

Supplementary Materials

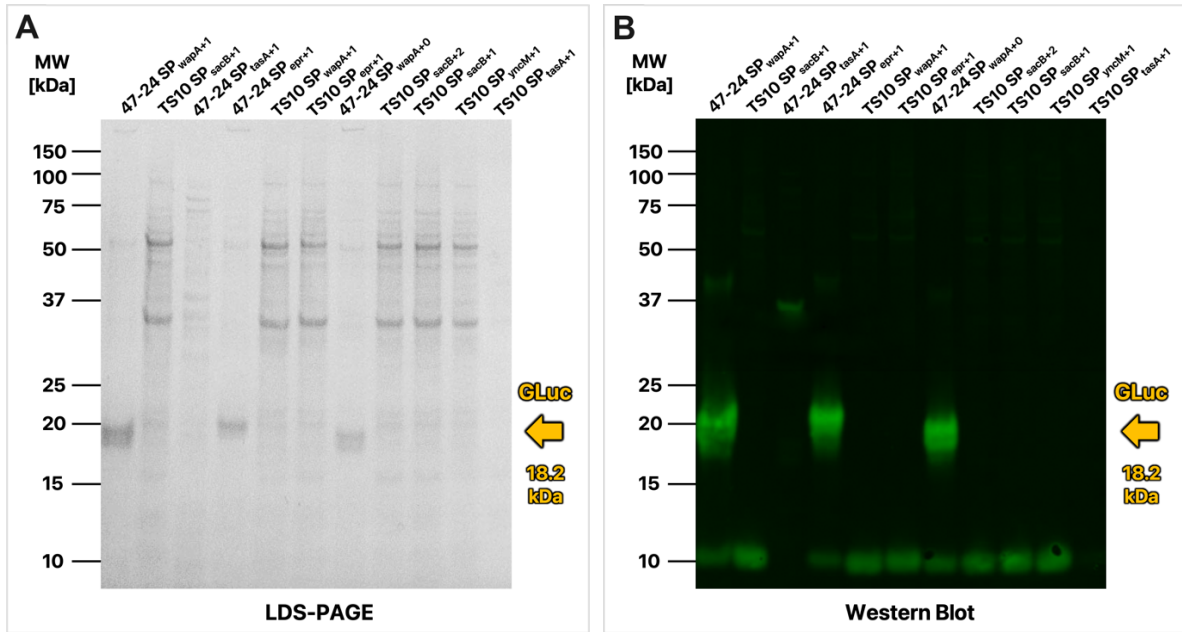
Let There Be Light: Genome-reduction Enables *Bacillus subtilis* to Produce Disulfide-bonded *Gaussia* Luciferase

