11. Supplementary

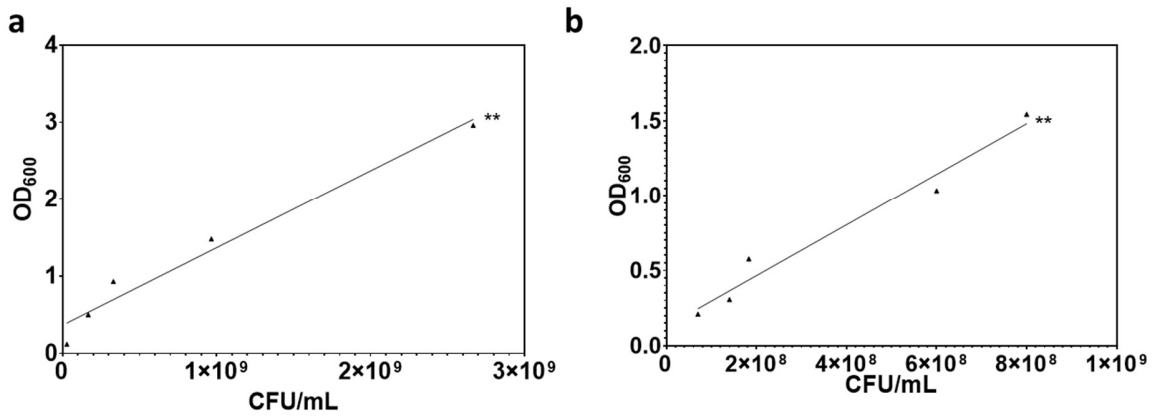

Figure 1: Additional calibration curves showing how the OD of a culture grown in LB depends on the number of CFU/mL present. Experimental points denote the average of three technical replicates with error bars representing the S.E.M. R^2 values were calculated through a simple linear regression (a: R^2 = 0.9838, b: R^2 = 0.9836. Correlation is statistically significant and calculated through Pearson's correlation coefficient (a: (r(3) = 0.9838, p = 0.0025), b: (r(3) = 0.9836, p = 0.0025)).

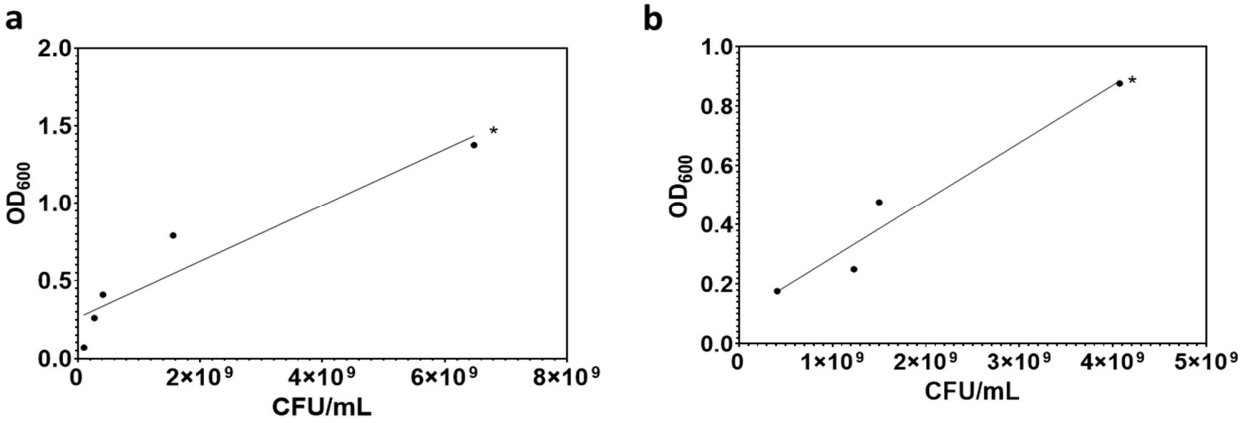

Figure 2: Additional calibration curves showing how the OD of a culture grown in M9 depends on the number of CFU/mL present. Experimental points denote the average of three technical replicates with error bars representing the S.E.M. R^2 values were calculated through a simple linear regression (a: R^2 = 0.8916, b: R^2 = 0.9740. Correlation is statistically significant and calculated through Pearson's correlation coefficient (a: (r(3) = 0.9442, p = 0.0157), b: (r(3) = 0.9740, p = 0.026)).

