telomeric sequences (14) has allowed the construction of linear artificial chromosomes (12,15). Linear artificial chromosomes containing all three components (ARS, CEN, TEL) required for proper chromosome segregation have been examined for their stability in mitosis. Results of these studies suggest that a fundamental determinant of mitotic stability is the length of linear DNA molecules; as the length of the linear chromosome increases (up to 137kb), the mitotic stability improves (12,13,16). In these studies the length of the artificial chromosome was modulated by the addition of phage lambda or yeast DNA.

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However, even the most stable of these artificial chromosome studied (137kb) was lost at a rate of $1.5\times10^{-3}$ per generation (13); this is one to two orders of magnitude less stable than an authentic yeast chromosome. The mitotic stability of artificial chromosomes greater than 150kb in size has not been thoroughly investigated.

Successful application of YACs for mapping the human genome is dependent on their structural stability as well as their mitotic stability. Given the abundance of repetitive elements in human DNA (17) and the ability of yeast to promote homologous recombination (18), YACs with human inserts may undergo intramolecular recombination resulting in rearranged clones. The manipulation and transformation of large fragments of human DNA might also induce strand damage which could stimulate repair processes leading to rearrangements in the transformed YAC molecule. In one study, a 1050kb YAC containing human DNA decreased in size when transformed into yeast (19). This clone retained both vector telomeric regions, but restriction enzyme digestion analysis indicated that the clone underwent internal deletions after being transformed into yeast. In addition, studies of the behavior of YACs containing tandem repeats from the human Y chromosome indicated that the majority of clones examined had restriction enzyme digestion patterns which either did not directly correspond to genomic DNA or were too unstable to allow a simple comparison (20).

We report here the construction of a pool of 65 YACs containing DNA obtained from a mouse/human microcell hybrid specific for human chromosome 4 (21). Chimeric mouse/human clones were present in the pool at a frequency of 15%. The mitotic stability of twelve clones was measured, and a correlation between size and stability was demonstrated. Measurement of the structural stability of these clones indicated that most of them had relatively low frequencies of at least one type of rearrangement.

## MATERIALS AND METHODS

### Strains, vectors, and media

The yeast strain used to transform the YACs was AB1380: *MATa, ade2-1, can1-100, lys2-1, trp1, ura3-1, his5, ile, gal, psi*$^+$. The cloning vector pYAC4 has been described (1). YAC B22F9 was provided by B.Weiffenbach (Collaborative Research Inc., Waltham, MA).

All media used in these experiments have been described in detail (22,23).

### Construction of the YAC library

In order to create a human chromosome 4 YAC library, we first isolated large DNA from a mouse/human hybrid cell line HA(4)A (21). A large size class of the DNA prepared from the cell line was selected from a sucrose gradient and was partially digested with *Eco*RI (BRL). The vector pYAC4 (1) was digested with *Bam*HI (BRL) to remove the *HIS2* region between the telomeres and was then completely digested with *Eco*RI and treated with calf intestinal phosphatase (Boehringer Mannheim) to prevent reclosure of the vector arms. The vector arms and large *Eco*RI-digested DNA were ligated overnight at 16°C, and the ligation products were transformed into competent AB1380 yeast cells via spheroplast transformation (24). The transformants were plated in selective regeneration top agarose. After five days, the transformants were repicked to small patches on medium lacking uracil or both uracil and tryptophan and grown for three days.

To screen for clones containing human DNA, patches were transferred to Zetabind nylon membranes, and cells lysed according to a variation of the patch lysis protocol by Tschumper and Carbon (25). The membranes were prehybridized approximately four hours at 65°C in 5×SSPE, 5×Denhardt's (50×= 1% BSA, 1% Ficoll, 1% PVP), 0.2% SDS, 2.5% sodium dextran sulfate, and 200μg sheared, sonicated and denatured salmon sperm DNA. Sheared, heat denatured, $^{32}$P-labeled total genomic human DNA was added to the prehybridization solution. After hybridization at 65°C overnight, the filters were washed four times with the final wash in 0.1×SSC, 0.1% SDS at 60°C. for 30 min. Filters were then exposed to Kodak XAR-5 film at −70°C. for several days. Clones that appeared to be positive were repicked as large patches to medium lacking tryptophan and uracil and retested by the hybridization protocol just described.

