

1 **Modular, inducible, and titratable expression systems for *Escherichia coli* and**
2 ***Acinetobacter baumannii***

3
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15
16 **Abstract**

17 Gene expression systems that transcend species barriers are needed for cross-species
18 analysis of gene function. In particular, expression systems that can be utilized in both model
19 and pathogenic bacteria underpin comparative functional approaches that inform conserved and
20 variable features of bacterial physiology. Here, we develop replicative and integrative vectors
21 alongside a novel, IPTG-inducible promoter that can be used in the model bacterium
22 *Escherichia coli* K-12 as well as strains of the antibiotic-resistant pathogen, *Acinetobacter*
23 *baumannii*. We generate modular vectors that transfer by conjugation at high efficiency and
24 either replicate or integrate into the genome, depending on design. Embedded in these vectors,
25 we also developed a synthetic, IPTG-inducible promoter, P_{abstBR} , that induces to a high level, but
26 is less leaky than the commonly used *trc* promoter. We show that P_{abstBR} is titratable at both the
27 population and single cell level, regardless of species, highlighting the utility of our expression
28 systems for cross-species functional studies. Finally, as a proof of principle, we use our
29 integrating vector to develop a reporter for the *E. coli* envelope stress σ factor, RpoE, and
30 deploy the reporter in *E. coli* and *A. baumannii*, finding that *A. baumannii* does not recognize
31 RpoE-dependent promoters unless RpoE is heterologously expressed. We envision that these
32 vector and promoter tools will be valuable for the community of researchers that study
33 fundamental biology of *E. coli* and *A. baumannii*.

34

35 **Importance**

36 *Acinetobacter baumannii* is a multidrug-resistant, hospital-acquired pathogen with the
37 ability to cause severe infections. Understanding the unique biology of this non-model bacterium
38 may lead to the discovery of new weaknesses that can be targeted to treat antibiotic-resistant
39 infections. Here, we provide expression tools that can be used to study gene function in *A.*
40 *baumannii*, including in drug-resistant clinical isolates. These tools are also compatible with the
41 model bacterium, *Escherichia coli*, enabling cross-species comparisons of gene function. We
42 anticipate that the use of these tools by the scientific community will accelerate our
43 understanding of *Acinetobacter* biology.

44

45 **Keywords:**

46 synthetic biology, gene expression, cloning, shuttle vector, Tn7 vector

47

48 **Introduction**

49 Historically, research in bacterial genetics focused on specific model organisms, such as
50 *Escherichia coli* K-12, due to a lack of techniques, tools, reagents, genome sequences, and
51 general knowledge of non-model bacteria (1, 2). As a result, much of our current understanding
52 about the basic physiology of Gram-negative bacteria comes from *E. coli* (3, 4). Although most
53 core cellular processes are likely conserved, gene function and regulation can vary subtly or
54 even dramatically across species boundaries (4, 5). Such deviation is obvious in pathogens
55 such as *Acinetobacter baumannii*, which has adopted many traits that are distinct from *E. coli* K-
56 12—most notably extreme antibiotic resistance (6-8). With advances in DNA sequencing and
57 synthesis as well as tools that democratize genetic analysis across species (e.g., CRISPR
58 approaches (9)), there now exists an enormous opportunity to shrink the knowledge and
59 technique gaps between model bacteria and clinically relevant pathogens. One simple approach
60 to bridge the gap would be to develop systems capable of assessing gene function in both
61 model and pathogenic bacteria, such that the function of any gene could be readily compared in
62 different strain or species backgrounds.

63 Here, we focus on genetic tools that function in the antibiotic-resistant pathogen, *A.*
64 *baumannii*. *A. baumannii* is considered an "urgent threat" by the Centers for Disease Control
65 and Prevention due to its ability to resist nearly all available antibiotic treatments (10). Although
66 some promising new anti-*Acinetobacter* compounds have recently been discovered (11, 12),
67 more work is needed in this area as *Acinetobacter* is adept at acquiring and developing new
68 resistance mechanisms (13-15). *A. baumannii* is poorly studied compared to *E. coli* K-12 and

69 even other Gram-negative pathogens such as *Pseudomonas aeruginosa*; however,
70 understanding the distinct physiology of *A. baumannii* is critical to developing new treatments
71 (16, 17). For instance, lipid A, an essential component of the outer membrane in most Gram-
72 negatives and a binding site for the antibiotic colistin (18), is not essential for viability in many *A.*
73 *baumannii* strains including clinical isolates (19). Further, regulation of stress pathways that
74 could play roles in antibiotic resistance, tolerance, or persistence is distinct in *A. baumannii*
75 compared to other γ -proteobacteria, as *A. baumannii* lacks conserved transcription factors such
76 as the stationary phase sigma (σ) factor, RpoS (20, 21).

77 Vectors that are capable of replicating in or integrating into *E. coli* and *A. baumannii*
78 have been previously described, but also share important limitations. Replicative shuttle vectors
79 typically use a high-copy, ColE1 origin of replication for *E. coli* and either the pWH1266 (22) or
80 pRSF1010 (23) origin for *A. baumannii*. The pWH1266 and pRSF1010 origins are compatible in
81 *A. baumannii*, enabling expression from two replicative vectors in the same cell (23). Integrative
82 vectors based on the site-specific transposon Tn7 insert DNA cargo into the genome
83 downstream of the *glmS* gene and have been used extensively in *E. coli* (24), *A. baumannii* (25-
84 27), and many other species (28, 29). However, many of these vectors were not designed to
85 contain easily swappable modules (e.g., different antibiotic markers) outside of standard
86 multiple cloning sites (MCS). Existing vectors typically employ inducible promoters that are
87 either native to or designed for use in *E. coli* (30, 31). These include *E. coli* native promoters
88 such as P_{lac} and P_{araBAD} that can be induced with IPTG or arabinose, respectively (22, 32), or
89 semi-synthetic promoters such as P_{tac} and P_{trc} which are IPTG-inducible (23). Unfortunately,
90 characteristics of these promoters pose challenges for precise control of expression. For
91 instance, P_{araBAD} expression cannot be titrated with sub-saturating concentrations of its inducer,
92 arabinose, due to "all or nothing" effects that result in a fraction of cells inducing at high level
93 while others show minimal activity (33-35). P_{tac} and P_{trc} are sufficiently leaky that genes placed
94 under their control often complement deletion phenotypes in the absence of inducer (30, 36,
95 37), and full induction often results in overexpression toxicity (38). A titratable promoter with less
96 leakiness and a lower maximal level of expression would be ideal for physiological expression
97 and gene function studies in *A. baumannii*.