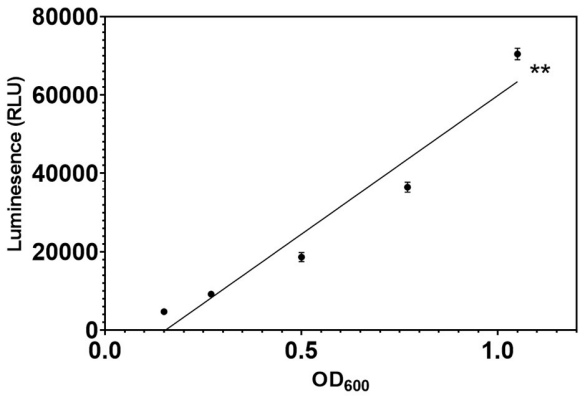

Figure 3: Luminescence given off by BacTiter-Glo™ Microbial Cell Viability Assay kit (*Promega*) is dependent on the OD of the sample. Experimental points denote the average of three technical replicates with error bars representing S.E.M. R^2 values were calculated through a simple linear regression (R^2 = 0.9439) Correlation is statistically significant and calculated through Pearson's correlation coefficient ($r(3) = 0.9715$, $p = 0.0057$).

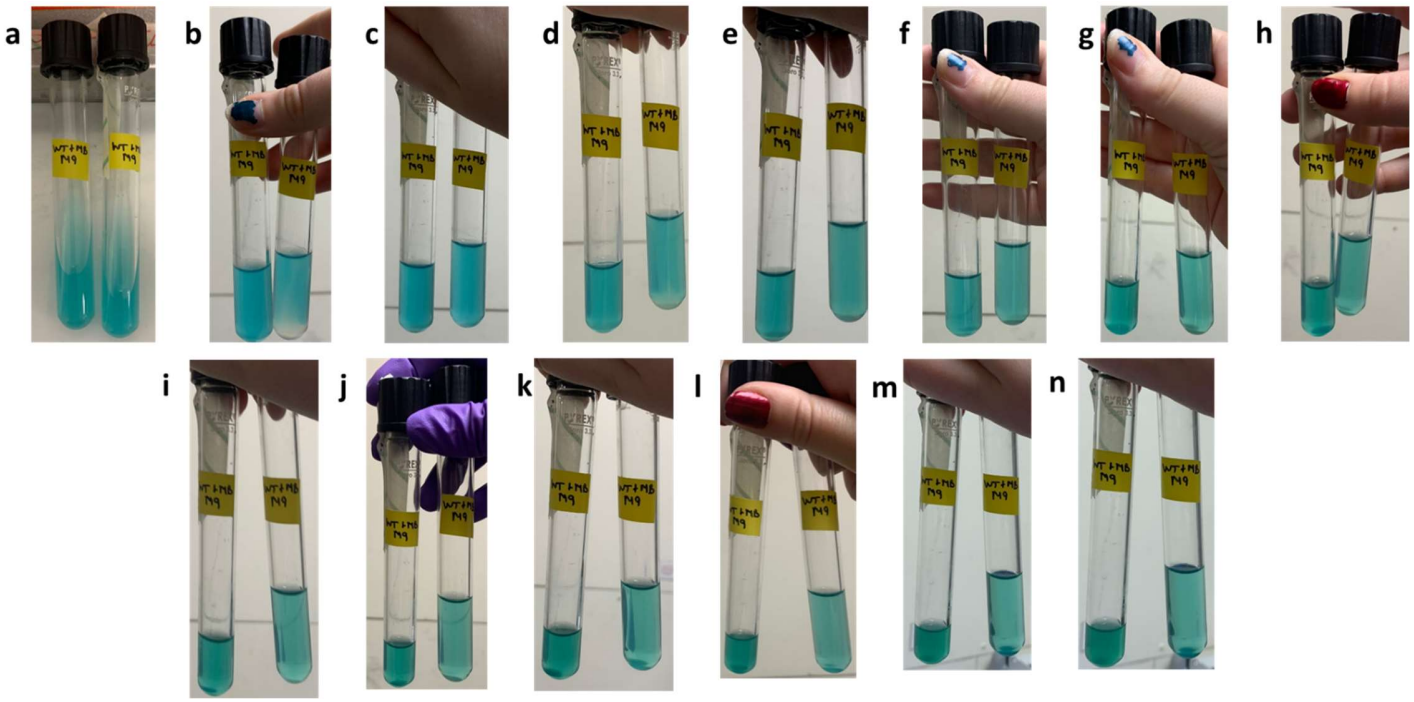

Figure 4: Appearance of WT cultures with the addition of Methylene Blue. The tubes were imaged at the following time points: Day 0 (**a**), Day 2 (**b**), Day 3 (**c**), Day 7 (**d**), Day 9 (**e**), Day 10 (**f**), Day 15 (**g**), Day 16 (**h**), Day 17 (**i**), Day 18 (**j**), Day 21 (**k**), Day 23 (**l**), Day 24 (**m**), and Day 25 (**n**). The open system is on the LHS and the closed system is on the RHS with the exception of **a** which is reversed. The blue colour became less intense in the closed system by day 7 however remained present throughout. The volume dropped in the open system due to evaporation losses.

