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4	Supporting Information for
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6	Release factor inhibiting antimicrobial peptides improve non-
7	standard amino acid incorporation in wild-type bacterial cells.
8	
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13	
14	The data that support the findings of this study are available from the
15	corresponding author.
16	

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32	
33	SI Methods
34	Reagents
35	Antibiotics and nsAAs were purchased from Sigma, except for AbK, which was purchased
36	from TOCRIS. Apidaecin 1b was purchased from AnaSpec. Api137 was purchased from NovoPro
37	Biosciences Inc. N-3-oxo-octanoyl-L-Homoserine lactone (NHL) was purchased from Cayman
38	Chemical and stock solutions with it were made in ethyl acetate (acidified with 0.01% acetic acid)
39	to 1 mg mL ⁻¹ . nsAA stock solutions were prepared in water with minimal base or acid, e.g. 0.3 M
40	KOH to prepare 0.2 M Bpa stock solution, except for Cou stock, which was prepared in DMSO
41	at concentrations of 100-200 mM. Aqueous apidaecin (to 5-20 mg mL ⁻¹) stocks and nsAA stock
42	solutions were filter-sterilized and stored at -20 °C before use. DNA oligonucleotides and gBlocks
43	were synthesized by IDT.

44 In Vitro Protein Translation Assay.

The recombinant MjBpaRS was prepared as previously described¹. $tRNA_{CUA}^{T,rr}$ was prepared 45 by in vitro transcription and purified as previously described². PURExpress® and PURExpress® 46 47 and Δ RF123 Kit were purchased from NEB and the cell-free translation experiments were set-up 48 following the manufacturer's instructions supplemented with 20 ng μ L⁻¹ linearized DNA templates 49 (T7-(UAG)₀-sfGFP, T7-(UAG)₁-sfGFP, or T7-(UAG)₂-sfGFP, see also below), MjBpaRS (to 10 μ M final) and $t_{RNA_{CUA}^{\tau_{vr}}}$ (to 5 μ M final) in 5 μ L reactions per condition. 4 μ L of these reaction 50 51 mixtures were transferred to a Corning® 384 Well flat bottom, low flange, white polystyrene assay and relative fluorescence units for sfGFP was measured at excitation/emission wavelengths of 485 52 53 nm/528 nm using a Biotek spectrophotometric plate reader at 37 °C over 8 hours. The signal values 54 were normalized to peak fluorescence magnitude within an experiment and the graph was plotted 55 indicating the standard deviation between repeats in shade. Graphs were plotted and analyzed in 56 Prism 8.2.1 for Windows, GraphPad Software, www.graphpad.com.

57

58 Growth Media and Growth Conditions.

Unless otherwise noted, cultures were grown in 2xYT medium (16 g L⁻¹ bacto tryptone, 10 59 60 g L^{-1} bacto yeast extract, 5 g L^{-1} sodium chloride) supplemented with antibiotics to retain the 61 plasmids. nsAA incorporation experiments with autoinduction (e.g. SI Figure 6) were done in 62 GMML minimal media [1× M9 (Sigma-Aldrich M-6030) /1 mM MgSO₄/0.1 mM CaCl₂/8.5 mM NaCl /5 µM Fe₂SO₄ /1% v/v glycerol /0.3 mM leucine] supplemented with 10% 2xYT, 0.05% 63 64 glucose and 0.05% arabinose. Typically, Agrobacterium tumefaciens C58 cells were grown in LB at 30 °C, C321 strains were grown at 34 °C, and the rest of the E. coli strains at 37 °C. To check 65 the sensitivity of a given species to the apidaecins, overnight cultures were adjusted to $OD_{600} \sim 0.5$ 66

and serially diluted (2 x 10^{-1} dilutions). 2 µL of each dilution was spotted on solid media (e.g. LB) containing Api137 of different concentrations up to 750 µg mL⁻¹.

69 **Growth Curves.**

70 Overnight cultures (grown in 2xYT) were diluted to $OD_{600} = 0.05$ into media with different 71 concentrations of apidaecins; either 2xYT (for experiments in SI Figure 2 and 9b) or into GMML 72 supplemented with appropriate antibiotics 10% 2xYT (final), 0.05% glucose and 0.05% arabinose 73 (for experiments in SI Figure 8b, 8d, 10g) in a Corning® 96 Well clear flat bottom plate. OD₆₀₀ 74 was recorded every minute using a Biotek spectrophotometric plate reader set to 30 °C with 75 continuous shaking over at least 18 hours. At least three technical and two biological repeats were 76 plotted (indicating the standard deviation between repeats in shade). Exceptions were the high 77 apidaecins concentrations where the availability of the peptide was limiting, e.g. 1280 μ g mL⁻¹ Apilb. The growth curves were analyzed in Prism 8.2.1 for Windows, GraphPad Software, 78 79 www.graphpad.com. The growth parameters were predicted by fitting the growth data to logistic 80 growth models and the two-tail P values were determined by t-test.

81 Lambda Phage lysis assay

82 In order to induce the C321 λ cI857 lysogens, freezer stocks of the cells were streaked on 83 LB agar plates and incubated overnight at 30 °C. Several colonies were screened for temperature 84 sensitivity at 42 °C. Parallel liquid cultures were set up in LB supplemented with 5mM MgSO4 at 85 30 °C. Overnight cultures from the temperature sensitive isolates were diluted 1:100 in the same 86 medium containing Api137 at indicated concentrations. Once the cells reached $OD_{600} \sim 0.1$ (grown at 30 °C with good aeration) the temperature was shifted to 42 °C for 15 min. The cells were then 87 diluted to $OD_{600} \sim 0.05$ in a Corning® 96 Well clear flat bottom plate. OD_{600} was recorded every 88 89 minute using a Biotek spectrophotometric plate reader set to 37 ° with continuous shaking.

91 Cloning and strain engineering

92 For routine PCR and Gibson assembly procedures Q5® High-Fidelity 2X Master Mix and 93 Gibson Assembly® Master Mix from NEB were used and primers were designed following the 94 manufacturer's instructions. (T7-(UAG)₂-sfGFP DNA template was generated by linearizing and 95 amplifying the pBAD-Ub-UAG-sfGFP 151UAG plasmid with primers Pril and Pri2 (SI Tables 96 1–2). The template was cleaned up and concentrated by phenol-chloroform extraction and ethanol 97 precipitation before use in cell-free translation experiments. Routinely, new plasmids were 98 constructed using parts from existing plasmids, e.g. p006-GFP-pBAD (Addgene Plasmid 99 #108315) as the plasmid backbone for the new pBAD-PopZ plasmids (SI Table 2), or gBlocks 100 (IDT) via Gibson assembly and cloning into NEB® 5-alpha Competent E. coli.

101 Of note, a shortened backbone from pDULE-ABK (Addgene Plasmid #49086, with total 102 vector size of 7590) was used to construct the new pDule plasmids, e.g. pDule-MbAbKRS-103 2xtRNA with total vector size of 4577 bp. The pDule-MbAbKRS-2xtRNA plasmid series contains 104 two copies of $tRNA_{CUA}^{p_{wl}}$ genes under proK and lpp promoters.

Simple site-directed mutagenesis of reporter plasmids, e.g. PopZ-(UAG)₂-sfGFP to PopZ (UAG)₆-sfGFP, were performed using Q5® Site-Directed Mutagenesis Kit from NEB® following
 the manufacturer's instructions.

108 Strains that are used in the nsAA incorporation experiments were generated by 109 transforming electrocomponent cells, BL21 (E. cloni EXPRESS BL21(DE3), Lucigen), DH10B 110 (E. cloni 10G, Lucigen) or other strains including *E. coli* Nissle 1917 and *A. tumefaciens* that are 111 made electrocompetent and handled as described^{3,4}. Cultures from at least 3 separate colonies were 112 frozen and used for the nsAA incorporation assays as biological replicates. Routinely, new sequences were verified via Sanger sequencing by Genewiz and NGS-based complete plasmid
sequencing by MGH DNA Core (SI Table 2).

115

116 nsAA Incorporation Assays.

