

#### Appendix IVa: Time courses experimental conditions

| Experiment            | Strain   | Timepoints | Sample label | Reference   | Ref label | Media   | Temp (°C) | Sample OD | Array batch number |
|-----------------------|--|------------|--------------|---|-----------|---------|-----------|-----------|--------------------|
| Elutriation I         | 972 h-   | 20         | Cy3          | Asynchronous 972 h-                                     | Cy5       | EMM     | 30        | 0.65      | 137 & 140          |
| Elutriation II        | 972 h-   | 20         | Cy5          | Asynchronous 972 h                                      | Cy3       | EMM     | 30        | 0.24      | 228 & 232          |
| Elutriation III       | 972 h-   | 20         | Cy5          | Asynchronous 972 h                                      | Cy3       | EMM     | 30        | 0.15      | 489                |
| cdc25 b&r I           | <i>cdc25-22 h-</i>   | 18         | Cy3          | Asynchronous <i>cdc25-22 h-</i>                         | Cy5       | YE      | 25        | 0.34      | 40 & 70            |
| cdc25 b&r I dye swap  | <i>cdc25-22 h-</i>   | 18         | Cy5          | Asynchronous <i>cdc25-22 h-</i>                         | Cy3       | YE      | 25        | 0.34      | 40 & 70            |
| cdc25 b&r II          | <i>cdc25-22 h-</i>   | 19         | Cy5          | Asynchronous <i>cdc25-22 h-</i>                         | Cy3       | EMM     | 25        | 0.19      | 292                |
| cdc25 elu + b&r       | <i>cdc25-22 h-</i>   | 22         | Cy5          | Asynchronous <i>cdc25-22 h-</i>                         | Cy3       | EMM     | 25        | 0.22      | 226, 228 & 232     |
| cdc10 elu + b&r       | <i>cdc10-129 h-</i>  | 22         | Cy5          | Asynchronous <i>cdc10-129 h-</i>                        | Cy3       | EMM     | 25        | 0.30      | 331 & 334          |
| cdc10::ura4           | <i>cdc10::ura4 leu1-32 ura4-D18 ade6-M210 + pREP1-HA-res1(l-192)</i> | 3          | Cy5          | Untreated cdc10::ura4                                   | Cy3       | EMM ade | 30        | 0.2       | 477                |
| cig1Δ cig2Δ puc1Δ elu | <i>cig1::ura4 cig2::ura4 puc1::ura4</i>                              | 6          | Cy5          | Asynchronous <i>cig1::ura4 cig2::ura4 puc1::ura4 h-</i> | Cy3       | EMM     | 30        | 0.15      | 477                |
| HU b&r                | 972 h-   | 4          | Cy3          | Untreated 972 h-  | Cy5       | EMM     | 32        | 0.08      | 489                |
| nda3                  | <i>nda3-KM311 h-</i>   | 3          | Cy5          | Untreated nda3  | Cy3       | EMM     |           | 0.23      | 477                |
| sep1 b&r              | <i>cdc25-22 sep1::ura4 h-</i>  | 20         | Cy5          | <i>cdc25-22 sep1::ura4 h-</i>                           | Cy3       | EMM     | 25        | 0.12      | 331 & 334          |
| wt b&r                | 972 h-   | 18         | Cy3          | 972 h-  | Cy3       | EMM     | 25        | 0.15      | 782 & 784          |

0.5 OD = 1 X 10<sup>7</sup>

## Appendix IVb: Mutant strains experimental conditions

| Experiment   | Strain   | Sample label | Reference          | Ref label | Media               | Temp (°C) | Sample OD | Array batch number |
|--------------|--|--------------|--------------------|-----------|---------------------|-----------|-----------|--------------------|
| ace2 #1      | <i>ace2Δ::kanMX6 ade6-M21?h-</i>                                 | Cy5          | 972 h-             | Cy3       | EMM ade             | 32        | 0.22      | 489-48             |
| ace2 #2      |  | Cy3          |                    | Cy5       |                     |           | 0.23      | 489-7              |
| ace2 #3      |  | Cy5          |                    | Cy3       |                     |           | 0.23      | 489-14             |
| ace2 #4      |  | Cy5          |                    | Cy3       |                     |           | 0.24      | 489-12             |
| ace2 #5      |  | Cy5          |                    | Cy3       |                     |           | 0.33      | 668-2              |
| ace2 sep1 #1 | <i>ace2Δ::kanMX6 sep1::ura4 ade6-M21? leu1-32</i>                | Cy5          | 972 h-             | Cy3       | EMM ura ade leu     | 30        | 0.26      | 668-9              |
| ace2 sep1 #2 |  | Cy5          |                    | Cy3       |                     |           | 0.32      | 668-10             |
| atf1 #1      | <i>atf1Δ::ura4 ura4-D18 h-</i>                                   | Cy5          | 972 h-             | Cy3       | YE                  | 25        | 0.18      | 596-21             |
| atf1 #2      |  | Cy5          |                    | Cy3       |                     |           | 0.21      | 599-2              |
| cdc10-C4 #1  | <i>cdc10-C4 leu1-32 h+</i>                                       | Cy5          | 972 h <sup>+</sup> | Cy3       | EMM                 | 25        | 0.23      | 489-10             |
| cdc10-C4 #2  |  | Cy5          |                    | Cy3       |                     |           | 0.24      | 489-11             |
| cdc10-C4 #3  |  | Cy3          |                    | Cy5       |                     |           | 0.23      | 334-19             |
| cdc10-C4 #4  |  | Cy5          |                    | Cy3       |                     |           | 0.24      | 334-17             |
| fhl1 #1      | <i>SPAC8C9.01Δ::kanMX6 h90</i>                                   | Cy3          | 972 h-             | Cy5       | EMM                 | 25        | 0.23      | 334-22             |
| fhl1 #2      |  | Cy5          |                    | Cy3       |                     |           | 0.23      | 334-11             |
| fhl1 #3      |  | Cy5          |                    | Cy3       |                     |           | 0.22      | 668-41             |
| fkh2 #1      | <i>SPBC16G5.15c::ura4 leu1-32 ura4-D18 ade6-M21? his7-366 h-</i> | Cy5          | 972 h-             | Cy3       | YE                  | 30        | 0.20      | 560-42             |
| fkh2 #2      |  | Cy5          |                    | Cy3       |                     |           | 0.20      | 560-45             |
| mbx1 #1      | <i>mbx1::kanMX6 leu1-32 ura4-D18 ade6-M210 his7-366 h-</i>       | Cy5          | 972 h-             | Cy3       | EMM leu ura ade his | 25        | 0.23      | 334-18             |
| mbx1 #2      |  | Cy3          |                    | Cy5       |                     |           | 0.22      | 334-20             |

