Identification of a Compound Origin of Replication at the HMR-E Locus in Saccharomyces cerevisiae*

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Eukaryotic chromosomal origins of replication are best defined in Saccharomyces cerevisiae. Previous analysis of yeast origins suggests that they are relatively simple structures comprised of three or four small DNA sequence elements contained within approximately 100–200-bp minichromosome maintenance regions (Gilbert, D. M. (1998) Curr. Opin. Genet. Dev. 8, 194–199). In contrast, the sequence elements that may comprise origins in multicellular eukaryotes are largely unknown. The yeast HMR-E region is both a chromosomal origin of replication and a silencer that represses transcription of adjacent genes through a position effect. The analysis presented here indicated that HMR-E had a novel DNA structure that was more complex than defined for other yeast origins, and thus revealed that there is variation in the structural complexity of yeast origins. In contrast to “simple” yeast origins, the origin at HMR-E consisted of at least three independent subregions that had the capacity to initiate replication. We have termed HMR-E a compound origin to reflect its structural complexity. Furthermore, only one origin within the compound origin was a silencer.

The regulation of DNA replication ensures that duplication of eukaryotic genomes occurs once per cell cycle. Initiation of replication is a primary control point in the regulation of replication (1–3). The ORC (origin recognition complex), Mcm2–7p (minichromosome maintenance), and Cdc6p (cell division cycle) proteins, which were identified in Saccharomyces cerevisiae and are required for initiation of replication, are conserved among eukaryotes, suggesting that at least some of the underlying mechanisms of regulating initiation are conserved. However, other features of replication do not appear to be conserved; in particular, the DNA structure of origins appears to differ among eukaryotes (4).

Eukaryotic origins of replication are best characterized in S. cerevisiae. In S. cerevisiae it is possible to define the elements that comprise origins by combining techniques that map origins to specific chromosomal regions with site-directed mutagenesis of those regions. This type of analysis, in addition to analysis of plasmid origins (ARS elements; autononomous replicating sequence), has led to the model that yeast origins are relatively simple structures comprised of an “A” element and two to three “B” elements that direct initiation to a single site (5–11). The A element corresponds to the 11-base pair (bp) ACS (ARS consensaus sequence), which binds ORC (12, 13). Some B elements are binding sites for transcriptional activator proteins, whereas other B elements may correspond to regions of easily unwound DNA (11, 14–19). In yeast, ORC is bound to origins throughout the cell cycle and is required for the regulated assembly and disassembly of an Mcm2–7p/Cdc6p-containing complex that limits initiation to once per cell cycle (1–3).

Some features of yeast origins appear to be conserved among eukaryotes. In particular, in Schizosaccharomyces pombe, Drosoophila melanogaster, Sciara coprophila, Tetrahymena thermophila, and mammalian cell lines, sites of initiation map to defined chromosomal regions, suggesting that sequences within these regions are required for initiation of replication (20–31). In cases where it has been possible to dissect these regions genetically, specific sequences have been shown to be required for initiation. For example, at least four S. pombe ARS elements contain distinct DNA regions that, when deleted, result in loss of initiation (24, 32–35). Additionally, recent genetic dissection of one mammalian origin, the human β-globin origin, indicates that initiation is dependent upon particular sequences at this locus (20). Thus, a requirement for specific sequences is a conserved feature of at least some eukaryotic origins.

Other features of yeast origins do not appear to be conserved among eukaryotes. For instance, origins in S. cerevisiae are approximately 100–200 bp in length, whereas those in S. pombe are 500 bp to 2.0 kb in size, and some origins in multicellular eukaryotes are several kilobases in length (32, 35–37). In addition, unlike the single site of initiation at yeast origins, multiple sites of initiation have been identified at origins in multicellular eukaryotes (30, 38–41). The human β-globin locus, for example, contains two to four sites of initiation within an 8.0-kb region (20). Furthermore, studies of the DHFR locus identified two potentially preferred sites of initiation, and several additional sites of initiation spread throughout a 60-kb region (30, 39, 41). Collectively, these data suggest that, although the proteins that regulate initiation of replication are conserved, the structure and complexity of origins may vary among eukaryotes.

