Supplementary Data Liang et al

Methods

Oligonucleotides used in the study are listed in Table S1. Polyphosphate extraction in Figure S2 was carried out using a GENECLEAN™ kit (MP Biomedicals Europe) as described [1]. Briefly, 4 M guanidine isothiocyanate (GITC)–50 mM Tris-HCl, pH 7.0 (GITC lysis buffer), prewarmed to 95°C was used to lyse pelleted cells, then 10% sodium dodecyl sulphate (SDS), 95% ethanol, and Glassmilk was added to adsorb polyphosphate, washing with New Wash buffer. Polyphosphate was eluted from the pellet by adding 50 □l of 50 mM Tris-HCl (pH 8.0) at 95°C for 2 min, recovery of polyphosphate was completed with two additional elutions.

Polyphosphate chain length in polyphosphate extracts (Fig S2) was visualised in polyacrylamide gels by 4',6-diamidino-2-phenylindol (DAPI) negative staining [2]. Gels were agitated for 30 min in 2 mg/mL DAPI in fixative at room temperature. Gels were then exposed to 365 nm light via a UV transilluminator for 2 – 20 min to induce specific photobleaching of polyphosphate bound to DAPI.

References

1. Ault-Riche D, Fraley CD, Tzeng C-M, Kornberg A. Novel Assay Reveals Multiple Pathways Regulating Stress-Induced Accumulations of Inorganic Polyphosphate in Escherichia coli. J Bacteriol. 1998;180:1841-1847.
2. Serafim LS, Lemos PC, Levantesi C, Tandoi V, Santos H, Reis MAM. Methods for detection and visualization of intracellular polymers stored by polyphosphate-accumulating microorganisms. Journal of Microbiological Methods. 2002;51:1-18.

Oligonucleotides used in this study

Table S1

Name	Sequence (5'-3')
PPK1-F	AGTGAGCTCATGGGTCAGGAAAAGCTATACATCGAAAAAGAACTC
PPK1-R	AATAAAGCTTTTATTCAGGTTGTTCGAG
PPX-F	GGATCCAAATGGAAGGACGTTTCCGT
PPX-R	GAATTCCCCGCAAAGTATTAAGCGG
ACYCDuetUP1	GGATCTCGACGCTCTCCCT
DuetDOWN1	GATTATGCGGCCGTGTACAA
DuetUP2Primer	TTGTACACGGCCGCATAATC
T7 Terminator Primer.	GCTAGTTATTGCTCAGCGG

Fig. S1

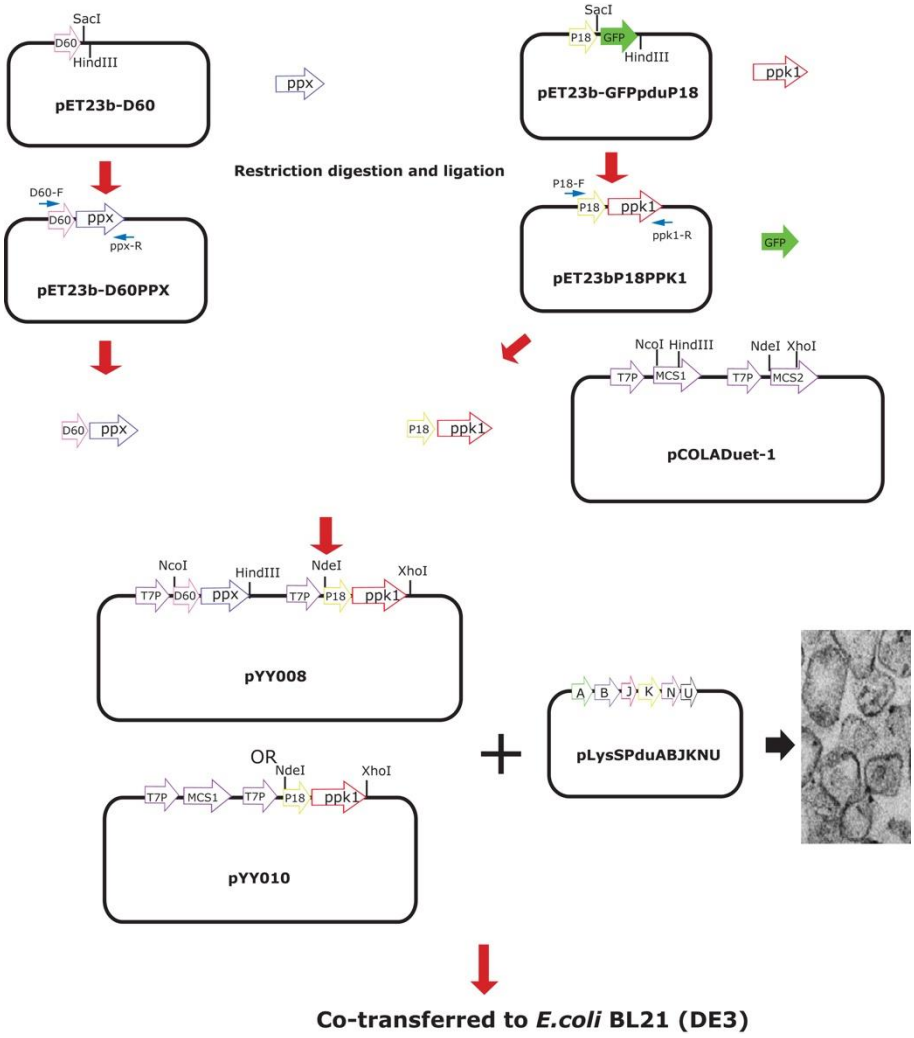
SacI
D60
HindIII
pET23b-D60
ppx
SacI
P18
GFP
HindIII
pET23b-GFPpduP18
ppk1
Restriction digestion and ligation
D60-F
D60
ppx
ppx-R
pET23b-D60PPX
P18-F
P18
ppk1
ppk1-R
pET23bP18PPK1
GFP
D60
ppx
P18
ppk1
NcoI HindIII
NdeI XhoI
T7P
MCS1
T7P
MCS2
pCOLADuet-1
NcoI
HindIII
NdeI
XhoI
T7P
D60
ppx
T7P
P18
ppk1
pYY008
OR
NdeI
XhoI
T7P
MCS1
T7P
P18
ppk1
pYY010
A
B
J
K
N
U
pLysSPduABJKNU
Co-transferred to E.coli BL21 (DE3)