Table 1: Tukey's multiple comparison was performed to analyse if there was a difference in the number of *E. coli* (determined through CFU/mL tests) present in WT, *Δlon,* and *Δlon*R cultures when grown for various times.

Days	Strains Compared	Predicted Mean Difference	95% Confidence Interval of difference	Significant	P Value
0	WT vs *Δlon*	12411111	-301369994 to 326192216	ns	0.9949
	WT vs *Δlon*R	-34766667	-385584607 to 316051274	ns	0.9685
	Δ*lon* vs Δ*lon*R	-47177778	-397995718 to 303640162	ns	0.9427
1	WT vs *Δlon*	-902476190	-1237922301 to -567030079	****	<0.0001
	WT vs *Δlon*R	-116309524	-486632003 to 254012956	ns	0.7267
	Δ*lon* vs Δ*lon*R	786166667	435348726 to 1136984607	****	<0.0001
0	WT vs *Δlon*	44391667	-219888119 to 308671452	ns	0.9133
	WT vs *Δlon*R	-16508333	-340183646 to 307166979	ns	0.9917
	Δ*lon* vs Δ*lon*R	-60900000	-384575312 to 262775312	ns	0.8925
5	WT vs *Δlon*	-331987000	-614834846 to -49139154	*	0.0178
	WT vs *Δlon*R	40575000	-337396333 to 418546333	ns	0.9636
	Δ*lon* vs Δ*lon*R	372562000	-10415794 to 755539794	ns	0.0581
0	WT vs *Δlon*	476515	-67045661 to 67998691	ns	0.9998
	WT vs *Δlon*R	-34573485	-102095661 to 32948691	ns	0.4396
	Δ*lon* vs Δ*lon*R	-35050000	-101087991 to 30987991	ns	0.4140
10	WT vs *Δlon*	-105157875	-183758806 to -26556944	**	0.0059
	WT vs *Δlon*R	-17070625	-90903344 to 56762094	ns	0.8437
	Δ*lon* vs Δ*lon*R	88087250	16758067 to 159416433	*	0.0119
0	WT vs *Δlon*	-4153333	-161712050 to 153405384	ns	0.9977
	WT vs *Δlon*R	-42036667	-199595384 to 115522050	ns	0.7882
	Δ*lon* vs Δ*lon*R	-37883333	-188109676 to 112343009	ns	0.8083
15	WT vs *Δlon*	-131678000	-289236717 to 25880717	ns	0.1150
	WT vs *Δlon*R	-166769167	-324327884 to -9210450	*	0.0364
	Δ*lon* vs Δ*lon*R	-35091167	-185317509 to 115135176	ns	0.8329
0	WT vs *Δlon*	-30166667	-196730465 to 136397131	ns	0.8963
	WT vs *Δlon*R	-72333333	-238897131 to 94230465	ns	0.5393
	Δ*lon* vs Δ*lon*R	-42166667	-208730465 to 124397131	ns	0.8081
20	WT vs *Δlon*	-335953333	-502517131 to -169389535	****	<0.0001
	WT vs *Δlon*R	-72053333	-238617131 to 94510465	ns	0.5418
	Δ*lon* vs Δ*lon*R	263900000	97336202 to 430463798	**	0.0014
0	WT vs *Δlon*	-17677778	-97167117 to 61811562	ns	0.8512
	WT vs *Δlon*R	-61411111	-140900451 to 18078228	ns	0.1573
	Δ*lon* vs Δ*lon*R	-43733333	-123222673 to 35756006	ns	0.3818
25	WT vs *Δlon*	28650000	-106548658 to 163848658	ns	0.8639
	WT vs *Δlon*R	-60209222	-192027379 to 71608935	ns	0.5121
	Δ*lon* vs Δ*lon*R	-88859222	-173836901 to -3881544	*	0.0387

Table 2: Tukey's multiple comparison was performed to analyse the differences between peptide concentrations present in the supernatant taken from either WT, *Δlon* or *Δlon*R cultures grown for 1, 5, 10, 15, 20 and 25 days.

