

**Table S1. Non-synonymous base changes in sequenced bypass mutants.**

| R6 Position <sup>a</sup> | R6 Base | SNP <sup>b</sup> | SNP Reads <sup>c</sup> | Freq. <sup>d</sup> | Gene <sup>e</sup> | Coding region change <sup>f</sup>                 | Amino acid change <sup>g</sup>                        | Strain <sup>h</sup> | Unique Gene |
|--------------------------|---------|------------------|------------------------|--------------------|-------------------|---|---|---------------------|-------------|
| 79856                    | T       | C                | 194                    | 89                 | spr0073, spr0074  | NP_357667.1:c.[185A>G];<br>NP_357668.1:c.[197T>C] | NP_357667.1:p.[His62Arg];<br>NP_357668.1:p.[Val66Ala] | SCT-4               | 1           |
| 137972                   | A       | G                | 182                    | 99                 | spr0129           | NP_357723.1:c.88A>G                               | NP_357723.1:p.Asn30Asp                                | ALT-4               | 2           |
| 172808                   | T       | A                | 99                     | 95                 | ribD              | NP_357758.1:c.952A>T                              | NP_357758.1:p.Ile318Phe                               | TXT-4               | 3           |
| 188052                   | T       | C                | 124                    | 98                 | cls               | NP_357774.1:c.323T>C                              | NP_357774.1:p.Phe108Ser                               | DET-5               | 4           |
| 204465                   | A       | G                | 186                    | 99                 | rpsE              | NP_357800.1:c.145A>G                              | NP_357800.1:p.Thr49Ala                                | MAT-5               | 5           |
| 206465                   | T       | C                | 126                    | 97                 | secY              | NP_357803.1:c.857T>C                              | NP_357803.1:p.Ile286Thr                               | GAT-1               | 6           |
| 237689                   | G       | A                | 218                    | 100                | proS              | NP_357837.1:c.916G>A                              | NP_357837.1:p.Ala306Thr                               | MTT-5               | 7           |
| 239704                   | G       | A                | 218                    | 100                | bglA              | NP_357838.1:c.978G>A                              | NP_357838.1:p.Met326Ile                               | FLT-4               | 8           |
| 298527                   | A       | G                | 110                    | 99                 | spr0297           | NP_357891.1:c.1576A>G                             | NP_357891.1:p.Ile526Val                               | DET-5               | 9           |
| 341113                   | G       | A                | 118                    | 91                 | gnd               | NP_357929.1:c.1381G>A                             | NP_357929.1:p.Asp461Asn                               | GAT-1               | 10          |
| 343401                   | T       | C                | 193                    | 98                 | mvk               | NP_357932.1:c.308T>C                              | NP_357932.1:p.Val103Ala                               | NVT-1               | 11          |
| 346404                   | G       | A                | 58                     | 94                 | fni               | NP_357935.1:c.520G>A                              | NP_357935.1:p.Val174Ile                               | NYT-2               | 12          |
| 362984                   | C       | T                | 58                     | 95                 | recD              | NP_357957.1:c.658G>A                              | NP_357957.1:p.Ala220Thr                               | NYT-2               | 13          |
| 368383                   | A       | G                | 143                    | 100                | mutS2             | NP_357962.1:c.1973A>G                             | NP_357962.1:p.Gln658Arg                               | MAT-5               | 14          |
| 395308                   | C       | G                | 171                    | 98                 | asp23             | NP_357993.1:c.188C>G                              | NP_357993.1:p.Ala63Gly                                | FLT-4               | 15          |
| 429371                   | G       | A                | 136                    | 99                 | spr0429           | NP_358023.1:c.49G>A                               | NP_358023.1:p.Ala17Thr                                | MAT-5               | 16          |
