Supporting Information (SI)

Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle exit during starvation in *Pseudomonas aeruginosa*

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Strain construction
Strains, plasmids, and primers used in this study are listed in Tables S1-3 respectively.

Plasmids
All plasmids were generated using Gibson cloning (1). Plasmids pLREX9, pLREX21, pLREX23, pLREX24, pLREX25, pLREX27, pLREX35, and pLREX36 are derivatives of suicide vector pMQ30 (2), generated by amplifying 1 kb of sequence upstream and downstream of the target gene from P. aeruginosa genomic DNA. For pLREX24, 1 kb of sequence upstream of phaC1 and 1 kb of sequence downstream of phaC2 were used, resulting in deletion of three intervening genes: phaC1, phaD and phaC2. For pLREX27, the last 13 bases of ppk1 were retained because of overlap with the c-terminus of the coding region of the exopolyporphatase ppx, transcribed from the opposite strand. Plasmid pLREX15 is a derivative of pMQ72 (2), generated by amplifying the coding sequence for ppk2B (PA14_33240) from P. aeruginosa genomic DNA using primers that insert a 6His tag, followed by the Tev cleavage site on the 5’ terminus of the protein. Plasmids pLREX60 and pLREX61 are derivatives of pMQ70 (2), generated by amplifying the coding sequence for ppk2A (PA14_01730) from P. aeruginosa genomic DNA using primers that insert a Flag tag, followed by a linker containing an 8 amino acid serine/glycine linker, on the 5’ terminus of the protein. Plasmids pLREX38, pLREX49, pLREX62, pLREX63, pLREX64, pLREX65, and pLREX66 are derivatives of pUC18R6K- mini-Tn7T-Gm (3). pLREX38 was generated by amplifying the ssb coding sequence and 1 kb upstream sequence from P. aeruginosa genomic DNA. pLREX49 was generated by amplifying the ParS<sup>glmMTI</sup> sequence from pFHC3228 (4), the upstream sequence containing the ssb promoter from P. aeruginosa genomic DNA, and the GFP-parB<sup>glmMTI</sup> chimera from pFHC2973 (4). pLREX62 was generated by amplifying the ParS<sup>glmMTI</sup> sequence from pFHC3228 (4), the ssb coding sequence and 1 kb upstream sequence from P. aeruginosa genomic DNA, and the GFP-parB<sup>glmMTI</sup> chimera from pFHC2973 (4). pLREX63 was generated by amplifying the ssb coding sequence and 1 kb upstream sequence from P. aeruginosa genomic DNA, and the GFP-parB<sup>glmMTI</sup> chimera from pFHC2973 (4). pLREX64 was generated by amplifying a fragment containing araC, and P<sub>ara</sub> Flag-ppk2A (PA14_01730) followed by the T1T2 transcriptional terminator from pLREX60, and a fragment containing ParS<sup>glmMTI</sup> P<sub>ssb</sub> ssb-mCherry GFP-parB<sup>glmMTI</sup> from pLREX62. pLREX65 was generated by amplifying a fragment containing araC, and P<sub>ara</sub> Flag-ppk2A<sup>D183A,R184A</sup> (PA14_01730) followed by the T1T2 transcriptional terminator from pLREX61, and a fragment containing ParS<sup>glmMTI</sup> P<sub>ssb</sub> ssb - mCherry GFP-parB<sup>glmMTI</sup> from pLREX62. pLREX66 was generated by amplifying a fragment containing araC, and P<sub>ara</sub> His-Tev-ppk2B (PA14_33240) followed by the T1T2 transcriptional terminator from pLREX15, and a fragment containing ParS<sup>glmMTI</sup> P<sub>ssb</sub> ssb-mCherry GFP-parB<sup>glmMTI</sup> from pLREX62.

Strains
All unmarked deletion strains, and strains in which the endogenous ppk2A is replaced by a ppk2A-mCherry chimera, were generated by trip parental conjugation with P. aeruginosa UCBPP-PA14, and then merodiploids were selected as described by Choi and Schweizer (3) on VBMM medium (3 g/L trisodium citrate, 2 g/L citric acid, 10g/L K<sub>2</sub>HO₄, 3.5 g/L
NaH\textsubscript{2}PO\textsubscript{4}, 1mM MgSO\textsubscript{4}, 100uM CaCl\textsubscript{2}, pH 7) containing 100 ng/mL gentamicin. Counterselection for homologous recombination events removing the endogenous copy of the gene in question was then performed on LB plates without NaCl and containing 20mM sucrose, followed by PCR. All strains with insertions at the attTn\textsuperscript{7} site were generated by tetraparental conjugation with \textit{P. aeruginosa} UCBPP-PA14, and then exconjugants were selected as described by Choi and Schweizer (3) on VBMM medium, and verified by PCR.

