

**Table S1.** Basic source information and accession numbers for deficient isolates and 148 Fha-producing isolates used for mutation identification.

Strain ID	Source	Year	Fha ECL <sup>a</sup>	Prn ECL <sup>b</sup>	Pt ECL <sup>c</sup>	Accession	EPS <sup>d</sup>	Duplication <sup>e</sup>	Reference
B199	FDA 105	1935	Neg	Pos	Pos	SRS2357476			This study
C958	USA: NJ	2001	Pos	Pos	Pos	SRS1832432			(1)
E024	USA: MD	2003	Pos	Pos	Pos	SRS1832551			(1)
E150	USA: OH	2003	Pos	Pos	Pos	SRS1832535			(1)
F011	USA: NE	2005	Pos	Pos	Pos	SRS1832396			(1)
F658	USA: CO	2008	Pos	Pos	Pos	SRS1832471			(1)
F670	USA: WA	2003	Pos	Pos	Pos	SRS1832508			(1)
H320	USA: FL	2009	Pos	Pos	Pos	SRS1832510			(1)
H627	USA: CA	2010	Pos	Pos	Pos	SRS1324858			(2)
H920	USA: WA	2011	Pos	Neg	Pos	SRS2357489			This study
I743	USA: MN	2013	Pos	Neg	Pos	SRS1832416	yes		(1)
I747	USA: MN	2012	Pos	Neg	Pos	SRS2357500	yes	B199	This study
I751	USA: CT	2012	Pos	Pos	Pos	SRS1832383	yes		(1)
I752	USA: CT	2013	Pos	Pos	Pos	SRS1832485	yes		(1)
I754	USA: CT	2013	Pos	Neg	Pos	SRS1832502	yes		(1)
I755	USA: NY	2013	Pos	Neg	Pos	SRS1832582	yes		(1)
I762	USA: NY	2013	Pos	Neg	Pos	SRS1832559	yes		(1)
I944	USA: CA	2013	Pos	Pos	Pos	SRS1832476			(1)
I955	USA: CA	2013	Pos	Neg	Pos	SRS2357474			This study
I958	USA: NM	2013	Pos	Neg	Pos	SRS2357499	yes		This study
I959	USA: OR	2013	Pos	Neg	Pos	SRS1832573	yes		(1)
I965	USA: CO	2013	Pos	Neg	Pos	SRS1832552	yes	J042	(1)
I968	USA: CO	2013	Pos	Neg	Pos	SRS1832479	yes		(1)
I975	USA: NY	2013	Pos	Neg	Pos	SRS1832398	yes		(1)
I976	USA: NY	2013	Pos	Neg	Pos	SRS1832434	yes		(1)
I977	USA: NY	2013	Pos	Neg	Pos	SRS1832511	yes		(1)
I978	USA: NY	2013	Pos	Neg	Pos	SRS1832523	yes		(1)
I979	USA: NY	2013	Pos	Neg	Neg	SRS1324875	yes		(3)
J010	USA: CT	2013	Pos	Neg	Pos	SRS1832483	yes		(1)
J012	USA: MN	2013	Pos	Neg	Pos	SRS1832494	yes		(1)
J013	USA: MN	2013	Pos	Neg	Pos	SRS1832413	yes		(1)
J014	USA: MN	2014	Neg	Neg	Pos	SRS2357481	yes		This study
J016	USA: MN	2013	Pos	Neg	Pos	SRS1832553	yes		(1)
J018	USA: MN	2013	Pos	Neg	Pos	SRS1832408	yes		(1)
J019	USA: MN	2013	Pos	Neg	Pos	SRS1832572	yes		(1)
J021	USA: OR	2013	Pos	Neg	Pos	SRS1832544	yes		(1)
J022	USA: OR	2013	Pos	Neg	Pos	SRS1832390	yes		(1)
J023	USA: OR	2013	Pos	Neg	Pos	SRS1832399	yes		(1)
J024	USA: NY	2013	Pos	Pos	Pos	SRS2357448	yes		This study
J027	USA: CO	2013	Pos	Neg	Pos	SRS1832594	yes		(1)
J029	USA: CO	2013	Pos	Pos	Pos	SRS2357509	yes	J042	This study
J030	USA: CO	2013	Pos	Neg	Pos	SRS1832564	yes		(1)
J042	NIH 325	1947	Neg	Pos	Pos	SRS2357480			This study