| 435792                   | G       | A                | 182                    | 95                 | spr0436           | NP_358030.1:c.324G>A                              | NP_358030.1:p.Trp108*                                 | NVT-1               | 17          |
| 498602                   | G       | A                | 91                     | 98                 | spr0499           | NP_358093.1:c.787G>A                              | NP_358093.1:p.Ala263Thr                               | GAT-1               | 18          |
| 517931                   | G       | A                | 51                     | 91                 | pnpA              | NP_358110.1:c.2236G>A                             | NP_358110.1:p.Gly746Ser                               | NYT-1               | 19          |
| 519232                   | G       | A                | 131                    | 98                 | spr0518           | NP_358112.1:c.565G>A                              | NP_358112.1:p.Ala189Thr                               | NYT-1               | 20          |
| 592126                   | A       | G                | 90                     | 99                 | zmpB              | NP_358175.1:c.680A>G                              | NP_358175.1:p.Asp227Gly                               | OHT-1               | 21          |
| 606409                   | T       | C                | 105                    | 100                | spr0592           | NP_358186.1:c.329T>C                              | NP_358186.1:p.Leu110Pro                               | MTT-5               | 22          |
| 649209                   | C       | T                | 86                     | 97                 | ctpA              | NP_358235.1:c.2224C>T                             | NP_358235.1:p.Leu742Phe                               | GAT-1               | 23          |
| 715042                   | G       | T                | 78                     | 99                 | thiJ              | NP_358307.1:c.442G>T                              | NP_358307.1:p.Gly148Cys                               | GAT-1               | 24          |
| 720738                   | G       | A                | 74                     | 100                | transposase_D     | NP_358312.1:c.131G>A                              | NP_358312.1:p.Arg44His                                | GAT-1               | 25          |
| 826714                   | A       | G                | 104                    | 99                 | spr0824           | NP_358418.1:c.562T>C                              | NP_358418.1:p.Tyr188His                               | ILT-1               | 26          |
| 965792                   | C       | T                | 112                    | 100                | rpoD              | NP_358573.1:c.512C>T                              | NP_358573.1:p.Ala171Val                               | DET-5               | 27          |
| 965792                   | C       | T                | 119                    | 98                 | rpoD              | NP_358573.1:c.512C>T                              | NP_358573.1:p.Ala171Val                               | MAT-5               | 27          |
| 965792                   | C       | T                | 152                    | 99                 | rpoD              | NP_358573.1:c.512C>T                              | NP_358573.1:p.Ala171Val                               | ALT-4               | 27          |
| 965792                   | C       | T                | 186                    | 99                 | rpoD              | NP_358573.1:c.512C>T                              | NP_358573.1:p.Ala171Val                               | NCT-2               | 27          |
| 966227                   | G       | A                | 59                     | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | NYT-2               | 27          |
| 966227                   | G       | A                | 70                     | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | TXT-4               | 27          |
| 966227                   | G       | A                | 96                     | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | ILT-1               | 27          |
| 966227                   | G       | A                | 126                    | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | GAT-1               | 27          |
| 966227                   | G       | A                | 152                    | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | MTT-5               | 27          |
| 966227                   | G       | A                | 228                    | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | NVT-1               | 27          |
| 966227                   | G       | A                | 248                    | 100                | rpoD              | NP_358573.1:c.947G>A                              | NP_358573.1:p.Arg316His                               | SCT-4               | 27          |

