# SUPPLEMENTARY INFORMATION

## Genetic Manipulation of *Desulfovibrio ferrophilus* and Evaluation of Fe(III) Oxide Reduction Mechanisms

Toshiyuki Ueki[a*], Trevor L. Woodard[a], and Derek R. Lovley[a,b#]

[a]Department of Microbiology, University of Massachusetts-Amherst, Amherst, MA, USA

[b]Institute for Applied Life Sciences, University of Massachusetts-Amherst

[*]Present address: Electrobiomaterials Institute, Key Laboratory for Anisotropy and Texture of Materials (Ministry of Education), Northeastern University, Shenyang 110819, China.

[#]Address correspondence to Derek R. Lovley, dlovley@umass.edu

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DFE_2355 -------------MSLVINNNLMAINAARNMGSAFSMLASSVSRLSSGLRVTSAADDAAGLAIRELMRSEIAAMHQGARN
DFE_2171 -------------MSLVINHNLMAMNAARNLTESYGRLSTSTRRLSSGLRVGTAADDAAGLAIRELMRSDIKTLHQGVRN
DFE_0942 -------------MSLVINNNLMAMNAARNLGNAYDALASSTNKLSSGLRINSAADDAAGLAIRELMRADISTLNQGVRN
DFE_0943 -------------MSLVINNNIVAMTAARNLGTAYDALSSSTNKLSSGLRITTAADDAAGLAVRELMRSDIAALDQGVRN
DFE_1910 MDAGTEAFMEESDMSMVINNNLMAMNAARNLSNSFGALSVSVRRLSSGLRIGTAADDAAGMAIREIMRSDIAALNQGVRN
DFE_0352 -------------MSLVINNNLMAANASRNLSQSYSALGTSIRRLSSGLRVGIAADDAAGLAVRELMRSDIAALGQGVRN
DFE_2105 -------------MSLVINNNLMAANAARNLSQSFGALGTSVRRLSSGLRIGTASDDAAGLAIRELMRSDIAALNQGVRN
                      **:***:*::* .*:**:  ::. *. *  :******:  *:*****:*:**:**::* :: **.**

DFE_2355 VNDAISLIQTADGAAQVIDEKLIRMKELAEQAATGTYNSDQRLIIDSEYQAMASEITRIANATDFNGIHLLNGNLSGEN-
DFE_2171 ANDAISMVQTADGALQVIDEKLIRMKELAEQAATGTYNSDQRLIIDSEYQAMASEITRIALATDFNQVHLLDGTLSGVVP
DFE_0942 ANDAISMIQTADGALQVVDEKLIRMKELAEQAATGTYSSAQRLIIDSEYQAMASEITRIANATDFNGIMLLDGNLSGTE-
DFE_0943 ANDAISMIQTADGALQVIDEKLIRMKELAEQAATGTYSSTQRLIIDSEYQAMASEITRIANATDFNGIYLLNGNLSS---
DFE_1910 ANDAISMLQTADGALAVIDEKLIRMKELAEQAATGTYNSDQRLILDSEYQAMASEITRIANATDFNGLYLLNGNLSS---
DFE_0352 ANDAISMIQTADGALQVIDEKLIRMKELAEQASTGTYNSDQRLIIDSEYQAMASEITRIANATDFNGIYLLNGNLSG---
DFE_2105 ANDAISMIQTADGALQVIDEKLIRMKELAEQGATGTYNSDQRLIIDSEYQAMASEITRIANATDFNGIYLLNGNLSS---
         .*****::******  *:*************.:****.* ****:*************** ***** : **:*.**.

