How to make a fluorescent sensor?

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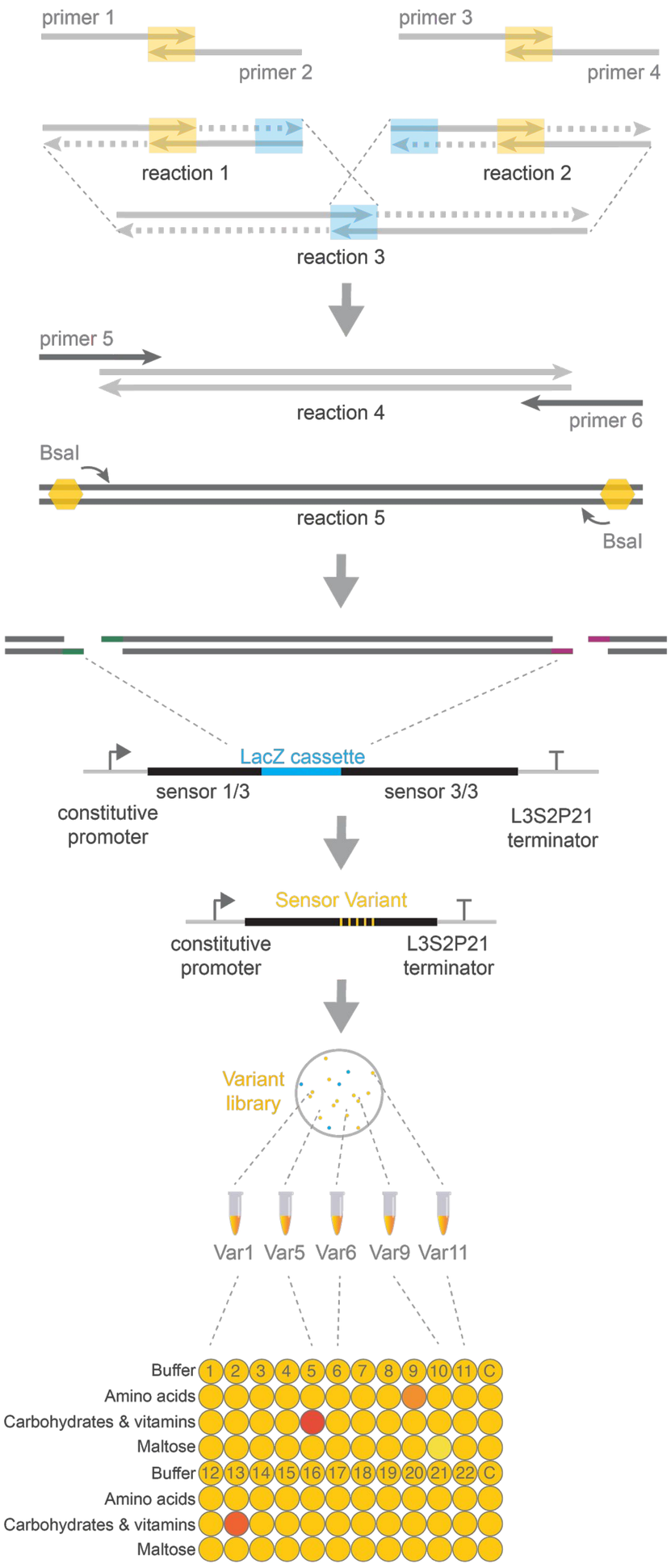
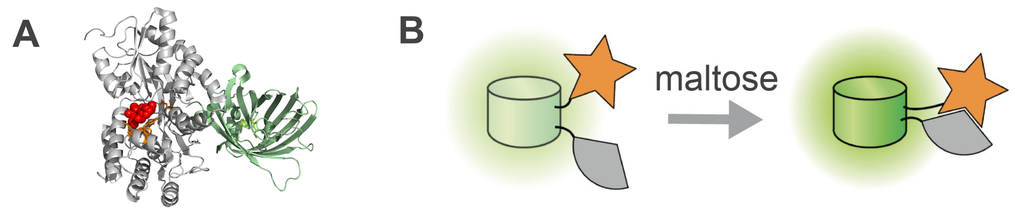


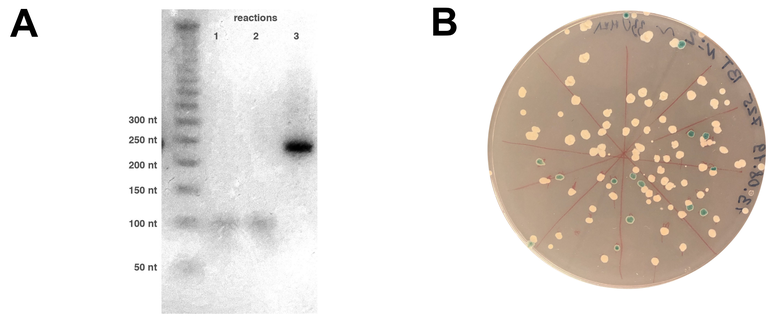
Fluorescent sensors are proteins that glow upon binding of specific chemical compounds. It is not easy to create such indicators but as they allow to detect chemicals *in vitro* and *in vivo,* they are valuable cell biology tools.

