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Technology Offer

Method for cell-free protein synthesis assays or other fluorescent assays in the context of cell-free protein synthesis

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An improved method to perform cell-free protein synthesis (CFPS) assays or other fluorescent assays in the context of CFPS with very low reaction mix volumes. Various additional advantages allow to significantly reduce costs and time.

Background

Cell-free protein synthesis, also known as *in vitro* protein synthesis or CFPS, uses the biological machinery of a cell without being confined with a living cell. As the *in vitro* synthesis environment does not require a cell wall or homeostasis conditions to maintain cell viability, CFPS comes with many advantages such as direct control and monitoring of reaction conditions, facilitated expression of cytotoxic proteins, incorporation of unnatural amino acids or co-translational solubilization of membrane proteins. Crucial components of the cell-free reaction include amino acids, a DNA or RNA template encoding for the desired protein, ribosomes, tRNA and an energy source.
In the context of CFPS, fluorescent assays are often used for fluorescent protein detection. To detect the amount of synthesized target protein, the protein of interest is encoded together with a fluorescent protein or subsequently labeled with a fluorophore to facilitate analysis.
The assays are usually performed in standardized plate formats, such as 96 well plates, that carry a high reaction volume per well and are therefore associated with high costs.

Technology

Our scientists have developed an improved method to perform CFPS, providing the following advantages:

- The design of the multi-well plate requires reaction mix volumes of 20 µl or less with the option of volumes as low as 3 µl.
- The outline of the plate also allows the introduction of one or more biochemical factors that can form an incremental gradient across multiple wells following a predetermined function.
- Using more than one biochemical factor, the introduction can also follow different functions, therefore allowing the formation of gradients across different matrices.
- The multi-well plate may also comprise dialysis membranes between the wells to allow diffusive exchange of molecules from within the cells.
- In additional steps, freeze-drying of the fluid after the introduction and reconstitution is possible which further streamlines and simplifies the usage of the assay. This step also enables the provision of a gradient of biochemical factors in advance of the experiment, allowing for a rapid testing of processes for optimized assay conditions as well as simplifies assessment of protein yield.
- Sealing of the wells using a transparent cover brings the liquid in contact with both well bottom and cover and hence reduces evaporation of the small reaction volumes. The method may also include the use of software to analyzing the protein yield in the wells.

Taken together, the method allows for significantly reduced costs and time when performing cell-free protein synthesis assays and/or fluorescent-assays in context with cell-free protein synthesis.

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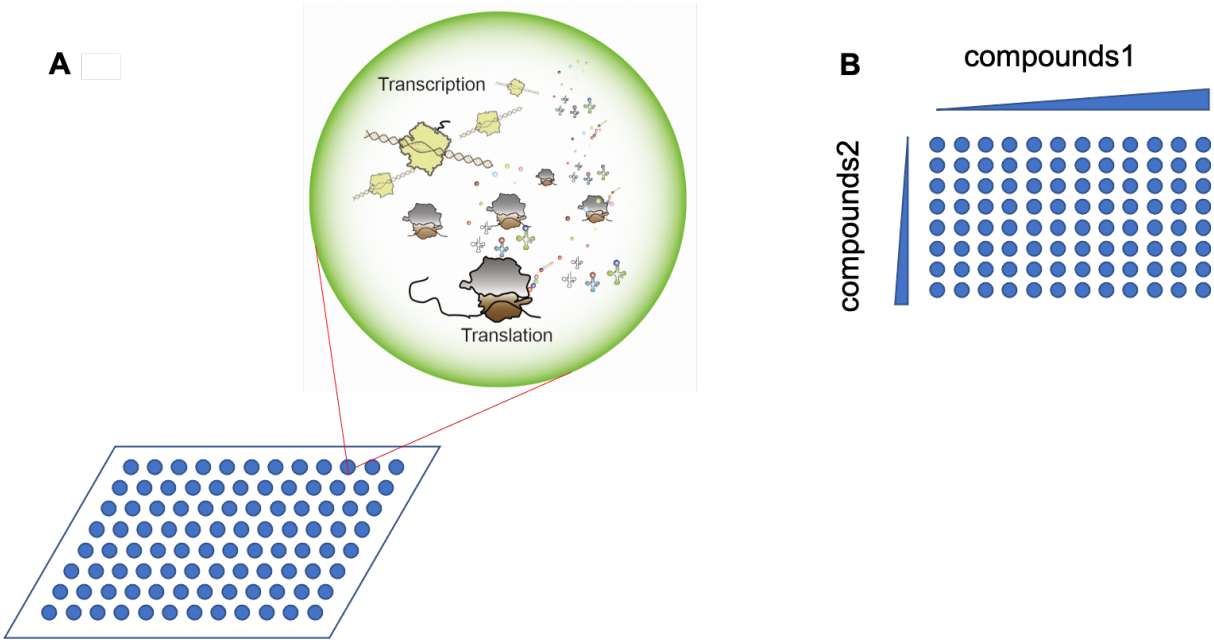

(A) Multi-well plate with cell free protein synthesis reaction in less than 3 µl
(B) View from above of a multi-well plate with concentration gradients of two different compounds

We are now looking for either a licensing partner, or a collaboration partner to further develop this project.

Patent Information

PCT application was filed on 01.02.2021 (EP21/052299)

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