

Supplementary Material for
Mutagenesis of *Klebsiella aerogenes* UreG to Probe Nickel Binding and Stabilization of
a UreG-UreE Complex

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Table S1: Plasmids and *E. coli* strains used in this study

Plasmid	Description	<i>E. coli</i> strain	Reference
pASK-IBA3plus	Plasmid for creating fusion proteins with a <i>Strep</i> -tag II (WSHPQFEK) at the C-terminus		IBA
pASK-IBA5plus	Plasmid for creating fusion proteins with a <i>Strep</i> -tag II at the N-terminus		IBA
pEC007	Modified pACT3 to encode UreE	DH5 α	(1)
pIBA3+G	Modified pASK-IBA3plus to encode UreG _{Str}	DH5 α or BL21 (DE3)	This work
pIBA5+G	Modified pASK-IBA5plus to encode UreG _{Str}	DH5 α or BL21 (DE3)	This work
pIBA3+GK20A, pIBA3+GE25A, pIBA3+GC28A, pIBA3+GD33A, pIBA3+GD49A, pIBA3+GE68A, pIBA3+GC72A, pIBA3+GH74A, pIBA3+GD80A, pIBA3+GS111A, pIBA3+GS115A, pIBA3+GD120A and pIBA3+GD127A	Modified pIBA3+G to encode the K20A, E25A, C28A, D33A, D49A, E68A, C72A, H74A, D80A, S111A, S115A, D120A and D127A variants of UreG _{Str}	BL21 (DE3)	This work
pKAU17	<i>K. aerogenes</i> urease gene cluster in pUC8		(2)
pKAUD2	pKAU17 modified to overexpress <i>ureD</i>		(3)
pKAUG-1	Modified pKAUD2 containing only <i>K. aerogenes ureG</i> . The plasmid was constructed by isolating a <i>KspI-EcoRI</i> fragment, forming blunt ends, and religating.	BL21 (DE3)	(4)
pKK17	<i>K. aerogenes</i>	DH5 α	(5)

	<i>ureDABCEFG</i> gene cluster inserted into pKK223-3		
pKKG	Modified pKK17 encoding UreG _{Str}	DH5α	This work
pKKGK20A, pKKGE25A, pKKGC28A, pKKGD33A, pKKGD49A, pKKGE68A, pKKGC72A, pKKGH74A, pKKGD80A, pKKGS111A, pKKGS115A, and pKKGD127A	Modified pKKG encoding the K20A, E25A, C28A, D33A, D49A, E68A, C72A, H74A, H74C, H74N, D80A, S111A, S115A, and D127A variants of UreG _{Str}	DH5α	This work

TABLE S2: Oligonucleotides used to generate UreG mutations

Purpose	Sequence
UreG mutation E25A	5' GTA AAA CCG CTC TGC TGG <u>CGG</u> CGC TGT GTA AAG CGA TG 3' 5' CAT CGC TTT ACA CAG CGC <u>CGC</u> CAG CAG AGC GGT TTT AC 3'
UreG mutation C28A	5' CT CTG CTG GAA GCG CTG <u>GCA</u> AAA GCG ATG CGC GAT AC 3' 5' GT ATC GCG CAT CGC TTT <u>TGC</u> CAG CGC TTC CAG CAG AG 3'
UreG mutation D33A	5' GTA AAG CGA TGC GCG <u>CGA</u> CCT GGC AGC TGG C 3' 5' G CCA GCT GCC <u>AGGTCG</u> CGC GCA TCG CTT TAC 3'
UreG mutation D49A	5' - GAC ATC TAT ACC AAA GAA <u>GCG</u> CAG CGC ATC CTC ACC GAA - 3'
UreG mutation E68A	5' - GAA CGC ATC GTC GGT GTG <u>GCG</u> ACC GGC GGC TGC CCG CAT - 3'
UreG mutation C72A	5' - GTC GGT GTG GAA ACC GGC GGC <u>GCG</u> CCG CAT ACG GCG ATC CGC GAA - 3'
UreG mutation H74A	5' - GAA ACC GGC GGC TGC CCG <u>GCA</u> ACG GCG ATC CGC GAA GAT - 3'
UreG mutation H74C	5' - GAA ACC GGC GGC TGC CCG <u>TGC</u> ACG GCG ATC CGC GAA GAT - 3'
UreG mutation H74N	5' - GAA ACC GGC GGC TGC CCG <u>AAT</u> ACG GCG ATC CGC GAA GAT - 3'
UreG mutation D80A	5' - CAT ACG GCG ATC CGC GAA <u>GCG</u> GCC TCA ATG AAC CTC GCC - 3'
UreG mutation S111A	5' - GAA AGC GGC GGC GAT AAC CTG <u>GCC</u> GCC ACC TTC AGC CCG GAG CTG - 3' 5' - CAG CTC CGG GCT GAA GGT GGC <u>GGC</u> CAG GTT ATC GCC GCC GCT TTC - 3'
UreG mutation S115A	5' - AAC CTG AGC GCC ACC TTC <u>GCC</u> CCG GAG CTG GCG GAT CTG - 3' 5' - CAG ATC CGC CAG CTC CGG <u>GGC</u> GAA GGT GGC GCT CAG GTT - 3'
UreG mutation D120A	5' C CGG AGC TGG CGG <u>CGC</u> TGA CCA TCT AC 3' 5' GT AGA TGG TCA <u>GCG</u> CCG CCA GCT CCG G 3'
UreG mutation D127A	5' CA TCT ACG TCA TCG <u>CGG</u> TGG CCG AAG GGG AG 3' 5' CT CCC CTT CGG CCA <u>CCG</u> CGA TGA CGT AGA TG 3'