

Supplementary Information.

The Glycyl-Radical Enzyme 2-Ketobutyrate Formate-Lyase, TdcE, Interacts Specifically with
the Formate-Translocating FNT-Channel Protein FocA

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Figure S1.

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PflD -----MTNRI SRLKTALFANTR-EISLERALLYTASHRQTEGEPVILRRAKATAYILEHVEISIRDE 61
PflF MTTLKLDTLSDRIKAHKNALVHIVKPPVCTERAQHYTEMYQQHLDKPIPVRRALALAHHLANRTIWKHD 70
PflB -----MSELNEKLATAWEGFTKGDWQNEVNVDRFIQKNYTPYEGDESFLAGATEATTTLWDKVMGKLE 65
TdcE -MKVDIDTSDKLYADAWLGFKGTDWKNEINVRDFIQHNYTPYEGDESFLAEATPATELWEKVMGIRIE 69
      .       . :       *       . . . : * :       : : :

PflD ELIAGNRTVKPRAGIMSEMPDYWLLKELDQFP----TRPQDRFAISEEDKRIYREELFPYWEKRSMKDF 127
PflF ELIIGNQASEVRAAPIFPEYTVSWIEKEIDDLA----DRPGAGFAVSEENKRVL-HEVCPWWRGQTVQDR 135
PflB NRTHAPVDFDFTAVASTITSHDAGYINKQLEKIVGLQTEAPLKRALIPFGGIKMIEGSCKAYNR---ELDP 132
TdcE NATHAPVDFDFTNIATTITAHDAGYINQPLEKIVGLQTDAPLKRALHPFGGINMIKSSFHAYGR---EMDS 136
      :       .       . :       . : : : :       *       .       . : :       . : :       *

PflD INGQMTDEVKAATNTQIF SIN-QTDKGQGHIIIDYPRLLNHGLGELVAQMQQHCQQQ-----PENHF 188
PflF CYGMFTDEQKGLLATGIIKAEGNMTSGDAHLAVNFPLLEKGLDGLREEVAERRSRINLTVLEDLHGEQF 205
PflB MKKIFTEYRKHQVDFVYTPDILRCRKSGLVLTGLPDAYGRGRIIGDYRRVALYG-----IDYL 193
TdcE EFEYLFDTLKRKHQVDFVYSPDMLRCRKSGLVLTGLPDGYGRGRIIGDYRRVALYG-----ISYL 197
      : : :       : : .       : :       * : : :       . : :

PflD YQAALLLEASQKHILRYAELAEATMAANCTDAQRREELLTIAEISRHN---AQHKPQTFWQACQLFWYMN 255
PflF LKAIIDIVLVAVSEHIERFAALAREMAATETRESRRDELLAMAENCDLI---AHQPPQTFWQALQCYFIQ 282
PflB MKDKLAQFTSLQADLENGVLEQTIRLREEIAEQHRALGQMKEMAAYGYDISGPATNAQEAIQWTFYGY 263
TdcE VRERELQFADLQSRLEKGEDLEATIRLREELAEHRHALLQIQEMAAYGFDISRPAQNAQEAVQWLYFAY 267
      :       :       . : .       *       :       . : : * : * .       . . : * * : :

PflD IILQYESNASSLSLGRFDQYMLPFY--QTSLTQGEDAAFLKELLES LWVKNDIVLLRSTSSARYFAGFP 323
PflF LILQIESNGHSVSFGRMDQYLYPYRRDVELNQTLDRHAIEMLHSCWLKLEVNKIRSGSHSKASAGSP 342
PflB LAAVKSQNGAAMSFRGTSTFLDVYIERDLKAG-KITEQEAQEMVDHLMKLRMVRFLRTPFYDELFSGDP 332
TdcE LAAVKSQNGGAMSLGR TASFLDIYIERDFKAG-VLNEQQAQELIDHFIMKIRMVRFLRTPFEDSLFSGDP 336
      :       . . * . : : * * : : :       . : :       * : : : * : : : * *

PflD TGYTALLGG---LTENGRSAVNVLSFLCLDAYQSVQ-LPQPNLGVRTNALIDTFFLMKTAETIRFGTGIP 389
PflF LYQNVTIGGQNLVDGQPMDAVNPLSYAILESCGRLR-STQPNSLVRYHAGMSNDFLDACVQVIRCGFGMP 411
PflB IWATEVIGG---MGLDGRTLVTKNSFRFLNTLYTMGPSPEPNMTILWSEKLPNFKKFAAKVS-IDTSSL 398
TdcE IWATEVIGG---MGLDGRTLVTKNSFRYLHTLHTMGPAPEPNLTILWSEELPIAFKKYAAQVS-IVTSSL 402
      . : * * : : * . * : * : :       . : * * : : * : :       . : .

