

rate was 2-4 times that of sample flow. The flow of the beads in the microchannel was monitored through an upright, bright-field microscope (DM 4000, LEICA Microsystems AG, Wetzlar, Germany) and a cooled CCD camera (ORCA-AG, Hamamatsu corporation, Bridgewater, N.J.). The enriched cell solution was collected in a microcentrifuge tube. The collected enriched cells were amplified by overnight growth in LB medium with 0.2% glucose. A second round of induction, labeling, negative CMACS depletion of SA binders, positive CMACS enrichment of target binders, and overnight growth was performed at a reduced cell concentration of 10^8 cells/mL and 10^7 beads/mL.

[0157] The initial frequency of cells that express target-binding peptides was quantified using flow cytometry after labeling the library with biotinylated target antibody conjugated with a fluorophore (SAPE). This measurement gave the combined frequency of target-binding peptides as well as unwanted subpopulation that simply binds to streptavidin on the magnetic beads. The frequency of SA-binding peptides was independently measured by incubating the library with SAPE. The difference of the two measurements gave the net frequency of target-binding population. Before CMACS, the frequency of target-binding cells was 0.03% (FIG. 9 top). After the first round of screening, the frequency of target cells reached 0.7% (FIG. 9 middle) and the second round enriched the target cells to 53.6% of the population (FIG. 9 bottom).

[0158] Note that FIG. 9 provides flow cytometric analysis of the CMACS selection. The fraction of target-binding population in the library was analyzed by flow cytometry after incubating them with fluorescently labeled target. The intensity of red fluorescence (x-axis) indicates the expression level of target-binding peptides on each cell. (a) Unselected library (b) Following one round of CMACS, 0.7% of the population exhibit target-binding peptides (c) 23.8% of the population exhibit target-binding peptides after two rounds.

[0159] Following the screening, the collected fraction was diluted and spread on agar plates to obtain colonies. Colonies were picked to individual wells of a 96-well microtiter plate, grown overnight in LB medium with 25 ug/ml chloramphenicol and 0.10% (v/v) glycerol, and then frozen. Template preparation and plasmid sequencing were then carried out by the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences at the University of Washington.

[0160] Cell library population analysis was performed with conventional FACS (FACSAria, BD Biosciences, San Jose, Calif.), which was carried out by growing, inducing, and labeling the library with biotinylated anti-FLAG antibody at a final concentration of 5 nM. The cells were then washed twice and incubated on ice with streptavidin-phycoerythrin (60 nM) for 45-60 min. Cells were washed once and resuspended in cold PBS at a final concentration of $\sim 10^6$ cells/mL and immediately analyzed by flow cytometry. Control samples were prepared in parallel with SAPE labeling, but without antibody labeling, to assay SA binding clones.

[0161] A total of 87 sequences were obtained from clones isolated in the second round of sorting. The sequences were aligned using the program AlignX (Invitrogen, Carlsbad, Calif.) employing the ClustalW algorithm. A clear consensus group (21 of 87) contained a strong motif of DYKxxD, the well-established critical motif of the FLAG epitope. The identification of the consensus motif validates the methodology of CMACS based epitope mapping. It is also apparent that the streptavidin binding clones were co-enriched and abundant, however, they are easily identified and excluded

from the pool of sequences at the data analysis stage because they present the known HPQ or HPM motif (31 of 87 sequences), as well as other known disulfide-constrained motifs (4 of 87). The remaining sequences displayed no consensus, most likely originating from non-specific binding during the screening process. The sequence analysis is in qualitative agreement with the enrichment factors as monitored by flow cytometry.

Example 3—Parallel Architecture

[0162] In order to achieve higher throughput, the use of parallel branch architecture can be used. This example presents a three-dimensional “channel circuit.”

[0163] In the example, multiple channels are fabricated in one chip. The microchannel design is optimized to achieve a uniform flow pattern in each of multiple sorting stations. One challenge in implementing a three-dimensional channel circuit is the fact that flow streams may have to cross each other to achieve the necessary routing. To address this challenge, multiple layers for fluid distribution are used, analogous to an over-pass in a highway, where the buffer is introduced and divided into several sub streams in one layer, while the sample is introduced and infused into several downstream channels in another layer. This way, only two microfluidic connections are required at the inlet.

[0164] In this example, the channels are 20 μm deep and about 1 mm wide, which means that the flow should always be fully developed laminar flow. One goal of this example is to design the channel structure so that the same flow pattern results in every single channel. With a relatively wide inflow channel, one can achieve the same flow velocity and distribution in each channel. Generally this means that the fluidic resistance in the branches should be significantly greater than of the trunk or parent branch, typically on the order of at least 10 \times greater and sometimes in the range of 100 \times greater.

[0165] In an embodiment **1001** depicted in FIG. 10A (a schematic view), a top layer **1002** includes a port **1004** for sample inlet, a port **1006** for buffer inlet, a port **1008** for waste outlet, and a port **1010** for collection outlet. Underlying top layer **1002** is a layer **1003** that includes a sample inlet **1005**, a buffer inlet **1007**. Sample inlet **1005** allows sample to pass through layer **1003** to an underlying layer having features for distributing sample into multiple streams. Layer **1003** also includes a channel **1009** for distributing buffer into multiple stream channels **1011** that direct the buffer to parallel sorting stations on a lower level. Layer **1003** further includes a channel for collecting the target collection from multiple collection stream channels **1015** from the sorting stations. A lower layer **1017** includes buffer inlets **1019** and multiple channels **1021** for distributing sample to multiple sorting stations **1023**. The sample channels **1021** receive sample distributed from a main sample channel **1025**, also located on lower layer **1017**. The main sample channel provides a central connection with the sample inlet port **1004**. Multiple waste outlet channels **1027** for receiving waste streams from the sorting stations are also provided on layer **1017**. Finally, a main waste collection channel **1029** is provided on layer **1017** for providing a central contact with waste port **1008** on the top layer.

[0166] To actually model this approach, the flow field of a device with five channels was modeled in FEMLAB 3.1 (Comsol). During the simulation the width of inflow channel and distance between each sorting station was optimized. The flow field was calculated with an incompressible Navier-