Clones hybridizing to human DNA were streaked onto medium lacking uracil, and a single colony was used to innoculate a culture for pulse field gel analysis. Agarose plugs were made by resuspending the pellet from a 3 ml culture with 0.2 ml SCE (1M sorbitol, 0.1M sodium citrate, 0.06M EDTA) containing 100mM β-mercaptoethanol (β-ME) and 100 U/ml zymolyase (100T, ICN Immunobiologicals). Each suspension was then mixed with 0.2 ml 1.5% LMP InCert agarose (FMC) in 0.5×TBE at 45°C and pipetted into a plug-forming mold. After cooling, sample plugs were individually incubated at 37°C overnight with 4 ml SCE containing 70mM β-ME. The SCE was then replaced with lysis solution (0.45M EDTA, 10mM Tris, 1% sodium N-lauroylsarcosinate, 0.5mg/ml proteinase K, pH 8.9), and the plugs were incubated overnight at 50°C to lyse the cells. Pulse field gels were run in 0.75% SeaKem agarose in the Bio-Rad CHEF apparatus at a 60 sec. pulse time for 14 hours followed by a 90 sec. pulse time for 8 hours. The gel was blotted to a GeneScreen Plus filter and hybridized with total human DNA as described above. YAC sizes were determined by comparison with the YAC HY-1 (1).

### Measuring the frequency of chimeras in the pool

To investigate the frequency of mouse/human chimeras in our pool, DNA was isolated from 65 yeast clones containing the YACs (23). After heat-denaturation, 4 μg of each YAC-containing yeast DNA were applied to a GeneScreen Plus membrane. In addition, the following controls were applied to the membrane: mouse DNA (25ng, 12 ng, and 5 ng), human DNA (150 ng, 50 ng), and AB1380 yeast DNA (2 μg). The DNA was crosslinked to the filter by exposure to 260 nm UV light and then hybridized with $^{32}$P-labeled total mouse DNA using conditions described above.

### Measuring the rate of artificial chromosome loss

Five independent cultures of each YAC-containing clone were started at a concentration of 20 cells/ml and were grown in nonselective medium (YPD) until saturation (approximately 23 generations). Various dilutions of the cultures were plated onto 3 types of media: COM medium (a complete array of auxotrophic requirements), URA medium (COM lacking uracil), and 5-FOA (5-fluoroorotic acid) medium (on which only uracil auxotrophs can grow) (26). Since one of the YAC vector arms contains a *URA3* marker, one would expect that a colony growing in the presence of 5-FOA contains cells that have either lost or mutated the *URA3* gene.

To calculate the frequency of loss of YACs, we divided the number of Ura$^-$ colonies (corrected for the number of Ura$^-$

Table 1. Mitotic and structural stability of YACs containing human chromosome 4 DNA.

| YAC | Size (kb) | From 5-FOA medium[a]: Rate $Ura^-$ [c] ($\times 10^{-4}$) | Fraction $Trp^+Ura^-$ [d] | Corrected rate[e] ($\times 10^{-4}$) | From complete medium[b]: Fraction $Trp^+Ura^-$ [f] | Fraction $Trp^-Ura^+$ [g] |
|---|---|---|---|---|---|---|
| IC4Y21 | 55 | 340 ± 130 | 0/161 | 340 | 0/434 | 1/434 |
| IC4Y26 | 75 | 530 ± 150 | 0/678 | 530 | 0/1080 | 0/1080 |
| IC4Y2 | 90 | 63 ± 37 | 1/409 | 63 | 1/524 | 0/524 |
| IC4Y27[h] | 102 | 150 ± 110 | 0/250 | 150 | 1/250 | 0/250 |
| IC4Y49 | 135 | 100 ± 84 | 0/250 | 100 | 0/222 | 0/222 |
| IC4Y47 | 147 | 28 ± 73 | 1/619 | 28 | 1/674 | 0/674 |
| IC4Y65 | 160 | 2.8 ± 4 | 8/286 | 2.8 | 0/665 | 0/665 |
| IC4Y12 | 195 | 32 ± 19 | 0/250 | 32 | 0/250 | 0/250 |
| IC4Y8 | 233 | 23 ± 18 | 20/759 | 22 | 1/961 | 0/961 |
| IC4Y11 | 257 | 39 ± 26 | 3/250 | 39 | 0/250 | 0/250 |
| IC4Y44 | 290 | 34 ± 17 | 1/250 | 34 | 1/250 | 0/250 |
| IC4Y42[h] | 290 | 200 ± 180 | 127/300 | 153 | 23/150 | 0/150 |

[a]YAC strains grown nonselectively to saturation were serially diluted and plated onto medium containing 5-FOA.