This study focused, in part, on the possibility that chromosomal origins in yeast may vary in complexity. In particular, we analyzed initiation of replication at the HMR silent mating-type locus of S. cerevisiae. The HMR-E silencer is a control region that is required for heterochromatin-mediated silencing of the mating-type genes at HMR. In addition to its role in gene

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) AF106619.

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‡ The abbreviations used are: bp, base pair(s); kb, kilobase pair(s); PCR, polymerase chain reaction.
silencing, an origin of replication maps to the HMR-E silencer. Furthermore, the ACS element of the silencer contributes to both silencing and initiation of chromosomal replication (42). Previous analysis also indicates that two distinct segments of HMR, each of which contains a different part of the HMR-E silencer, have ARS activity (43). Although only some ACS elements are origins in their native chromosomal context, this observation raises the possibility that HMR-E may be more complex than other yeast origins. In this report, we investigated whether HMR-E contained more than one origin. The work presented here revealed that HMR-E is a compound origin that contains three regions which can independently function as chromosomal and plasmid origins of replication. This result suggests that some S. cerevisiae origins are more complex than the ‘simple’ origins described previously. Furthermore, one of the three independent origins was a silencer, whereas the other two were not.

**EXPERIMENTAL PROCEDURES**

Construction of a Plasmid to Measure ARS Activity and Mitotic Stability—Prior analysis indicated that HMR-E had a property referred to as centromere antagonism, which can interfere with mitotic stability assays. Centromere antagonism is experimentally defined as the reduced mitotic stability of a plasmid containing both HMR-E and a centromere relative to one containing only HMR-E (44, 45). Although its molecular basis is not clear, we hypothesize that centromere antagonism may result from local action of the silencer interfering with centromere function. Previous plasmids used in mitotic stability assays contained HMR-E silencer sequences adjacent to centromere and marker sequences. In this study, a new test plasmid was constructed to minimize centromere antagonism, so that mitotic stability values would accurately reflect ARS activity. This plasmid, pDR1149, contained one polynucleotide for the insertion of potential ARS elements, and a second polynucleotide, on the opposite side of the plasmid, which contained URA3 and CEN6. This placement maximized the distance between ARS elements and CEN6. To determine whether pDR1149 was free of centromere antagonism, a 2.2-kb HMR-E fragment was inserted into the polynucleotide and its mitotic stability was determined. This plasmid was 3-fold more stable than a derivative that contained HMR-E, but not CEN6. Therefore, centromere antagonism was either minimized or eliminated in pDR1149.

The sequence of pDR1149 has been submitted to GenBank (AF106619) and, hence, is only briefly described here. The polynucleotide of pUC18 was replaced by the Bluescript SK+ polynucleotide, and a second polynucleotide, comprised of PmaI, PacI, AscI, and FseI restriction sites, was inserted opposite to it using the following oligonucleotides: 5'-GGCGCGCCTTTTGGTGAAGGCCTTCCCTCGCGCCGCG-3' and 5'-GGCGGGCCGCTTTGATAGCGTATCTTTAACCTAAACAAGGGG-3' inserted opposite to it using the following oligonucleotides: 5'-GGCGCGCCTTTTGGTGAAGGCCTTCCCTCGCGCCGCG-3'.