## Supplemental Material

J043	NIH 326	1947	Neg	Neg	Neg	SRS2357496			This study
J066	USA: CT	2013	Pos	Neg	Pos	SRS2357446	yes		This study
J068	USA: CT	2013	Pos	Neg	Pos	SRS1832376	yes		(1)
J072	USA: OR	2013	Pos	Neg	Pos	SRS1832591	yes		(1)
J073	USA: OR	2013	Pos	Neg	Pos	SRS1832580	yes		(1)
J074	USA: NM	2013	Pos	Neg	Pos	SRS2357487	yes		This study
J076	USA: NM	2013	Pos	Neg	Pos	SRS1832426	yes		(1)
J077	USA: MN	2013	Pos	Neg	Pos	SRS2357470	yes		This study
J078	USA: MN	2013	Pos	Neg	Pos	SRS2357461	yes		This study
J081	USA: MN	2013	Pos	Neg	Pos	SRS2357475	yes		This study
J083	USA: CO	2013	Pos	Neg	Pos	SRS2357507	yes	J042	This study
J085	USA: CO	2013	Pos	Neg	Pos	SRS2357490	yes	J042	This study
J090	USA: CO	2013	Pos	Neg	Pos	SRS1832414	yes		(1)
J091	USA: CO	2014	Pos	Neg	Pos	SRS1832574	yes		(1)
J093	USA: DE	2014	Pos	Neg	Pos	SRS1832593			(1)
J094	USA: GA	2014	Pos	Neg	Pos	SRS2357488	yes		This study
J096	USA: CO	2014	Pos	Neg	Pos	SRS1832404	yes		(1)
J097	USA: CO	2014	Pos	Neg	Pos	SRS1832445			(1)
J098	USA: NM	2014	Pos	Pos	Pos	SRS1832571	yes		(1)
J099	USA: OR	2014	Pos	Neg	Pos	SRS1832442	yes		(1)
J100	USA: OR	2014	Pos	Neg	Pos	SRS1832595	yes		(1)
J103	USA: MN	2014	Pos	Neg	Pos	SRS1832389	yes		(1)
J104	USA: MN	2014	Pos	Neg	Pos	SRS1832505	yes		(1)
J105	USA: MN	2014	Pos	Neg	Pos	SRS1832563	yes		(1)
J106	USA: MN	2014	Pos	Neg	Pos	SRS2357467	yes		This study
J107	USA: MN	2014	Pos	Neg	Pos	SRS1832545	yes		(1)
J108	USA: MN	2014	Pos	Neg	Pos	SRS2357443	yes		This study
J109	USA: MN	2014	Pos	Neg	Pos	SRS2357462	yes		This study
J110	USA: MN	2014	Pos	Neg	Pos	SRS1832458	yes		(1)
J111	USA: MN	2014	Pos	Neg	Pos	SRS2357435	yes		This study
J112	USA: NY	2014	Pos	Neg	Pos	SRS2357458	yes		This study
J113	USA: NY	2014	Pos	Neg	Pos	SRS2357451	yes		This study
J114	USA: NY	2014	Pos	Neg	Pos	SRS2357477			This study
J115	USA: NY	2014	Pos	Neg	Pos	SRS1832492	yes		(1)
J131	USA: CA	2014	Pos	Neg	Pos	SRS2357456			This study
J148	USA: OR	2014	Pos	Neg	Pos	SRS2357472	yes		This study
J149	USA: OR	2014	Pos	Neg	Pos	SRS1832528	yes		(1)
J150	USA: OR	2014	Pos	Neg	Pos	SRS2357485	yes		This study
J151	USA: OR	2014	Pos	Neg	Pos	SRS2357454	yes		This study
J152	USA: MN	2014	Pos	Neg	Pos	SRS1832452	yes		(1)
J153	USA: MN	2014	Pos	Neg	Pos	SRS2357447	yes		This study
J154	USA: MN	2014	Pos	Neg	Pos	SRS2357468	yes		This study
J155	USA: MN	2014	Pos	Neg	Pos	SRS1832422	yes		(1)
J157	USA: MN	2014	Pos	Neg	Pos	SRS2357437	yes		This study
J159	USA: MN	2014	Pos	Neg	Pos	SRS1832386	yes		(1)
J160	USA: MN	2014	Pos	Neg	Pos	SRS2357466	yes		This study
J161	USA: MN	2014	Pos	Neg	Pos	SRS1832503	yes		(1)

