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Development of a coupled assay for the ultrahigh-throughput screening of 2'-deoxyribosyltransferases using droplets microfluidics

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PURPOSE OF THE ABSTRACT

Nucleoside analogues are an important class of compound with antiviral, anticancer, or antibacterial activity. Due to the growing environmental concern, nowadays, the enzymatic synthesis of these compounds is gaining ground over the traditional chemical methodologies in the pharma industry. In this contest, one of the enzymes that can be exploited for the synthesis of these non-natural compounds are 2'-deoxyribosyltransferases (NDTs) [1].

Although in recent years many NDTs have been described capable of synthesizing some of these nucleoside analogues, their catalytic efficiencies are far from those shown towards natural substrates, rendering their industrial application not economically attractive.

In this regards, new enzymes with improved activities towards these non-natural substrates are needed.

Nowadays, there are two main direct route that can be followed to identify candidate proteins with improved activities towards desired substrates: the screening of naturally occurring enzymes by functional metagenomics or the screening of man-made variants by directed evolution.

In both cases, the success of screening campaigns relies on the fraction of genetic diversity that can be sampled and analysed.

Microfluidics, allows the creation and use of picoliter water-in-oil (w/o) droplets as miniature compartments for enzyme assays that can be screened at ultrahigh-throughput (uHTP) resulting in a concomitant reduction of screening time (>107 per day), reagent consumption (50 µL per library) and costs (10€/library) [2].

In our study we present the development and optimization of a transferase/oxidase/peroxidase fluorogenic coupled assay to perform uHTP screening of 2'-deoxynucleotidyl transferases activity in droplets [3].

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

nucleosides | microfluidics | coupled assay

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