Implen Journal Club | October Issue

Welcome to our October issue of the #Implen #JournalClub in 2021.

This month's Implen NanoPhotometer® Journal Club: Halloween Edition is being kicked off with highlighting the work of Yang et al. published in the Journal of Insect Molecular Biology, which was the first detailed report of the characterization and expression profiling of Halloween genes in spiders

with the identification of the Halloween genes spook (spo), disembodied (dib), shadow (sad) and shade (shd) in the wolf spider. Ecdysteroids are key growth hormones known to regulate moulting, metamorphosis and reproduction in arthropods. The biosynthesis of Ecdysteroids is catalyzed by cytochrome P450 monooxygenases (CYP450's) which are encoded by Halloween genes including spo, phm, dib, sad and shd. The wolf spider exhibited the temporal expression role of the four Halloween genes in concurrence with spider-ling moulting with steady increase followed by a rapid decline in expression once moulting was completed, which is the typical expression pattern of Halloween genes in insects. Spatially, the four Halloween genes were highly expressed in spiderling abdomen and in the ovaries of female adults. Phylogenetic analysis grouped arachnid and insect Halloween gene products into two CYP450 clades, the CYP2 clan (spo and phm) and the mitochondrial clan (dib, sad, and shd). This study revealed the different ecdysteroid biosynthesis pathways in spiders and insects. The NanoPhotometer® was used in this study to determine the RNA purity and concentrations.

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Next, Implen NanoPhotometer® Journal Club is exploring a new method for extracting DNA from skeleton bones. Lodha et al. reported in the Journal of Forensic Science International, a novel approach of PCR ready-human DNA extraction method from skeletal remains using copper nanoparticles (CuNPs) for personnel identification. This has been shown to be a rapid, cost effective, sensitive and non-hazardous method requiring very small quantities of blood and skeletal remains. The surface plasmon resonance (SPR) of the synthesized CuNPs solution showed absorption maxima at 582 nm which remained the same even after months of storage. The applicability of this approach was first tested in blood samples and extended to skeletal remains' samples also. This method yields good quality DNA ready for PCR reactions from small quantities of blood and skeletal remains. Consequently, even small quantities of nanoparticles could be potentially utilized for a highly efficient isolation of DNA from skeletal remains as well as from ancient archaeological bones to study the genetic information for getting information in relation to past populations such as linguistics, culture and morphology. Archaeology, anthropology and forensic science will all benefit

from the improved DNA analysis of skeletal remains. The NanoPhotometer® P-Class was used in this study to evaluate isolated DNA. The extracted DNA samples were diluted up to the range of 50–75 ng/µl to maintain an average of 200 ng/reaction for PCR reaction of all samples to provide linear results. To check the quality of the DNA, the TE buffer was used as the blank and absorbance was measured at wavelengths of 260 and 280 nm (A260 and A280, respectively) and estimated the DNA purity using the absorbance quotient (A260/A280). An absorbance quotient value of 1.8 < ratio (R) < 2.0 is considered to be good purified DNA, while a ratio of <1.8 is indicative of protein contamination, while a ratio of >2.0 indicates RNA contamination.

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Continuing with this month's COVID-19 issue of the NanoPhotometer® Journal Club, we are exploring the challenging technique of grafting being used to create 'Frankenstein' combinations of cucumber and pumpkin. Graft compatibility is a prerequisite for grafting. Rena et al. showed in a study published in the Journal of Plant Physiology and Biochemistry that a number of metabolic, physiological and hormonal responses are involved in graft compatibility in cucumber leaves including metabolic processes, nutrient transport, signal transduction, plant hormone signal transduction, transcription factors, oxidation-reduction processes, and defense responses. Using cucumber (Cucumis sativus L.) grafted onto pumpkin (Cucurbita L.) rootstocks with different degrees of graft compatibility, an integrative analysis of mRNA and miRNA expression and regulatory networks was conducted by using RNA-Seq and sRNA-Seq. Overall, there were 223 genes and 30 miRNAs that were differentially expressed between the two graft combinations with different degrees of graft compatibility. The results suggest that compatible rootstocks might possess a greater ability for cell proliferation and a more efficient carbohydrate metabolism that promotes plant growth. In contrast, incompatible grafts induced multiple defense response-related genes and various transcription factors, likely in response to stress. This study has demonstrates the fine-tuning and tight regulation of genes and miRNAs that are differentially expressed play important roles in plant graft compatible/incompatible responses advancing the understanding of the underlying molecular

mechanisms and revealed numerous mRNA and miRNA candidates for more in-depth studies into the graft compatibility process. The NanoPhotometer® was utilized in this study to determine the RNA concentration and integrity.

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In the final issue of this October's Implen NanoPhotometer® Journal Club: Halloween Edition, we will be featuring studies investigating the intriguing properties of the golden spider web. Golden orb-weaving spiders (Trichonephila clavata), a genus of araneomorph spiders, produce bright yellow thread that absorbs light with wavelengths less than 500 nm. Spider silk is a natural fiber with remarkable strength, toughness, and elasticity that is attracting attention as a biomaterial of the future. Golden orb-weaving spiders construct large, strong webs using golden threads. Owing to its outstanding properties and biocompatible and biodegradable proteinaceous nature, spider silk has attracted attention as a potential tool in the commercial production of synthetic threads for industrial and medical applications. Fujiwara et al. characterized the pigment of the golden-orb weaving spider's dragline silk by coupling liquid chromatography with high resolution ESI-MS, APCI-MS, and UV detector, and further confirming the structure by MS/MS spectrometric analysis, identifying xanthurenic acid as the major pigment in the golden dragline spider silk. Furthermore, xanthurenic acid was shown to exhibit a slight antibacterial effect on the growth. The NanoPhotometer® was used in this study to monitor the growth of wild-type Escherichia coli (W3110) and Bacillus subtilis (str. 168) OD600 every hour.

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