

**Table S1 : Plasmids used in this study.**

| Plasmid           | Description  | Source or Reference                 |
|-------------------|--|-------------------------------------|
| YIp5'5            | <i>CENV URA3</i>   | Ferguson <i>et al.</i> <sup>i</sup> |
| Yp-CN1            | YIp5'5 ChrIV: 456,853 → 472,955 <sup>a</sup> [ <i>ARS1</i> ACS: 462,593] <sup>a</sup>                  | This study                          |
| Yp-CN2            | YIp5'5 ChrX: 688,409 → 676,641 <sup>a</sup> [ <i>ARS121</i> ACS: 683,933] <sup>a</sup>                 | This study                          |
| Yp-CN3            | Yp-CN1 <i>ars1</i> (-1046 → 954) :: <i>ARS121</i> (-920 → 1080) <sup>b</sup>                           | This study                          |
| Yp-CN4            | Yp-CN2 <i>ars121</i> (-920 → 1080) :: <i>ARS1</i> (-1046 → 954) <sup>b</sup>                           | This study                          |
| Yp-CN5            | Yp-CN1 $\Delta$ <i>TRP1</i> promoter (-395 → -1) <sup>c</sup>  | This study                          |
| Yp-CN6            | Yp-CN2 <i>ars121</i> (-233 → 140) :: <i>ARS1</i> (-107 → 269) <sup>b</sup>                             | This study                          |
| Yp-CN7            | Yp-CN5 <i>ars1</i> (-107 → 269) :: <i>ARS121</i> (-233 → 140) <sup>b</sup>                             | This study                          |
| Yp-CN7B           | Yp-CN1 <i>ars1</i> (-107 → 269) :: <i>ARS121</i> (-233 → 140) <sup>b</sup>                             | This study                          |
| Yp-CN7C           | Yp-CN1 <i>ars1</i> (-107 → 269) :: <i>ARS121</i> (140 → -233) <sup>b</sup>                             | This study                          |
| Yp-CN8            | Yp-CN5 <i>ars1</i> (-86 → 18) :: <i>ARS121</i> (-86 → 18) <sup>b</sup>                                 | This study                          |
| Yp-CN9            | Yp-CN2 <i>ars121</i> (-86 → 18) :: <i>ARS1</i> (-86 → 18) <sup>b</sup>                                 | This study                          |
| Yp-CN10           | Yp-CN5 <i>ars1</i> <sub>A,B1</sub> (-32 → 18) :: <i>ARS121</i> <sub>A,B1</sub> (-32 → 18) <sup>b</sup> | This study                          |
| Yp-CN11           | Yp-CN2 <i>ars121</i> <sub>A,B1</sub> (-32 → 18) :: <i>ARS1</i> <sub>A,B1</sub> (-32 → 18) <sup>b</sup> | This study                          |
| Yp-CN12           | Yp-CN5 <i>ars1</i> <sub>B2</sub> (-86 → -27) :: <i>ARS121</i> <sub>ATR</sub> (-86 → -27) <sup>b</sup>  | This study                          |
| Yp-CN13           | Yp-CN2 <i>ars121</i> <sub>Mcm1 sites</sub> :: <i>ARS121</i> <sub>scrambled Mcm1 sites</sub>            | This study                          |
| Yp-CN14           | Yp-CN2 <i>ars121</i> <sub>'B2'</sub> :: <i>ARS121</i> <sub>inverted 'B2'</sub>                         | This study                          |
| YCp101            | <i>CENV LEU2 ARS1</i> (ChrIV: 462,352 → 463,193) <sup>a</sup>  | Reference 21                        |
| YCp121            | <i>CENV LEU2 ARS121</i> (ChrX: 685,748 → 679,017) <sup>a</sup>   | Reference 21                        |
| Yp- <i>ARS1</i>   | YIp5'5 ChrIV: 462,360 → 462,732 <sup>a</sup> [ <i>ARS1</i> (-107 → 269) <sup>b</sup> ]                 | This study                          |
| Yp- <i>ARS121</i> | YIp5'5 ChrX: 684036 → 683665 <sup>a</sup> [ <i>ARS121</i> (-233 → 140) <sup>b</sup> ]                  | This study                          |

<sup>a</sup> Chromosomal coordinates.

