

Detection and isolation of auto-reactive human antibodies from primary B cells

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Supplementary figures

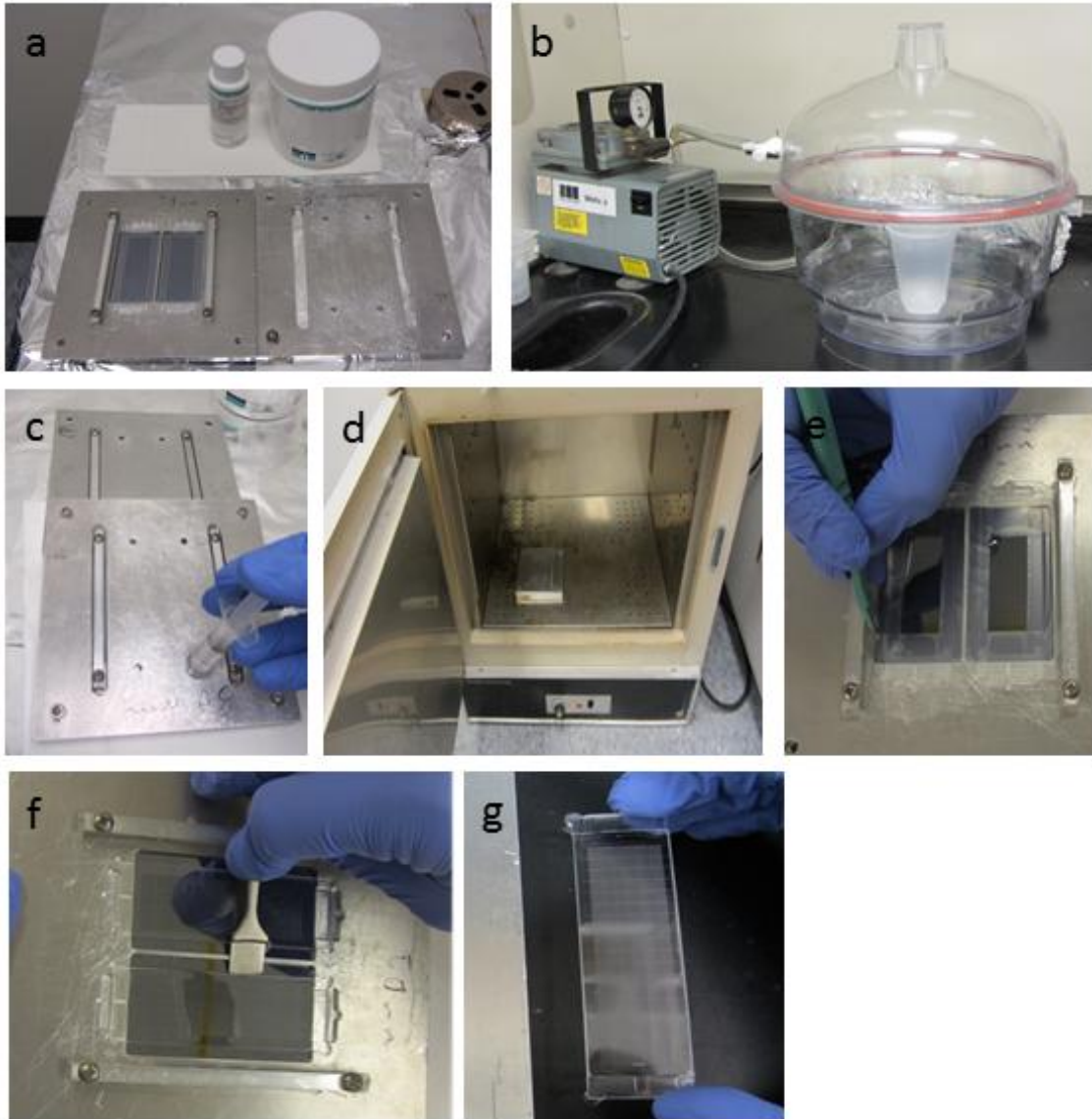


Figure S1. Preparation of nanowell arrays. A “master” template, patterned using photolithography, is used as a mold to print the elastomeric rubber (PDMS) array that conforms to the dimensions of a standard microscopy slide (25 x 75 mm) (a). After mixing Sylgard 184 silicone elastomer base and curing agent (10:1 weight ratio, respectively) with a plastic knife, the mixture is degassed under vacuum for 1 h at room temperature (b) and the mix is poured over the master (c). The PDMS is cured for 2 h at 80°C in the oven (d) and the excess of PDMS was cut around the array with a sharp scalpel (e) and gently peeled off of the master (f, g). A detailed video protocol for the preparation of nanowell arrays is outlined in our recent publication¹.

