## Supplementary Table - Sequence fidelity of NeoChrs

Mutation identified in the neochromosomes as compared to the *in silico* design and with the most relevant parental strain. The \* indicates mutations which are the same in two separate transformations and therefore probably resulting from the template DNA and not during the *in vivo* assembly. Non-synonymous mutations are indicated in bold.

|  |  |  |
| --- | --- | --- |
| **Position** | **Region** | **Mutation type** |
| **NeoChr25 (IMF27)** | | |
| 8648 | *pTKL1* | C to CT |
| 14466 | SHR BQ | C to CT |
| 20137\* | *pTEF2 (mTurquoise 2)\** | CAT to C\* |
| 26993 | *pHHF2 (EcAroB)* | AT to A |
| 46676\* | *pCWP2 (EcTyrB)\** | A to G\* |
| 52732 | SHR AE | G to GT |
| 66608 | SHR N | CA to C |
| 73854\* | *tENO2\** | C to A\* |
| 86809 | *pPFK2* | GA to G |
| 90753 | SHR M | GC to G |
| 90762 | SHR M | AT to A |
| **NeoChr26 (IMF29)** | | |
| 14306 | *tGND1* | CT to C |
| **15181** | ***RKI1*** | **C to A (Glu-129-Gln)** |
| 20137\* | *pTEF2 (mTurquoise2)\** | CAT to C\* |
| 22220 | SHR DF | TC to T |
| 22223 | SHR DF | TC to T |
| 46676\* | *pCWP2 (EcTyrB)\** | A to G\* |
| 57795 | SHR DL | A to AG |
| 64374 | SHR Q | T to TG |
| 66632 | *pPYK1* | CT to C |
| 73854\* | *tENO2\** | C to A\* |
| 73864 | *tENO2* | GT to G |
| 73925 | SHR B | T to C |
| 73926 | SHR B | A to T |
| 73928 | SHR B | G to A |
| 78556 | *pPGI1* | C to A |
| 88608 | *pHIS3* | GA to G |
| 90398 | *pGPM1* | C to CTA |
| **NeoChr30 (IMF41) as compared to NeoChr26 (IMF29)** | | |
| 8648 | *pTKL1* | C to CT |
| 35602 | *tPGM2 (coEcAroL)* | CT to C (In T stretch) |
| 67393 | *pSeTPI1 (At4CL3)* | G to GT (In T stretch) |
| 71689 | *tMDH1 (coAtCHS3)* | GA to G (In A stretch) |
| 73063\* | *pSkADH1 (AtCHI1)\** | AT to A\* |
| 73209\* | *pSkADH1 (AtCHI1)\** | CT to C (In T stretch)\* |
| 81107 | *tFUM1 (coAtANS)* | CG to C |
| **82459\*** | ***coAtANS\**** | **Insertion of 46 bp\*** |
| 115179 | *pPFK2* | G to GAA (In A stretch) |
| **NeoChr31 (IMF42) as compared to NeoChr25 (IMF27)** | | |
| 57432 | Chunk 16AB | A to AC |
| 64168 | *tLAT1 (AtPAL1)* | TAA to T (In A stretch) |
| 67393 | *pSeTPI1 (At4CL3)* | G to GT (In T stretch) |
| **67753** | ***At4CL3*** | **A to G (Thr-15-Ala)** |
| 70927 | *coAtCHS3* | G to A (Leu-155-Leu) |
| 70930 | *coAtCHS3* | A to G (Arg-156-Arg) |
| 73063\* | *pSkADH1 (AtCHI1)\** | AT to A\* |
| 73209\* | *pSkADH1 (AtCHI1)\** | CT to C (In T stretch)\* |
| **82459\*** | ***coAtANS\**** | **Insertion of 46 bp\*** |
| **NeoChr33 (IMF47) as compared to NeoChr31 (IMF42)** | | |
| 48856 | *pCWP2 (coEcTyrB)* | A to G |
| 50730 | *tMDH1 (coAtCHS3)* | A to AT (In T stretch) |
| 85431 | SHR EB | AT to A |
| 85439 | SHR EB | TG to T |
| 85458 | SHR EB | GA to G |
| 96995 | *tPGK1* | T to A |