98 In this work, we generate useful reagents for gene function studies in *A. baumannii* and
99 *E. coli*. We create modular vectors that replicate or integrate in both species, and carry the
100 novel promoter P_{abstBR} , which can be induced and titrated with IPTG. In a proof of principle
101 experiment, we combine all three reagents to probe the activity of the *E. coli* envelope stress σ
102 factor, RpoE, in both species.

103

104 **Results and Discussion**

105 **Modular replicative and integrating vectors for *E. coli* and *A. baumannii***

106 We sought to construct a modular set of replicative and integrative vectors that could be
107 used to examine gene function in *A. baumannii* and *E. coli*. Our shuttle vector (Fig. 1a)
108 replicates in *E. coli* using the medium copy origin, p15A (20-30 copies per cell (39)), and in *A.*
109 *baumannii* using the low copy origin pWH1266 (~9 copies per cell (40)). Our integrating vector
110 (Fig. 1a) inserts into the genomes of *E. coli* and *A. baumannii* downstream of *glmS* using the
111 Tn7 transposase (provided on a separate plasmid (9, 28)). Both vectors have an antibiotic
112 module flanked by XhoI sites for easily swapping resistance markers using Gibson assembly
113 (41). Here, we have provided hygromycin, apramycin, and kanamycin versions of both
114 replicative and integrative vectors. We note that hygromycin and apramycin are attractive
115 resistance markers for studying multidrug-resistant pathogens given that neither antibiotic is
116 used against *A. baumannii* clinically (25, 42). FRT sites in the integrative vector allow for
117 optional FLP recombinase-mediated excision of the antibiotic marker (43, 44). The cloning
118 module, or multiple cloning site (MCS), has several restriction sites for cloning genes of interest
119 (Fig. 1b and 1c). Although other sites can be used, we recommend cloning into NcoI because it
120 contains a translation start codon (ATG) in alignment with a strong upstream ribosome binding
121 site (RBS) taken from the classic expression vector pTrc99a (45). The promoter module exists
122 between AatII and NcoI sites for the replicating vector and SpeI and NcoI sites for the
123 integrating vector. We provide these vectors with a novel, IPTG-inducible promoter (P_{abstBR} ,
124 described below), but other promoters and RBSs of interest can be readily swapped into the
125 module. Additionally, both the replicative and integrative vectors can be used in the same strain
126 as multiple markers are available and only one vector replicates, ruling out compatibility issues.

127 We next determined the efficiency of transfer for both vectors into *E. coli* and *A.*
128 *baumannii*. Both vectors contain *oriT* sites, enabling transfer by conjugation from *E. coli* cells
129 that are auxotrophic for diaminopimelic acid (DAP⁻) to DAP⁺ recipient bacteria followed by
130 antibiotic selection to recover only vector-containing recipients. Additionally, both vectors can be
131 transferred by electroporation into competent recipient cells, if desired. To quantify efficiency of
132 transfer by conjugation, we mated DAP⁻ *E. coli* donor cells (*E. coli* K-12 WM6026) with model
133 strains of *E. coli* K-12 (BW25113) and *A. baumannii* (ATCC 17978). We found that both vectors
134 were transferred at efficiencies consistent with use in downstream experiments ranging in scale
135 from individual genes to large libraries (Fig. S1a and S1b). Transfers of both the replicative and
136 integrative vectors were highly efficient in *E. coli* (>10⁻¹ efficiencies for both vectors) and *A.*

137 *baumannii* ($>10^{-2}$ and 10^{-4} efficiencies for replicative and integrative vectors, respectively).
138 Importantly, our observed transfer efficiencies were on par with those needed for library
139 construction for genome-scale experiments (9). We note that we observed instances of
140 unintended integration of the Tn7 vector backbone in both *E. coli* and *A. baumannii* (i.e., co-
141 integrates (46)). The presence of such co-integrates in recipient colonies can be tested by
142 screening for the *ampR/bla* gene (which confers carbenicillin resistance) present in the vector
143 backbone. We patched 40 transconjugants for each organism, and while the frequency of
144 integration with the vector backbone was relatively low ($\leq 3/40$ for each), we recommend testing
145 transconjugants to verify insertion accuracy (Fig. S1c). Taken together, we have created
146 modular replicative and integrative vectors for *E. coli* and *A. baumannii* that can be transferred
147 at efficiencies that are useful for a variety of applications.

148

149 **A tightly regulated, IPTG-inducible promoter for *E. coli* and *A. baumannii***

150 We sought to develop an IPTG-inducible promoter with low leakiness and high
151 expression for *A. baumannii*. We previously found that a broadly utilized synthetic promoter in *E.*
152 *coli*, P_{LacO-1} , was unstable when used to express a toxic protein in *A. baumannii* (dCas9) (27).
153 When we selected for mutants with stable expression of dCas9, we found that *lacO* repeats in
154 the promoter had collapsed, creating a new IPTG-regulated promoter (Fig. 2a, *Acinetobacter*
155 Suppressor of Toxicity or P_{abst}). We hypothesized this promoter was weaker due to its success
156 at repressing toxicity. To measure promoter activity in *A. baumannii*, we cloned P_{abst} upstream
157 of a gene encoding Superfolder Green Fluorescent Protein (*sfgfp*) in our replicative vector (Fig.
158 2b). Our measurements confirmed that P_{abst} expression was very weak, with less than 2-fold
159 increase in expression at saturating levels of inducer. This weak activity is likely due to
160 divergence between the P_{abst} -35 element (TTATAA) and the consensus σ^{70} -35 (TTGACA),
161 especially at the -33 position (A versus G, respectively).