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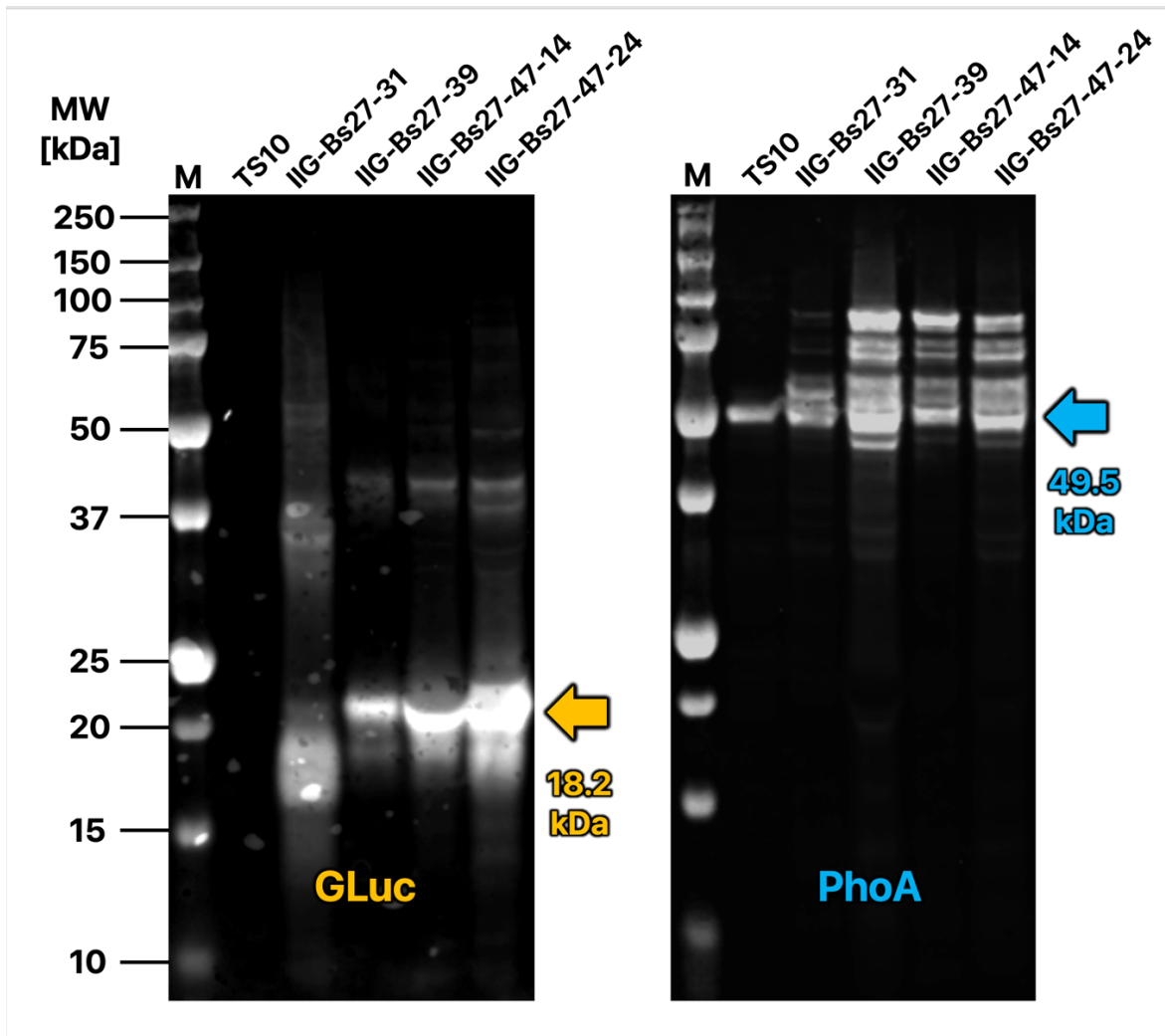
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Supplementary Figure S1: Signal peptide screening for GLuc secretion by *B. subtilis*. Cells of *B. subtilis* TS10 or IIG-Bs27-47-24 producing GLuc with N-terminal signal peptide fusions as indicated were separated from the growth medium by centrifugation after 16 h of growth in LB medium at 37°C. Subsequently, proteins in the supernatant fractions were separated by LDS-PAGE, and visualized by staining with Coomassie (A) or by Western blotting using specific antibodies against GLuc (B). The expected position of mature GLuc in the LDS-PAGE and Western blot is indicated with an arrow.



Supplementary Figure S2: Benchmark of different genome-minimized strains producing GLuc or PhoA. Culture supernatant samples from four different genome-minimized *B. subtilis* strains and the reference strain TS10, producing either GLuc or PhoA, were separated by LDS-PAGE and analyzed Western blotting with specific antibodies against GLuc or PhoA, respectively. As opposed to the blots shown in Fig. 2B in the main manuscript, these blots were not incubated with an antibody against TrxA to visualize possible cell lysis.

Supplementary Table S1: Oligonucleotides used in this study.

