

**Table 5. Determination of the sequence of events in P-cluster maturation**

Line	Assay components					Activity*				
	Av1 <sup>†</sup>	NifZ	Av2 <sup>wt</sup>	ATP	FeMoco	C <sub>2</sub> H <sub>4</sub> formation under C <sub>2</sub> H <sub>2</sub> /Ar	H <sub>2</sub> formation under Ar	NH <sub>3</sub> formation under N <sub>2</sub>	H <sub>2</sub> formation under N <sub>2</sub>	Average, %
1	Av1 <sup>ΔnifB</sup>	–	–	–	+	1,014 ± 29 (100)	998 ± 31 (100)	633 ± 6 (100)	223 ± 34 (100)	100
2	Av1 <sup>ΔnifZΔnifB (+Av2/ATP)</sup>	–	–	–	+	491 ± 6 (48)	514 ± 15 (52)	320 ± 6 (51)	100 ± 5 (45)	49
3	Av1 <sup>ΔnifZΔnifB (+Av2/ATP)</sup>	+	–	–	+	470 ± 25 (46)	479 ± 20 (48)	287 ± 2 (45)	106 ± 9 (48)	47
4	Av1 <sup>ΔnifZΔnifB (+Av2/ATP)</sup>	+	–	+	+	466 ± 16 (46)	470 ± 15 (47)	310 ± 22 (49)	112 ± 4 (50)	48
5	Av1 <sup>ΔnifZΔnifB (+NifZ)</sup>	–	–	–	+	518 ± 28 (51)	523 ± 66 (52)	330 ± 32 (52)	106 ± 6 (48)	51
6	Av1 <sup>ΔnifZΔnifB (+NifZ)</sup>	–	+	–	+	479 ± 35 (47)	526 ± 10 (53)	324 ± 12 (51)	94 ± 21 (42)	46
7	Av1 <sup>ΔnifZΔnifB (+NifZ)</sup>	–	+ <sup>‡</sup>	+ <sup>§</sup>	+	803 ± 17 (79)	772 ± 42 (77)	585 ± 37 (92)	201 ± 10 (90)	85

Activities of C<sub>2</sub>H<sub>4</sub> formation under C<sub>2</sub>H<sub>2</sub>/Ar, H<sub>2</sub> formation under Ar, NH<sub>3</sub> formation under N<sub>2</sub>, and H<sub>2</sub> formation under N<sub>2</sub> are expressed as nmol per min per mg of protein. Percentages relative to Av1<sup>ΔnifB</sup> (line 1) are given in parentheses. Average activities are expressed as percentages only.

\*The lower detection limits were 0.01, 0.02, 0.001, and 0.02 nmol per min per mg of protein for C<sub>2</sub>H<sub>4</sub> formation under C<sub>2</sub>H<sub>2</sub>/Ar, H<sub>2</sub> formation under Ar, NH<sub>3</sub> formation under N<sub>2</sub>, and H<sub>2</sub> formation under N<sub>2</sub>, respectively

<sup>†</sup>Note that both Av1<sup>ΔnifZΔnifB (+Av2/ATP)</sup> and Av1<sup>ΔnifZΔnifB (+NifZ)</sup> can be activated in assays containing NifZ, Av2<sup>wt</sup>, and MgATP to an average substrate reduction activity of 82% and 87%, respectively.

<sup>‡</sup>No P-cluster formation was observed if Av2<sup>wt</sup> was replaced by Av2<sup>M156C</sup>; whereas Av2<sup>E146D</sup> was fully competent as a replacement for Av2<sup>wt</sup> in P-cluster formation. These results are consistent with those from Table 3 (lines 8-9).

<sup>§</sup>No P-cluster formation was observed if ATP was replaced by ADP, AMPPNP, or ATPγS. These results are consistent with those from Table 3 (lines 3-5).