

Figure S1. Schematic of conventional plates described in section 2.2. **(a)** A ø6-cm plate in which test solution and standard (control) solution were spotted on T₁ and C₁, respectively. **(b)** A ø10-cm plate in which test solution was spotted on T₁ and T₂ and standard solution was spotted on C₁ and C₂.

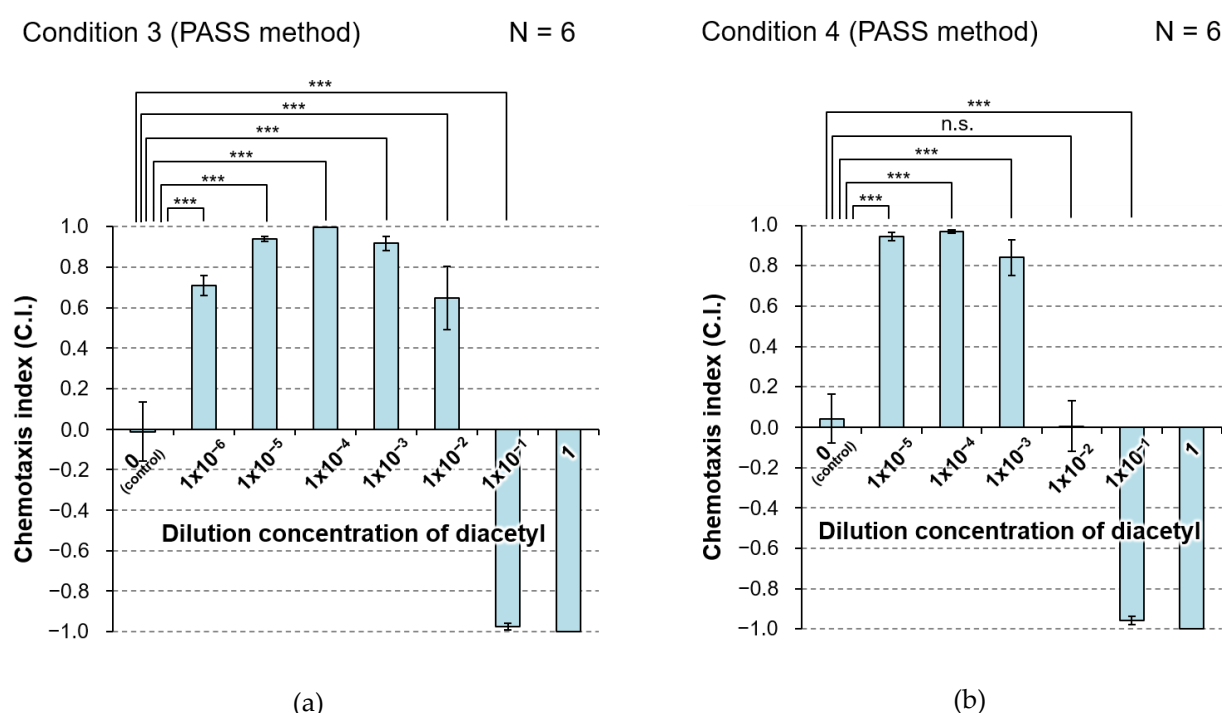


Figure S2. Result of PASS to evaluate chemotaxis of *C. elegans* to a graded series of concentrations of diacetyl. **(a)** Result of PASS under condition 3 in Table 1. **(b)** Result of PASS under condition 4 in Table 1. The maximum concentration of diacetyl was 1 (undiluted solution) and the minimum diluted concentration was 1×10^{-6} for **a** or 1×10^{-5} for **b**. Light-blue bar and error bar represent the mean and SEM of chemotaxis index (C.I.) of six independent assays under the same condition. All data were analyzed by one-way ANOVA at $p < 0.05$, $p < 0.01$, and $p < 0.001$ (***) significance levels by comparison to the control group. Note that the data of the undiluted solution with no variance were excluded from the analysis. n.s., not significant; N, the number of assays. The details of the p -values are shown in Table S1.