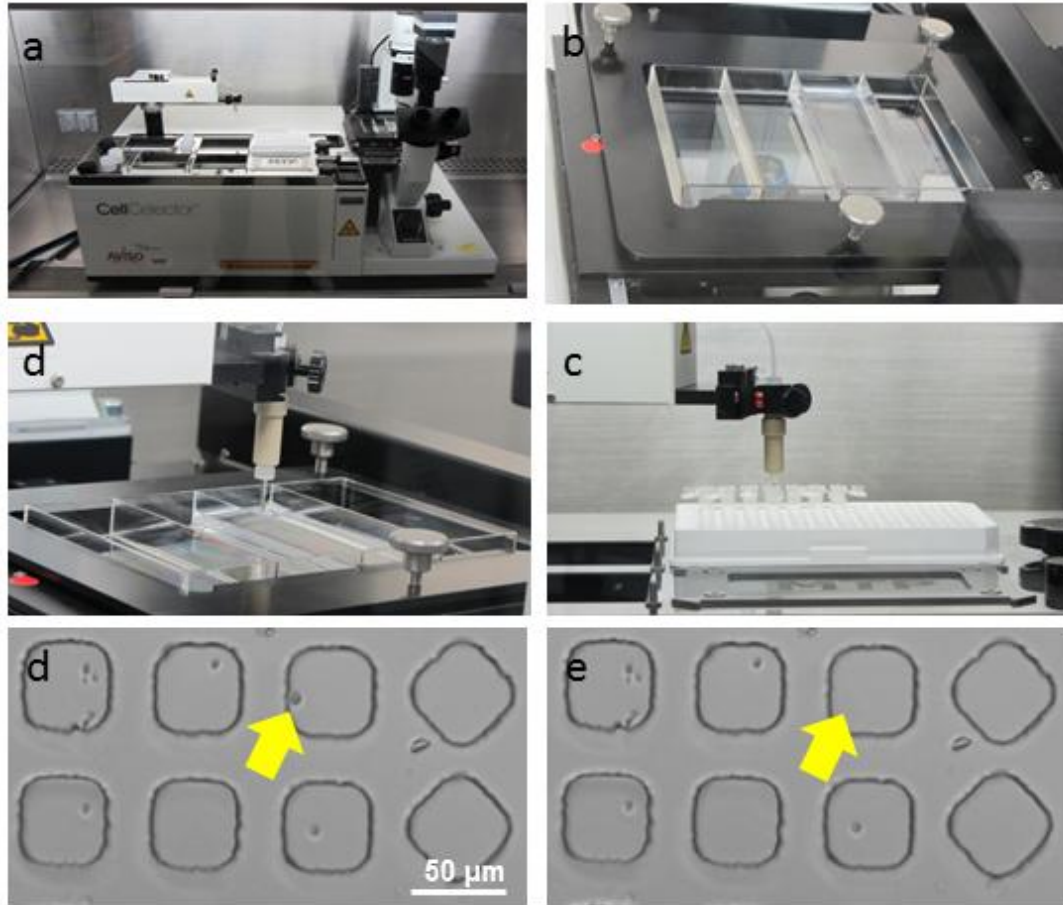


Figure S2. Retrieval of autoreactive B cells for amplification of variable regions. The CellCelector micromanipulator (ALS, Jena, Germany) is prepared as follows: Sterile 0.1 mL PCR tubes containing RT buffer (5x) in sterile nuclease-free water are placed on a rack in the receiving receptacle (a). The 4-well plate containing the nanowell array is placed on the motorized stage in the CellCelector microscope (a, b). After loading the .CSV file containing the locations of autoreactive B cells and calibrating the CellCelector, automated picking and transfer of the desired B cells is started (c, d). This process is monitored on the microscope and the images before and after picking were recorded (e,f).

Reference:

1. Liadi, I., Roszik, J., Romain, G., Cooper, L.J. & Varadarajan, N. Quantitative high-throughput single-cell cytotoxicity assay for T cells. *J Vis Exp*, e50058 (2013).