We set out to reconfigure the maltose sensor to make it bind other substrates.



Crystal structure (A) and mechanism of action (B) of maltose sensor

We selected five positions at the **maltose binding site** (E111, E153, P154, Y155, F156) to **introduce random amino acids** and test whether or not sensor specificity would change.

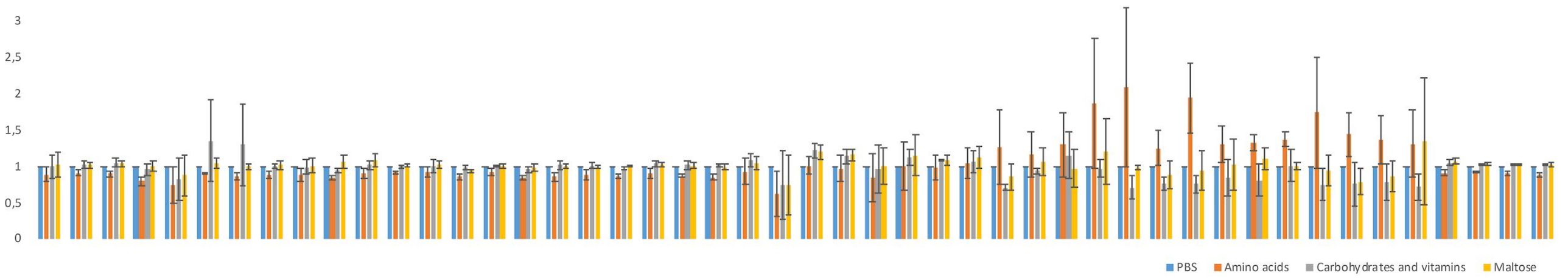


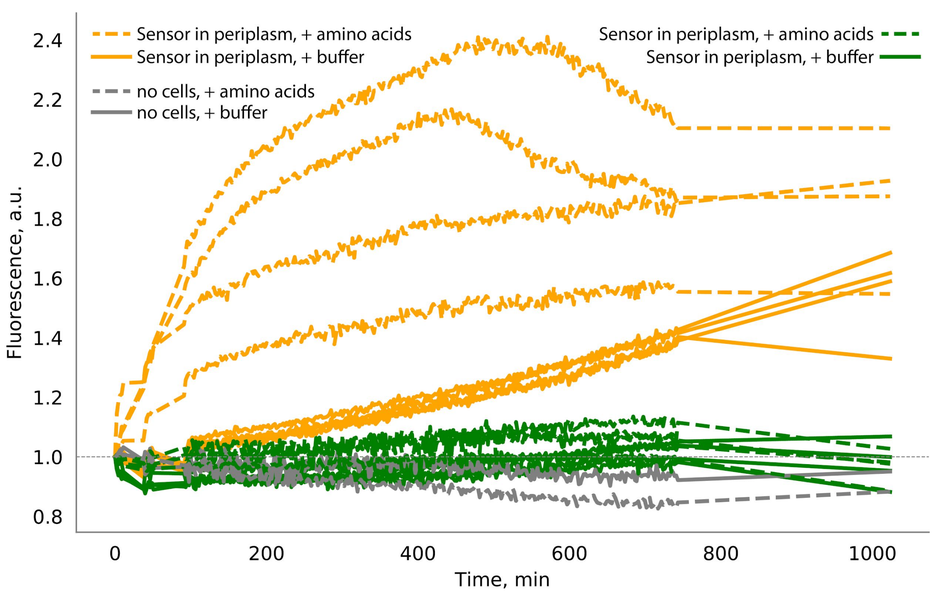
Gel electrophoresis of synthesized maltose sensor gene fragments. E.coli colonies grown after transformation with plasmid library.

We **synthesized a library of variants** of gene coding for maltose sensor. We cloned the library into two *E. coli* expression vectors. We used blue-white screening to select colonies carrying plasmids with correct inserts. In one of the vectors, sensor variants were produced in the **cytoplasm** while the other one directed proteins to the **periplasm**.

Have mutations changed specificity of the maltose sensor?

We tested performance of 100 mutant variants against a mixture of various carbohydrates, vitamins, amino acids and maltose. None of the variants we tested reacted to addition of maltose, carbohydrates and vitamins. However, addition of amino acids resulted in increase of fluorescence 1.5-2.0–fold in some variants.





Interestingly, addition of amino acids to bacteria expressing periplasmic seensors triggers an increase in fluorescence intensity that lasts for several hours. The nature of this phenomenon is unclear, but may be related to the *E. coli* physiological response to amino acid influx.

Conclusions

We identified variants of maltose sensors with specificity towards amino acids. Their development towards amino acid sensors should rely upon further testing of purified protein samples against individual amino acids.

We identified an unknown effect of amino acids increasing brightness of fluorescent proteins expressed in periplasm.

The method we tested allows obtaining reproducible data on fluorescent sensors.