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Supplemental Data

A Synthetic Genetic Edge Detection Program

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1. Quick Edge Detection Protocol

Here, we present an easy-to-follow protocol for bacterial edge detection using minimal supplies. The image that appears on the plate is stable for many months at 4°C.

Equipment Required:

- 37ºC shaking incubator
- Benchtop Microcentrifuge
- Stirplate
- 42ºC heat block with 50mL conical adapter
- 36ºC still incubator with hole in top
- Kodak Ektagraphic projector (or other broad wavelength light source)
- 34x24mm slide with printed image
- 650nm bandpass filter (Edmund Optics catalogue #43-189)
- Focusing Lens

Solutions:

- LB + 0.1M HEPES pH = 6.6
- LB + 0.1M HEPES pH = 8.0
- LB + 0.1M HEPES pH = 6.6 + 0.3mg/mL Sgal + 0.5mg/mL Ferric Ammonium Citrate + 1% Seaplaque agarose

Day 1

1. Prepare 2 solutions of LB media supplemented with 0.1M HEPES buffer. pH one batch to 6.6 and one to 8.0. Autoclave.
2. Inoculate 3mL LB + 0.1M HEPES pH = 8.0 + 50ug/mL Ampicillin, 50ug/mL Kanamycin and 34ug/mL Chloramphenicol with a colony of JW3367c transformed with plasmids pED_L3, pPLPCB and pCph8.
3. Grow in 37ºC shaking incubator (250rpm) to saturation (OD_{600} ~ 3.0-3.7; approximately 14 hours).

Day 2

4. Add 50mg Ferric Ammonium Citrate (Sigma #15-1030), 30mg Sgal (Sigma #S9811) and 1g Seaplaque low melt agarose (Cambrex #CLNZR006) to 100mL of the LB + 0.1M HEPES pH=6.6 solution in a 150mL screw cap Pyrex bottle with a stirbar.
5. Autoclave.
6. Carefully place the hot bottle with the molten agarose media solution onto a stirplate. Stir gently for no more than 10 minutes, being sure not to allow the media to cool below 50ºC.
7. Add 15mL of the molten agarose solution to a 50mL sterile conical tube in a 42ºC heat block and allow to cool for to 42°C (~1 hour).
8. Add 500µL of the starter culture to a microcentrifuge tube (1.7mL). Spin at 13k rpm in a small tabletop centrifuge for 1 min to pellet the cells. Remove the supernatant media. Resuspend the pellet in 500µL fresh (unbuffered) LB media.
9. Pellet and resuspend twice more.
10. Add Ampicillin, Kanamycin and Chloramphenicol (concentrations as in step 2) to the 42ºC molten agarose. Promptly inoculate the molten agarose with 150µL of the triple

washed bacterial suspension. Immediately mix well, but be careful not to introduce bubbles into the media.

**Note: The bacterial seeding density directly determines the contrast of the final image. Contrast can therefore be optimized by altering the initial seeding density.*

11. Immediately pour the inoculated media into a 90mm sterile Petri Dish and tilt the dish if necessary to ensure evenness of the media.
12. Allow the inoculated agarose solution to solidify at room temperature for 1 hour.
13. After the agarose slab has set, cover the top of the Petri Dish with cellophane and poke several slits with a razor blade.
14. Place the agarose slab in the incubator, under the image.
15. Incubate at 36ºC for 24 hours.