DFE_2355 SAHVGSALVPTGPLKVHFGSGNDCSEDYYYISINNATASALGVGNQSA-----AGAGFSISTQSAAQFALDAIKNAIVSK
DFE_2171 TDHDGSGMQPTGPMKIHFGTGNDSSEDYYFISIGGATASHFGLGLGSDGAASNFVGGQTISTQSLAQAALAALDQAIISK
DFE_0942 ASHDGSGMSATGPLKVHFGTGNDSTEDYYYVTIGDCTASALGVGNSAAV----GSAGYTISTQSAAQSALDAINNAIVSK
DFE_0943 DTHTGSGLSSTGKMKIHFGTANDSAEDYYYVQIGNSTASALGVGNSAAA----GSAGFTISTQSAAQNALDAITNAIVSK
DFE_1910 STHDGSGLTPSGKMKIHFGTANDSAEDYYYIQIGNATASALGVGNQAV-----AGAGFAISTQSAAQNALDGINDAIVSK
DFE_0352 STHTGSGLVSSGKLKVHFGTGNDSAEDYYYVQINTSTASAMGVGNQAAS----TSAGFTISTQSAAQNALDGIKNAIVSK
DFE_2105 DTHTGSGLVSTGKMKVHFGTANDSSEDYYYIRIDTSTASALGVGNQATS----TSAGFTISTQSAAQNALDGIKNAIVSK
           * **.:  :* :*:***:.**.:****:: *. .*** :*:*  :        .* :***** ** ** .: :**:**

DFE_2355 DKIRANLGALENRLY-AVTNLEIMAENLQASESRISDVDVALEMTQFVRAQIMSQAAVAMLAQANSLPRMAMQLIGG-
DFE_2171 DKIRANLGALQNRLENTITNLQIQSENLQAAESRISDVDVALEMTEFVRNQILTQSATAMLAQANSLPRLAMQLIGGG
DFE_0942 DNIRAHLGAMQNRLSNTISNLEIQAENLQAAESRISDVDVATEMTEYSKQQIITQSATSMLAQANSLPQLALQLLGG-
DFE_0943 DNIRANLGALQNRLSNTVSNLEIQAENLQAAESQISDVDVATEMTEYSRQQIISQSATSMLAQANSLPQLALSLLG--
DFE_1910 DKIRAGLGAMQNRLENTITNLQIQAENLSASESRISDVDVALEMTNFVKQQILSQAATAMLAQANSLPRMAMQLLGG-
DFE_0352 DKIRAALGGIQNRLENTISNLQIQAENLQAAESRISDVDVSLEMTEFVRNQIMSQSAVAMLAQANSLPRMAMSLLGG-
DFE_2105 DKIRANLGALQNRLENTISNLQIQSENLQAAESRISDVDVAIEMTEFVRQQIKSSAAVSMLAQANSLPRMAMQLLGG-
         *:*** **.::***  :::**:* :***.*:**:******: ***:: : ** :.:*.:*********::*:.*:*
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Figure S1. Putative flagellins (FliC) in *D. ferrophilus*.

| Organism | fliC gene | Promoter |
|---|---|---|
| *D. ferrophilus* | *Dfe_0352* | *FliA* |
|  | Dfe_0942 | unknown |
|  | Dfe_0943 | unknown |
|  | Dfe_1910 | FliA |
|  | Dfe_2105 | FliA |
|  | Dfe_2171 | RpoN |
|  | Dfe_2355 | unknown |
| *D. desulfuricans* | *Dde_1501* | *unknown* |
|  | Dde_1502 | unknown |
|  | Dde_1570 | FliA |
|  | Dde_1709 | RpoN |
| *D. vulgaris* | *Dvu_1441* | *RpoN* |
|  | Dvu_2082 | FliA |
|  | Dvu_2444 | unknown |

Figure S2. *fliC* genes in *Desulfovibrio* species.

