SUPPORTING INFORMATION

Design and Selection of a Synthetic Feedback Loop for Optimizing Biofuel Tolerance

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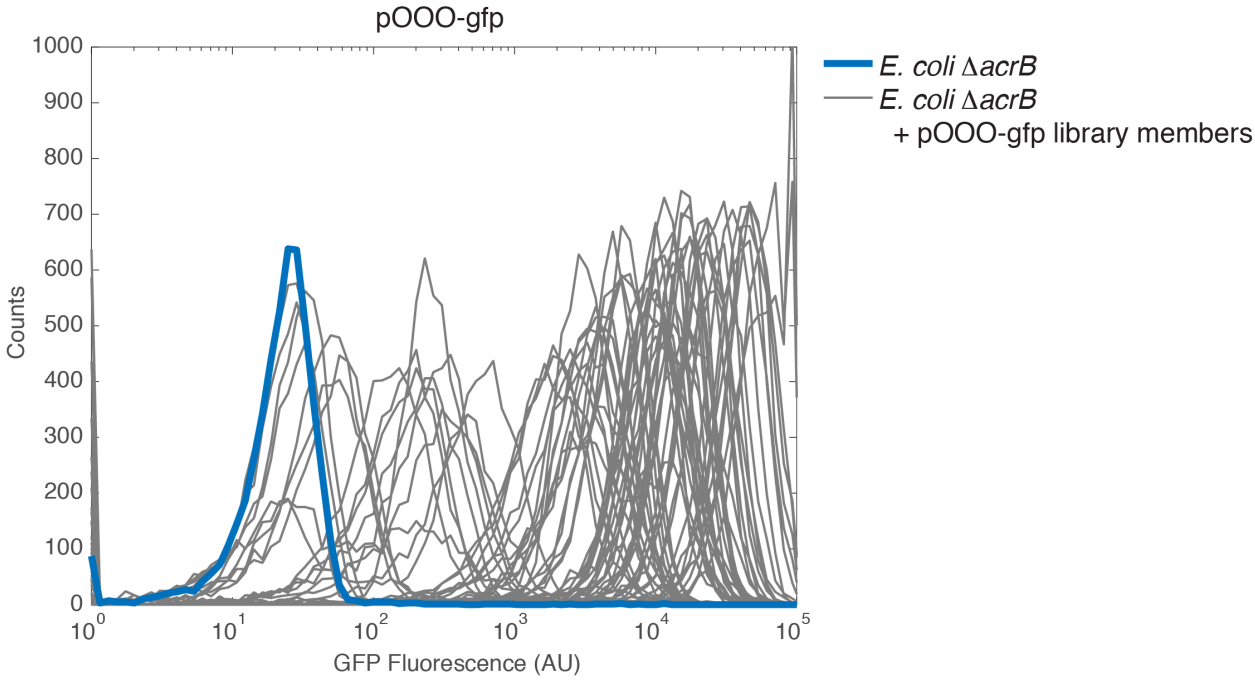

Figure S1
Distributions of fluorescence levels from cultures in Fig. 1G, with control for autofluorescence. Gray lines show colonies picked at random from the pOOO-gfp library.

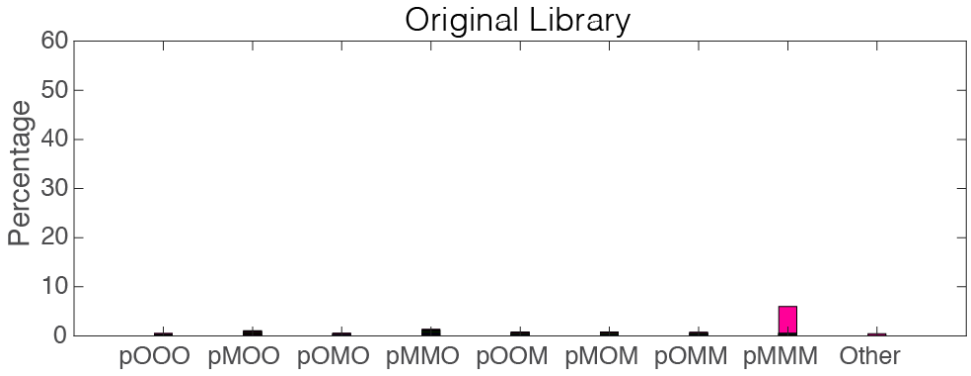

Figure S2
Stacked bar chart showing repeated sequences within the initial library, before selection. Magenta is most abundant, cyan second, and so on.