**RESULTS AND DISCUSSION**

Previous characterization of the DNA sequence elements that comprise the HMR-E origin and silencer focused primarily on the identification of the minimal set of elements required for silencing and for initiation of replication (42–45). Deletion of an 868-bp region of HMR, referred to here as HMR-E, removes both silencer and origin function (42–45). Replacement of HMR-E with a 138-bp subregion of HMR-E, containing a perfect match to the ACS and two transcription factor binding sites, restores silencing and initiation of replication (42). The 138-bp subregion can, therefore, restore silencing and for initiation of replication (42, 45). Deletion of an 868-bp region of HMR, referred to here as HMR-E, removes both silencer and origin function (42–45). Replacement of HMR-E with a 138-bp subregion of HMR-E, containing a perfect match to the ACS and two transcription factor binding sites, restores silencing and initiation of replication (42, 45). The 138-bp subregion can, therefore, restore silencer and origin function independent of other HMR-E regions.

In addition to the perfect 11/11 bp ACS at the 138-bp silencer, several 9/11 or 10/11 bp near-matches to the ACS reside in the vicinity of the silencer (44). Analysis of the 868-bp HMR-E region identified 27 9/11 or 10/11 bp near-matches to the ACS, including nine cases in which the near-matches overlapped by 5 or more base pairs (data not shown). This observation led us to question whether regions other than the 138-bp silencer contribute to initiation of replication of HMR-E. For this purpose, some of the near-matches used may be components of additional, unidentified origins within HMR-E. Alternatively, the near-matches may contribute to initiation at the previously identified 138-bp silencer, much as B elements contribute to initiation at other yeast origins. An additional possibility is that the near-matches may play no role in initiation.
To determine whether regions of HMR-E other than the 138-bp silencer contributed to initiation, HMR-E was divided into three subsections: the previously defined 138-bp silencer (HMR\_AE::138), the 520-bp region to the left of the 138-bp silencer, the L allele (HMR\_AE::L), and the 207-bp region to the right of the 138-bp silencer, the R allele (HMR\_AE::R). An additional construct included all of HMR-E except for the 138-bp silencer, the L+R allele (HMR\_AE::L+R). To explore the possible role of these subregions in replication, each was tested for the ability to support autonomous replication of a plasmid (ARS activity), as measured by high frequency transformation. Each HMR-E allele was flanked by approximately 1.3 kb of HMR DNA to avoid any potential influence from heterologous plasmid DNA. In this context, a plasmid that lacked HMR-E (pDR1155) was not capable of high frequency transformation, whereas one that contained the 138-bp silencer (pDR1161) was (Fig. 1A). These data indicated that the DNA flanking HMR-E did not contain an ARS element and confirmed previous observations that the 138-bp silencer is an ARS element. To determine whether HMR-E contained an ARS element in addition to the 138-bp silencer, the L+R allele (pDR1167) was tested for ARS activity. Similar to the 138-bp silencer, the L+R allele was capable of high frequency transformation, which revealed that HMR-E is comprised of at least two separable ARS elements. To map more precisely the additional ARS elements, the L allele (pDR1163) and the R allele (pDR1165) were tested for ARS activity independent of one another. Both alleles were capable of high frequency transformation (Fig. 1A). Thus, HMR-E contained at least three separable ARS elements.

Origins of replication in yeast exhibit a wide range of initiation efficiency, with some origins initiating replication in greater than 85% of cell cycles and others in less than 10% of cell cycles (54). To assess the relative initiation efficiency of the three HMR-E ARS elements, the mitotic stability of plasmids containing each of the individual alleles was determined. Wild-type HMR-E (pDR1159) was the most efficient origin (Fig. 1A). The 138-bp (pDR1161) and L+R alleles (pDR1167) were comparable to one another and were slightly less efficient than wild-type. The L allele (pDR1163) was less efficient than the 138-bp silencer, and the R allele (pDR1165) was the least efficient plasmid-based origin (Fig. 1A). Taken together, these results indicated that the three HMR-E ARS elements vary in their efficiency of initiation.

