

Generation of bright autobioluminescent bacteria by chromosomal integration of the improved *lux* operon *ilux2*

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Supplementary Tables

Supplementary Table S1. Plasmid combinations used for error-prone mutagenesis. pGEX(-) was used as the vector backbone.

Plasmid for error-prone mutagenesis				Plasmid containing the remaining lux genes			
Insert	Promoter	Origin of replication	Resistance	Insert	Promoter	Origin of replication	Resistance
<i>iluxAB</i>	lac	p15Aori	ampicillin	<i>iluxCDEfrp</i>	tac	ColE1	kanamycin
<i>iluxCDE</i>	lac	p15Aori	ampicillin	<i>iluxABfrp</i>	tac	ColE1	kanamycin
<i>ilux frp</i>	tac	p15Aori	ampicillin	<i>iluxCDABE</i>	tac	ColE1	kanamycin

Supplementary Table S2. Mutations identified during the screening of *luxAB* in addition to the mutations in *ilux*.

Gene	Mutations
<i>iluxXA</i>	L24M, I76V, Q86H, M139L, K283R, N289S, I298T, Y360-
<i>iluxXB</i>	T28S, Q95H, D112G, K193R, K238N, Y276F, M306T, V311A, D317A, H323L

Supplementary Table S3. Mutations contained in *ilux2* in addition to the mutations in *ilux*.

Gene	Mutations
<i>ilux2C</i>	T10N, N27T, N45T, I47T, E54D, D74N, S77P, L132I, D135G, T216I, Y248N, K345N, V348A, K414E, T445K
<i>ilux2D</i>	N3K, I90T, K106N, N108S, M112V, N165K, D168V, K234R, V251A, D274N, E300V

Supplementary Table S4. Primer sequences.

Name	Sequence (5'→3')
KanR SpeI fwd	GTTGATACTAGTATGAGCCATATTCAACGG
KanR Ascl rev	GTTGATGGCGCGCCTTAGAAAACTCATCGAG
pGEX(-) SpeI rev	GTTGCAACTAGTACTCTTCCTTTTTCAATATTATTG
pGEX(-) Ascl fwd	GTTGATGGCGCGCCCTGTCAGACCAAGTTTACTCA
pGEX(-) +lac BamHI rev	TCCGGGGATCCTGTTTCCTGTGTGAAATTGTTATCCGCTCA CAATTCCACACCAACATACGAGCCGGAAGCATAAAGTGTA ACAGCTCATTTTCAAGT
pGEX(-) BamHI fwd	GGATAACAATTTACACAGGAAACAGGATCC
p15Aori BglII fwd	ATCTTCAGATCTTTGAGATCGTTTTGGTCT
p15Aori AvrII rev	TTGATCCCTAGGTTTCCATAGGCTCCGCCC
pGEX(-) BglII rev	AACTAGAGATCTGAAGATCCTTTGATCTTTTCTAC
pGEX(-) AvrII fwd	GTTGCACCTAGGAACGCCAGCAACGCGGCC
pGEX(-) Ascl tac fwd	TTGATAGGCGCGCCTTGACAATTAATCATCGGCTCGTATAA
pGEX(-) Ascl tac rev	GTTACTGGCGCGCCAGCTCATTTTCAAGATATTTGCCAG
attTn7 fwd	GATGCTGGTGGCGAAGCTGT
luxC EcoRI rev	GTTGATGAATTCACTTTTTACCTATTATGGGACAAATACAAG GAAC
frp Sall fwd	GTAAGTGTGACCTAAGGAGAAAGAAATGGTGAAGATACA G
attTn7 rev	GATGACGGTTTGTACATGGA

Supplementary Figures

>ilux2C

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>*ilux2B*

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TGCCAGAAGGTAA

Figure S1. Nucleotide sequences of the *ilux2* genes. The sequences of *ilux2A*, *ilux2B* and *ilux2frp* are identical to *iluxA*, *iluxB* and *iluxfrp*, respectively. The sequence of *ilux2E* is the same as in the wild-type *lux* operon.

