Automation of *E.coli* cell based assay for CRE1/AHK4 receptor characterization

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Abstract (200 words or less)

Histidine kinase CRE1/AHK4 is a plant hormone receptor providing cytokinin signalling across the membrane structures in the plant cell. Functional studies of histidine kinases are crucial for understanding many signal processes in plants as well as in prokaryotes where these signalling systems dominate. Possibility of expression of CRE1/AHK4 receptor in *E. coli* allows studying responses of this receptor to various potential ligands. Artificial signalling pathway in *E. coli* depending on the receptor function leads to expression of reporter gene encoding enzyme beta-galactosidase. Beta-galactosidase is able to hydrolyse an artificial substrate to form a measurable product. The amount of formed product is proportional to the amount of added hormone. We used liquid handling robotics to automate and miniaturise an existing method [1] for response measurement. Advantages of the method are lower consumption of all solutions and fast, walk away setup. The performance of the method was examined and compared with current state of the art solution. Using the improved method we studied response of the CRE1/AHK4 receptor with a several ligands in the 384-well microtitration plate. We confirmed usability of the method and obtained new data for further receptor functional studies.

[1] *Spíchal L., et al. (2004): Two Cytokinin Receptors of Arabidopsis thaliana, CRE1/AHK4 and AHK3, Differ in their Ligand Specificity in a Bacterial Assay, Plant Cell Physiol. 45(9): 1299–1305*