Not all ARS elements are origins of replication in their native chromosomal contexts (55, 56). To establish if any of the HMR-E subregions could function as chromosomal origins, each of the above HMR-E alleles was integrated into the chromosome in place of the wild-type HMR-E locus and subjected to two-dimensional gel electrophoresis method (50). The replication intermediates of genomic regions that contain an origin are bubble-shaped, whereas the replication intermediates of genomic regions that do not contain an origin are fork-shaped. Bubble- and fork-shaped replication intermediates each yield distinctive arc patterns when separated on two-dimensional agarose gels and subjected to DNA blot analysis. The presence of a bubble arc indicates that the chromosomal region examined contains an origin, whereas the absence of such an arc indicates that the chromosomal region does not contain an origin. As described above, yeast origins vary in the efficiency with which they initiate replication (54); consequently, regions that contain origins typically give rise to both bubble and fork arcs (50).

As reported previously, chromosome-derived restriction fragments from strains that contained either the wild-type HMR-E allele (DRY434) or the 138-bp silencer (DRY1285) gave rise to bubble arcs, confirming that both are chromosomal origins of replication (42) (Fig. 1B). Restriction fragments from strains that contained the L+R allele (DRY1293) also gave rise to a bubble arc, which indicated the presence of at least two independent chromosomal origins within HMR-E (Fig. 1B). Furthermore, analysis of the individual L (DRY1287) and R alleles (DRY1283) indicated that each gave rise to a bubble arc. These data revealed that HMR-E is composed of at least three subregions that could function independently as chromosomal origins of replication.

In each of the HMR-E alleles described above, the spacing among the various HMR-E subregions and the flanking DNA varied. To determine whether each of the three subregions of HMR-E was an origin when native spacing was maintained relative to each other and the flanking DNA, each subregion was replaced with DNA from the LYS2 gene (Fig. 2A). This DNA lacked any near-matches to the ACS and was typical of the AT/GC content of the yeast genome. Furthermore, the LYS2 DNA was derived from the coding region of the gene and was, thus, unlikely to contain any cryptic regulatory elements.

The HMR-E allele in which all three subregions were replaced with LYS2 (HMR\_LYS2\_A) DNA did not have ARS activity when inserted into a plasmid (pDR1198) (Fig. 2A). Similarly, chromosome-derived restriction fragments from strains that contained this allele integrated in place of the wild-type HMR-E locus (DRY1779) did not give rise to a bubble arc (Fig. 2B). These results indicated that the DNA used to maintain spacing did not function as an ARS element or a chromosomal origin. Both the 138-bp silencer (HMR\_LYS2\_A::138) and the L+R allele (HMR\_LYS2\_A::L+R) with native spacing were ARS elements when inserted into a plasmid (pDR1206 and pDR1202, respectively) (Fig. 2). Restriction fragments from strains containing either of these chromosomal alleles

| HMR-E sequences | HMR-E alleles | HFT | Mitotic Stability |
|-----------------|---------------|-----|------------------|
| vector          | -             | N/A |                  |
| wild-type HMR-E | +++           | 0.0488 |                |
| \(\Delta E\)     | -             | N/A |                  |
| \(\Delta E::138\)| +++           | 0.0635 |                |
| \(\Delta E::L-R\)| +++           | 0.0652 |                |
| \(\Delta E::L\)  | +++           | 0.0175 |                |
| \(\Delta E::R\)  | +++           | 0.2193 |                |

FIG. 1. Three subregions of HMR-E were ARS elements and chromosomal origins. A, HMR sequence diagrams represent the DNA present in each plasmid or chromosomal allele; lines represent flanking HMR DNA, open boxes the subregion(s) of HMR-E present and gaps the subregions absent. Plasmids analyzed were (top to bottom) pDR1149 (vector), pDR1159 (HMR-E), pDR1155 (\(\Delta E\)), pDR1161 (\(\Delta E::138\) bp silencer), pDR1167 (\(\Delta E::L-R\)), pDR1163 (\(\Delta E::L\)), and pDR1165 (\(\Delta E::R\)). High frequency transformation assays (HFT) were scored as ‘—’ for no transformants, each ‘+’ indicates approximately 1.0 \(\times\) 10^4 transformants/\mu g of DNA, and N/A is not applicable. Mitotic stability assays are as described under “Experimental Procedures.” B, two-dimensional gel analysis of replication intermediates from isogenic strains DRY 1285 (\(\Delta E::138\) bp silencer), DRY1293 (\(\Delta E::L-R\)), DRY1287 (\(\Delta E::L\)), and DRY1283 (\(\Delta E::R\)). The HMR-E fragments analyzed were 3995, 4557, 4373, and 4061 bp, respectively. Arrows indicate the presence of bubble arcs.

