Supplementary Materials

An automated system for interrogating the evolution of microbial endosymbiosis

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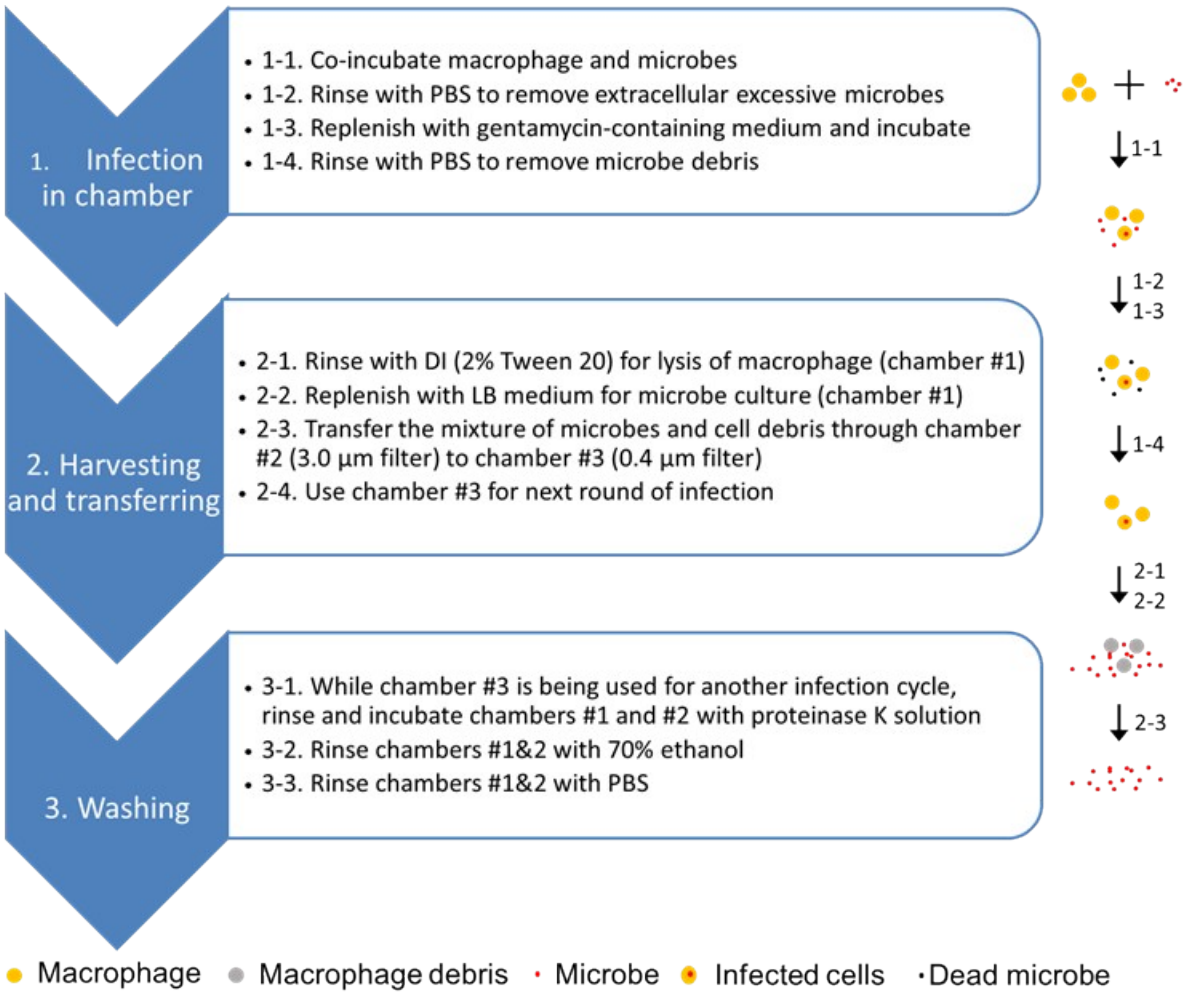
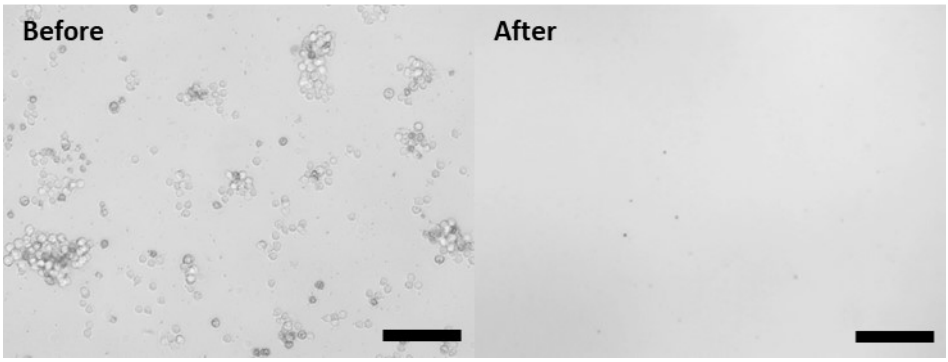
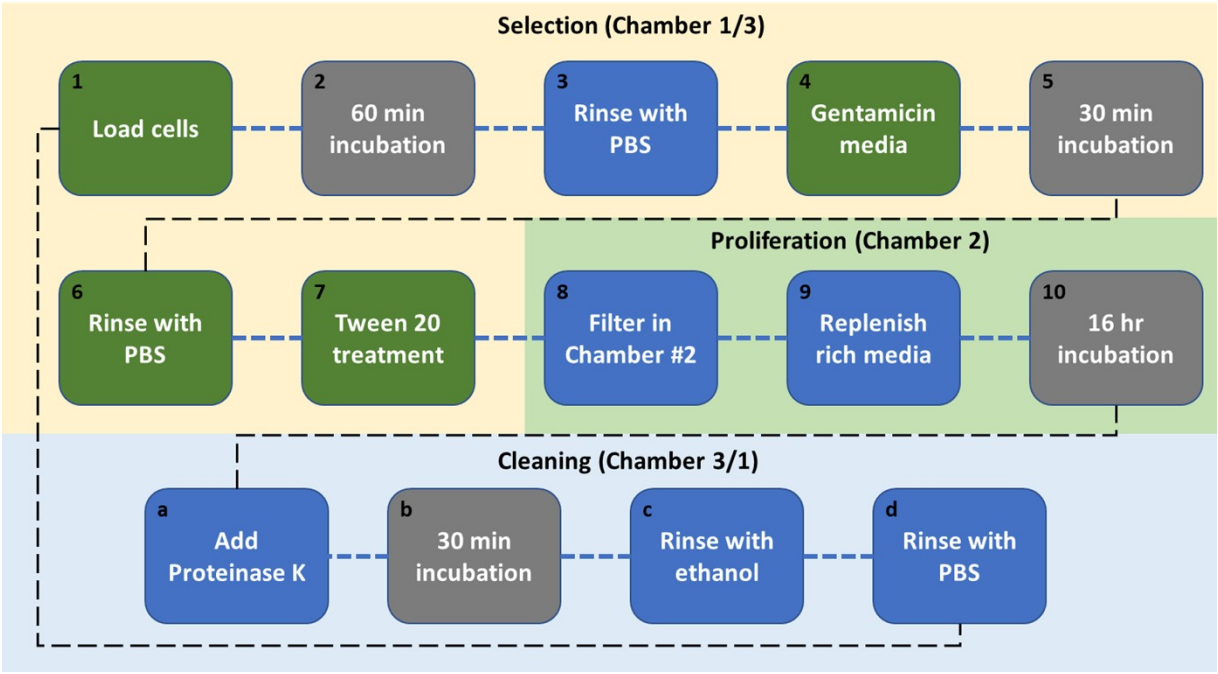