117 nsAA incorporation was quantified using at least 3 biological and technical replicates as described previously⁵ with the following modifications: in general, strains harboring indicated 118 119 reporter and aaRS/tRNA plasmids were inoculated from frozen stocks in biological triplicates 120 and grown to confluence overnight in 96-well deep well plates. Experimental cultures were 121 inoculated at 1:10 dilutions of the overnight in 96-well deep well plates containing 100 µL of 122 either 2xYT, GMML, or LB (for A. tumefaciens) media supplemented with antibiotics, inducers 123 and nsAAs (or no nsAA). Cells were incubated shaking at their optimal temperatures. In 124 experiments with apidaecins, the peptide (or water control) was added at around OD_{600} of 0.5– 125 0.8, typically after 2–4 h of growth, as adding the peptide earlier was either toxic or did not result 126 in an improvement in nsAA incorporation. The cells were then further incubated until the 127 cultures reached confluency (16–24 h). Cells were then centrifuged (5,000 g for 3 min), the 128 supernatant was removed by decanting the plates and the pellets were washed with 1 mL PBS at 129 least once (three times for experiments with Cou) and resuspended in 1 mL PBS. 100 µL of the 130 cell suspensions were transferred to a Corning® 96 Well clear flat bottom, black polystyrene 131 plates and absorbance at 600 nm (i.e. OD_{600}) and relative fluorescence units (RFU, with 132 excitation/emission wavelengths of 485 nm/528 nm for sfGFP and 390 nm/450 nm for Cou) 133 were measured using a Biotek spectrophotometric plate reader. The read data were blanked and 134 further processed as reported in figures, e.g. the reporter fluorescence was normalized by the 135 OD_{600} reading to obtain RFU/ OD_{600} . Typically, these individual intensity values were further

normalized to the highest average signal within an experiment and the data were plotted andanalyzed in Prism 8.2.1 for Windows.

•

138	Specifically, in experiments leading to Figures 2c and SI Figures 4c-d 5a, 6a we used
139	cells harboring the reporter plasmid pZE21/Ub-UAG-sfGFP_151UAG (ColE1 origin, KanR,
140	under Tet induction) and pEVOL aaRS/tRNA _{CUA} plasmids (p15A origin, CmR, tRNA genes
141	constitutively expressed, aaRS genes under arabinose induction) as previously described ⁵ . In
142	these experiments, arabinose (to final 0.2%) was present throughout, including the initial
143	inoculation of the frozen stocks, because the constitutive expression of the aaRS genes did not
144	affect the growth. After the cells were diluted into 2xYT supplemented with arabinose,
145	kanamycin (to final 25 μ g mL ⁻¹), chloramphenicol (to final 12.5 μ g mL ⁻¹), and nsAAs, at around
146	OD_{600} of 0.5–0.8, typically after 2–4 h of growth, sfGFP expression was induced by the addition
147	of anhydrotetracycline (100 ng mL ⁻¹ final) together with the addition of the apidaecin peptides.
148	
149	For experiments with A. tumefaciens, overnight cultures of A. tumefaciens cells harboring
150	the plasmids pTD114_sfGFP-1ATG (pBBR1 origin, GmR, under NHL induction) and
151	pYW15c_MjBpaRS (pSa origin, AmpR, MjBpaRS and $tRNA_{CUA}^{\tau_{MT}}$ genes constitutively expressed
152	under PN25 and proK promoters, respectively) were diluted 1:10 in LB supplemented with
153	gentamycin (to final 125 μ g mL ⁻¹), carbenicillin (to final 125 μ g mL ⁻¹) and Bpa (or no nsAA). At
154	around OD ₆₀₀ of 0.5–0.8 (after 3–4 h of growth at 30 °C), sfGFP expression was induced by the
155	addition of NHL (1 μ g mL ⁻¹ final) together with the addition of the apidaecin peptide.
156	
157	For experiments leading to Figures 2d, 3, 4 and SI Figures 6b–g, 7, 8a, 8c, 9c, 10c and

158 **10d** the new auto-inducible reporter system was used. In these experiments *E. coli* cells harbored

159	two plasmids: an auto-inducible reporter plasmid (ColE1 origin, KanR, under arabinose
160	induction), and a pDule aaRS/tRNA _{CUA} plasmid (p15A origin, TcR, aaRS and tRNA genes
161	constitutively expressed, under GlnS and proK promoters, respectively). In any strain the
162	reporter plasmid was either of the pBAD-Ub-UAG-sfGFP_151UAG, pBAD-PopZ-(UAG) ₀ -
163	sfGFP, pBAD-PopZ-(UAG) ₂ -sfGFP, pBAD-PopZ-(UAG) ₆ -sfGFP or pBAD-PopZ-(UAG) ₈ -
164	sfGFP. The aaRS/tRNA _{CUA} plasmid was either of the pDule-MjBpaRS, pDule-MjCouRS, pDule-
165	Sc5OHWRS, pDule-MbAbKRS-2xtRNA, pDule-MbAbKRS-2xtRNA-RBS1-api1b, pDule-
166	MbAbKRS-2xtRNA-RBS2-api1b, pDule-MbAbKRS-2xtRNA-RBS1-apiB5, pDule-MbAbKRS-
167	2xtRNA-RBS1-apiB8, and pDule-MbAbKRS-2xtRNA-RBS1-apiC3) (SI Table 2). In these
168	experiments, overnight cultures were grown in 2xYT supplemented with 25 μ g mL ⁻¹ kanamycin
169	and 5 μ g mL ⁻¹ tetracycline. The cells were then directly diluted 1:10 in GMML supplemented
170	with kanamycin (to final 12.5 μ g mL ⁻¹), tetracycline (to final 2.5 μ g mL ⁻¹), glucose (to final
171	0.05%), arabinose (to final 0.05%), and nsAAs (or no nsAA). In conditions involving
172	exogenously added peptides, at around OD ₆₀₀ of 0.5–0.8 (after 3–4 h of growth at 37 $^{\circ}$ C)
173	apidaecin peptides were added. Otherwise, the cells were incubated until the cultures reached
174	confluency (16–24 h) and they were as detailed above.

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176 Protein Sequence Analysis by LC-MS/MS

Excised gel bands were cut into approximately 1 mm³ pieces. Gel pieces were then subjected to a modified in-gel trypsin digestion procedure ⁶. Gel pieces were washed and dehydrated with acetonitrile for 10 min. followed by removal of acetonitrile. Pieces were then completely dried in a speed-vac. Rehydration of the gel pieces was with 50 mM ammonium bicarbonate solution containing 12.5 ng/µl modified sequencing-grade trypsin (Promega, Madison, WI) at 4°C. After 45 min., the excess trypsin solution was removed and replaced with 50 mM ammonium bicarbonate solution to just cover the gel pieces. Samples were then placed in a 37°C room overnight. Peptides were later extracted by removing the ammonium bicarbonate solution, followed by one wash with a solution containing 50% acetonitrile and 1% formic acid. The extracts were then dried in a speed-vac (~1 hr). The samples were then stored at 4°C until analysis.

188 On the day of analysis, the samples were reconstituted in 5 - 10 ul of HPLC solvent A 189 (2.5% acetonitrile, 0.1% formic acid). A nano-scale reverse-phase HPLC capillary column was 190 created by packing 2.6 µm C18 spherical silica beads into a fused silica capillary (100 µm inner 191 diameter x \sim 30 cm length) with a flame-drawn tip⁷. After equilibrating the column each sample 192 was loaded via a Famos auto sampler (LC Packings, San Francisco CA) onto the column. A 193 gradient was formed, and peptides were eluted with increasing concentrations of solvent B 194 (97.5% acetonitrile, 0.1% formic acid). As peptides eluted, they were subjected to electrospray 195 ionization and then entered into an LTO Orbitrap Velos Pro ion-trap mass spectrometer (Thermo 196 Fisher Scientific, Waltham, MA). Peptides were detected, isolated, and fragmented to produce a 197 tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence 198 protein identity) were determined by matching protein databases with the acquired fragmentation 199 pattern by the software program, Sequest (Thermo Fisher Scientific, Waltham, MA)⁸. All 200 databases include a reversed version of all the sequences and the data was filtered to between a 201 one and two percent peptide false discovery rate. 202

Image acquisition and quantification.

204 For imaging, bacterial cells were resuspended in minimal volume of 1 x PBS. 1 μ L of this 205 cell suspension was spotted to the coverslips (typically, 24X50 mm coverslips; #1.5) and an 8x8-206 mm wide, 2-mm thick PBS-agarose pad (SeaKem LE Agarose) was laid on top of the cells. Phase 207 and fluorescence images were acquired using a Nikon Ti2 Eclipse inverted microscope equipped 208 with a Plan Apo Lambda DM 60X (1.4 NA, Ph3) oil objective and Andor Zyla sCMOS camera. 209 NIS-Element AR software was used for image acquisition. For quantitative comparisons, the 210 samples were imaged in the same session with the same image conditions across. Image processing 211 was performed in FIJI. Images were scaled, cropped and rotated without interpolation. Linear 212 adjustment was performed to optimize contrast and brightness of the images. Figure construction 213 was performed in Adobe Illustrator. The relative fluorescence units of Cou and sfGFP signal intensities were quantified using a FIJI plugin, MicrobeJ⁹, where cells were identified in the phase 214 215 contrast channel with width limit from 0.3 to 2 µm and length above 1 µm. Fluorescence intensities 216 at the cell poles and the rest of the cell body then quantified and averaged within individual cells 217 (N> 100) using the 'polarity' mode in MicrobeJ. Violin plots (Figure 3e and SI Figure 7c) were 218 plotted and analyzed in Prism 8.2.1 for Windows,

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220 Partial recoding by multiplex automated genome engineering (MAGE)

