Table S2. Primers used in the study

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| Primer | sequence (5ʹ - 3ʹ) | usage |
| Primers used to generate constructs for targeted gene replacement of *RPL22*α, *RPL22***a,** *RPL39*α and *RPL39***a**. In low case are sequences complementary to plasmid pRS426, underlined are sequences complementary to the NAT primers. In italics are indicated restriction sites |
| JOHE43231 | gtaacgccagggttttcccagtcacgacgTGCTAAATCTTGAACTAGC | *RPL22*α KO 5' F |
| JOHE43669 | GCTTATGTGAGTCCTCCCGTGTCTACAGATGTTCCTGC | *RPL22*α KO 5' R |
| JOHE43670 | CTCGTTTCTACATCTCTTATGAGTAGCTTTAAGGACG | *RPL22*α KO 3' F |
| JOHE43234 | gcggataacaatttcacacaggaaacagcTTGTCTCGCTGATATGTTG | *RPL22*α KO 3' R |
| JOHE44325 | TGCTAAATCTTGAACTAGC  | *RPL22*α F for split marker  |
| JOHE44326 | TTGTCTCGCTGATATGTTG  | *RPL22*α R for split marker |
| JOHE43816 | TGGGTTGACTGGCTCTTGTC | Internal *RPL22*α F; sequencing complementation plasmid |
| JOHE43817 | ATATAATATCAGCGGTGT | Internal *RPL22*α R; sequencing complementation plasmid |
| JOHE43235 | GGTAACAGGAACAGACGTG | *RPL22*α KO F screening |
| JOHE43236 | ATGTTCATCATATGCTGAGG | *RPL22*α KO R screening |
| JOHE43255 | gtaacgccagggttttcccagtcacgacgATAGCTGCACTCAACTTACC | *RPL39*α KO 5' F |
| JOHE43677 | GCTTATGTGAGTCCTCCCAGCCGATGAGCACACTTACC | *RPL39*α KO 5' R |
| JOHE43678/GI | CTCGTTTCTACATCTCTTGTCGAACCAAGCTCAACC | *RPL39*α KO 3' F |
| JOHE43258 | gcggataacaatttcacacaggaaacagcGAGAACAGGAATTGATTCC | *RPL39*α KO 3' R |
| JOHE44331 | ATAGCTGCACTCAACTTACC | *RPL39*α F for split marker  |
| JOHE44332 | GAGAACAGGAATTGATTCC | *RPL39*α R for split marker |
| JOHE43812 | AAGGCATCGCGAATACAACC | Internal *RPL39*α F |
| JOHE43813 | TAGATGTTAGCACGAAACCG | Internal *RPL39*α R |
| JOHE43259 | CACATTGACTACTTATGACC | *RPL39*α KO F screening |
| JOHE43260 | ATGTTCTGATCGTAATCTGC | *RPL39*α KO R screening |
| JOHE43832 | gtaacgccagggttttcccagtcacgacgcactgcatcaatcgaacgc | *RPL39***a** KO 5' F |
| JOHE43833 | GCTTATGTGAGTCCTCCCcgaacagatatatagcaagc | *RPL39***a** KO 5' R |
| JOHE43834 | CTCGTTTCTACATCTCTTcgtgcagtataacgccaagc | *RPL39***a** KO 3' F |
| JOHE43835 | gcggataacaatttcacacaggaaacagccttcgtcaatggacatgatg | *RPL39***a** KO 3' R |
| JOHE44335 | cactgcatcaatcgaacgc | *RPL39***a** F for split marker  |
| JOHE44336 | cttcgtcaatggacatgatg | *RPL39***a** R for split marker |
| JOHE43810 | CACAACAACATAATCACACG | Internal *RPL39***a** F |
| JOHE43811 | GCAAGTGTACGTGAGCAGA | Internal *RPL39***a** R |
| JOHE43836 | acatgaggacagattggcgt | *RPL39***a** screening F |
| JOHE43837 | ctcacggtacgtattctgc | *RPL39***a** screening R  |
| JOHE43844 | gtaacgccagggttttcccagtcacgacgcttcacctcaagctctgcg | *RPL22***a** KO 5' F |
| JOHE43845 | GCTTATGTGAGTCCTCCCttagcgcagcatccacaacc | *RPL22***a** KO 5' R |
| JOHE43846 | CTCGTTTCTACATCTCTTgaactctttcgagaacttcc | *RPL22***a** KO 3' F |