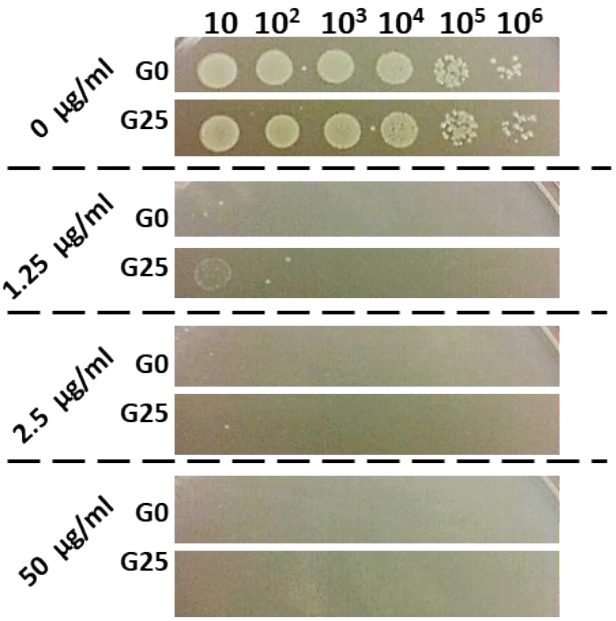
Supplemental Figure 1: Workflow of the presented SEER system operation. The entire SEER microfluidic workflow composes of three major sections, 1) co-incubation and infection; 2) bacterial cells harvesting, cultivation, and transfer of cells into the next round of evolutionary assay; 3) washing and cleaning of chambers to remove cell residuals. Specifically, in the first section the microbes and macrophages were introduced into the SEER device (vertical flow through a 0.4 µm membrane), trapped, and co-incubated for bacterial internalization into host cells. Afterwards, PBS was applied in lateral flow direction to remove the excessive extracellular microbes, followed by gentamicin-containing cell culture media introduction to kill any non-internalized bacterial cells. During the incubation period, the internalized microbes will be selected with environmental pressure. In the second section, the intracellularly surviving microbes were released through host cell lysis with lysis buffer and guided to the second chamber for amplification as well as separation from host cell debris. Once amplified, the total population was introduced into the third chamber, in which the next cycle of the evolutionary assay started. Meanwhile, in the third section, two idle used chambers were treated with lysis enzyme and ethanol to completely clean the entire chamber for a new round of evolution experiments.



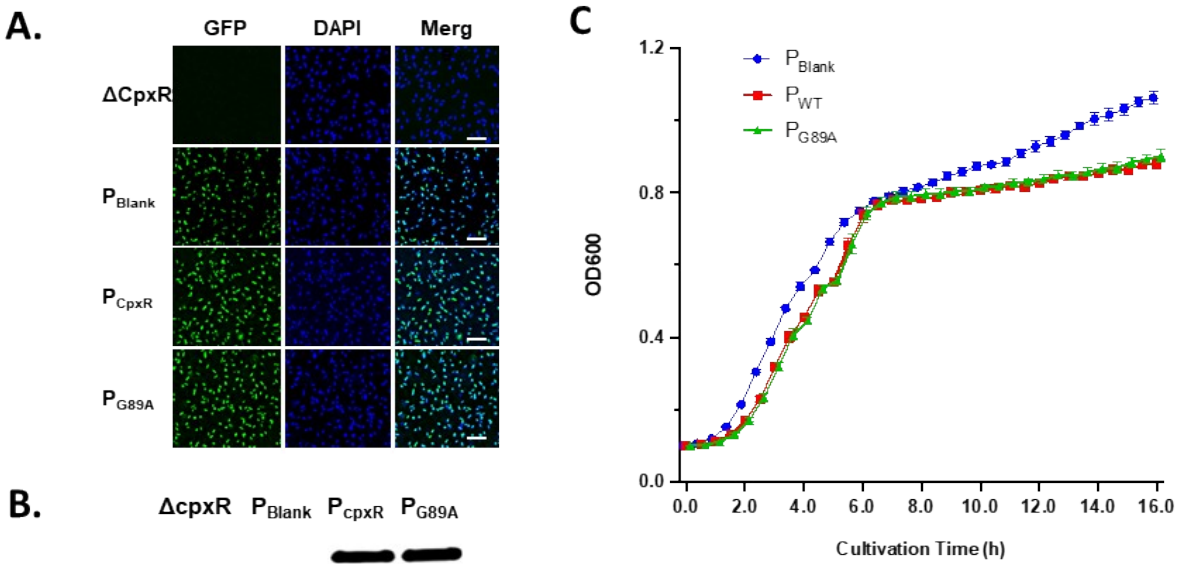
Supplemental Figure 2: Cleaning of standby chambers after a round of evolutionary assay. Macrophage residues could be effectively removed after incubating with Proteinase K at 37 ˚C for 30 mins. Scale bar: 100 μm.