[b]YAC strains grown nonselectively to saturation were serially diluted and plated onto complete medium.

[c]Rates are the geometric mean of five independent cultures.

[d]Colonies from 5-FOA medium were replica plated to medium lacking tryptophan to determine the fraction of cells that retained the TRP1 gene.

[e]The rate of URA3 loss was corrected for the percentage of $Ura^-$ colonies that did not lose the entire YAC. These corrections only affected IC4Y8 and IC4Y42.

[f]Nonselectively grown cells containing YACs were analyzed for loss of URA3 gene function by replica plating colonies to medium lacking uracil.

[g]Nonselectively grown cells containing YACs were analyzed for loss of TRP1 gene function by replica plating colonies to medium lacking tryptophan.

[h]Chimeric mouse/human YAC.

$Trp^+$ colonies, see below) by the total number of colonies (colonies growing on COM medium). Rates were calculated from the frequencies according to Drake (27).

### Measuring the frequency of rearrangements involving the *URA3* gene

Two types of $Ura^-$ cells were examined. First, $Ura^-$ colonies were detected by replica plating colonies on COM to medium lacking uracil. Second, Ura-colonies were directly isolated by spreading cells grown nonselectively onto medium containing 5-FOA. The proportion of $Trp^+$ and $Trp^-$ colonies in the population of $Ura^-$ colonies was then determined. The frequency of $Trp^-$ $Ura^-$ colonies was interpreted as a measure of the complete loss of the YAC, whereas the frequency of $Trp^+$ $Ura^-$ was interpreted as a measure of a rearrangement occurring between the *URA3* arm of the vector and the insert. In some cases, $Trp^+$ $Ura^-$ and $Trp^-$ $Ura^-$ colonies were analyzed for the presence of YAC vector DNA (pBR322) or human DNA by hybridization to labeled probes.

## RESULTS

### Isolation of YACs containing human chromosome 4 DNA

As part of an effort to clone human chromosome 4 into YACs, we have used a mouse/human hybrid cell line HA(4)A. This cell line contains a full complement of mouse chromosomes, but the only human chromosome present is a chromosome 4 which contains a neomycin resistance gene (21). The total human DNA probe used to screen the YAC library detects human repetitive sequences in the YAC inserts. Simple repetitive elements, such as the short interspersed repeated element Alu, are abundantly dispersed in the genome, appearing as frequently as every 10kb (28). Given the abundance and diversity of repeated sequences,

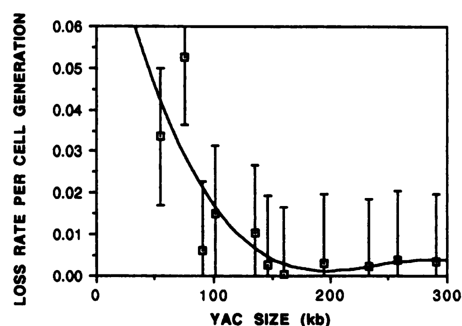


**Figure 1.** Size versus mitotic stability of YAC clones. Plot of the size of each YAC vs. mitotic stability; the smooth curve is best fit polynomial of power 3. [$y=9.34\times10^{-2} - 1.19\times10^{-3}s + 4.97\times10^{-6}s^2 - 6.62\times10^{-9}s^3$, $R^2=0.69$; y=loss rate, s=size in kb].

we would expect that greater than 99% of YACs larger than 50kb should hybridize to labeled total human DNA. Using the methods described, we isolated 65 YACs containing human chromosome 4 DNA. The clones derived from the mouse/human hybrid cell line vary in size from 30kb to 290kb with the average size being 108kb. In order to isolate these 65 YACs, 6058 transformants were screened. Therefore, the frequency of human clones detected in the pool is 1.07%.