| JOHE43847 | gcggataacaatttcacacaggaaacagcaagatgagtctaacatgcc | *RPL22***a** KO 3' R |
| JOHE44339 | cttcacctcaagctctgcg | *RPL22***a** F for split marker  |
| JOHE44340 | aagatgagtctaacatgcc | *RPL22***a** R for split marker |
| JOHE43814 | TTGGGTTGTGGATGCTGCG | Internal *RPL22***a** (F); sequencing complementation plasmid  |
| JOHE43815 | TGGCAACGACACTATGAGGC | Internal *RPL22***a** (R); sequencing complementation plasmid |
| JOHE43848 | atgaaaggtacttggtgagg | *RPL22***a** screening F |
| JOHE43849 | cattaccagcgtagatcgg | *RPL22***a** screening R  |
| JOHE43364/ai006 | GAAGAGATGTAGAAACGAG | NAT amplification |
| JOHE43665/ai290 | GGGAGGACTCACATAAGC | NAT amplification |
| JOHE44324 | TTCCAGCCGGAGTAAGAGACG | NAT-R split marker |
| JOHE43667/ai37 | ATGGCGGCCGCCACTCTTGAC | NAT-F split marker |
| Primers used to score mitochondrial inheritance and *MAT* identity  |
| JOHE44507/Da20 | GACACTACACAAGATGCCTC | Mitochondrial inheritance |
| JOHE44508/Da3 | GCAATAGCATATACCATCCCG | Mitochondrial inheritance |
| JOHE39201 | CTAACTCTACTACACCTCACGGCA | *MAT***a** |
| JOHE39202 | CGCACTGCAAAATAGATAAGTCTG | *MAT***a** |
| JOHE39203 | GGCTGCAATCACAGCACCTTAC | *MAT*α |
| JOHE39204 | CTTCATGACATCACTCCCCTAT | *MAT*α |
| Primers used to clone the *RPL22***a** and *RPL22*α genes within pSDMA57 |
| JOHE44538/GI | CC*ATCGAT*tcttgggtactggattggac | Cloning *RPL22*α F |
| JOHE44539 | CC*ATCGAT*aacagcatgtagtattgtcg | Cloning *RPL22*α R |
| JOHE44540 | CC*ATCGAT*agtcgtgccatcagcatattcg | Cloning *RPL22***a** F |
| JOHE44541 | CC*ATCGAT*tcgcggacagtataagagtg | Cloning *RPL22***a** F |
| M13 F | GTAAAACGACGGCCAGT | sequencing  |
| M13 R | CAGGAAACAGCTATGAC | sequencing |
| JOHE43966 | AGCCAACGCTATGTCCTGAT | Internal NEO R – sequencing  |
| Primers used for RT-qPCR of *RPL22***a** and *RPL22*α during mating  |
| JOHE50205/GI | GCCGCTAAGAACGCTGCCTCC | *RPL22***a** F  |
| JOHE50206 | CCTTCTGGACAGCGACGACA | *RPL22***a** R |
| JOHE50207 | ACTACCAAGAACGCAGCCGCT | *RPL22*α F |
| JOHE50208 | AGGCTTGCCGTCGACCTTGAT | *RPL22*α R |
| JOHE50209 | ACTCCAAGGCGGCCATTATGC | *GDP1* F |
| JOHE50210/GI | CCTTGGAGCTTTTGTCCGTTC | *GDP1* R |
| JOHE50590/GI | tgccgcgtttgagaagttc | c*RPL22*α F |
| JOHE50591/GI | agaaggggatttgagaggta | c*RPL22*α R |
| PCR amplify the *CAS9* gene |
| JOHE45941 | GGTGACGCTGTGAGAGTGG | Cas\_F |
| JOHE45942 | GGGCCCCTCTTCACGTGG | Cas\_R |
| Universal PCR for assembly U6 and sgRNA fragments |
| JOHE45943 | TTTGCATTAGAACTAAAAACAAAGCA | U6\_F |
| JOHE50185 | AACAGTATACCCTGCCGGTG | U6 promoter\_R |
| JOHE50186 | GTTTTAGAGCTAGAAATAGCAAGTT | sgRNA\_scaffold\_F |
| JOHE45944 | TAAAACAAAAAAGCACCGACTCGGTGCC | sgRNA\_scaffold\_R  |
| Primers used to generate strains GI228 (5ʹ Δ *RPL22***a**)**.** Bridge primers for assembly sgRNA cassette. In red sequences that overlap with the U6 promoter; in blue sequences that overlap with the sgRNA scaffold; in bold the sgRNA guide sequences. |