Oligonucleotide Name	Sequence
TS101	tacgGATAAATAGGAAATGAGACT
TS102	AAACAGTCTCATTTCCTATTTATC
TS103	CGACGGCCAACGAGGtaatatgttacatggccatcaaacat
TS104	gctagcggcaaattccgcatgatgt
TS105	ggaattggcgtagctttttattttaaaaaattg
TS106	atattgggtgggaggatttcgtgccg
RH001	AGCTTAGGCCAGTCGAAAG
RH002	CAGCTAGGAGGTGACTGAAG
RH003	ACCGAGCGTTCTGAACAAATCC
pBSMul1.fw	GATATCTCCTCCTTATTATGTAAATCGCTCC
pBSMul1.rev	TCTAGAGGTCGAAATTCACCTCGAAAGC
GLuc JN.fw	TAAGGAGGAGATATCAAACCAACTGAAAAC
GLuc JN.rev	ATTTGCACCTCTAGATTAATCACCACCGCACCTTTATTTGTCCACTTG
eprJN.fw	GTTTTCAGTTGGTTTGCTGTTTTGCGCATGAGCGAGAGGgcctatgg
eprJN.rev	CCTCTCGCTCATGCGAAACCAACTGAAAAC
epr+1AAJN.rev	GTTTTCAGTTGGTTTGCTGTTTTGCGCATGAGCGAGAGGgcctatgg
epr+2AAJN.rev	GTTTTCAGTTGGTTTGCTGTTTTGCGCATGAGCGAGAGGgcctatgg
epr+3AAJN.rev	GTTTTCAGTTGGTTTGCTGTTTTGCGCATGAGCGAGAGGgcctatgg
epr-GLucJN+0AA.fw	CCTCTCGCTCATGCGAAACCAACTGAAAAC
epr-GLucJN+1AA.fw	CCTCTCGCTCATGCGAAAAACCAACTGAAAACAATGAAGATTTCAACATTGTAGCTG TAGC
epr-GLucJN+2AA.fw	CCTCaTCGCTCATGCGAAAAACCAACTGAAAACAATGAAGATTTCAACATTGTA GCTGTAGC
epr-GLucJN+3AA.fw	CCTCTCGCTCATGCGAAAAACAGCAAACCAACTGAAAACAATGAAGATTTCAACATT GTAGCTGTAGC
WapAJN.fw	TAAGGAGGAGATATCATGAAAAAAGAAAGAGGGCGAAACTTTAAAAGGTTTCATTGC AGC
wapA+0AAJN.rev	CATTGTTTTCAGTTGGTTTGCTAGTACATCGGCTGGCACTAATGAAATCATTAAAGC C
wapA+1AAJN.rev	CATTGTTTTCAGTTGGTTTTTTGCTAGTACATCGGCTGGCACTAATGAAATCATTAAA GCC
wapA+2AAJN.rev	CATTGTTTTCAGTTGGTTTAGATTTTGCTAGTACATCGGCTGGCACTAATGAAATCATT AAAGCC

