

Supplemental Table 1. *E. coli* chromosomal integration sites obtained using a RAM-targetron library with randomized target site recognition sequences.

| Target gene | Target gene function | Integration site | Annotation |
|-------------|---|--------------------|---------------------|
| | intergenic region | 3553453o | |
| | intergenic region | 3643872o | |
| | intergenic region | 3773856o | |
| | intergenic region | 3851225i | |
| | intergenic repeats | 2902301o | |
| agaZ | putative tagatose 6-phosphate kinase 2 | 3276627o | |
| argE | acetylornithine deacetylase | 4151651i | |
| b1452 | putative receptor | 1521597i | |
| b2520 | unknown | 2647574i | |
| b3814 | unknown | 3998023o | |
| bisC | biotin sulfoxide reductase | 3711727o | |
| cdh | CDP-diacylglycerol phosphotidylhydrolase | 4107960i | |
| cyaA | adenylate cyclase | 3988770o | |
| deaD | ATP-independent RNA helicase | 3304206o | |
| deoB | phosphopentomutase | 4617331o | |
| ebgA | evolved beta-D-galactosidase, alpha subunit | 3220453o, 3221497i | |
| eutH | putative transport protein | 2565489i | |
| fadB | 4-enzyme protein | 4027811i | |
| flhA | flagellar structural component | 1962889i | |
| fliC | flagellin | 2000818i | |
| fpr | ferredoxin-NADP reductase | 4111553o | 2 different introns |
| frvB | transport of small molecules (carbohydrates, organic acids, alcohols) | 4089543i | |
| FrwD | PTS system fructose-like IIB component 2 | 4144880i | |
| glnL | histidine protein kinase sensor for GlnG regulator | 4052894i | |
| gltS | transport of small molecules (amino acids & amines) | 3826182o | |
| hofQ | putative transport protein | 3517567i | |
| hsdR | endonuclease R | 4582348o | |
| hybF | may modulate levels of hydrogenase-2 | 3138104o | |
| ilvB | acetolactase synthase I | 3849472i | |
| ilvD | dihydroxyacid dehydratase | 3952908i | |
| ilvG_2 | acetolactate synthase II | 3949387o | |
| ilvY | positive regulator for ilvC | 3955169i | |
| katG | catalase | 4132746i | |
| malM | periplasmic protein of mal regulon | 4247678i | |
| malT | positive regulator of mal regulon | 3552527i | |
| metL | aspartokinase II and homoserine dehydrogenase II | 4129267i | |
| modF | ATP-binding component of molybdate transport system | 792101o | |
| nfnB | oxygen-insensitive NAD(P)H nitroreductase | 604329i | |
| nrdE | ribonucleoside-diphosphate reductase 2, alpha subunit | 2801360o | |
| nrfA | periplasmic cytochrome C | 4286662i | |
| ppiA | peptidyl-prolyl cis-trans isomerase (rotamase A) | 3489568o | |
| ppx | exopolyphosphatase | 2624459o | |
| ptsI | pEP-protein phosphotransferase system enzyme I | 2533081o | |
| recQ | ATP-dependent DNA helicase | 4003683o | |
| rhaB | rhamnulokinase | 4094828o | |
| rhsA | rhsA protein in rhs element; Transposon-related functions | 3759887o | |
| sela | selenocysteine synthase | 3758861o, 3757911i | |

| | | | |
|------|---|--------------------|---------------------|
| selB | selenocysteinyl-tRNA-specific translation factor | 3756822i | |
| ubiB | ferrisiderophore reductase/flavin reductase | 4024371o | |
| ugpB | sn-glycerol 3-phosphate transport system; periplasmic binding protein | 3589389o | |
| uhpB | sensor histidine protein kinase | 3847413i | |
| wecE | putative regulator | 3973830o, 3973099o | |
| wzxE | putative cytochrome | 3974108i | |
| yfbS | putative transport protein | 2408241i | |
| yfeN | putative sugar hydrolase | 2523510o | |
| yggM | unknown | 3096577o, 3097264o | |
| ygjE | unknown | 3206400i | |
| ygjO | putative enzyme | 3232638i | |
| yhcD | putative outer membrane protein | 3362694o | |
| yhcP | unknown | 3384118o | |
| yhiQ | unknown | 3640733o | 5 different introns |
| yhjK | unknown | 3681887i | |
| yhjS | putative protease | 3694887o | |
| yhjW | unknown | 3706205i | |
| yiaM | unknown | 3742411i | |
| yiaY | putative oxidoreductase | 3754545o | |
| yibK | unknown | 3778886i | |
| yicO | unknown | 3840463o, 3840519i | |
| yidJ | unknown, similar to arylsulfatases | 3854924o | 2 identical introns |
| yidR | unknown | 3866768i | |
| yidZ | putative transcriptional regulator LYSR-type | 3890889o, 3890949i | |
| yieG | putative membrane / transport protein | 3893761i, 3893459i | |
| yieK | putative isomerase | 3896317i | |
| yieL | putative xylanase | 3897502o | |
| yigB | putative phosphatase | 3994809o | |
| yigZ | unknown | 4030395o | |
| yihO | putative permease | 4063262o | |
| yihU | putative dehydorgenase | 4070425i | |
| yjbC | unknown | 4228328i | |
| yjgR | unknown | 4487174i | |
| yqjB | unknown | 3246378o | |
| yraR | unknown | 3296369o | |
| ysgA | unknown | 4013570i | |

Integration sites are numbered according to the genome sequence of *E. coli* K-12 MG1655 (Genbank accession number U00096), followed by o or i, indicating the outside (presented) or inside DNA strand.