| JOHE50781 | gtaacgccagggttttcccagtcacgacggcttcaaatgcgttcagtc | 5ʹ Δ KO *RPL22***a** F |
| JOHE45730 | GCTTATGTGAGTCCTCCCttgtcatgatggacgaagga | 5ʹ Δ KO *RPL22***a** R |
| JOHE45731 | tccttcgtccatcatgacaaGGGAGGACTCACATAAGC | NAT F |
| JOHE50782 | actcgatcaaccacgagcttGAAGAGATGTAGAAACGA | NAT R |
| JOHE50783 | TCGTTTCTACATCTCTTCaagctcgtggttgatcgagt | 3ʹ Δ KO *RPL22***a** F |
| JOHE50784 | gcggataacaatttcacacaggaaacagcatgcatgcacgtctgaaaag | 3ʹ Δ KO *RPL22***a** R |
| JOHE50785 | *CACCGGCAGGGTATACTGTT***gatctcgaattgcccctagt***GTTTTAGAGCTAGAAATAGCAAGTT* | gRNA  |
| JOHE50833/GI | cctttcgcctagtcagcatc | Internal F |
| JOHE50834/GI | ccctctccctcgagttatcc | Internal R  |
| JOHE50801/GI | ctccttaaatcggccatcag | 5' screening F |
| JOHE43849 | cattaccagcgtagatcgg | 3' screening R |
| Assembly of the *SH2* HDR template. Bridge primers for assembly sgRNA cassette. In red sequences that overlap with the U6 promoter; in blue sequences that overlap with the sgRNA scaffold; in bold the sgRNA guide sequences. revcom, sgRNA target sequences are reverse complementary to the positive DNA. |
| JOHE46291 | *CACCGGCAGGGTATACTGTTG***TAATCCCTCCGCGCTTGCCG***GTTTTAGAGCTAGAAATAGCAAG* | sgRNA\_SH2\_352\_revcom |
| JOHE46292 | *CACCGGCAGGGTATACTGTTG***GACCGACAGCGCACCCTCTT***GTTTTAGAGCTAGAAATAGCAAG* | sgRNA\_SH2\_212\_revcom |
| JOHE46293 | ATAATACGAACGAAACGGTGTGA | *SH2*\_Left homology arm\_F |
| JOHE46294 | GTGACTGGGAAAACCCTGGCGTCCAAACTTTTATTTAAGACATCAGC | *SH2*\_Left homology arm \_Reverse |
| JOHE46295 | TCCTGTGTGAAATTGTTATCCGCTCATAATTTTCCATGATTGATTCTGTC | *SH2*\_Right homology arm \_F |
| JOHE46296 | GCTCGGAATCTAGCACAATCTACC | *SH2*\_Right homology arm \_Reverse |
| JOHE46301 | CGCTTTCCGTATCCTGACC | *SH2*\_ Left homology arm \_inner\_F |
| JOHE46302 | GAGCGATAAGTAAAAAGTGAGGAAC | *SH2*\_Right homology arm \_inner\_R |
| Assembly of the HDR template used for replacing *RPL22α* with *RPL22***a** |
| JOHE46303 | GCCTGTCTTCACACTTCATACCTGG | *RPL22*α\_Left homology arm\_F |
| JOHE46304 | GATATGAGAAAAGGAAACGCACCATTGTGTCTACAGATGTTCC | *RPL22*α\_Left homology arm\_R |
| JOHE46305 | CTGAGGAGGATGAACTCGCCTAAATGAGTAGCTTTAAGGACGGTTGC | *RPL22*α\_Right homology arm\_F |
| JOHE46306 | CCACGGGATTCCATTATGACC | *RPL22*α\_Right homology arm\_R |
| JOHE46307 | CCTCGTCTAGGATTTGCTTCTGGAC | *RPL22*α\_Left homology arm\_inner\_F |
| JOHE46308 | ACTGGACGGATTGATCTTGGTG | *RPL22*α\_Right homology arm\_inner\_R |
| JOHE46309 | ATGGTGCGTTTCCTTTTCTCATATC | *RPL22***a**\_1\_F |
| JOHE46312 | GATACCTCTTGGAGAAGGGGATTTG | *RPL22***a**\_412\_Split\_R |
| JOHE46311 | GTGAACGATTCTGTCTTCGACCTC | *RPL22***a**\_198\_Split\_F |
| JOHE46310 | TTAGGCGAGTTCATCCTCCTCAG | *RPL22***a**\_600\_R |
| JOHE46508 | AAATTCCGCCTTTTGATTGCTG | Sequencing validation of changing *RPL22*α to **a**  |