To determine whether any known chromosome 4-specific sequences were present in the YAC pool, nitrocellulose filters containing patches of each YAC strain were consecutively hybridized with the following radioactively-labeled chromosome 4 probes: ADH3 (4q21-23), and D4S119, F11, D4S187, D4S163 and D4S139 (all at 4q34-35). Hybridization of IC4Y45 with D4S119 allowed the mapping of this 56kb YAC to 4q34-4q35.

### Frequency of mouse/human chimeras in the YAC pool

The frequency of mouse/human chimeras in our pool was analyzed by hybridization to radioactively labeled total mouse DNA. 10 of the 65 clones analyzed contain mouse DNA, indicating that the frequency of chimeras in the pool is at least 15% (see Discussion).

### Mitotic stability of YACs improves with increased size

The stability of YACs in mitosis was determined using an assay that measures the rate at which the YAC is lost (see Materials and Methods). The frequency of loss of a YAC may be measured as the ratio of the number of $Ura^-$ colonies (i.e., colonies growing in the presence of 5-FOA) to the total number of colonies (i.e., colonies growing on COM), corrected for YACs which lost only the *URA3*-containing arm and remained $Trp^+$ (Table 1). The rates of loss of twelve YACs were calculated from the observed frequencies (27) (Table 1, Figure 1). In the YACs analyzed, rates of loss per cell per generation range from $5.3\times10^{-2}$ to $2.8\times10^{-4}$. We have also analyzed a 360kb YAC containing human chromosome 4 DNA that was generously provided by B.Weiffenbach (see Materials and Methods). Although the 360kb clone appears to have a lower rate of loss ($1.4\times10^{-4}$ loss events per cell per generation) than the most stable YAC constructed here (IC4Y65, loss rate = $2.8\times10^{-4}$), the difference is not significant at the 95% confidence level by the Student T test.

### Structural stability of YACs in the pool

The specific rearrangement we monitored involves the loss of the *URA3* gene located on the right arm of the YAC vector. This event was easily detected because it confers resistance to 5-FOA (see Materials and Methods). The same cultures used to determine the frequency of YAC loss were utilized to measure this specific rearrangement (i.e., loss of the *URA3* gene and retention of the *TRP1* gene). Two types of experiments were performed: 1)total cells were examined for the presence of $Trp^+$ $Ura^-$ cells (as well as $Trp^-$ $Ura^+$ cells), and 2)$Ura^-$ cells (selected on medium containing 5-FOA) were tested to determine if they were $Trp^+$ (Table 1). One may infer that $Trp^-$ $Ura^-$ cells have lost the YAC completely; hybridization of four $Trp^-$ $Ura^-$ colonies from each of twelve YACs confirmed that none contained pBR322 DNA. We conclude that such cells have completely lost the YAC. In contrast, $Trp^+$ $Ura^-$ cells should have retained part of the YAC and, therefore, represent one type of rearrangement. Hybridization of a total of 40 $Trp^+$ $Ura^-$ colonies (originally isolated on 5-FOA) from the twelve YACs confirmed that these cells contain pBR322 DNA. These data indicate that the frequency of rearrangements leading to the loss of the *URA3* arm is, in general, quite low (Table 1). Exceptions include the YACs IC4Y8, IC4Y42, and IC4Y65.

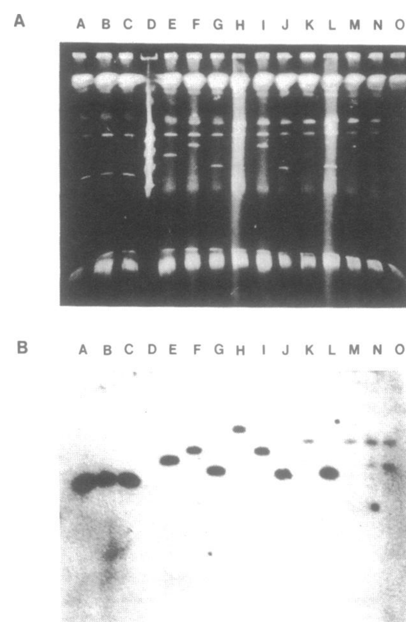


**Figure 2.** CHEF gel analysis of chimeric IC4Y42 $Trp^+Ura^-$ colonies. A) Four independent IC4Y42 cultures were grown nonselectively, and $Trp^+Ura^-$ colonies were analyzed from each culture. Lanes A,B,C—colonies from culture 1; Lane E—colony from culture 2; Lanes F,G,I,J—colonies from culture 3; Lanes K,M,N,O—colonies from culture 4. CONTROLS: Lane D—lambda ladder; Lane H—original IC4Y42 (290kb); Lane L—YAC HY-1 (130kb). B) Southern blot of CHEF gel. Probe was $^{32}P$-labeled total human DNA. Lanes are as described for Panel A.

