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Microfluidic-free encapsulation of bacteria for isothermal DNA amplification

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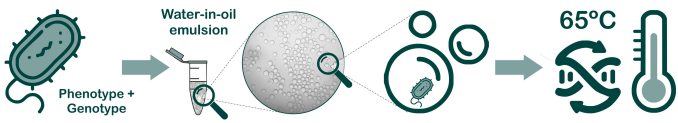
Linking genotype (a nucleic acid that can be replicated) and phenotype (a functional trait, such as a binding or catalytic or regulatory activity) in a unique compartment is a key aspect of protein functional analysis, because emulates the natural genotype-to-phenotype linkage that results from the compartmentalization of genes in cells. This connection is easy to obtain when the number of samples is below 100, but when the number of samples rises to the order of millions, a technical problem appears. A solution to this limitation is the miniaturization and parallelization of the process, associated with the formation of aqueous nanocompartments, like aqueous droplets in oil phases. This methodology converts the analysis into ultra-high-throughput, since is possible to obtain compartments of small sizes as 100 µm and volumes of nearly 1 nanolitre. This high capacity (>1010 droplets in 1 ml of emulsion), the ease of preparing emulsions and their high stability over a broad range of temperatures, pH and salt concentrations, makes this methodology ideal for compartmentalizing biochemical and genetic assays. In this sense, the nanocompartmentalization of bacteria is an optimal tool for in vitro evolution analysis, with the only condition that it is possible to confine a single bacteria per compartment. To do this in a conventional system the water-in-oil emulsion formation parameters, must be considered. In this study, parameters like bacterial DO600 and vortex speed where evaluated in order to obtain particles of average size of 100µm containing a bacteria or none inside. Bacterias expressing a DNA polymerase were used to study the isothermal DNA amplification inside these particles, by DNA recovery and fluorescence detection. Here we also report on preliminary studies towards microfluidic generation of microdroplets. A PMMA chip with a cross-junction was used to obtain W/O emulsion, with fluids fed by syringe pumps.



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