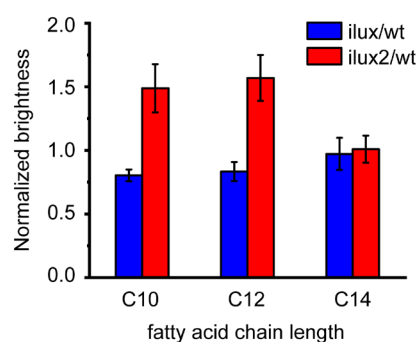


Figure S2. Comparison of aldehyde production of LuxCDE wt, iLuxCDE and iLux2CDE from saturated fatty acids of different chain length. Top10 cells expressing *luxCDE wt*, *iluxCDE* or *ilux2CDE* from pGEX(-) Kan were incubated in LB medium containing 300 μ M decanoic, dodecanoic or tetradecanoic acid for 5 min. Subsequently, Top10 cells expressing *iluxABfrp* from pGEX(-) Kan were added to detect the aldehyde produced by LuxCDE by bioluminescence. Imaging was performed at room temperature. For each fatty acid, the bioluminescence signal was normalized to LuxCDE wt. Error bars represent SD of 4 different clones.

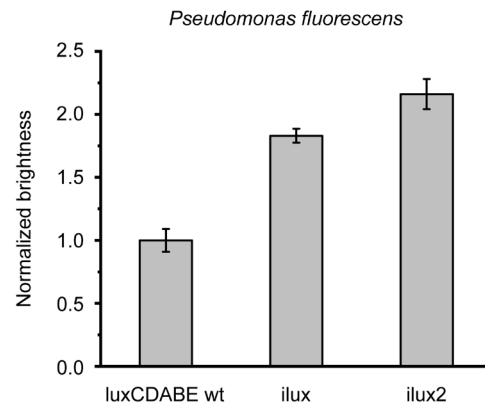


Figure S3. Comparison of *luxCDABE wt*, *ilux* and *ilux2* in *Pseudomonas fluorescens*. The indicated *lux* operons were expressed in *P. fluorescens* from the vector pJOE7771.1 at 30 °C. The bioluminescence signal was normalized to the *luxCDABE wt* operon. Error bars represent SD of 5 different clones.