### Structural instability of IC4Y42

Examination of the structural stability of several YACs in the pool revealed that IC4Y42, a mouse/human chimera, frequently appeared to lose all or a portion of the *URA3* vector arm (Table1). CHEF gel analysis of several independent $Trp^+$ $Ura^-$ IC4Y42 derivatives demonstrated that several different size classes exist (Figure 2A). Interestingly, the sizes of the rearranged YACs from independent cultures were different. Even within a single culture it was possible to observe YACs of more than one size. This suggests that, if specific sequences were stimulating the rearrangements, there were several such sequences present in the clone. Southern analysis of the rearranged IC4Y42 clones indicated that all retained some human DNA (Figure 2B). Rehybridization of the same blot with radioactively labeled total mouse DNA indicated that both the original IC4Y42 clone and its rearranged products contained mouse DNA (data not shown).

To determine which sequences in the *URA3* vector arm of IC4Y42 were deleted in the rearranged products, a blot containing the same DNAs as the blot shown in Figure 2 was consecutively rehybridized with two DNA fragments in the *URA3* pYAC4 vector arm. The location of these DNA fragments is diagrammed in Figure 3. Hybridization of the blot with the 774bp *AvaI-SalI* pYAC4 fragment revealed that only the original, unrearranged IC4Y42 and HY-1, a positive control (1), contained this DNA sequence (Figure 4A). However, hybridization with the 1kb

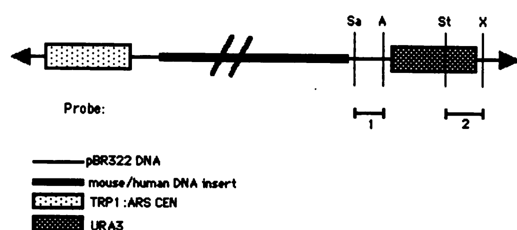


**Figure 3.** Schematic representation of IC4Y42. The left vector arm contains *TRP1*, ARS and CEN, and the right vector arm contains the *URA3* gene. Telomeres are represented by filled-in triangles, and the mouse/human DNA insert is depicted by a thick line. Thin lines represent pBR322 DNA. The bars below the right vector arm indicate the fragments of DNA used as probes in Figure 4. This diagram is not to scale. Restriction enzyme abbreviations: Sa-*Sal*I, A-*Ava*I, St-*Stu*I, X-*Xho*I.

*Xho*I-*Stu*I pYAC4 fragment indicated that the IC4Y42 deletion derivatives contained at least part of this sequence (Figure 4B). These hybridization analyses limited the possible sequences in the *URA3* vector arm which were recombining with the mouse/human insert.

## DISCUSSION

### Mouse/human chimeras

The hybrid cell line HA(4)A contains a 30:1 ratio of mouse DNA to human DNA. Therefore, one would expect that if clones were formed randomly, approximately 3% of the clones isolated from a transformation would contain human DNA. The YACs containing human DNA arose at a frequency of 1.07%. The difference between the observed and expected number of human clones is highly significant (Chi-square = 96.1, $p<.005$). This difference may be due to the relatively small average size of the human clones (108kb) resulting in a failure to detect them due to lack of repeated sequences.

High frequencies of chimeric inserts in YACs have been found in YAC pools derived from total human DNA. For example, 50% of the clones from the Center for Genetics in Medicine at Washington University School of Medicine YAC library are chimeras (29). On the contrary, hybridization of a filter containing 50 human-specific YACs isolated from a hamster-human cell line revealed that none of these clones were hamster/human chimeras (30).

