AGAROSE DROPLET MICROFLUIDICS FOR HIGHLY PARALLEL AND EFFICIENT EMULSION PCR

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ABSTRACT

An agarose droplet method was developed for highly parallel and efficient single copy emulsion PCR. By capitalizing on the unique thermal-sensitive sol-gel switching property of agarose, PCR is efficiently performed in agarose solution droplets, which is gelated to agarose beads after PCR to capture amplicons to maintain the monoclonality of each droplet. This method allows high throughput generation of uniform droplets and enables high PCR efficiency, making it a promising platform for many single copy genetic studies.

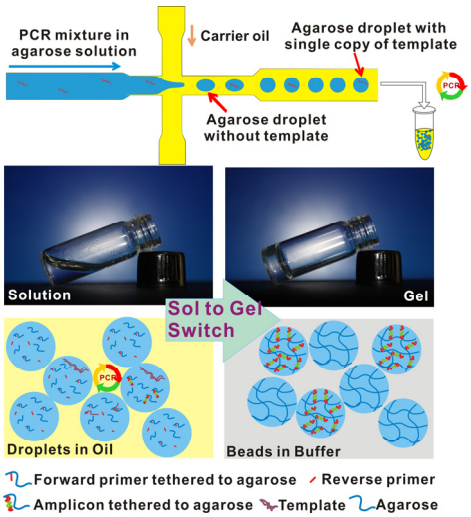
KEYWORDS: Emulsion PCR, Agarose, Hydrogel, Droplet, Genetic analysis

INTRODUCTION

Emulsion PCR (ePCR) for single DNA molecule amplification have received increasing interest because they offer the advantage of massively parallel colonal amplification of DNA templates, which allows the identification and quantitation of rare mutant gene within large populations[1-2] and enables new generation of ultra-high throughput DNA sequencing technologies[3-4]. However，traditional emulsion PCR methods suffer from some fundamental limitations because they rely on polydispersed droplets as reactors and microbeads as amplicon capturing matrix [5]. Large size variation of droplets leads to inefficient and nonuniform amplification of long DNA amplicons and the use microbeads causes poor PCR efficiency and limited amplicon length due to the steric hindrance effect and charge repulsion on the solid surface. To address these challenging problems, we have developed an agarose droplet microfluidic method for highly parallel and efficient emulsion PCR. This method allows high-throughput generation of uniform droplets and enables high PCR efficiency, making it a promising platform for many single copy genetic studies.

DESIGN

Scheme 1 shows our agarose emulsion droplet microfluidic method. We use microfabricated emulsion generator to produce highly uniform monodispersed nanoliter emulsion droplets of an agarose solution with PCR reagent in carrier oil. The droplet generator consists of a microfabricated nozzle for controlled injection of a stream of agarose solution into a sheath oil flow. By adjusting the relative flow rates, precise control of the emulsion droplet size can be achieved. DNA template molecules are introduced into the PCR mix in a statistically diluted concentration so that on average there will be no more than one template in one droplet. During PCR process, agarose remains in liquid phase, where PCR can take place with high efficiency. After PCR amplification, solution form of agarose droplet can be switched to solid gel phase by simply cooling the solution below gelling point. Once solidified, agarose beads will remain solid unless the temperature hits above 56°C. As a result, DNA products amplified in the droplet will remain its monoclonity even after oil phase is removed. The polony beads can be used for downstream sequencing or genotyping applications.



Scheme 1: Schematics of the agarose emulsion droplet microfluidic method for single copy genetic analysis. Statistically diluted templates are encapsulated into uniform nanoliter agarose-in-oil droplets, which are then thermally cycled for PCR amplification. Droplets with DNA template will produce amplicons physically attached to agarose matrix after PCR.

