# DNA Encoded Biotechnologies for Informative Cancer Diagnostics

Thesis by

Gabriel Abner Kwong

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*Here, then are the true characteristics of objectivity…Objectivity does not demand that we estimate man's significance in the universe by the minute size of his body, by the brevity of his past history or his probable future career. It does not require that we see ourselves as a mere grain of sand in a million Saharas. It inspires us, on the contrary, with the hope of overcoming the appalling disabilities of our bodily existence, even to the point of conceiving a rational idea of the universe which can authoritatively speak for itself. It is not a counsel of self-effacement, but the very reverse—a call to the Pygmalion in the mind of man.*

- Michael Polanyi, 1958, *Personal Knowledge*

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# Abstract

This thesis describes the development of DNA-encoded, multi-parametric, sensing platforms for informative cancer diagnostics. In the first part of this thesis, I will present a technology called "DNA-encoded antibody library (DEAL)." In this approach, computationally derived, orthogonal ssDNA sequences are conjugated to antibodies specific for protein targets and cell surface markers. The resulting collection of conjugates is applied to a biological sample of interest, binds to their cognate antigens, and is detected after the complexes are hybridized to a glass substrate printed with spatially distinct complementary DNA sequences. By using DNA assembly, the DEAL platform enables the simultaneous detection of the major classes of biological molecules, namely nucleic acids, proteins and cells.

The second part of this thesis focuses on the development of a cell sorting platform that can detect antigen-specific T cells called "Nucleic Acid Cell Sorting (NACS)." In NACS, ssDNA encoding is used to assemble peptide major histocompatability complexes (p/MHC) on glass substrates by hybridization to cDNA microarrays. These assembled peptide/MHC microarrays are then used to sort mixed populations of antigen-specific T cells. This spatially encoded scheme addresses the widespread desire for methods that allow the multiplexed detection of antigen-specific T cells. The sensitivity and selectivity of NACS is similar to flow cytometry, demonstrated in key experiments with T cells derived from multiple sources, including endogenous and TCR-engineered T cells collected from cancer patients. Finally, this platform is used to

monitor the persistence of cancer-specific T cells in peripheral blood collected from a patient undergoing T cellular immunotherapy.

Lastly, a scheme for the detection of cell surface markers is presented. In this approach, DEAL and NACS conjugates prepared with UV labile ssDNA oligonucleotides are allowed to bind to target cell samples in solution. The ssDNA tags are released in solution by UV-induced photocleavage. The presence and expression of the cognate antigen is determined by collecting the pool of reporter ssDNA tags followed by exponential amplification by PCR. A DEAL conjugate specific for the oncogene EGFR was used to determine the expression level of EGFR in a low-passage brain tumor primary cell line. The feasibility of using ssDNA-p/MHC complexes for detecting unique TCRs was also demonstrated. Finally an experimental flow is described for integration with second generational high-throughput sequencing platforms for global and quantitative surface-ome profiling.

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