162 To generate a new promoter with higher activity but without repeating *lacO* elements, we
163 used site-directed mutagenesis to replace the P_{abst} -35 sequence with a consensus -35 (Fig.
164 2a). We found that the new promoter, P_{abstBR} (*Acinetobacter* Suppressor of Toxicity with Better
165 Regulation), showed significantly higher induction than P_{abst} (~150-fold; Welch's *t*-test, $p=0.003$)
166 in *A. baumannii* (Fig. 2b). P_{abstBR} also showed ~3-fold reduced leakiness compared to P_{trc} , a
167 popular IPTG-inducible promoter used in both *E. coli* (30) and *A. baumannii* (23); although
168 induction at saturating levels of IPTG was somewhat lower (~3-fold) than P_{trc} . With reduced
169 leakiness and a more physiologically appropriate expression range, P_{abstBR} has advantages for
170 complementation and expression with reduced toxicity (36, 47).

171

172 **P_{abstBR} expression is titratable at the population and single cell level**

173 Investigators frequently titrate promoter activity to determine expression-phenotype
174 relationships and avoid toxic overexpression. To determine if P_{abstBR} expression is titratable at
175 the population level, we induced expression of P_{abstBR}-sfgfp at varying concentrations of IPTG
176 from both our replicative and integrative vectors in *E. coli* K-12 BW25113 and *A. baumannii*
177 ATCC 17978 (Fig. 3a and 3b). We found that P_{abstBR} was titratable in all tested contexts.
178 Plasmid-borne P_{abstBR} showed similar patterns of IPTG induction in both *E. coli* and *A.*
179 *baumannii* and had ~10-fold higher level of maximal expression compared to an integrated
180 copy. Unexpectedly, Tn7 integrated P_{abstBR} showed a higher apparent level of expression in *A.*
181 *baumannii* compared to *E. coli* at nearly every concentration of IPTG, including saturating
182 concentrations (Fig. 3b). In addition to 17978, the *A. baumannii* field uses strains ATCC 19606
183 and AB5075 as antibiotic susceptible and resistant models, respectively. To test P_{abstBR}
184 titratability in those strain backgrounds, we again expressed P_{abstBR}-sfgfp at varying IPTG
185 concentrations (Fig. S3). As expected, we found that P_{abstBR} was titratable at the population
186 level.

187 Inducible promoters can erroneously appear to be titratable at the population level due to
188 varying subpopulations of fully induced cells, as is seen in systems with active transport and
189 feedback of inducer molecules (e.g., arabinose and P_{araBAD} (33)). To rule out this possibility, we
190 measured induction of P_{abstBR}-sfgfp at varying concentrations of IPTG in single cells using flow
191 cytometry (Fig. 4a and 4b). We measured P_{abstBR} expression from replicative vectors as we
192 reasoned that variations in plasmid copy number would be more likely to have a subpopulation
193 effect. We found that P_{abstBR} was fully titratable at the single cell level in *E. coli* K-12 BW25113
194 and *A. baumannii* ATCC 17978. Distributions of sfGFP fluorescence were unimodal at all IPTG
195 concentrations in both species, consistent with relatively uniform induction of P_{abstBR} at the single
196 cell level. Although increasing concentrations of IPTG fully shifted the sfGFP distributions in *A.*
197 *baumannii*, the distributions were wider than those seen in *E. coli* for unknown reasons (Fig.
198 4b). One possibility to explain increased expression variation in *A. baumannii* is simply that the
199 pWH1266 origin has intrinsically greater plasmid copy number variation than p15A, although
200 testing plasmid copy number at the single cell level is fraught with challenges (48). We conclude
201 that P_{abstBR} is titratable at the single cell level, enabling gene function studies with precise levels
202 of expression.

203

204 **Modular vectors and P_{abstBR} enable gene regulation studies in *E. coli* and *A. baumannii***

205 As a proof of principle to demonstrate the utility of our P_{abstBR} vector set in studying gene
206 function, we investigated RpoE promoter activity in *E. coli* and *A. baumannii*. RpoE, also known
207 as σ^E , is an extracytoplasmic function (ECF) σ factor that regulates the envelope stress
208 response in *E. coli* and related γ -proteobacteria (49-52). Species as distant from *E. coli* as
209 *Pseudomonas aeruginosa* have a functional ortholog (AlgU, 66% identity) that recognizes the
210 same DNA sequence as RpoE (53); however, a BLAST search of the *A. baumannii* genome
211 recovered no hits for RpoE. To determine if *A. baumannii* recognizes RpoE-dependent
212 promoters, we cloned the autoregulated *rpoE* promoter (P_{rpoE}) from *E. coli* into our integration
213 vector upstream of a gene encoding monomeric Red Fluorescent Protein (*mrfp*) as a reporter.
214 We integrated this construct into both *E. coli* and *A. baumannii* and found P_{rpoE} was only active
215 in *E. coli* (Fig 5a and 5b). To determine if the promoter could be recognized in *A. baumannii* in
216 the presence of RpoE, we cloned the *rpoE* gene into our replicating vector under the control of
217 P_{abstBR} . We found that expression of RpoE in *A. baumannii* was sufficient to drive expression
218 from P_{rpoE} (Fig 5a). This suggests that *A. baumannii* has no RpoE activity and that no other
219 factors in *A. baumannii* can recognize RpoE promoters. As expected, we also found that
220 overexpression of RpoE in *E. coli* resulted in increased P_{rpoE} activity (Fig. 5b). Importantly, these
221 results demonstrate the ability to utilize our integrative and replicative expression systems
222 together, in the same strain, to better understand biology and gene function in both *E. coli* and
223 *A. baumannii*.

