SUPPORTING INFORMATION

Influence of Cofactor Regeneration Strategies on Preparative-Scale, Asymmetric Carbonyl Reductions by Engineered *Escherichia coli*

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dkgA gene knockout and strain characterization

The method of Derbise *et al*. was used to delete the complete coding region of the dkgA gene from the chromosome of *E. coli* BL21(DE3).[1] Overlap extension PCR was used to locate the FRT-kan-FRT cassette (amplified from plasmid pKD13) between 500 bp homologous regions immediately adjacent to the *dkgA* gene (Supporting Information Scheme 1). The assembly was used to transform *E. coli* strain BL21(DE3) (pKD46), which expressed the λ red recombinase under control of an arabinose-inducible promoter. Cells were grown at 37°C to eliminate plasmid pKD46 (due to its temperature-sensitive replicon) with selection for kanamycin resistance, yielding a single colony. The results of several PCR amplifications confirmed that the *dkgA* gene had been deleted precisely and replaced by a kanamycin resistance gene, as designed. This resulting strain was designated BL21(DE3)ΔdkgA::kan. The kanamycin resistance gene was removed by recombination to leave a single FRT site at the original *dkgA* locus (designated *E. coli* BL21(DE3) ΔdkgA). The growth rate of BL21(DE3) ΔdkgA was identical to that of the parent BL21(DE3) in rich medium under aerobic conditions (data not shown).

Plasmid maintenance by *serA* complementation

As an alternative to maintaining plasmids by antibiotic resistance, we also explored an alternative strategy in which a plasmid-borne *serA* gene complemented a chromosomal deletion in the host strain to restore serine prototrophy.[2] Two *serA*-containing plasmids that overexpressed dehydrogenase Gcy1 from identical promoters were tested. In one (pBC0087), *serA* expression was driven by the native promoter while the other plasmid (pBC0464) contained a mutated *serA* promoter that significantly decreased SerA expression. *E. coli* strains containing

one of these *serA* plasmids or an analogous plasmid that utilized antibiotic selection were grown in minimal medium and induced. While all three strains grew at identical rates, a native *serA* promoter on the plasmid decreased Gcy1 specific activity in the crude by an order of magnitude as compared to otherwise identical plasmids that used either low-level SerA expression or antibiotic resistance for selection (data not shown). These results demonstrated that an exogenous dehydrogenase can be overexpressed without antibiotic selection and also suggest that a gene involved in cofactor regeneration could replace the chromosomal *serA* locus to allow both plasmid maintenance and cofactor supply.

Experimental section

Deletion of the dkgA *gene in BL21(DE3).*

To obtain genomic DNA, cells were harvested from a 500 mL culture of *E. coli* strain BL21(DE3) by centrifugation (4,000 × *g* for 10 min at 4°C). After resuspending in 250 mL of minimal salt solution (93 mM KP_i, 76 mM $(NH_4)_2SO_4$, 3.3 mM sodium citrate, 4 mM $MgSO_4$), the cells were collected by centrifugation as described above. Cells were resuspended in 10 mL of minimal salt solution, then an equal volume of cell lysis solution was added (100 mM Tris-Cl, 100 mM EDTA, 10 mM NaCl, 0.5% SDS, pH 8.0) and the suspension was mixed gently by inversion. Proteins were removed by phenol extraction and the DNA was precipitated by EtOH. After drying, the pellet was dissolved in 2 mL of TE buffer (pH 7.5) and extracted with equal volumes of 1 : 1 phenol-chloroform followed by chloroform, then the DNA was precipitated by EtOH. The dried pellet was dissolved in TE buffer (pH 8.0) and DNA concentration was estimated by UV absorbance (A_{260} / A_{280} = 2.1).

A cassette containing the kanamycin resistance gene flanked by FRT sites was amplified from plasmid pKD13[1] using primers pKD13F (GTG TAG GCT GGA GCT GCT TC**)** and pKD13R (ATT CCG GGG ATC CGT CGA CC). Agarose gel electrophoresis confirmed that the expected 1.3 kb fragment had been produced. The PCR product was digested with *Dpn*I overnight (to linearize template DNA), then the 1.3 kb band was purified by low-melt agarose. The purified material was used as a template for five additional PCR amplifications under the same conditions. After precipitation with EtOH, the 1.3 kb product was purified by low-melt agarose.