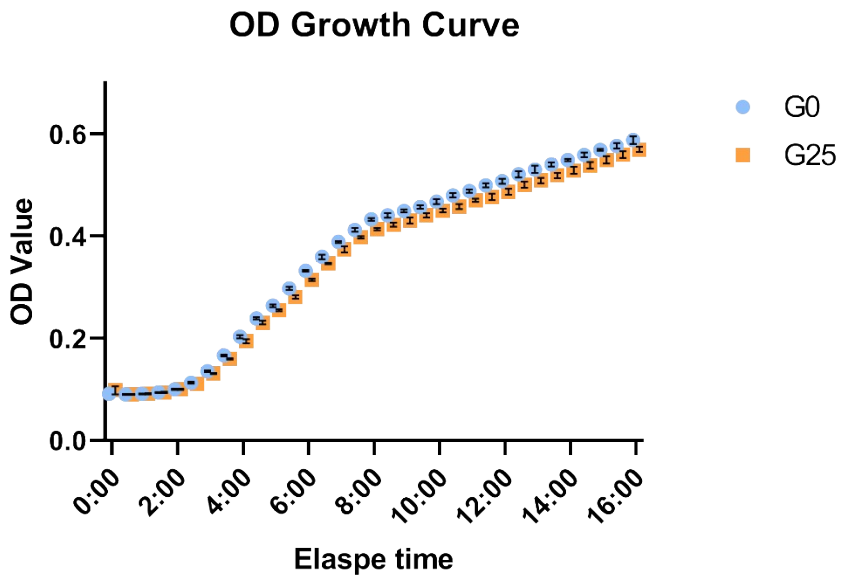
Supplemental Figure 3: Workflow diagram of LabVIEW control system. Green blocks indicate lateral flow control, blue blocks represent vertical flow control, and gray blocks do not need operation.



