**Table S1.*****Ba* target description, primer sequences, and 5’ fluorescent labels.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Ba* Targets** | **Position Ames Ancestor** | **Forward Primer/Reverse Primer** | **Amplicon Size (bp)** | **Gene Category: Function** |
| pXO1*\_lef* | 151,111-150,762 | FAM-CAGCTTAAGGAACATCCCACAGAC/ TGAATTATGTCATCTTTCTTTGGCTCA | 350 | Virulence: pXO1-encoded virulence factor (lethal factor) |
| pXO1*\_ger*XB | 137,700-138,160 | JOE-AAGGGATGATTTATCCAGCCGC/ TGCTGAAATGGGTAAGAAAACATGAG | 461 | Sporulation: pXO1-encoded spore germination response protein, *B. subtilis* *ger*XB homologue [[1](#_ENREF_1), [2](#_ENREF_2)] |
| *ssp*F | 47,540-47,881 | TMR-GTTGCAACGTGTGTCGTATAGC/ GCTGTTCTGCAATTTCTATAGCACG | 342 | Sporulation: small-acid soluble spore protein F, *B. cereus* homologue [[3](#_ENREF_3)], conserved in *Ba* |
| *spo*VT | 58,273-58,694 | FAM-TCGAGAAGGGGACCCATTAGAA/ CAGCAGTATTGACCGCTTTGTG | 422 | Sporulation: stage V sporulation protein T, transcriptional regulator AbrB homologue [[4](#_ENREF_4)], conserved in *Ba* |
| GBAA0872 | 881,262-880,841 | ROX-TTCTTACTGGCCAAACCGTCTT/ TTCGATTTAAAAATGCGGCGGA | 422 | Metabolism: N-acetylmuramoyl-L-alanine amidase [[5](#_ENREF_5)], cell wall peptidoglycan metabolism enzyme, highly conserved protein in *Ba* |
| *hem*L | 4,269,357-4,268,901 | ACTTGTTTAGGTAAAGTAATYGGTGGT/ FAM-TCGCTTCAATATCAGCATCACTATGTA | 457 | Metabolism: glutamate -1-semialdehyde-2,1-aminomutase (key enzyme in heme biosysnthesis pathway) [[6](#_ENREF_6)] |
| *bas*B, GBAA0871 | 880,178-880,666 | ROX-TCAAGCGCCAGAAGGTTATGAGTT/ GGAAGCTGTTGAGCATGAGAGGTA | 385-615 | Metabolism: LPXTG-motif containing surface-cell wall protein, sortase A substrate [[7](#_ENREF_7)], gene harbors internal VNTR-like repeat [[8](#_ENREF_8)]. |
| *pbp*1A | 2,179,564-2,180,018 | TMR-GAGCGTACCGGATACAACAGAA/ CTTATTGACCATTATTAGCAGGGGC | 456-533 | Metabolism: penicillin-binding protein 1A, cell-wall synthesis enzyme, gene harbors internal VNTR-like repeat[[8](#_ENREF_8)] |
| *yih*Y-GBAA0410 | 428,604-428,942 | ROX-CTTGGCGGCGAAATTAATGGTT/ TTCCATTCGGTGTTATTGGGCA | 339-340 | Metabolism: Ribonuclease BN homologue YihY and ATPase (GBAA0410), amplicon spans 3’ ends of ORFs and intergenic region (IGS), highly conserved in *Ba* |
| *cod*Y | 3,643,422-3,643,057 | FAM-TAAAATTGCTGACCGCGTAGGA/ GTCGAAAATGCGAACAATGTAAAACT | 366-367 | Virulence: regulatory protein, required for toxin gene expression and full virulence[[9](#_ENREF_9)], amplicon spans 3’ end of *cod*Y and adjacent IGS containing indels. |

Positions of amplicon boundaries based on Ames Ancestor chromosome and pXO1, AE017334.2 and AE017336.2, respectively, and amplicon lengths based on *in silico* range observed in *Ba* whole genome strains are also noted.

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