Reply to 'No beneficial fitness effects of random peptides'

To the Editor — We agree that Knopp and Andersson[1] (as well as Weisman and Eddy[2]) make a valid point that we had not considered in our original analysis. We have repeated some growth experiments with the pure vector with and without isopropylthiogalactoside (IPTG), as described in ref. [1]. We can confirm that the majority of the parallel runs show that cells harbouring the vector grow more slowly under IPTG induction, but we see also some variation.

Our experimental set-up was designed to identify the fraction of random sequences (RNA or protein) that could have any biological effect. Searching for frequency changes in the mixture of clones under conditions in which the RNAs/peptides are expressed thus remains a valid approach. Hence, our general results, namely that a large fraction of random sequences has bioactivity, is undisputed. But in light of the results above, the question of the fraction of clones with positive fitness effects is not satisfactorily answered. For a number of clones the positive effect could indeed be due to a double negative effect, that is, an inhibition of a deleterious effect of the vector to the cells[1,2]. However, the range of positive effects for different clones is broad. For example, the results of the deep sequencing experiment shown in Fig. 3 of our paper[3] indicate that at high sequencing depth a group of positive clones comes up that has a much higher fold change than the other positive clones. Among the three clones studied in further detail[3], clones 4 and 32 belong to the latter class, while clone 600 belongs to the class with high fold change.

Table 1 summarizes the growth increase for these clones across the four cycles of the experiment, together with inferred selection coefficients. Clones 4 and 32 have selection coefficients (s) of 0.14 and 0.16 respectively, which correspond to the vector effects described in ref. [1] ($s = 0.12–0.14$). However, clone 600 has a much higher selection coefficient ($s = 0.45$), which could not be explained by the vector effect alone. Knopp and Andersson report in their validation experiment that clone 600 did not have an effect at all, which is in contrast to the highly repeatable effect in our experiments. Hence, this suggests that the experimental conditions are not fully comparable and that further experiments will be required to understand this discrepancy. This observation implies also that the measurement of the possible vector effect will require deeper analysis.

Table 1 | Growth increase and selection coefficients

Clone	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Selection coefficient
PEPNR00000000004	1.00	1.87	2.40	3.70	0.14
PEPNR00000000032	1.00	1.95	2.82	4.51	0.16
PEPNR00000000600	1.00	1.33	6.67	41.50	0.45

Average numbers of reads were calculated for each cycle from the read table of experiment 7 (available in Dryad https://doi.org/10.5061/dryad.6f356). Read numbers were then normalized to the read numbers of cycle 1, that is, the values of cycles 2–4 show the relative increase compared with cycle 1. The selection coefficient is calculated based on the fold change in cycle 4 and the assumption of a total of ten cell divisions between cycle 1 and cycle 4, analogous to ref. [1].

We note that in a recent study on the effects of random peptides on *Arabidopsis* development[4], the authors found that one of three peptides analysed in detail had an early flowering phenotype, which could also be interpreted as a candidate for a positive fitness effect. Hence, although beneficial mutations are rare in mutation accumulation lines[5], the introduction of a complete new molecule into the cell may have a higher likelihood of having a beneficial effect. It has been shown that random sequences can convey a specific function (adenosine triphosphate binding[6] and nickel tolerance[7]), although only at a very low frequency. However, in our (and the *Arabidopsis*[4]) experiment, we are not targeting only a single function, but any possible interaction within the cell. ❐

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Competing interests

The authors declare no competing interests.