224

225 **Conclusion**

226 Here, we have provided modular vectors that replicate and integrate into *E. coli* and *A.*
227 *baumannii*, and a titratable, IPTG-inducible promoter, P_{abstBR} . We envision that our vectors will
228 be valuable for complementation studies, particularly for comparing the function of genes in *E.*
229 *coli* to those found in *A. baumannii*. We predict that our tools will allow for precise tuning of gene
230 expression to achieve physiological or somewhat higher levels of expression while avoiding
231 toxicity from extreme high-level overexpression. As such, our vectors could also be used for
232 expressing gene fusions with fluorescent proteins for localization studies. The high integration
233 efficiencies make library scale experiments possible, as we have previously shown for Tn7-
234 based CRISPRi work (9). Given the host ranges of our vector components, we expect our
235 vectors to be broadly useful for gene function studies in *Acinetobacter* species not tested here,
236 including multidrug-resistant isolates.

237

238 **Materials and Methods**

239 **Strains and growth conditions.** Strains are listed in Table S1. *Escherichia coli* and
240 *Acinetobacter baumannii* were grown in Lennox lysogeny broth (LB) at 37°C shaking in a flask
241 at 250 rpm, in a culture tube on a rollerdrum at max speed, in a 96-well plate shaking at 900
242 rpm, or in a plate reader shaking (Tecan Infinite Mplex or Tecan Sunrise). Culture medium was
243 solidified with 1.5% agar for growth on plates. Antibiotics were added when necessary: 100
244 µg/mL ampicillin (amp), 30 µg/mL kanamycin (kan), 50 µg/mL apramycin (apr), and 150 µg/mL
245 hygromycin (hyg) for *E. coli* and 150 µg/mL carbenicillin (carb), 60 µg/mL kanamycin (kan), 100
246 µg/mL apramycin (apr), 150 µg/mL hygromycin (hyg) for *A. baumannii*. Diaminopimelic acid
247 (DAP) was added at 300 µM to support growth of *E. coli* dap⁻ donor strains. IPTG (isopropyl b-
248 D-1-thiogalactopyranoside) was added at varying concentrations from 0 to 1 mM as indicated in
249 the figures or figure legends. Strains were preserved in 15% glycerol at -80°C. Plasmids were
250 propagated in *E. coli* strain BW25141 *att_{Tn7}::acrIIA4* (sJMP3053) or in *E. coli* strain DH10B
251 (sJMP1) for DNA extraction and analysis or in *E. coli* strain WM6026 *att_{Tn7}::acrIIA4* (sJMP3257)
252 for conjugation.

253
254 **General molecular biology techniques.** A complete list of plasmids and oligonucleotides are
255 listed in Tables S2 and S3. Oligonucleotides were synthesized by Integrated DNA Technologies
256 (Coralville, IA). Plasmid DNA was purified using GeneJet Plasmid Miniprep kit (Thermo) or the
257 Purelink HiPure Plasmid Midiprep kit (Invitrogen K210005). PCR was performed according to
258 manufacturer directions using Q5, OneTaq, or Phusion DNA Polymerases (NEB). DNA was
259 digested with restriction enzymes from NEB. PCR products were purified with DNA Spin and
260 Concentrate kit (Zymo Research) following manufacturer instructions or gel-purified from kit
261 (Zymo Research). Plasmids were assembled using NEBuilder HiFi DNA assembly kit (NEB).
262 DNA was quantified on a Nanodrop Lite or Qubit. Plasmids and recombinant strains were
263 sequenced via Sanger sequencing by Functional Biosciences or Oxford Nanopore sequencing
264 by Plasmidsaurus.

265
266 **Construction of replicative expression vectors.** Details for construction of expression vectors
267 are listed under “Construction/notes” for corresponding vectors (Table S2). Briefly, base
268 replicative expression plasmid construction was performed using HiFi assembly with: (i) p15A
269 origin of replication and *oriT* from pJMP3262, (ii) pWH1266 origin of replication from pJMP3347,
270 (iii) pTrc99a plasmid base including *lacI* and MCS from pJMP3067, and (iv) *kanR* marker from
271 pJMP3341 to create plasmid pJMP3649. To swap the promoters, pJMP3649 was cut with AatII

272 and NcoI enzymes and HiFi assembled with gblocks containing the desired promoters, to create
273 plasmids pJMP3651 (P_{abst} , *kanR*) and pJMP3653 (P_{abstBR} , *kanR*). To swap the resistance
274 markers, pJMP3653 was cut with XhoI enzyme and HiFi assembled with gblocks containing the
275 desired resistance markers, to create plasmids pJMP3664 (P_{abstBR} , *aprR*) and pJMP3665
276 (P_{abstBR} , *hygR*). To test expression of genes from these vectors, the *kanR* versions of the vectors
277 with P_{trc} , P_{abst} , and P_{abstBR} (pJMP3649, pJMP3651, and pJMP3653, respectively) were cut with
278 NcoI and BamHI enzymes and HiFi assembled with the *sfgfp* gene amplified from pJMP2748 to
279 create plasmids pJMP3650, pJMP3652, and pJMP3654.

280
281 **Construction of P_{abstBR} .** Site-directed mutagenesis of the P_{abst} promoter was performed by
282 single-primer high-fidelity Phusion PCR using pJMP3407 and oJMP2167. The PCR product was
283 treated with DpnI, electroporated into sJMP3053, and selected on kan to make plasmid
284 pJMP4481 containing the P_{abstBR} promoter. The mutation was verified by whole-plasmid
285 sequencing with Plasmidsaurus.

286
287 **Conjugative-based transfer of expression vectors.** *Replicative vector:* Donor Dap⁻ *E. coli*
288 mating strain containing desired replicative expression vector and recipient strain (*A. baumannii*
289 or *E. coli*) were both scraped off an agar plate into LB at OD600 of ~3. Strains were mixed at
290 equal ratios, placed on a 0.45 µm filter on an LB plate, and incubated upright at 37°C for ~3 hrs.
291 Filters were vortexed in LB media to remove cells and plated onto LB plates supplemented with
292 appropriate antibiotic.

293 *Tn7 integrating vector:* Conjugation was performed similarly to above, except with the
294 addition of a donor Dap⁻ *E. coli* strain carrying a Tn7 transposase plasmid (tri-parental mating)
295 for *E. coli*, *A. baumannii* ATCC 17978, and AB5075 strains. For *A. baumannii* ATCC 19606,
296 quad-parental mating was performed, using an additional Dap⁻ donor *E. coli* strain (sJMP4061)
297 harboring a helper plasmid that contains extra mating machinery to improve efficiency. Tn7
298 matings were performed for ~4 hrs before plating on LB plates supplemented with appropriate
299 antibiotic.