Day 3

16. Remove the plate and place at 4ºC overnight.

2. Supplemental Figures

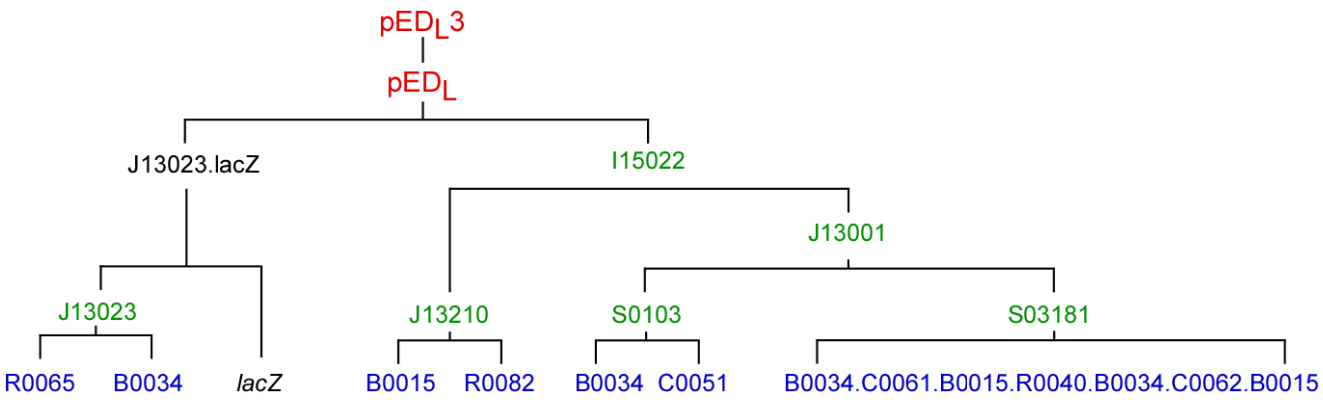

Figure S1. Construction of the edge detector plasmid, pED_L3. Basic Biobrick parts (blue) are combined using standard assembly (Knight, 2002) to make composite Biobricks (green). Composite Biobricks are combined and eventually yield J13023.lacZ and I15022. These two segments are combined in pSB4A3 to make the prototype edge detector plasmid, pED_L (red). The RBS preceeding the cI gene is replaced by the weaker 'RBS3' (Weiss, 2001), to yield the final edge detector plasmid pED_L3. Note that the *lacZ* gene (black) is not a Biobrick as it contains a forbidden EcoRI restriction site.

