

Strain	Description	Reference
RK103	MG1655 $\Delta ccm$	(2)
RK111	MG1655 $\Delta ccm$ <i>cyt c4</i> :His6 chromosomal integrate	(1)

Plasmid	Short name	Description	Reference
pRGK385	pSysI $\Delta GH$	pGEX <i>ccmABCDEF</i> :His6	(3)
pRGK386	pSysI	pGEX <i>ccmABCDEF</i> :His6GH	(3)
pRGK387	pSysI $\Delta EGH$	pGEX <i>ccmABCDEF</i> :His6	(3)
pRGK388	--	pBAD <i>ccmF</i> :His6GH	(3)
pRGK402	pSysI $\Delta FGH$	pGEX <i>ccmABCDE</i>	(1)
pRGK427	pSysI $\Delta GH$ delAB	pGEX <i>ccmCDEF</i> :His6	This work
pRGK428	pSysI $\Delta GH$ mutE	pGEX <i>ccmABCDE</i> (His130Ala) <i>F</i> :His6	This work
pRGK429	pSysI $\Delta GH$ P-His1Ala	pGEX <i>ccmABCDEF</i> (His173Ala):His6	This work
pRGK430	pSysI $\Delta GH$ TM-His1Ala	pGEX <i>ccmABCDEF</i> (His261Ala):His6	This work
pRGK431	pSysI $\Delta GH$ P-His2Ala	pGEX <i>ccmABCDEF</i> (His303Ala):His6	This work
pRGK432	pSysI $\Delta GH$ TM-His2Ala	pGEX <i>ccmABCDEF</i> (His491Ala):His6	This work
pRGK433	pSysI $\Delta GH$ Dbl P-His	pGEX <i>ccmABCDEF</i> (His173Ala/His303Ala):His6	This work
pRGK434	--	pBAD <i>ccmF</i> (His173Ala):His6GH	This work
pRGK435	--	pBAD <i>ccmF</i> (His173Gly):His6GH	This work
pRGK436	--	pBAD <i>ccmF</i> (His173Cys):His6GH	This work
pRGK437	--	pBAD <i>ccmF</i> (His173Met):His6GH	This work
pRGK438	--	pBAD <i>ccmF</i> (His173Tyr):His6GH	This work
pRGK439	--	pBAD <i>ccmF</i> (His303Ala):His6GH	This work
pRGK440	--	pBAD <i>ccmF</i> (His303Gly):His6GH	This work
pRGK441	--	pBAD <i>ccmF</i> (His303Cys):His6GH	This work
pRGK442	--	pBAD <i>ccmF</i> (His303Met):His6GH	This work
pRGK443	--	pBAD <i>ccmF</i> (His303Tyr):His6GH	This work

Primer	Sequence (5'-3')	Plasmid Constructed
delABloop_BgIII_Fw d	GCCAGA TCTATGTGGAAAACTG	pRGK427
delABloop_BgIII_Rev	GATAGA TCTACGCGGAACCA GATC	pRGK427
ccmE_H130A_Fw d	GCGAAA GAA GTGCTGGCGAAA GCCGATGAAAACTATACGCC	pRGK428
ccmE_H130A_Rev	GGCGTATAGTTTTCATCGGCTTTCCGCCA GCACTTCTTTTCGC	pRGK428
ccmF_H173A_Fw d	CCGGGGCTGATCTTCGCTCCGCCTCTGCTTT	pRGK429 pRGK434
ccmF_H173A_Rev	AAAGCAGAGGCGGAGCGAA GATCAGCCCCGG	pRGK429 pRGK434
ccmF_H173G_Fw d	CCGGGGCTGATCTTCGGTCCGCCTCTGCTTT	pRGK435
ccmF_H173G_Rev	AAAGCAGAGGCGGACC GAA GATCAGCCCCGG	pRGK435
ccmF_H173C_Fw d	GATCCGGGGCTGATCTTCTGTCCGCCTCTGCTTTATA	pRGK436
ccmF_H173C_Rev	TATAAAGCAGAGGCGGACAGAA GATCAGCCCCGGATC	pRGK436
ccmF_H173M_Fw d	GGATCCGGGGCTGATCTTCATGCCGCCTCTGCTTTATATGG	pRGK437
ccmF_H173M_Rev	CCATAAAGCAGAGGCGGCA TGAAGATCAGCCCCGGATCC	pRGK437
ccmF_H173Y_Fw d	CGGGGCTGATCTTCTATCCGCCTCTGCTT	pRGK438
ccmF_H173Y_Rev	AAAGCAGAGGCGGATA GAAGATCAGCCCCG	pRGK438
ccmF_H303A_Fw d	GGCGTGCTGGTATCGGTA GCCGCGTTTGCG	pRGK431 pRGK433 pRGK439
ccmF_H303A_Rev	CGCAAACGCGGCTACCGATA CCA GCA CGCC	pRGK431 pRGK433 pRGK439
ccmF_H303G_Fw d	GGCGTGCTGGTATCGGTA GCCGCGTTTGCG	pRGK440
ccmF_H303G_Rev	CGCAAACGCGCCTACCGATA CCA GCA CGCC	pRGK440
ccmF_H303C_Fw d	CGGCGTGCTGGTATCGGTA TGCGCGTTTGCGT	pRGK441
ccmF_H303C_Rev	ACGCAAACGCGCATACCGATA CCA GCA CGCCG	pRGK441
ccmF_H303M_Fw d	CGGCGTGCTGGTATCGGTAATGGCGTTTGCGTCTGA	pRGK442
ccmF_H303M_Rev	TCA GACGCAAACGCCATTACCGATA CCA GCA CGCCG	pRGK442
ccmF_H303Y_Fw d	GGCGTGCTGGTATCGGTA TGCGTTTGCGTCTG	pRGK443
ccmF_H303Y_Rev	CAGACGCAAACGCATATACCGATA CCA GCA CGCC	pRGK443
ccmF_H261A_Fw d	GGACTGCGCTGATGGCCTCA CTGGCGGTCA	pRGK430
ccmF_H261A_Rev	TGACCGCCAGTGAGGCCATGCGCGCAGTCC	pRGK430
ccmF_H491A_Fw d	GGGATGGTGGCGGCTGCCCTTGGGCTGGC	pRGK432
ccmF_H491A_Rev	GCCA GCCAAAGGGCAGCCGCCACCATCCC	pRGK432

## REFERENCES

- (1) San Francisco, B., Bretsnyder, E. C., Rodgers, K. R., and Kranz, R. G. (2011) Heme ligand identification and redox properties of the cytochrome c synthetase, CcmF. *Biochemistry* 50, 10974-85.
- (2) Feissner, R. E., Richard-Fogal, C. L., Frawley, E. R., Loughman, J. A., Earley, K. W., and Kranz, R. G. (2006) Recombinant cytochromes c biogenesis systems I and II and analysis of haem delivery pathways in *Escherichia coli*. *Mol Microbiol* 60, 563-77.
- (3) Richard-Fogal, C. L., Frawley, E. R., Bonner, E. R., Zhu, H., San Francisco, B., and Kranz, R. G. (2009) A conserved haem redox and trafficking pathway for cofactor attachment. *Embo J* 28, 2349-59.