E. coli genomic DNA was used as the template in PCR amplifications of *ca*. 500 bp fragments immediately adjacent to the *dkgA* gene. The upstream reaction used primers UpF (GGC GCT GGC GTA CCG CAG GAC) and UpR-pKD13F (GAA GCA GCT CCA GCC TAC ACA CGT TCC TCC TTT ATA TGA ACT CA) and the downstream reaction used primers DownF-pKD13R (GGT CGA CGG ATC CCC GGA ATC ATG CAA ATT CTC CCG GTG GCG G) and DownR (TTT GGG TTA TCG CAA CGC TTT CTA). The underlined portions are complementary to the ends of the FRT-kan-FRT cassette; the remaining portions are complementary to *E. coli* genomic DNA.

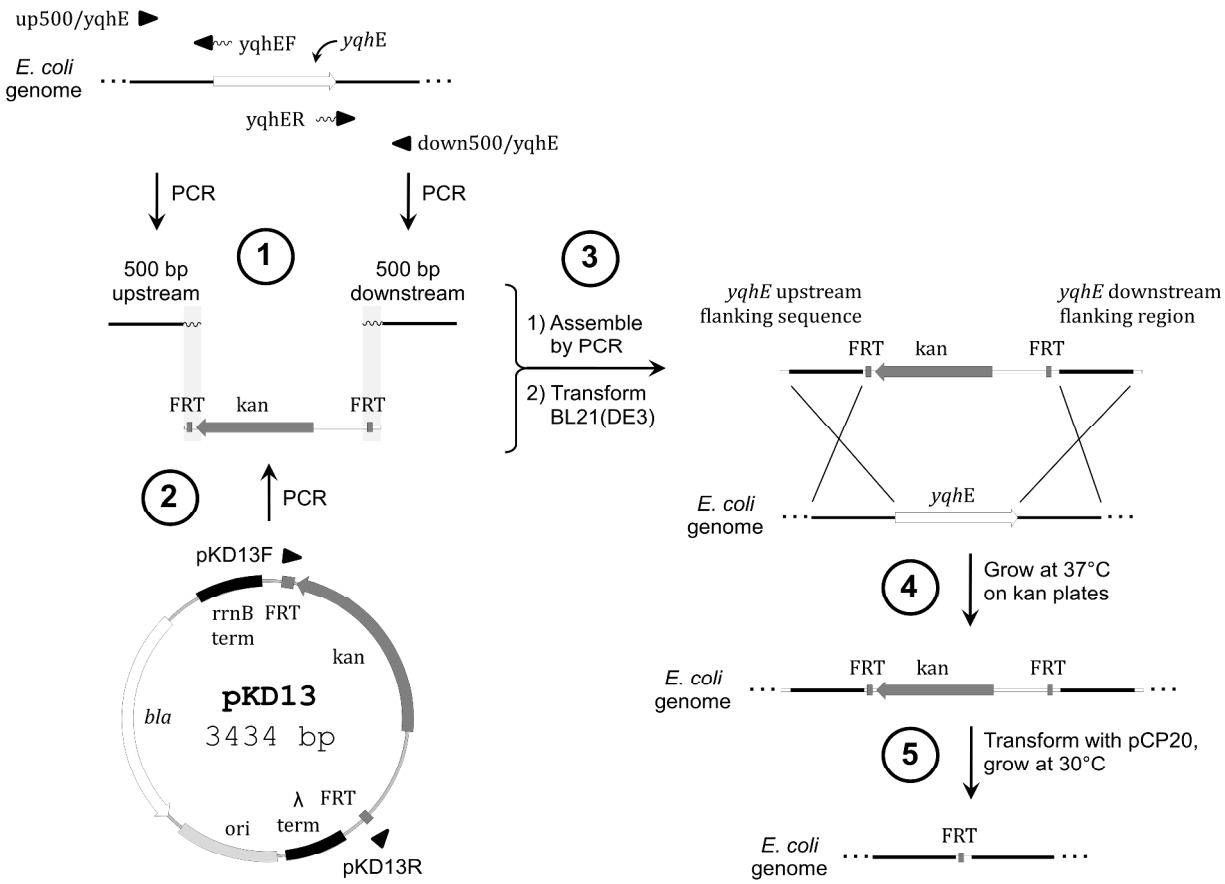
The linear DNA required to knock out the *dkgA* gene was assembled by mixing equal portions of the upstream and downstream PCR products (*ca*. 200 ng) along with the FRT-kan-FRT cassette (*ca*. 1,000 ng) and primers UpF and DownR. PCR amplification was carried out with 1 : 3 *Pfu* : *Taq* DNA polymerases. The reaction mixture was diluted 1 : 100 into a new PCR amplification mixture with primers UpF and DownR. The desired 2.3 kb fragment was isolated by low-melt agarose, then this was used as a template for PCR amplifications with primers UpF and DownR. The final product was purified by low-melt agarose.

