

Table S2. Primers used in this study

| Primers | Sequence | Used for |
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| 5'-pU6-SacI | CCGAGCTCAAGTAAGCAGAAGCACGC | To clone U6-sgRNA(UPRT) into pSAG-Ble-SAG |
| 3'-gRNA-SacI | GCGAGCTCAAAAAGCACCGACTCGGTGCCACT TTTTCAAGTTGATAACGGACTAGCCTTATTTAA CTTGCTATTTCTAGCTCTAAAACGCTCTCACAAT CGAGACGCCAACTTGACATCCCCA | |
| 5'-CAS9-NsiI | CCATGCATCTAGACAAAATGGACAAGAAGTACA GCATCG | To clone CAS9-NLS-GFP into pSAG1-U6::sgUPRT |
| 3'-CAS9-PacI | GCTTAATTA ACTACTTGTACAGCTCGTCC | |
| 5'-UpgRNA | GATCCGCTTCTCTTGTACTGC | PCR3 |
| 3'-DngRNA | AAGCAGGTGCAGCGGACAAG | |
| UPRT-seq | GGAAGCATGCAGCCATATACG | To sequence the gRNA targeting region of <i>UPRT</i> gene in FUDR resistant mutants |
| 5'-B4-UpgRNA | GGGG ACA ACT TTG TAT AGA AAA GTT GAGCTACTCCTGTGTCTGAGTG | To produce PCR product for BP cloning making pDONR-P4P1r-UpgRNA; to get the amplicon <i>uprt::DHFR*-I</i> for transfection |
| 3'-B1r-UpgRNA | GGGG AC TGC TTT TTT GTA CAA ACT TGATCTTGGAATAGAAGGAACTCC | To produce PCR product for BP cloning making pDONR-P4P1r-UpgRNA |
| attB1-DHFR-F | GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC AAG CTT CGC CAG GCT GTA AAT C | To produce PCR product for BP cloning making pDONR-P1P2-DHFR* |
| attB2-DHFR-R | GGGG AC CAC TTT GTA CAA GAA AGC TGG GTC GGA ATT CAT CCT GCA AGT GCA TAG AAG | |
| 5'-B2r-DngRNA | GGGG ACA GCT TTC TTG TAC AAA GTG GCGAGTCGATGGAAAGCG | To produce PCR product for BP cloning making pDONR-P2rP3-DngRNA |
| 3'-B3-DngRNA | GGGG AC AAC TTT GTA TAA TAA AGT TGCTTACGCAGCAGTGACCATG | To produce PCR product for BP cloning making pDONR-P2rP3-DngRNA; to get the amplicon <i>uprt::DHFR*-I</i> for transfection |

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| attB4 UPRT 5' KO - forward | GGGGACAAC TTTGTATAGAAAAGTTGTCCCGCT GTGCCTAGTATCG | To produce PCR product for BP cloning making pDONR-P4P1r-U5UPRT |
| attB1r UPRT 5' KO - reverse | GGGGACTGCTTTTTTTGTACAAACTTGTTTTAGAA GCCCTGTGGA | |
| attB2r UPRT 3' KO - forward | GGGGACAGCTTTCTTGTACAAAGTGGTCGTCTCT AGTTTTTTTGACAGAC | To produce PCR product for BP cloning making pDONR-P2rP3-U3UPRT |
| attB3 UPRT 3' KO - reverse | GGGGACAAC TTTGTATAATAAAGTTGTCGACGT CAACTGTACGAC | |
| UPRT 5' KO - forward | GCTGTAGAGTTGCCACCC | to get the amplicons <i>uprt::DHFR*-D</i> and <i>uprt::ROP18</i> for transfection |
| UPRT 3' KO - reverse | GTACGACATCCGAGTGAA | |
| 5'-UpUPRT | TGCGACTGCATGCGAAAGTAG | PCR1 |
| 3'-InDHFR | TACCAGTCATGGACGAGATCG | PCR1, PCR4, PCR6 |
| 5'-InDHFR | ACACGCATGTCTACACGAACC | PCR2, PCR5, PCR7 |
| 3'-DnUPRT | GCTACTAACAAGTGACGACG | PCR2 |
| 5'-UPRT-OT1 | GGCGTCTCGATTGTGAGACGGTTTTAGAGCTAG AAATAGC | Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT to UPRT-OT1 |
| 5'-UPRT-OT2 | GGCGTCTCGATTGTGAAGGCGTTTTAGAGCTAG AAATAGC | Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT to UPRT-OT2 |
| 5'-ROP18-gRNA | TACGCGTACCGTCGTCCGAAGTTTTAGAGCTAG AAATAGC | Q5 mutagenesis changing the gRNA in pSAG1::CAS9-U6::sgUPRT to gRNA-ROP18 |
| 3'-ROP18-gRNA | AACTTGACATCCCCATTTAC | Generic reverse primer for Q5 mutagenesis in pSAG1::CAS9-U6::sgUPRT |
| 5'-B4-UpROP18 | GGGG ACA ACT TTG TAT AGA AAA GTT G ACAGGAAACGCCAACAGACG | To produce PCR product for BP cloning making pDONR-P4P1r-UpROP18; to make the amplicon <i>rop18::DHFR*</i> for transfection |
| 3'-B1r-UpROP18 | GGGG AC TGC TTT TTT GTA CAA ACT TGAGAGGTGGCCGCTGTACCG | To produce PCR product for BP cloning making pDONR-P4P1r-UpROP18 |
| 5'-B2r-DnROP18 | GGGG ACA GCT TTC TTG TAC AAA GTG | To produce PCR product for BP cloning |

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| 5'-B2r-DnROP18 | GGGG ACA GCT TTC TTG TAC AAA GTG GTGGGTTTAGCGACTCTTCTCC | To produce PCR product for BP cloning making pDONR-P2rP3-DnROP18 |
| 3'-B3-DnROP18 | GGGG AC AAC TTT GTA TAA TAA AGT TGAAGCATAGTCTCATCGGTGG | To produce PCR product for BP cloning making pDONR-P2rP3-DnROP18; to make the amplicon <i>rop18::DHFR*</i> for transfection |
| 5'-UpUP18 | GATCTTCAGCAGCCTGGTTC | PCR6 |
| 3'-DnDn18 | CAAACCAATCACGACCTCTG | PCR7 |
| 5'-InROP18 | AAGTTCTATCGCGCCACTCG | PCR8 |
| 3'-InROP18 | TTTGCCGATGACGCATCCAG | |
| 5'-UpU5UPRT | GATAGGGTTACCCTGGTACTC | PCR4 |
| 3'-DnU3UPRT | GGTTCGACACTGGTCAGATG | PCR5 |
| pIMC1-Rev | GGGGACAACCTTTGTATAGAAAAGTTGGGTGGGT GAGAAAGGAGGGAACAAC | PCR9 |
| U3DHFR-For | CCGCACCACCTAAGTGTAACC | PCR10 |
| DHFR-20nt-Fw | CCTTTTATTCCAAGATCTGTAAGCTTCGCCAGGC TGAAA | PCR amplification of the DHFR* cassette with 20 bp <i>UPRT</i> homologous arms on each side for transfection |
| DHFR-20nt-Rv | CACTTTCCATCGACTCGCCAGGAATTCATCCTGC AAGTGCATAG | |
| DHFR-0nt-Fw | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAA GCTTCGCCAGGCTGTAAATC | PCR amplification of the DHFR* cassette for transfection |
| DHFR-0nt-Rv | GGGGACCACTTTGTACAAGAAAGCTGGGTCCGA ATTCATCCTGCAAGTGCATAGAAG | |