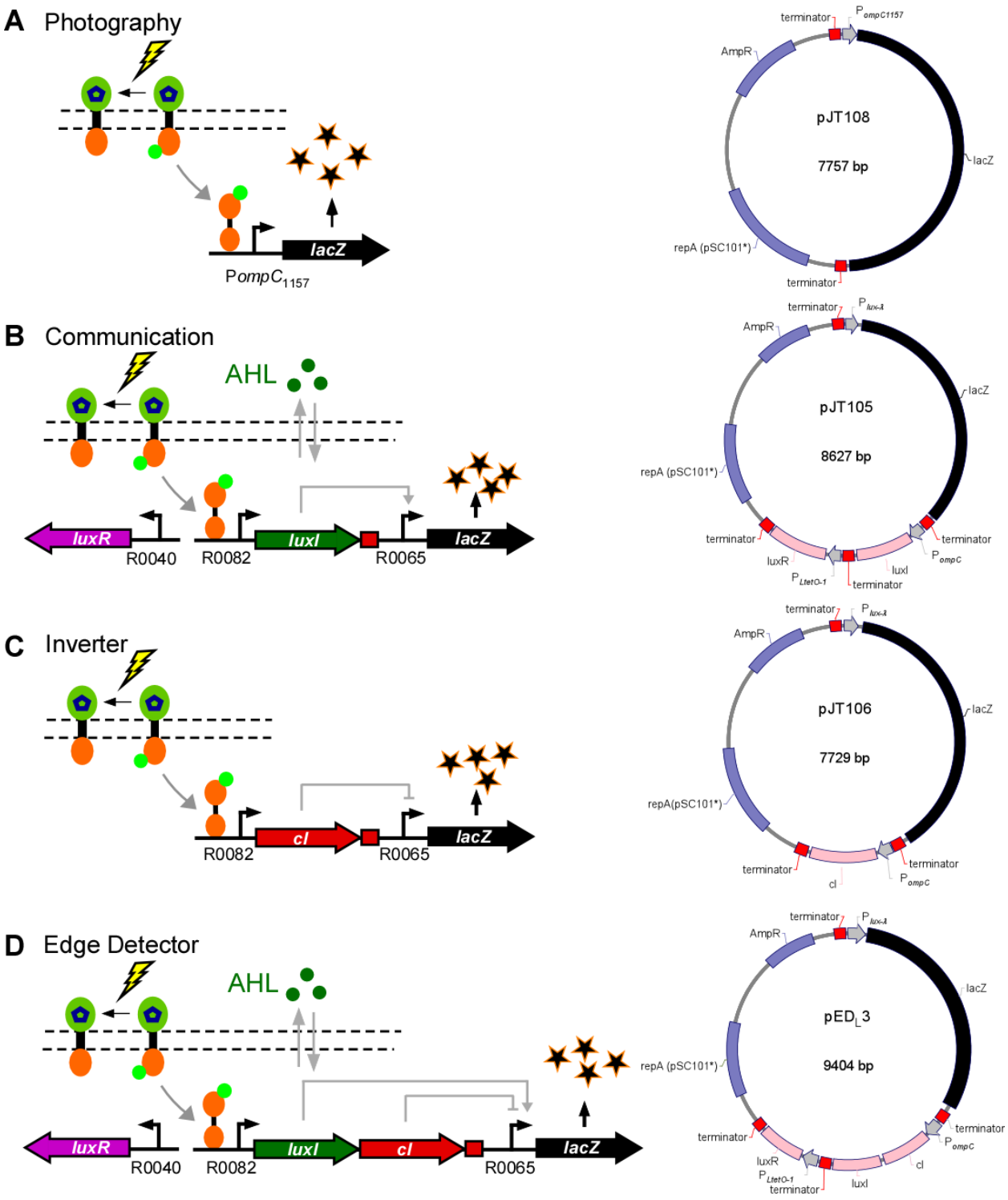

Figure S2. The genetic circuit diagrams and associated plasmids from **Figure 3** are shown for (A) photography, (B) cell-cell communication, (C) inverter, and (D) the full edge detector. The plasmids are combined with the pPLPCB(Gambetta and Lagarias, 2001) and pCph8(Levskaya et al., 2005), containing the phycocyanobilin (PCB, blue hexagons) biosynthesis pathway and chimeric light sensor (green-orange protein), respectively. All terminators (red boxes) are the

tandem *E.coli rrnB*:Bacteriophage T7 TE terminator (BBa_B0015). The orange protein is OmpR, which is phosphorylated by the light sensor, and is expressed by the host strain. The BioBrick numbers are provided for the promoters.

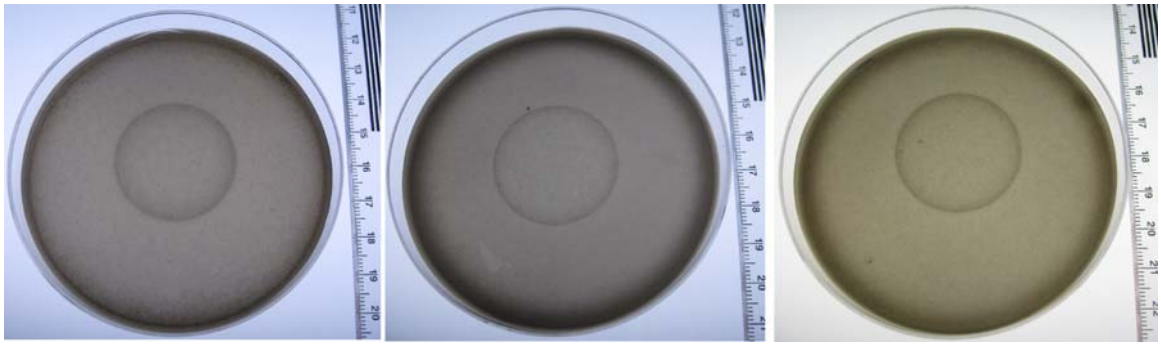

Figure S3. The three 36mm circle plate plates used to determine the edge intensity profiles in **Figure 5a** and average edge width reported in the main text.