**Media and Growth conditions**
For nitrogen starvation experiments, strains were grown at 37\textdegree C shaking in MOPS-buffered minimal media overnight (40mM sodium succinate, 22mM NH\textsubscript{4}Cl, 43mM NaCl, 2.2mM KCl, 1.25mM NaH\textsubscript{2}PO\textsubscript{4}, 1mM MgSO\textsubscript{4}, 0.1mM CaCl\textsubscript{2}, 7.5\mu M FeCl\textsubscript{2}·4H\textsubscript{2}O, 0.8\mu M CoCl\textsubscript{2}·6H\textsubscript{2}O, 0.5\mu M MnCl\textsubscript{2}·4H\textsubscript{2}O, 0.5\mu M ZnCl\textsubscript{2}, 0.2\mu M Na\textsubscript{2}MoO\textsubscript{4}·2H\textsubscript{2}O, 0.1\mu M NiCl\textsubscript{2}·6H\textsubscript{2}O, 0.1\mu M H\textsubscript{3}BO\textsubscript{3}, and 0.01\mu M CuCl\textsubscript{2}·2H\textsubscript{2}O, 50mM MOPS, pH 7.2). 10-25mL cultures at OD\textsubscript{500} = 0.0125 to 0.025 were grown in 250mL Erlenmeyer flasks at 37\textdegree C shaking to OD\textsubscript{500} = 0.4 to 0.6, then spun down at room temperature in 50mL conical tubes at 5000xG, and resuspended to OD\textsubscript{500} = 0.4 in nitrogen-limited MOPS-buffered minimal media (Identical to MOPS-buffered media, but with 1mM NH\textsubscript{4}Cl instead of 22mM) in clean Erlenmeyer flasks and grown at 37\textdegree C shaking. Time 0h = cells collected immediately before being spun down and shifted to nitrogen-limited medium.

**RNA Extraction**
Total RNA was extracted from \~10\textsuperscript{9} cells using the RNeasy Mini Kit (Qiagen), including the optional on-column DNase treatment step. 5-10 \mu g per sample were subsequently treated with Turbo DNase Free (Ambion) according to the manufacturer’s instructions. Absorbance at 260 nm was used to quantify purified RNA, and qPCR of the purified RNA was used to verify thorough removal of free of genomic DNA.

**qRT-PCR**
The iScript cDNA synthesis kit (BioRad) was used to convert 1 \mu g DNase-treated total RNA to cDNA. 1/100\textsuperscript{th} of the reaction mix (representing 10 ng of total RNA) was subsequently used as template for each qRT-PCR reaction using the iTaq SYBR Green reaction mix (BioRad) and 500 nM each of forward and reverse primers. Samples were run using a 40 cycle program with an annealing temperature of 60\textdegree C on a Real Time 7500 PCR Machine (Applied Biosystems). Samples were assayed in biological triplicate. Threshold cycle values of OprI were used as an exogenous control. Expression values are reported relative to the expression value of the same gene in exponential growth phase (0h). Primers were designed using Primer3 and are listed in Table S3.

**Granule statistics (TEM)**
For Figure 3, global analysis of granule volume and count are shown, combining all cells from multiple independent experiments for a given timepoint (n = 4 experiments for 1h, n = 3 for 1.5h, n = 2 for 2h, n = 4 for 3h, n = 3 for 6h, n = 2 for 24h). Average of means from individual data sets: Granules/cell: 11.7±2.5 at 1h; 7.1±1.8 at 1.5h, 5.4±0.3 at 2h, 3.7±1.9 at 3h, 5.6±1.7 at 6h, 6.7±0.9 at 24h. Granule volume: 0.0020±.0001\mu m\textsuperscript{3} at 1h, 0.0048±0.0015 \mu m\textsuperscript{3} at 1.5h, 0.0096±0.0057 \mu m\textsuperscript{3} at 2h, 0.0129±0.0049 \mu m\textsuperscript{3} at 3h, 0.0137±0.0013 \mu m\textsuperscript{3} at 6h, 0.0081±.0006 \mu m\textsuperscript{3} at 24h.
 Simulation of Granule Positioning

Model for the simulation (See Fig S6a): mEnd = user-defined constraint on the minimum distance (in microns) from cell ends. For the first granule, lowerBound1 and upperBound1 are defined by the sum of the radius of granule 1 (r1) and a user-defined minimum distance for granules from cell ends (mEnd). The allowed region for the origin of granule 1: between lowerBound and (cell Length minus upperBound). For the second granule, lowerBound2 and upperBound2 defined by the sum of the radius of granule 2 (r2) and the user-defined minimum distance for granules from cell ends. A user-defined minimum distance between granules (mGran) constrains the origins of granules 1 and 2 to be at least r1+mGran+r2 apart.

