**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Done.

2. Please define all abbreviations before use.  
3. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.  
4. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Done.

5. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

We added additional details to several steps and specifically expanded section 6, describing more precisely the microfluidic setup. Also, we added a few sections to the supplementary information, *e.g.* a PCR protocol or details on the assembly of a heated stage.

6. 1.2.1: How large is the petri dish?  
7. 1.2.2: What volume of isopropyl alcohol is added?  
8. 2.1: Please ensure that conditions and primers are listed PCR.  
9. 2.1.1: Please add more details here. Also please specify the volume of NaCl added.  
10. 3.3.3: Please specify the previous steps repeated here.  
11. Please specify incubation conditions throughout.  
12. Please number the figures in the sequence in which you refer to them in the manuscript text.

All of this information was added to the protocol.

13. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary.

We split several steps into multiple sub-steps.

14. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.  
15. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.  
16. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

We highlighted 2.75 pages including all relevant steps of the sections 1. Chip fabrication, 3. Photolithography, 5. Compartmentalized gene expression. Together, these three sections contain the basics of the chip fabrication and patterning as well as an application of the technique in the context of cell-free gene expression. In contrast to our original manuscript, we now highlighted section 5 (and not section 6 on the microfluidic device), because we consider the simpler setup more accessible to researchers who have little experience with biochips and microfluidics (see also reviewer 3, comment 5).

17. Please reference all data and figures in the manuscript. Please reference Figure 2 and supplemental file in the manuscript.

Done.

18. Discussion: Please also discuss any limitations of the technique.

We added a discussion of the factors limiting the size of systems of gene brushes.

19. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.  
20. Reference 23: Please include the journal name.  
Done.

**Reviewers' comments:**  
  
We thank all three reviewers for their positive assessment and constructive comments.

**Reviewer #1:**  
Pardatscher et al. describe a method for photolithographically patterning long dsDNA templates on a surface followed by integrating this surface with a microfluidic device and conducting cell-free expression experiments. The method should be useful for a variety of researchers interested in photolithographically pattern DNA on a surface and scientist working in the area of cell-free synthetic biology. Overall the manuscript is well written and is instructive. Below are a few small comments suggestions to improve the manuscript.  
  
Is the DIS oligo with a space commercially available? Providing more detail on the sequence and chemical moieties for this primer would be helpful.

Yes, it is commercially available. DNA sequences, modifications and the company providing them are given in the supplementary information (section 2).

Section 2 is very short. Purify how?

We added some more details to this section. Additionally, we added section 4 (PCR) to the supplementary information, which gives details specifically on the PCR, kit-specific purification, and devices used.  
  
Section 3.2.1, how are masks prepared? If bought, what type of masks are they: chrome, printed photomasks?  
Provide CAD designs for the microfluidic chip, DNA patterning mask, etc. (CAD drawings for the mechanical assembly would be potentially useful if available)

We now mention that we used printed photomasks and refer to a supplementary PDF containing the masks for both types of photolithography (PDMS master mold fabrication and projection lithography on Bephore chips). Also, we added section 5 (Lithography masks) to the supplementary information, which explains the PDF file in more detail.

As indicated in the “Note” at the beginning of section 5, the holder was not 3D-printed, but made from plastics, screws, a drill *etc.*  
  
Section 6: What flow rates are used?

Flow rates between 0.5 to 5 µl per hour were used (now mentioned in step 6.4.4).  
  
Figure 1:  
- unclear what the green star shape is and how the green oligo is attached to the dsDNA template. Include a description of the Pink, green and red stars directly in the Figure?  
We now mention in the caption that the purple and green stars represent the photocleavable modification and fluorophores, respectively. We also mention the triethylene glycol spacer between oligo and dsDNA template.

Figure 2, 3, 4:  
- quality and size of the images are poor / small so it is difficult to make out the details. Quality may be due to the pdf quality generated for review…

At the given resolution, the images show all the relevant details.  
  
  
**Reviewer #2:**  
Manuscript Summary:  
In this paper, authors disclose a manufacturing scheme for genetic biochips that includes technical details on how to immobilize genes on a structured surface in a microfluidic bioreactor system using a simple-to-use lithographic technique based on DNA strand displacement reactions. This setup facilitates the integration of compartmentalized genes with the microfluidic system that allows for a continuous supply with gene expression reagents while simultaneous removing wastes. The detailed technical steps presented in this paper should be useful and beneficial to other researchers in the community. I recommend publication of the manuscript in JoVE.  
  
**Reviewer #3:**  
The authors present a protocol for nucleic acid lithography that I consider to be of huge interest to the field. In combining biochemistry, optics and microfluidic elements into their procedure, I consider this an excellent example for a JOVE manuscript. Interdisciplinary contributions like this one require high proficiency in several diverse areas to reproduce the results, an movie tutorial can be invaluable in this regard. I thus recommend accepting this well written manuscript, providing following concerns are addressed:  
  
1.) Figures 2 and 3 are actually identical to the ones already published in the SI of reference 1. Would this incur copyright problems?

No. The copyright agreement mainly affects the main paper. Regarding the supporting information, Wiley was only granted a **non-exclusive** right to publish and re-distribute.

Wiley copyright transfer agreement, section A1: “To the extent that any Supporting Information is submitted to the Journal for online hosting, Wiley-VCH is granted a perpetual, non-exclusive license to host and disseminate this Supporting Information for this purpose.”

2.) Step 2.1. The PCR method and the method for DNA concentration determination should me more detailed. Please specify the used kits and instrumentation in the materials table. How was the DNA purified (column, gel, plasmid DNA digest) to remove excess biotin-primers? How was the DNA concentration determined? Please provide details, on how nM DNA concentrations where computed. For instances, does this number refer only to the DNA, or are biotin and fluorescent-probe included in the computation as well? Does the fluorescent probe interfere with A280 determination? Are there other means for quality control? Anything that will help the reader to correctly set-up their favourite system should be included.

Similar to reviewer 1: We added some more details to this section. Additionally, we added section 3 (PCR) to the supplementary information, which gives details specifically on the PCR, kit-specific purification, and devices used.

Also, please briefly explain addition of 1M NaCl. The effect of NaCl concentration on resulting brush density has been well documented and that information is linked in the cited manuscripts already. A new-comer to the field may struggle un-necessarily to find that connection.

We now explain the addition of NaCl in more detail and cite a paper by the Bar-Ziv group, which discusses the effect of salt concentration on DNA brushes.

3.) While the DNA preparation section in the current draft is overly short, the PDMS replica process in turn seams overly lengthy and maybe shortened.

We shortened section 4 (PDMS devices) and refer to a review paper by McDonald *et al.* for additional information.

4.) The authors should include their mask designs as well as the STL files for the 3D printed jig to facilitate reproducing the results by interested readers.

Similar to reviewer 1: We now mention that we used printed photomasks and refer to a supplementary PDF containing the masks for both types of photolithography (PDMS master mold fabrication and projection lithography on Bephore chips). Also, we added section 5 (Lithography masks) to the supplementary information, which explains the PDF file in more detail.

As indicated in the “Note” at the beginning of section 5, the holder was not 3D-printed, but made from pieces of 3.5 – 5 mm thick polyvinyl chloride (PVC) plastics, screws *etc.*

5.) detailed comments:  
Figures 2, 3,4 : please annotate for each section (A,B,C..) which Step from the protocol is shown.

Figure captions now refer to the corresponding protocol steps or sections.

line 133: Please include how much time each Section (sum of the steps) requires, maybe a table..

We added section 1 (Time schedule) with such a table to the supplementary information.

line 181: Please specify yellow foil type and source in the Table of Materials

Done.

line 319: I strongly recommend to include Section 5 into the movie shot, as there are several steps, such as the alignment in Step 5.3 that can be challenging to reproduce from the text/figure alone. This is in particular the case for Step 5.3.5 (line 381).

In contrast to our original manuscript, we now highlighted section 5 (and not section 6 on the microfluidic device), because we consider the simpler setup more accessible to researchers who have little experience with biochips and microfluidics.

Given the authors note that this step requires some experience (also noted in the discussion), it would be great if the two cases of two-low and two-high a clamping pressure could be distinguished from each other. Fig 6b is referred to, but the figure 6 caption does not mention the over-pressure case. Please include fluorescence and bright field microscopy images. It is unclear to me what the authors mean by 'diffraction pattern' and providing an image would be nice. Are these Newton ring patterns, in which case interference may be the overall more popular description?

We included section 6 (Compartmentalization – Troubleshooting) into the supplementary information, which shows exemplary images for the two cases (and another one for an air bubble) and gives indications on how to find the right settings. The stripe patterns in Figure S1B are indeed interference patterns similar to Newton’s rings.

6.) other edits:  
There are still some typos in the script, please proof-read carefully..  
lines 67/73/77/78/..: TX/TL vs. TXTL please chose one consistent name  
line 294: .. and dry it a ...

Done.