

**Supplemental Material**

Theresa D. Ho & Craig D. Ellermeier

Department of Microbiology  
University of Iowa  
51 Newton Rd  
Iowa City, IA 52242

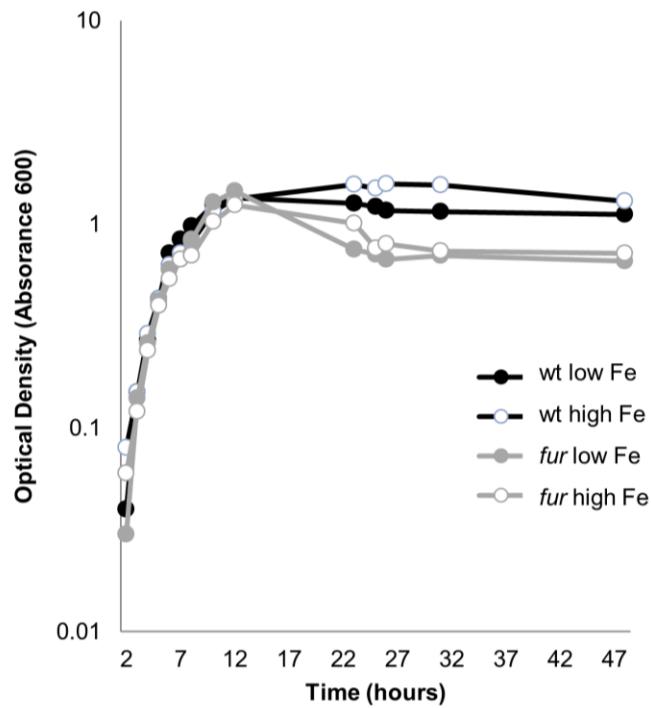
Running title: Fur repression in *C. difficile*

Keywords: iron acquisition, gene expression, *Clostridium difficile*

**Table S1. List of Oligonucleotide Primers.**

| Primer Name | Use   | Sequence 5'-3'  |
|-------------|---|---|
| RP34        | qRT PCR in <i>mldA</i>                                    | acagaaccacttattggcaaatgg  |
| RP35        | qRT PCR in <i>mldA</i>                                    | gagagtctaattccctttcatagcc   |
| CDEP3202    | 5' <i>fur</i> for <i>in vitro</i> protein synthesis       | gcgaattaatacgactcactataggcttaagtataaggaggaaaaatatg<br>gcaaatacaatggatttattaaaag |
| CDEP3203    | 3' <i>fur</i> for <i>in vitro</i> protein synthesis       | aaaccctccgttagagaggggtatgctagtattattcttctgaatcgctact<br>cc                      |
| CDEP3221    | 5' <i>fur</i> for <i>in vitro</i> protein synthesis       | gcgaattaatacgactcactataggcttaagtataaggagg                                       |
| TE485       | Test DNA contamination of RNA                             | aaggtaaccatctaagtctattctagg   |
| TE486       | Test DNA contamination of RNA                             | aaggtaacctacttttacagttcc  |
| TE2125      | <i>fur</i> targetron IBS1                                 | aaaaaaagcttataattatcccttagattacgacagtgtgcgccagatagggtg                          |
| TE2126      | <i>fur</i> targetron EBS1d                                | cagattgtacaaatgtggtgataacagataagtgcacagtcttaacttacccct<br>ttgt                  |
| TE2127      | <i>fur</i> targetron EBS2                                 | tgaacgcaagttctaattcgattnaatctcgatagaggaaagtgtct                                 |
| TE2247      | 5' P <sub>cd2992</sub> for EMSA                           | atataaaatagattattagtaagatgtatgcatt  |
| TE2248      | 3' P <sub>cd2992</sub> for EMSA                           | ctattttatcaattttcaaataaccatctagacttataaagggtgaaactttct                          |
| TE2280      | 3' end of <i>fur</i>                                      | tagggatccttattcttctgaatcgcttac  |
| TE2281      | 5' P <sub>fur</sub> for EMSA                              | taggctagcccattaaatgtaaaagcattatagtatataatctg                                    |
| TE2596      | pRPF185 + P <i>fur</i> <sub><i>C. difficile</i></sub> ITA | gaattctgcatcaagctagcccttattatgtaaaagcattatagtatataatctg                         |
| TE2597      | pRPF185 + <i>fur</i> <sub><i>C. difficile</i></sub> ITA   | ctcccttactgcaggagcttattcttctgaatcgcttactccattaca                                |
| TE2721      | 5' end of <i>fur</i>                                      | tgaccattcacatctaagttagtaaagaaatctatgatttagtttaggg                               |
| TE2723      | 5' P <sub>cd1477</sub> for EMSA                           | cgattttatataaaaatcaaatcgag  |
| TEQ005      | qRT PCR in <i>csfU</i>                                    | cgagggtgataatgtgaatgtatgcgg   |
| TEQ006      | qRT PCR in <i>csfU</i>                                    | acatggcgataacaataacaatagatgc  |
| TEQ009      | qRT PCR in <i>rpoB</i>                                    | aagagctggattcgaagtgcgtga  |
| TEQ010      | qRT PCR in <i>rpoB</i>                                    | accgatatttggccctctggagt   |
| TEQ057      | qRT PCR in <i>feoB2</i>                                   | cattggctggcaatccaaatac  |
| TEQ058      | qRT PCR in <i>feoB2</i>                                   | ccagttcccaatgtctaa  |
| TEQ059      | qRT PCR in <i>feoA3</i>                                   | gattgctgcactggatgtataa  |
| TEQ060      | qRT PCR in <i>feoA3</i>                                   | gcaaggctaaaggctctaaatc  |
| TEQ061      | qRT PCR in <i>feoB1</i>                                   | gttataggttcccagtggtag   |
| TEQ062      | qRT PCR in <i>feoB1</i>                                   | cttgcaagttcaactgcctt  |
| TEQ065      | qRT PCR in <i>fhuD</i>                                    | agttggctacagaagtatgtgaa   |
| TEQ066      | qRT PCR in <i>fhuD</i>                                    | aacttgccaacactggtaaataag  |
| TEQ077      | qRT PCR in <i>cd2992</i>                                  | gtaacctgtccatgtacacaattaag  |
| TEQ078      | qRT PCR in <i>cd2992</i>                                  | cacccttctgttcagcaaactc  |
| TEQ079      | qRT PCR in <i>cd1889</i>                                  | ttctggaggatgtaaagcaaagg   |
| TEQ080      | qRT PCR in <i>cd1889</i>                                  | cctgctgtggctcatctaata   |
| TEQ081      | qRT PCR in <i>cd1647</i>                                  | gctgggttaggaatgagcatag  |
| TEQ082      | qRT PCR in <i>cd1647</i>                                  | gctgcataatgttagcacct  |
| TEQ083      | qRT PCR in <i>cd1087</i>                                  | accagttattatgccacagga   |
| TEQ084      | qRT PCR in <i>cd1087</i>                                  | gctcctaaaggctcagacatac  |
| TEQ085      | qRT PCR in <i>cd0591</i>                                  | ggtctcttccctgtcaaataaa  |
| TEQ086      | qRT PCR in <i>cd0591</i>                                  | acataactgaaccagccgtatt  |
| TEQ087      | qRT PCR in <i>fldX</i>                                    | gtggctgaagggtgtaaagtt   |