## Supplemental Material

J162	USA: GA	2014	Pos	Neg	Pos	SRS1832496	yes	(1)
J163	USA: GA	2014	Pos	Neg	Pos	SRS2357505	yes	This study
J164	USA: GA	2014	Pos	Neg	Pos	SRS2357493		This study
J165	USA: NY	2014	Pos	Neg	Pos	SRS2357498	yes	This study
J170	USA: CO	2014	Pos	Neg	Pos	SRS2357455	yes	This study
J171	USA: CO	2014	Pos	Neg	Pos	SRS2357473		This study
J172	USA: CO	2014	Pos	Neg	Pos	SRS2357457	yes	This study
J173	USA: CO	2014	Pos	Neg	Pos	SRS2357464	yes	This study
J174	USA: CO	2014	Pos	Neg	Pos	SRS1832526	yes	(1)
J175	USA: CO	2014	Pos	Neg	Pos	SRS1832447	yes	(1)
J177	USA: CO	2014	Pos	Neg	Pos	SRS2357504	yes	This study
J178	USA: CO	2014	Pos	Neg	Pos	SRS1832443	yes	(1)
J179	USA: CO	2014	Pos	Neg	Pos	SRS1832388	yes	(1)
J191	USA: IN	2014	Pos	Neg	Pos	SRS1832498		(1)
J192	USA: OR	2014	Pos	Neg	Pos	SRS1832518	yes	(1)
J193	USA: CO	2014	Pos	Neg	Pos	SRS1832531	yes	(1)
J194	USA: CO	2014	Pos	Neg	Pos	SRS1832550	yes	(1)
J195	USA: CO	2014	Pos	Neg	Pos	SRS2357450	yes	This study
J197	USA: CO	2014	Pos	Neg	Pos	SRS1832375	yes	(1)
J198	USA: CO	2014	Pos	Pos	Pos	SRS1832436	yes	(1)
J199	USA: CO	2014	Neg	Neg	Pos	SRS2357506	yes	This study
J201	USA: CO	2014	Pos	Neg	Pos	SRS1832412	yes	(1)
J202	USA: CO	2014	Pos	Neg	Pos	SRS2357444	yes	This study
J203	USA: GA	2014	Pos	Neg	Pos	SRS2357503	yes	This study
J204	USA: GA	2014	Pos	Pos	Pos	SRS2357478		This study
J205	USA: GA	2014	Pos	Neg	Pos	SRS2357438		This study
J206	USA: NY	2014	Pos	Neg	Pos	SRS1832392	yes	(1)
J207	USA: NY	2014	Pos	Neg	Pos	SRS2357486	yes	This study
J208	USA: NY	2014	Pos	Neg	Pos	SRS1832477	yes	(1)
J209	USA: NY	2014	Pos	Neg	Pos	SRS2357491	yes	This study
J210	USA: NY	2014	Pos	Neg	Pos	SRS1832549	yes	(1)
J222	USA: MN	2014	Pos	Neg	Pos	SRS2357469	yes	This study
J223	USA: MN	2014	Pos	Neg	Pos	SRS2357440	yes	This study
J224	USA: MN	2014	Pos	Neg	Pos	SRS2357479	yes	This study
J225	USA: MN	2014	Pos	Neg	Pos	SRS1832401	yes	(1)
J226	USA: MN	2014	Pos	Neg	Pos	SRS2357436	yes	This study
J227	USA: MN	2014	Pos	Neg	Pos	SRS2357483	yes	This study
J228	USA: MN	2014	Pos	Neg	Pos	SRS2357441	yes	This study
J229	USA: MN	2014	Pos	Neg	Pos	SRS2357449	yes	This study
J230	USA: MN	2014	Pos	Neg	Pos	SRS2357508	yes	This study
J246	USA: VT	2014	Pos	Neg	Pos	SRS2357452		This study
J247	USA: VT	2014	Pos	Neg	Pos	SRS2357471		This study
J248	USA: VT	2014	Pos	Pos	Pos	SRS2357501		This study
J276	USA: OR	2014	Pos	Neg	Pos	SRS1832384	yes	(1)
J277	USA: OR	2014	Pos	Neg	Pos	SRS1832538	yes	(1)
J286	USA: WI	2014	Pos	Neg	Pos	SRS2357465		This study
J288	USA: WI	2014	Pos	Neg	Pos	SRS2357460		This study

