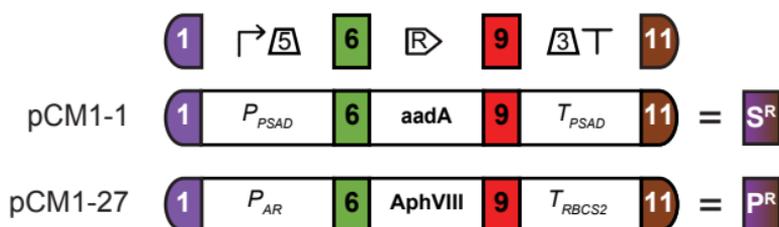


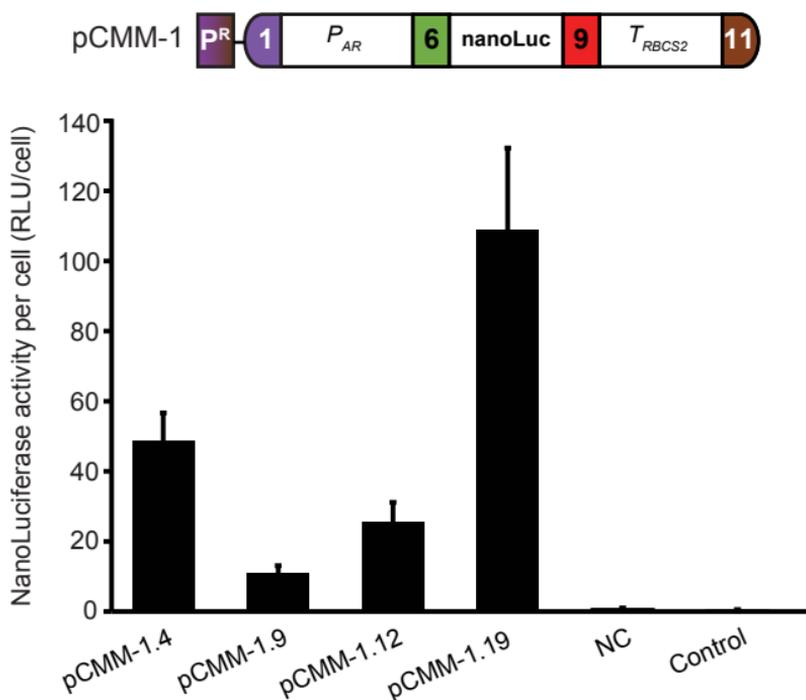
**Supplementary Figure 1. MoClo assembly workflow reflecting the abstraction hierarchy.**

We followed the original MoClo syntax<sup>22</sup> updated<sup>30</sup>. After design of a gene part controlled in silico for full compatibility until level M assembly, the part is cloned with *Bpil* (equivalent to *BbsI*) into the appropriate level 0 plasmid (Spectinomycin bacterial resistance). After quality control (QC) by restriction and sequencing, the resulting clone is registered in the part database as a pCM0-xxx (where pCM stands for plasmid Chlamydomonas Moclo). To generate the desired Transcriptional Unit (TU), the compatible parts are assembled with *BsaI* into the appropriate level 1 plasmid (Ampicillin bacterial resistance). After QC by restriction, the clone is registered as pCM1-xxx in the module database. Finally, to assemble a device, up to 6 modules at a time are assembled with the corresponding end-linker<sup>22</sup> by *Bpil* into a level M or 2 plasmid (Spectinomycin or kanamycin bacterial resistance, respectively). After QC by restriction, the clone is registered as pCMM-xxx in the device database. New assembly from this device can be performed to assemble more modules to the device<sup>22</sup>. Parts are represented in SBOL2.0 visual code<sup>69</sup> (see also Fig. 1).

a.