EXPERIMENTAL

Emulsion generator was constructed from two glass wafers. The glass channels were rendered hydrophobic with octadecyltrichlorosilane (OTS) treatment. The droplet PCR cocktail was prepared by thoroughly mixing 2% agarose, 0.1 U/μL Hot-start Taq polymerase, buffer, 0.2 mM dNTPs each, 0.4μM primers (5'-agarose-(C18)2-TAAGCACGAACTCAGCCAGAACGA-3', 5'-CAAGCTTTGCC ACACCACGGTATT-3') amplifying a 101bp sequence of lambda DNA fragment and specified amounts of template. The thermal cycling conditions were as follows: 94°C for 3 min, 25 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec, followed by a single final extension for 5 min at 72°C in a peltier thermal cycler. The amplified emulsions were solidified to agarose beads by cooling to 4°C. After removing the oil phase by acetone, isopropanol and deionized water sequentially, the agarose beads were stained by dispersing in 100 μL deionized water with 6 μL 20× SYBR Green. Agarose beads were then analyzed using a flow cytometer with a 488 nm excitation source.

RESULTS AND DISCUSSION

A microfabricated agarose droplet generator that produces uniform, nanoliter-volume agarose emulsion droplets for genomic analysis was developed and evaluated. Uniform agarose droplets at the range of 50 to 200 μm were easily obtained by controlling the microfluidic-chip channel dimension, flow rates of aqueous or oil phase (Figure 1).

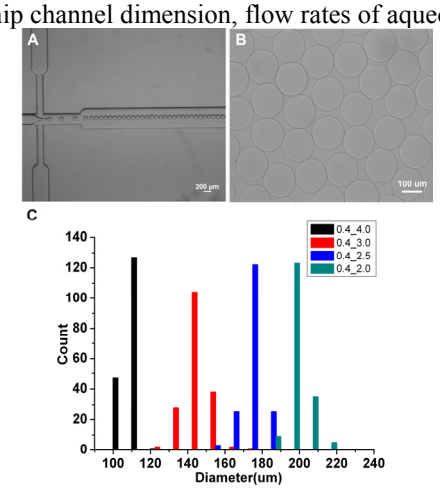


Figure 1：A) Generation of agarose droplet on chip. B) Agarose beads in water. C) Size distribution of agarose droplet with different oil flow rates (4.0 mL/hr~2.0 mL/hr) while keeping the flow rate of aqueous at 0.4 mL/hr.

Single copy DNA can be amplified efficiently in agarose droplets. Figure 2 shows FACS analysis images of agarose beads after amplification from template concentration of (A) 0 and (B) 0.5copy/droplet. Droplets with no DNA were low in fluorescence while droplets with 0.5 copy/droplet concentration showed a biomodal distribution. As indicated by FACS results for the sample with 0.5 copy/droplet，about 45 percent of the population was high in fluorescence, matching well with theoretical calculation. The results clearly indicate the capability of the method for single copy genetic amplifications. Further analysis indicated single copy DNA can be efficiently amplified (>90% amplification efficiency) in agarose droplets.

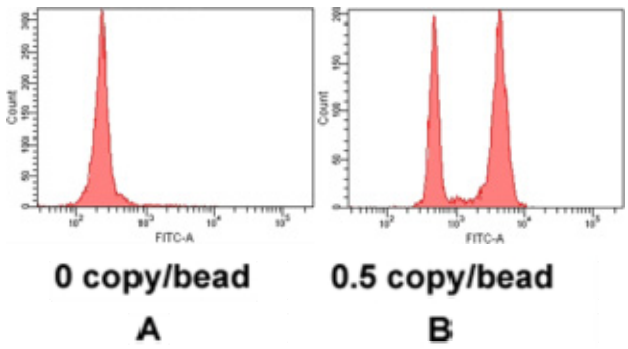


Figure 2: Results of single copy agarose droplet PCR. FACS analysis of agarose beads after amplification from template concentration of (A) 0 and (B) 0.5 copy/bead.

CONCLUSION

An agarose droplet method was developed for highly parallel and efficient single copy emulsion PCR in this study. Our agarose droplets significantly increase the efficiency of generating positive droplets because there is no need for compartmentalization of limiting diluted microbeads. Agarose droplets, once solidified, change to agarose beads, can be easily processed and used for downstream applications such as sequencing, FACS analysis or long term storage etc. The agarose droplet method reported here allows uniform, massively parallel, highly efficient monoclonal amplification and

holds great potential for a variety of applications such as single cell expression study, rare mutant detection, as well as next generation high throughput sequencing.

ACKNOWLEDGEMENTS

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