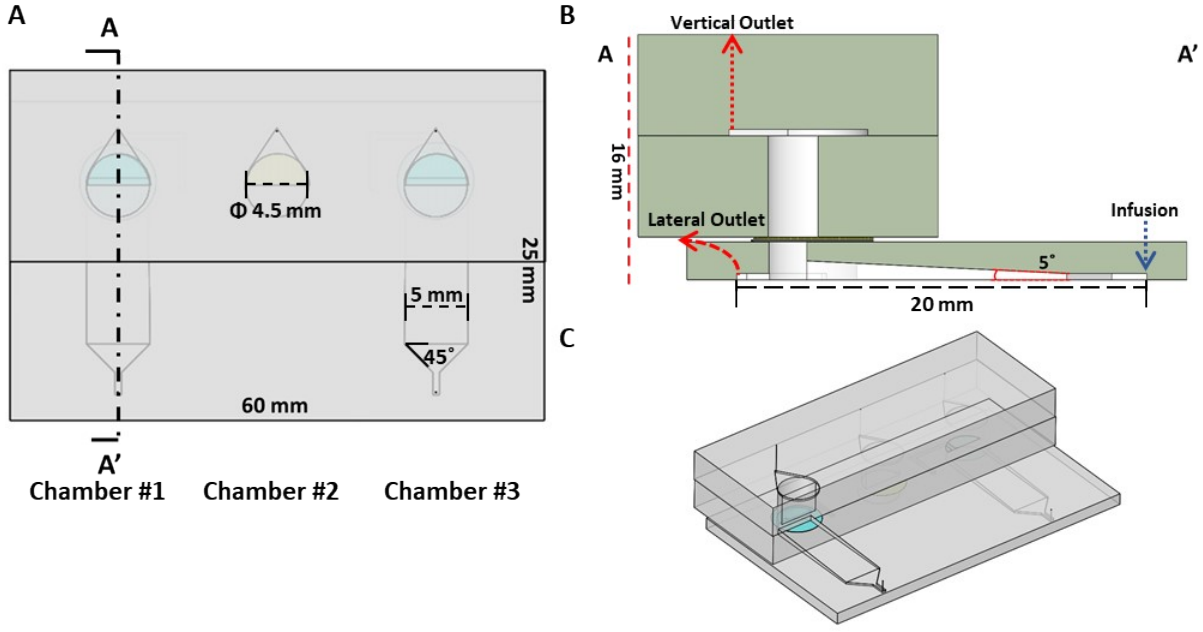
Supplemental Figure 4: Gentamicin sensitivity test to validate the functionality of proposed screening method. Evolved strain (G25) exhibited limited sensitivity against gentamicin, and can not survive under treatment condition.



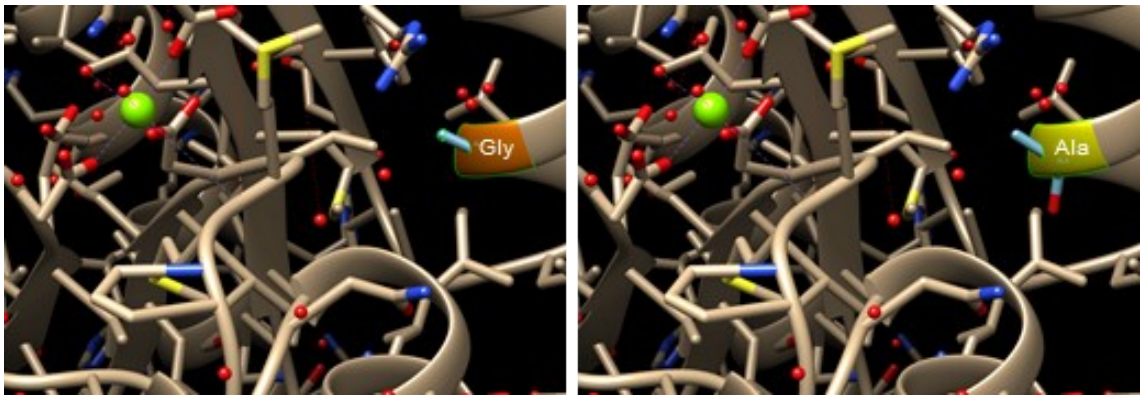
Supplemental Figure 5: Biological validation of the constructed *Escherichia coli* strains. A. GFP expressed in constructed *E. coli* strains. **B.** Western blot showing *cpxR* and *cpxR* (G89A) expression in the engineered *E. coli* strains P_{CpxR} and P_{G89A} separately introduced with plasmids. **C.** Bacteria expressing *cpxR* or *cpxR* (G89A) displayed same growth rate in LB medium, but they grew slower than the bacteria carrying a blank plasmid.