221 Previously designed MAGE oligos were ordered from IDT with standard desalting and 2 222 phosphorothioate bonds at each terminus (**SI Table 1**).¹⁰ A master stock solution with a mixture 223 of these 13 oligos to reach final concentrations of 30 μ M for each oligo. As a negative control, 224 MAGE-Neg control was prepared to 400 μ M. pORTMAGE protocol was performed as

previously described¹¹. Briefly, an overnight culture of cells harboring pORTMAGE-3 plasmid

(Addgene Plasmid # 72678) was diluted 100-fold into 20–30 mL 2xYT + kanamycin (to final 25 226 227 μ g mL⁻¹), and grown at 30 °C with aeration until mid-log growth was achieved (OD600 ~0.55– 0.65). Lambda Red was induced in a shaking water bath (42 °C, 300 rpm, 15 minutes), then 228 229 induced culture tubes were cooled rapidly on ice for at least 5 minutes. Electrocompetent cells 230 were prepared at 4 °C by pelleting 10 mL of culture (centrifuge at 16,000 g at 5 min) and 231 washing the cell pellet twice with 1 mL ice cold deionized water (dH₂O) and eventually 232 resuspending cells in 250 μ L cold dH₂O. 55 μ L of the cells were mixed with 5 μ L of the oligo 233 mixture. Cells were transferred to 0.1 cm cuvettes, electroporated (BioRad GenePulserTM, 1800 234 V, 200 Ω , 25 μ F), and then immediately resuspended in 0.5 mL SOC medium. The cells were 235 allowed to recover 1 h at 30 °C at 250rpm. To select for Api 137 resistant colonies, 100 µL of 236 these cells were plated on LB + Api 137 (750 μ g mL⁻¹). For continued MAGE cycling 4.5 mL 237 2xYT + kanamycin (to final 25 µg mL⁻¹) were added and cultures were recovered to mid-log 238 phase before being induced for the next cycle. Colonies on LB + Api 137 plates appeared after as 239 little as 2 MAGE cycles with the mixture of the 13 oligos, but not with the negative control. The 240 resistant isolates were tested for their ability to incorporate nsAAs after the pORTMAGE 241 plasmid was cured. The presence of codon replacements were confirmed using allele-specific 242 colony PCR using primer sets specific for 13 genes and following the MASC-PCR protocol as 243 described¹⁰.

244

245 Library Generation Flow Cytometry and Cell Sorting.

Apidaecin peptide library was constructed using pDule-MbAbKRS-2xtRNA-RBS1-api1b as the template. First, the plasmid backbone was linearized using primers Pri3 and Pri4. The library insert sequence, Ultra1, was acquired as one 200 bp PAGE purified Ultramer (IDT). Ultra1 (to 249 final 1 µM) was further amplified by Pri5 and Pri6. The insert (~125 ng) is assembled into the 250 backbone (~400 ng) in a 150µL Gibson assembly reaction (NEB 50 °C, 1h). The product was then 251 cleaned and concentrated by ethanol precipitation and the entire product was electro-transformed 252 into E. cloni 10G SUPREME (Lucigen) cells that already harbored the pBAD-Ub-UAG-253 sfGFP 151UAG plasmid. After cells were recovered in SOC for 1 h, overnight cultures were set 254 up by adding 4 mL 2xYT supplemented with kanamycin (to final 25 µg mL⁻¹) and tetracycline (to final 5 µg mL⁻¹) at 37 °C with aeration. In parallel, dilutions were plated to estimate the library 255 256 size. 50 colonies were randomly selected and sequenced (Genewiz) in order to estimate library 257 diversity and quality.

258 The library was directly diluted 1:10 in GMML supplemented with kanamycin (to final 259 12.5 µg mL⁻¹), tetracycline (to final 2.5 µg mL⁻¹), glucose (to final 0.05%), arabinose (to final 260 0.05%), and BocK (to 2 mM). After the cells were incubated overnight at 37 °C, they were 261 washed twice with 1 mL 1 x PBS and diluted for fluorescence activated sorting in a Sony 262 MA900 Cell Sorter. Cells displaying the top ~0.0005% and ~0.02% of fluorescence activation 263 (~2,000 cells) were collected into 2xYT. After 30 min recovery, dilutions of the recovery were 264 plated on LB + tetracycline (to final 10 μ g mL⁻¹). The next day, 30 colonies from each sort were 265 sequenced. Library variants of interest were grown overnight, miniprepped, and retransformed 266 into E. cloni 10G cells for further analysis in nsAA incorporation assays.

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306

SI Figures



309

310 SI Figure 1. Apidaecins improved nsAA incorporation in a cell-free translation system. a) In the

cell-free protein translation system PURE, addition of purified MjBpaRS/ $tRNA^{T_{VIF}}_{CUA}$ pair and their 311

- cognate nsAA, **Bpa**, expressed an in-frame amber containing sfGFP construct (T7-(UAG)₁-312
- 313 sfGFP) comparably to a construct without ambers (T7-(UAG)₀-sfGFP). Api137 inhibited cell-
- free translation at concentrations higher than 80 µg mL⁻¹. b) At the same concentrations, Api137, 314
- a synthetic apidaecin analog, promoted nsAA-dependent increase of T7-(UAG)₁-sfGFP signal 315
- significantly better than the naturally occurring Api1b. Maximum relative fluorescence units 316
- (RFU) for each condition are shown on right panels. ****, P <0.0001 317



SI Figure 2. Apidaecins are toxic to different Gram-negative bacteria where RF1 function is essential. In a dose-manner, apidaecins inhibited growth of different Gram-negative bacteria in liquid media. This inhibition typically manifested itself as a reduction in final cell mass or as a prolonged doubling time (right panels). In general, Api137 was a more potent inhibitor than Api1b, and RF1+ strains (including C321.RF1) was more sensitive than C321. Δ RF1. Among *E*. coli strains tested, Nissle 1937 was the strain that was the most sensitive to apidaecins followed by BL21, DH10B, MG1655, C321.RF1 and C321.ΔRF1. ****, *P* <0.0001; **, *P* ≤ 0.007; *, *P* = 0.0116



336 SI Figure 3. Apidaecins inhibit colony formation of different E. coli strains where RF1 function

is essential. In LB solid media, Api137 (down to 125 µg mL⁻¹) was toxic to cells from different

E. coli strains, except the recoded MG1655 lacking native UAGs and RF1 (C321.ΔRF1).

ECNR2gO* is the MG1655 non-recoded and RF1+ parental strain of C321.



- 344 SI Figure 4. Apidaecins confer conditional phage resistance and improve nsAA incorporation in
- 345 *E. coli* cells with redundant RF1 functionality. a) In liquid media, C321. Δ RF1 is resistant to λ
- 346 phage-induced lysis and to Api137. b) In a dose dependent manner, Api137 rescued lysis of
- 347 C321.RF1 (λ cl857) cells upon λ phage lytic cycle induction. c) Api1b stimulated AbK-
- dependent sfGFP signal increase in C321.RF1, but not in C321.ΔRF1 cells expressing
- 349 MbAbKRS / $t_{RNA_{CUA}^{P_{W}l}}$ system. d) Api1b improved **5OH-** and **Bpa**-dependent sfGFP signal
- increase in C321.RF1 expressing Sc5OHWRS/ $t_{RNA_{CUA}}^{T_{rn}}$ and MjBpaRS/ $t_{RNA_{CUA}}^{T_{vr}}$ systems. ****,
- 351 *P* < 0.0001
- 352



354 SI Figure 5. Exogenously added apidaecins improve nsAA-dependent sfGFP signal increase in

different bacteria. a) in a dose-dependent manner and with minimal effect in final cell density (manifested by the drop of the relative final OD_{600} compared to no drug controls), Api1b

improved AbK-dependent sfGFP signal increase in *E. coli* BL21 cells, b) *A. tumefaciens*

358 (constitutively expressing MjBpaRS/ $tRNA_{CUA}^{T,rr}$) expressed (UAG)₁-sfGFP optimally at 50 μ M

359 **Bpa**. c–d) Tandem mass spectrometry (MS/MS) fragmentation analysis of the trypsin digested

360 (UAG)₁-sfGFP construct from *A. tumefaciens* (grown in LB) confirmed **Bpa** incorporation at the

361 expected amber position. c) A MS-MS spectra for a representative **Bpa**-containing peptide. d)

362 Relative abundances are based on ion count of detected relevant peptides with **Bpa** and the other

363 natural amino acids.