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was a relatively inefficient origin of replication B reproducibly gave rise to a weakly detectable bubble arc (Fig. 2).

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sequences present in each construct; lines represent flanking HMR DNA, open boxes the region(s) of HMR-E present, and solid boxes the subregion(s) substituted with LY52 DNA. Plasmids analyzed were (top to bottom): pDR1149 (vector), pDR1159 (HMR-E), pDR1198 (HMR-LYS2), pDR1206 (HMR-LYS2::138 bp silencer), pDR1202 (HMR(LYS2)::L+R), pDR1196 (HMR(LYS2)::L), and pDR1204 (HMR(LYS2)::R). High frequency transformation assays (HFT) were scored as “+” for no transformants, each “+” indicates approximately 1.0 × 10^4 transformants/μg of DNA, and N/A is not applicable. Mitotic stability values are as described under “Experimental Procedures.” B, 9-h exposures of blots of two-dimensional gel analysis of replication intermediates from isogenic strains DRY434 (wild-type HMR-E), DRY1779 (HMR(LYS2)), DRY1781 (HMR(LYS2)::138 bp silencer), DRY1783 (HMR(LYS2)::L+R), DRY1785 (HMR(LYS2)::L), and DRY1787 (HMR(LYS2)::R). All HMR-E fragments analyzed were approximately 4.7 kb. Arrows indicate the presence of bubble arcs. C, 19-h exposures of blots from strains DRY1779, DRY1785, and DRY1787 as shown in panel B above.

(DRY1781 and DRY1783, respectively) gave rise to bubble arcs. Hence, in the context of wild-type spacing, HMR-E was com- prised of at least two separable origins. Both the L (HMR(LYS2)::L) and R (HMR(LYS2)::R) plasmid-based alleles with native spacing also had ARS activity. In addition, chromoso- mical restriction fragments containing either of these two alleles gave rise to bubble arcs (DRY1785 and DRY1787, re- spectively) (Fig. 2). HMR-E, therefore, contained a minimum of three subregions that were independent origins of replication in two plasmid contexts as well as two chromosomal contexts.

The R allele (DRY1787) in which spacing was maintained reproducibly gave rise to a weakly detectable bubble arc (Fig. 2B and data not shown), which suggested that the R subregion contained an inefficient origin. This idea was supported by mitotic stability assays, which indicated that the R allele (pDR1204) was a relatively inefficient origin of replication when contained on a plasmid (Fig. 2A). Similarly, mitotic sta- bility assays of the L+R (pDR1202), 138-bp silencer (pDR1206), and L (pDR1196) alleles with native spacing indicated that each was a less efficient origin than wild-type HMR-E (pDR1159).

The observation that HMR-E contained three subregions that could initiate replication raised the possibility that HMR-E might also contain multiple subregions that could repress transcription. The ability of the three HMR-E subregions to function as silencers was assessed by a mating type assay. Haploid MATα cells in which HMRa is silent display the α mating phenotype, whereas haploid MATα cells in which HMRa is not silent display the non-mating phenotype. Among the series of chromosomal HMR-E alleles that either lacked or maintained wild-type spacing, only the alleles that contained the 138-bp silencer could repress transcription (Fig. 3 and data not shown). Thus, although the L and R subregions were origins, they were not silencers. The HMR-E compound origin, therefore, contained only one silencer element.

