Picodroplet Mass Spectrometry for Miniaturized High Throughput Analysis of Synthetic Biology Microbial Clones


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Introduction

We report the progress towards a high throughput, lab-on-a-chip single analysis methodology for characterizing synthetic biology clone libraries. Recent advances in DNA assembly have greatly improved a synthetic biologist’s ability to design and efficiently build multiple gene pathway libraries in a combinatorial fashion. The large number of strains and permutations in these libraries leads to an enormous demand for high-throughput analysis methods to discover the desired phenotype(s). Mass Spectrometry (MS) is a powerful and high throughput screening method. However, standard MS (e.g., electrospray ionisation, ESI) suffers from drawbacks (e.g. sample preparation for droplets, matrix effects, and low throughput). A current gold standard method, Agilent’s RapidFire MS system can screen one sample every 1-7 seconds, which equates to 10-12 samples per 24 hour day. One of the major drawbacks of microplates (MTP) based screening is the high volumes of reagents required for phenotypic screening of large synthetic biology libraries. Picodroplets are a new, lab-on-a-chip based technology, involving the use of ultrahigh flow rates and generation statistics to analyse large numbers of samples per day. Picodroplets have recently developed a high throughput ESI-MS system based on its proprietary picodroplet technology (1), which can test up to 200,000 biomolecular samples/day. With the development of this tool, synthetic biologists will now be able to true fulfill the Design – Build – Test – Analyse cycle. Thus, they can discover the best engineered clones for their specific purpose, e.g. green manufacture of chemical feedstocks, biofuels, high value chemicals, enzymes, food additives, colourants, fragrances, plant protection organisms and more novel medicines. An overview of picodroplet workflows for synthetic biology is outlined below.

Encapsulation of bacteria in Picodroplets

As all bacteria is a droplet, picodroplets can be used to store them, as any bacteria found on the surface of the droplet would affect the MS spectrum. In the picodroplets, the bacteria are encapsulated. These ‘live’ picodroplets also contain a unique mass tag to distinguish them from the mass spectrum from the bacteria free droplets. Sampling the live picodroplets in the MS and storing the bacteria enables them to be used in subsequent experiments. The bacteria are encapsulated in the picodroplets within a water in oil emulsion, which is then dispersed into a microfluidic channel. The picodroplets will exit the microfluidic channel at the outlet of the microchannel. The picodroplets will then be directed into the MS separator. The bacteria can only be captured in the EIC channel. The other channels are air laden with air laden droplets. The bacteria will then be directed to the MALDI target. The bacteria will then be ionised and the ES mass spectrum will be measured. The bacteria can now be retrieved from the MALDI target by changing the ionisation mode to negative ions from positive ions. The bacteria can then be used in other experiments. There are a few examples of encapsulated bacteria in the literature. In briefly, it shows that these bacteria that can be captured by the EIC channel.

Optical & MS Coding Strategy 

The optical & MS coding strategy involves the optical and MS components. The optical code is a sequential number that is used to help the software identify and extract the optical data. The optical code is comprised of five digits. The first digit is the picodroplet number, the second digit is the picodroplet size, the third digit is the picodroplet type, the fourth digit is the picodroplet presence, and the fifth digit is the picodroplet absence. Each data point represents one scan. The optical code will be used to identify the optical data. The MS code is a sequential number that is used to help the software identify and extract the MS data. The MS code is comprised of five digits. The first digit is the MS channel, the second digit is the MS polarity, the third digit is the MS mass range, the fourth digit is the MS mass range, and the fifth digit is the MS mass range.

References & Acknowledgements

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