Supplementary Table S1. $^{1}D_{HN}$ values of the imino groups of native *E. coli* $tRNA^{Val}$ obtained from different external alignment media

	External Alignment Media					
Assignment	Pf1[a]	C_8E_5/1-octanol[b]	fd	fd-9ah8	DMPC/DHPC	DMPC/DHPC/SDS
G1	-12.2	-11.3	-3.0	-3.9	-19.2	-1.7
G2	6.5	10.0	2.4	3.0	ND[c]	0.6
G3	9.8	14.2	4.2	4.3	ND[c]	1.2
U4	4.6	4.0	1.5	2.8	9.5	-0.3
G5	-21.2	-24.2	-2.4	-4.9	-60.7	-3.6
U7	-6.5	-5.0	-1.2	-2.5	ND[c]	-2.5
s^4U8	-0.8	-1.0	0.5	-0.6	-3.7	0.0
G10	-12.9	-20.9	ND[c]	ND[c]	ND[c]	3.6
U12	1.0	-3.7	-0.3	-0.5	-7.5	-0.6
G15	-23.6	-31.0	-3.6	-5.9	-49.4	-3.4
G18	-16.6	-18.6	-4.4	-6.9	ND[c]	-3.7
G22	-7.5	-8.5	-0.4	-1.0	-23.6	-1.3
G24	-8.4	-11.4	-1.6	-2.7	-25.6	-2.4
U29	-20.1	-26.5	-2.0	-4.4	-54.9	-3.4
G39	-15.3	-16.6	-2.9	-5.3	-36.7	-3.8
G40	-24.4	-30.7	-4.9	-9.0	ND[c]	-2.5
G42	-5.7	-9.9	-1.5	-2.4	-16.4	-0.8
m^7G46	-23.6	-25.0	-7.9	-10.7	ND[c]	-4.9
G49	25.1	28.1	5.8	9.2	ND[c]	3.6
G50	17.9	25.3	1.8	5.0	ND[c]	1.0
G52	-25.3	-23.8	-4.7	-8.4	ND[c]	-3.9
G53	5.5	0.1	1.3	3.7	ND[c]	-0.6
T54	4.1	7.6	1.1	1.7	16.4	2.1
Ψ55(N3)	14.9	20.9	4.8	6.8	42.7	2.4
G63	0.3	3.0	-3.9	-3.7	ND[c]	-0.3
U64	20.6	30.1	4.6	7.6	ND[c]	2.7
U67	-17.5	-19.9	-3.5	-5.8	-48.1	-3.0
R_P[d]		0.98	0.91	0.96	0.98	0.83

[a] $^{1}D_{HN}$ values from (Mollova, et al. 2000).

[b] $^{1}D_{HN}$ values from (Vermeulen, et al. 2005).

[c] $^{1}D_{HN}$ values not determined.

[d] Pearson's correlation coefficient between $^{1}D_{HN}$ values from each of the different alignment media and Pf1.

Supplementary Table S2. $^1J_{HN}(B_0)$ and MSA-induced $^1D_{HN}(B_0)$ values for the imino groups of native E. coli $tRNA^{Val}$

	$^1J_{HN}(B_0)$[a]					$^1D_{HN}(B_0)$[c]		
Assignment	$^1J_{HN}(500)$	$^1J_{HN}(600)$	$^1J_{HN}(800)$	$^1J_{HN}(900)$	$^1J_{HN}(0)$	$^1D_{HN}(800)$	$^1D_{HN}(900)$	$^1D_{HN}(800,900)$
G1	-87.63	-88.46	-86.59	-85.46	-88.91	-2.32	-3.45	-2.53
G2	-88.33	-88.89	-88.16	-87.87	-88.72	-0.56	-0.85	-0.37
G3	-89.14	-89.96	-89.39	-89.66	-89.18	0.22	0.48	1.01
U4	-87.47	-86.73	-87.94	-88.94	-86.60	1.33	2.33	1.37
G5	-87.78	-88.41	-87.37	-86.20	-88.63	-1.27	-2.43	-1.84
U7	-87.68	-86.88	-86.59	-86.03	-88.24	-1.65	-2.21	-1.69
s^4U8	ND[d]							
G10	-88.83	-89.28	-88.76	-87.94	-89.30	-0.54	-1.36	-0.36
U12	-87.20	-86.34	-87.07	-87.60	-86.80	0.27	0.81	0.51
G15	-88.81	-88.72	-87.45	-85.72	-90.16	-2.72	-4.44	-2.46
G18	ND[d]							
G22	-88.37	-89.08	-89.00	-88.84	-88.29	0.71	0.54	0.86
G24	-87.03	-87.38	-85.63	-84.53	-88.36	-2.73	-3.83	-2.20
U29	-85.89	-84.56	-84.59	-85.29	-85.96	-1.38	-0.67	-1.29
G39	-87.45	-86.97	-86.44	-85.82	-88.09	-1.65	-2.27	-1.78
G40	-87.27	-87.51	-86.03	-84.61	-88.57	-2.53	-3.96	-2.84
G42	-86.65	-87.29	-86.94	-86.44	-86.87	0.07	-0.43	-0.49
m^7G46	-86.22	-85.53	-83.87	-82.93	-87.69	-3.82	-4.76	-3.62
G49	-88.99	-89.37	-89.16	-88.92	-89.10	0.06	-0.19	0.11
G50	-89.59	-90.85	-90.67	-90.70	-89.37	1.30	1.32	1.07
G52	-86.85	-87.22	-85.95	-83.99	-88.17	-2.22	-4.18	-2.92
G53	-87.87	-84.67	-86.38	-86.43	-87.64	-1.26	-1.21	-1.18
T54	-87.41	-86.49	-86.75	-87.01	-87.40	-0.66	-0.39	-0.26
Ψ55(N3)	-91.53	-91.70	-92.58	-92.34	-91.05	1.54	1.29	1.28
G63	-87.66	-88.65	-88.32	-87.60	-87.84	0.48	-0.24	0.08
U64	-89.78	-89.48	-90.17	-91.79	-88.90	1.27	2.88	1.76
U67	-84.53	-83.60	-83.59	-83.36	-84.84	-1.25	-1.48	-1.76
Standard deviation (Hz)	0.30[b]	0.72[b]	0.31[b]	0.83[b]	1.15[f]	1.19[g]	1.42[g]	1.85[g]

[a] One-bond imino group splittings determined at four magnetic field strengths. Reported values are the average of the two experimental methods described in Materials and Methods.

[b] Standard deviation between of the $^1J_{HN}(B_0)$ values determined from two method (see Materials and Methods).

[c] $^1D_{HN}(800)$ and $^1D_{HN}(900)$ were determined by subtracting $^1J_{HN}(0)$ from $^1J_{HN}(800)$ and $^1J_{HN}(900)$, respectively.

[d] Values not determined.

[f] Standard deviation of $^{1}J_{HN}(0)$ determined from weighted linear regression analysis.

[g] Error in $^{1}D_{HN}(B_0)$ values calculated using standard error propagation analysis.