

## Mitochondrial DNA as a Biomarker of Disease Detection

The need for genetic testing is fueled by the advent of personalized medicine, discovery of disease biomarkers, and the pace of technological advances applied to the diagnosis of symptomatic individuals (including cancer diagnostics), carrier screening, prenatal testing, and newborn screening. Additionally, in the field of inherited genetic disorders, coordinated efforts between NIST, the American College of Medical Genetics, the Centers for Disease Control and Prevention, and the Eurogentest community has increased the number of standards for genetic testing, e.g. Fragile X syndrome. There is a national effort to identify biomarkers for the early detection of cancer. For example, mitochondrial DNA mutations have been reported in neurodegenerative diseases, sudden infant death syndrome, some forms of cancer, and age-related disorders. The accumulation of mutations is like a molecular clock predicting the time of disease and its progression. Results at NIST suggest that a relatively simple diagnostic test using a DNA microarray "chip" could provide early detection of some cancers.

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A research team from NIST, the National Cancer Institute (NCI), and Tetracore has developed a rapid, high-throughput sequencing protocol to detect mutations in the mitochondrial genome. The project is very timely because the human genome has been sequenced and published, and currently there is great demand for the use of genomic markers for diagnostic applications. Affymetrix has developed a microarray chip that has improved the data collection and interpretation of mitochondrial genomic data. We have used this chip to analyze the mitochondrial DNA isolated from various tumors and bodily fluids (i.e. blood and urine). The  $\approx 40,000$  hybridization spots that were obtained were scanned in  $\approx 10$  minutes and the sequence information compiled in a database. Mutations in the cancer cells are identified according to the nucleotide change and according to gene location. Further studies have been initiated with staff at Genesis Genomics and at the Environmental Protection Agency. We plan to also validate the results using different cancer population sets (to rule out bias) and to evaluate the sensitivity of chip-based DNA sequencing methods for forensic applications.

This work was supported in part by the Early Detection Research Network (EDRN) of the National Cancer Institute, and in close collaboration with NCI's Biomarkers Research Group.

**Future work includes:** Cross-validation of different population sets and parallel analysis of mitochondrial expression array.

### Publications:

C.D. O'Connell, D.H. Atha, and J.P. Jakupciak. "Standards for validation of cancer biomarkers." *Cancer Biomarkers*, 1 (2005) 233-239.

J.P. Jakupciak. "New Microarray Improves Sensitivity of Cancer Detection by Pinpointing Small Changes in mtDNA." *Affymetrix Microarray Bulletin* (2005) 1, 23-26.

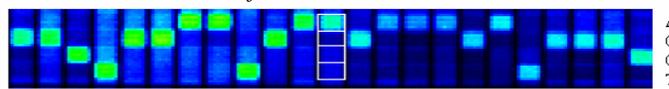
J.P. Jakupciak, M. Markowitz, D. Ally, S.Srivastava, W. Wang, A. Maitra, P.E. Barker, D.Sidransky, and C.D. O'Connell. "Mitochondrial DNA as a Cancer Biomarker." *J. Mol. Diagnostics*, (2005) 7, 258-267.

J. P. Jakupciak and C.D. O'Connell. "Standards and Standardization of Molecular Diagnostics. as Part V: "Quality Assurance in Molecular Diagnostic Laboratories." In *Molecular Diagnostics for the Clinical Laboratorian*, Second Edition, Editor: G. Tsongalis, Humana Press, Totowan, NJ, 2005, pp. 243-246.

J.P. Jakupciak and C.D. O'Connell. "NIST Physical Standards for DNA-Based Medical Testing", in the *Encyclopedia of Medical Genomics and Proteomics*, Editors: J. Fuchs and M. Podia, Marcel Dekker, New York, 2005, pp. 929-933.

### Advances in Mitochondrial DNA Sequencing

- Optimized *high-throughput* Resequencing Array: 105 mitochondrial genomes from *Stage I and II* tumors completely sequenced (provided by Dr. Sidransky).
- *Early cancer* associated mutations detected in 88% of patients.
- *Non-invasive* samples (BAL, urine, serum) contained the identical mutations as observed in the primary tumor tissue.
- Assay results are *easy* to interpret. Assay is technically *facile*. Assay format is readily *transferable to clinical* use.



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