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**Title: "Droplet based Microfluidic Approach and Microsphere-PCR Amplification for single stranded DNA Amplicons"**

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**Editorial comments:**

C1. Please proofread; there are still some grammar and usage errors.

[Response] English edit certificate is attached.



C2. Abstract: “Corrected by simple pipetting” isn’t clear; are you referring to the centrifuging/removal of supernatant step to get rid of dsDNA? This is also unclear in the last paragraph of the Results.

[Response]

This sentence is now changed as shown in below (Line 30-31):

* “~~corrected by pipetting from”

The result part (Line 289~290) is now modified as shown in below:

* Therefore, ssDNA amplicons can be obtained by pipetting without centrifugation step.

C3. 1.1: Can you provide the CAD file as supplemental information, or at least provide a schematic with more details/dimensions? You mentioned a reference in your response to Reviewer 2 ("Integrated Microfluidic Selex Using Free Solution Electrokinetics"), but that does not appear to describe the same device.

[Response] We provided the CAD file. See supporting information.

C4. 5.1/6.1: By the following reagents, do you mean the reagents in Tables 4/6 (respectively)?

[Response] we put the Note (Line 181) information. Table 4 shows the reagent information for the asymmetric PCR. Table 6 describes the reagent for the microsphere-PCR.

C5. Please highlight 2.75 pages or less of the Protocol that you want filmed; this will enable us to write a script that will guide the filming of your procedure. 2.75 pages is our limit due to filming time and video length limitations.

[Response] The part of protocol is highlighted with yellow color.

C6. Figure 1: Please separate numbers and units in the Figure itself (e.g., 10 mm instead of 10mm).

[Response] We separate the numbers and units in the Figures.

C7. Figure 2: Please explain the schematics in the legend. What excitation/emission wavelengths are used for the microscopy here?

[Response] The figure legend for Figure 2 is now modified. Cy3 wavelength is described in 7.2 (Line 231).

* Figure 2. Fluorescent readout of DNA hybridization on the surface of microsphere. 5’-Acrydite-modified DNA probe (Ap) were capable of hybridizing with complementary Cy3 labeled DNA probes (cAp).

C8. Figures: ‘Figure 1/Figure 2/etc.’ are still in the Figures themselves; please remove and additionally remove excess whitespace.

[Response] Figures are now changed and attached with TIFF format.

C9. Tables: Please remove all embedded tables from the manuscript and instead upload each individually as .xls/.xlsx files to your Editorial Manager account (there is an option to upload a ‘Table’, distinct from the Table of Materials).

[Response] All tables are now removed from the manuscript and they are now upload each individually as xlsx files.

C10. Discussion: Please include more information to help future readers decide whether this protocol fits their needs and to help them replicate it--critical steps, common troubleshooting procedures, limitations (of the protocol presented here, not other ones), and future directions.

[Response] Discussion is now improved.

In addition to the editorial comments, we corrected SI units according to the Jove author instructions. For example,

* ml 🡪 mL
* μl 🡪 μL
* hr 🡪 h