SAMPLE PREPARATION FOR SINGLE-CELL WHOLE CHROMOSOME ANALYSIS

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ABSTRACT

In this work we present an integrated system for whole chromosome analysis of single bacterium. Using whole genome barcoding techniques, which offer direct and rapid microscopic visualization of the entire genome in one field-of-view, we aim to rapidly identify individual bacterium. We are developing our device to achieve the crucial, and difficult process of isolating a bacterium, removing the DNA in one piece and transferring it to a nano-channel for visualisation. In order to achieve control over the bacteria we encapsulate them in agarose, using flow focusing. The encapsulated bacteria can then be transported in microchannels to proximity with the nanochannels and then chemically lysis can be performed. Following lysis the intact genome can be extracted and transferred to the meandering nanochannel for analysis. We believe this device holds the potential to significantly decrease analysis times for single cell, whole genome analysis with the potential of opening up for automated, high-throughput genome analysis in microfluidic systems.

KEYWORDS

Melting mapping, bacteria encapsulation, chromosome analysis, nanochannels

INTRODUCTION

The recent advent of whole genome barcoding techniques [1-3] applicable to use together with direct visualization of DNA in nanofluidic devices [4, 5] opens up for a range of applications in medicine and biology based on the characterization of large-scale genomic rearrangements and identification of pathogens. One important roadblock in this context is sample preparation. Here we present the development of a comprehensive system to extract intact chromosomal DNA from individual bacteria, and the transfer of this DNA to nanochannels for characterization.

EXPERIMENTAL

The bacteria are encapsulated in low-melting point agarose droplets, formed using flow focusing [6], see Figure 1 A and B. We use a fluorinated oil with added surfactant (Picosurf™) as the continuous phase and keep the drop-forming device at a temperature of 40°C, which ensures that the agarose remains melted and with a viscosity that facilitates drop formation. The temperature is subsequently lowered and the gelified agarose droplets are extracted from the oil phase. Extraction is performed using a sugar solution with a density less than the oil, which 1.9 g ml^{-1}, but greater than the agarose which is added on top of the agarose/oil emulsion. The agarose capsules cream to the top of the sugar solution and can then be collected and washed. Figure 1 C shows encapsulated, GFP expressing *E. coli*.

Lysis was performed using a DNA isolation kit (PureLink) from Invitrogen containing proteinase K and RNase A. After transfer of the bacteria-containing capsules to 0.5x TBE buffer, DNA was extracted using an applied DC field at 7.5v cm^{-1} as shown in Figure 1 D.

DNA can then be transported into a meandering nanochannel, designed to maximize the length of DNA that can be imaged simultaneously. Each meander can hold 6 Mbp of DNA stretched to 50% extension (1 mm), which can then be imaged within one field of view. Figure 1 D shows an entire chromosome (5.7 Mbp) from *Schizosaccharomyces pombe* stained with YOYO-1 at a concentration of 1 dye molecule per 6 bp. Any fragments from the lysis, large enough to clog the nanochannel, remain trapped within the agarose capsules.

The final step of the procedure is to perform a whole genome barcoding [1-3] for identification. Figure 1 F shows a 1Mbp segment of *S. pombe* stained with YOYO-1 to a ratio of 1 dye molecule to 6 bp subjected to melting mapping [2].

RESULTS AND DISCUSSION

At present, encapsulation, lysis, DNA extraction and genome barcoding have been optimized separately with the results shown in Figure 1. We are currently working to integrate these steps into a single device with pathogen-in/genome-barcode-out functionality. The difficulties are those of fabricating large-scale microchannels and nanochannels in the same device, of transporting fluids between channels of such disparate scales and also of temperature control for both the encapsulation and the meltmapping steps. We aim to present our progress towards this goal at the conference.

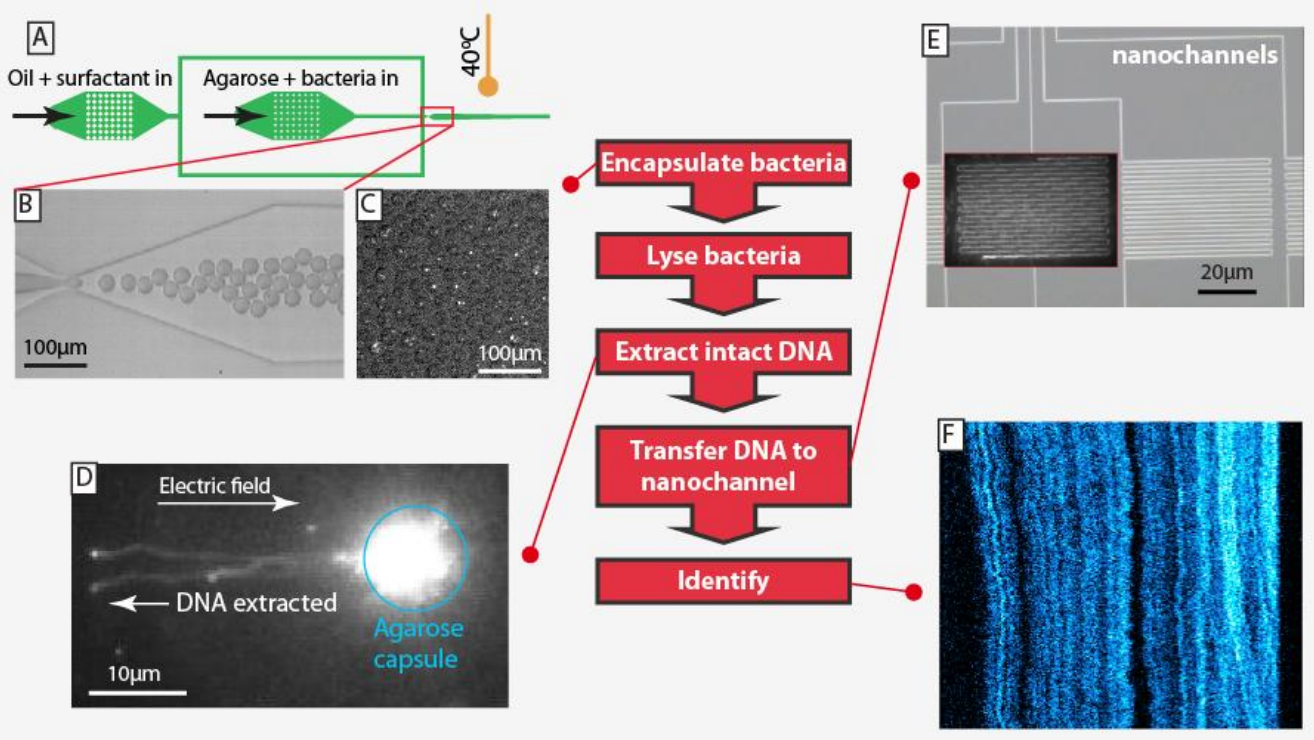

Figure 1: (A) A microfluidic device is used to create agarose drops in oil. (B) Bacteria (in this case E. coli) are encapsulated in the agarose phase of the emulsion. (C) After gelification the bacteria can be lysed. Most of the cell debris is trapped in the agarose capsule but the DNA can be extracted using electrophoresis, as shown in (D). The intact DNA can be transferred to a nanochannel (this image shows S. pombe) (E) where it is linearized and can be imaged using for example meltmapping,(F) which again shows S. Pombe.

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