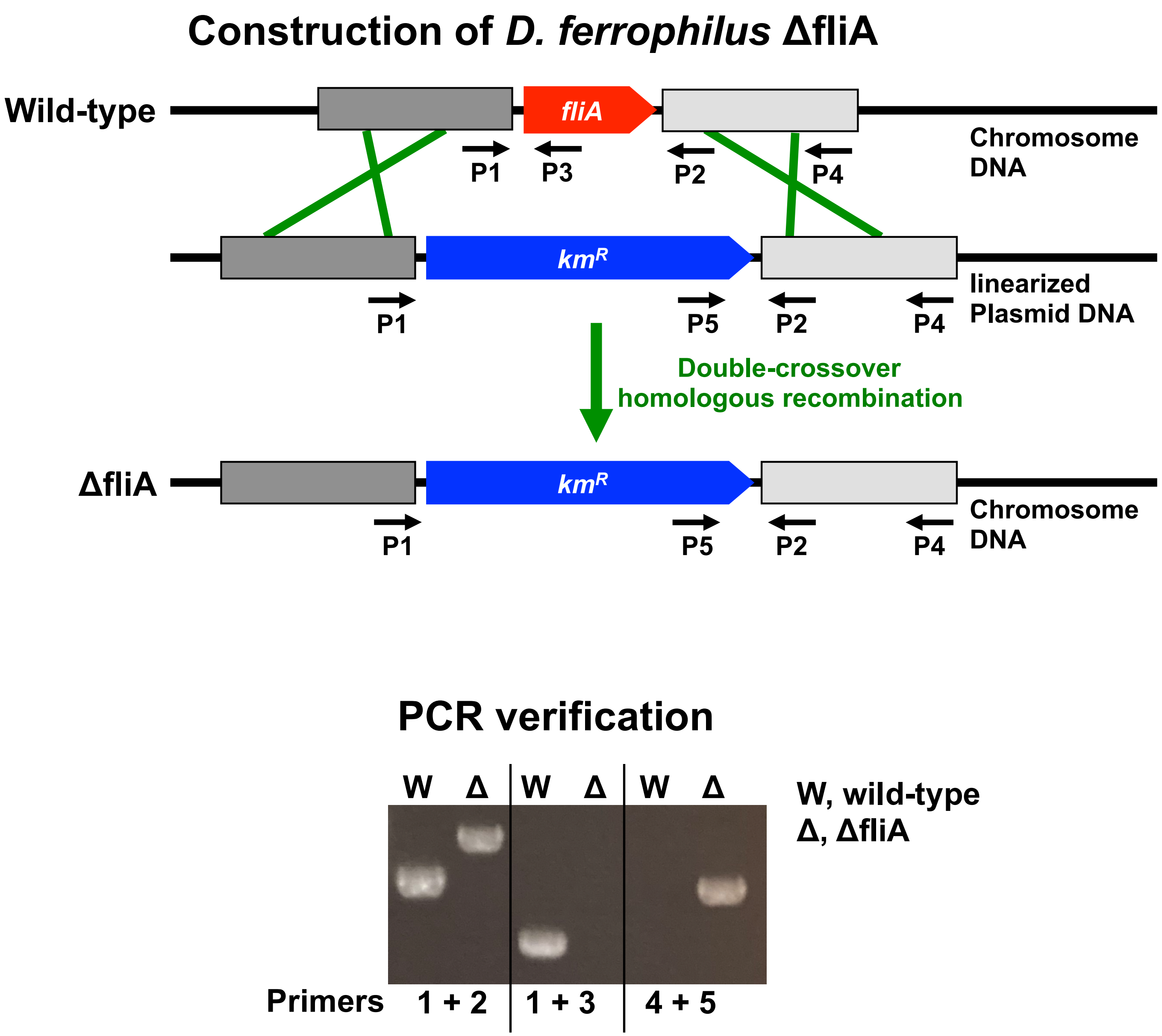