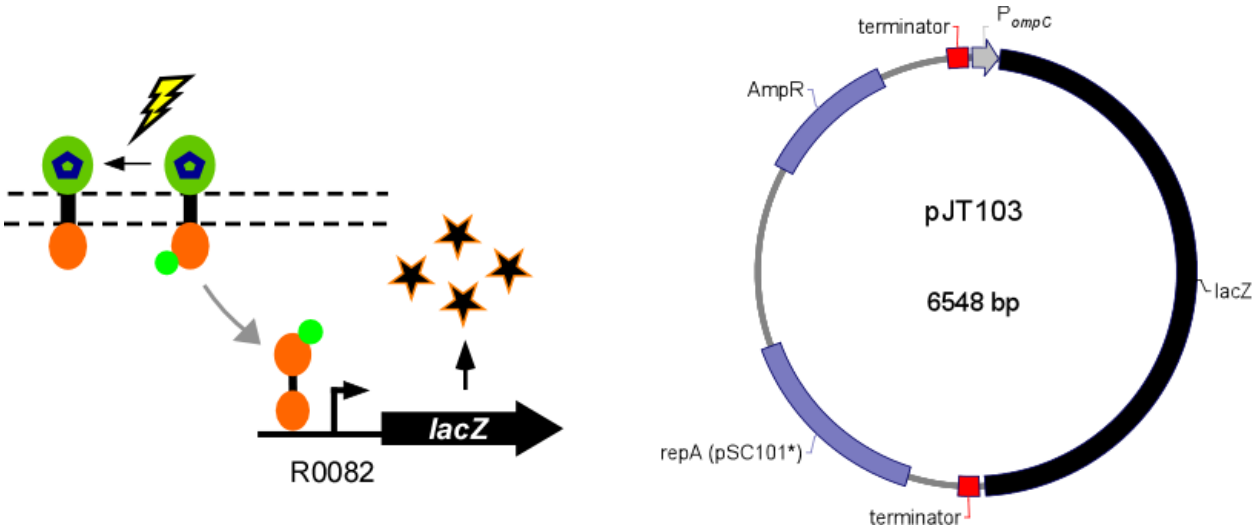

Figure S4. The genetic circuit diagram and associated plasmid map of the light transfer function measuring construct, pJT103. The plasmid is combined with the pPLPCB(Gambetta and Lagarias, 2001) and pCph8(Levskaya et al., 2005), containing the phycocyanobilin (PCB, blue hexagon) biosynthesis pathway and chimeric light sensor (green-orange), respectively. The orange protein is OmpR, which is phosphorylated by the light sensor, and is expressed in the host strain. R0082 is the P_{ompC} promoter.

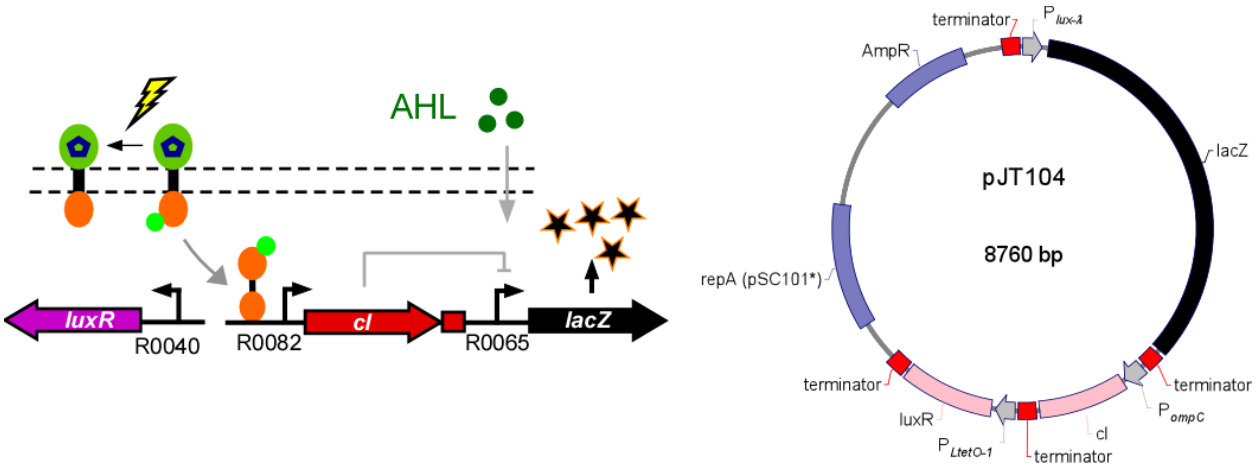

Figure S5. Schematic Diagram and plasmid map of the genetic circuit for the calculation of the two-dimensional transfer function for $P_{lux\text{-}\lambda}$ (R0065) in **Figure 2b.** Light controls CI abundance and AHL is added exogenously. Not shown are the PCB biosynthetic genes *ho1* and *pcyA* (plasmid pPLPCB) and *cph8* (plasmid pCph8).

