G14 A Method for the Separation and Isolation of Intact Single Cells From Paraffin- Embedded, Formalin-Fixed Tissue Using Laser-Dissection Microscopy

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After attending this presentation, attendees will gain an understanding of the methods used to separate, identify, and isolate single cells from paraffin-embedded tissue blocks for the purposes of single-cell studies or assays, as well as the specific methodologies employing laser-dissection microscopy, and the success rate using this method in capturing single cells.

This presentation will impact the forensic science community by providing results from replicate experiments in an area with applications to histology, pathology, DNA analysis, and molecular genetics. This presentation will add to research being carried out in forensics by demonstrating a reliable process by which single cells from archival paraffin-embedded tissues can be isolated for the purposes of single-cell assays.

The separation and isolation of single cells from formalin-fixed, paraffinized tissue was necessary in a recent study into the effects of mitochondrial heteroplasmy at a cellular level. While the results from the study were inconclusive due to an issue in the SNP primer design, the methodology for the removal of paraffin, the mashing of the tissue to free cells, and the use of laser-dissection microscopy was seen to be highly effective at the isolation of intact single cells from a previously formalin-fixed and paraffinized tissue source. The use of laser-dissection microscopy is a commonly accepted approach for cell isolation along with flow cytometry and the use of optical tweezers.[1-3]

The cells were freed from the tissue using a combination of heated water washes to melt the paraffin, followed by the mashing of the tissue block between the frosted ends of microscope slides. The frosted glass acted as a grinding surface to release cells from the intact tissue, while the remaining space between the slides allowed the lateral migration of the intact cells without further mechanical breakdown of the cell structure. The mashed tissue and cells were then sieved to separate the cells from the gross tissue, after which the cells were counter-stained with a combination of a mitochondrial-specific fluorescent dye (MitoTracker® Green FM) and Nuclear Fast Red and then affixed to the laser dissection microscope slides.

The use of laser-microscopy on such cells following separation and fluorescent and optical staining was critical for the proper identification of intact single cells. The methodology used in the process of laser dissection was highly controlled to ensure the proper isolation of a single cell, which was shown to be highly reproducible. To ensure collection, the cells were ablated from the microscope slide and into a collection buffer ensconced within the one ml collection tube cap. The capture buffer was not only a support medium to trap the isolated cell, but also contained the appropriate volume of amplification buffer to send the single cell directly to a one-step amplification which simultaneously lysed the cell and amplified the cellular mtDNA. The resulting detection of the SNP base in question demonstrated an overall collection efficiency of 94.6%, with 681 out of 720 collected cells yielding a signal upon fluorescent detection.

The results of the study demonstrated that the methodology for separating and isolating the cells from the paraffinized tissue was reliable and reproducible. The use of the laser dissection microscopy was effective under the controlled conditions for collecting single cells and was highly specific for single cells selected on a slide when other cells and cellular debris were present within the same viewing field. The confirmation of the single cells was based on the downstream testing and resolution of the SNP base from the mtDNA, which confirmed not only the presence of a captured cell but the presence of only one cell compared to clusters of cells.

References:

1. Pflugradt, R., et al., A novel and effective separation method for single mitochondria analysis. Mitochondrion, 2011. 11(2): p. 308- 314.
2. Lutz-Bonengel, S., et al., Single lymphocytes from two healthy individuals with mitochondrial point heteroplasmy are mainly homoplasmic. Int J Legal Med, 2008. 122(3): p. 189-197.
3. Reiner, J.E., et al., Detection of Heteroplasmic Mitochondrial DNA in Single Mitochondria. PLoS ONE, 2010. 5(12).

Isolation, Single-Cells, Laser Dissection

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