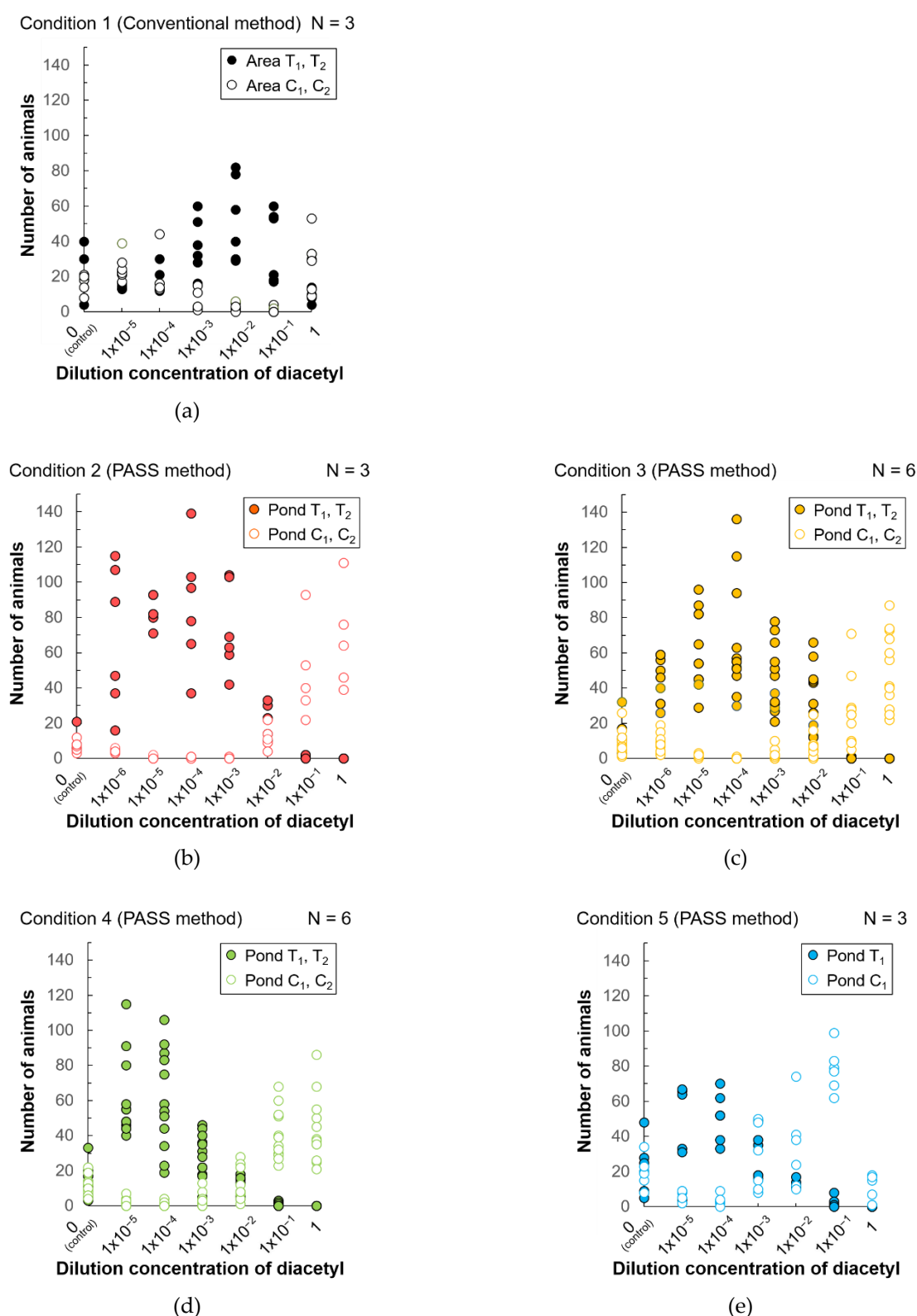


Figure S3. Number of *C. elegans* individuals trapped in the test area/ponds and control area/ponds in chemotaxis assays of diacetyl. (a) Result of conventional chemotaxis assay under condition 1 corresponding to Figure 5. (b) Result of PASS under condition 2 corresponding to Figure 6a. (c) Result of PASS under condition 3 corresponding to Figures 6b and S2a. (d) Result of PASS under condition 4 corresponding to Figures 6c and S2b. (e) Result of PASS under condition 5 corresponding to Figure 6d. Closed circle represents the number of animals trapped in the area/ponds of the test side, T₁ and T₂, and open circle represents the number of animals trapped in the area/ponds of the control side, C₁ and C₂ at each of three or six independent assays. N, the number of assays.

Table S1. The *p*-values from comparisons between the control group and each dilution group for the results shown in Figures 5, 6, and S2.

Dilution Figure		1×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}	1×10^{-2}	1×10^{-1}	1
Figure 5			0.1933	0.7336	0.0051	0.0006	0.0004	0.1028
Figure 6	a	< 0.000001	< 0.0000001	< 0.0000001	< 0.0000001	0.0063	< 0.0000001	Not applicable
	b	0.0004	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0001	Not applicable
	c		< 0.0001	< 0.0001	0.0002	0.8512	< 0.0001	Not applicable
	d		0.0031	0.0012	0.6585	0.1767	0.0010	Not applicable
Figure S2	a	< 0.000001	< 0.00000001	< 0.000000001	< 0.00000001	< 0.00001	< 0.00000001	Not applicable
	b		< 0.00000001	< 0.00000001	< 0.0000001	0.7515	< 0.00000001	Not applicable