- 365 SI Figure 6. a) The Sc5OHWRS/ $tRNA_{CUA}^{T_{rn}}$ in LB results in high back-ground (no nsAA) signal
- 366 occluding the effects of apidaecins. b) The new autoinducable reporter system in GMML
- 367 minimal media results in strong signal (+ nsAA) over back-ground (no nsAA). In a dose
- 368 dependent manner, apidaecins improve nsAA-dependent sfGFP expression in both BL21 (a-d)
- and DH10B (e–f) expressing PopZ-(UAG)₂-sfGFP reporter and Sc5OHWRS/ $t_{RNA_{CUA}}^{T_{rn}}$ (a,b,g),
- 370 or MbAbKRS $/_{tRNA_{CUA}^{p_{\nu}l}}(c,e)$, or MjBpaRS $/_{tRNA_{CUA}^{T_{\nu}r}}(d,f)$ systems. b) In BL21 cells expressing
- 371 the Sc5OHWRS/ $_{tRNA_{CUA}}^{\tau_{rm}}$ and PopZ-(UAG)₂-sfGFP, Api137, or Api1b improved sfGFP signal
- up to ~4, or ~5 fold. c) In BL21 cells expressing the MbAbKRS $/_{tRNA_{CUA}^{P_{3}J}}$ and PopZ-(UAG)₂-
- 373 sfGFP, Api137 improved OD_{600} normalized sfGFP signal up to ~10 fold. d) In BL21 cells
- 374 expressing the MjBpARS/ $t_{RNA_{CUA}}^{\tau_{vr}}$ and PopZ-(UAG)₂-sfGFP, Api137, or Api1b improved
- 375 sfGFP signal up to ~13, or ~14 fold. e) In DH10B expressing the MbAbKRS $/_{tRNA_{CUA}^{p_{nl}}}$ and
- 376 PopZ-(UAG)₂-sfGFP, Api137 improved sfGFP signal up to ~3 fold. f) In DH10B expressing the
- 377 MjBpARS/ $tRNA_{CUA}^{T_{VT}}$ and PopZ-(UAG)₂-sfGFP, Api137 improved sfGFP signal up to ~2 fold. g)
- In DH10B expressing the Sc5OHWRS/ $t_{RNA_{CUA}}^{\tau_{rn}}$ and PopZ-(UAG)₂-sfGFP, Api137, or Api1b
- 379 improved sfGFP signal up to ~16, or ~3 fold. ****, P < 0.0001; ***, $P \le 0.005$; **, $P \le 0.0076$.
- 380



383 SI Figure 7. Apidaecins improve specific, multi-site incorporation of Cou. a) Bulk measurements of DH10B expressing MjCouRS/ $t_{RNA_{CUA}^{T_{VT}}}$ and PopZ-(UAG)₂-sfGFP show that 384 385 Api137 treatment increases spectrally distinct sfGFP and Cou signals to the comparable extend. 386 b) Micrographs showing subcellular signals from **Cou** (false colored in red) and PopZ-(UAG)₆-387 sfGFP fusion (false colored in green) colocalized at the poles of the DH10B E. coli cells imaged 388 in phase, DAPI, and EGFP channels and overlayed on phase (false colored in blue) c) Violin 389 plots of single cell quantification by light microscopy showed that Api137 improved both Cou 390 and sfGFP signals comparably. An exception was DH10B cells expressing PopZ-(UAG)₆-sfGFP 391 treated with highest concentration of Api137 tested (100 µg mL⁻¹). Under these conditions, the 392 **Cou** signal improvement was ~2 fold, but the sfGFP signal improvement was ~5 fold. The scale bars are 2 μ m. ****, P < 0.0001; ***, $P \le 0.004$; **, P = 0.0035. 393



396 SI Figure 8. a) Three ribosome binding sequences of different strenghts (RBS2>RBS1>RBS3) 397 were designed using the RBS calculator tool (https://salislab.net/software/)¹². Arabinose operated 398 autoinduction of *api1b* improved **BocK** incorporation up to ~37 fold while showing significant 399 toxicity depending on the gene's RBS-strengh. b) Growth curves (left), generation times 400 (middle) and final OD (right) of BL21 cells auto-inducibly expressing MbAbKRS and ParaB-RBS1-apilb or ParaB-RBS2-apilb. Exogenously added Apilb further sensitized apilb expressing 401 cells but did not affect the growth of MbAbKRS control cells. 1.25 µg mL⁻¹ Api1b reduced the 402 growth rate of MbAbKRS control cells only 2% but it reduced the growth rate of apilb 403 404 expressing cells ~30%. Growth of these *apilb* expressing cells were completely inhibited in the 405 presence of higher Api1b concentrations tested (>10 µg mL⁻¹). c) In-cell autoinduction of *api1b* 406 genes had a dramatically less effect on nsAA incorporation in DH10B compared to BL21. d) 407 P_{araB}-RBS1-apilb or P_{araB}-RBS2-apilb expression did not inhibit the growth of DH10B. The 408 RBS sequences are RBS1: GGAGGTAAAAA, RBS2:GGAGTTAAGGAGGTAAAAA, and RBS3: GGAGGTAAAAAATGCCCGTTTTTAAGGAGGTAAAAA. ****, P <0.0001; **, P ≤ 409 0.007. 410 411



414 SI Figure 9. RF1 inhibition by apideacins can facilitate recoding efforts toward improved nsAA 415 incorporation. a) Cells recoded by a mixture of oligos specifically targeting essential genes 416 formed colonies on Api137 containing selective plates. Same experimental set-up with a random 417 oligo did not result into spontanously resistant mutants. b) Growth curves (left) and generation 418 times (right) of 'partially recoded' DH10B and MG1655 in the presence of different [Api137] 419 show that partially recoded strains are more apidaecin-resistant than the wild-type parents (right, 420 from SI Figure 2). c) Partially recoded MG1655 cells incorporated BocK more efficiently than the wild-type even in the absence of Api137. ****, P < 0.0001; **, $P \le 0.0082$; *, $P \le 0.0366$. 421



423 SI Figure 10. In-cell autoinduction allows evolution of new apidaecin-like peptides that show 424 improved nsAA incorporation and decreased cell toxicity. a-b) Enriched apiaecin-like peptide 425 variants from the loose (a) or stringent (b) sorts do not converge to common sequences. c-d) In-426 cell autoinduction of variants from the stringent sort improved BocK incorporation but also 427 affected the cell growth to different extends in DH10B cells. e-f) a Neighbor-joining 428 phylogenetic tree without distance corrections (e), and multiple sequence alignment of relevant 429 apidaecin variants (f) generated using Clustal Omega Multiple Sequence Alignment tool¹³. g) Growth curves (left), generation times (middle) and final OD (right) of DH10B cells auto-430 inducibly expressing MbAbKRS and *apiB5*, *apiB8*, *apiB10*, or *apiC3*. ****, *P* <0.0001; *, *P* = 431 432 0.0113. 433

434 SI Tables

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Pri1	GTAATACGACTCACTATAGGGGTTAACTTTAAGAAGGAGATATACAT
D	
PfIZ	
Ultral	
	AGAGCACCIGGAAGAATTIACTAIGGIGRVCIICDSCCAGDBSGGI
D :0	
Pri3	
Pri4	AAAATCITGAAGCICICATCAAAG
Pri5	GATACTGAACTTTCAAAACAGATCTTCCGGGTGGATAAAAATCTCT
	GCTTG
Pri6	CCAGAAACTCTTTGATGAGAGCTTCAAGATTTTCCCGAGTACATCC
	CG
MAGE-Neg control	T*C*ATGTTGCTTCATGTGATCTGGATAGCGGGAAAAACATTGTACA
	TACAGAGTAGTTACGAGAGTTGGCCATGGTACTGGGAGCTTGCCA
	g^{t} t*taaagccggaataatatttgaccaaatgttcggccagcca
MAGE_murF_TAA	gtactacctcttcca*t*g
	g*t*cgtaggatttaaataagagtccaggcctgatgagacgtgacaagcgtcacatcaggcatcggtgcacaat
MAGE pgpA TAA	tacgacagaataccca*g*c
	g*a*taatgccttatccggtctacagtgcaggtgaaacttaaactattacacgtccagcagcagacgcgtcggat
MAGE sucB TAA	cttccagcaactctt*t*g
	a*a*agtattatccgaaaaatcgagcgacagattgctcactcaggtgcctttacttac
MAGE lolA TAA	gccttgcggcggg*g*t
	$a^{*}g^{*}cgacattcatgactccatcaatcgaacgctgccgcgcgtaattagttgccagaagccagcaaggttagt$
MAGE lpxK TAA	tgcgtaagcagtttcg*c*t
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MAGE fabH TAA	gtgaatccaccgccaa*a*g
	c*a*taggcgtaaatgcaccctgtaaaaaaaaaaaaaaaa
MAGE hemA TAA	atattcagecettcgt*t*a
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MAGE hda TAA	gaatgotragett*a*e
	a*a*caacaacacaataaaaaaaaaaaaaaaaaaaaaaa
MAGE mreC TAA	
MAGE coop TAA	
	auguuggauggaag u u
MAGE other TAA	
MAGE_ape_TAA	
MAGE_yaff_IAA	cgcaaggactgacgc*c*a

435 **SI Table 1**. Sequences of key oligonucleotides.