<sup>b</sup> Coordinates relative to the first base of the essential A element's A-rich strand.

<sup>c</sup> Coordinates relative to the *TRP1* ORF.

Additional reference:

- i. Ferguson, B.M., Brewer, B.J., Reynolds, A.E. and Fangman, W.L. (1991) A yeast origin of replication is activated late in S phase. *Cell*, **65**, 507-515.

**Table S2 : Yeast strains used in this study.**

| Strain  | Genotype  | Source or Reference                 |
|---------|---|-------------------------------------|
| 8534-8C | A364 <i>MAT<math>\alpha</math> his4<math>\Delta</math>34 ura3-52 leu2-3, -112</i>                   | Reference 21                        |
| M46-3C  | A364 <i>MAT<math>\alpha</math> leu2-3, 112 his3-11, -15 ura3-52 mcm2-1</i>                          | Reference 21                        |
| mcm1-1  | A364 <i>MAT<math>\alpha</math> ura3-52 leu2-3,-112 his3-11,-15 mcm1-1</i>                           | Reference 21                        |
| DBY2056 | S288C <i>MAT<math>\alpha</math> ura3-52 ade2-1 lys2-801 leu2-3,112</i>                              | Hennessy <i>et al.</i> <sup>i</sup> |
| DBY2028 | S288C <i>MAT<math>\alpha</math> mcm5-461</i>  | Hennessy <i>et al.</i> <sup>i</sup> |
| AY925   | W303 <i>MAT<math>\alpha</math> ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d2 Gal+</i> | Tanaka <i>et al.</i> <sup>ii</sup>  |
| orc2-1  | W303 <i>MAT<math>\alpha</math> orc2-1</i>   | Tanaka <i>et al.</i> <sup>ii</sup>  |
| orc5-1  | W303 <i>MAT<math>\alpha</math> orc5-1</i>   | Tanaka <i>et al.</i> <sup>ii</sup>  |
| cdc6-1  | W303 <i>MAT<math>\alpha</math> cdc6-1</i>   | Tanaka <i>et al.</i> <sup>ii</sup>  |
| dbf4-1  | W303 <i>MAT<math>\alpha</math> dbf4-1</i>   | Tanaka <i>et al.</i> <sup>ii</sup>  |
| cdc17-1 | W303 <i>MAT<math>\alpha</math> cdc17-1</i>  | Tanaka <i>et al.</i> <sup>ii</sup>  |
| pri2-1  | W303 <i>MAT<math>\alpha</math> pri2-1</i>   | Tanaka <i>et al.</i> <sup>ii</sup>  |

## Additional references:

- i. Hennessy, K. M., Clark, C. D., and Botstein, D. (1990) Subcellular localization of yeast CDC46 varies with the cell cycle. *Genes & Development*, **4**, 2252-2263.
- ii. Tanaka, T., and Nasmyth, K (1998) Association of RPA with chromosomal replication origins requires an Mcm protein, and is regulated by Rad53, and cyclin- and Dbf4-dependent kinases. *Embo J*, **17**, 5182-5191.