300 Ten-fold serial dilutions were spotted (10 µL) on LB and LB with antibiotic. Transfer
301 efficiencies were calculated as transformants or transconjugants (colony forming units or CFUs
302 on selective plates) divided by total cells (CFUs on LB only).

303
304 **Promoter activity assays.** Promoter activities were assayed using the sfGFP expression
305 vectors. Promoter-*sfgfp* or empty vector strains were grown to saturation in LB supplemented

306 with appropriate antibiotic and IPTG inducer, washed several times with 1xPBS to remove all
307 media, and GFP fluorescence and OD₆₀₀ were measured in a Tecan Infinite Mplex plate reader.
308 Values were normalized to OD₆₀₀ readings and were background-subtracted using empty vector
309 cells.

310
311 **Flow cytometry.** Cells containing either a P_{abstBR} -*sfgfp* vector or empty vector control were
312 grown in LB supplemented with kan and varying concentrations of IPTG to saturation overnight
313 in tubes. Cells were formaldehyde fixed, washed, and resuspended in 1xPBS. GFP
314 fluorescence was measured by flow cytometry on a LSR Fortessa instrument (BD Biosciences)
315 at 100,000 events/sample. Data were analyzed in FlowJo (FlowJo, LLC) using singlet gates and
316 dead cell or debris exclusion gates, as previously described (54).

317
318 **Data availability.** Plasmids and their sequences are available from Addgene under accession
319 numbers xxxx-xxxx (note: accession #s pending). R code for data analysis and graphs can be
320 found at <https://github.com/jasonpeterslab/Aba-Eco-expression-systems-2024>. Data available
321 on request.

322
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330
331 **Competing Interest**

332 None.

333
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Figure 1 Modular replicative and integrative expression vectors. (A) Circular plasmid map and features of the replicative shuttle vector containing both *E. coli* and *A. baumannii* origins of replication (top) and the Tn7 expression vector containing a transposon that will integrate into the chromosomal *att*_{Tn7} site (bottom). Available antibiotic resistance cassettes (AbR) are listed. Maps are adapted from SnapGene (GSL Biotech). **(B and C)** Linear maps showing the modular promoter region and multiple cloning sites (MCS) for the replicative plasmid and Tn7 vector. NcoI site provides an ATG start codon optimally proximal to a strong ribosome binding site (RBS).

Figure 2 P_{abstBR} promoter construction and expression. (A) Promoter sequences showing the homologous recombination event in *lacO* repeat regions (red) of the P_{LlacO-1} sequence that produces P_{abst}, which contains a -35-like region (yellow). Site-directed mutagenesis reverts the -35 region back to consensus (orange) to create P_{abstBR}. **(B)** Dot plots showing sfGFP fluorescence from replicative vectors containing *sfgfp* under P_{abst}, P_{abstBR}, or P_{trc} promoters in *A. baumannii* ATCC 17978 with no IPTG (left) or 1 mM IPTG (right). Values were normalized to empty vector controls, and sample means are represented by a solid horizontal line (n=3). Asterisks and ns indicate significant and not significant sample differences, respectively (Welch's *t*-tests; p-values < 0.05).

Figure 3 Titration of P_{abstBR} expression at the population level. Titration of expression from **(A)** the replicative plasmid or **(B)** the Tn7 transposon. Plots shown are normalized sfGFP levels expressed from P_{abstBR} across IPTG concentrations for *E. coli*

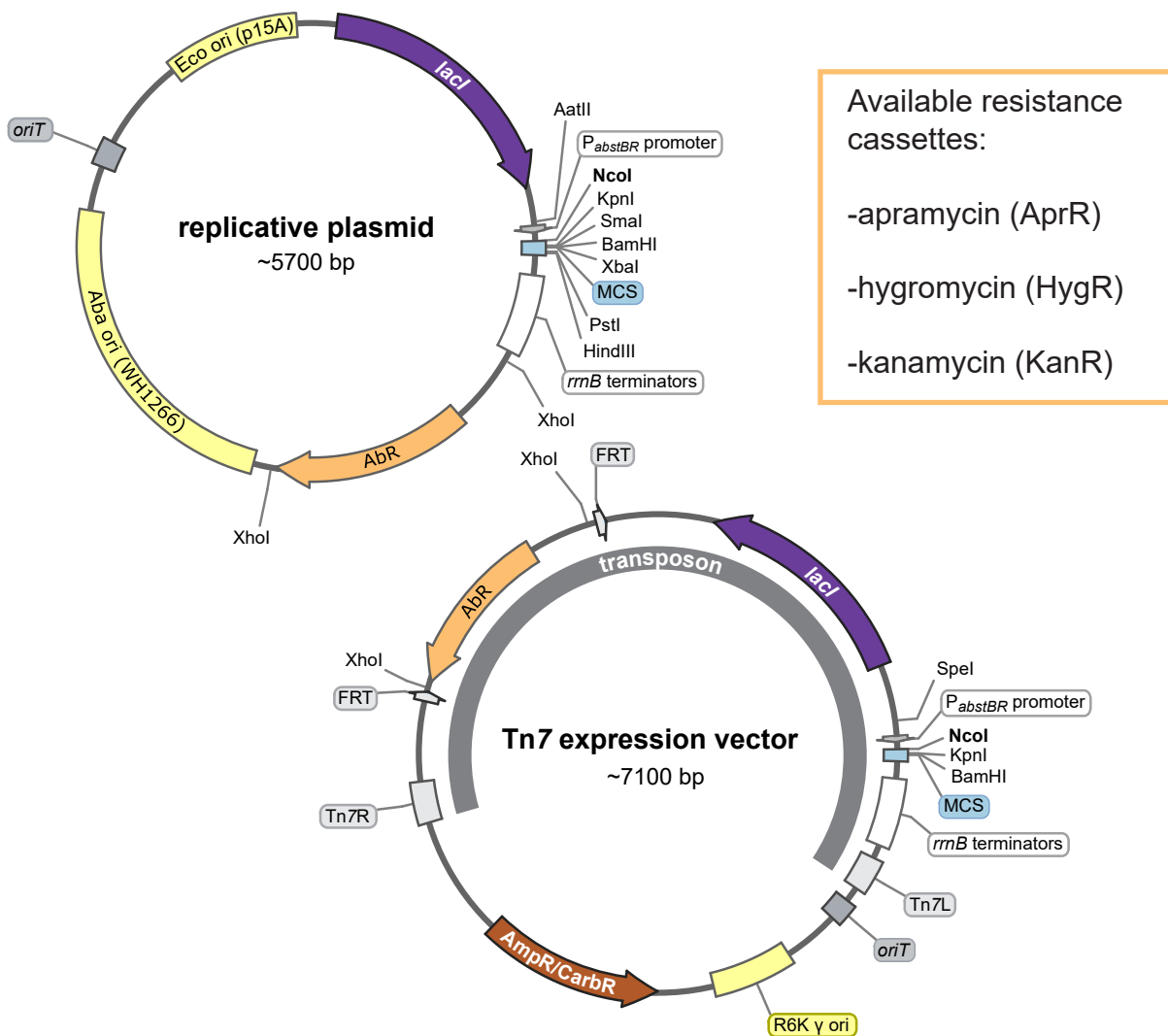
BW25113 and *A. baumannii* ATCC 17978. Error bars represent standard deviation (n=3 for replicative vector, n=6 for Tn7 transposon).

