Editor, *Journal of Visualized Experiments* April 19, 2019

Dear Dr. Wu,

Thank you for modifying the manuscript. Please find below the changes made to the revised manuscript according to the editorial comments.

Editorial comments:

1. *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

The revised manuscript has been proofread and should be free of spelling or grammar errors.

2. Please avoid long steps/notes (more than 4 lines).

In order to keep below the 4 lines upper limit for each step, we removed information that does not pertain to the "step-by-step" approach:

"The script should contain a list of mask files that will be displayed onto the synthesis substrates for photodeprotection and corresponds, from top to bottom, to the 3′□5′ direction of oligonucleotide synthesis. The first line of the script therefore shows which mask will be displayed after the coupling of the first phosphoramidite (first T of the T5 linker)."

We have also split single steps into two or three separate steps:

1.9 into 1.9 and 1.10

2.1 into 2.1 and 2.2

2.3 into 2.3 and 2.4

4.11 into 4.11, 4.12 and 4.13

6.1 into 6.1 and 6.2

6.5 into 6.5 and 6.6

9.5 into 9.5 and 9.6

The whole protocol section should still under the 10 pages limit.

3. Figure 3: Please provide a title for the whole figure in figure legend.

Figure 3 now has the following title: "Photographs of the microarray photolithography optical and synthesis setup"

4. Figure 5: Please provide a title for the whole figure in figure legend.

Figure 5 now has the following title: "Hybridization assays to the 25mer DNA and RNA sequences synthesized in situ on microarrays."

5. The highlighted protocol steps are over the 2.75 page limit (including headings and spacing). Please highlight fewer steps for filming.

The following steps have been removed from the future video section:

"3.3 Prepare a solution of 1% (w/w) imidazole in DMSO by dissolving 11 g of imidazole into 1 L of dry DMSO. Shake well until completely dissolved. Attach the solution to the auxiliary port of the DNA synthesizer"

"3.4 For the synthesis of libraries, prepare a solution of 1% (w/v) β-carotene in dichloromethane by dissolving 100 mg β-carotene in 10 ml dichloromethane. Shake well in an amber glass bottle then wrap in aluminum foil"

"4.8 Set the coupling time for DNA phosphoramidites (cycles A, C, G and T) to 15 s, to 120 s for rU phosphoramidite (cycle 8) and to 300 s for rA, rC and rG phosphoramidites (cycles 5, 6 and 7). For library preparation, set the coupling of the base-sensitive, cleavable dT monomer to 2 × 120 s."

The remaining highlighted sections of the protocol should now be around 2.5 pages long.

6. Please do not abbreviate journal titles for references.

The bibliography should now display the full names of the journals.