

TABLE 3-continued

<u>List of DNA sequences used for spatial encoding of antibodies</u>			
Sequence Name	Sequence	SEQ ID NO	T <sub>m</sub> (50 mM NaCl) ° C.
GG	5'- AAAAAAAAAACTCTGTGAAGTGCATCGGT-3'	38	57.8
GG'	5' NH3-AAAAAAAAAACCGATGACAGTTCACAGAG-3'	39	57
HH	5'- AAAAAAAAAAGAGTAGCCTTCCCGAGCATT-3'	40	59.3
HH'	5' NH3-AAAAAAAAAATGCTCGGAAGGCTACTC-3'	41	58.6

\*All amine-terminated strands were linked to antibodies to form DNA-antibody conjugates using SFB/SANH coupling chemistry as described by R. Bailey et al.<sup>1</sup> Codes AA-HH were used in the experiment which examined fresh whole blood from a healthy volunteer. Codes A-M were used for the molecular analyses of cancer patient serum samples.

### Example 12

#### Barcoded Array for Detecting a Biological Profile: Quantitative Protein Profiling in Cancer Patients

[0223] The blood barcodes measured throughout the experiments illustrated in Example 10 were unique for each patient.

[0224] FIGS. 26 to 28 show quantitation and clustering of cancer patient barcode data obtained using a barcode array designed as exemplified in Example 8. In particular, FIG. 26 shows layout of the barcode array used in this study. Strand M denotes the reference (control). FIG. 27 illustrates a plot showing quantitation of fluorescence signals of all proteins (left axis) detected as shown in FIG. 21A for all cancer patients (from left: B01-B11, P01-P11, S01 and S02). S01 and S02 are two unknown serum samples. FIG. 28 shows an exemplary manual clustering of cancer patients derived on the basis of protein patterns. First, all prostate cancer patients are clearly identified by PSA. Second, both breast and prostate cancer patients exhibit possible subpopulations with distinct cytokine profiles.

[0225] The fluorescence signals intensity for all the patient samples are plotted in FIG. 26. The cancer marker, PSA, clearly distinguishes between the breast and prostate cancer patients, and allowed for the unknown samples, S01 and S02, to be assigned to prostate cancer patients. Applicants then performed a manual clustering of patients on the basis of protein signals and generated the map schematically illustrated in FIG. 27 to assess the potential of this technology for patient stratification. This approach is only going to be as good as the biomarker panel itself, and the number of serum samples profiled is small. Nevertheless, the results are encouraging. For example, the measured profiles of breast cancer patients can be classified into three subsets—non-inflammatory, IL-1 $\beta$  positive, and TNF- $\alpha$  positive. The prostate cancer patient data exhibits a generally higher level of inflammation, and those inflammation-positive samples can also be classified as shown in FIG. 27. An interesting observation is the lack of IL-10 signal for most patients. IL-10 is a cytokine production suppressor that functions as an anti-inflammatory mediator, and its absence may reflect deviation from normal immune homeostasis in local tumor sites. Applicants have initiated studies involving a larger number of proteins and a much larger number of blood samples. Researches have been focused on developing technologies for multi-

plexed measurement of cytokines, and serum cytokine profiling has shown relevance in cancer diagnostics and prognostics. The results described above have clearly demonstrated that integrated platforms can be applied to the multiparameter analysis of human health-relevant proteins.

[0226] The principal goal behind developing the integrated platform was to be able to measure the levels of a large number of proteins in human blood within a few minutes of sampling that blood, so as to avoid protein degradation that can occur when plasma is stored. In a typical 96 well plate immunoassay, the biological sample of interest is added, and the protein diffuses to the surface-bound antibody. Under sufficient flow conditions, diffusion is no longer important, and the only parameter that limits the speed of the assay is the protein/antibody binding kinetics (the Langmuir isotherm), thus allowing the immunoassay to be completed in just a few minutes.

### Example 13

#### Barcoded Array for Detecting a Biological Profile: Human Plasma Proteome

[0227] Use of a barcoded array was tested to verify improved sensitivity for plasma protein assays.

[0228] The human plasma proteome is comprised of three major classes of proteins—classical plasma proteins, tissue leakage proteins, and cell-cell signaling molecules (cytokines and chemokines). Cell-cell signaling molecules are biologically informative in a variety of physiological and pathological processes, i.e. tumor host immunity and inflammation.

[0229] The results of a first series of experiments performed by the Applicants are illustrated in FIG. 29, wherein a detection of target protein other than cytokines TNF- $\alpha$ , and Interleukins such as IL-6, IL-10 is shown. In particular, FIG. 29, shows detection of molecules such as CRP, C3 and plasminogen associated with biological profile such inflammation response (CRP), complement system (C3) and liver toxicity response (CRP and plasminogen).

[0230] The results of a second series of experiments performed by the Applicants is summarized in the diagram of FIG. 30, showing a schematic of human plasma proteome (refer to N. L. Anderson and N. G. Anderson, *Molecular & Cellular Proteomics* 11, 845, 2001).

[0231] As shown in FIG. 30, the concentration range of plasma proteins spans 12 orders of magnitude and the lowest