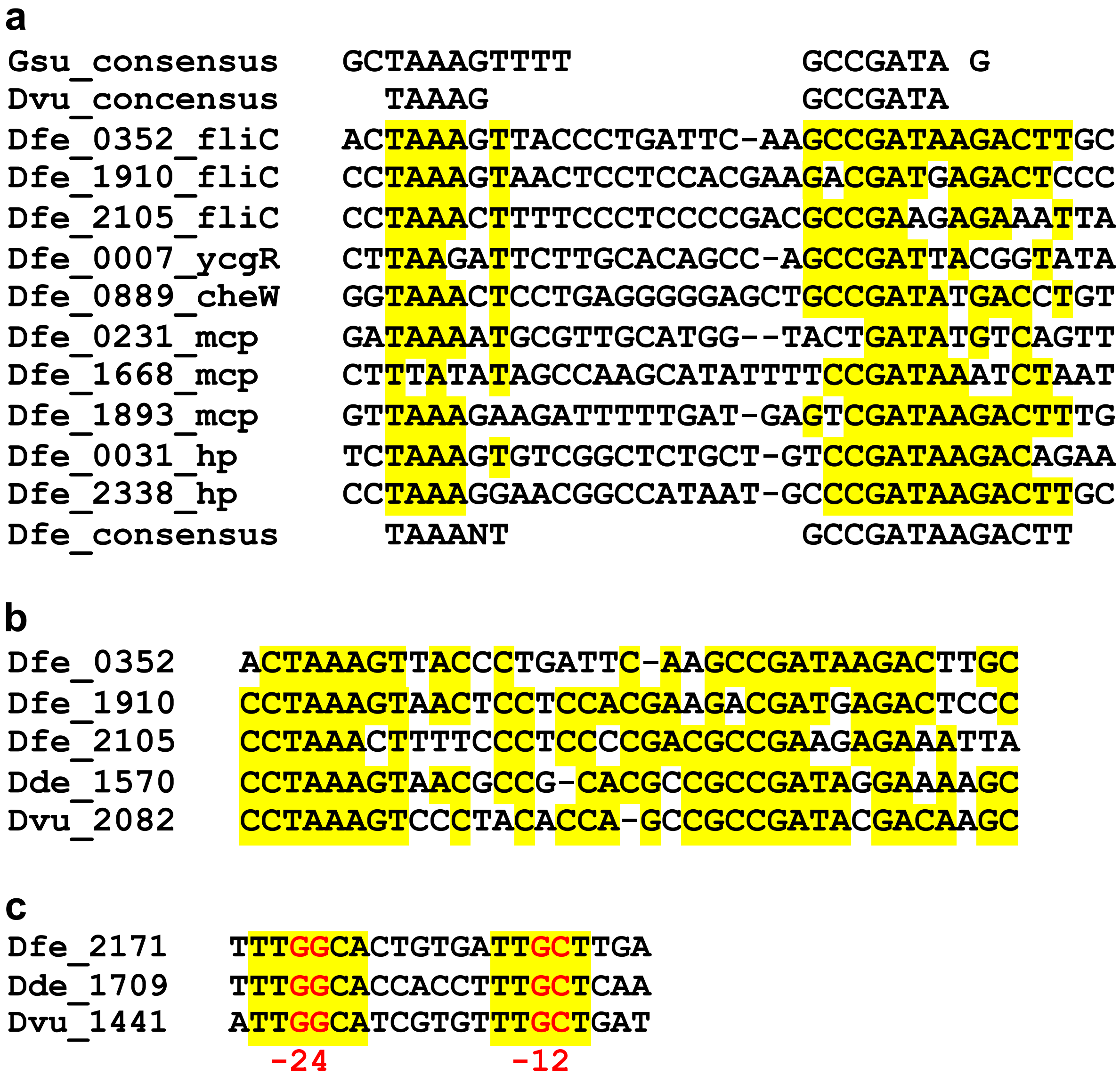