**Table S3 : Loss rates for *ARS1* and *ARS121* plasmids in various strains at a range of temperatures.**

| Strain          | Temperature <sup>2</sup> | Rate of plasmid loss <sup>1</sup> |            |
|-----------------|--------------------------|-----------------------------------|------------|
|                 |                          | Yp-CN1                            | Yp-CN2     |
| A364 wild-type  | 23.0                     | 0.0 ± 0.1                         | 0.0 ± 0.3  |
|                 | 30.0                     | 0.8 ± 0.6                         | 0.0 ± 0.6  |
| <i>mcm2-1</i>   | 23.0                     | 7.8 ± 0.7                         | 1.1 ± 0.0  |
|                 | 25.5                     | 8.9 ± 1.1                         | 0.7 ± 0.4  |
|                 | 27.5                     | 15.4 ± 0.4                        | 0.9 ± 0.2  |
|                 | 30.0                     | 26.4 ± 0.9                        | 3.5 ± 0.2  |
|                 | 32.5                     | 31.4 ± 1.4                        | 9.5 ± 0.9  |
|                 | <i>mcm1-1</i>            | 23.0                              | 6.7 ± 0.0  |
|                 | 30.0                     | 12.3 ± 0.9                        | 5.3 ± 0.4  |
| S288C wild-type | 23.0                     | 2.0 ± 0.7                         | 1.1 ± 0.1  |
|                 | 32.5                     | 0.4 ± 0.2                         | 0.0 ± 0.5  |
| <i>mcm5-461</i> | 23.0                     | 3.1 ± 1.8                         | 0.0 ± 2.0  |
|                 | 25.5                     | 4.1 ± 1.5                         | 0.0 ± 1.5  |
|                 | 27.5                     | 6.0 ± 1.9                         | 0.0 ± 1.7  |
|                 | 30.0                     | 13.5 ± 2.1                        | 2.8 ± 2.1  |
|                 | 32.5                     | 17.2 ± 1.9                        | 4.5 ± 2.5  |
| W303 wild-type  | 23.0                     | 0.2 ± 0.1                         | 0.0 ± 0.1  |
|                 | 30.0                     | 0.9 ± 0.1                         | 0.3 ± 0.1  |
| <i>orc2-1</i>   | 23.0                     | 10.0 ± 0.6                        | 0.2 ± 0.0  |
|                 | 30.0                     | 32.1 ± 1.1                        | 13.3 ± 0.4 |
| <i>orc5-1</i>   | 23.0                     | 13.3 ± 2.3                        | 3.7 ± 0.4  |
|                 | 30.0                     | 23.1 ± 1.9                        | 8.8 ± 0.8  |
| <i>cdc6-1</i>   | 23.0                     | 0.9 ± 0.4                         | 0.2 ± 0.4  |
|                 | 30.0                     | 27.7 ± 0.1                        | 9.1 ± 0.1  |
| <i>dbf4-1</i>   | 23.0                     | 1.0 ± 0.2                         | 0.3 ± 0.5  |
|                 | 30.0                     | 5.3 ± 0.1                         | 6.8 ± 0.9  |
| <i>pri2-1</i>   | 23.0                     | 1.6 ± 0.3                         | 1.6 ± 0.4  |
|                 | 30.0                     | 20.2 ± 0.9                        | 16.0 ± 0.4 |
| <i>cdc17-1</i>  | 23.0                     | 0.7 ± 0.0                         | 0.4 ± 0.1  |
|                 | 30.0                     | 4.7 ± 0.1                         | 4.7 ± 0.2  |

<sup>1</sup> % per generation.

<sup>2</sup> °C.

**Table S4 : Loss rates in wild-type cells for Yp-CN1 and Yp-CN2 derived chimaeric plasmids at 30 °C.**

| Plasmid | Rate of plasmid loss <sup>1</sup> |
|---------|-----------------------------------|
| Yp-CN1  | 0.8 ± 0.6                         |
| Yp-CN2  | 0.0 ± 0.6                         |
| Yp-CN3  | 1.2 ± 1.2                         |
| Yp-CN4  | 0.8 ± 0.3                         |
| Yp-CN5  | 0.1 ± 0.1                         |
| Yp-CN6  | 1.3 ± 0.8                         |
| Yp-CN7  | 0.2 ± 0.3                         |
| Yp-CN7B | 2.1 ± 0.7                         |
| Yp-CN7C | 1.8 ± 0.7                         |
| Yp-CN8  | 1.0 ± 0.0                         |
| Yp-CN9  | 0.4 ± 0.3                         |
| Yp-CN10 | 1.5 ± 0.1                         |
| Yp-CN11 | 0.7 ± 0.0                         |
| Yp-CN12 | 0.6 ± 0.0                         |

<sup>1</sup> % per generation.