B199

Supplemental Material

J294	USA: MN	2014	Pos	Neg	Pos	SRS2357502	yes	This study
J295	USA: CT	2014	Pos	Neg	Pos	SRS2357463	yes	This study
J296	USA: CT	2014	Pos	Neg	Pos	SRS1832450	yes	(1)
J298	USA: CO	2014	Pos	Neg	Pos	SRS2357459	yes	This study
J299	USA: CO	2014	Pos	Neg	Pos	SRS2357492	yes	This study
J300	USA: CO	2014	Pos	Neg	Pos	SRS1832500	yes	(1)
J301	USA: CO	2014	Pos	Neg	Pos	SRS2357484		This study
J302	USA: CO	2014	Pos	Neg	Pos	SRS2357482		This study
J303	USA: CO	2014	Pos	Neg	Pos	SRS2357495		This study
J304	USA: GA	2014	Pos	Neg	Pos	SRS2357497	yes	This study
J305	USA: NY	2014	Pos	Neg	Pos	SRS1832490	yes	(1)
J306	USA: NY	2014	Pos	Neg	Pos	SRS2357439	yes	This study
J308	USA: NY	2014	Pos	Neg	Pos	SRS2357442	yes	This study
J309	USA: NY	2014	Pos	Neg	Pos	SRS2357494	yes	This study
J310	USA: NY	2014	Pos	Neg	Pos	SRS2357445	yes	This study
J311	USA: NY	2014	Pos	Neg	Pos	SRS1832373		(1)
J365	USA: CA	2014	Pos	Neg	Neg	SRS2357453		This study

<sup>a</sup> Fha production measured by ECL assay.

<sup>b</sup> Prn production measured by ECL assay.

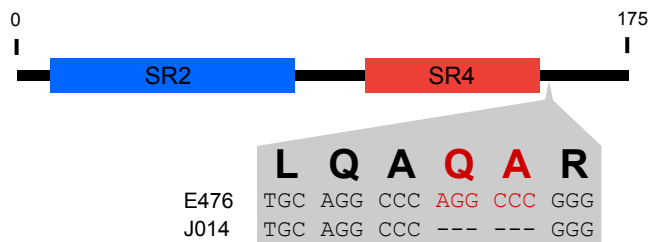
<sup>c</sup> Pt production measured by ECL assay.

<sup>d</sup> Isolates collected through Enhanced Pertussis Surveillance (4).

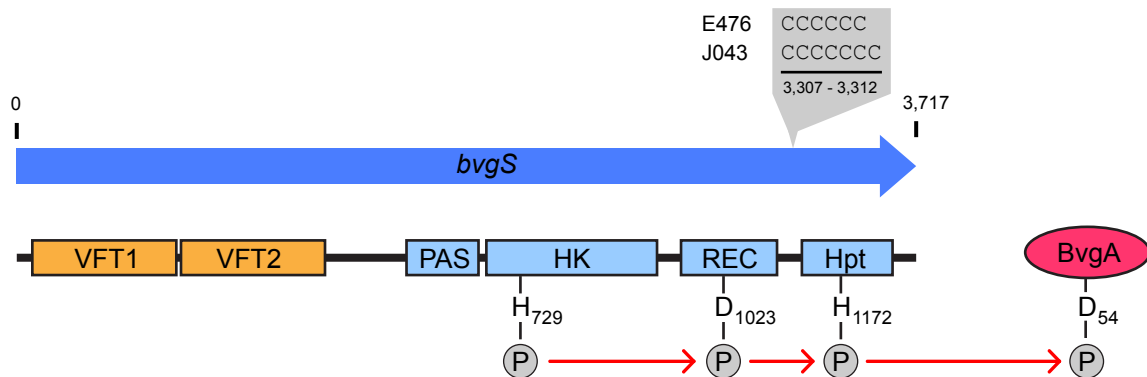
<sup>e</sup> Putative duplications inferred from high sequencing read coverage in same genomic region as observed in Fha-deficient isolates B199 or J042.