To determine the best mEnd and mGran parameters, we analyzed all combinations of mEnd and mGran from 0 to 0.6 μm in 0.1 μm increments, see heat map in Figure 4b, as well as Figure S6. For each combination of parameters, we modeled 229,000 cells. We then used the non-parametric Kolmogorov-Smirnov (KS) test to compare the experimentally observed distribution to the modeled distributions. For the KS testing, we combined the positions of both granules into a single bi-modal distribution. A heat map of the resulting KS test statistics, and the resulting p-values are consistent, indicating that a minimum distance from cell ends of 0.3 μm and between granules of 0.2 μm gives the best fit.

Simulation procedure: The matlab script ‘peas2pod_simulation.m’, included at the end of the supplement, takes the following input values: cells = number of cells to simulate; muLength = mean cell Length (μm), sigmaLength = standard deviation of distribution of cell lengths (μm), muDiameter = mean diameter of granules (μm), sigmaDiameter = standard deviation of distribution granule diameters (μm), mEnd = minimum distance of edge of granule from cell end (μm), mGran = minimum distance from edge of granule1 to edge of granule2 (μm).

This function generates a population of a specified number of cells with a normal distribution of cell lengths specified by input mean and standard deviation values, and also generates two populations of granules, each with a normal distribution of granule diameters using mean and standard deviation values from the EM data. Each cell is screened to make sure that both granules can fit into the cell length given the constraints of minimum distance from ends and between granules; if both granules do not fit, then the function randomly re-generates a new length and new granule diameters using the input mean and standard deviation values for cell length and granule diameter. For each cell, the function positions granule 1 randomly, but excluding it from the cell ends by input mEnd + radius of granule 1. The function then does the same for granule 2. The function then checks that the position of granule 2 satisfies the following condition: it must be at least radius granule1+mGran+radius granule2 away from granule 1. If it doesn't satisfy this condition, the function randomly re-positions one of the granules within the cell (which granule is determined by a coin toss) and again checks that this new pair of granules satisfies both mEnd and mGran. The function repeats this process until the mEnd and mGran constraints are satisfied, and then goes on to the next cell.
Figure S1. Elemental analysis of putative polyphosphate granules by Energy Dispersive X-ray Spectroscopy (EDS). A) Top Panel: Scanning emission EM image of cell after line analysis. Bottom Panel: Overlay of line analysis for phosphorous (green), oxygen (red), magnesium (blue), and calcium (cyan). B) Line analysis transecting two polyphosphate granules shows elevated counts of phosphorous and oxygen relative to cell background.
Figure S2. TEM images of polyphosphate mutants under nitrogen starvation. Scale bar = 0.5 μm A) 3h WT cell, B) 3h ΔpolyP cells (Δppk1Δppk2AΔppk2BΔppk2C), C) 3h Δphb cell lacking both polyhydroxyalkanoate synthases, Δ(phaC1-phaC2). D) 3h Δ(p)pGpp cell lacking both (p)pGpp synthases, ΔrelAΔspoT. E) 24h WT cells. F) 24h, ΔpolyP cells. G) 24h, Δphb cells. H) 24h, Δ(p)pGpp cell. I) 3h WT cell fixed with paraformaldehyde before drying on EM grid. J) 3h triple mutant cell Δppk2AΔppk2BΔppk2C. K) 3h triple mutant cell Δppk1Δppk2BΔppk2C. L) 3h Δppk1Δppk2A double mutant cell. M) 6h WT cell showing measurement of cell and granule dimensions. N) 6h WT cell showing measurement with satellite polyP granules.
Figure S3. Control for specificity of GFP-ParB<sup>MTT1</sup>-parS<sup>MTT1</sup>-interaction. Scale bar = 2 μm
A) Top Panel: All four strains carrying both GFP-ParB<sup>MTT1</sup> under control of the SSB promoter and parS<sup>MTT1</sup> at the attT7 site. Exponential phase cells grown in MOPS minimal media. 2nd Panel: All four strains carrying GFP-ParB<sup>MTT1</sup> under control of the SSB promoter at the attT7 site, without a parS<sup>MTT1</sup> site, exponential phase. 3rd Panel: All four strains carrying both GFP-ParB<sup>MTT1</sup> under control of the SSB promoter and parS<sup>MTT1</sup> at the attT7 site. 24h nitrogen-starved cells. Bottom Panel: All four strains carrying GFP-ParB<sup>MTT1</sup> under control of the SSB promoter at the attT7 site, without a parS<sup>MTT1</sup> site. 24h nitrogen-starved cells. B) Fraction of cells lacking the parS<sup>MTT1</sup> site with GFP foci after 24h nitrogen starvation (As in 3<sup>rd</sup> panel of A).
Figure S4. Changes in granule number and size. A) Total number of granules per cell. All granules shown in black circles, granules constituting 95% of total granular volume per cell in grey diamonds. Granule diameter for all granules (white bars) and granules constituting 95% of total granular volume (grey bars). B) 1h, mean granule diameter for all granules of 0.062± 0.02 μm. C) mean granule diameter for all granules of 1.5h, 0.092± 0.037 μm. D) 2h, mean granule diameter for all granules of 0.115± 0.052 μm. E) 3h, mean granule diameter for all granules, bimodal distribution, 0.052± 0.019 μm and 0.188± 0.050 μm. F) 6h, mean granule diameter for all granules, bimodal distribution 0.056± 0.020 μm and 0.21± 0.060 μm; G) 24h, 0.104± 0.057 μm.
