# Seeing Deletions in "3D"

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There are approximately 1000 to 10,000 mitochondria in a human cell. These organelles function to provide energy to the cell but encode a genome separate from the nuclear genome. Deletions in the mitochondrial genome are associated with several pathogenic conditions and are believed to contribute to the aging process. Deletions must be present in the majority of mitochondria in a cell before these phenotypes are manifested; however, it is unclear if these deletions arise early and accumulate through a process of clonal expansion, or if there is a continual generation and accumulation of deletions over time. In a recent report published in*Aging Cell* Drs. Sean D. Taylor and Jason H. Bielas (Public Health Sciences and Human Biology) and their collaborators demonstrate a sensitive deletion detection assay and provide support for the hypothesis that mitochondrial genome deletions arise early and are propagated by clonal expansion.

Current methods for the detection of deletions in genomes are labor intensive and susceptible to various artifacts that reduce their accuracy. PCR-based methods are susceptible to template switching or preferential amplification of short amplicons, while Southern blotting or next generation sequencing techniques are not sensitive enough to detect early deletion events against the background of wildtype genomes. To address these issues, Taylor et al. developed a very sensitive three step process known as Digital Deletion Detection to characterize rare deletion events (3D; see figure). In this method, samples are first enriched for deleted genomes by endonucleolytic digestion. This step destroys wildtype templates but spares templates with deletions that remove these endonuclease recognition sequences. These enriched templates are then subjected to single molecule droplet PCR scaled so that each droplet contains either no template or at most a single genome. This single molecule amplification prevents many PCR artifacts such as template switching to bias amplification. Finally, the deletions are either quantified by droplet digital PCR, which directly counts droplets containing an amplified template, and therefore a deletion, or the reactions are subjected to next generation sequencing to characterize unique deletions. Using 3D methodology, Taylor *et al*. were able to detect deletions as rare as 1 event in 100 million genomes, and deletion detection was accurate over 8 orders of magnitude. Furthermore, the mean fluorescence intensity of droplets was inversely proportional to the size of the amplicon (smaller amplicons were brighter), allowing the researchers to determine whether samples had a single deletion or a complex mixture of deletions by flow cytometry of the droplets.

Taylor, *et al*. used this 3D technique to characterize the deletion dynamics of two sites in the mitochondrial genome. Both deletions increased in frequency with respect to the age of the patient; the “common deletion” increased over 300-fold between samples of 15-year-olds and 80-year-olds, while a deletion at the ND1/ND2 site increased approximately 25-fold. Interestingly, the frequency of the ND1/ND2 deletion was more strongly correlated with age than the “common deletion” ($R^2$ = 0.812 vs. 0.453). Finally, Taylor, *et al*. subjected the amplification products to deep sequencing to determine whether the deletions were clonal or heterogeneous. They found that the “common deletion” site was generally dominated by a single deletion while there was a heterogeneous distribution of deletion sizes at the ND1/ND2 site. However, even at the ND1/ND2 site most of the deletion load was dominated by a few discrete deletions representing 1 to 10% of the total deletion burden individually. Taken together, these data support the hypothesis that mitochondrial genome deletions arise early and are propagated by clonal expansion or preferential replication.

Mitochondria are key organelles in cellular metabolism, but the maintenance of their genomes is poorly understood. With the sensitive new Digital Deletion Detection assay, it is now possible to identify the origin of deletions in the mitochondrial genome and define how they accumulate over time. "The same principles used for the detection of rare deletion events also apply to the detection of rare nuclear variants or the detection of disease biomarkers," said Dr. Taylor, making 3D technology a powerful new tool to investigate genetic changes in both normal and disease states.

Taylor SD, Ericson NG, Burton JN, Prolla TA, Silber JR, Shendure J, Bielas JH. 2013. Targeted enrichment and high-resolution digital profiling of mitochondrial DNA deletions in human brain. *Aging Cell*. Epub ahead of print. doi: 10.1111/acel.12146.