Figure S3. Construction of *D. ferrophilus* ΔfliA strain.



a

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Gsu_consensus   GCTAAAGTTTT           GCCGATA G
Dvu_concensus     TAAAG               GCCGATA
Dfe_0352_fliC   ACTAAAGTTACCCTGATTC-AAGCCGATAAGACTTGC
Dfe_1910_fliC   CCTAAAGTAACTCCTCCACGAAGACGATGAGACTCCC
Dfe_2105_fliC   CCTAAACTTTTCCCTCCCCGACGCCGAAGAGAAATTA
Dfe_0007_ycgR   CTTAAGATTCTTGCACAGCC-AGCCGATTACGGTATA
Dfe_0889_cheW   GGTAAACTCCTGAGGGGAGCTGCCGATATGACCTGT
Dfe_0231_mcp    GATAAAATGCGTTGCATGG--TACTGATATGTCAGTT
Dfe_1668_mcp    CTTTATATAGCCAAGCATATTTTCCGATAAATCTAAT
Dfe_1893_mcp    GTTAAAGAAGATTTTTGAT-GAGTCGATAAGACTTTG
Dfe_0031_hp     TCTAAAGTGTCGGCTCTGCT-GTCCGATAAGACAGAA
Dfe_2338_hp     CCTAAAGGAACGGCCATAAT-GCCCGATAAGACTTGC
Dfe_consensus     TAAANT              GCCGATAAGACTT
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b

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Dfe_0352   ACTAAAGTTACCCTGATTC-AAGCCGATAAGACTTGC
Dfe_1910   CCTAAAGTAACTCCTCCACGAAGACGATGAGACTCCC
Dfe_2105   CCTAAACTTTTCCCTCCCCGACGCCGAAGAGAAATTA
Dde_1570   CCTAAAGTAACGCCG-CACGCCGCCGATAGGAAAAGC
Dvu_2082   CCTAAAGTCCCTACACCA-GCCGCCGATACGACAAGC
```

c

```
Dfe_2171   TTTGGCACTGTGATTGCTTGA
Dde_1709   TTTGGCACCACCTTTGCTCAA
Dvu_1441   ATTGGCATCGTGTTTGCTGAT
              -24         -12
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Figure S4. Promoter region of genes for flagellar biosynthesis and motility.

(a) Putative FliA-dependent promoter elements in genes for flagellar biosynthesis and motility in *D. ferrophilus*. fliC, flagellin; ycgR, flagellar protein YcgR; cheW, chemotaxis protein W; mcp, methyl-accepting chemotaxis protein; and hp, hypothetical protein.

(b) Putative FliA-dependent promoter elements in *fliC* genes in *Desulfovibrio* species.

(c) Putative RpoN-dependent promoter elements in *fliC* genes in *Desulfovibrio* species. -24/-12 elements are indicated in red.

Gsu, *G. sulfurreducens*; Dfe, *D. ferrophilus*; Dde, *D. desulfuricans*; Dvu, *D. vulgaris*.

Highly conserved sequences are highlighted in yellow.