wapA+3AAJN.rv	CATTGTTTTAGTTGGTTTTGTAGATTTGCTAGTACATCGGCTGGCACTAATGAAATC ATTAAGCC
wapA-GLuc+0JN.fw	GCCAGCCGATGTACTAGCAAAACCAACTGAAAAAATGAAGATTTCAACATTGTAGC TGTAGCTAGC
wapA-GLuc+1JN.fw	GCCAGCCGATGTACTAGCAAAAAACCAACTGAAAAAATGAAGATTTCAACATTGT AGCTGTAGCTAGC
wapA-GLuc+2JN.fw	GCCAGCCGATGTACTAGCAAAATCTAAACCAACTGAAAAAATGAAGATTTCAACAT TGTAGCTGTAGCTAGC
wapA-GLuc+3JN.fw	GCCAGCCGATGTACTAGCAAAATCTacaAAACCAACTGAAAAAATGAAGATTTCAAC ATTGTAGCTGTAGCTAGC
sacBJN.fw	TAAGGAGGAGATATCATGAACATCAAAAAGTTTGCAAAAAAAGCAACAGTATTAACC TTTACTaccgc
sacBJN.rv	TCATTGTTTTAGTTGGTTTTCGCAAACGCTTGAGTTGCGCCTCC
sacB+1AAJN.rv	TCATTGTTTTAGTTGGTTTTTTTCGCAAACGCTTGAGTTGCGCCTCC
sacB+2AAJN.rv	TCATTGTTTTAGTTGGTTTTCTTTTCGCAAACGCTTGAGTTGCGCCTCC
sacB+3AAJN.rv	TCATTGTTTTAGTTGGTTTTCGTTTTCTTTTCGCAAACGCTTGAGTTGCGCCTCC
sacB_GLucJN.fw	GCAACTCAAGCGTTTGCGAAACCAACTGAAAAAATGAAGATTTCAACATTGTAGCT GTAGCTAGC
sacB_GLuc+1JN.fw	GCAACTCAAGCGTTTGCGaaaAAACCAACTGAAAAAATGAAGATTTCAACATTGTAG CTGTAGCTAGC
sacB_GLuc+2JN.fw	GCAACTCAAGCGTTTGCGAAAGAAAAACCAACTGAAAAAATGAAGATTTCAACATT GTAGCTGTAGCTAGC
sacB_GLuc+3.fw	GCAACTCAAGCGTTTGCGAAAGAAACGAAACCAACTGAAAAAATGAAGATTTCAAC ATTGTAGCTGTAGCTAGC
yncMJN.fw	CATAATAAGGAGGAGATATCATGGCGAAACCACTATCAAAGGGGGAATTTTGG
yncM.rv	CTTCATTGTTTTAGTTGGTTTTGCTTTTTCGCACTTGAGCGTCTGCCG
yncM+1JN.rv	CTTCATTGTTTTAGTTGGTTTTGCTGCTTTTTCGCACTTGAGCGTCTGCCG
yncM+2JN.rv	CTTCATTGTTTTAGTTGGTTTTGGATGCTGCTTTTTCGCACTTGAGCGTCTGCCG
yncM+3JN.rv	CTTCATTGTTTTAGTTGGTTTTCTCGGATGCTGCTTTTTCGCACTTGAGCGTCTGCCG
yncM_GLucJN.fw	CAAGTCGCAAAAGCAAAACCAACTGAAAAAATGAAGATTTCAACATTGTAGCTGTA GC
yncM_GLuc+1AAJN.fw	CAAGTCGCAAAAGCAGCAAAACCAACTGAAAAAATGAAGATTTCAACATTGTAGCT GTAGC
yncM_GLuc+2AAJN.fw	CAAGTCGCAAAAGCAGCATCAAACCAACTGAAAAAATGAAGATTTCAACATTGTA GCTGTAGC
yncM_GLuc+3AAJN.fw	CAAGTCGCAAAAGCAGCATCCGAGAAACCAACTGAAAAAATGAAGATTTCAACATT GTAGCTGTAGC
tasAJN.fw	TAAGGAGGAGATATCATGGGTATGAAAAAGAAATTGAGTTTAGGAGTTGCTTCTGCA

	GC
tasAJN.rv	TGTTTTCAGTTGGTTTTGCCCATGTTCTCCTCCAACCTAAAGCTAATCCTAG
tasA+1AAJN.rv	TGTTTTCAGTTGGTTTTGCTGCCCATGTTCTCCTCCAACCTAAAGCTAATCCTAG
tasA+2AAJN.rv	TGTTTTCAGTTGGTTTAAATGCTGCCCATGTTCTCCTCCAACCTAAAGCTAATCCTAG
tasA+3AAJN.rv	TGTTTTCAGTTGGTTTGTTAAATGCTGCCCATGTTCTCCTCCAACCTAAAGCTAATCCT
	AG
tasA_GLucJN.fw	GGAGGAACATGGGCAAACCAACTGAAAACAATGAAGATTTCAACATTGTAGCTGT AGCTAGC
tasA_GLuc+1AAJN.fw	GGAGGAACATGGGCAGCAAACCAACTGAAAACAATGAAGATTTCAACATTGTAGC TGTAGCTAGC
tasA_GLuc+2AAJN.fw	GGAGGAACATGGGCAGCATTAAACCAACTGAAAACAATGAAGATTTCAACATTGTA GCTGTAGCTAGC
tasA_GLuc+3AAJN.fw	GGAGGAACATGGGCAGCATTAAACAAACCAACTGAAAACAATGAAGATTTCAACATT GTAGCTGTAGCTAGC
pBSMullcol.fw	GTGGTTGCCGAAAGAGCGAAAATGCCTC
pBSMullcol.rev	GGAGCCTTTAATTGTATCGGTTTATCAGCTTGC

Supplementary Table S2: Non-processed read-out data of the GLuc and PhoA activity assays in Figures 1, 2 and 4.

