

The Social Amoeba in Forensic Science: Microbial Fingerprinting and Epigenetics

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This presentation will examine the potential utility of the social amoeba, *Dictyostelium discoideum*, in forensic science casework, research, and education. Attendees will learn how *D. discoideum* can be used in microbial soil fingerprinting and as a model organism for epigenetic, proteomic, and transcriptome analyses, using both traditional sequencing and portable nanopore sequencing devices. This overview will also feature recent case studies demonstrating the use of *D. discoideum* in each of these analysis areas.

This presentation will impact the forensic science community by providing the basic knowledge and competence needed to begin incorporating *D. discoideum* into research or training programs. This presentation will be of special relevance to forensic scientists interested in environmental characterization and/or gene expression studies, and also to forensic science educators seeking engaging, cost-effective options for student laboratory experiences.

Social amoebae (*Dictyostelium sp.*) can be useful tools in forensic biology, both for soil-based environmental characterization, and as model organisms for development of new techniques for genetic, epigenetic, transcriptomic, and proteomic studies. *Dictyostelium*-derived protocols for gene expression analysis can be developed at a much more rapid and affordable rate than exclusively human-based studies. Later, when optimized for human samples, these protocols may provide information about tissue/fluid type, subject age estimation, and other markers of epigenetic effects on gene expression. This information can facilitate sample classification, and can provide details that help to narrow the candidate pool for subject identification when a DNA database match is not obtained.

Dictyostelia is a genus containing ~120 species of eukaryotic amoebae (a.k.a. slime molds) found in the soil and leaf litter of terrestrial ecosystems, worldwide. The best-studied of these, *D. discoideum*, has long been a popular model organism for genetic research due to its short life cycle, multiple developmental pathways, and numerous orthologs to human disease genes. *D. discoideum*'s six-chromosome, 34 Mb haploid genome is abundant in trinucleotide tandem repeats and also encodes ~12,000 proteins that vary in expression throughout the life cycle. These single-celled, asexually-reproducing amoebae can, under certain conditions, also reproduce sexually, having three different sexes that can each reproduce only with a partner of a different sex class. Additionally, when experiencing nutrient stress, the multiple genetic lineages will aggregate into a multicellular slug, cooperatively migrate to a better food patch, then produce a fruiting body that releases spores which will mature into new, unicellular offspring. Each of these stages can be readily induced and observed in colonies grown on simple agar plates in the laboratory.

Collection of wild-dwelling colonies for laboratory study is also quick and inexpensive, and available right in many researchers' back yards. Colonies can be frozen to pause research, and will resume activity when thawed. The above qualities combine to provide an ideal organism for multiple areas of forensic biology research and, especially, for hands-on forensic student research that is extraordinarily flexible, budget-friendly, and does not typically require the time-consuming process of obtaining Institutional Review Board (IRB) approval before each study can commence.

Case studies that will be briefly summarized for illustrative purposes include: (1) the harvesting and genetic characterization of wild *Dictyostelium* colonies from different mock crime scene locations; (2) isolation and comparison of (reverse-transcribed) messenger RNA and select expressed proteins under varying conditions and throughout the life cycle; (3) proteins useful for taxonomic classification by amino acid sequence; and (4) assessment of highly-economical, portable nanopore sequencing devices, compared to established sequencing systems, for conducting these types of studies.

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