Figure 4 Titration of P_{abstBR} expression at the single-cell level. Titration of expression in **(A)** *E. coli* BW25113 or **(B)** *A. baumannii* ATCC 17978. Ridgeline plots depict overlapping density plots of sfGFP fluorescence for cells induced at different IPTG concentrations, measured by flow cytometry and expressed from the replicative expression vector under control of P_{abstBR} . EV are empty vector (no GFP) control samples in 1 mM IPTG.

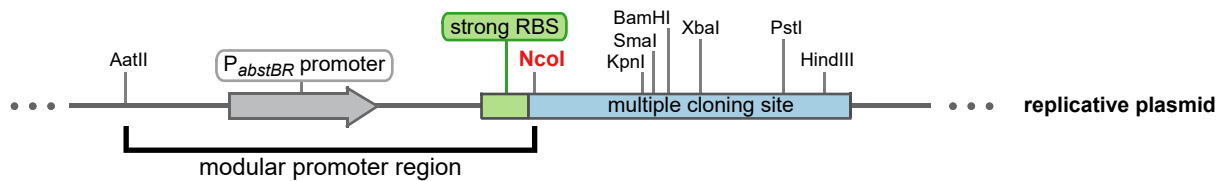
Figure 5 Modular integrative and replicative vectors facilitate a functional reporter assay. **(A)** Graphical depiction of reporter assay experiments. Strains contain an mRFP reporter under control of the *E. coli*-native *rpoE* promoter (P_{rpoE}) in the att_{Tn7} site (constructed using the Tn7 vector) and either a P_{abstBR} -*rpoE* overexpression vector or empty vector control (replicative plasmid). **(B and C)** Bar graphs of mRFP fluorescence from P_{rpoE} with and without expression of RpoE *in trans* from the replicative plasmid in *E. coli* or *A. baumannii*. As RpoE is native to *E. coli*, the *E. coli* strains also carry a copy of the *rpoE* gene on the chromosome. Fluorescence is normalized to no mRFP controls, and individual data points and standard deviation are displayed (n=6). Asterisks and ns indicate significant and not significant sample differences, respectively (Welch's *t*-tests; p-values < 0.05)