3. DNA sequences

Biobrick	Description
BBa_B0015	*rrnB*/TE tandem transcription terminator
BBa_B0034	strong ribosome binding site
BBa_C0051	*cI* open reading frame with LVA degradation tag
BBa_C0061	*luxI* open reading frame with LVA degradation tag
BBa_C0062	*luxR* open reading frame
BBa_R0040	$P_{Ltet\text{-}O1}$ promoter (constitutive in the absence of Tet repressor)
BBa_R0065	$P_{lux\text{-}\lambda}$ LuxR::AHL activated, cI repressed promoter
BBa_R0082	P_{ompC} promoter
BBa_J13023	Composite of R0065 and B0034
BBa_J13210	Composite of B0015 and R0082
BBa_S0103	Composite of B0034 and C0051
BBa_S03181	Composite of B0034, C0061, B0015, R0040, B0034, C0062, B0015
BBa_J13001	Composite of S0103 and S03181
BBa_I15022	Composite of J13210 and J13001

Biobrick sequences

BBa_B0015

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ctttcgttttatctgttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcctttct
gcgtttata

Promoters

Constitutive Promoter (BBa_R0040)
tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac

LuxR+AHL activated – CI repressed promoter (BBa_R0065; LuxR operator underlined, cI operators in bold)
TAAGCACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGAA**TAACACCGTGCGTGTTGA**CTA
TTT**TACCTCTGGCGGTGATA**

P_{ompC} promoter BBa_R0082 (OmpR operators underlined)
TCCCTTGCATTTACATTTTGAAACATCTATAGCGATAAATGAAACATCTTAAAAGTTTTAGTATCATATTCGTGTTG
GATTATTCTGCATTTTTGGGGAGAATGGACT

$P_{ompC1157}$ (region upstream of *lacZ* from the genome of RU1012. Includes complete R0082 sequence. OmpR operators underlined)
TCCCTTGCATTTACATTTTGAAACATCTATAGCGATAAATGAAACATCTTAAAAGTTTTAGTATCATATTCGTGTTG
GATTATTCTGCATTTTTGGGGAGAATGGACTTGCCGACTGATTAATGAGGGTTAATCAGTATGCAGTGGCATAAAAA
AGCAAATAAAGGCATATAACAGAGGGTTAATAACATGAAAGTTAAAGTACTGTCCCTCCTGGTCCCAGCTCTGCTGG
TAGCAGGCGCAGCAAACGCTGCTGAAGTTTACAACAAAGACGGCAACAAATTAGATCTGTACGGTAAAGTAGACGGC
CTGCACTATTTCTCTGACAACAAAGATGTAGATGGCGACCAGACCTACATGCGTCTTGGCTTCAAAGGTGAAACTCA
GGTTACTGACCAGCTGACCGGTTACGGCCAGTGGGAATATCAGATCCAGGGCAACAGCGCTGAAAACGAAAACAACT
CCTGGACCCGTGTGGCATTCGCAGGTCTGAAATTCCAGGATGTGGGTTCTTTCGACTACGGTCGTAACTACGGCGTT
GTTTATGACGTAACTTCCTGGACCGACGTACTGCCAGAATTCGGTGGTGACACCTACGGTTCTGACAACTTCATGCA
GCAGCGTGGTAACGGCTTCGCGACCTACCGTAACACTGACTTCTTCGGTCTGGTTGACGGCCTGAACTTTGCTGTTC
AGTACCAGGGTAAAAACGGCAACCCATCTGGTGAAGGCTTTACTAGTGGCGTAACTAACAACGGTCGTGACGCACTG
CGTCAAAACGGCGACGGCGTCGGCGGTTCTATCACTTATGATTACGAAGGTTTCGGTATCGGTGGTGCGATCTCCAG
CTCCAAACGTACTGATGCTCAGAACACCGCTGCTTACATCGGTAACGGCGACCGTGCTGAAACCTACACTGGTGGTC
TGAAATACGACGCTAACAACATCTACCTGGCTGCTCAGTACACCCAGACCTACTGAAGCGGCGCACGAAAAACGCGA
AAGCTAAAGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTTCACCGGATAAAGAGCAGCTAC
TGGTGGTTAACCTTTCCGGTCGCGGCGATAAAGACATCTTCACCGTTCACGATATTTTGAAAGCACGAGGGGAAATC
TG

Other sequences

lacZ ORF
ATGATAGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA
TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG
GCGAATGGCGCTTTGCCTGGTTTCCGGcACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCC
GATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTgACCTATCCCAT
TACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCT
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CGCGGTGATGGTGCTGCGcTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCC
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GCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCG
ATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTG
GTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGA
AAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATG
GTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGT
TCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATAT
TGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAA
CGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCT
AATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGA

CACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAAT
GGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGT
AACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGGCTTCGTCTGGGA
CTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATA
CGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCA
AAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCA
TAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATG
TCGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAACTCTGGCTCACA
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CAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCG
CATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCA
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GAATTCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAAAAAGCGGCCGCTCGAGTCTAA

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