|         |   |   |     |     |                     |                       |                         |       |    |
|---------|---|---|-----|-----|---------------------|-----------------------|-------------------------|-------|----|
| 966344  | G | A | 140 | 100 | rpoD                | NP_358573.1:c.1064G>A | NP_358573.1:p.Arg355His | OHT-1 | 27 |
| 966344  | G | A | 141 | 100 | rpoD                | NP_358573.1:c.1064G>A | NP_358573.1:p.Arg355His | FLT-4 | 27 |
| 966367  | C | T | 87  | 99  | rpoD                | NP_358573.1:c.1087C>T | NP_358573.1:p.Leu363Phe | NYT-1 | 27 |
| 1072831 | T | C | 190 | 99  | lacC                | NP_358667.1:c.847A>G  | NP_358667.1:p.Asn283Asp | FLT-4 | 28 |
| 1091608 | G | A | 161 | 99  | spr1094             | NP_358687.1:c.569C>T  | NP_358687.1:p.Ala190Val | NCT-2 | 29 |
| 1102165 | T | C | 165 | 93  | spnII-interrupted-C | NP_358694.1:c.587A>G  | NP_358694.1:p.Asp196Gly | NCT-2 | 30 |
| 1119841 | G | A | 91  | 99  | glnP                | NP_358713.1:c.184G>A  | NP_358713.1:p.Gly62Ser  | NYT-1 | 31 |
| 1128529 | T | C | 242 | 100 | smc                 | NP_358719.1:c.2146A>G | NP_358719.1:p.Lys716Glu | NCT-2 | 32 |
| 1128802 | C | T | 45  | 98  | smc                 | NP_358719.1:c.1873G>A | NP_358719.1:p.Ala625Thr | NYT-2 | 32 |
| 1143200 | C | T | 119 | 94  | topA                | NP_358734.1:c.613G>A  | NP_358734.1:p.Glu205Lys | NYT-1 | 33 |
| 1212019 | G | A | 193 | 98  | ABC-N/P             | NP_358809.1:c.1535C>T | NP_358809.1:p.Ala512Val | NCT-2 | 34 |
| 1233121 | T | C | 104 | 99  | spr1236             | NP_358829.1:c.1003A>G | NP_358829.1:p.Asn335Asp | GAT-1 | 35 |
| 1253298 | C | T | 145 | 99  | pstB                | NP_358847.1:c.529G>A  | NP_358847.1:p.Ala177Thr | ALT-4 | 36 |
| 1299392 | C | A | 41  | 100 | spr1307             | NP_358900.1:c.130G>T  | NP_358900.1:p.Ala44Ser  | NYT-2 | 37 |
| 1375972 | C | T | 236 | 99  | asnC                | NP_358990.1:c.1138G>A | NP_358990.1:p.Asp380Asn | SCT-4 | 38 |
| 1401571 | C | T | 109 | 98  | dapB                | NP_359007.1:c.334G>A  | NP_359007.1:p.Gly112Ser | GAT-1 | 39 |
| 1464129 | C | T | 107 | 95  | relA                | NP_359080.1:c.1029G>A | NP_359080.1:p.Trp343*   | OHT-1 | 40 |
| 1467154 | G | A | 119 | 100 | pepO                | NP_359084.1:c.1643C>T | NP_359084.1:p.Ala548Val | DET-5 | 41 |
| 1483714 | T | C | 114 | 98  | divIVA              | NP_359098.1:c.692A>G  | NP_359098.1:p.Glu231Gly | DET-5 | 42 |
| 1519669 | A | G | 49  | 98  | axe1                | NP_359131.1:c.728T>C  | NP_359131.1:p.Ile243Thr | NYT-2 | 43 |
| 1520053 | C | T | 115 | 97  | axe1                | NP_359131.1:c.344G>A  | NP_359131.1:p.Arg115Gln | MTT-5 | 43 |
| 1541906 | G | A | 175 | 99  | natB                | NP_359154.1:c.427C>T  | NP_359154.1:p.Gln143*   | OHT-1 | 44 |
| 1542870 | A | G | 127 | 100 | natA                | NP_359155.1:c.353T>C  | NP_359155.1:p.Val118Ala | NYT-1 | 45 |
| 1558537 | C | T | 124 | 98  | pppL                | NP_359170.1:c.284G>A  | NP_359170.1:p.Arg95Lys  | MAT-5 | 46 |
| 1616338 | G | A | 165 | 99  | ABC-NBD             | NP_359236.1:c.650C>T  | NP_359236.1:p.Pro217Leu | DET-5 | 47 |
| 1656423 | C | T | 157 | 100 | fatD                | NP_359276.1:c.401C>T  | NP_359276.1:p.Thr134Ile | ALT-4 | 48 |
| 1675970 | C | T | 44  | 100 | amiC                | NP_359298.1:c.958G>A  | NP_359298.1:p.Ala320Thr | NYT-2 | 49 |
| 1688914 | C | T | 182 | 91  | msmR                | NP_359306.1:c.776C>T  | NP_359306.1:p.Ala259Val | NVT-1 | 50 |
| 1754472 | C | T | 166 | 100 | endA                | NP_359371.1:c.229G>A  | NP_359371.1:p.Ala77Thr  | FLT-4 | 51 |
| 1777381 | A | G | 119 | 99  | spr1806             | NP_359398.1:c.425T>C  | NP_359398.1:p.Val142Ala | NYT-1 | 52 |
| 1883328 | T | C | 148 | 100 | hipO                | NP_359497.1:c.737A>G  | NP_359497.1:p.His246Arg | GAT-1 | 53 |

a. Nucleotide position in R6 genome

b. Single nucleotide polymorphism (SNP) found in bypass mutant

c. Number of sequence reads containing SNP

d. % of reads with SNP base

e. Gene in R6 containing SNP

f. Accession number: base change

g. Accession number: amino acid change

h. Suppressor mutant strain in which the SNP is found