|        |   |                                  |
|--------|---|----------------------------------|
| TEQ088 | qRT PCR in <i>fldX</i>  | tagtgctctgctcccataga             |
| TEQ089 | qRT PCR in <i>cd1485</i>  | cctcttaaagatgtgattggattatgg      |
| TEQ090 | qRT PCR in <i>cd1485</i>  | cgaatatgtgcctcggtttgt            |
| TEQ091 | qRT PCR in <i>cd2499</i>  | gattgggtggcatgaaagaatgg          |
| TEQ092 | qRT PCR in <i>cd2499</i>  | caactgcacatgtggcttcct            |
| TEQ093 | qRT PCR in <i>cd3118</i>  | aaccatggaaagatgctgaaac           |
| TEQ094 | qRT PCR in <i>cd3118</i>  | tccttcgttaacttgccttctt           |
| TEQ097 | qRT PCR in <i>cd1745A</i>   | tgaagagacttctagccttagga          |
| TEQ098 | qRT PCR in <i>cd1745A</i>   | actatagggtcattaagaggagcta        |
| TEQ099 | qRT PCR in <i>fur</i>   | cgcccacaaagaagagacaatag          |
| TEQ100 | qRT PCR in <i>fur</i>   | ttcaggacagtcaaccctaac            |
| TEQ105 | qRT PCR in <i>cd0627A</i>   | agcatgtccaagaagtgtatc            |
| TEQ106 | qRT PCR in <i>cd0627A</i>   | caaatcccacatccaacacataaaa        |
| TEQ107 | qRT PCR in <i>cd2214</i>  | aggcaggtttacatccaacata           |
| TEQ108 | qRT PCR in <i>cd2214</i>  | agtggtatgtctaaaggcagtgc          |
| TEQ121 | qRT PCR in <i>tcdA</i>  | ggagaagtcaagtatattgctctg         |
| TEQ122 | qRT PCR in <i>tcdA</i>  | cagtggtagaaaggattcaactatagcc     |
| TEQ123 | qRT PCR in <i>tcdB</i>  | aaggaatatcttagttacagaagtattagagc |
| TEQ124 | qRT PCR in <i>tcdB</i>  | gcagtgtcattattgacctcca           |
| TEQ129 | qRT PCR in <i>cd1477</i>  | aggaggatattatgatgccatt           |
| TEQ130 | qRT PCR in <i>cd1477</i> , 3'<br><i>P<sub>cd1477</sub></i> for EMSA | aaagtccctcgcttatacacttcc         |



**Figure S1. A *fur* mutant does not exhibit a growth defect.** Wild type and *fur* mutant *C. difficile* strains were grown overnight in high iron containing TY. Overnight cultures were washed in PBS and diluted 1:50 in low (DP, closed circle) or high (Fe, open circle) iron containing TY medium. Optical densities (OD<sub>600</sub>) of the wild type (black line) and *fur* mutant (gray line) cultures were measured over time.