Single cell antibody production and isolation VyCAP

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Abstract

We have developed an innovative technology to measure and analyze the production of antibodies at the single cell level, followed by the isolation of the cells that produce the antibody of interest In first step, single cells are forced into individual wells. In total, there are 6400 microwells available in an area of 8 x 8 mm. Next, the antibody production of each of the cells is measured using ELISA based fluorescence detection. Once the cell that produced the antibody of interest is identified, this cell can be isolated for clonal expansion or isolation of its DNA/RNA.



Single cells in single wells

VyCAP's microwell chip



- VyCAP's microwell chip contains 6400 micro-wells in an area of 8 x 8 mm.
- Fluids enters the microwells and exits through the pore in the bottom.
- Fluidic forces pulls the cell in the microwell towards the pore in the bottom.
 Once a cell has landed on the pore, the fluid flow stops and no other cell will enter anymore

Forced single cell distribution



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Attach the membrane for antibody capture

Capture the antibodies produced by a single cells





Single cells in individual wells

- An activated membrane is attached to the bottom of the microwell chip
- The produced antibodies diffuse towards to the membrane where these are captured on the membrane

Remove the membrane





Isolate the single cell; expand; isolate RNA

Isolate the cells by Punching the cell out of the microwell



- After overnight incubation the membrane with the captured antibody produced by the single is removed from the membrane
- The antibodies are collected in spot with a diameter of approximately 70μm
- Common ELISA labelling is used to identify the collected antibodies

The location of the spots on the membrane are correlated to the microwell number in which the cell resides



clonal expansion and selection of the

Isolate the Ig genes for cell line generation

- After the microwell number in which the cell resides that produced the antibody is identified the cell is isolated from the microwell using VyCAP's Puncher system
- The single cell can be expanded
- The DNA / RNA sequences of the Ig genes can be isolated => generate antibody producing cell lines
- The Puncher platform is a very versatile platform for measuring the production and secretion of antibodies. High quality fluorescence images of the cell in the microwell ensure that a single cell is isolated.
- The produced antibody can be detected after 4 hours of incubation. Current practices that are based on 96 well culture plates require 3 weeks of incubation before sufficient antibodies are produced to obtain an ELISA intensity that can be detected.
- One microwell chip contains 6400 single cells; this equals 250 500, 96 well plates