Comparison	Mean Difference	95% Confidence Interval of difference	Significant	P Value
LGA-1 WT vs. LGA-1 *Δlon*	35.26	-206.7 to 277.2	ns	>0.9999
LGA-1 WT vs. LGA-1 *ΔlonR*	-36.74	-278.7 to 205.2	ns	>0.9999
LGA-1 WT vs. LGA-5 WT	-223.1	-465.0 to 18.82	ns	0.0985
LGA-1 WT vs. LGA-10 WT	-178.2	-420.1 to 63.70	ns	0.3748
LGA-1 WT vs. LGA-15 WT	84.00	-157.9 to 325.9	ns	0.9964
LGA-1 WT vs. LGA-20 WT	36.15	-205.8 to 278.1	ns	>0.9999
LGA-1 WT vs. LGA-25 WT	105.3	-136.7 to 347.2	ns	0.9668
LGA-1 *Δlon* vs. LGA-1 *ΔlonR*	-72.00	-313.9 to 169.9	ns	0.9994
LGA-1 *Δlon* vs. LGA-5 *Δlon*	-303.2	-545.1 to -61.26	**	0.0041
LGA-1 *Δlon* vs. LGA-10 *Δlon*	-232.2	-474.1 to 9.704	ns	0.0715
LGA-1 *Δlon* vs. LGA-15 *Δlon*	-74.44	-344.9 to 196.0	ns	0.9998
LGA-1 *Δlon* vs. LGA-20 *Δlon*	-173.6	-415.5 to 68.37	ns	0.4184
LGA-1 *Δlon* vs. LGA-25 *Δlon*	-111.7	-353.6 to 130.2	ns	0.9456
LGA-1 *ΔlonR* vs. LGA-5 *Δlon*R	-13.56	-255.5 to 228.4	ns	>0.9999
LGA-1 *ΔlonR* vs. LGA-10 *Δlon*R	-2.518	-244.4 to 239.4	ns	>0.9999
LGA-1 *ΔlonR* vs. LGA-15 *Δlon*R	-111.0	-353.0 to 130.9	ns	0.9482
LGA-1 *ΔlonR* vs. LGA-20 *Δlon*R	-121.2	-363.1 to 120.7	ns	0.8998
LGA-1 *ΔlonR* vs. LGA-25 *Δlon*R	-181.9	-423.9 to 60.00	ns	0.3420
LGA-5 WT vs. LGA-5 *Δlon*	-44.81	-286.7 to 197.1	ns	>0.9999
LGA-5 WT vs. LGA-5 *Δlon*R	172.8	-69.11 to 414.7	ns	0.4255
LGA-5 WT vs. LGA-10 WT	44.89	-197.0 to 286.8	ns	>0.9999
LGA-5 WT vs. LGA-15 WT	307.1	65.18 to 549.0	**	0.0034
LGA-5 WT vs. LGA-20 WT	259.3	17.33 to 501.2	*	0.0256
LGA-5 WT vs. LGA-25 WT	328.4	86.44 to 570.3	**	0.0013
LGA-5 *Δlon* vs. LGA-5 *Δlon*R	217.6	-24.30 to 459.6	ns	0.1187
LGA-5 *Δlon* vs. LGA-10 *Δlon*	70.96	-171.0 to 312.9	ns	0.9995
LGA-5 *Δlon* vs. LGA-15 *Δlon*	228.7	-41.74 to 499.2	ns	0.1807
LGA-5 *Δlon* vs. LGA-20 *Δlon*	129.6	-112.3 to 371.6	ns	0.8434
LGA-5 *Δlon* vs. LGA-25 *Δlon*	191.5	-50.45 to 433.4	ns	0.2654
LGA-5 *Δlon*R vs. LGA-10 *Δlon*R	11.04	-230.9 to 253.0	ns	>0.9999
LGA-5 *Δlon*R vs. LGA-15 *Δlon*R	-97.48	-339.4 to 144.4	ns	0.9835
LGA-5 *Δlon*R vs. LGA-20 *Δlon*R	-107.6	-349.6 to 134.3	ns	0.9599
LGA-5 *Δlon*R vs. LGA-25 *Δlon*R	-168.4	-410.3 to 73.56	ns	0.4692
LGA-10 WT vs. LGA-10 *Δlon*	-18.74	-260.7 to 223.2	ns	>0.9999

