Manuscript No: JoVE60185
Manuscript title: Generation of heterogeneous drug stress gradients across cancer populations on the microfluidic Evolution Accelerator for real-time observation
First and corresponding author: Ke-Chih Lin

Reviewer #1:
Manuscript Summary:
The authors describe a sound protocol to prepare an evolution accelerator as a cancer cell ex vivo model.

Major Concerns:
The protocol is sound. no major concern.

Minor Concerns:
1. Practically, it will be nice if the authors can elaborate the necessity or the function for the other two stacks of PDMS(reservoir & capping layer). Was it because the tubings were not directly inserted into device? as in methods 3.4.5
2. in methods 3.4.7. For this device sealing after seeded cells approach, avoidance or clearance of microbubbles are essential because during sealing, any bubble entrapped will be significant to shear off cells or cause obstruction to the flow. Is there particular operation to avoid this. It may be critical for JOVE viewers when performing the protocol.
3. in the methods 2.2. The authors state " cell lines were transfected with cytoplasmic-labeled fluorescent marker, such that PC3-EMT express nuclear GFP and PC3-EPI express nuclear mCherry" . If the label is nuclear such as the H2B-GFP used in their paper cited as 11, then nuclear-labeled fluorescent marker will be more precise. However, after reviewing their figure 5 and 6, the fluorescent seems to be indeed cytoplasmic. It will be helpful for authors to clarify. Perhaps it's actually EF1alpha-promoted cytoplasmic fluorescent protein expression? Addition of Addgene entry of the plasmid they used for transfection will also be beneficial for interested JOVE viewer.

Authors' response: Thank you very much for your thorough reading of our work and your suggestion. We really appreciate your feedback.

(1) The description in the protocol has been modified:
"3.4.6. Prime the tubing and insert the steel pin into each PDMS chip through the capping layer. Fill up the reservoir layer and wet the PDMS pattern layer pattern with media. The reservoir layer works as an on-chip bubble trap to prevent air bubbles from getting into the microfluidic pattern."

(2) The description in the protocol has been modified:
"3.4.10. Place chip directly on top of the gas-permeable culture membranes (with cells already adhered to the membranes). In order to avoid entrapping microbubbles in the microfluidic pattern, dispense 1 mL prewarmed and degassed media into the 35-mm gas-permeable culture dish before assembly, and then make sure that the chip approaches the liquid surface with a 15-degree tilt angle."

(3) Thank you for pointing it out. This is actually a typo. There is no H2B label used in this paper. The description in the protocol has been modified as follows:
“2.2. Cell lines were transfected with cytoplasmic-labeled fluorescent markers for better visualization, as described previously[12], such that PC3-EMT expresses cytoplasmic GFP and PC3-EPI expresses cytoplasmic mCherry. Note that the technology is also compatible with any fluorescent-labeled cells as well as brightfield imaging of unlabeled cells.”