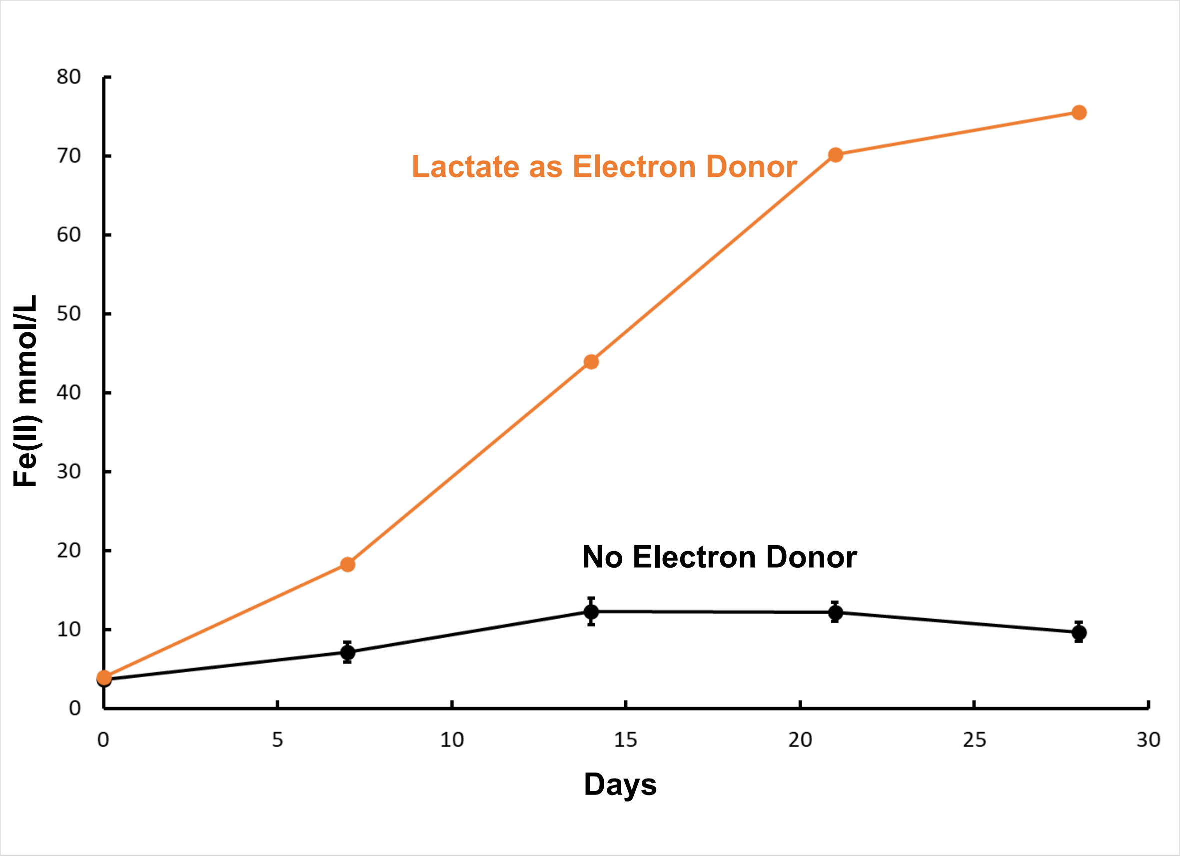

Figure S5. Lack of Fe(III) reduction in the absence of lactate. No electron donor data represents the mean and standard deviation of three replicate cultures.

Table S1. Primers used in this study.
Restriction enzyme sites are underlined. Restriction enzymes used to linearize plasmids for electroporation were indicated in bold.