| Assembly of the HDR template used for replacing *RPL22***a**with the entire *RPL22α* |
| JOHE46313 | CCAGTCGTGCCATCAGCATATTC | *RPL22***a**\_Left homology arm\_F,also used for spanning validation |
| JOHE46314 | GAAAAAATAAAACGCACCATTGTGTCTACAGATGTTTC | *RPL22***a**\_Left homology arm\_R |
| JOHE46315 | GCTGAGGAGGATGAACTCGCTTAATTCCGTATCTTGTATGGGTAGGGC | *RPL22***a**\_Right arm\_F |
| JOHE46316 | CTCGTTCACGAAAGTTCAGTGGC | *RPL22***a**\_Right arm\_R, also used for spanning validation |
| JOHE46317 | GCTGACTGATGCGACTTTAGACGTG | *RPL22***a**\_Left arm\_inner\_F |
| JOHE46318 | GGTACGATAGAAGCGAGCAAAGGC | *RPL22***a**\_Right arm\_inner\_R |
| JOHE46319 | ATGGTGCGTTTTATTTTTTCTTTTTGA | *RPL22*α\_1\_F |
| JOHE46322 | GGAAGTGAGGACGATCTTGGCA | *RPL22*α\_403\_Split\_R |
| JOHE46321 | CGTCCCCGTGAACGATTCTGTC | *RPL22*α\_196\_Split\_F |
| JOHE46320 | TTAAGCGAGTTCATCCTCCTCAGC | *RPL22*α\_616\_R |
| Assembly of the HDR template used for replacing *RPL22***a**with *RPL22α*1-268-*RPL22***a**253-600 |
| JOHE46532 | CCGTCAACCTTGATGCGGTCGTGGAGGAACTTC | *RPL22*α\_\_N terminal\_R |
| JOHE46533 | CTCCACGACCGCATCAAGGTTGACGGCAAGCCC | *RPL22***a**\_C terminal\_F |
| JOHE46538 | TTTCAACATGGCATCAAGCTATCC | Sequencing the entire *RPL22***a** ORF\_F |
| Bridge primers for assembly sgRNA cassette. Bridge primers for assembly sgRNA cassette. In red sequences that overlap with the U6 promoter; in blue sequences that overlap with the sgRNA scaffold; in bold the sgRNA guide sequences. revcom, sgRNA target sequences are reverse complementary to the positive DNA. |
| JOHE46323 | CACCGGCAGGGTATACTGTTG**CGTTTGCAATCAGGATTACA**GTTTTAGAGCTAGAAATAGCAAG | *RPL22*α\_62\_revcom |
| JOHE46324 | CACCGGCAGGGTATACTGTTG**CAAGTACTTCAAGGTTGATC**GTTTTAGAGCTAGAAATAGCAAG | *RPL22*α\_565 |
| JOHE46327 | CACCGGCAGGGTATACTGTTG**AGTTGCAATGAGGGTTACAT**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**\_47\_revcom |
| JOHE46328 | CACCGGCAGGGTATACTGTTG**CCCATACAAGATACGGAATT**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**2\_618\_revcom |
| JOHE46329 | CACCGGCAGGGTATACTGTTG**TGTATCTACACGTTACTAAC**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**\_487\_revcom |
| JOHE46534 | CACCGGCAGGGTATACTGTTG**GTTTCTCCACGACCGCGTCA**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**\_236 |
| JOHE46535 | CACCGGCAGGGTATACTGTTG**GCAGCGTTCTTAGCGGCGGA**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**\_151\_revcom |
| JOHE46536 | CACCGGCAGGGTATACTGTTG**GGAGGCAGCGTTCTTAGCGG**GTTTTAGAGCTAGAAATAGCAAG | *RPL22***a**\_155\_revcom |
| Primers used for PCR amplifying probes for Southern blot verification of mutants |
| JOHE41547 | CGATACGGCTTACCGTTACAG | NAT\_F |
| JOHE41548 | GAGCTTGCTCTCCGTCAGATG | NAT\_R |
| JOHE41551 | GAAGGGACTGGCTGCTATTG | NEO\_F |
| JOHE41552 | GAACTCGTCAAGAAGGCGATA | NEO\_R |