SUPPLEMENTARY INFORMATION

Design of experiment approach for efficient multi-parametric drug testing using a *Caenorhabditis elegans* model

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Supplementary Table 1. Full model matrix of coded values of the experimental parameters temperature (X_1), *E. coli* concentration (X_2) and doxycycline concentration (X_3), their interactions and quadratic values.

	I	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
1	1	0	0	0	0	0	0	0	0	0
2	1	1	0	0	0	0	0	1	0	0
3	1	0.5	0.866	0	0.433	0	0	0.25	0.749956	0
4	1	0.5	0.289	0.816	0.1445	0.408	0.235824	0.25	0.083521	0.665856
5	1	-1	0	0	0	0	0	1	0	0
6	1	-0.5	-0.866	0	0.433	0	0	0.25	0.749956	0
7	1	-0.5	-0.289	-0.816	0.1445	0.408	0.235824	0.25	0.083521	0.665856
8	1	0.5	-0.866	0	-0.433	0	0	0.25	0.749956	0
9	1	0.5	-0.289	-0.816	-0.1445	-0.408	0.235824	0.25	0.083521	0.665856
10	1	-0.5	0.866	0	-0.433	0	0	0.25	0.749956	0
11	1	0	0.577	-0.816	0	0	-0.47083	0	0.332929	0.665856
12	1	-0.5	0.289	0.816	-0.1445	-0.408	0.235824	0.25	0.083521	0.665856
13	1	0	-0.577	0.816	0	0	-0.47083	0	0.332929	0.665856

Supplementary Table 2. Coefficients of the quadratic polynomial model in Equation 1.

b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_1^2	b_2^2	b_3^2
95.861	-17.8661	-1.451	27.163	0.914	-5.893	8.588	2.097	3.577	-6.789

Temperature controller

A Peltier thermoelectric module was used to set the temperature experienced by the worms inside the microfluidic chip placed in the thermal incubator. A pair of heat sink and fan was glued to each surface of the Peltier module using a thermal grease, to enhance the heat exchange. Larger heat sink and fan were chosen for the hot Peltier surface, to facilitate the heat dissipation and prevent overheating. A temperature sensor (AD22100, Analog Devices, US) was placed in contact with the glass side of the microfluidic chip, to sense the temperature in the thermal incubator, ultimately experienced by the worms in the microfluidic device. An Arduino microcontroller board (Arduino UNO, Ivrea, Italy) was used to read the signal from the temperature sensor and set the power to the Peltier thermoelectric module,

in a PID (proportional integral derivative) closed-loop configuration. Generally, A PID controller reads the sensor signal and computes the desired output by calculating and summing the proportional, integral and derivative responses. The output control signal *u(t)* can be therefore defined as follows:

$$u(t) = K_p e(t) + K_i \int_{t_0}^{t} e(t)dt + K_d \frac{de(t)}{dt}$$

K_p, K_i and K_d are respectively the gains of the proportional, integral and derivative components. *e(t)* is the difference between the set-point value and the output signal. The proportional, integral and derivative gains were tuned using the open-loop step response method by Ziegler and Nichols (1) and were then experimentally optimized. After calculating the three gains, the PID control operations were committed to a dedicated Arduino library. The electric circuit was provided with an integrated H-bridge (BTS 7960B, Infineon technologies, Germany), to change the direction of the current depending on the heating or cooling mode of the Peltier element. The default value of the set-point, 20 °C, could be increased or decreased by 0.5 °C by the user, pressing two dedicated buttons. The temperature set-point and the actual temperature were displayed on a LCD.