Figure S5. Granule spacing on long axis of the cell. A) Left: Demograph of all granules in 3h-starved cells imaged by TEM. Right: Demograph of all 2-granule cells in 3h-starved cells. B) Histogram of relative position of granules along long axis of cells imaged by TEM as in (A). Top Left: All cells, Top Right: 2-granule cells, 1st granule 0.32±0.07, 2nd granule 0.68±0.08, Bottom Left: 3-granule cells, 1st granule 0.27±0.06, 2nd granule 0.49±0.10, 3rd granule 0.73±0.06. Bottom Right: 4-granule cells, 1st granule 0.24±0.1, 2nd granule 0.42±0.10, 3rd granule 0.60±0.10, 4th 0.78±0.06 C) As in (A), but imaging only size-ranked granules contributing to 95% or more of total granular volume per cell. D) As in (B), but for size-ranked granules contributing to 95% or more of total granular volume per cell. Top Left: All cells. Top Right: 2-granule cells, 1st granule 0.24±0.06, 2nd granule 0.68±0.08, Bottom Left: 3-granule cells, 1st granule 0.26±0.06, 2nd granule 0.50±0.09, 3rd granule 0.74±0.06, Bottom Right: 4-granule cells, 1st granule 0.24±0.06, 2nd granule 0.43±0.09, 3rd granule 0.61±0.08, 4th granule 0.78±0.05.
Figure S6. Simulation of polyP granule positioning on long axis of the cell. A) Model for the simulation. mEnd = user-defined constraint on the minimum distance (in \( \mu m \)) from cell ends. For the first granule, lowerBound1 and upperBound1 defined by the sum of the radius of granule 1 (r1) and a user-defined minimum distance for granules from cell ends (mEnd). The allowed region for the origin of granule 1: between lowerBound and (cell Length minus upperBound). For the second granule, lowerBound2 and upperBound2 defined by the sum of the radius of granule 2 (r2) and the user-defined minimum distance for granules from cell ends. A user-defined minimum distance between granules (mGran) constrains the origins of granules 1 and 2 to be at least r1+mGran+r2 apart. B) Heat map of KS-test statistic from comparing the experimentally observed distribution of granules in 2-granule cells to a simulation of randomly positioning granules along the long axis of the cell, with two added constraints: First, a minimum distance between granules and cell ends (mEnd) and second, a minimum distance between 1st and 2nd granule (mGran). Symbol ǂ indicates parameter space where the model is statistically indistinguishable from the data. C) Top panel: Histogram of relative granule positions (blue for granule 1, yellow for granule 2) along long axis of cells from a simulation of 229,000 cells in which the only constraint is that granules cannot overlap along long axis of the cell (mEnd = 0 \( \mu m \), mGran = 0 \( \mu m \)). Bottom panel: Histogram of combined granule 1 and granule 2 relative granule relative positions, this combined distribution was used for KS-testing. D) As in (C), but mEnd = 0.6 \( \mu m \), mGran = 0 \( \mu m \). E) As in (C), but mEnd = 0 \( \mu m \), mGran = 0.6 \( \mu m \). F) As in (C), but mEnd = 0.6 \( \mu m \), mGran = 0.6 \( \mu m \). G) As in (C), but mEnd = 0.3 \( \mu m \), mGran = 0.2 \( \mu m \).
Figure S7. Granule spatial organization imaged by DAPI fluorescence. A) Top panel: Granules stained with DAPI in 3h-starved cells (scale bar 3 µm). Bottom panel: Example cells with 2 granules (scale bar 2 µm). B) Left panel: Demograph of DAPI fluorescence in 3h-starved cells; Right panel: Demograph of DAPI fluorescence in 3h-starved 2-granule cells, C) Histograms of relative position of granules along the long axis of cells imaged by DAPI in 3h-starved cells. Top left panel: all granules, Top right panel: 2-granule cells, 1st granule 0.32±0.08 (blue), 2nd granule 0.67±0.09 (yellow); Bottom left panel: 3-granule cells, 1st granule 0.25±0.07 (blue), 2nd granule 0.50±0.08 (yellow), 3rd granule 0.74±0.08 magenta; Bottom right panel: 4-granule cells, 1st granule 0.23±0.07 (blue), 2nd granule 0.41±0.07 (yellow), 3rd granule 0.60±0.06 (magenta), 4th granule 0.80±0.06 (green). D) Granules imaged with DAPI (yellow) and co-localization with Ppk2A-mCherry chimera (magenta). Scale bar 2 µm. E) Ppk2A::P-pk2A-mCherry imaging in cells lacking the other three known polyphosphate kinases (∆ppk1 ∆ppk2B∆ppk2C). Scale bar 2 µm.
Figure S8. Effects of individual polyphosphate kinases on cell cycle exit. WT black circles; ΔpolyP (Δppk1Δppk2AΔppk2BΔppk2C) blue diamonds; Δppk2AΔppk2BΔppk2C purple squares/dashed line; Δppk1Δppk2A magenta circles/dashed line; Δppk1Δppk2BΔppk2C green triangles/dashed line. A). Average cell length as a function of time after induction of nitrogen starvation. Mean and SD from at least 3 independent experiments, variance analyzed using a one-way ANOVA. Significant differences between strains at the same timepoint are marked with upper case letters based on a post hoc Tukey test. Strains at the same timepoint marked with different letters have significantly different means, P <0.05. B) Cell counts (CFU) after the shift to nitrogen-limited medium (note that 6h timepoint does not have error bars because it was not done in triplicate). C) Fraction of cells with >1 GFP-ParB<sup>mt1</sup> focus as a function of time after induction of nitrogen starvation. D) Granule diameter at 3h nitrogen-starved cells. Box represents two middle quartiles separated by the median. Means: WT 0.207±0.043 μm, Δppk2AΔppk2BΔppk2C 0.185±0.069, Δppk1Δppk2BΔppk2C 0.117±0.041. Variance analyzed using a one-way ANOVA, significant differences between strains are marked with upper case letters based on a post hoc Tukey test. Strains marked with different letters have significantly different means, P <0.05.
Table S1: Strains

