

1 **Supplemental Fig. 1.** A phylogenetic tree based on the conserved intein motifs listed in
2 Supplemental Table 1 was generated using MrBayes (7) in the Geneious software
3 package. The 148 inteins selected for analysis included all Class 3 inteins, the 2 Class 1
4 WCT motif inteins, 29 inteins with the WCT motif Cys^{F:4} instead of the more common
5 Asp^{F:4} in the absence of the other WCT motif residues, various types of DNA helicase
6 inteins, and phage, prophage or viral inteins. Probability values at each node are
7 expressed as consensus support percentages. The scale bar represents 0.3 substitutions
8 per site. Class 3 inteins are indicated in red, as is the *Arthrobacter species* FB24 DnaB
9 intein that begins with Gly¹, but lacks Cys^{F:4}. Further biochemical analysis is required to
10 determine the Class of the *Arthrobacter species* FB24 DnaB intein.

11 Class 1 WCT inteins are indicated in blue. Intein names are defined in the InBase
12 database (<http://www.neb.com/neb/inteins.html>) (14) with the following additional
13 suffixes: T1, Class 1 intein, T3, Class 3 intein; P, phage or prophage intein; V, viral
14 intein; T1a, DnaB Class 1 intein present at the DnaB-a insertion site; T1b, DnaB Class 1
15 intein present at the DnaB-b insertion site; T1c, DnaB Class 1 intein present at the DnaB-
16 c insertion site; T1d, DnaB Class 1 intein present at the DnaB-d insertion site; T1e, DnaB
17 Class 1 intein present at the DnaB-e insertion site; T1f, DnaB Class 1 intein present at the
18 DnaB-f insertion site; T1h, DnaB Class 1 intein present at the DnaB-h insertion site; T1C,
19 Class 1 intein with Cys^{F:4}; T1CP, Class 1 intein from a phage or prophage that has Cys^{F:4};
20 T1WCT, Class 1 intein with the WCT motif.