| Name | Sequence | Restriction enzyme |
|---|---|---|
| Kanamycin-resistance gene | | |
| km-F | TCTGAATTCCTGACGGAACAGCGGGAAGTC | EcoRI |
| km-R | TCTAAGCTTTCATAGAAGGCGGCGGTGGAATC | HindIII |
| km-V | CTTGACGAGTTCTTCTGAGC | |
| | | |
| Deletion of DFE_1249 (ΔfliA) | | |
| fliA-up1 | ATCTCTAGACGAAGCTCGATGAAGCC | **XbaI** |
| fliA-up2 | TCTGAATTCCAGGGCTCAGTCTTGG | EcoRI |
| fliA-do1 | TCTAAGCTTCACAACTCCACTCTCAAGC | HindIII |
| fliA-do2 | TCTGGTACCAGGAATCGGATCTTGTC | KpnI |
| fliA-P1 | TGCGAACGGCCGTAATCAAC | |
| fliA-P2 | TACCGTCATCGGCTTCCACG | |
| fliA-P3 | GTGACCTCCATGATTTCGTC | |
| | | |
| Deletion of DFE_1797-1801 (Δpil5) | | |
| pil5-up1 | TCTCTCGAGCTGATGCTGTTCACCAAG | **XhoI** |
| pil5-up2 | TCTAAGCTTGTTGGGACCGTCGGAAC | HindIII |
| pil5-do1 | TCTGAATTCGCACCCAAGTTGCAATAACC | EcoRI |
| pil5-do2 | ATCTCTAGATGGCAACAACGTTGTCGC | XbaI |
| pil5-P1 | TTCCGTAGGTAGAATGTCCG | |
| pil5-P2 | CTCTTGTCATCGGTTACTGC | |
| pil5-P3 | CCGTATTCGATGCTGCACTG | |
| | | |
| Deletion of DFE_1987-1988 (Δspp) | | |
| spp-up1 | TCTGGTACCTAGATCTGTATCGGTAGC | KpnI |
| spp-up2 | TCTAAGCTTGGAATGAAGTGGTTGCATC | HindIII |
| spp-do1 | TCTGAATTCCAACGGCAATACCCAG | EcoRI |
| spp-do2 | ATCTCTAGACAGGAGGCAATCTCTGC | **XbaI** |
| spp-P1 | AGCCATCTGATTCACTGCAC | |
| spp-P2 | GTGTCGCTGCTGCATCAATC | |
| spp-P3 | CTTGGACTTGATCAGCGTGC | |
| | | |
| Deletion of DFE_1992 (Δlpp) | | |
| lpp-up1 | TCTGGTACCAAGCGCTAAACACTTAGAC | KpnI |
| lpp-up2 | TCTAAGCTTACTACGTTCCGCTGATGC | HindIII |
| lpp-do1 | TCTGAATTCCTGCCCTAACACGG | EcoRI |
| lpp-do2 | ATCTCTAGATAGTACTCGGGATTGTCG | **XbaI** |
| lpp-P1 | ACCGTTGATGTACAATCCAG | |
| lpp-P2 | TGAACTTGGGCATTGCTGTC | |
| lpp-P3 | GTGTGTCATAGGCTTGCACG | |
| | | |
| Deletion of DFE_0448-0450 (Δ448-450) | | |

|  |  |  |
|---|---|---|
| 448950-up1 | TCTGGTACCAGCCCAAACTACTGCGTG | KpnI |
| 448950-up2 | TCTAAGCTTCGGCCTCGCAGGCTGCAC | HindIII |
| 448950-do1 | TCTGAATTCGGCTGCCATAACAAGCAAC | EcoRI |
| 448950-do2 | ATCTCTAGACGTTTACCGTGTGCCGC | **XbaI** |
| 448950-P1 | AGATCTATCGCCTGATGGAC |  |
| 448950-P2 | ATCAGTCTTATGGAGCGCAG |  |
| 448950-P3 | ATTGACGGCAACCAATGCAG |  |
| Deletion of DFE_0461-0462 (Δ461-462) |  |  |
| 4612-up1 | TCTGGTACCGGTTGTTGTCGTCATG | KpnI |
| 4612-up2 | TCTAAGCTTGTCAGCAGGTGTATGCC | HindIII |
| 4612-do1 | TCTGAATTCTGCAATGCCATAAGGGATAC | EcoRI |
| 4612-do2 | ATCTCTAGACTGTTGTGCATGGTGTGAC | **XbaI** |
| 4612-P1 | AAGTCCGCAGGCCAGGATAC |  |
| 4612-P2 | AAGTGTCCGCCACATACAAC |  |
| 4612-P3 | TTATCCGAGGAATGAGCATC |  |
| Deletion of DFE_0464-0465 (Δ464-465) |  |  |
| 4645-up1 | TCTGGTACCTATGCACTGCGGTTCGAC | KpnI |
| 4645-up2 | TCTAAGCTTGCCAGCCAGGCCAATACC | HindIII |
| 4645-do1 | TCTGAATTCAAGTTTGTTGCCACGGC | EcoRI |
| 4645-do2 | ATCTCTAGAGGTATAACAGCTGTAGTCGC | **XbaI** |
| 4645-P1 | GCAGATATTGATGTTCGGAC |  |
| 4645-P2 | TCCAGTACATACGGGCATTC |  |
| 4645-P3 | GTCCGTAATCAGCAGATTGG |  |