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<th>Name</th>
<th>Genotype</th>
<th>Source</th>
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<td><em>P. aeruginosa</em> UCBPP-PA14</td>
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<td>(F− λ− Δ(gpt-proA)62 leuB6 glnV44(AS) araC14 galK2(Oc) lacY1 Δ(mcrC-mrr) rpsL20(StrR) xylA5 mtl-1 recA13 hsdS20), pRK2013</td>
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<td>This study</td>
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<td>P. aeruginosa UCBPP-PA14 attTn7:: mini-Tn7T-GmR (P_{ssb;ssb-mCherry})</td>
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<td>P. aeruginosa UCBPP-PA14 Δppk1 Δppk2a Δppk2b Δppk2c ΔrelA ΔspoT attTn7:: mini-Tn7T-GmR (P_{ssb;ssb-mCherry})</td>
<td>This study</td>
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<td>LR169;DKN1756</td>
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<td>LR173;DKN1758</td>
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<td>LR227;DKN1760</td>
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| LR228;DKN1761 | ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX60 | This study |
| LR229;DKN1762 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX61 | This study |
| LR245;DKN1763 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX63 | This study |
| LR282;DKN1764 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX64 | This study |
| LR283;DKN1765 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX65 | This study |
| LR284;DKN1766 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pLREX66 | This study |
| LR285; DKN1767 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pFHC3228 | (4) |
| LR286; DKN1768 | E. coli TOP10(F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15  
ΔlacX74 recA1 araD139 Δ(ara leu)  
7697 galU galK rpsL (StrR) endA1 nupG), pFHC2973 | (4) |
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<td>pMQ30</td>
<td>Allelic replacement vector</td>
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<td>pMQ70</td>
<td>Expression vector with $P_{ara}$ and $araC$</td>
<td>Amp&lt;sup&gt;R&lt;/sup&gt; (2)</td>
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<td>pMQ72</td>
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<td>Gm&lt;sup&gt;R&lt;/sup&gt; (3)</td>
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<td>pFHC3228</td>
<td>Source of $parS^{pMT1}$ DNA binding sequence</td>
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<td>pFHC2973</td>
<td>Source of GFP-$ParB^{pMT1}$</td>
<td>Amp&lt;sup&gt;R&lt;/sup&gt; (4)</td>
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<td>pLREX9</td>
<td>$ppk2A$ (PA14_01730)::$ppk2A$-mCherry; pMQ30 derivative</td>
<td>Gm&lt;sup&gt;R&lt;/sup&gt; This study</td>
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<td>pLREX15</td>
<td>$P_{ara}$ His-TEV-$ppk2B$ (PA14_33240) expression vector; pMQ72 derivative</td>
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<td>pLREX21</td>
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<td>pLREX23</td>
<td>$ppk2C$ (PA14_19410) deletion vector; pMQ30 derivative</td>
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<td>pLREX24</td>
<td>($phaC1-phaC2$) (PA14_66820, PA14_66830, PA14_66840) deletion vector; pMQ30 derivative</td>
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<td>pLREX35</td>
<td>$spoT$ deletion vector; pMQ30 derivative</td>
<td>Gm&lt;sup&gt;R&lt;/sup&gt; This study</td>
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<td>$relA$ deletion vector; pMQ30 derivative</td>
<td>Gm&lt;sup&gt;R&lt;/sup&gt; This study</td>
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<td>pLREX38</td>
<td>pUC18T-mini-Tn7T-G&lt;sup&gt;R&lt;/sup&gt; $P_{ssb}$.ssb-mCherry; pUC18R6K-mini-Tn7T-Gm derivative</td>
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<td>pLREX63</td>
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<td>pUC18T-mini-Tn7T-G&lt;sup&gt;R&lt;/sup&gt; $P_{ara}$ Flag-$ppk2B$ (PA14_33240) $ParS^p_{pMT1}$ $P_{ssb}$ mCherry GFP-$parB$&lt;sup&gt;pMT1&lt;/sup&gt;; pUC18R6K-mini-Tn7T-Gm derivative</td>
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AAAACAGACGCGCATCGCTGGCTGAG

