

Supplementary Table 1: List of mutants and designated *spo0A* mutations, ordered by mutation type. For alignments, *spo0A* (GBAA_4394) from *B. anthracis* strain Ames Ancestor or *B. subtilis* str. 168 served as template. MS = missense, NS = nonsense, INS = insertion, DEL = deletion, DUP = duplication, FS = frameshift.

SPO Mutants	No. of isolates	Mutation type	Nucleotide change(s)	Amino acid change	Amino acid in <i>B. subtilis</i>
107	1	MS	A32G	D11G	D11
22	1	MS	T158C	L53P	L53
12	1	MS	T473C and A673G	I158T and N225D	I157, N228
31, 121	2	MS	C596A	A199E	A202
23, 26	2	MS	G623A	R208H	R211
91, 117	2	MS	C631T	R211C	R214
126	1	MS	C640A	R214C	R217
5, 75	2	MS	C647T	A216V	A219
102, 124	1	MS	G646A	A216T	A219
119	1	MS	C740T	S247F	S250
101, 106, 115, 133	4	MS	C527T	A176V	A179
108, 110	2	MS	A574G	K192E	K195
54	1	NS	C145T	Stop after K48	K48
55, 128, 129	3	NS	C235T	Stop after R78	K78
132	1	NS	G742T	Stop after S247	S250
111	1	SIL	A156G	V52V	V52
116	1	DEL(3 bp)	TAT position 168-170	Deletion of I57	I57
114	1	DEL(1 bp)	C148	FS after Q49	D49
LL44*	1	DEL (2116 bp)	Starting after A156	Stop after L55	L55
120	1	DEL (1 bp)	T192	FS after G64	G64
105	1	DEL (7 bp)	TTCATAT position 306-312	FS after Y104	Y104
18	1	DEL (4 bp)	GCGG position 368-371	FS after V122	V122
46	1	DEL (5 bp)	TAATA position 606-610	FS after N203	N206
63	1	INS (1 bp)	G after position 39	FS after K13	R13
7	1	INS (1 bp)	A after position 282	FS after K96	K96
112	1	DUP (7 bp)	TTCATAT after position 305	FS after I106	I106
127	1	DUP(80 bp)	409-569 duplicated	FS after I190	I193

* LL44 was derived from parental strain UM44-1C9 in an independent experiment.