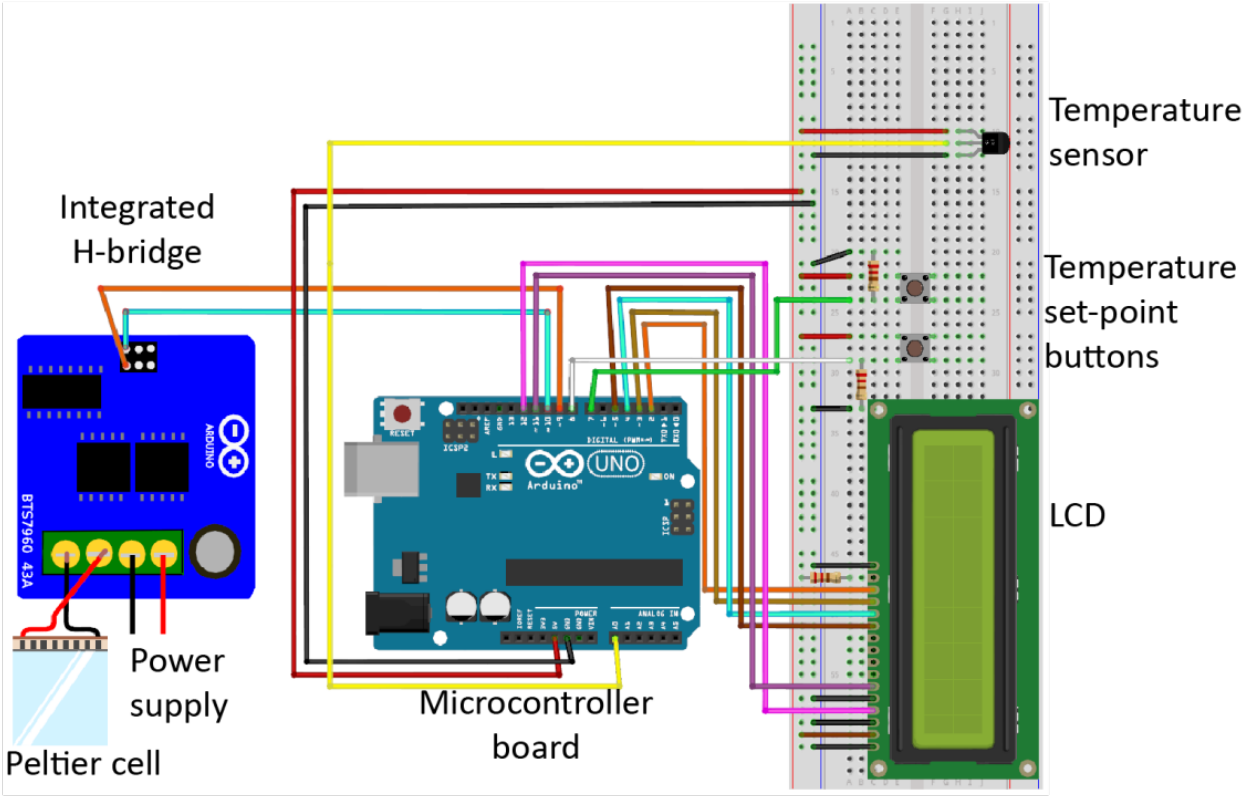

Figure S1. Schematic of the electric circuit designed to control the temperature into the thermal incubator. According to the signal measured from a temperature sensor, a microcontroller sets the power given to an H-bridge connected to a Peltier cell, in a closed-loop PID configuration. The temperature set-point can be adjusted by the user through two dedicated buttons, and is displayed on a LCD with the actual measured temperature.

Normality and ANOVA tests

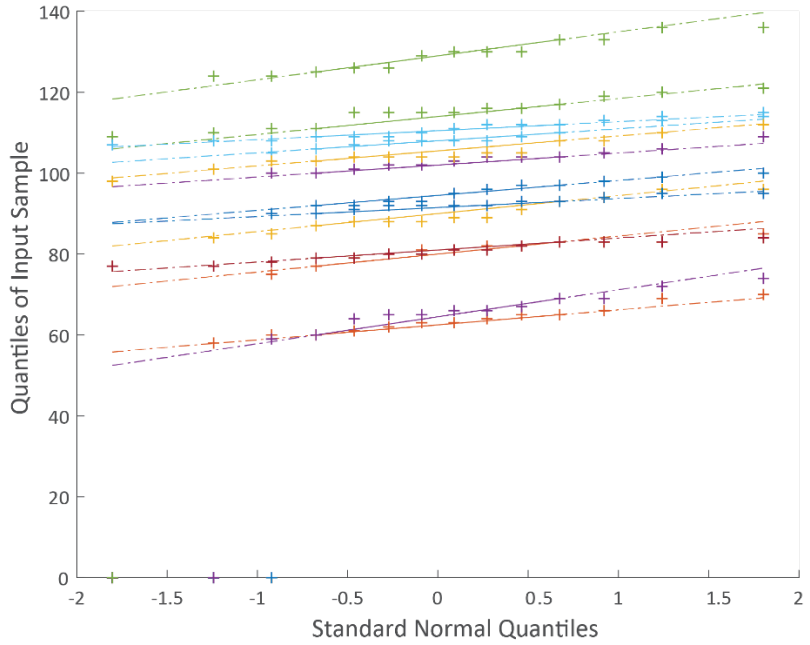

Figure S2. Quantile-quantile plot of the quantiles of the sample data (e.g. 13 experimental runs) versus normal distribution of the sample. The theoretical quantiles values from a normal distribution. The approximately straight lines indicate that the distributions of our datasets are normal.

Supplementary Table 3. Adjusted p-values from 2-way ANOVA test with Tukey correction for multiple comparisons. The p-values were graphically reported as * p ≤0.05, ** p ≤0.01.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1		**	**	**	**	**	**	0.4644	**	**	**	**	**
2			**	**	**	**	0.999	**	**	**	**	**	**
3				**	**	**	**	0.7438	**	**	**	**	**
4					**	**	**	**	**	0.9893	**	**	**
5						**	**	**	**	**	**	**	*
6							**	**	**	0.0682	**	**	0.9912
7								**	**	**	**	**	**
8									**	**	**	**	**
9										**	0.6262	**	**
10											**	**	**
11												**	**
12													**
13													

The analysis of variance (ANOVA) is a procedure meant to assign variance to different sources and to decide whether the variation arises within or among different population groups. ANOVA is therefore used to determine the validity of a model in relation to experimental data. The coefficients of the model are related to the factors targeted by the set of experiments and are then varied during the experiments. The residue is due to unidentified factors that are not controlled and thus have changed during the experiments.

For each factor, the total variance of the observations is given by the total Sum of Squares (SS), which is the sum of squares of the deviations of all the observation y_i from their mean y_m. SS can therefore be quantified as $SS = \sum_{i=1}^{n}(y_i - y_m)^2$.
The Degree of Freedom (DoF) represents for each factor the number of values (or levels) that are free to vary in one experiment. Having chosen here a Doehlert design of experiment, each factor assumes a precise value in each experiment. Therefore the number of DoF for each factor is 1.
The Mean Square (MS), gives information about the total variance of the observations taking into account the DoF. It is therefore defined as $MS = {SS}/{DoF}$.
For each factor, the Fisher ratio (F) is the ratio between the MS and the MS of the error, or residue. The *p*-value is the probability to obtain a given F by chance. The smaller the *p*-value, the higher is the significance of the considered factor. The standard criteria for accepting a factor as significant is typically $p < 0.05$.

References

1. Ziegler JG, Nichols NB. Optimum Settings for Automatic Controllers. J Dyn Syst Meas Control. 1993 Jun 1;115(2B):220–2.