| Experiment   | Strain   | Sample label | Reference                | Ref label | Media               | Temp (°C) | Sample OD | Array batch number |
|--------------|--|--------------|--------------------------|-----------|---------------------|-----------|-----------|--------------------|
| meu3 #1      | <i>meu3::kanMX6 ade6-M21?h-</i>                            | Cy5          | 972 h-                   | Cy3       | EMM ade             | 32        | 0.23      | 489-49             |
| meu3 #2      |  | Cy3          |                          | Cy5       |                     |           | 0.23      | 489-8              |
| meu19 #1     | <i>meu19::kanMX6 ade6-M21?h-</i>                           | Cy5          | 972 h-                   | Cy3       | EMM ade             | 32        | 0.22      | 489-50             |
| meu19 #2     |  | Cy3          |                          | Cy5       |                     |           | 0.25      | 489-9              |
| pcl1 #1      | <i>pcl1::ura4 h-</i>                                       | Cy5          | 972 h-                   | Cy3       | YE                  | 25        | 0.24      | 489-17             |
| pcl1 #2      |  | Cy5          |                          | Cy3       |                     |           | 0.21      | 560-46             |
| prr1 #1      | <i>prr1Δ::his7 his7? h-</i>                                | Cy3          | 972 h-                   | Cy5       | EMM                 | 25        | 0.23      | 334-21             |
| prr1 #2      |  | Cy5          |                          | Cy3       |                     |           | 0.22      | 334-12             |
| sep1 #1      | <i>sep1Δ h-</i>  | Cy5          | 972 h-                   | Cy3       | EMM                 | 25        | 0.24      | 489-13             |
| sep1 #2      |  | Cy3          |                          | Cy5       |                     |           | 0.25      | 334-23             |
| sep1 #3      |  | Cy5          |                          | Cy3       |                     |           | 0.24      | 334-14             |
| sep1 #4      |  | Cy5          |                          | Cy3       |                     |           | 0.23      | 668-5              |
| fhl1 sep1 #1 | <i>SPAC8C9.01Δ::kanMK6 sep1::ura4 ade6-M21? leu1-32 h-</i> | Cy5          | 972 h-                   | Cy3       | EMM ura ade leu     | 30        | 0.23      | 668-39             |
| fhl1 sep1 #1 |  | Cy5          |                          | Cy3       |                     |           | 0.23      | 668-27             |
| ace2 OE #1   | <i>leu1-32 h- pREP3X-ace2</i>                              | Cy5          | <i>leu1-32 h- pREP3X</i> | Cy3       | EMM, 15 µM thiamine | 32        | 0.06      | 596-3              |
| ace2 OE #2   |  | Cy5          |                          | Cy3       |                     |           | 0.12      | 560-31             |
| fhl1 OE #1   | <i>leu1-32 h- pREP3X-fhl1</i>                              | Cy5          |                          | Cy3       |                     | 32        | 0.03      | 596-4              |
| fhl1 OE #2   |  | Cy5          |                          | Cy3       |                     |           | 0.08      | 560-47             |
| fkh2 OE #1   | <i>leu1-32 h- pREP3X-fkh2</i>                              | Cy5          |                          | Cy3       |                     | 32        | 0.07      | 596-20             |
| fkh2 OE #2   |  | Cy5          |                          | Cy3       |                     |           | 0.04      | 596-10             |
| sep1 OE #1   | <i>leu1-32 h- pREP3X-sep1</i>                              | Cy5          |                          | Cy3       |                     | 32        | 0.23      | 596-22             |
| sep1 OE #2   |  | Cy5          |                          | Cy3       |                     |           | 0.17      | 596-19             |

0.5 OD = 1 X 10<sup>7</sup>