Fig. S2

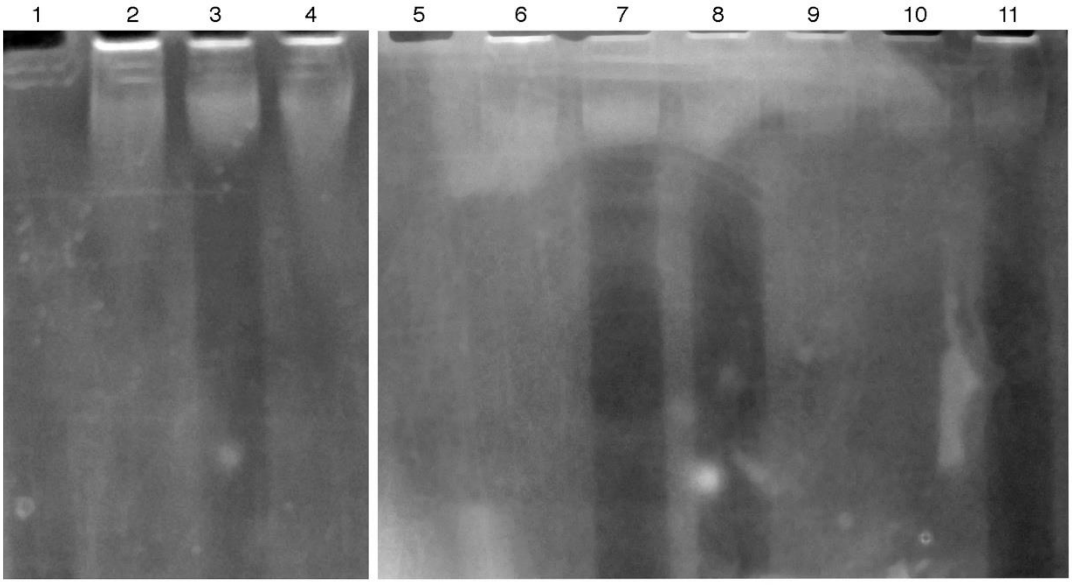

Figure Legends

Figure S1 Vector Cloning Strategy

Figure S2 Size distribution of whole cell extracted polyphosphate. DAPI PAGE gel of polyphosphate extractions from *E. coli* quantified in Figure 3. Polyphosphate associated with DAPI is bleached by prior UV exposure and appears as a dark vertical band. High molecular weight shadows below the inoculation well correspond to lipid (extracts treated with micrococcal nuclease to remove DNA). Lane 1, 5 Sodium phosphate glass Type 45 (Sigma)
Lane 2,3,4 Time zero extractions. Lane 6,7,8 time 24 hours Lane 9,10,11 time 48 hours. Lane 2,6,9 *E.coli* BL21 (DE3) control. Lane 3,7,10 *E. coli* BL21 (DE3) pML1 (*p18ppk1*). Lane 4,8,11 E.coli BL21 (DE3) pML1 (*p18ppk1*) pLysSPduABJKNU .