E. coli BL21(DE3) (pKD46) cells were prepared for *dkgA* gene knockout by diluting an overnight culture (grown at 30°C in LB medium containing 100 μg / mL ampicillin) 100-fold into 50 mL of SOB medium supplemented with 100 μg / mL ampicillin. After shaking for 1 h at room temperature, arabinose was added to a final concentration of 1 mM. The culture was chilled on ice for 20 min when the $O.D._{600}$ reached 0.55, then cells were prepared for electroporation as usual. The PCR-assembled cassette described above (2 μL) was used to transform a 40 μL aliquot of competent cells in a 0.2 cm cuvette (2.5 kV, 25 μF, 200 Ω). After dilution with 600 μL of SOC medium, the suspension was transferred to a 15 mL test tube and shaken at 37°C before spreading on an LB plate containing 25 μg / mL kanamycin. After incubating at 37°C, a single colony was obtained. PCR reactions were carried out directly on cells (dispersed from a sterile loop into 4 – 8 μL of sterile water). PCR amplifications contained 2 μL of cell suspension along with 10 U of *Taq* DNA polymerase, 200 μM of each dNTP and 200 ng of each primer using the same temperature program as described above. The results of several PCR amplifications confirmed that the desired chromosomal gene replacement had occurred in this colony (Supporting Information Table 1). Primers used are listed above, in addition to UpF550 (TGC GCG CCA ACG TCA TGT GGG; anneals 550 bp upstream from the start of the *dkgA* gene), DownR550 (ATC TTT AAA GAT ACG AAA GCG CAC; anneals 550 bp downstream from the end of the *dkgA* gene) and k2 (CGG TGC CCT GAA TGA ACT GC; anneals within the kanamycin resistance gene). This strain was designated BL21(DE3) ΔdkgA::kan. The kanamycin resistance gene was removed by transforming this strain by electroporation with pCP20. Transformants were spread on LB plates containing 100 μg / mL ampicillin. A single colony was picked and grown to saturation in 3 mL of LB liquid medium at 37°C. Dilutions were spread on LB plates containing no antibiotic, kanamycin (25 μg / mL) or

ampicillin (100 μg / mL). These were grown at 37°C to eliminate pCP20. No colonies were observed on the ampicillin-containing plate, very few on the kanamycin-containing plate and a lawn appeared on the plate lacking antibiotic. A single colony was chosen from the last plate and analyzed by PCR, which revealed that the FRT-kan-FRT cassette had been replaced by an FRT site (Table 3). This final strain was designated BL21(DE3) ΔdkgA.

Supporting Information Table 1. PCR characterization of parent and *dgkA* knockout strains. The parent strain is BL21(DE3) (pKD46) and the knockout strains were designated BL21(DE3) ΔdkgA::kan and BL21(DE3) ΔdkgA.

Primers	Strain	Predicted size (kb)	Observed size (kb)
UpF, DownR	Parent	0.80	0.80
	ΔdkgA::kan	no band	background
Up500, Down500	Parent	1.8	1.8
	ΔdkgA::kan	2.3	2.3
UpF550, DownR550	Parent	1.9	1.9
	ΔdkgA::kan	2.4	2.4
	ΔdkgA	1.2	1.2
UpF550, DownR	Parent	1.4	1.4
	ΔdkgA::kan	no band	no band
Up500, k2	ΔdkgA::kan	1.2	1.2
Up550, k2	ΔdkgA::kan	1.2	1.2



Supporting Information Scheme 1

Supporting Information references

(1) Derbise, A.; Lesic, B.; Dacheux, D.; Ghigo, J. M.; Carniel, E. *FEMS Immunol. Med. Microbiol.* **2003**, *38*, 113.

(2) Li, K.; Frost, J. W. *Biotechnol. Progr.* **1999**, *15*, 876.