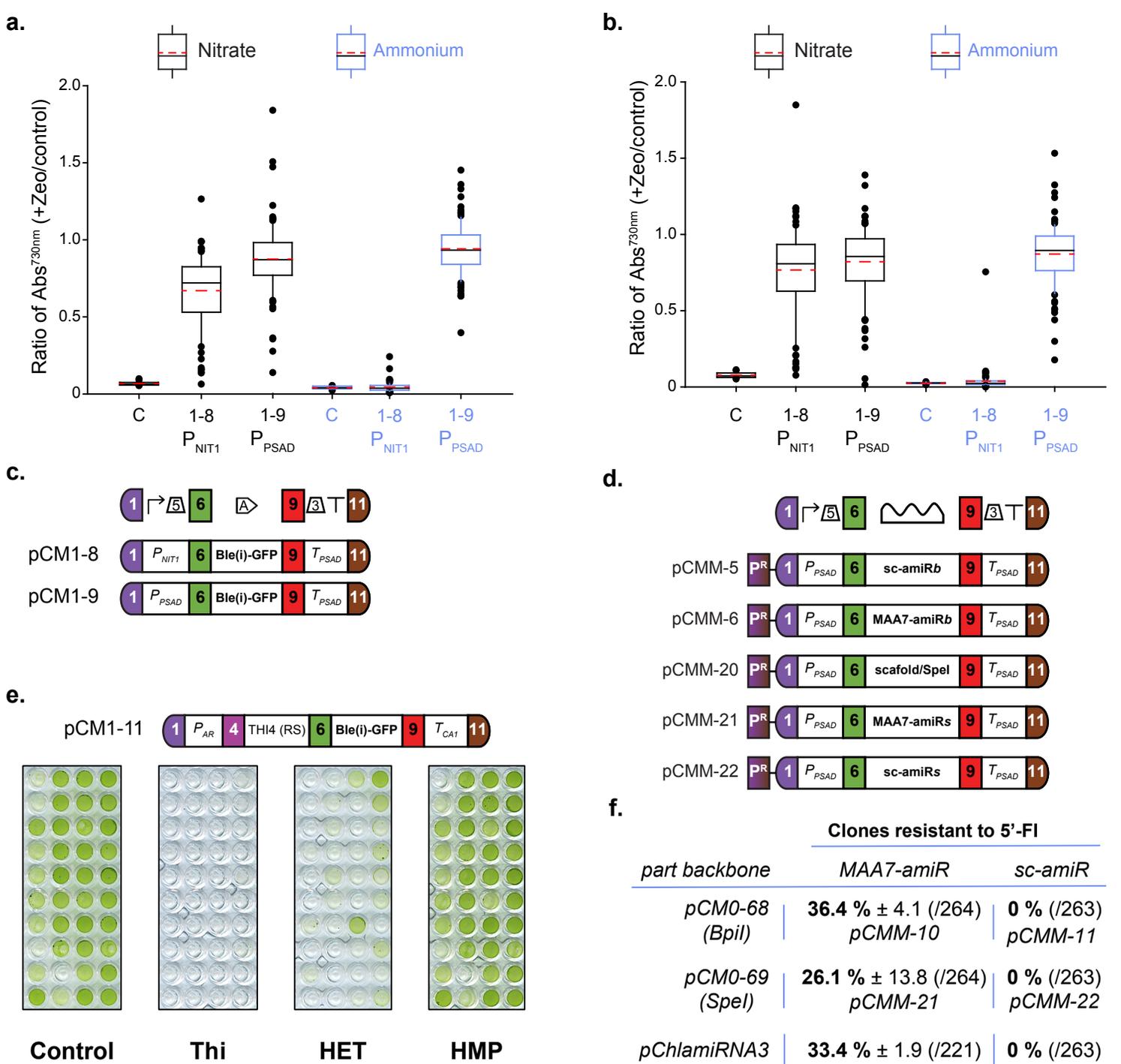
b.



### Supplementary Figure 2. Variability of NanoLuc expression in pCMM-1 transformants.

a. Schemes of the spectinomycin ( $S^R$ ) and paromomycin ( $P^R$ ) resistance modules.

b. NanoLuc activity averaged ( $N=3$ , mean  $\pm$  SEM) per line and expressed as RLU (Relative Luminescence Unit) per cell (same data as in Fig. 3b) of four independent NanoLuc-expressing transformants, one non-expressing clone (noted NC) and the recipient strain (CC-4425, noted control).



### Supplementary Figure 3. Control of gene expression, complementary data.

**(a-b)** Results from the two additional transformation assays performed independently from that presented in Fig. 4a. Control of gene expression by the nitrogen source. Zeocin resistant colonies (*Ble*) selected after transformation of CC-1690 cells by each of the two represented modules (“1-8” stands for pCM1-8 and “1-9” for pCM1-9,) were grown in TAP-nitrogen ± zeocin supplemented with either 7.5 mM (NH<sub>4</sub>)Cl (ammonium, blue) or 4 mM KNO<sub>3</sub> (nitrate, black) and their growth was followed (absorbance at 730 nm). The plot shows the ratio between growth in the presence and absence of zeocin (C is non-transformed CC-1690). Results presented (N = 16 for CC-1690 “C” and N=86 for all others) correspond to two out of three independent transformations (the other is shown in Fig. 4a). The box and whisker plots show the 10th (lower whisker), 25th (base of box), 75th (top of box) and 90th (top whisker) percentiles. The line within the box is the median, the dashed red line is the mean. Outliers are plotted as individual data points.

**(c)** Modules used to generate data presented in figure 4a and in **a** and **b**.

**(d)** Modules used to generate results presented in figure 4d and in **f**.

**(e)** Module used to generate results presented in Figure 4c (top) and image of the cultures used to generate these data. Cultures in TAP+zeocin supplemented or not (control) with 10 μM of thiamine (Thi), 10 μM of 4-methyl-5-(2-hydroxyethyl) thiazole (HET), or 10 μM of 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP).

**(f)** Percentage of transformants resistant (± SD) to the metabolic drug 5-fluoroindole (5'-FI, used at 20 μM). The CC-1690 strain was transformed with devices (indicated under each result, design in d) containing the amiRNA sequence targeting the *MAA7* gene or a scrambled sequence as a negative control, cloned into the amiRNA backbone with *Bpil* or *SpeI*, corresponding to parts pCM0-79 and pCM0-80, respectively. The pChlamiRNA3 construct containing the same miRNA backbone was used as a positive control. Average of three independent transformations, the total number of transformants screened for 5'-FI resistance in each experiment is indicated in parenthesis.