PflD QIFNDEVVVP AFLNRGVSLEDARDYSVVGCVLSIPGRT-YGLHDIAMFNLLKVMIEICLHENEGNAA--- 455
PflF AFNND EIVIP EFKLGE PQDAYDYAAIGCIETAVGGKGYRCTGMSFINFARVMLAALEGGHDATSGKV 481
PflB QYENDDLMRPDFNND-----DYAIACCVSPMIVGKQMQFFGAR--ANLAKTMLYAINGGVDEKCLK-- 456
TdcE QYENDDLMRPDFNSD-----DYAIACCVSPMIVGKQMQFFGAR--ANLAKTMLYAINGGVDEKCLK-- 460
      * * : : . * .       * * : * : : * : :       * : : : . . . .

PflD -----LTYEGLLEQIRAKISHYITLMEVGSNICDIGHRDWAPVPLSSFISDCLEKGRDITD 512
PflF FLPQEKALSAGNFNNFDEVMDAWDTQIRYTRKSIEIEYVVDTMLEENVHDI LCSALVDDCIERAKSIKQ 551
PflB -----MQVGPKSEPIKGDVLDYDEVMERMDHFMWDLAKQYITALNI IHYMHDKYSYEASLMA 513
TdcE -----IQVGPKTA PLMDDVLDYDKVMDSLDHFMDWLAVQYISALNI IHYMHDKYSYEASLMA 517
      . : *       . . *       :       : *       . :

PflD GGARYNFSGVQGGIGIANLSDSLHALKGMVFEQQRLSFDLSSVLKANFATPEGEKVRARLINRFEKYGND 582
PflF GGAKYDWSGLQVGIANLGNLSAAVKLVFEQGAIGQQQLAALADDGDLTHEQLRQLRLINGAPKYGND 621
PflB LHDR-DVIRTMACGIAGLSVAADSLSAIKYAKVKPIRDEDGLAIDFEIEG-----EYPQFGNN 570
TdcE LHDR-DVYRTMACGIAGLSVATDLSAICYARVKPIRDEGLAVDFEIDG-----EYPQYGN 574
      : :       * * * . * : : : : :       : :       . : : : * * : : * * : :

PflD IDEVDNISAE LLRH YCKEVEKYQ NPR-----GGYFTPGSYTVAHVPLG SVVGATPDGRFAGEQLADGG 646
PflF DDTVDTLARAYQTYIDELKQYHNPRYGRGPVGGNYAGTSSISANVPFGAQT MATPDGRKAHTPLAEG- 690
PflB DPRVDDLAVDLVERFMKKIQKLH TYR-----DAIPTQSVLTITSNVYVGKKTGNTPDGRRAGAFPFGP- 633
TdcE DERVDSIACDLVERFMKKIKALPTYR-----NAVPTQSILTITSNVYVGKKTGNTPDGRRAGTFPAPG- 637
      * * :       . : : : :       . *       . .       . : : * *       . * * * * * : . *

PflD LSPMLGQDAQGPTAVLKS VSKLDNTLLSNGTLLNVKFTPATLEGEAGLRK-----LADFLRAFTQLKL- 709
PflF ASPASGTDHLGPTAVIGSVGKLP TAA ILGGVLLNQKLN PATLENESDKQK-----LMI LLRTFFEVHKG 754
PflB ANPMHGRDQK GAVASLTSVAKLPFAYAKDGISYTF SIVPNALGKDDEV RKTNLAGLMDGYFHHEASIEGG 703
TdcE ANPMHGRDRK GAVASLTSVAKLPFTYAKDGISYTF SIVPAALGKEDPV RKTNLVGLLDGYFHHEADVEGG 707
      . * * * * . * : * * * * : . * . . : * * : * : : * : : : : :

PflD QHIQFNVNADTLREAQRRPQDYAGLVVRVAGYSAFFVELSKEIQDDIIRRTAHQL- 765
PflF WHIQNIVSRETL L DAKKHPDQYRD LVVRVAGYSAFF TALSPDAQDDI IARTEHML- 810
PflB QHLNVNVMNREMLLDAMENPEKYPQLTIRVSGYAVRFNSLTKEQQQDVITRTFTQSM 760
TdcE QHLNVNVMNREMLLD AIEHPEKYPNLTIRVSGYAVRFNALTREQQQDVISRTFTQAL 764
      * : : * : : . * : * : * : * * : * : * * * : * : * * * *

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Figure S1. Multiple alignment of the four GREs PflB, PflD, PflF and TdcE from *Escherichia coli*. The alignment was performed with the Clustal 2.1 algorithm. The 5 amino acid residues of PflB identified by chemical cross-linking to interact with FocA, and which are conserved in TdcE, are shown in red; their adjacent, similar amino acids are shown in bold. Other amino acid residues in PflB identified to cross-link with FocA are shown as a bold green amino acid in the PflB sequence. The black bar signifies the amino acids residues adjacent to the glycine residue that is converted to a glycy radical. * indicates identical amino acids; : indicates similar amino acids; and . indicates functionally related amino acids.

Table S1. Amino acid identity between the four GRE's PflB, PflD, PflF and TdcE from *E. coli*. The amino acid identities were calculated based on the amino acid alignment shown in Fig. S1.

	PflB	TdcE	PflD
TdcE	78.82		
PflD	22.28	21.27	
PflF	22.19	23.61	38.53