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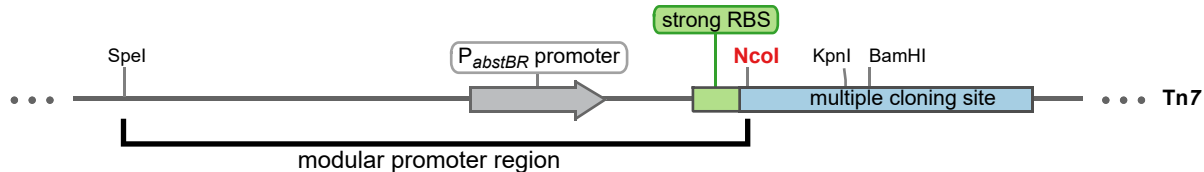
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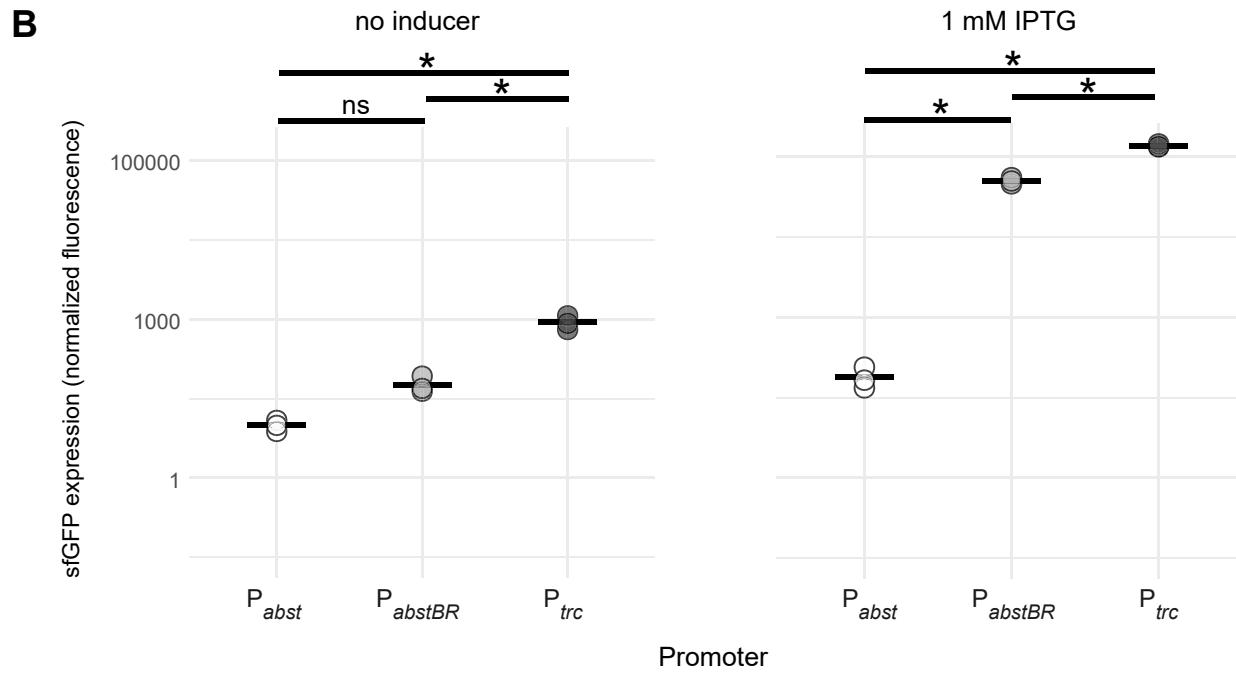
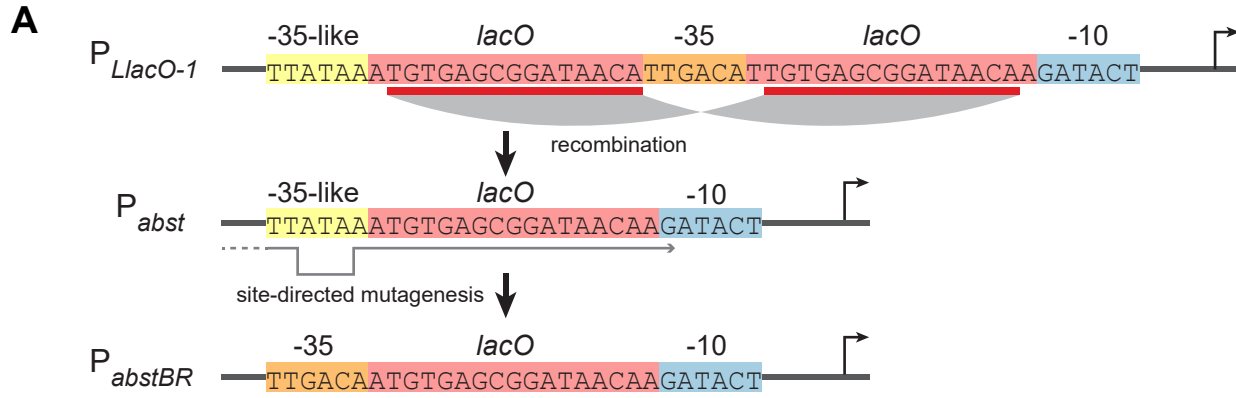


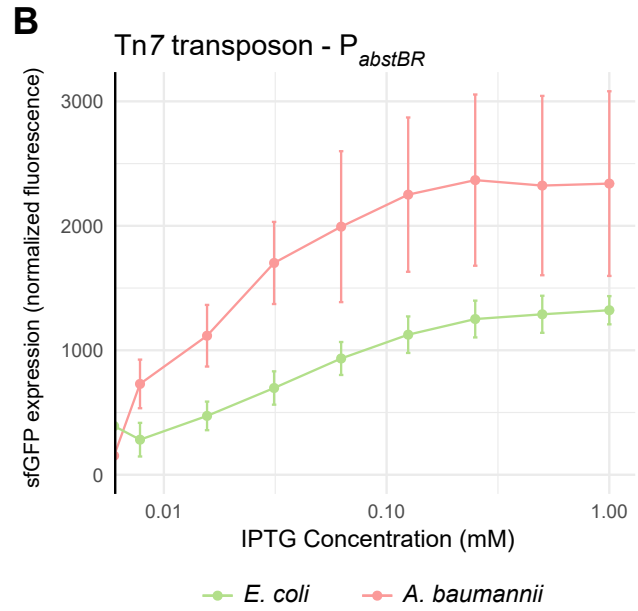
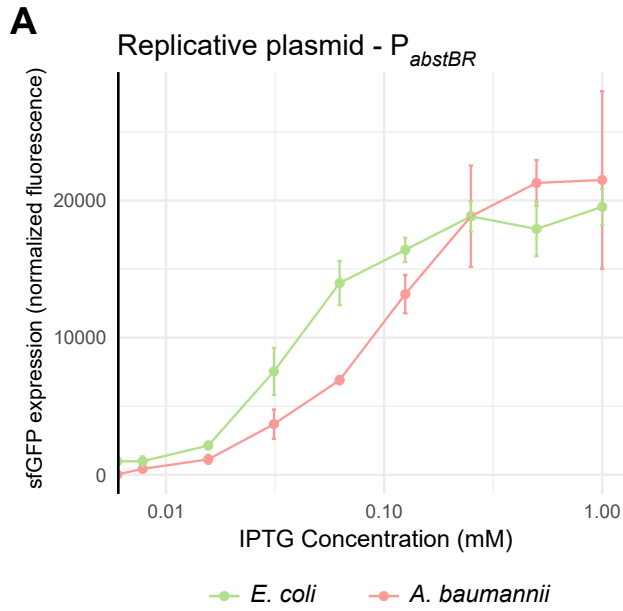
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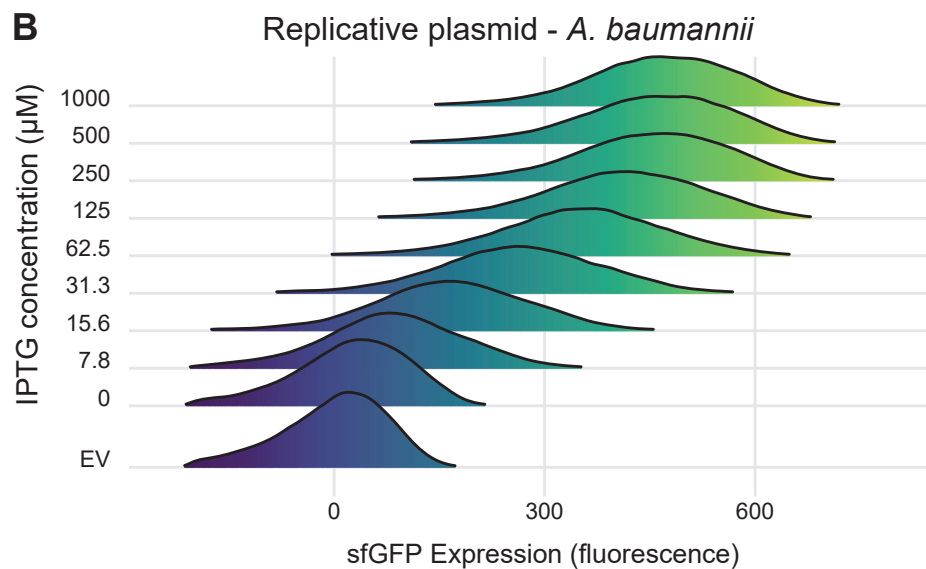
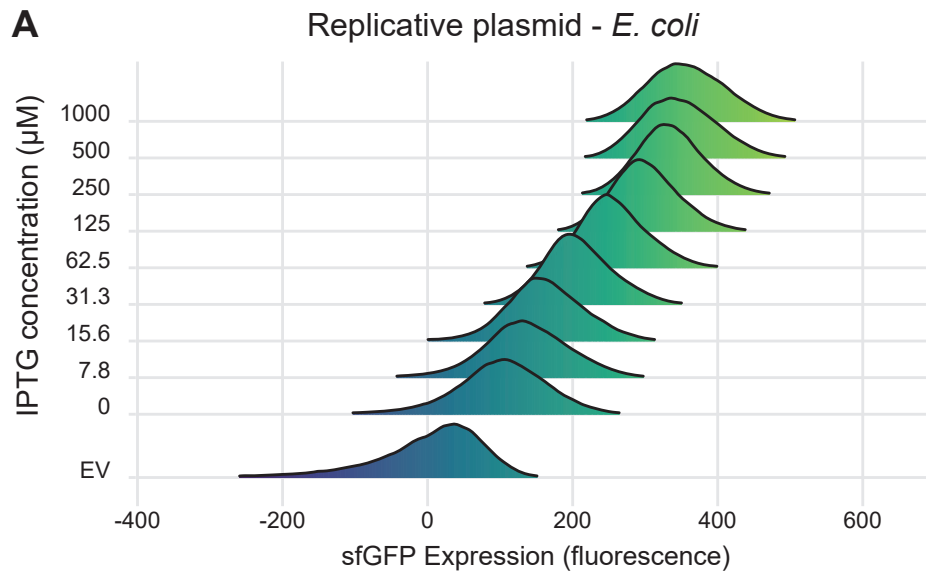


