**Title:** Pathological Society Equipment Grant EG 2020 1242

**Name and address of Grant Holders:**

Dr Hannah Crane

Professor Sherif El-Khamisy

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**Background and aims:**

The El-Khamisy research group is based in the School of Biosciences at the University of Sheffield. The laboratory space is used by nine PhD students and a post-doctoral researcher, and also trains BSc and MSc students. In addition, we collaborate with researchers from the Bateson Centre (a large MRC funded zebrafish research facility) and Sheffield Institute for Translational Neuroscience (SITraN; part of the Sheffield NIHR Biomedical Research Centre for neurology) who frequently use our facilities. Professor El-Khamisy is also the Head of the Institute of Cancer Therapeutics at Bradford University, and this is an important collaboration providing access to cancer drug development pipelines.

The laboratory studies DNA damage and its repair and over the past few years have expanded the repertoire of experiments to include advanced genomics techniques including RNA-seq, ChIP-seq and DRIP-seq. These methods have allowed us to provide mechanistic insights across the genome, however they require precise quantification of input nucleic acids and prepared sequencing libraries prior to sequencing to ensure robust, reproducible results.

**Equipment purchased:**

The equipment grant from the Pathological Society has allowed us to purchase a Qubit Flex Fluorometer (total cost £5,215.15) which we received in the laboratory in May 2021. This equipment allows for highly accurate quantification of DNA, RNA and protein. Up to eight samples can be processed simultaneously, which allows for high throughput of samples. The Qubit is very sensitive and allows for accurate quantification, even when the sample concentration is low. This is very important for certain techniques used in the laboratory, such as ChIP-Seq, where accurate quantification of low concentration samples is required.

The Qubit has been used on a weekly basis by PhD students and a postdoctoral researcher in the laboratory to quantify DNA, RNA and protein. It is simple to calibrate and use and has provided accurate results. The Qubit will also be made available to other researchers across the School of Biosciences and is available to support another academic pathologist in training, who is likely to make use of this equipment during the final year of his PhD.

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**Results of representative research work:**

The Qubit has provided accurate quantification of nucleic acids, which has been essential in the development of sequencing libraries. For example, the Qubit has been used to quantify input nucleic acids and subsequent sequencing libraries for ChIP-Seq. The Qubit has also been used to quantify nucleic acids prior to qPCR and preparation of sequencing libraires in a project investigating the role of a novel protein in DNA repair pathways. The Qubit is also able to accurately quantify protein and provides results comparable to the currently used Bradford assay (Figure 1).



Figure 1: Graph showing protein concentrations quantified by Bradford assay and Qubit on 8 samples.

**Conclusions:**

We are very grateful to the Pathological Society for this grant which has allowed us to purchase a Qubit Flex Fluorometer for the laboratory. This equipment has been essential for accurate quantification of nucleic acids and protein, and we anticipate data which has arisen from its use will be used in multiple publications in the future.

**How closely have the original aims been met:**

The original aims have been met in full.

**Outputs:**

To follow