- 437 SI Table 2. Sequences of new constructs. Promoter sequences blue highlight; RBS sequences –
- 438 purple highlight; relevant ORFs red; stop codons underlined; terminator sequences green
- 439 highlight. In-frame amber stop codons are highlighted in black

Name	Sequence
Т7-	GTAATACGACTCACTATAGGGGTTAACTTTAAGAAGGAGATATACATATGCAGATTTTTGTGAAGACTTTAACAGGTAAGACGATT
(UAG)	ACCCTGGAGGTGGAGTCCTCGGACACCATCGATAATGTAAAATCAAAAATCCAAGATAAGGAAGG
ofGED	CGTCTGATTTTCGCAGGTAAACAACTGGAGGATGGTCGCACGCTTTCGGACTACAACATCCAGAAAGAA
SIGIT	TTCTGCGTCTGCGTGGAGGAGGAGGATTGTTTGTTGTGCAGGAGCTTGCATCCAAGGGCGAGGAGCTCTTTACTGGCGTAGTACCAATTCT
	CGTAGAGCTCGATGGCGATGGCGATGGCGATGGCGAGGGCGAGGGCGATGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGGCGAGGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGCGAGGCGAGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGGGG
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	AAGCGCGATCACATGGTACTCCTGGAGTTTGTAACTGCAGCAGGCATTACTCATGGCATGGATGAGCTCTATAAGCTCGAGCACC
	ACCACCACCACCACCACTAA
pTD114 sf	GTAAAACGACGGCCAGTGAGCGCGCGCG <mark>TAATACGACTCACTATAGG</mark> GCGAATTGGAGCTCACGTGCAGATCTGCACATAGCCACA
GFP-	CCCTGAATGAGATGTTTTCTCTCCGCTACGTTTCTTGGGCTAGCCCG <mark>AAAGAGGAGAAA</mark> TTAACTATGGCATCCAAGGGCGAGGA
	GCTCTTTACTGGCGTAGTACCAATTCTCGTAGAGCTCGATGGCGATGTAAATGGCCATAAGTTTTCCGTACGCGGCGAGGGCGAG
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nVW150	CATGGCTCGAGAAATCATAAAAAATTTATTTGCTTGGGGGGGATAACAATTATAATAGGATCAATTGTGAGCGGATAACAATT
M:DroDS	TCACACAGAATTCATTAAAGAGGAGAAATTAACTATGGATGAATTCGAGATGATCAAGCGTAATACATCTGAAATCATCAGGGA
мјбраћб	AGAGGAATTACGTGAGGTGTTGAAAAAAGATGAGAAATCCGCTGGCATTGGATTTGAGCCTTCCGGTAAGATTCATCTTGGGCAC
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	TCAGAAGGGAGAACTTGACGAAATTCGCAAGATTGGCGATTACAACAAGAAGGTATTTGAGGCGATGGGACTGAAGGCGAAGTA TCTTTATGGCTCACCTTTTCAGTTGGATAAGGACTACACTTTAAATGTATATCGTCTGGCTTTAAAGACTACCCTGAAGCGTGCGC GCCGCTCGATGGAGCTTATCGCGCGTGAGGACGACAAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGACGTCGCCGTGGGGCGGAATGGAGCAGCGTAAAATCCACATGTTAGCTCGTGAGTTACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATGAGTTCATCCAAAGGCAACTTTATCGCAGTG GATGATAGTCCTGAAGAGATTCGCGCCAAGATTAAAAAGGCCTATTGTCCCGCCGGAGGTGATCGAGGGGAATCCTATTATGGAAA TCGCCAAATACTTCCTGGAATATCCTTTAACAGACTACAACGTCCAGAGAAGTTTGGAGGAGACCTGACGGTAAATTCGTACGAAGA GCTTGAATCCCTGTTTAAGAACAAAGAACTGCACCCGATGGACGTGAGAGAAGTTGGAGGAGACCTGACGGTAAATTCGTACGAAGA GCTTGAATCCCTGTTTAAGAACAAAGAACTGCACCCGATGGACCTGAAAAACGCCGTAGCCGAAGAGCTTATCAAAATTTAGAG CCAATCCCTAAGCGTCTT <u>TAA</u> CTGCAGTTTCAAACGCTAAATTGCCTGAGAATTCC <mark>AAAAAGCCCGCACCTGACGGCACCTGACGGCCTTTTCCCACGGCCTTTTCCCGCCGATCAAAAGGCCCTGACGGCCTTTTCCCCCGCAACTTTCCCCCCCGATGCCGACCTGACAGTGCCGGGCCTTTTCCCCCCCC</mark>
	TCAGAAGGGAGAACTTGACGAAATTCGCAAGATTGGCGATTACAACAAGAAGGTATTTGAGGCGATGGGACTGAAGGCGAAGTA TCTTTATGGCTCACCTTTTCAGTTGGATAAGGACTACACTTTAAATGTATATCGTCTGGCTTTAAAGACTACCCTGAAGCGTGCGC GCCGCTCGATGGAGCTTATCGCGCGTGAGGACGACAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGACGTCGCCGTGGGCGGAATGGAGCAGCGTAAAATCCACATGTTAGCTCGTGAGTACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATCACACATGTTAGCTCGTGAGTACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATGAGTTCATCCAAAAGGCAACTTTATCGCAGTG GATGATAGTCCTGAAGAGATTCGCGCCCAAGATTAAAAAGGCCTATTGTCCCGCCGGAGGTGGTCGAGGGGAATCCTATTATGGAAA TCGCCAAATACTTCCTGGAATATCCTTTAACCATCAAACGTCCAGAGAAGTTTGGAAGAGCCTGACGGTAAATTCGTACGAAGA GCTTGAATCCCTGTTTAAGAACAAAGAACTGCACCCGATGGACCTGAAAAACGCCGTAGCCGAAGAGCTTATCAAAATTTTAGAG CCAATCCCTAAGCGTCTT <u>TAA</u> CTGCAGTTTCAAACGCTAAATGCCTGAGAATTC <mark>AAAAAGCCCCGCACCTGACGGCGTTTTCCAAAGGCCTTTTCCTTTCCGCCGATCAAAAGGCCTTTTCCTTTTCCTTTTTTTT</mark>
	TCAGAAGGGAGAACTTGACGAAATTCGCAAGATTGGCGATTACAACAAGAAGGTATTTGAGGCGATGGGACTGAAGGCGAAGTA TCTTTATGGCTCACCTTTTCAGTTGGATAAGGACTACACTTTAAATGTATATCGTCTGGCTTTAAAGACTACCCTGAAGGCGAAGGC GCCGCTCGATGGAGCTTATCGCGCGTGAGGACGAAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAAGCGTGCGC GCCGCTCGATGGAGCTTATCGCGCGTGGGGCGGAAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGACGTCGCCGTGGGCGGAAAACCCAAAAGTAGCCGAAAATCCACATGTTAGCTCGTGAGTTACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATCACACATGTTAGCTCGTGAGGAAACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATGAGTTCATCCAAAAGGCAACTTTATCGCAGTG GATGATAGTCCTGAAGAGAATTCGCGCCCAAGATTAAAAAGGCCTATTGTCCCGCCGGAGGGAG
	TCAGAAGGGAGAACTTGACGAAATTCGCAAGATTGGCGATTACAACAAGAAGGTATTTGAGGCGATGGGACTGAAGGCGAAGTA TCTTTATGGCTCACCTTTTCAGTTGGATAAGGACTACACTTTAAATGTATATCGTCTGGCTTTAAAGACTACCCTGAAGGCGAAGGCG GCCGCTCGATGGAGCTTATCGCGCGTGAGGACGACAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGACGTCGCCGTGGGCGGAAAACCCAAAAGTAGCCGAAGTGATCTATCCAATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGACGTCGCCGTGGGCGGAATGGAGCAGCGTAAAATCCACATGTTAGCTCGTGAGTTACTTCCCAAAAA GGTGGTCTGTATCCACAATCCTGTTCTTACAGGGCTGGACGGTGAAGGCAAAATGAGTTCATCCAAAAGGCAACTTTATCGCAGTG GATGATAGTCCTGAAGAGAATTCGCGCCCAAGATTAAAAAGGCCTATTGTCCCGCCGGAGGTGGTCGAGGGGAATCCTATTATGGAAA TCGCCAAATACTTCCTGGAATATCCTTTAACCATCAAACGTCCAGAGAAGTTGGAAGGAGACCTGACGGGAAATCCTATTATGGAAA GCTTGAATCCCTGTTTAAGAACAAAGAACTGCACCCGATGGACCTGAAAAACGCCGTAGCCGAAGAGCTTATCAAAATTTAGAG CCAATCCGTAAGCGTCTT <u>TAA</u> CTGCAGTTTCAAACGCCAACGCGCAGCTGACGGCGACCTGACGGGCGGCTT TTTTTTGGACCTTTAACTATGAGAGGATTGCACGGCTAACTAA
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	TCAGAAGGGAGAACTTGACGAAATTCGCAAGATTGGCGATTACAACAAGAAGGTATTTGAGGCGATGGGACTGAAGGCGAAGTA TCTTTATGGCTCACCTTTTACGTGGATAAGGACTACACTTTAAATGTATATCGTCGGCGTTTAAAGACTACCCTCAAGGGAAGGCGAAAGTACCT CACATTATCTTGGTGTTGACCGCGGTGGGCGGAAGGCGAAAACCCCAAAGTGATCATCACAATGATGATCATCCCAACATCATGCAAGTGAATACCT CACATTATCTTGGTGTTGCACGCGCGGGGGCGGAATGGAGCGCGAAAGTGGCCAAATGAGTTCATCCCAACAGGAATACCT CACATTATCTGGGGTTGACCGCGCGGGCGGACGGGACG

GGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTC ${\tt CCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCG}$ CATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCCGCCAACACCCGCTGACGCG ${\tt CCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACC}$ GTCATCACCGAAACGCGCGAGGCAGGCGTCCATCAGCTCGCCCCGATCTTCGGGCAGTGAGGCGGCAATGACTGCCGCCTTTTCC TGCCTTTCGGTCTTGGGTGGTGCTTTCTGCTCACGGCGTCGGCTCCGGCTCAATACTCGTGGGGCAGCATGGCTACCGTGTGCGA ACGGTCGCCATCGGTCACGATCAGCACTCGCACACCGTGCGGGTGGTAAACCGACACGATGGTGCCGCACTCCTTCACGGCTTCC TCGTTGGCCTCGCGCTGCGCATCGTCCACGTCGCCCCAGTCCCCGGAAACGTGGCGGTCGATCAGCGGCCCTGCTACGCCCACGT GTTCTGCCAACATAACGGCGGATGCTTCATCACGGGAAAGCACCGGAAACACGGCGACCTCATGGGCCAAAAGCCACATTGAAC GGGTCATGCCATCAGTGAAACACCGCGAAATGGCCCAGAATCATGGTCGGGCTTACGTCAGCAAGCGGAACGGGGCTGTCGA TGCCCACGGCAAAATGCGCGGCGCTCGACGGATATTTCCGGTCTTTAGGCATGTCCTGCTTCAACGGGCAGGCTTGAGAATCCTC CATAAAGGCGTCAGGATGGTTCGGCACGGGCAGCCGCCAAACAACCTCATTCCAAGCATTCGGCACGATTTGAGGCAATTCGAG GGGCAACCCGCTCGACTGCGCAAGCTCTTTATCAAGCCGCCGAATTTCCAGCCAAGCGCGACACCGCCGCCGTAGCAACATCAGC ATCGTTAGCGCCCATGCTCTCGGCCTGCGCCGGTTTTTTCCATCTGCCGCTGTCGTTGATGTTGCGCGGCCTGTGGGTTGCGGACG ATTCCGGCGCGACCGTGCGGATAACTGATTTCCGCATGGTCGTTCATCTTCACTTTCTCCGCGCCCCGAAACAGCGGCGCGTTCATG CAGAATGATCGTGCCTGGGCGGCTATCGTCGGCCTGAATGACATAGCTATTGGTGCGACCGATCACACGGCCCCGGTAAGTTTCG CCTTTCTACGTCGCGCTCAAGCCGGTTGGCGTTGGTGCTCTTAGTGGCGTCTGGCGTGGTTTCTAGCTCTTTGCGGGGTCTCTTGTAG GCAGGCGCATGGTTTCCGCTTCATTCTGGCGCGACTGCTCTTGCAGCTTCCGGCGTTCGTCTTTCTCCTGCTCGCGGCGCGTGTCG GTCATGGTCTTTCCTCCATCGTGTCAAGCTCGCTTCGCGTGCCATAGTGCTCGCGCCGTTCAACGGCGTTGCCGTCCTCCACTACG TTGCGGCCCTTCGGGTGACTCGGCGCTGTGCGCTATGGCGATCTGGAAGTGTCGGGACGAGGGGCGTCCCGGCGGTAAATTCGAC GTGCCTGCGGCGCGTCGAACAAGGGGCGTGCCCGGTGTCAGTTCGTGGCATTTTTCGAGGCGCGACGCCATTTCCAAGGCTCCTG AATAACGGTAAGCCCAGGCCATGCCTTCTTGAACCATGATCGCATTGATGTTGGTGAGTTGTGTTTGGCCGCCGGGGGTATTGCAA CGGCGCGTAAACGACCCCAAGAGTGCGGCCATACCGATCAACCTCTTTTTCGGTCACTTGAACCTCTTGGCGAAAGGTCAAGTCG GCGAGCCGTTGGCGAGCACGGGAGCCGAAGGCTTGGCCGCTTCCCGGTGCGTCAATATCGGCCAATCTCACGCGGATGGTCTGAC GGTTCACCAAAAACGTCGATAGTGTCACCGTCAAGGATTCGGACGACTTCACCCCGGAAGTCGGCCCAAGCGGGCACACTGACGA TTAGGACGACAGCGGCCGCGACCGCGCAAGGGCGGCAAGGGCGCTTTTCATTGTTTGCCTCCTGTTTTCAAGACGGCTGTGAGA GATAGCCTCGTTATCGCGTTGGGTGAGCTTTTCCATGCAGCGGTTTAGTTCTTCGCCGCTGCGGCGTTTCACTTCGGCAAGCTGGT CGGCCAGCTTGTCGCGCTCGCGTTCCATAGGTTCGAGCTGATTCACTCGTTCGCGGAGCTGGTCGTTTTCGCGGGTGAAGGTGTCG GCTAGTTCGATTGCTTCGGCAAGCTGCTGGCTGATGGCCGCTTTGTCGGCCTCGATCTGTTCCGATCTTCGTCAAACCGGGCGTT GGCGTGCGCCAGGGCGATAGCCCATAGCGCATTGCCAAGCTCGGCAAGATGCTCGTTGACTGCAACCGGCAATGGGTCTGATGA GGGCAGGGTGGCGGTCTTGCGGTTTTTCCATTCAGCCATTGCATCGGAAATGGTTGTGAAGCTACCGCTTCCGAGTTTCTTGCGCA CGGCGGCCAAAGTGGGCCGGATGCCTTCGGCGTCCAGTTCGTCGGCTGCTCGCCAAATGTCTTGTTTAGTGATTGCCATTCTTGCG GGCCTCTGTACTGTAGTATGTTGTATGATACTACAACAACAACTTAACAAGAGCCATCTTGGAATCTGGTGTCTCTGCGCCCTA TAATTCTGGAACAGCTACTTTCCGAACGACTCCTGCGTTGATCGGAAATCCAGAAGCCCGAGAGGTTGCCGCCTTTCGGGCTTTTT CTTTTTCAAAAAAAAAATTTATAAAACGATCTGTTGCGGCCGCCGCGGGTTGTGGGCAAAGGCGCTCGACGGTGGGCAACCGCTTG GAAAAGCTAATTGAACATACTACTTGCTGTAACTACTTGCCGGAGCGAGGGGTGTTTGCAAGCTGTTGATCTGAAAGGGCTATTA GCGTTCTCACGTGCCTTTTTGATTAGCGATTTCACGTGACCTTATTAGCGATTTCACGTACTCCGATTAGCGATTTCACGTACCCTG ATTAGCGATTTCACGTGGATAGTTTTTGGAGCGGGCCGGAAAGCCCCGTGAATCAAGGCTTTGCGGGGGCATTAGCGGTTTCACGT GGATAACTACCCTCTATCCACAGGCTTCCGGGGGATAAAAAGCCCGCTCGACGGCGGGCTGTTGGATGGGAAGGCTTGACCAAG CCAAGCGTAGCGTTGGCCTGGTCAAGTCGGAGGGGGGGCCGATGCGAGCGCCCTTGCCGGGTGCGCGGGGGACATGCAGGCGTGT ACGCGATAGGTCAGCCACGTGTAAATGTCCATCGCAAGCGGAGACTGCCGCAAGGCATGCAGGTAGTCGATTCGGATAGGAACC GGTGAGCGGGTGACTTCCTCGAAGAAATCGCCTGTGAGGGTGAGGGTGCTATCCCATAGCGCCCGATCTTCTGGCCGCTTGGGAT TCCAGAATAGAAAAGCGCGCTTGGCAATGACGACGTTCTCAATGCCGAAGTCATTGCCTTGCTCGCCGGCAAGCGAAATCATGG ATGAAAACAGGCGTTGCGCCTGATTGCGAAGGGTGGCCGTGTAACGGCCATCGGTGTGCATTCCGAGCCTTTGTAGAAATTCCGA ACCGTAGGGCAGGCCGATGCTCGGCTTGCCCATGATCGACAAGGTGACGATGCCATTGGTGCGCTCAAAGTAGCTGGTCTTGGGG TCGGTGTGGGGCATGGTCGCTTGCACAAGGCAACGGGCCATGTAGCCGACTAAGCCAGCTTCGCGGGCATCCTCCATTTCGAGCG CGAGGCTCGTCTTGATGATCTCGTTGATACGATGGCCGGGGGGCTTTGTTGTTCTTAGGCATGTTGTTCCCCCCCGGCATGGTGATGGTTGGTCTAGTGTTTGTGGGTTTGATGTTCCGGCGTTTGATGAACAGGCGCAAGGTGTGAGGGCTGACGCCTAACAACTCGGCT GCGCGACTTTGCGGCAAGCCAAGGTTCACGTATGCCTGTACTTCATCAATACGGCTGTCCAGGCTTCAAGGCGCTCGATTTGCTGCC GTGCAGCCAAGCCAAGCACGGTTGCCATGATGTCGCTTTGTAGGCTGCCGTCCATGATGATCTTCTGTTTGGTCACATGGACGATT AGGCCGCGCTCGCCGCTTTGAGAATTTCCAAGGCGGCGAGGGCGGAACCGGCAATGCGCGTAATCTCCGGCGTCAGTAGC ACGTCGCCACGCTCGGCCTTTTCGATGATTGCTCCGAGCTTGCGCCAGTCCTTTGCTCTGCTCGGCAATTTCTTCCTCGATC TGTAGCGGCGCGAAGCCTTTGGCGTTCGCGTATTCGAGCAAACCGTATTTTTGGTTTTCCGGGTCTTGGCCGTCACGCGAAACCCG GAGATAGGCATAGTATTTTGGCATTTGCAGGGAAAACGTCAGATTCGGTTAAACATGCCTCATTCTAGCGCAGATTAAATAGGAA TTAAATACCCTGTTGCGGTATAGATAAAACGTTGGTTTGTTCTGCCCTATGAGCGTACAAAAAAGGCCGGGTGAGTGGCCCGGCC TTCGTTTAGGTGCTGAATAGGATTGGTTCTGGTGCCAGCCTCATGAGAAGCGCGTCATAAAACCACATGAGGGCCGACGACCAA GGCCGACGCCTGCGACCGATAGCATGATGTGGGTCTTATTGGCCGAGTCCAGCCCAAGCCACATGATCGGTAGGGTGATGAGAC