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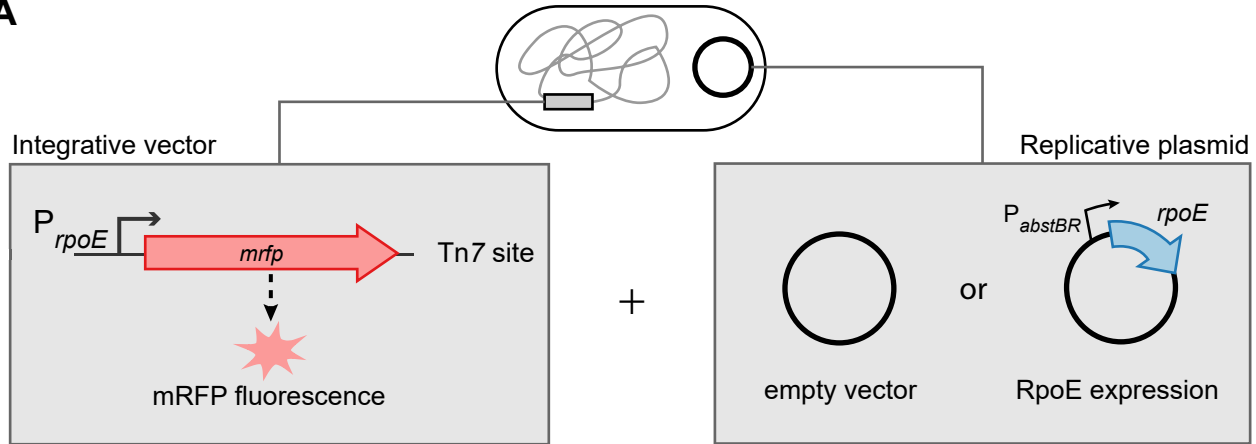






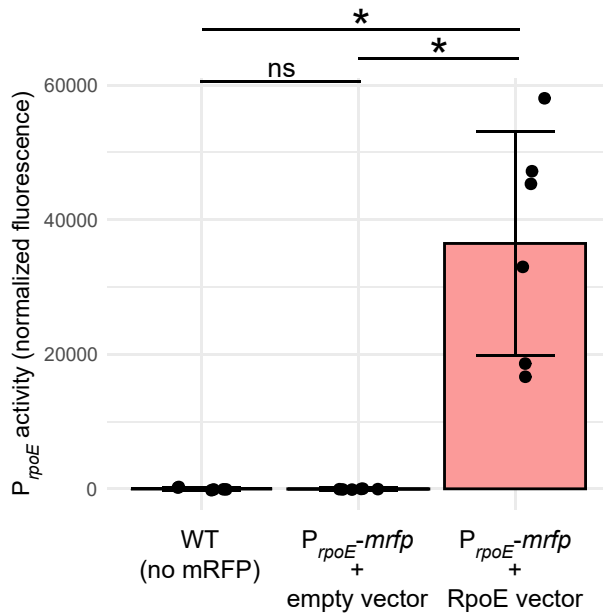


A



B

A. baumannii P_{rpoE} reporter assay



C

E. coli P_{rpoE} reporter assay