Two sources of chimeric YACs (i.e., YACs containing two noncontiguous genomic DNA sequences) have been proposed. First, chimeric molecules might result from ligation of two DNA molecules prior to transformation into yeast. Second, as has been proposed by Green and Olson (29), they could arise from recombination events occurring between regions of homology in two different molecules after transformation. If chimeras occur by random coligation of DNA fragments, a human fragment would have a 30-fold greater probability of interacting with a mouse fragment than with another human fragment (there is 30 times more mouse DNA than human DNA in the hybrid cell). If chimeras in our library formed at the 50% rate observed in the Washington University library (29), then one would have expected approximately 50% of the human clones to also contain mouse DNA. If, on the other hand, most chimeras arise from recombination in the yeast cell, one would predict that mouse/human chimeras would occur at a lower frequency than chimeras in YAC libraries derived solely from human DNA, since mouse DNA is not completely homologous to human DNA.

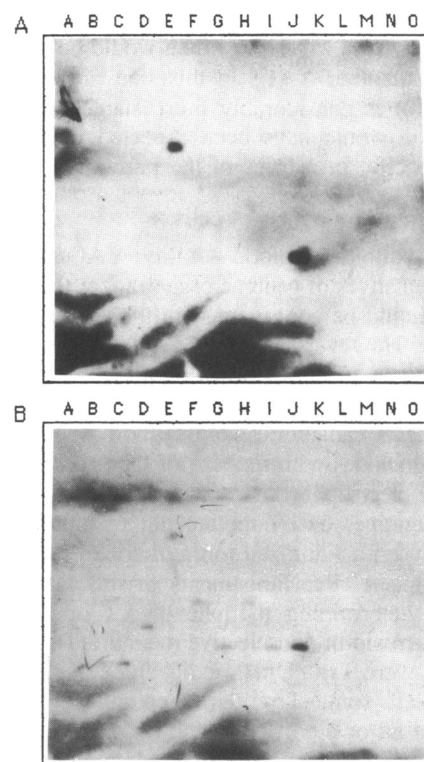


**Figure 4.** Southern analysis of chimeric IC4Y42 $Trp^+$ $Ura^-$ deletion derivatives. Four independent IC4Y42 cultures were grown nonselectively, and $Trp^+$ $Ura^-$ colonies were analyzed from each culture by Southern blotting. Lanes A,B,C—colonies from culture 1; Lane D—colony from culture 2; Lanes F,G,H,I—colonies from culture 3; Lanes K,L,M,N—colonies from culture 4. Controls: Lane E—original IC4Y42 (290kb); Lane J—HY-1 (130kb). A) Probe was $^{32}$P-labeled 774bp *Ava*I-*Sal*I fragment of pYAC4. B) Probe was $^{32}$P-labeled 1kb *Xho*I-*Stu*I fragment of pYAC4.

Our data indicate that 15% (10/65) of the YACs are detectable mouse/human chimeras; this is significantly different (Chi-square = 31.15, $p<.005$) than the 50% expectation under the hypothesis of coligation. This suggests that *in vivo* recombination makes a significant contribution to chimeric YACs, consistent with the Green and Olson hypothesis. Of course, it is possible that other YACs in our library contain mouse DNA that was not detectable due to lack of repeated sequences. We note, however, that mouse/human chimeras may be less likely to cause problems in mapping the human genome as it is possible to distinguish (and may be possible to separate) mouse from human DNA at a later stage.

### Correlation between size and mitotic stability of YACs

For YACs to be an optimal tool in cloning and mapping the human genome, they should be able to properly replicate and segregate in mitosis. Our results display a relationship between the size of an artificial chromosome and its mitotic stability (Figure 1). The mitotic stability of a YAC containing human chromosome 4 DNA improves as the size of the clone increases. This trend is seen up to 175kb; clones ranging in size from 175 to 290kb appear to have similar loss rates. The 360kb YAC B22F9 is the most stable of all, suggesting that additional stability may be acquired as the size increases beyond 300kb. However, it will be necessary to analyze more clones in this size range to verify this hypothesis. Two clones do display loss rates that are

different than expected for their size. IC4Y42 (290kb) is less stable (rate of loss = $1.5\times10^{-2}$) than would be anticipated by comparison with other YACs in this size range. In contrast, IC4Y65 (160kb) is considerably more stable (rate of loss = $2.8\times10^{-4}$) than would have been expected. These variations may reflect specific properties of the particular YAC.

**Rearrangements among $Ura^-$ cells**

In addition to exhibiting mitotic stability, YACs should also be faithful representatives of genome organization; the human DNA in the YAC should be congruent with the sequence that exists in the genome. However, *S.cerevisiae*, the host for the YACs, is capable of recombining homologous repeated sequences resulting in inversions and deletions (18).