Abbreviations: Pos, positive; Neg, negative.

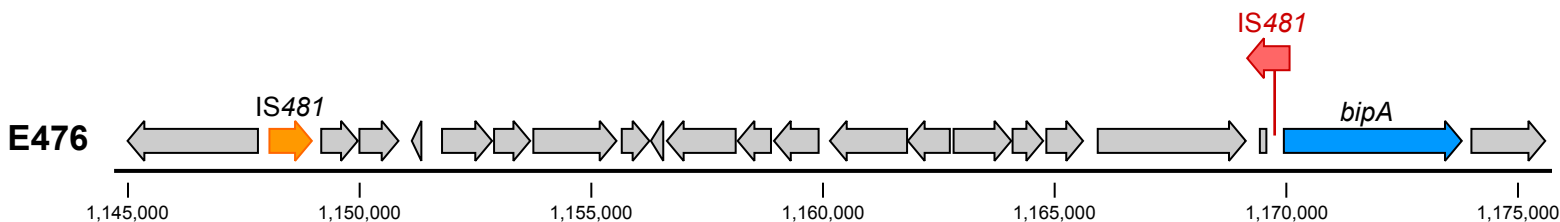
a.



b.



c.



**Figure S1.** [a] Predicted domain structure of the ECF-sigma factor (RD\_14675) and the in-frame deletion of two amino acids at position 160 observed in J014. [b] The multidomain structure of BvgS includes two periplasmic venus flytrap (VFT) domains connected to the cytosolic PAS, histidine kinase (HK), receiver (Rec), and histidine phosphoryl transfer (Hpt) domains. An insertion with a homopolymeric tract upstream of the Hpt domain was observed in J043 which likely disrupted phosphorelay to BvgA. [c] Predicted gene organization near *bipA* in the reference strain E476 (CP010964). *IS481* insertion in the 5'-UTR (red) and subsequent inversion with an existing *IS481* copy ~20 kb upstream (orange) disrupted the complex *bipA* promoter region in J042.

## References

1. Weigand MR, Peng Y, Loparev V, Batra D, Bowden KE, Burroughs M, Cassidy PK, Davis JK, Johnson T, Juieng P, Knipe K, Mathis MH, Pruitt AM, Rowe L, Sheth M, Tondella ML, Williams MM. 2017. The History of *Bordetella pertussis* Genome Evolution Includes Structural Rearrangement. *J Bacteriol* **199**:e00806-00816.
2. Bowden KE, Weigand MR, Peng Y, Cassidy PK, Sammons S, Knipe K, Rowe LA, Loparev V, Sheth M, Weening K, Tondella ML, Williams MM. 2016. Genome Structural Diversity among 31 *Bordetella pertussis* Isolates from Two Recent U.S. Whooping Cough Statewide Epidemics. *mSphere* **1**:e00036-00016.
3. Williams MM, Sen K, Weigand MR, Skoff TH, Cunningham VA, Halse TA, Tondella ML, Group CDCPW. 2016. *Bordetella pertussis* Strain Lacking Pertactin and Pertussis Toxin. *Emerg Infect Dis* **22**:319-322.
4. Skoff TH, Baumbach J, Cieslak PR. 2015. Tracking Pertussis and Evaluating Control Measures through Enhanced Pertussis Surveillance, Emerging Infections Program, United States. *Emerg Infect Dis* **21**:1568-1573.