LRPR350 Construction of pLREX36, relA deletion vector
CGAGCGCGCGTGCTTCTGCCTCGCCCTTTGCTACCTCT
TTACCGACGCTGGCTGAG

LRPR351 Construction of pLREX36, relA deletion vector
GTTAAACGACGGCCAGTGCCAAGCTTCCGCGCCGGATCGC
CGTGCTGAG

LRPR352 Construction of pLREX36, relA deletion vector
GAATCAGCCGAAGATCCAACGATAGAGGTACCGAGCTCGA
ATTCGTAATCATG

LRPR353 Construction of pLREX35, spoT deletion vector
GGGTGAACCCTTGCCGGGCATACGCAGCTGACCCGCTTTTT
CCTGTGTCATC

LRPR354 Construction of pLREX35, spoT deletion vector
GATGACACAGGAAAAAGCGGGTCAGCTGCGTATGCCCGG
AAGGGTTCACCC

LRPR355 Construction of pLREX35, spoT deletion vector
GTTGTAAAACGACGGCCAGTGCCAAGCTTCTCCGGCGCCGGCAGACCAGCCTG

LRPR356 Construction of pLREX35, spoT deletion vector
ATGATTACGAATTCGAGCTCGGTACCGACCAGGCCATTGGC
CTGGAACTC

LRPR389 Construction of pLREX38, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
AGCTAATTCGATCATGCATGAGCTCACTAGTTACTTGTACA
GCTCGTCCATGCCGCCGG

LRPR390 Construction of pLREX38, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
AGGCCTTCGCGAGGTACCGGGCCCAAGCTTTGTGGGTACGC
GCCCCACGAATCAGG

LRPR391 Construction of pLREX38, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
CTTGCTCACGCCCGAGCCCCCGCCGCTGCCGCCGAACGGAA
TGTCGTCGTCGAAGCTGTC

LRPR392 Construction of pLREX38, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
CATTCCGTTCGGCGGCAGCGGCGGGGGCTCGGGCGTGAGC
AAGGGCGAGGAGGATAAC

LRPR435 Construction of pLREX62, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
CGGCATGGACGAGCTGTACAAGTAAAGGAGGATATACATA
TGTCTAAAGGTGAAGAACTG

LRPR436 Construction of pLREX62, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
GAGCGCTTTTGAAAGCTAATTCGATCATGCATGAGCTCACTA
GTTACTCACTGTAGATTTCG