Supplementary Data for Fig. 1: GLuc Activity Assay (Light Emission at 485 nm)						
Signal peptide	Cell Pellet Read 1 [RLU]	Cell Pellet Read 2 [RLU]	Cell Pellet Read 3 [RLU]	Supernatant Read 1 [RLU]	Supernatant Read 2 [RLU]	Supernatant Read 3 [RLU]
[Blank]	5	8	13	5	8	13
SP _{WapA+0}	3547	4174	3920	47106	53303	47537
SP _{WapA+2}	5129	4910	5029	16016	16783	16708
SP _{TasA+1}	3008	2990	3399	287	297	374
SP _{Epr+1}	2807	2989	3354	57346	55438	60947

Supplementary Data for Fig. 2C: GLuc Activity Assay (Light Emission at 485 nm)							
<i>B. subtilis</i> Strain	Cells Read 1 [RLU]	Cells Read 2 [RLU]	Cells Read 3 [RLU]	Supernatant Read 1 [RLU]	Supernatant Read 2 [RLU]	Supernatant Read 3 [RLU]	OD ₆₀₀
[Blank]	4	11	9	26	25	63	-
TS10	48	52	53	9566	10717	10351	3.75
IIG-Bs 27-31	341	332	316	25561	24509	24232	3.42
IIG-Bs 27-39	622	583	689	44854	46494	46609	4.10
IIG-Bs 27-47-14	874	793	832	82518	97991	107080	1.97
IIG-Bs 27-47-24	1439	1396	1554	26	25	63	1.54

Supplementary Data for Fig. 2D: PhoA Activity Assay (Optical Density at 405 nm)							
<i>B. subtilis</i> Strain	Cell Pellet Read 1	Cell Pellet Read 2	Cell Pellet Read 3	Supernatant Read 1	Supernatant Read 2	Supernatant Read 3	OD ₆₀₀
[Blank]	0	0	0	0	0	0	-
TS10	1.0522	0.9207	1.0928	1.2783	1.207	1.2748	3.825
IIG-Bs 27-31	0.9749	1.1705	1.0493	1.3828	1.4115	1.4034	3.0965
IIG-Bs 27-39	0.8755	0.6474	0.8746	1.8981	1.9098	1.9239	1.8915
IIG-Bs 27-47-14	1.0272	1.1473	0.9891	1.1623	1.1616	1.1758	3.034
IIG-Bs 27-47-24	0.4032	0.4138	0.3974	0.8829	0.8826	0.8841	1.313

Supplementary Data for Fig. 2E: GLuc Activity Assay (Light Emission at 485 nm)						
Sample Type	Cells Read 1 [RLU]	Cells Read 2 [RLU]	Cells Read 3 [RLU]	Supernatant Read 1 [RLU]	Supernatant Read 2 [RLU]	Supernatant Read 3 [RLU]
[Blank]	10	5	8	20	22	14
Native	245	3412	2844	173198	187108	196076
DTT treated	224	298	250	20	22	14

Supplementary Data for Fig. 4A: GLuc Activity Assay (Light Emission at 485 nm)							
<i>B. subtilis</i> Strain	Cells Read 1 [RLU]	Cells Read 2 [RLU]	Cells Read 3 [RLU]	Supernatant Read 1 [RLU]	Supernatant Read 2 [RLU]	Supernatant Read 3 [RLU]	OD ₆₀₀
IIG-Bs27-39							
[Blank]	4	5	11	7	6	6	-
WT	1495	1606	1703	65781	60791	66323	5.56
ScDsbA	302	327	342	7340	7550	8802	4.77
ScDsbA	172	199	190	65	63	45	5.56

Δ BdbCD	939	956	1026	37435	33937	34845	4.70
Supplementary Data for Fig. 4B: PhoA Activity Assay (Optical Density at 405 nm)							
<i>B. subtilis</i> Strain	Cells Read	Cells Read	Cells Read	Supernatant	Supernatant	Supernatant	OD ₆₀₀
IIG-Bs27-39	1	2	3	Read 1	Read 2	Read 3	
[Blank]	-	-	-	0.082	0.081	0.081	-
WT	-	-	-	0.999	1.019	0.999	1.11
ScDsbA	-	-	-	0.097	0.097	0.098	3.16
ScDsbA	-	-	-	0.09	0.09	0.094	2.93
Δ BdbCD	-	-	-	0.102	0.103	0.103	2.16

RLU, relative light units