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	TCAGGTATAAATGGGCTCGCGATAATGTTCAGAATTGGTTAATTGGTTGTAACACTGACCCCTATTTGTTTATTTTTCTAAATACA
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	TGACTCATGACCAAAAATCCCTTAACGTGAGTTACGCGCGGCGGCGGCGGCGCGCGC
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	AGGGCTACCAACTCTTTTTTCCGAAGGTAACTGGCTTCAGCAGGCGCGGGAGCGCAGGTACCGAATACTGTTCTCAGTGGTGGCGCGGTA
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Pon7-	ATGTCCGATCAGTCTCAAGAACCTACAATGGAGGAAATCCTCGCCTCCATTCGACGCATCATCTCGGAGGATGACGCGCCGGCGG
(UAC)	AGCCTGCGGCCGAAGCGGCGCCCCCCGCCGCCGCGGAACCCGAACCTGAACCGGTGTCGTTCGACGACGACGAGGTTCTGGAATTGA
(UAU)2-	CGGATCCGATCGCGCCCGAGCCCGAGCTGCCGCCGCTGGAGACTGTCGGCGACATCGACGTCTATTCGCCGCCGGAACCTGAGTC
SIGFP	GGAACCGGCCTACACGCCGCCGCCGCCGGCGGCTCCGGTGTTTGATCGCGACGAAGTCGCCGAGCAGCTGGTCGGCGTTTCGGCCGCC
ORF	TCGGCCGCGCGAGCGCCTTCGGCAGCCTGAGCTCGGCCCTGCTGATGCCCAAGGACGGTCGGACGCTGGAAGACGTCGTACGC
(rest is	GAGCTGCTGCGCCCGCTGCGCCCGGACCAGAACCTGCCGGCCCCGCGACCAAGGTTGAGGAAGGA
same as	
pBAD-	
PopZ-	TTTTTCCCCCTATCCAGATCACATGACATGACATGACTTTTTAAGTCCCGCAATGCCAGAGGCCACTGCACAAGGCCACTATTA
(UAG) ₀ -	GCTTTAAGGATGATGGCACCTATAAGACTCGCGCAGAGGTAAAGTTTGAGGGCGATACTCTCGTAAATCGCATTGAGCTCAAGGG
sfGEP)	CATTGATTTTAAGGAGGATGGCAATATTCTCGGCCATAAGCTGGAGTATAATTTCAATTCCCATAATGTATACATTACCGCAGATA
51011)	AGCAAAAGAATGGCATTAAGGCGAATTTTAAGATTCGCCATAATGTGGAGGATGGCTCCGTACAACTCGCAGATCATTATCAACA
	AAATACTCCAATTGGCGATGGCCCAGTACTCCTCCCAGATAATCATTATCTCTCCACTCAATCCGTGCTCTCCAAAGATCCAAATG
	AGAAGCGCGATCACATGGTACTCCTGGAGTTTGTAACTGCAGCAGGCATTACTCATGGCATGGATGAGCTCTATAAGCTCGAGCA
PopZ-	A IGLEGATE A GLEGATE A LA A IGLAGUA A LE LOCETA LA LEGATE A LA LEG
$(UAG)_{6}$ -	CGCATCGATCGACCGCGCCCCCCCCCCCCCCCCCCCCCC
sfGFP	GGAACCGGCCTACACGCCGCCGCCGCCGCCGCGGCTGTTGATCGCGACGAAGTCGCCGAGCAGCTGGCGCGCTTTCGGCGCGC
ORF	TCGGCCGCGCGAGCGCCTTCGGCAGCCTGAGCTCGGCCCTGCTGATGCCCAAGGACGGTCGGACGCTGGAAGACGTCGTACGC
(rest is	GAGCTGCTGCGCCCGCTGCTC <u>AAG</u> GAGTGGCTGGACCAGAAC <u>CTG</u> CCG <u>CGC</u> ATC <u>GTC</u> GAG <u>ACCA</u> AGGTTGAGGAAGAAGTGCAG
same as	CGTATCTCTCGGGGACGCGGC <mark>TAG</mark> GGTGGTGGTGGTTCTGGTACC <mark>TAG</mark> GGC <mark>TAG</mark> GGCTAGGCATCCAAGGGCGAGGAGCTC
nBAD-	TTTACTGGCGTAGTACCAATTCTCGTAGAGCTCGATGGCGATGTAAATGGCCATAAGTTTTCCGTACGCGGCGAGGGCGAGGGCG
PDAD-	ATGCAACTAACGGCAAGCTCACTCTCAAGTTTATTTGTACTACTGGCAAGCTCCCAGTACCATGGCCAACTCTCGTAACTACTGG
POPZ-	ACCIA IGGEGTACAATGTTTTCCCGETATCAGATCACATGAAGCAACATGATTTTTTAAGTCCGCCAATGCCCGGAAGCCAGGGGTATG
$(UAG)_0$ -	ACAAGAGGGCACTATTAAGGATGATGATGGCAGGATGGCAAGACTGCGCAGAGGTAAAGTTGAGGGGAGGATAACTGCGCAGA
stGFP)	TA UNACCEGCAGATAAGCAAAAAGAATGACATAAGGCATAAGCTGCATAACCEGCATAACGCAGAGATGACCECGAAGCTCCCTACAAC
	TCGCAGATCATTATCAACAAAATACTCCAATTGGCGATGGCCCAGTACTCCTCCCAGATAATCATTATCTCTCCACTCAATCCGTG
	CTCTCCAAAGATCCAAATGAGAAGCGCGATCACATGGTACTCCTGGAGTTTGTAACTGCAGCAGGCATTACTCATGGCATGGATG
	AGCTCTATAAGCTCGAGCACCACCACCACCACCACCACCAC
PopZ-	ATGTCCGATCAGTCTCAAGAACCTACAATGGAGGAAATCCTCGCCTCCATTCGACGCATCATCTCGGAGGATGACGCGCCGGCGG
(UAG)	AGCCTGCGGCCGAAGCGGCGCCCCCCGCCGCCGGAACCCGAACCTGAACCGGTGTCGTCGACGACGAGGTTCTGGAATTGA
sfGFP	CGGATCCGATCGCGCCCGAGCTGCCGCGCGCGCGGGGGGCGCCGCGCGGCGCGCGGGACCTGAGTC
ORF	
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same as	GGCGAGGAGCTCTTTACTGGCGTAGTACCAATTCTCGTAGAGCTCGATGGCGATGTAAATGGCCATAAGTTTTCCGTACGCGGCG
pBAD-	AGGGCGAGGGCGATGCAACTAACGGCAAGCTCACTCTCAAGTTTATTTGTACTACTGGCAAGCTCCCAGTACCATGGCCAACTCT
PopZ-	CGTAACTACTCTGACCTATGGCGTACAATGTTTTTCCCGCTATCCAGATCACATGAAGCAACATGATTTTTTTAAGTCCGCAATGC