LRPR437 Construction of pLREX62, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
GAGCGCTTTTGAAAGCTAATTCGATCATGCATGAGCTCACTA
GTACTCACTGTAGATTTCG

LRPR442 Construction of pLREX49, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
GAGCGCTTTTGAAAGCTAATTCGATCATGCATGAGCTCACTA
GTACTCACTGTAGATTTCG

LRPR443 Construction of pLREX49, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
GAGCGCTTTTGAAAGCTAATTCGATCATGCATGAGCTCACTA
GTACTCACTGTAGATTTCG

LRPR444 Construction of pLREX49, att Tn7:: mini-Tn7T-Gm^P_{ssb} mCherry
CAACCTCTTCGTAATCTGATCGACATGAGCTACG
CCCACGAATCAGGATC

LRPR561 opp qPCR F
AGCACGCCACTCCAAAGGAAC

LRPR562 opp qPCR R
CAGAGGTTCGTGCTGCTTG

LRPR555 recA qPCR F
GCCCTGGAAATCACCGACAT

LRPR556 recA qPCR R
TTGATCTCAGGCTTGGTA

LRPR553 lexA qPCR F
CCAGGAACTCGGCTCAGT

LRPR554 lexA qPCR R
GTTCTGTTCGCGCGAGGATC

LRPR611 Construction of pLREX64, CAGGACGCCCGCCATAAACTGACGCCAGATCAGTGATCAGGATC
| Construction of pLREX64, attTn7:: mini-Tn7T-Gm<sup>8</sup> P<sub>ara</sub> Flag-ppk2A P<sub>ssb</sub>-mCherry GFP-<i>parB</i><sup>MT1</sup> | CCGAAGAGCATCTTTTTTG |
| Construction of pLREX64, attTn7:: mini-Tn7T-Gm<sup>8</sup> P<sub>ara</sub> Flag-ppk2A P<sub>ssb</sub>-mCherry GFP-<i>parB</i><sup>MT1</sup> | CAAAAAAAGATGCTCTCTCGCATCCTGGATCATGCTGGCA GTTTATGGCGGCGTCCTG |
| Construction of pLREX64, attTn7:: mini-Tn7T-Gm<sup>8</sup> P<sub>ara</sub> Flag-ppk2A P<sub>ssb</sub>-mCherry GFP-<i>parB</i><sup>MT1</sup> | AGGCCCTTCGCGAGGTACCGGGCCTTTTATGACAACT TGACGGCTACATCATCAC |
| Construction of pLREX49, attTn7:: mini-Tn7T-Gm<sup>8</sup> parS<sup>EMT1</sup> P<sub>ssb</sub>-mCherry GFP-<i>parB</i><sup>MT1</sup> | GGATGCTGAACCTTAGGAGAACGTCAGGAGGATATACATA TGCTAAAGGAGACCA |
| Construction of pLREX49, attTn7:: mini-Tn7T-Gm<sup>8</sup> parS<sup>EMT1</sup> P<sub>ssb</sub>-mCherry GFP-<i>parB</i><sup>MT1</sup> | CAGTTCTTCCCTTAGACATATGTATATCCTCCTGACGTTC TCCTAAGGTTCAGCATCC |
function [twopeas,twopeasDemograph] = peas2pod_simulation(cells,muLength,sigmaLength,muDiameter,sigmaDiameter,mEnd,mGran)
%cells = number of cells to simulate
%muLength = mean cell length
%sigmaLength = standard deviation of distribution of cell lengths
%muDiameter = mean diameter of granules
%sigmaDiameter = standard deviation of distribution granule diameters
% mEnd = minimum distance of edge of granule from cell end
% mGran = minimum distance from edge of granule1 to edge of granule2

%this function generates a population of a specified number of cells with
%a normal distribution of cell lengths specified by input mean and standard
%deviation vaules generates two population of granules, each with a normal
%distribution of granule diameters using mu and sigma from EM data
%for each cell, positions granule 1 randomly, excluding from the ends
%by input mEnd + radius of granule 1
%do the same for granule 2, then check that the position of granule 2
%satisfies the following condition: it must be at least
%radius granule 1+mEnd+radius granule2 away from G1
%if it doesn’t satisfy this condition, roll the dice again until it does,
%then go on to the next cell

twopeas(i,1) = cell number
%twopeas(i,2) = cell length
%twopeas(i,3) = diameter granule 1
%twopeas(i,4) = diameter granule 2
%twopeas(i,5) = P1, position of center of granule 1
%twopeas(i,6) = P2, position of center of granule 2
%twopeas(i,7) = position of center of granule nearest to near end of cell
%twopeas(i,8) = position of center of granule furthest from near end of cell
%twopeas(i,9) = normalized position of center of near granule
%twopeas(i,10) = normalized position of center of far granule

twopeas = zeros(cells,12);