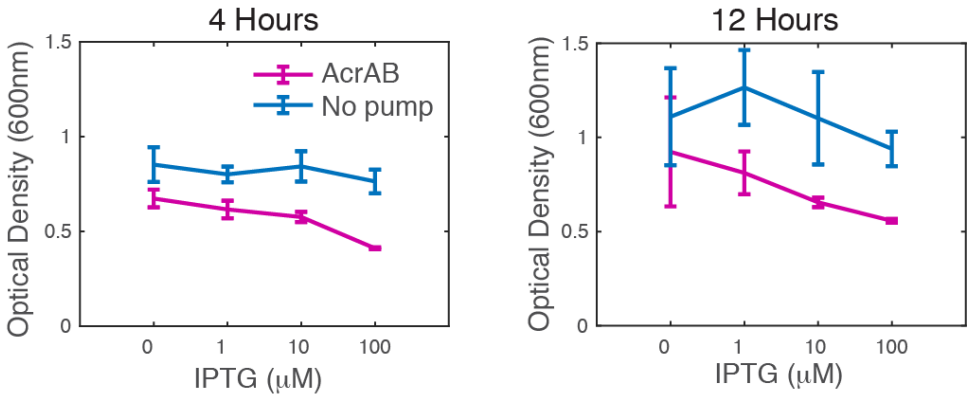

Figure S3
Overexpression toxicity measurements in *E. coli* Δ*acrB* with and without *acrAB* complementation. Data shown are from t = 4 or 12 hours after inoculation into selective medium. Error bars show standard deviations for n = 3 biological replicates.

Table S1
List of synthetic promoter sequences. MexR binding sites are shown in bold, -35 and -10 sites are underlined, degenerate bases are shown in italics.

Promoter	Sequence
pOOO	GGATCGAGTTATCAAAAAGAGTATT*KHMW*TAAAGTCTAACCTATAGGA*DDHT*TACAGCCATCGAGAGGGACACGGCGAAGAATTCAAAAG
pMOO	GGATCGAG**AGTTGACCTTATCAACC**TT*KHMW*TAAAGTCTAACCTATAGGA*DDHT*TACAGCCATCGAGAGGGACACGGCGAAGAATTCAAA
pOMO	GGATCGAGTTATCAAAAAGAGTATT*KHMW***AGTTGACCTTATCAACC**GA*DDHT*TACAGCCATCGAGAGGGACACGGCGAAGAATTCAAAAG
pOOM	GGATCGAGTTATCAAAAAGAGTATT*KHMW*TAAAGTCTAACCTATAGGA*DDHT***AGTTGACCTTATCAACC**ACACGGCGAAGAATTCAAAAG
pMMO	GGATCGAG**AGTTGACCTTATCAACC**TT*KHMW***AGTTGACCTTATCAACC**GA*DDHT*TACAGCCATCGAGAGGGACACGGCGAAGAATTCAAA
pOMM	GGTCGAGTTATCAAAAAGAGTATT*KHMW***AGTTGACCTTATCAACC**GA*DDHT***AGTTGACCTTATCAACC**ACACGGCGAAGAATTCAAAAG
pMOM	GGATCGAG**AGTTGACCTTATCAACC**TT*KHMW*TAAAGTCTAACCTATAGGA*DDHT***AGTTGACCTTATCAACC**ACACGGCGAAGAATTCAAA
pMMM	GGATCGAG**AGTTGACCTTATCAACC**TT*KHMW***AGTTGACCTTATCAACC**GA*DDHT***AGTTGACCTTATCAACC**ACACGGCGAAGAATTCAAA

AGTTGACCTTATCAACC = MexR binding site
TT*KHMW* = -35 site
GA*DDHT* = -10 site

Table S2
List of all sequences that represent 10% or more of the sequenced samples.

Initial Library	None
Treatment: No Pinene	None
Treatment: Constant Pinene	Replicate 1 Class: pMMM; -35 = TTTAAT; -10 = GATTAT; 46.9% of sequences Class: pMOM; -35 = TTTTCA; -10 = GAGTCT; 15.2% of sequences Class: pOOM; -35 = TTTACT; -10 = GAGGTT; 14.0% of sequences Replicate 2 Class: pOMM; -35 = TTTCCA; -10 = GATTTT; 34.9% of sequences Class: pMMM; -35 = TTTAAT; -10 = GATTTT; 13.3% of sequences Replicate 3 Class: pOMM; -35 = TTGAAA; -10 = GAGGCT; 23.8% of sequences Class: pMMM; -35 = TTTAAA; -10 = GAATCT; 16.3% of sequences
Treatment: Varying Pinene	Replicate 1 Class: pOMO; -35 = TTGTAA; -10 = GATTAT; 10.2% of sequences Class: pMOM; -35 = TTTTCA; -10 = GAGTCT; 26.0% of sequences Replicate 2 Class: pOMM; -35 = TTGAAA; -10 = GAGTAT; 30.4% of sequences Class: pMOM; -35 = TTGTCT; -10 = GATTCT; 17.2% of sequences Replicate 3 Class: pOMM; -35 = TTGAAA; -10 = GAAGAT; 42.6% of sequences Class: pMOM; -35 = TTTTCA; -10 = GAGTCT; 24.7% of sequences