

Additional file 1. PhoA and LacZ enzymes activities of *E. coli* cells carrying pHKU1601 series plasmids.

Plasmid	Fusion ^a	PhoA activity rate ^b	LacZ activity rate ^c	Cell density PhoA ^d	Cell density LacZ ^e	Relative PhoA activity ^f	Relative LacZ activity ^g	Strength index ^h	Location ⁱ
pHKU1601-034	D34	0.0000	0.0064	0.1681	0.1713	0.0001 ±0.0082	0.1089 ±0.0068	-6.993	C
pHKU1601-052	G52	0.0000	0.0070	0.1454	0.1493	0.0001 ±0.0050	0.1357 ±0.0084	-7.213	C
pHKU1601-062	T62	0.0000	0.0097	0.1501	0.1373	0.0001 ±0.0060	0.2066 ±0.0143	-7.633	C
pHKU1601-076	G76	0.0000	0.0034	0.1640	0.1548	0.0001 ±0.0074	0.0641 ±0.0141	-6.464	C
pHKU1601-090	K90	0.0000	0.0078	0.1501	0.1385	0.0001 ±0.0067	0.1633 ±0.0090	-7.398	C
pHKU1601-100	M100	0.0000	0.0033	0.1803	0.1602	0.0001 ±0.0042	0.0599 ±0.0117	-6.395	C
pHKU1601-114	G114	0.0458	0.0111	0.2450	0.2109	0.4351 ±0.0167	0.1527 ±0.0114	1.047	P
pHKU1601-126	A126	0.0619	0.0335	0.2573	0.2533	0.5600 ±0.0754	0.3850 ±0.0228	0.375	P
pHKU1601-138	E138	0.0000	0.0420	0.2766	0.2123	0.0001 ±0.0065	0.5769 ±0.0466	-8.660	C
pHKU1601-150	A150	0.0000	0.0382	0.1973	0.2299	0.0001 ±0.0088	0.4840 ±0.0130	-8.485	C
pHKU1601-163	Q163	0.0000	0.0459	0.2501	0.2128	0.0001 ±0.0081	0.6284 ±0.0406	-8.746	C
pHKU1601-172	I172	0.0000	0.0723	0.3168	0.2639	0.0001 ±0.0102	0.7988 ±0.0420	-8.986	C
pHKU1601-191	A191	0.0336	0.0064	0.1672	0.1431	0.4677 ±0.0492	0.1303 ±0.0121	1.278	P
pHKU1601-203	V203	0.0063	0.0083	0.2050	0.1690	0.0712 ±0.0070	0.1428 ±0.0124	-0.697	C
pHKU1601-220	V220	0.0000	0.0069	0.1865	0.1522	0.0001 ±0.0073	0.1330 ±0.0106	-7.193	C
pHKU1601-240	G240	0.0000	0.0045	0.1698	0.1450	0.0001 ±0.0044	0.0896 ±0.0072	-6.798	C
pHKU1601-252	L252	0.0000	0.0040	0.1779	0.1578	0.0001 ±0.0057	0.0745 ±0.0091	-6.613	C
pHKU1601-266	G266	0.0371	0.0020	0.1804	0.1548	0.4785 ±0.0661	0.0384 ±0.0107	2.523	P
pHKU1601-284	S284	0.0615	0.0069	0.1484	0.1331	0.9647 ±0.1112	0.1513 ±0.0172	1.853	P
pHKU1601-296	G296	0.0000	0.0460	0.2352	0.2158	0.0001 ±0.0049	0.6213 ±0.0374	-8.734	C
pHKU1601-313	K313	0.0000	0.0378	0.1612	0.1340	0.0001 ±0.0048	0.8222 ±0.0375	-9.015	C
pHKU1601-322	I322	0.0000	0.0449	0.1883	0.1677	0.0001 ±0.0062	0.7800 ±0.0436	-8.962	C
pHKU1601-337	Y337	0.0616	0.0085	0.1435	0.1349	1.0000 ±0.1665	0.1829 ±0.0134	1.699	P
pHKU1601-358	S358	0.0637	0.0041	0.1611	0.1348	0.9216 ±0.1059	0.0884 ±0.0069	2.345	P
pHKU1601-379	A379	0.0104	0.0000	0.1646	0.1437	0.1472 ±0.0106	0.0001 ±0.0067	7.294	P
pHKU1601-400	V400	0.0506	0.0019	0.1445	0.1273	0.8150 ±0.0309	0.0435 ±0.0062	2.931	P
pHKU1601-421	K421	0.0302	0.0011	0.1233	0.1285	0.5709 ±0.0587	0.0254 ±0.0072	3.114	P
pHKU1601-442	A442	0.0473	0.0025	0.1374	0.1281	0.8011 ±0.0191	0.0572 ±0.0143	2.639	P
pHKU1601-454	S454	0.0222	0.0000	0.1501	0.1348	0.3441 ±0.0112	0.0001 ±0.0166	8.143	P
pHKU1601-466	T466	0.0000	0.0140	0.1527	0.1381	0.0001 ±0.0090	0.2952 ±0.0288	-7.990	C
pHKU1601-479	M479	0.0176	0.0423	0.1872	0.1740	0.2194 ±0.0154	0.7083 ±0.0873	-1.172	C
pHKU1601-493	Y493	0.0105	0.0306	0.1415	0.1374	0.1736 ±0.0086	0.6481 ±0.0424	-1.317	C
pHKU1601-502	G502	0.0046	0.0377	0.1568	0.1566	0.0687 ±0.0078	0.7006 ±0.0572	-2.323	C
pHKU1601-520	S520	0.0078	0.0249	0.1633	0.1512	0.1106 ±0.0141	0.4803 ±0.0209	-1.468	C
pHKU1601-532	A532	0.0104	0.0545	0.1757	0.1588	0.1381 ±0.0076	1.0000 ±0.0754	-1.980	C
pHKU1601-552	D552	0.0144	0.0336	0.1438	0.1201	0.2338 ±0.0101	0.8142 ±0.0745	-1.248	C

^a Position of the last residue of Deh4p followed by reporter.

^b Average PhoA activity rate was determined by the conversion of *p*-nitrophenylphosphate (PNPP) to *p*-nitrophenol. Formation of *p*-nitrophenol was measured by absorbance at 405 nm as described in Methods.

^c Average LacZ activity rate was determined by the conversion of *o*-nitrophenylgalactoside (ONPG) to *o*-nitrophenol. Formation of *o*-nitrophenol was measured by absorbance at 420 nm as described in Methods.

^d and ^e Average cell density used in assaying PhoA or LacZ activity was determined by absorbance at 600 nm as described in Methods.

^f and ^g PhoA and LacZ activities are presented as mean \pm S.E. which were obtained by linear regression through at least 20 data points. To normalize PhoA activities, the maximum PhoA activity recorded in the experiment (pHKU1601-337) was transformed to 1 or 100% and PhoA activities of other samples were expressed as a percentage relative to this maximum activity. The same procedure was applied to normalize LacZ activities using the activity from pHKU1601-532 as the maximum. Exception was required when a normalized activity value was zero. In this case an arbitrary small value, 0.0001, was assigned to prevent logging a zero or undefined number in calculating the strength index.

^h and ⁱ A positive value for the strength index indicates that the reporter ended in the periplasm (P) and a negative value suggests that the reporter ended in the cytoplasm (C). The strength index was defined as $\text{Ln}(\text{normalized PhoA activity}/\text{normalized LacZ activity})$.