Supplemental Figure 6: **OD Growth curve measurement.** Evolved G25 strains has insignificant change of doubling time in LB rich culture media, indicating that the reproliferating steps can maintain the relative abundancy of evolved resistant strain during the cultivation.



Supplemental Figure 7: **3D detailed diagram of the presented SEER device. A.** Detailed dimension of the SEER device. **B.** Side view of the SEER device. **C.** The isometric view of the assembled SEER platform.



Supplemental Figure 8: Bioinformatic analysis of evolved strain. The mutation at position 89 from glycine to alanine may affect the magnesium binding pocket. Relevant residues within 5.0 angstroms radius around magnesium atom, involved in the Mg interaction, are displayed using the UCSF Chimera. Protein depicted as ribbons with interacting side chains depicted as sticks colored by element. Mutated residues are highlighted and labeled as Gly and Ala.

Supplemental Table 1: Comparison of existing microfluidic cell manipulation techniques.

	Properties	Limitations
Physical filtration (membrane-based)	• High throughput • Label free • High selectivity • Passive method • Low system complexity	
Magnetic beads	• High throughput • High separation efficiency	• Requires labelling • Requires external magnetic field
Hydrodynamics	• Label free • High throughput • Passive method	• Sensitive fluidic geometry • Moderate selectivity
Acoustophoresis	• Label free • High throughput • High selectivity	• Requires hard material • Sensitive fluidic geometry • Heat generation • Requires external power source
Dielectrophoresis	• Label free • High selectivity	• Low throughput • Requires external power source

Supplemental Table 2: Comparison of drug sensitivities between naïve and evolved strains.

	Naïve strain	Evolved strain	Differential
Ampicillin	2.5 µg/mL	5.0 µg/mL	Yes
Kanamycin	5.0 µg/mL	7.5 µg/mL	Yes
Gentamycin	0 µg/mL	1.25 µg/mL	Yes
Streptomycin	1.25 µg/mL	2.5 µg/mL	Yes
Rifampin	0.625 µg/mL	0.625 µg/mL	No
Sodium dodecyl sulfate (SDS)	1.0% (v/v)	1.0% (v/v)	No
Hydrogen Peroxide	1 mM	1 mM	No

Supplemental Table 3: Details of the *Escherichia coli* SNPs analysis.

Detailed information can be found in an additional excel data sheet: Supplemental Table 3.

Supplemental Table 4: Primers used for CpxR cloning and CpxR mutant G89A generation.

Primer	Sequence (5'-3')	Gene Name
CpxR_F	GAGGTCGACGGTATCGATACATTTGCTCCCAAAATCTTTCTG	CpxR
CpxR_R	CTCTAGAACTAGTGATTATCAAGCATAATCTGGAACATCATATGGATATGAAGCAGAAAC	
CpxR_G89A_F	CTTGATCGCGTTCTCGCCCTTGAGCTGGGCGC	CpxR_G89A
CpxR_G89A_R	GCGCCCAGCTCAAGGGCGAGAACGCGATCAAG	

Supplemental Table 5: *Escherichia coli* strains used in this study.

Strain name	Parent Strain	Knock-out gene	Inserted plasmid	CGSC number*	Reference
WT	None	None	None	7636	Keio Collection#
ΔcpxR	WT	*cpxR*	None	10800	Keio Collection#
ΔcpxA	WT	*cpxA*	None	10799	Keio Collection#
ΔcyoD	WT	*cyoD*	None	8583	Keio Collection#
ΔcoxR	WT	*coxR*	None	10892	Keio Collection#
P_{Blank}	ΔCpxR	*cpxR*	pBBR1MCS6Y-Blank	NS	This study
P_{CpxR}	ΔCpxR	*cpxR*	pBBR1MCS6Y-CpxR	NS	This study
P_{G89A}	ΔCpxR	*cpxR*	pBBR1MCS6Y-CpxR(G89A)	NS	This study

*CGSC, The Coli Genetic Stock Center at Yale
: Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol 2:1-11