LGA-10 WT vs. LGA-10 *Δlon*R	139.0	-103.0 to 380.9	ns	0.7656
LGA-10 WT vs. LGA-15 WT	262.2	20.30 to 504.1	*	0.0227
LGA-10 WT vs. LGA-20 WT	214.4	-27.56 to 456.3	ns	0.1323
LGA-10 WT vs. LGA-25 WT	283.5	41.55 to 525.4	**	0.0095
LGA-10 *Δlon* vs. LGA-15 *Δlon*	157.8	-112.7 to 428.3	ns	0.7457
LGA-10 *Δlon* vs. LGA-20 *Δlon*	58.67	-183.3 to 300.6	ns	>0.9999
LGA-10 *Δlon* vs. LGA-25 *Δlon*	120.5	-121.4 to 362.4	ns	0.9036
LGA-10 *Δlon*R vs. LGA-15 *Δlon*R	-108.5	-350.4 to 133.4	ns	0.9570
LGA-10 *Δlon*R vs. LGA-20 *Δlon*R	-118.7	-360.6 to 123.3	ns	0.9137
LGA-10 *Δlon*R vs. LGA-25 *Δlon*R	-179.4	-421.3 to 62.52	ns	0.3641
LGA-15 WT vs. LGA-15 *Δlon*	-123.2	-393.7 to 147.3	ns	0.9513
LGA-15 WT vs. LGA-15 *Δlon*R	-231.8	-473.7 to 10.15	ns	0.0726
LGA-15 WT vs. LGA-20 WT	-47.85	-289.8 to 194.1	ns	>0.9999
LGA-15 WT vs. LGA-25 WT	21.26	-220.7 to 263.2	ns	>0.9999
LGA-15 *Δlon* vs. LGA-15 *Δlon*R	-108.6	-379.1 to 161.9	ns	0.9840
LGA-15 *Δlon* vs. LGA-20 *Δlon*	-99.11	-369.6 to 171.4	ns	0.9936
LGA-15 *Δlon* vs. LGA-25 *Δlon*	-37.26	-307.7 to 233.2	ns	>0.9999
LGA-15 *Δlon*R vs. LGA-20 *Δlon*R	-10.15	-252.1 to 231.8	ns	>0.9999
LGA-15 *Δlon*R vs. LGA-25 *Δlon*R	-70.89	-312.8 to 171.0	ns	0.9995
LGA-20 WT vs. LGA-20 *Δlon*	-174.4	-416.4 to 67.48	ns	0.4099
LGA-20 WT vs. LGA-20 *Δlon*R	-194.1	-436.0 to 47.85	ns	0.2468
LGA-20 WT vs. LGA-25 WT	69.11	-172.8 to 311.0	ns	0.9996
LGA-20 *Δlon* vs. LGA-20 *Δlon*R	-19.63	-261.6 to 222.3	ns	>0.9999
LGA-20 *Δlon* vs. LGA-25 *Δlon*	61.85	-180.1 to 303.8	ns	>0.9999
LGA-20 *Δlon*R vs. LGA-25 *Δlon*R	-60.74	-302.7 to 181.2	ns	>0.9999
LGA-25 WT vs. LGA-25 *Δlon*	-181.7	-423.6 to 60.22	ns	0.3439
LGA-25 WT vs. LGA-25 *Δlon*R	-323.9	-565.9 to -82.00	**	0.0016
LGA-25 *Δlon* vs. LGA-25 *Δlon*R	-142.2	-384.1 to 99.70	ns	0.7353

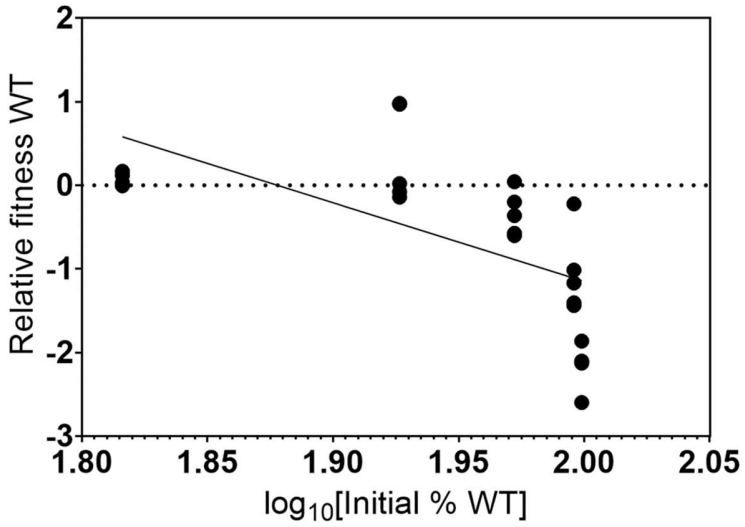

Figure 5: Relative fitness of the WT E. coli is dependent on the initial proportion of the WT in the population. Relative fitness is calculated through comparing the growth rates of the WT relative to that of the Δlon. Data (n = 5 except for 70% initial percentage where n = 4) is fitted with a simple linear regression.