

to single channels for analysis, samples can be split into many channels using the MOV routers and followed by multiple-EXPAR.

### 13. RiboMaker Reactions Performed in a NanoBioProcessor

The RiboMaker detection systems is based upon abortive initiation of RNA polymerase (RNAP) transcription, termed Abscription™, using Artificial Promoter Complexes (APCs) and nucleotide analogs called RiboLogs™. The APCs provide an initiation site for RNAP polymerase to generate 50-450 trinucleotide abortive products/min/site. Detection can be by MS analysis, fluorescence, chemiluminescence, or other methods well known to one skilled in the art. For DNA or RNA analysis, the APCs can have flanking sequences that provide specificity for the target site probe. RiboLogs with different mass units can identify which site is bound. By binding multiple APCs to different portions of a sequence to be interrogated, a fingerprint of RiboLogs can provide additional specificity information for biodefense, which can help eliminate false positives and false alarms. For proteins, an APC unit can be linked to an antibody. The RiboMaker detection is claimed to be fast, linear, and less sensitive to inhibition than PCR.

The RiboMaker reaction is accomplished on a NanoBio-Processor microchip such as the one in FIG. 13. The addition of a single APC reagent followed by single reaction mix requires two mixing steps. If the RiboMaker sample is captured on a bead, the bead is through the IMS Input (FIG. 13) into the reaction chamber, which optionally has a weir or magnet to trap the bead. The APCs are added using one of the reagent channels. The RiboLogs are added from a second reagent channel. If necessary, the reaction is moved back and forth between pumps A and B.

### 14. Microchip CMS Array Design

An embodiment of a 16 channel microchip 270 is shown in FIG. 23. The actuation lines 271 for the valves and pumps are shown running vertically and terminating at vias on the bottom of the microchip where external actuation lines can be connected. The cycle sequencing mixture is supplied via a syringe pump to a channel 272 on the left and water or buffer to regenerate the microchip is supplied in a channel 273 on the right. Both these "service" channels are multiplexed to feed all 16 channels and have on-chip pumps or valves 274 respectively to control the flow. This microchip is constructed as a four layer device from glass wafers and PDMS membrane.

### 15. A Complete MINDS System

To create the complete MINDS System, the instrumentation from the Core MINDS system is modified: 1) A bead service channel is added and interfaced with a bead sorting method to deliver individual beads; 2) The resistive heater design and electrode ring on the microchip interface device is altered to the microchip; 3) Microchip modifications to ensure that single beads are loaded and unloaded repeatedly.

A design of a MINDS microchip is shown in FIG. 24. The microchip is similar to the Core MINDS microchip shown in FIG. 22 except that a bead service channel leads 330 to the input line, the sample volume is decreased 4-fold to 25 nL, and a weir is formed in the cycle sequencing chamber to trap the bead. Single beads are input through the Input channel. The weir is etched to only 2 μm, which requires an additional mask and fabrication steps.

The single bead is pumped into the cycle sequencing chamber with only the channel leading towards the electrode and to the affinity capture chambers flowing. The weir stops the movement of the bead. Once a bead is loaded, 25 nL of cycle sequencing mixture with primers for both forward and reverse paired-end reads are pumped by on-chip pumps into the reaction chamber. The valves adjacent to the chamber are closed and the temperature cycled. Following cycling, the cycle sequencing products in the cycle sequencing mixture are pumped into electrode reservoir 6, electrophoresed into two sample cleanup chambers, and processed essentially as described above, with each paired-end read injected into separate separation channels. The valve leading to waste is opened and the bead flushed into the waste channel by the wash line. The separation regeneration occurs as described above.

Single beads are fed into each channel by 1) manipulating a microfluidic string of beads, that are well separated, and moving them into each channel serially or in parallel, 2) feeding from a "bin" of beads in each channel and dispensing them one at a time into the cycle sequencing reactor, or 3) magnetically manipulating individual beads or pickup onto the end of capillaries for "pick-and-place" manipulation. For the string of beads approach, beads are well separated spatially from the next by a bolus of liquid, possibly immiscible such as FluorInert (3M). We have previously successfully used boluses of FluorInert in cycle sequencing and PCR reactions. The bead string is moved together into rough positions. A valve then closes on the circulating bead service channel, and flow is diverted through an individual cycle sequencing chamber long enough to move the bead into the loading channel. A valve on the loading channel is closed, the valve on the bead service channel opened, and the next bead is placed into the next channel. Parallel variations are also possible and can minimize loading time. Optical bead sensors can also assist in helping regulate timing and feed flows.

The MINDS system use valves and pumps with laser drilled test holes of 50 μm to decrease pump volumes of several nanoliters. Alternately, valves with 250 μm holes are partially opened with partial "strokes" on each cycle. The valves surrounding the chambers are pulsed to move the bead in the chamber or external ultrasonic mixing is applied. Surface interactions are ameliorated by additives with surface modifications applied as needed.

For direct injection, the sample cleanup matrix is positioned in line with the separation channel. As shown in FIG. 44, this design has the familiar elements of cycle sequencing chamber for a bead and sample cleanup except the sample cleanup chambers are moved to the cathode side of the separation channel. Cycle sequencing samples are be electrophoresed on the sample cleanup matrix and contaminants removed into the cathode chamber which is flushed if needed. The clean samples are in a sharpened band on the sample cleanup matrix, and are released by heating the chambers and separations started. This volumetrically injects a sharp band onto the separation channel. Therefore, all of the sample collected on each sample cleanup matrix is analyzed as opposed to the "heart cut" found in typical twin T injections where the loading of the twin T only allows a fraction of the sample to be analyzed.

What is claimed is:

1. A method for processing and analyzing an analyte comprising the steps of:
  - a) concentrating a polypeptide-containing analyte in a macroscale module by: