**NextGen Protocol on Fabricating the CometChip**

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**Synopsis:** This protocol details how to fabricate the 96-well CometChip.

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1. Cut a piece of GelBond® film to roughly the dimensions 80 mm x 110 mm.
2. Make a 1% (w/v) normal melting point agarose solution in 1X PBS; heat until fully dissolved.
   1. Note: Make a 20 mL volume agarose per CometChip.
   2. Note: 3% (w/v) gels are used for MalariaCometChip (Xiong et al., 2020).
3. Pour 2 mL molten normal melting point agarose on the lid of a rectangular petri plate lid. Immediately, affix the pre-cut GelBond® film hydrophilic side up to the rectangular petri plate lid.
4. Pour ~13 mL molten normal melting point agarose on the hydrophilic side of the GelBond® film. Immediately, gently overlay the PDMS stamp with an array of micropillars on top of the molten agarose gel.
   1. Note: the PDMS stamp will generate an array of microwells with 40-50 µm in both diameter and depth in a grid with 240 µm between wells.
   2. Note: overlay the PDMS stamp down at an angle to avoid any bubbles between the agarose gel and the stamp.
   3. Note: overlay the PDMS stamp such that a space remains on one side of the stamp to add 1X PBS in step 6.
5. Allow the CometChip to gelate at room temperature for 15 minutes.
6. Add ~5 mL 1X PBS around the edges of the PDMS stamp to facilitate its removal.
7. Gently remove the stamp, avoiding horizontal movement during removal to prevent collapse of the microwells.
8. Use tweezers to remove the CometChip gel from the rectangular petri plate lid.
9. Use a paper towel or razor to clean off any extra gel on the sides and bottom (hydrophobic side) of the CometChip.
10. Place the CometChip on a glass plate slightly larger than a 96-well plate (180 mm x 110 mm with 3 mm glass works well).
11. Inspect the CometChip under a bright field microscope for quality control, making sure the array of microwells is well formed.
12. Gently press a bottomless 96-well plate upside-down onto the CometChip on the glass plate.
13. Secure the bottomless 96-well plate onto the CometChip using four 1.5” binder clips one on each side pressing against the first row of wells.
14. The CometChip is now ready to be loaded with cells.

**Publications:**

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