See also: Hindson et al. 2011. High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number.*Analytical Chemistry*. 83, 8604-8610.

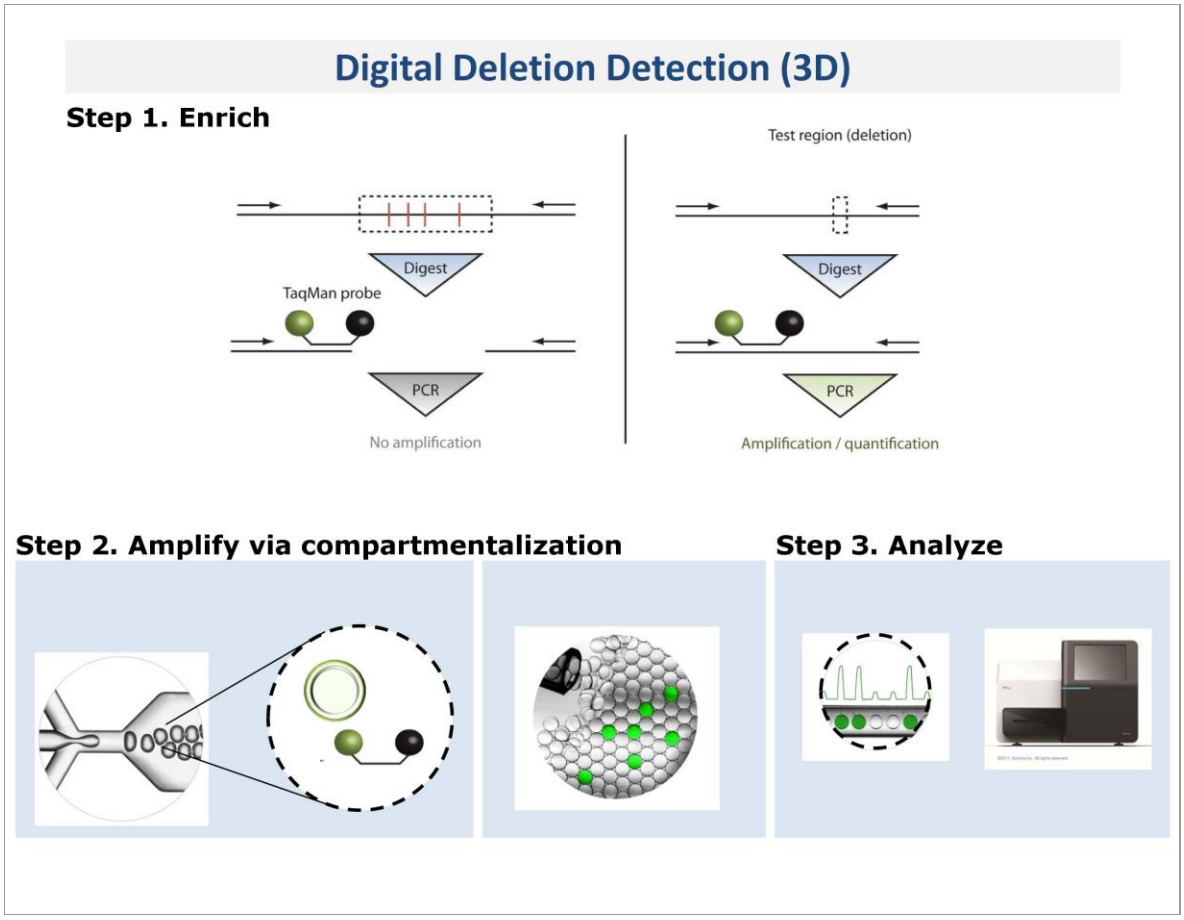


*Image courtesy Dr. Sean D. Taylor*

Schematic of the Digital Deletion Detection protocol. Samples are 1) enriched by endonucleoyltic digestion, 2) amplified by digital droplet PCR, then 3) characterized by a variety of methods.