The results presented here revealed that HMR-E contained at least three contiguous subregions that could direct initiation of replication. The organization of this origin region contrasts with previously defined yeast origins, which have “simple” structures and span approximately 100–200 bp (7–11). We refer to HMR-E as a compound origin to reflect its relatively large size and structural complexity. Our data indicated that origins in yeast may be simple or compound, and thus, yeast origins may vary in structural complexity. In addition, this work established that only one origin within the compound origin has the unique properties required for gene silencing.

At least two models can account for the structural complexity of the compound origin. One model is that the compound HMR-E origin consists of a cluster of three or more autono- mous, simple origins. By this model, each autonomous origin is likely to “compete” with the others for initiation, similar to origins experimentally placed adjacent to one another (57, 58). At the other extreme, the compound origin at HMR-E could consist of a single complex origin rather than a collection of simple origins. By this model, the various subregions of HMR-E could interact, such that each contributes to the overall effi- ciency of initiation of that genomic region.
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REFERENCES

1. Diffley, J. F. (1996) Genes Dev. 10, 2819–2830
2. Dutta, A., and Bell, S. P. (1997) Annu. Rev. Cell Dev. Biol. 13, 283–332
3. Newlon, C. S. (1997) Cell 91, 717–720
4. Gilbert, D. M. (1998)Curr. Opin. Genet. Dev. 8, 194–199
5. Gerbi, S. A., and Blobel, G. (1998)Science 279, 95–98
6. Walker, S. S., Malik, A. K., and Eisenberg, S. (1991) Nucleic Acids Res. 19, 6255–6262
7. Marahrens, Y., and Stillman, B. (1992) Science 255, 817–823
8. Foss, M., McNally, F. J., Laurenson, P., and Rine, J. (1993) Science 262, 1838–1844
9. Schulter, D. E., Rine, J., and Kornberg, R. D. (1988) Nucleic Acids Res. 8, 210–225
10. Huq, Y., and Kowalski, D. (1996) Nucleic Acids Res. 24, 816–823
11. Schwert, D. E., and Kowalski, D. (1992) EMBO J. 11, 3375–3386
12. Natale, D. A., Umek, R. M., and Kowalski, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2654–2658
13. Natale, D. A., Umek, R. M., and Kowalski, D. (1993) Nucleic Acids Res. 21, 559–560
14. Tadajim, M. I., Rodenwald, L. W., Kolman, J. L., and Wahl, G. M. (1998) Science 281, 1095–1099
15. Clyne, R. K., and Kelly, T. J. (1995) EMBO J. 14, 6348–6357
16. Delidakis, C., and Kafatos, F. C. (1989) EMBO J. 8, 891–901
17. Dobbs, D. L., Shain, W. L., and Benbow, R. M. (1994) Nucleic Acids Res. 22, 2479–2491
18. Minkley, J. D., Zhu, J., Carlson, D. L., Sharma, K., and Huberman, J. A. (1994) EMBO J. 13, 3638–3647
19. Marahrens, Y., and Stillman, B. (1992) Nature 357, 126–134
20. Aladjem, M. I., Rodewald, L. W., Kolman, J. L., and Wahl, G. M. (1998) Science 281, 559–560
21. Tadajim, M. I., Rodenwald, L. W., Kolman, J. L., and Wahl, G. M. (1998) Science 281, 1095–1099
