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Reduced occurrence of chimeric YACs in recombination-deficient hosts

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Yeast artificial chromosomes (YACs) with inserts averaging hundreds of kilobases in size have proven very useful for the construction of physical maps of large genomes (1). However, YACs are not ideal large-insert clones. For example, the frequent occurrence of chimeric inserts complicates the use of YACs for physical mapping, walking from insert ends, and determination of contiguous DNA sequence. Recombination within the yeast host strain between two YACs or YAC fragments has been suggested as a mechanism to account for the surprisingly high rates of chimerism (40–60%) in most genomic YAC libraries (2).

To determine if the genetic background of the yeast host strain contributes to the formation of chimeric YACs, the same YAC ligation mixture was introduced into three isogenic yeast hosts differing only in their recombination abilities. Strain CGY2872 (ATCC90436) carries a *LEU2* insertion at the *Bgl*II site of the *RAD52* gene, and strain CGY2897 (ATCC90437) carries the same insertion together with an insertion/replacement of the *Cla*I to *Stu*I region of the *RAD1* gene with *ADE2* in the parental host strain CGY2570 (ATCC90435) (genotype *MATa ura3–52 trp1-*Δ63 lys2-Δ202 his3-Δ200 ade2–1(oc) leu2-Δ1 ψ^+) (3). Defects in *RAD52* and *RAD1* were confirmed by measuring the loss of resistance to methyl methanesulfonate (MMS) and ultraviolet light (UV) respectively (3). Both genes appear to be implicated in mitotic recombination (4, 5). The two genes are in different epistatic groups, and mutations in both genes had been reported to exhibit additive or synergistic effects on several recombination-related properties (4).

To prepare YACs, human genomic DNA was partially digested with *Eco*RI and then ligated to YAC vector pCGS966 arms (6). DNA was size fractionated before and after ligation by preparative pulsed field gel electrophoresis (CHEF), selecting for fragments greater than 400 kb, and introduced into competent spheroplasts (6). YAC clones were selected for the presence of both the *URA3* and *TRP1* arms. CGY2872 transformed about 4-fold, and CGY2897 about 8-fold, less efficiently than did the isogenic Rad$^+$ parent CGY2570.

CHEF gel Southern blots of resulting colony-purified YACs were probed with human DNA to determine if multiple YACs or YAC fragments were present in the same cell. The frequency of chimeric YACs was measured by fluorescence *in situ* hybridization (FISH) of YACs to human premetaphase spreads. YACs that hybridized to more than one location were assumed to be chimeric. Probes for FISH were prepared by random-priming (BRL kit) a slice of an agarose block DNA preparation, or in the event of multiple YACs per cell, a pasteur pipet stab from one YAC band on a CHEF gel. YACs were chosen randomly for FISH except that care was taken to select a few from each of the two size ranges shown in Table 1.

The results of FISH analysis of YACs from all three host strains are shown in Table 1. Fewer chimeric YACs were found in the Rad$^-$ hosts (8 of 76 examined, or 10%) than were found in the isogenic Rad$^+$ parent CGY2570 (17 of 36 examined, or 47%). These values are statistically significant ($P < 0.001$, df = 1). The frequency of chimeric YACs in the Rad$^+$ parent is similar to that reported previously for YACs in Rad$^+$ hosts (eg., 43% by Selleri *et al.* (7) based on FISH analysis; 52% by Bates *et al.* (8) based on mapping insert-end STSs). There was no significant difference between the two Rad$^-$ hosts (4 out of 29 examined, or 14% chimerism in the rad1rad52 host vs. 4 out of 47, or 9% in the rad52 host), suggesting that RAD52 is the more important determinant in chimerism. Neil *et al.* (9) have previously shown that RAD1 also has no significant effect on YAC insert stability while mutations in RAD52 are effective in some cases.

The effect of YAC size on the frequency of chimerism was also examined. YACs of 360 to 450 kb ('average sized YACs' resulting from the gel size selection used and the fact that smaller YACs transform yeast more efficiently) and YACs of >450 kb ('large YACs') were considered separately. In both size ranges, the chimera frequency of YACs in the Rad$^-$ strains remained significantly lower than that of YACs in the Rad$^+$ parent, although the difference was less dramatic in the large YAC category. This is consistent with the observation of Selleri *et al.* (7) that larger YACs are more frequently chimeric than are smaller YACs, and may reflect the fact that many large YACs result from recombination of small YACs or YAC fragments within the yeast host.

We also found that chimeric YACs in Rad$^+$ hosts frequently consisted of DNA segments from more than two different genomic locations. For example, 9 of the 17 chimeric YACs in the Rad$^+$ background hybridized to three or more locations per YAC, whereas all 8 chimeric YACs in the Rad$^-$ hosts hybridized to only two locations each. These Rad$^-$ clones remained MMS sensitive, and thus, the chimerism of YACs in Rad$^-$ hosts is not a consequence of reversion of the Rad$^-$ phenotype, but may result from residual recombination or from co-ligation of non-contiguous fragments. Since the same YAC ligation mixture was used for all transformations, any chimeric regions due to pretransformation events should, on average, be

Table 1. Frequency of chimeric YACs

YAC Size (kb)	CGY2897 (rad1rad52) # Chimeric / # Tested	Percent Chimeric[a]	CGY2872 (rad52) # Chimeric / # Tested	Percent Chimeric[a]	CGY2570 (wild-type) # Chimeric / # Tested	Percent Chimeric
>450	4/12	33%	2/23	9%	12/20	60%
360–450	0/17	0%	2/24	8%	5/16	31%
Total (≥360)	4/29	14%	4/47	9%	17/36[b]	47%

[a]The significance level, determined by χ^2 analysis, of the difference in the chimera frequency observed in Rad$^-$ hosts compared to the wild-type host is $P < 0.01$, df = 1, except for CGY2897 YACs>450 kb ($P < 0.1$, df = 1) and for CGY2872 YACs 360–450 kb ($P < 0.02$, df = 1).
[b]9 of 17 samples hybridized to 3 or more chromosomal locations by FISH.

the same length in all three strains. Therefore, even if small non-contiguous fragments were undetected by FISH, these results still indicate that fewer chimeras were found in the Rad$^-$ hosts.

Both of these Rad$^-$ strains appear to be excellent hosts for preparing YAC libraries low in chimeric clones. However, CGY2897 may be preferable since it appears to have a significantly lower intraplasmid mitotic recombination frequency and transformation-associated recombination/deletion frequency as measured by the methods of Larionov *et al.* (10; and V.Larionov, personal communication). For these reasons, we are currently developing a human genome YAC library in this host.

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