$(UAG)_0$ -	CAGAGGGCTATGTACAAGAGCGCACTATTAGCTTTAAGGATGATGGCACCTATAAGACTCGCGCAGAGGTAAAGTTTGAGGGCG
sfGEP)	ATACTCTCGTAAATCG <u>CAT</u> TGAGCTCAAGGGCATTGATTTTAAGGAGGATGGCAATATTCTCGGCCATAAGCTGGAGTATAATTT
51011)	CAATTCCCATAATGTATAGATTACCGCAGATAAGCAAAAGAATGGCATTAAGGCGAATTTTAAGATTCGCCATAATGTGGAGGA
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nDule-	GCGCCGGTTAAGGCTAAACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAGTTGGTAGC
MiDroDG	TCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTCGTTTTCAGAGCAAGAGATACGCGCAGACCAAAACGATCTCAA
мјбрако	GAAGATCATCTTATTAATCAGATAAAATATTTCTAGATTTCAGTGCAATTTATCTCTTTCAAATGTAGCACCTGAAGTCAGCCCCAT
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	GAGAGECTICAACCCAGICAGCICCTICCGGIGGGCCGGGGCAGAGACIAICGICGCCGCACIIIAIGACIGICIICIIICI
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	A A TACCCECTE A CONTEXA A A CETECEEE A A A A ATTECETE A CETE A CETETA A CITETA CE A A GA A CIEGA A TOTETETE A A
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pDule-	GTTCGCGACGCGAGGCTGGATGGCCTTCCCCATTATGATTCTTCTCGCTTCCGGCGGCATCGGGATGCCCGCGTTGCAGGCCATGC
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SCOUHWK	GCTGATCGTCACGGCGATTTATGCCGCCTCGGCGAGCACATGGAACGGGTTGGCATGGATTGTAGGCGCCGCCCTATACCTTGTC
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$+DNA^{Trn}$	GTATAAGATCATACGCCGTTATACGTTGTTTACGCTTTG AGGAATCCCATATGTCAAATGATGAGACTGTTGAGAAAGTTACGCA
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	ΓΕΑΓΑΛΟΕΙ ΤΟΤΕΙ Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο
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<i>tRNA_{CUA}</i>	GCTCGAGCGTTTTATCTGTTGTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCTGTCCCTCCTGTTCAGCTAC
gene)	TGACGGGGTGGTGCGTAACGGCAAAAGCACCGCCGGGACATCAGCGGCAGGGGGGGG
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	A = C = C = A = C = C = C = C = C = C =
	CTACGCAGTCAGCACCGTGTATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGATA
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	ATGAGGGCTTGTTTCGCGGGGGGGGGGGCGGGGGGCGGGGGGCGGGGGCATCTCCTCGCATGCACCATTCC
	I I GUUGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU
	CCTGTCGCTTGCGGTATTCGGAATCTTGCACGCCCTCGCTCAAGCCTTCGTCACTGGTCCCCGCCACCAAACGTTTCGGCGAGAAGC
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	CCACAAAA ICAAGCACCAIGAGGICICAAGAAGIAAAA IA IACAIIGAAA IGGCGIGIGGAGACCCAICIIGIIGIGAAAAAIC
	A GUAGET IGLAGAACAGUCUAGAGCAT ICAGACATCATAGTACAGAAAAACC IGCAAACGATGTAGGGTT ICGGACGAGGATATC
	AATAA ITTETEAAAAA CAACQAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	COCATEGA A A TCA A CTCCA A ATTCGTCTGTCTCCGCATCGCTCCTCTCACATACGACCACATCACATCGCTTGACTAGGATCGACTGAG
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pDule-	TAACTATATGCGAAAACTCGATAGGATTTTACCAGGCCCAATAAAAATTTTCGAAGTCGGACCTTGTTACCGGAAAGAGTCTGAC
MbAbKRS	GGCAAAGAGCACCTGGAAGAATTTACTATGGTGAACTTCGCTCAGATGGGTTCGGGATGTACTCGGGAAAATCTTGAAGCTCTCA
-2xtRNA	TCAAAGAGTTTCTGGACTATCTGGAAATCGACTTCGAAATCGTAGGAGATTCCTGTATGGTCTTTGGGGATACTCTTGATATAATG
DDC1	CACGGGGACCIGGAGCITICIICGGCAGICGICGGCCAGITICICIIGAIAGAGAAIGGGGIAIIGACAAACCAIGGAIAGGI
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$+ P N \Lambda^{P v l}$	GCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTA
	AGGAGGTAAAAAATGGGTAATAATCGCCCCGTATACATTCCTCAGCCACGCCCGCC
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	THECA FAGUE FEEDECECCUC IGACAAGUA TEACGAAA TEAGUGE TEAAA TEAGIGU IGUGAAACUCGACAAGGACTA TAAAG
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	TCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACCACTGGCAGCAGCACCACTG
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	ΓΕΙΟΟΑΙΟΙΟΟΛΟΙΟΟΛΟΙΟΟΙΟΟΛΟΛΟΙΟΙΟΙΟΙΟΙΟΙΟΙΟ
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pDule- MbAbKRS- 2xtRNA- RBS2-api1b (rest is same as pDule- MbAbKRS- 2xtRNA- RBS1- api1b)	GAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGA AAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTATTATCCATAAGATTAGCGGATCCTACCTGACG CTTTTTATCGCAACTCTCTACTG TTTCTCCATACCGGTTTTTAAGGAGTTAAGGAGGTAAAAAATGGGTAATAAT
pDule- MbAbKRS- 2xtRNA- RBS1- apiB5 (rest is same as pDule- MbAbKRS- 2xtRNA- RBS1- api1b)	GAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGA AAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTAGCATAAGATTAGCGGGATCCTACCTGACG CTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTAAGGAGGTAAAAAAATGAAAAATGAAAAATAATGCACCCATATACGTACCAC AACCACGCCCGCCGCCACCACAACTA <u>TAA</u> TTCGCACACTGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGCTCGAGCGTTTTA TCTGTTGTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCTGTCCCTCCTGTTCAGCTACTGACGGGGTGGTGC GTAACGGCAAAAGCACCGCCGGGACATCAGCGCTAGCGGAGTGTATACTGGCTTACTATGTTGGCACTGA
pDule- MbAbKRS- 2xtRNA- RBS1- apiB8 (rest is same as pDule- MbAbKRS- 2xtRNA- RBS1- api1b)	GAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGA AAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTATCCATAAGATTAGCGGATCCTACCTGACG CTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTAAGGAGGTAAAAAATGGAAAATAATGGAACCCCATATACGTATCAG GACCACGCCCGCCGCCACCCAGAATA <u>TGA</u> TTCGCACACTGTGGAGCAAAAGGCCAGGAAAATGGAAAATAATGGAACCCCATATACGTATCAG GACCACGCCCGCCGCCACCCAAGAATA <u>TGA</u> TTCGCACATGTGGAGCAAAAGGCCAGGAAAAGGCCAGGAACCGCTCGAGCGTTTTA TCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCTGTCCCTCCTGTTCAGCTACTGACGGGGGTGGTGC GTAACGGCAAAAGCACCGCCGGGACATCAGCGCTAGCGGAGTGTATACTGGCTTACTATGTTGGCACTGA
pDule- MbAbKRS- 2xtRNA- RBS1- apiB10 (rest is same as pDule- MbAbKRS- 2xtRNA- RBS1- api1b)	GAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGA AAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTATTATCCATAAGATTAGCGGATCCTACCTGACG CTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTAAGGAGGTAAAAAATGGCAAATAATACACCCGTATACGTATCAC AACCACGCCCGCCGCACCCAAAAATA <u>TAA</u> TTCGCACATGTGAGCAAAAGGCCAGGCAAAAGGCCAGGAACCGCTCGAGCGTTTTA TCTGTTGTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCTGTCCCTCCTGTTCAGCTACTGACGGGGGGGG
pDule- MbAbKRS- 2xtRNA- RBS1- apiC3 (rest is same as pDule- MbAbKRS- 2xtRNA- RBS1- api1b)	GAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACC CCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGA AAAGTCCACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATAGCATAACAAAAGTGCAAAAAGCGGGATCCTACCTGACG CTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTAAGGAGGTAAAAAATGGCAAATAATGCACCCGTATACGTACCAA AACCACGCCCGCCGCACCCAAGAATATGGTTCGCACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGCTCGAGCGTTTTA TCTGTTGTTGTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCTGTCCCTCCTGTTCAGCTACTGACGGGGTGGTGC GTAACGGCAAAAGCACCGCCGGGACATCAGCGCTAGCGGAGTGTATACTGGCTTACTATGTTGGCAC <u>TGA</u>