22. Clyne, R. K., and Kelly, T. J. (1995) EMBO J. 14, 6348–6357
23. Delidakis, C., and Kafatos, F. C. (1989) EMBO J. 8, 891-901
24. Dobbs, D. L., Shain, W. L., and Benbow, R. M. (1994) Nucleic Acids Res. 22, 2479–2491
25. Minkley, J. D., Zhu, J., Carlson, D. L., Sharma, K., and Huberman, J. A. (1994) EMBO J. 13, 3638–3647
26. Marahrens, Y., and Stillman, B. (1992) Nature 357, 126–134
27. James, C. D., and Leffak, M. (1986) Mol. Cell. Biol. 6, 976–984
28. Lewis, E. B., Kafer, J. D., Mathog, D. R., and Celniker, S. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8403–8407
29. Liang, C., Spitzer, J. D., Smith, H. S., and Gerbi, S. A. (1993) Genes Dev. 7, 1072–1084
30. Wang, S., Dijkwel, P. A., and Hamlin, J. L. (1998) Mol. Cell. Biol. 18, 39–50
31. Zhao, J., and Stillman, B. (1994) Mol. Cell. Biol. 14, 7643–7651
32. Theis, J., and Newlon, C. S. (1997) Mol. Cell. Biol. 17, 5473–5484
33. Bell, S. P., and Stillman, B. (1992) Nature 357, 126–134
34. Buchanan, A. R., Kimmerly, W. J., Rine, J., and Kornberg, R. D. (1988) Mol. Cell. Biol. 8, 210–225
35. Huq, Y., and Kowalski, D. (1996) Nucleic Acids Res. 24, 816–823
36. Buchman, A. R., Kimmerly, W. J., and Hamlin, J. L. (1990) Cell 61, 1075–1087
37. Rivier, D. H., and Rine, J. (1992) Science 256, 659–663
38. Brand, A. H., Micklem, G., and Nasmyth, K. (1987) Cell 51, 709–719
39. Kimmerly, W. J., and Rine, J. (1987) Mol. Cell. Biol. 7, 4225–4237
40. McNally, F. J., and Rine, J. (1993) Mol. Cell. Biol. 13, 5648–5659
41. Jones, J. S., and Prakash, L. (1990) Yeast 6, 363–366
42. Gietz, R. D., and Sugino, A. (1988) Gene (Amst.) 74, 527–534
43. Fox, C. A., Loo, S., Dillin, A., and Rine, J. (1995) Genes Dev. 9, 911–924
44. Fox, C. A., Loo, S., Dillin, A., and Rine, J. (1995) Genes Dev. 9, 911–924
45. Loo, S., Fox, C. A., Rine, J., Kobayashi, R., Stillman, B., and Bell, S. (1995) Mol. Cell. Biol. 6, 741–756
46. Brewer, B. J., and Fangman, W. L. (1987) Cell 51, 463–471
47. Ehrenhofer-Murray, A. E., Rivier, D. H., and Rine, J. (1997) Genetics 145, 923–934
48. Gietz, R. D., and Schiestl, R. H. (1991) Yeast 7, 253–263
49. Friedmann, K. L., Brewer, B. J., and Fangman, W. L. (1997) Genes Cells 2, 667–678
50. Dubey, D. D., Davis, L. R., Greenfeder, S. A., Ong, L. Y., Zhu, J. G., Broach, J. R., Newlon, C. S., and Huberman, J. A. (1991) Mol. Cell. Biol. 11, 5346–5355
51. Sherman, F. (1991) Methods Enzymol. 194, 3–21
52. Ehrenhofer-Murray, A. E., Rivier, D. H., and Rine, J. (1997) Genetics 145, 923–934
53. Gietz, R. D., and Schiestl, R. H. (1991) Yeast 7, 253–263
54. Friedman, K. L., Brewer, B. J., and Fangman, W. L. (1997) Genes Cells 2, 667–678
55. Dubey, D. D., Davis, L. R., Greenfeder, S. A., Ong, L. Y., Zhu, J. G., Broach, J. R., Newlon, C. S., and Huberman, J. A. (1991) Mol. Cell. Biol. 11, 5346–5355
56. Fangman, W. L., and Brewer, B. J. (1991) Annu. Rev. Cell Biol. 7, 375–402
57. Brewer, B. J., and Fangman, W. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3418–3422
58. Marahrens, Y., and Stillman, B. (1994) EMBO J. 13, 3395–3400
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