Rearrangements can be detected by comparing the sizes of YACs from subclones with the size of the original YAC using pulse-field gel electrophoresis, comparing the YAC restriction map to the genome, or by monitoring recombination events occurring between a site distal to the markers in the YAC and a site in the insert. Rearrangements of this latter type were examined by determining the phenotypes of $Ura^-$ colonies isolated after growth in nonselective medium (Table1). Most of these colonies were $Trp^-$ $Ura^-$, indicating complete loss of the YAC. However, some $Trp^+$ $Ura^-$ colonies were observed, suggesting that all or a portion of the *URA3* gene was deleted. Only two of the nonchimeric YACs containing human DNA (IC4Y8, IC4Y65) showed substantial frequencies of rearrangement of the *URA3*-containing arm (Table 1).

Overall there appears to be little correlation between loss rates and rearrangement rates. Interestingly, two of the most stable nonchimeric YACs with respect to mitotic segregation (IC4Y8 and IC4Y65) appeared to have the highest rearrangement frequencies (Table 1). If a correlation can be made, it appears to be an inverse relationship; more rearrangement events appear to occur in the YACs that are more mitotically stable. One interpretation is that due to their size, the larger YACs have a greater probability of containing sequences capable of being recombined. In fact, there have been numerous reports of gene deletions arising from recombination events involving Alu repeats (31–34). An alternative possibility is that the absolute rearrangement rates among the YAC clones studied may be similar. However, if rearrangements occurred at low frequency, they would be undetectable in clones which had high loss rates. That is, small YACs lost at high frequency might generate too many $Trp^-$ $Ura^-$ cells (from loss) for the $Trp^+$ $Ura^-$ rearranged cells to be detected. For example, if IC4Y26 (75kb) rearranged at the same absolute rate as IC4Y8 (233kb) (i.e., $20/759\times.0023$) one would expect to find only 1 $Trp^+$ $Ura^-$ colony per 886 $Ura^-$ cells (since the rate of loss of IC4Y26 is $5.3\times10^{-2}$).

One hypothesis for the events which lead to $Trp^+$ $Ura^-$ cells is that they reflect intrachromosomal recombination events occurring between sequences in the insert DNA and sequences distal to or within the *URA3* gene in the YAC vector arm. Such an event would delete part or all of the *URA3* gene and result in a shortened chromosome that is $Trp^+$ $Ura^-$. The only sequences distal to the *URA3* gene are 1.8kb of pBR322 DNA and the YAC telomere which contains *Tetrahymena* $C_4A_2$ and yeast $C_{1-3}A$ repeats (35, 36; Figure 3). A human sequence that is a candidate for recombining with the telomere repeats is the $(CA)_n$ repeat, repeated $5\times10^4$ times and found on average once every 30–60kb in the human haploid genome (37, 38).

In the one case (IC4Y42) where we have tested the hypothesis that human $(CA)_n$ repeats recombine with YAC telomeric sequences, the data suggest that this type of recombination event did not occur. All 12 of the rearranged $Trp^+$ $Ura^-$ clones derived from IC4Y42 contained pBR322 DNA located proximal to the telomere (see Figure 3). We conclude that, at least in the case of a YAC that has a very high frequency of rearrangement (23/150), human $(CA)_n$ sequences do not appear to be responsible for the rearrangement.

**Implications for genomic analysis**

These studies indicate that the rate of loss of YACs containing human DNA is high even for YACs as large as an authentic yeast chromosome. Selection for a single marker on one arm of the YAC may not be sufficient due to the possibility for rearrangement during growth. Clearly, even in the presence of selection for both arms, the possibility exists for internal rearrangements. We are in the process of constructing strains which will allow us to accurately measure the rates of such events.

In addition, our studies support the argument that use of a rodent/human hybrid cell line may be more advantageous than total human DNA as a source for generating chromosome-specific YAC pools. Most mouse/human chimeras isolated should be easily identified as both the mouse and human genomes contain unique collections of repetitive elements. To detect human/human chimeras, 26 of the YACs described here were analyzed by fluorescence *in situ* hybridization. This analysis indicates that none hybridize to more then one location on chromosome 4 (C.Wijmenga, personal communication).

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