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Detection of Sequence Variations Within Populations Using Non-Symmetric PCR at the Near Digital Level

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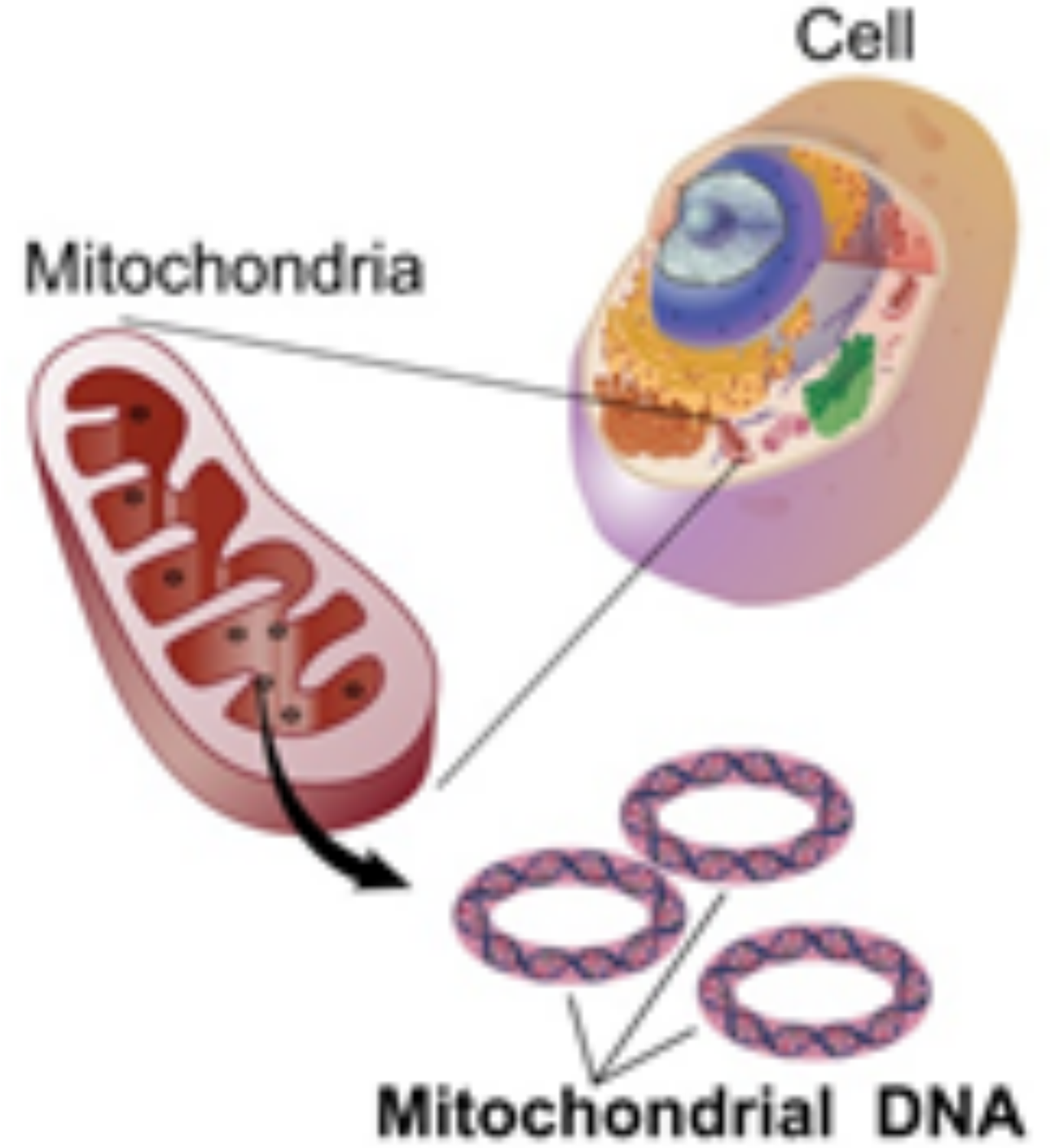
Technology description



The technologies for licensing are novel methods for asymmetric amplification and fluorescence detection of the mutational load in nucleic acid target sequences. These methods utilize single-tube, multiplex polymerase chain (PCR) reactions on mixed samples which each contain one or more target specific primer pairs for DNA amplification. These reactions also contain 1 or more probe pair sets that hybridize to sites within the target and each have covalently attached to it either a fluorescent compound or a non-fluorescent complementary quencher moiety. The Signaling Probe will not fluoresce unless bound to the amplified single-strand target sequence and its signal is eliminated

whenever both the fluorescent and quencher probes are bound to their adjacent sites on the target sequence.