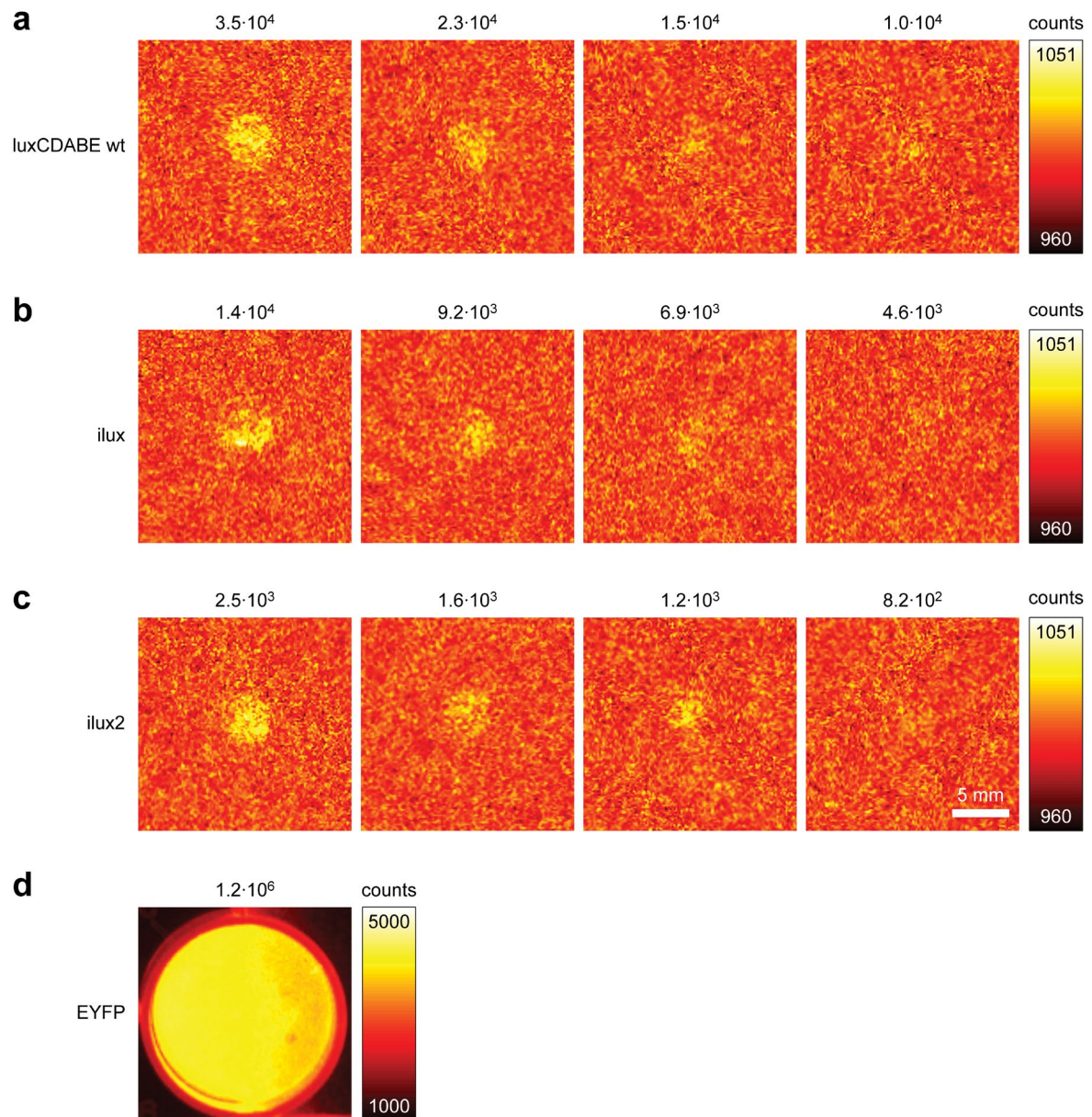


Figure S4. Detection of Top10 strains chromosomally labeled with (a) *luxCDABE wt*, (b) *ilux*, (c) *ilux2* or (d) EYFP on mashed potatoes. The indicated numbers of colony-forming units were applied in 1 μ l PBS onto mashed potatoes in 24-well plates. Imaging was performed at room temperature. Bioluminescence from the *luxCDABE wt*, *ilux* and *ilux2* strains was imaged with an exposure time of 10 min. Fluorescence of the EYFP strain was excited at 520 nm and imaged with an exposure time of 1 s.

Supplementary Movies

Movie S1 (separate file). *E. coli* Top10 strain chromosomally labeled with *ilux2* grown on cucumber. A slice of cucumber was inoculated with $1 \cdot 10^6$ colony-forming units (cfu). Bioluminescence images were taken at room temperature with an Amersham Imager AI 600 RGB using an exposure time of 1 min per image.

Movie S2 (separate file). *E. coli* Top10 strain chromosomally labeled with *ilux2* grown on potato. A slice of raw potato was inoculated with $1 \cdot 10^6$ cfu. Bioluminescence images were taken at room temperature with an Amersham Imager AI 600 RGB using an exposure time of 1 min per image.

Movie S3 (separate file). *E. coli* Top10 strain chromosomally labeled with *ilux2* grown on mashed potatoes. A dish filled with mashed potatoes was inoculated with $5 \cdot 10^5$ cfu. Bioluminescence images were taken at room temperature with an Amersham Imager AI 600 RGB using an exposure time of 5 s per image.

Movie S4 (separate file). *E. coli* Top10 strain chromosomally labeled with *ilux2* grown on egg yolk. A dish filled with raw egg yolk was inoculated with $5 \cdot 10^5$ cfu. Bioluminescence images were taken at room temperature with an Amersham Imager AI 600 RGB using an exposure time of 5 s per image.

Movie S5 (separate file). *E. coli* Top10 strain chromosomally labeled with *ilux2* grown in milk. A dish filled with UHT milk was inoculated with $3 \cdot 10^6$ cfu. Bioluminescence images were taken at room temperature with an Amersham Imager AI 600 RGB using an exposure time of 1 min per image.