%generate a cell with two granules specified by distribution of lengths
%and granule diameter specified by input mu and sigma
%but pre-screen to make sure that the granules can fit into the cell length
%given the constraints of minimum distance from ends and between granules
%if it doesn't, then re-generate new granule diameters and length
%this method will start to skew towards longer cells and smaller granules
%as the constraints of minimum distance from cell ends and between granules
%increases
for i=1:cells m = i;

    while m == i;
    lengths = normrnd(muLength,sigmaLength); granule1 = normrnd(muDiameter,sigmaDiameter); granule2 = normrnd(muDiameter,sigmaDiameter);

        if (granule1+granule2+mEnd+mGran)<lengths m = m+1;
        else clear lengths granule1 granule2 end

end

twopeas(i,1) = i; twopeas(i,2) = lengths; twopeas(i,3) = granule1;
    twopeas(i,4) = granule2;
% r1, r2 = radiii of granules 1 and 2 r1 =
    granule1/2;
r2 = granule2/2;
% specify a region1 in which center of granule 1 can reside, by defining
% the lower and upper bounds using specified minimum distance from cell ends
% the radius of the granule, and cell length lowerBound1
    = mEnd+r1;
    upperBound1 = lengths-(mEnd+r1); region1 =
    upperBound1 -lowerBound1;
% randomly position center position of granule 1 (P1) in the specified allowed
% region1
    initialP1 = rand * region1;
% repeat the process of defining the region1 and randomly positioning granule 2
% using radius of granule 2 lowerBound2 =
    mEnd+r2; upperBound2 = lengths-(mEnd+r2);
    region2 = upperBound2 -lowerBound2; initialP2 =
    rand * region2;

% now determine if positions P1 and P2 satisfy the criteria that they are
% at least the minimum distance specified by the user (mGran) apart
% if they are not, then randomly re-position one of the granules (which one
% determined by a coin toss), and re-test if conditions satisfied j = i;

    while j == i;

        if initialP2 > initialP1... || (initialP2-r2)-(initialP1+r1) > mGran P1 =
            lowerBound1+initialP1;
P2 = lowerBound2+initialP2; twopeas(i,5) = P1;
twopeas(i,6) = P2; j = j+1;
elseif initialP1 > initialP2...
& (initialP1-r1)-(initialP2+r2) > mGran
P1 = lowerBound1+initialP1;
P2 = lowerBound2+initialP2; twopeas(i,5) = P1; twopeas(i,6) = P2;
j = j+1; else
flipcoin = randi([0,1]); if
flipcoin == 0 ...
initialP1 = rand * region1; elseif
flipcoin == 1 ...
initialP2 = rand * region2;
end
end
end
end
%sort the granules such that granule 1 is always closest to the near end of
%the cell
for k = 1:cells
if twopeas(k,5) > twopeas(k,6) twopeas(k,7) =
twopeas(k,6); twopeas(k,8) = twopeas(k,5);
twopeas(k,9) = twopeas(k,4); twopeas(k,10) =
twopeas(k,3);
else
twopeas(k,7) = twopeas(k,5); twopeas(k,8) =
twopeas(k,6); twopeas(k,9) = twopeas(k,3);
twopeas(k,10) = twopeas(k,4);
end
k = k+1;
end

% normalize to generate relative positions as well for l = 1:cells
twopeas(l,11) = (twopeas(l,7)/twopeas(l,2));
twopeas(l,12) = (twopeas(l,8)/twopeas(l,2));
end

% convert data into format for running demograph program (TEM version)
twopeasDemograph = zeros((2*cells),5);
j = 1;
for i=1:cells
twopeasDemograph(j,1) = i; twopeasDemograph(j,2) = 2;
twopeasDemograph(j,3) = twopeas(i,2);
twopeasDemograph(j,4) = twopeas(i,7);
twopeasDemograph(j,5) = twopeas(i,9);
twopeasDemograph((j+1),1) = 0;
twopeasDemograph((j+1),1) = 0;
twopeasDemograph((j+1),3) = 0;
twopeasDemograph((j+1),4) = twopeas(i,8);
twopeasDemograph((j+1),5) = twopeas(i,10); i = i+1;
j = j+2;
end

figure
h = histogram(twopeas(:,11), 'FaceAlpha',0.5, 'FaceColor',[0 0 1]);
hold on
h = histogram(twopeas(:,12), 'FaceAlpha',0.5, 'FaceColor',[1 1 0]); end
SI REFERENCES