Mutations in mitochondrial DNA (mtDNA) can result as a side-effect of age, environmental hazards, genetic susceptibility, diet, drug exposure or a combination of causes. These mutations have been correlated with human diseases such as diabetes, Huntington's, cancer, Parkinson's, bipolar disorder, chronic fatigue syndrome, amyotrophic lateral sclerosis and Alzheimer's. However, no specific point mutation has been linked to the onset of a disease. It is hypothesized that the buildup of random mutations over time in the multiple genomes of the mitochondria leads to dysfunction of the organelle and then onset of disease. In order to be able to observe the accumulation of mutations over time, current techniques require analysis of typically 1 to <10 mtDNA molecules which is expensive to carryout.

The technologies for licensing are novel methods for asymmetric amplification and fluorescence detection of the mutational load in nucleic acid target sequences including non-nuclear DNA (e.g. mtDNA, chloroplast DNA, episomal DNA) as well as RNA, cDNA, and genomic DNA. These methods utilize single-tube, multiplex polymerase chain (PCR) reactions on mixed samples which each contain one or more target specific primer pairs for DNA amplification. These reactions also contain 1 or more probe pair sets that hybridize to adjacent sites within the target, though probe pairs need not be next to each other, and each have covalently attached to it either a fluorescent compound ("Signaling Probe") or a non-fluorescent complementary quencher moiety (e.g. dabcyl or Black Hole Quencher; "Quencher Probe"). The Signaling Probe will not fluoresce unless bound to the amplified single-strand target sequence. The signal is eliminated by the fluorophore and quencher moiety whenever both probes are bound to their adjacent sites on the target sequence.

The mutational load for the target is determined by analyzing the differences in fluorescence for the hybridization curves. Signals can be acquired for analysis either as the reaction temperature is decreased (annealing) or increased (melting). The Signaling Probes and Quencher Probes are both mismatch tolerant and can hybridize to target sequences that contain 1 or more substitutions where probes having greater variation from the target's complementarity lowers the melting temperature (T_m) of the probe-target hybrid. The T_m differences can be used to differentiate the mutations accumulating in the target DNA sequence.

Osborneet al. (2013) "AZT Treatment Increases mtDNA Mutations in HepG2 and CCD-1112Sk Cells." J AIDS Clin Res 4: 250. doi: 10.4172/2155-6113.1000250

Osborneet al. (2014) "Palm Fruit Juice Mitigates AZT Mitochondrial Genotoxicity and Dose-Dependent Cytotoxicity." J AIDS Clin Res 5: 400. doi:10.4172/2155-6113.1000400

Application area

The methods allow single-tube asymmetric PCR amplification and fluorescent detection of mutational loads within non-nuclear and genomic DNA contained in complex, mixed samples. Though the methods are broadly applicable to diagnostic and analytical assays, our invention has been enabled for the detection of mitochondrial DNA (mtDNA) mutations within: cytochrome c oxidase subunit 2 (CO2),

NADH dehydrogenase subunit 1 (ND1) and the hyper variable 2 (HV2) of human mitochondria. The mtDNA of mice, rats, and the Nile rat (used to study diabetes) have also been amplified using this technology for various studies.

Advantages

·Allows for amplification of targets and detection of multiple mutations in a single PCR reaction
· Overcomes limitations of prior analysis methods which can obscure the presence of mutations due to target DNA having methylated stretches of nucleic acids
· Novel methods for identifying mutational loads in target DNA sequences using single-tube, asymmetric PCR reactions on mixed biological samples (specifically enabled for mtDNA)
· Mutations are detected by analysis of the temperature-dependent fluorescence signatures created by the annealing or melting of signal/quencher probe pairs to the single-stranded target
· Methods are broadly applicable to human and animal genomic and other complex DNA samples

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