

Construction of inducible expression vector pDNLS-22n containing *P. aeruginosa* PAO1 ChiC

Novia Chan, Sagar Pannu, Lena Xiong, David Yeung

Department of Microbiology and Immunology, University of British Columbia, Canada

SUPPLEMENTAL MATERIALS

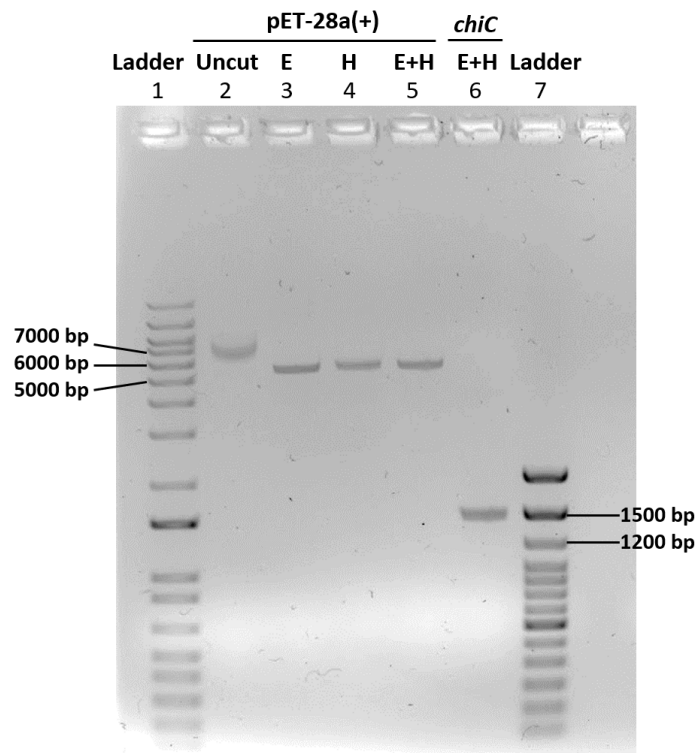


Figure S1. Agarose gel electrophoresis of digested pET-28a(+) and *chiC* PCR product demonstrates successful EcoRI and HindIII digestion. Purified pET-28a(+) and *chiC* were digested for 20 minutes at 37°C (Figure 1, Step 2). The restriction enzymes were heat inactivated, and 100 ng of DNA were loaded into each well for agarose gel electrophoresis. Lane 1 and 7: 1 Kb Plus DNA Ladder and 100 bp DNA Ladder, respectively. Lane 2: undigested pET-28a(+). Lane 3: EcoRI digested pET-28a(+). Lane 4: HindIII digested pET-28a(+). Lane 5: EcoRI and HindIII digested pET-28a(+). Lane 6: EcoRI and HindIII digested *chiC* PCR product. Linearized pET-28a(+) is expected to be 5369 bp. Digested *chiC* is expected to be ~1.4 to 1.5 kb. E: EcoRI. H: HindIII.

Table S1. Sequences of primers used for PCR amplification, colony PCR, and Sanger sequencing are shown below. The R1F/R1R primers from Bodykevich *et al.* (7) and the 2eta-chiC (2 η) primers were used for PCR amplification of the *chiC* gene from purified pGKMS21. 2eta-chiC primers were also used for colony PCR on the *chiC* gene from pGKMS21 and pDNLS-22n. The universal T7 promoter and terminator (T7F/T7R) and internal *chiC* forward and reverse (intF/intR) primers were designed for Sanger sequencing of pDNLS-22n.

Primer	Sequence (5' to 3')
R1F	GAAATGATCAGGATCGACTTTTCCC
R1R	AAGCGCAGCGGCCCGCCAGAG
2eta-chiC (2 η) forward	AGCTCGAATTCATGATCAGGATCGACTT
2eta-chiC (2 η) reverse	CATGTAAGCTTTCAGCGCAGCGG
Universal T7 promoter (T7F)	TAATACGACTCACTATAGGG
Universal T7 terminator (T7R)	GCTAGTTATTGCTCAGCGG
Internal <i>chiC</i> forward (intF)	CAAGTACGTGCCTTATCTGCAG
Internal <i>chiC</i> reverse (intR)	GAAGTCTTCTTTCATCGCGTCG